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# Influence of Cadmium on Certain Biological Activities in a Cadmium-Tolerant Fungi

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## ABSTRACT

*Aspergillus carbonarius* and a strain of *Penicillium* are able to grow on Harrold's agar media amended with different concentrations of cadmium chloride up to 2.5% (w/v). Considerable quantities of cadmium were absorbed by both fungi. *A.carbonarius* absorbed more cadmium than the *Penicillium sp.* did, under the same culturing conditions. In the presence of cadmium, the determined cellular contents of proteins, lipids, and carbohydrates were extraordinary high, whereas the activities of certain enzymes, lipases, amylases, and proteases were inhibited. The fungal rate of growth and sporulations were mostly suppressed. Conidiations were inhibited at lowest concentrations. At 1% Cd $Cl_2$, *A.carbonarius* produced malformed conidiophores, whereas the *Penicillium sp.* was less affected. At higher concentrations conidiophores production were entirely suppressed and several hyphal swellings were produced.

**Index Entries:** Cadmium toxicity; morphological distortions; cadmium-tolerant fungi; amylases; lipases; proteases; conidiogenesis; *Aspergillus carbonarius;* strain of *Penicillium.*

## INTRODUCTION

Tolerance of fungi to a variety of heavy metals has been reported by several investigators (*1,2*). It is evident that fungi can withstand levels of these metals far in excess of those tolerated by higher plants. Even in

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uncontaminated habitats fungi tend to have greater internal levels of heavy metals than angiosperms (*3,4*).

However, it was concluded that heavy metal pollutants inhibit a number of fungal biological activities including respiration, mycelial growth, spore production, and germination. Such effects are mediated by environmental factors including pH, humidity, temperature, clay minerals, cation exchange capacity, inorganic cations, anions, and organic matter. Although numerous in vitro studies of these factors were considered, there are few field studies of the effects of particular sources of pollution on fungal ecology. Nevertheless, pollution-sensitive fungi, e.g., *Sporobolomyces roseus* or fungi tolerant towards heavy metals, e.g., *Aureobasidium pullulans* may be used as an indicator species, however, the use of an organism sensitive to a pollutant as an indicator species is of limited value (*5*).

The growth of several ectomycorrhizal fungi on Hagem's agar was mostly inhibited by 350 μg cadmium/mL (*6*). On the other hand, the action of several heavy metals: Se, Co, Te, An, Hg, and Cd on fungal growth was investigated. Mercury was found to be the most toxic element. It was also reported that selenium, tellurium, and cadmium showed an inhibitory action on the growth and conidiogenesis of *Aspergillus fischeri* and a strain of *Alternaria*. They also concluded that the significant increase in proteins and carbohydrates content of the fungi possibly a regulatory mechanism to control the sorption of heavy metals (*7*). Recently, the growth of *Aspergillus terreus* on very low concentrations of cadmium appeared unaffected, whereas conidiophore production was inhibited and abnormal conidiophores were produced at high concentrations (*8*).

The uptake of cadmium in *Saccharomyces cerevisiae* and *Rhodotorula rubra* was the same but *Saccharomyces* cells were much more sensitive to cadmium than *Rhodotorula* cells. In both strains the effect of Cd on protein synthesis and on transport of glucose or adenine through membranes was low, and the effect on RNA and ribosome synthesis was high (*9*).

In this study the influence of cadmium on certain metabolic activities in a cadmium tolerant fungi has been considered aiming to proceed via the understanding of cadmium metabolism in fungi.

## MATERIALS AND METHODS

### *Organisms*

A previously identified cadmium-tolerant fungi *Aspergillus carbonarius* and *Penicillium sp.* were isolated from Sinai soil at Egypt. The organisms were photographed using a phase contrast light microscope (ZEISS 47/30 112-9902).

### Medium

Harrold's medium was chosen for the study according to a preliminary investigation on several media. The medium was amended with different concentrations of either cadmium chloride, zinc sulphate, or mercuric chloride. The pH was adjusted to 6.4.

