

uplift of the coast during the Terminal Pleistocene period. Reactivation of the boundary fault which trends almost NE and projecting into the sea near Mamallapuram could have been responsible for the neotectonic activity. This gains support from the data that Holocene to Terminal Pleistocene beach rocks and oyster bed deposits are globally a regional formation and that during this period the 'sea level' was several meters ( $120 \pm 20$  m) below the present position<sup>5,6</sup>. Since the Terminal Pleistocene period the coastal geomorphology in the study area has not changed drastically but for meagre channel shifts in the tidal flat zone. From the present study, as the results reveal, it is possible to offer a first order interpretation of the <sup>14</sup>C dates and data, but the interplay among the elements of neotectonism, global sea level change and sedimentation makes interpretation all the more difficult.

1. Katupotha, J. J., *Natnl. Sci. Coun. Sri Lanka*, 1994, **22**, 157-187.
2. Reineck and Singh, I. B., *Depositional Sedimentary Environments*, Springer, 1980.
3. Satyanarayana Rao, K., *Central Marine Fish. Res. Inst. Bull.*, 1974, **25**, 4-39.
4. Stuiver, M. and Braziunas, T. F., *Radiocarbon*, 1993, **35**, 137-189.
5. Pirazzoil, P. A., *World Atlas of Holocene Sea Level Changes*, Elsevier, Amsterdam, 1991, pp. 1-291.
6. Badve, R. M., Rajshekhar, C., Kumaran, K. P. N. and Kamble, V. V., *Curr. Sci.*, 1997, **72**, 168-170.

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## Isolation of a cadmium tolerant *Curvularia* sp. from polluted effluents

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A fungus exhibiting high level of tolerance to cadmium was isolated from polluted effluents by selection on cadmium-containing agar medium. The fungus, identified as *Curvularia lunata*, showed high tolerance towards cadmium in both solid and liquid media ( $I_{50}$  30-60 mM). The mechanism of tolerance is shown to be due to most of cadmium (90%) being accumulated on mycelial surface, extractable by EDTA. Metal-chelate affinity chromatography of cell-free extracts showed that most of the cadmium was in ionic form, not associated with any specific proteins. Biosorbent prepared by alkali extraction of mycelia of *Curvularia* bound metal ions up to 6% of biosorbent (w/w).

METAL toxicities have received widespread attention because of increasing number of toxic metals being released into the environment, their extended persistence and toxicity to a wide variety of organisms. Cadmium has been recognized as one of the most toxic elements and its mobilization into the biosphere has been accelerated by rapid industrialization<sup>1</sup>. Hence this aspect received wide attention with respect to its mechanisms of transport, toxicity and resistance in micro-organisms. In general, cadmium is reported to be toxic at relatively low concentrations to micro-organisms<sup>2</sup>. The most tolerant fungi which include species of *Rhizopus*, *Trichoderma*, *Penicillium* and *Cunninghamella* were found to grow at 1000 ppm of cadmium, while sensitive ones grew between 10 and 100 ppm (ref. 3). *Penicillium lilacinum* which comprised 23% of the total fungi isolated from polluted mine drainage was reported to be tolerant up to 10,000 ppm of cadmium<sup>4</sup>. But in the above cases the mechanism of tolerance/resistance has not been investigated. In case of yeast the maximum concentration tolerated was found to be 600 ppm of cadmium on solid media<sup>5</sup>.

Heavy metal resistance in fungi has been investigated in greater detail in mutants isolated in the laboratory<sup>6-8</sup> by gradual adaptation on toxic metal ion containing media or by mutagenesis<sup>9,10</sup>. A number of metal-resistant fungi isolated from polluted environment have also been reported<sup>10,11</sup>, but the mechanism of resistance in most cases was not studied. Resistance to heavy metals in micro-organisms can be due to any one of the two broad mechanisms (i) transport block which restricts the entry of toxic metals and/or, (ii) intracellular sequestration

into vacuoles or binding to specific proteins, viz. metallothioneins<sup>12</sup>.