### Preparation of Fungal Cell Free Extract

The harvested mycelia were homogenized with an approximately equal volume of 60% (v/v) ethanol using a MSE homogenizer. The slurry was centrifuged at 6000 rpm for 10 min. The supernatant was collected and concentrated using coarse Sephadex G25.

### Enzymes Assay

Proteases and amylases were assayed according to the method of Salle (*10*), whereas lipases activity was determined using the tributyrine clearing zone assay (*11*).

### Protein Determination

Protein was determined quantitatively with the folin-Phenol reagent using bovine serum albumin as a standard protein (*12*).

### Lipid Determination

Lipids were determined quantitatively using the sulpho-phosphovanillin method (*13*). Cholesterol was used as a standard lipid.

### Carbohydrate Determination

Carbohydrates were determined quantitatively using the anthrone reagent (*14*). Sucrose was used as a standard carbohydrate.

### Cadmium Determination

Cadmium was determined using dithiol/Loral solution reagents (*15*). Cadmium chloride was used as a standard cadmium containing compound.

## RESULTS

Although cadmium, zinc, and mercury occupy the same group in the periodic table, their action on microorganisms is quite different. Mercury showed complete inhibition of the fungal growth; no growth was detected on Harrold's, Cazapek Dox, and malt extract media. However,

cadmium chloride was less inhibitory, particularly at low concentrations. On the other hand, no harmful action of zinc sulphate was noticed on both fungi even at high concentrations not exceeding 2% (w/v).

### Cadmium Uptake and Fungal Responses

*Aspergillus carbonarius* absorbed cadmium more than the *Penicillium sp.* did. Although maximum quantity of cadmium content in *A.carbonarius* extract was detected at 1% Cd $Cl_2$ containing medium, minimum cadmium content was determined at 2% Cd $Cl_2$. Therefore, the absorbed quantities of cadmium are not dependent on the supplied cadmium concentration to the growth media (Table 1). Presumably several factors are influencing cadmium absorption by the fungal cells.

Data in Table 1 shows that mycelial dry weights of both fungi decreased with increasing Cd $Cl_2$ concentrations in the growth media. However, reasonable quantities of the mycelial dry weights were obtained in the presence of relatively high concentration of Cd ions. Alternatively, the detected quantities of proteins, carbohydrates, and lipids were increased considerably in the presence of Cd ions in the medium.

Although maximal quantity of mycelial dry weights of *A. carbonarius* and *Penicillium sp.* were obtained on cadmium free media, the maximum quantities of carbohydrates, proteins, and lipids in both fungi were obtained at 0.5% (w/v) and at 0.1% (w/v) Cd $Cl_2$, respectively. Interestingly, the determined quantities of proteins, carbohydrates, and lipids in *A. carbonarius* extracts were generally higher when cultivated on cadmium containing media than on cadmium free media.

The obtained results may give the impression that the increase in carbohydrates, proteins, and lipids contents in both fungi are presumably a response towards the presence of cadmium compound in the growth environment. The elevated contents of proteins and lipids may perhaps bind the absorbed $Cd^{+2}$, whereas such high quantities of carbohydrates may equilibriate the osmotic pressure exerted by cadmium compounds in the external environment. Or, probably their accumulation is an indication of disorders in certain metabolic activities.

### Antimicrobial Activities of the Produced Pigmented Metabolites by A. carbonarius

*A. carbonarius* produced heavy and black colored pigments when cultivated on cadmium containing media. It was expected that such pigmented metabolites could have anti-microbial activities. So the antimicrobial activities of the pigmented metabolites were assayed against: *Bacillus subtilis, B.mycoides, B.cereus, E. coli,* and *Salmonella typhosa*. Unfortunately, no antimicrobial activities were detected on such restricted scale.