In an effort to isolate a cadmium-tolerant species of fungi from polluted environment, samples of highly polluted industrial effluent collected from an effluent treatment plant (Jeedimetla, Hyderabad) were plated on 2% agar in basal medium<sup>8</sup> containing cadmium (10 mM) and incubated at  $28 \pm 1^\circ\text{C}$  up to seven days. From the few colonies (4–5) which appeared, one of them which exhibited better ability to grow was picked up and subcultured for further studies. The cadmium-tolerant fungal species was identified as *Curvularia lunata*. Cadmium toxicity in *Curvularia* was studied first on solid medium. Figure 1 shows that this fungus was able to grow at very high concentrations of cadmium (up to 60 mM). However growth inhibition was observed with an  $I_{50}$  (50% growth inhibition of colony diameter) of about 30 mM cadmium at five days. Cadmium tolerance was then quantitated in liquid medium<sup>8</sup> and results show that  $I_{50}$  (50% growth inhibition measured on dry weight basis at five days) occurred at about 60 mM. Cadmium uptake values determined by atomic absorption spectrophotometry of acid digested mycelia<sup>13</sup> indicate increase in cadmium uptake with increase in its medium concentration and at  $I_{50}$  value (60 mM derived from Figure 2) about  $1200 \mu\text{g}$  cadmium  $100 \text{ mg}^{-1}$  dry wt of mycelia was accumulated (Figure 2). In order to quantitate cadmium that is associated with cell surface (adsorbed) and intracellular fractions, surface-bound cadmium was leached by floating mycelia (grown previously in 20 mM cadmium for five days) in 10 ml EDTA solution (10 mM) for 5 min. Cadmium remaining with mycelia was assumed to be intracellular. Table 1 indicates that most of the cadmium (90%) is associated with cell surface (EDTA leachable). Further, cell-free extracts of mycelia grown in cadmium (20 mM) contained only 10% of total cadmium in the 10,000 g supernatant and the remaining cadmium was accounted in the insoluble fraction containing cell wall debris. In further experiments the association of cadmium in cell-free extracts with any specific protein was investigated by DEAE-cellulose column chromatography and metal-chelate affinity chromatography. The results indicated that most of the cadmium was found to be in the ionic form and was not associated with any proteins. Cadmium resistance in yeast has been investigated in greater detail and in most cases binding by specific metallothionein and other proteins was found to be the major mechanism of resistance<sup>14,15</sup>. To check whether metal was precipitated in the form of sulphides on the surface, presence of metal sulphides was tested by the method of King and Morris<sup>16</sup> and was found to be absent.

Since biosorption of toxic metal ions has excellent potential for decontaminating polluted effluents, biosorbent from mycelial biomass of *Curvularia* was pre-

pared by alkali extraction<sup>17</sup>. The binding capacity was tested by suspending biosorbent for 1 h in solutions containing cadmium and copper (50 mM) ions. Table 2