Table 1
Cadmium, Proteins, Carbohydrates, and Lipids Content of *Aspergillus carbonarius* and *Penicillium sp.* Cultivated on Harrold's Liquid Medium Supplemented with Different Concentrations of Cadmium ($CdCl_2$) for 7 Days at 28 ± 2°C

| Organism | Cadmium chloride Concentration, % w/v | Mycelial dry weight, mg × ml$^{-1}$ medium | Cadmium content, µg × g$^{-1}$ | Protein µg × g$^{-1}$ dry weight | Carbohydrates, µg × g$^{-1}$ dry weight | Total lipids, µg × g$^{-1}$ dry weight |
|---|---|---|---|---|---|---|
| *Aspergillus carbonarius* | 0.0 | 75.00 | 0.0 | 233.3 | 83.0 | 216.6 |
| | 0.1 | 69.50 | 288 | 273.4 | 173.0 | 461.3 |
| | 0.5 | 51.25 | 424 | 653.7 | 283.0 | 853.6 |
| | 1.0 | 37.50 | 513 | 426.7 | 266.7 | 830.0 |
| | 1.5 | 35.75 | 489 | 419.5 | 244.7 | 629.4 |
| | 2.0 | 18.50 | 260 | 365.3 | 202.7 | 506.7 |
| *Penicillium sp.* | 0.0 | 82.75 | 0.00 | 283.9 | 148.0 | 376.1 |
| | 0.1 | 37.25 | 200 | 604.0 | 275.0 | 671.1 |

### *Influence of Cd on the Biosynthesis of Certain Common Enzymes*

The activities of amylases, proteases and lipases which produced exogenously and those biosynthesized endogenously by *A.carbonarius* and Penicillium sp. were determined (Table 2).

The detected activities of both endogenously synthesized and exogenously produced considered enzymes were inhibited by the presence of cadmium. Marked suppression was detected with increasing cadmium concentrations. This may be attributed to the drastic influence of Cd ions on the enzymes. However, it is not clear whether the inhibition is because of an inhibition of their activities or on their productivities. However, it remains to be determined.

Such inhibitory action on those enzymes may perhaps clarify the increased levels of proteins, carbohydrates, and lipids in the presence of cadmium as a metabolic disorder leading to their accumulation.

### *Influence of Cadmium on the Morphological Characteristics of* A. carbonarius *and* Penicillium *sp.*

*A.carbonarius* is characterized by its rapid growth and abundant sporulation when cultivated on control medium. The morphology of the aerial mycelia of *A. carbonarius* and *Penicillium sp.* grown in the presence of different concentrations of cadmium chloride are illustrated (Figs. 1,2).

Generally, the fungal growth and sporulation were mostly suppressed by the presence of cadmium chloride (Fig. 1). Although the fungus was able to grow at high concentrations of cadmium up to 2.5% (w/v), its conidiation was distorted drastically. At 1% Cd $Cl_2$ agar medium, the fungus failed to give conidial heads and consequently no sterigmata. However, it gave stunted, short, thick, and trunk-like structures that represent conidiophores. Those distorted forms of conidiophores were carrying few numbers of solitary conidia, some of them were carried on single enlarged sterigmata like structure. Further increase in Cd $Cl_2$ concentrations leads to complete distortion. Such conidiophores like structures were disappeared completely and relatively few chlamydospores were noticed on media amended with more than 1% (w/v) Cd $Cl_2$. Moreover, abundant hyphal swellings were noticed at 2.5% Cd $Cl_2$.

Although the fungus failed to grow at 2.5% Cd $Cl_2$ containing liquid medium, it grew on such concentration when Cd $Cl_2$ was supplemented into agar medium. Presumably, the free ionic activity of cadmium ions in liquid medium is much higher than in agar medium, or the availability of Cd ions in agar medium is lower than in liquid medium.

Interestingly, *Penicillium sp.* was able to grow on agar medium up to 2.5% Cd $Cl_2$, whereas on liquid medium it failed to grow at 1% or more. This may support our thought that solid environment decreases metal ions activities. So the harmful effect of cadmium in agar medium on fun-

Table 2
Activities of Certain Enzymes Biosynthesized by *Aspergillus carbonarius* and *Penicillium sp.* Cultivated on Harrold's Media Amended with Different Concentrations of Cadmium Chloride for 7 Days at 28 ± 2°C[a]

| Organism | Cd $Cl_2$, % w/v | Amylases | | Proteases | | Lipases | |
|---|---|---|---|---|---|---|---|
| | | Endogenous | Exogenous | Endogenous | Exogenous | Endogenous | Exogenous |
| *Aspergillus carbonarius* | 0.0 | 15 | 17.0 | 14 | 15 | 12 | 13 |
| | 0.1 | 13 | 14.0 | 13 | 14 | 10 | 11 |
| | 0.5 | 11 | 12.0 | 12 | 13 | 9 | 10 |
| | 1.0 | 10 | 11.0 | 10 | 11 | 8 | 9 |
| | 1.5 | 9 | 9.5 | 8 | 9 | 7 | 8 |
| | 2.0 | 8 | 9.0 | 7 | 8 | 6 | 7 |
| *Penicillium sp.* | 0.0 | 14 | 15.0 | 13 | 14 | 12 | 13 |
| | 0.1 | 10 | 11.0 | 12 | 13 | 11 | 12 |

[a]Control diameter of each hole in the substrate media was 5 mm. Data are expressed as mean diameters of the hydrolyzed zones of substrates by their specific enzymes.