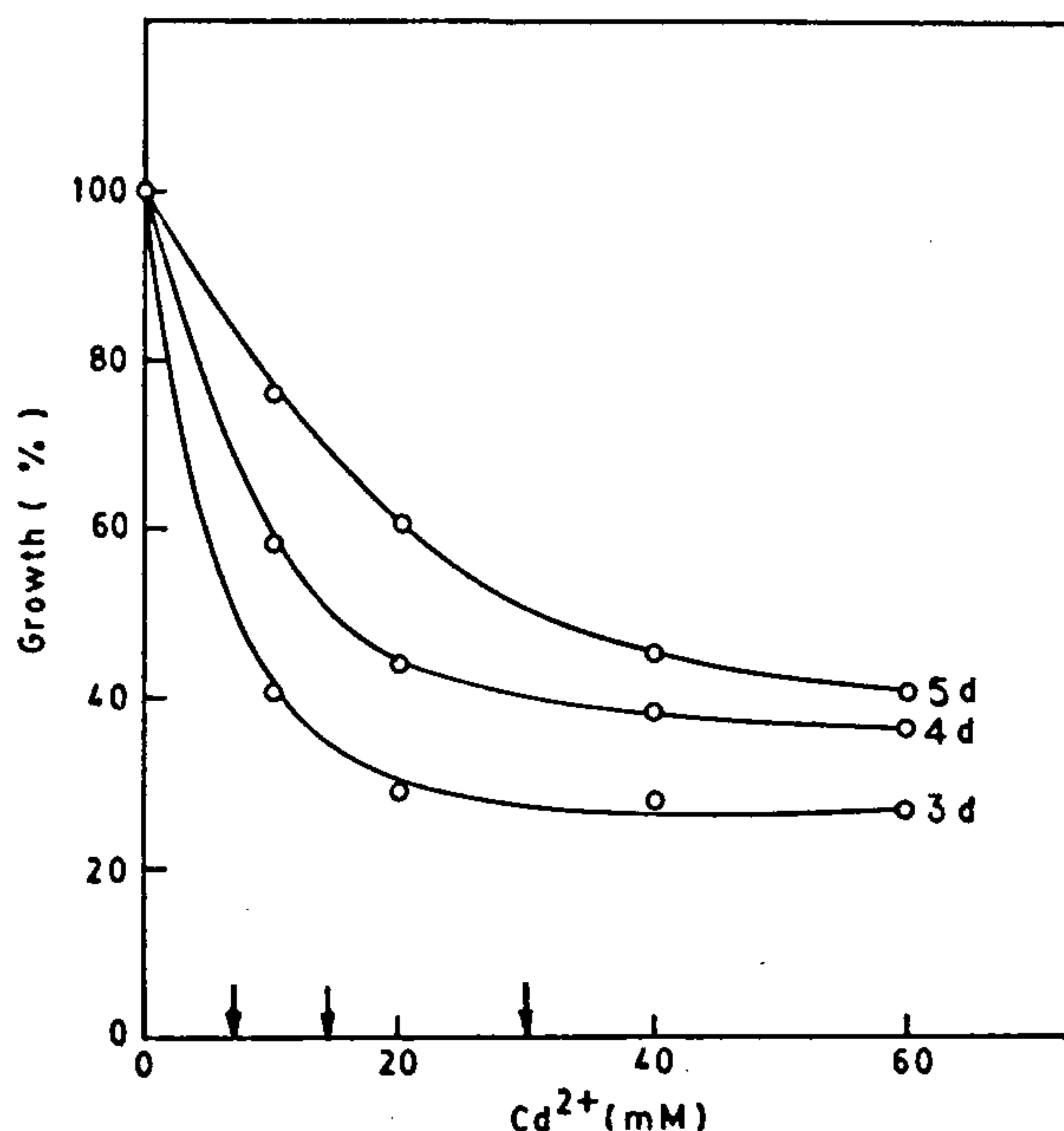


Figure 1. Cadmium toxicity in *Curvularia lunata*. The conidiospores were inoculated on agar medium containing indicated concentrations of cadmium. Colony growth was determined by measuring diameter at different time intervals. Control growth (100%) without cadmium is 4, 5.2 and 6 cm for 3, 4 and 5 days respectively.