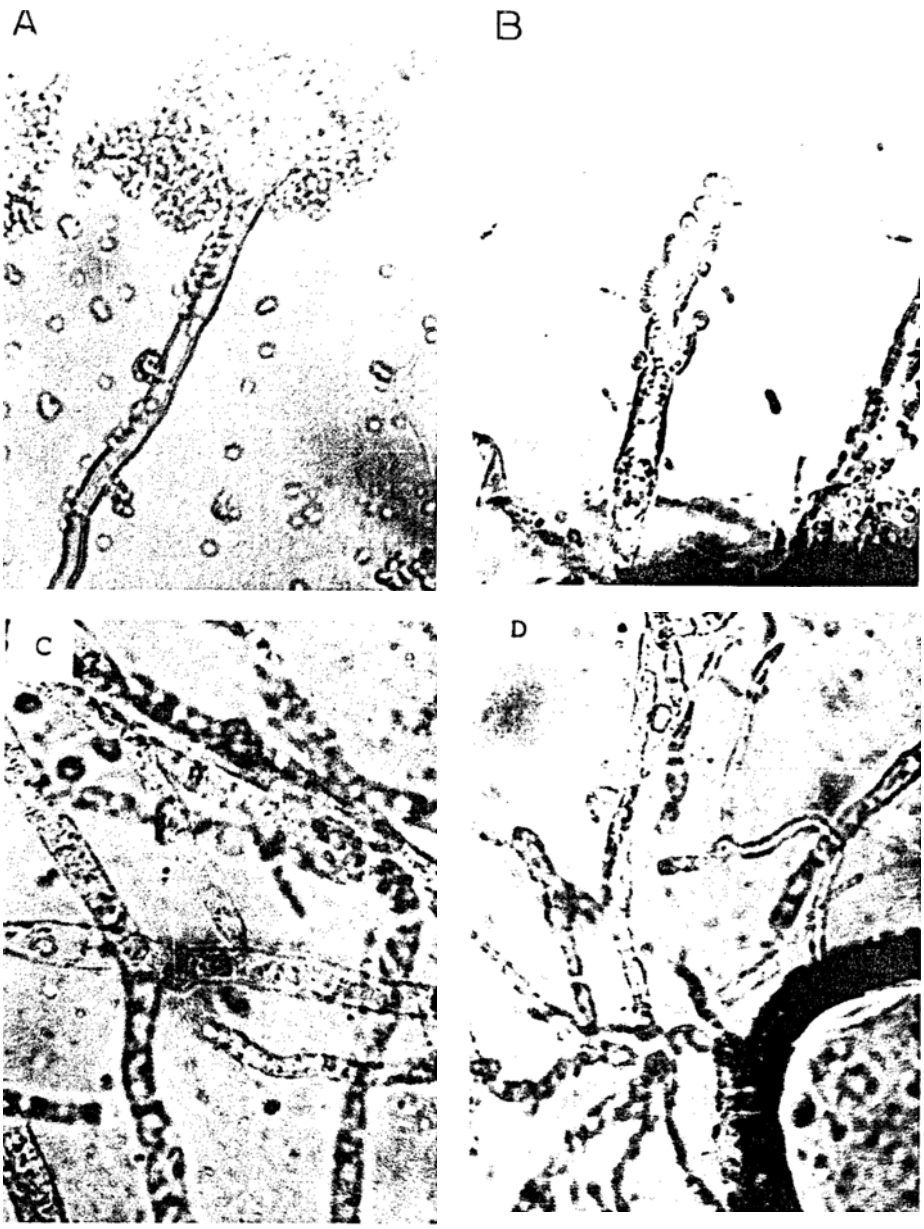


Fig. 1. Microphotographs of *A. carbonarius* grown on Harrold's agar media supplemented with different concentrations of Cd $Cl_2$%. A, Cadmium free medium: **B,** 1%; **C,** 1.5%; and **D,** 2.5% (X = 800).