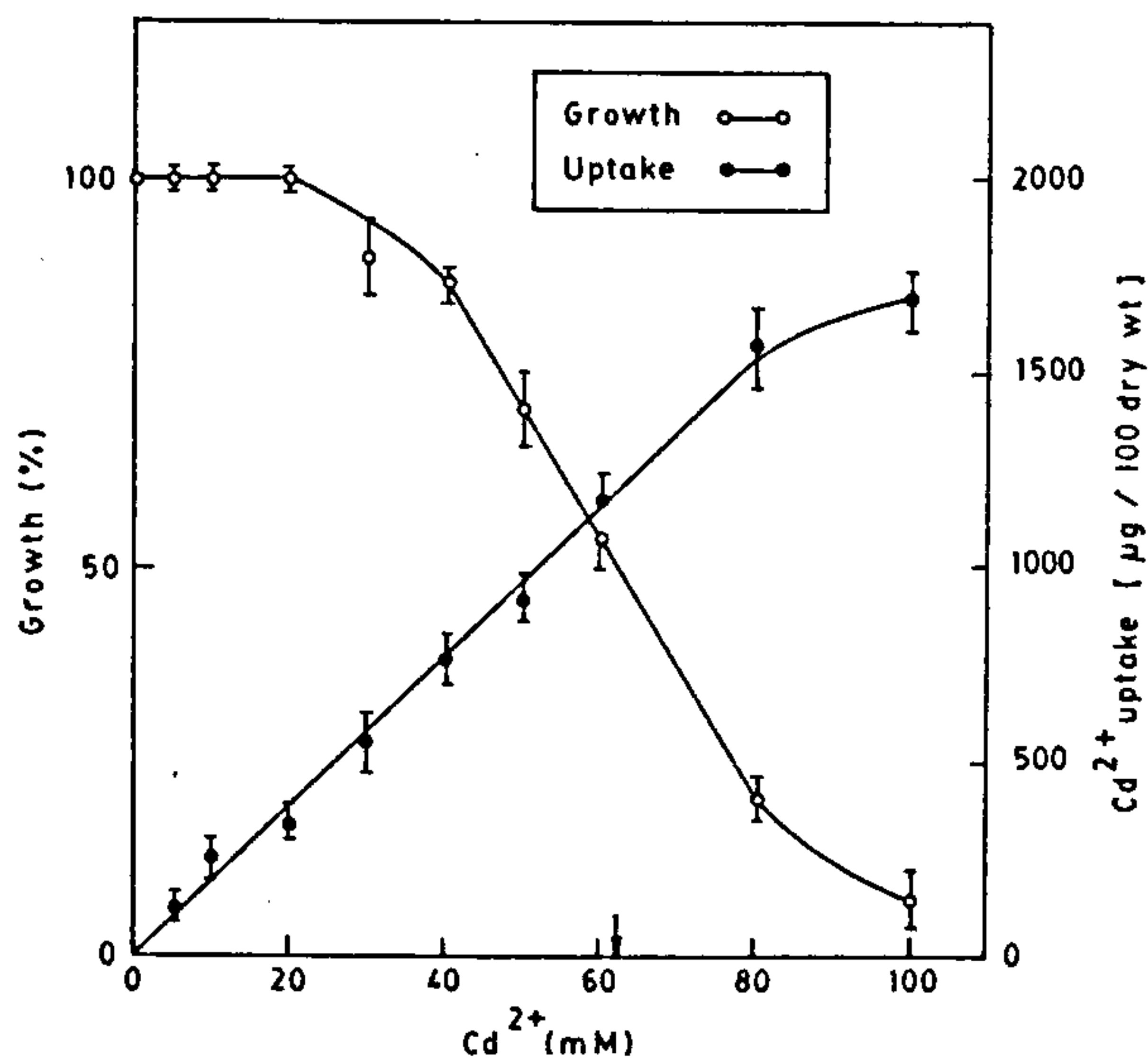


Figure 2. Cadmium toxicity in *Curvularia lunata*. The conidiospores ( $10^7$ ) were inoculated in 10 ml basal medium containing indicated concentrations of cadmium. The mycelia were harvested at 5 days and dried to record growth. Cadmium content was estimated by atomic absorption spectrophotometry after acid digestion. For details see text. The arrow mark indicates 50% growth inhibition. Control dry wt (100%) =  $82 \pm 2 \text{ mg}$ .



**Table 1.** Distribution of cadmium between cell surface and intracellular fractions

Cadmium ( $\mu\text{g } 100 \text{ mg}^{-1}$ ) [%]		
Surface-bound fraction (EDTA leached)	Intracellular fraction	Total
43 $\pm$ 21 [89.7%]	28.5 $\pm$ 5 [10/3%]	271 $\pm$ 26 [100%]

*Curvularia lunata* was grown for 5 days in 20 mM cadmium-containing medium. Mycelia were washed thoroughly with distilled water and resuspended in 10 ml of EDTA (10 mM) solution for 5 min to remove the surface-bound cadmium. Cadmium was estimated by atomic absorption spectrophotometry. Values shown are averages from three separate experiments, each in duplicate ( $\pm$  SD).

**Table 2.** Metal binding by biosorbent of *Curvularia* sp.

Metal ion	Metal bound (mg 100 mg <sup>-1</sup> dry weight)
Cadmium	6.0 $\pm$ 0.5
Copper	3.2 $\pm$ 0.4

Biosorbent (100 mg dry wt) was suspended in 20 ml solution containing 50 mM metal ion. After 1 h incubation, biosorbent was washed with distilled water and bound metals were eluted with 0.1 N HCl and estimated by atomic absorption spectrophotometry. Values shown are averages from three separate experiments, each in duplicate ( $\pm$  SD).

indicates that biosorbent binds 6.0 mg cadmium/100 mg dry weight and 3.2 mg copper/100 mg dry weight. This is about 3–6% of dry weight of the biosorbent.