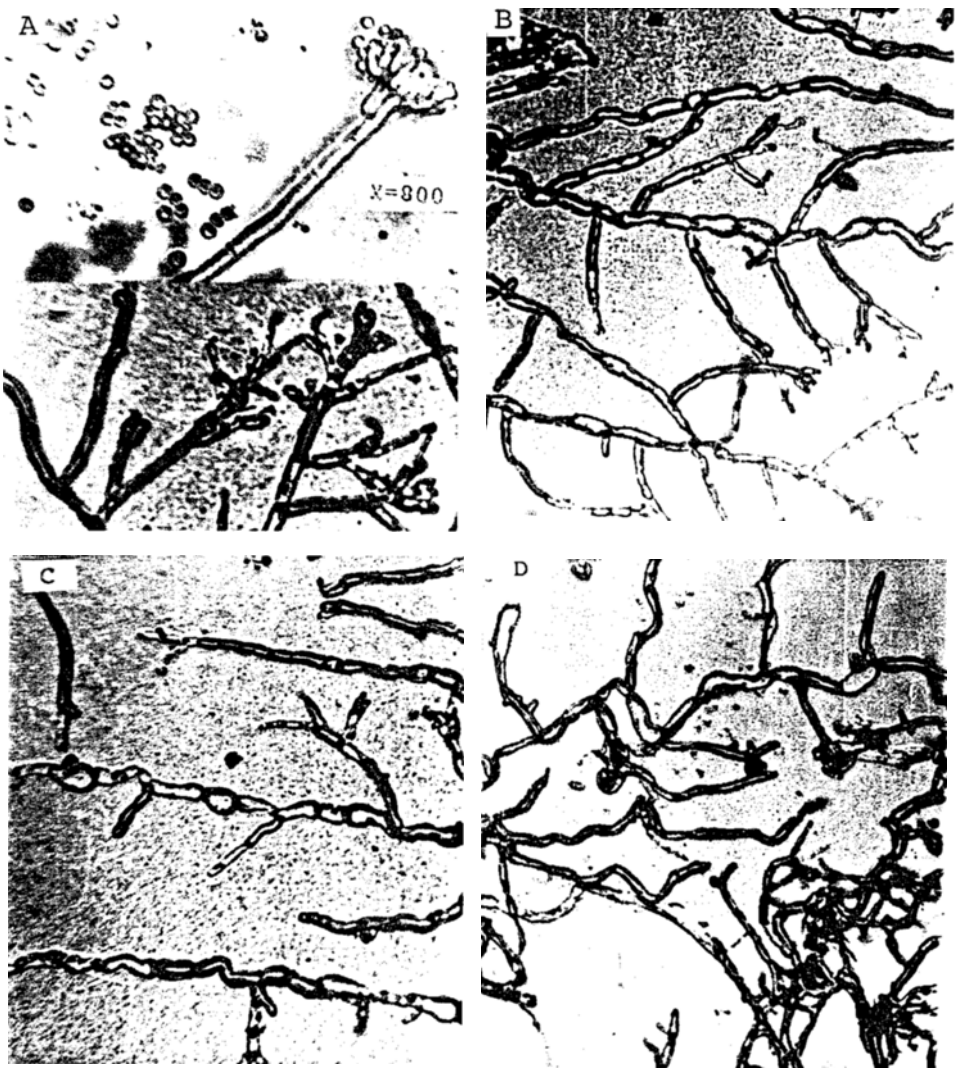


Fig. 2. Microphotographs of *Penicillium sp.* cultivated on Harrold's agar media provided with different concentrations of Cd $Cl_2$%. A, Cadmium free medium; **B,** 0.5%; **C,** 1%; and **D,** 2.5%. X = 500 otherwise stated.

gal hyphae is less than that in liquid medium. Control cultures of *Penicillium sp.* sporulated abundantly. Although sporulation was reduced sharply at 0.5% Cd $Cl_2$, stipes and phialides appeared normal (Fig. 2). At 1% Cd $Cl_2$ agar, stipes appeared unaffected, whereas no phialides were detected. Moreover, abundant hyphal swellings were noticed. Sporulation was entirely suppressed.

At 2.5% Cd $Cl_2$, tips of fungal hyphae appeared muddled up. Amazingly, few stipes were detected as well.

## DISCUSSION

Cadmium is one of several trace metals existing in nature in small quantities and having no known nutritive value, but is capable of producing toxic effects. It has been described as a cytotoxic agent with a particular affinity towards sulfhydryl groups and to a certain extent for hydroxyl groups and ligands containing nitrogen. Cadmium is also a potent inhibitor of several enzyme systems (*16,17*).

Although, *A. carbonarius* and the *Penicillium sp.* were able to tolerate the presence of cadmium in the growth media up to 2.5% (w/v) when grown on a synthetic laboratory agar medium, possibly higher concentrations of cadmium can be tolerated by such fungi when living normally in soil as several physical and chemical factors may occur. It was reported that several environmental factors associated with fungal growth in soil, such as the binding of the metal ions by organic chelating agents or its adsorption by clay minerals or organic matter, may serve to mitigate the inhibitory and toxic effects of cadmium (*18*).

It is evident from the foregoing results the actual absorption of cadmium in a considerably high levels, particularly in *A. carbonarius*. Therefore, it may be considered as a highly cadmium-tolerant fungus.

The inhibition of *A. carbonarius* and the *Penicillium sp.* growth and sporulations consistent with the held views that the inhibition of growth and sporulation by concentrations of heavy metals that permit vegetative growth seems a widespread phenomenon in microorganisms (*5–8, 19–22*).

On the other hand, some effects of sublethal doses of cadmium on the studied fungi were reflected on the morphological distortions, inhibition of several enzymes, as well as extraordinary accumulation of lipids, proteins, and carbohydrates. The morphological distortions of several microorganisms cultivated in the presence of cadmium were also reported by several investigators (*7,8,20,21,23*). Moreover, cadmium is known to induce serious malformations in animals as well (*24–26*).

Although *A. carbonarius* and the *Penicillium sp.* absorbed considerable quantities of cadmium into their cells, it seems quite possible that they were able to control the passage of $Cd^{+2}$ into their cells. The absorbed quantities in both fungi were very low in comparison with the quantities supplied into the media. Nevertheless, the presence of several elemental ions in the growth media may possibly interact with cadmium, reducing its toxicity and therefore protecting the fungi against its harmful action.

Although cadmium–elements interactions have not been established in microorganisms, the biochemical basis for the detoxification of cadmium in animals by several elements is becoming clear. The protective effects appear to be connected with changes in the chemical reactivity and distribution of cadmium in the animal (*27–31*).

The channeling of the absorbed cadmium within *A.carbonarius* and the strain of *Penicillium* remains to be clarified.

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