In the present study the cadmium-tolerant *C. lunata* though selected initially for its growth in 10 mM cadmium, was found to grow even up to 10-fold higher concentrations of cadmium in liquid media, with accompanied growth inhibition. Further, when the tolerant capacity was quantitated systematically, 50% growth inhibitory concentration in solid and liquid media was found to be 30 mM and 60 mM respectively. The surprising feature was that cultures displayed better tolerance in liquid medium when compared to solid medium. Investigation into the mechanism of tolerance has clearly shown that most of the metal does not enter the cell, but is bound to cell surface in liquid media, while the same could not be carried out on solid medium. This mechanism is rather unique and has been demonstrated in cases of copper and cadmium-tolerant strains of yeast (reviewed by Ashida<sup>11</sup>). However in the above cases production of hydrogen sulphide which precipitates these metals at the cell surface was shown to be the mechanism of resistance. In the present study such a possibility was excluded. It should be noted that the present isolate of *C. lunata* was isolated from highly polluted effluents containing undefined number of toxic agents. The concentration of cadmium in these effluents was not high enough to suggest that the present isolate is a cadmium-adapted culture. However, other possibilities of a mutation caused by a pollutant other than cadmium cannot be excluded. The terms *resistance* and *tolerance* have

been used interchangeably in the literature. Recently, Gadd<sup>10</sup> defined 'resistance' as the ability of an organism to survive metal toxicity by mechanisms produced in direct response to metals; while 'tolerance' may be defined as the ability of an organism to survive metal toxicity by means of intrinsic properties and/or environmental modification of toxicity. In the present work the absence of any specialized mechanism induced in response to cadmium, like elaboration of specific cadmium-binding proteins suggest that the present isolate is most likely a *tolerant* fungus.

The intrinsic property of *C. lunata* to bind more than 90% of cadmium to surface of mycelia has significance in bioremediation of toxic metal ions from polluted effluents. This has been demonstrated by biosorbent preparations which bind high levels of copper and cadmium, up to 6% of biomass (w/w). Biosorbent preparations using fungi were shown to have excellent potential in removing toxic metal ions from polluted effluents<sup>18–20</sup>.

- Babich, H. and Stotzky, G., *Adv. Appl. Microbiol.*, 1978, **23**, 55–117.
- Trevors, T. T., Stratton, G. W. and Gadd, G. M., *Can. J. Microbiol.*, 1986, **32**, 447–464.
- Babich, H. and Stotzky, G., *Appl. Environ. Microbiol.*, 1977, **33**, 681–695.
- Tatsuyama, K., Egawa, H., Senmaru, H., Yamamoto, H., Ishioka, S., Tamatsukuri, T. and Saito, K., *Experientia*, 1975, **31**, 1037–1038.
- Gadd, G. M., *J. Appl. Bacteriol.*, 1983, **54**, 57–62.
- Maruthi Mohan, P. and Sivarama Sastry, K., *Biochem. J.*, 1983, **212**, 205–210.
- Sajani, L. S. and Maruthi Mohan, P., *Biometals*, 1997 (in press).
- Venkateswerlu, G. and Sivarama Sastry, K., *Biochem. J.*, 1973, **132**, 673–677.
- Rama Rao, V. S. K. V., Wilson, H. G. and Maruthi Mohan, P., *Biometals*, 1997, **10**, 147–156.
- Gadd, G. M., *New Phytol.*, 1993, **124**, 25–60.
- Ashida, J., *Annu. Rev. Phytopathol.*, 1965, **3**, 153–174.
- Mehra, R. K. and Winge, D. R., *J. Cell Biochem.*, 1991, **45**, 1–11.
- Sastry, K. S., Raman, N. and Sarma, P. S., *Anal. Chem.*, 1962, **34**, 1302–1304.
- Murasugi, A., Wada, C. and Hayashi, Y., *J. Biochem. (Tokyo)*, 1981, **90**, 1561–1564.
- Murasugi, A., Wada, C. and Hayashi, Y., *Biochem. Biophys. Res. Commun.*, 1981, **103**, 1021–1028.
- King, T. E. and Morris, R. O., *Methods Enzymol.*, 1967, **10**, 634–641.
- Akthar, N. Md., Sastry, K. S. and Maruthi Mohan, P., *Biotechnol. Lett.*, 1995, **17**, 551–556.
- Akthar, N. Md. and Maruthi Mohan, P., *Curr. Sci.*, 1995, **69**, 1028–1030.
- Akthar, N. Md., Sastry, K. S. and Maruthi Mohan, P., *Biometals*, 1996, **9**, 21–28.
- Volesky, E., *FEMS Microbiol. Rev.*, 1994, **14**, 291–302.

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