Table 2 *Effect of incubation period on tricalcium phosphate and rock phosphate solubilization by A. niger As3*

Incubation period (days)	Tricalcium phosphate			Rock phosphate		
	Control pH	pH after growth	P_2O_5 solubilization (mg/50 ml)	Control pH	pH after growth	P_2O_5 solubilization (mg/50 ml)
3	5.0	3.35	6.80	5.0	4.35	5.10
7	4.9	3.20	12.40	4.8	3.65	7.20
10	4.8	3.10	9.50	4.8	3.80	10.20
13	4.8	3.20	8.10	4.7	4.0	8.50
CD at 5%	NS	0.065	0.392	NS	0.082	0.561

Cultures were isolated from different samples of rhizospheres of wheat, gram, garden soils and compost materials (Instructional Farm, N. D. University of Agriculture and Technology) and they were grown on solid medium containing $Ca_3(PO_4)_2$ as described by Pikovskaya[6]. Of several isolates, only ten fungal and three bacterial cultures were isolated on the basis of clear zone formation around the colonies. Different isolates were characterized and designated as As1, As2, As3, As4, (from compost), As5, As6, (wheat rhizosphere), Ts1, Ts2, (garden soil), Pf1, Pf2 (gram rhizosphere) and gram negative bacteria 1 and 2 from gram rhizosphere.

All the cultures were screened in liquid medium of Pikovskaya using tricalcium phosphate (0.25 g/50 ml) and incubated for seven days at 28 ± 1°C. An uninoculated control was also kept. Among the efficient isolates, *Aspergillus niger* As3 was found to be most effective for maximum solubilization of tricalcium phosphate (table 1).

A separate experiment was conducted to evaluate the effect of *A. niger* As3 on phosphate solubilization of rock phosphate and tricalcium phosphate equivalent to 50 mg P_2O_5 were added separately in Pikovskaya's broth (pH 5) and incubated for 13 days at 28 ± 1°C. Supernatants were collected by filtration through Whatman No 42 filter paper and soluble phosphate was determined by the methods described by Jackson[7]. The percentage of phosphorous was calculated considering P_2O_5 content of rock phosphate as 20%. All the experiments were repeated four times with six replications.

Among the 13 isolates, *A. niger* As3 was found most efficient and solubilized 26.9 mg P_2O_5/50 ml that is equivalent to 23.5%. Tricalcium phosphate was preferred over rock phosphate by this isolate (table 2). Maximum content of phosphate solubilization enhanced by incubation period (7 to 10 days) in both the cases. However, organic acids production by *A. niger* in growth medium resulted decrease in pH (from 5 to 3.7 on average). Organic acids production and lowering of pH attributed to the phosphate solubilization[8]. However, lowering of pH is not a major factor for phosphate solubilization as evident by data presented in table 1. There is a need to isolate better strains which can solubilize more of phosphate and also able to grow during summer seasons (around 37–45°C).

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BIOSORPTION POTENCY OF HEAVY METALS BY SOME FUNGI

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MICROBES tolerating heavy metals and capable of sequestering them under natural conditions have

been reported[1-3] from different ecosystem. For example, *Micrococcus luteus* and *Azotobacter* sp., have been shown to remove lead from the growth media[4] and *Penicillium* sp. is reported to accumulate zinc[5]. According to Vatsala[6], older cells of *Rhodospirillum* sp. absorb lead and nickel faster than the younger cells. Based on these observations, *Trichoderma harzianum* and *Rhizoctonia solani* were tested in the present study using mycelium and spores/sclerotia for their ability to uptake heavy metals from the medium.

T. harzianum and *R. solani* were cultured on potato dextrose agar (PDA) and maintained for a week for further studies.

Zinc sulphate, nickel sulphate, cobalt chloride, copper sulphate, lead acetate and cadmium chloride were used to yield the solutions of 10, 50 and 100 mg/l ionic concentration. The pH was adjusted to 6.5 using 0.1 N HCl/NaOH. Forty ml of the prepared medium was poured into a series of conical flasks and plugged with cotton and sterilized.

Five mm uniform mycelial discs of *T. harzianum* and *R. solani* were plugged from 2-day-old cultures maintained on PDA and transferred separately into flasks containing the medium. Similarly 5 mm uniform discs of spores and mycelia in the case of *T. harzianum* and sclerotia and mycelia in the case of *R. solani* were obtained from 8-days-old cultures and transferred to the metal amended medium.

Similarly inoculated cultures on PD broth unamended with metals served as control.

Cultures both in metal amended and unamended media were incubated at 22±2°C for 15 days under alternate cycles of NUV/darkness.

On 15th day the cultures were filtered through Whatman No. 1 filter paper using a suction pump and simultaneous washing using glass distilled water several times. The cultures were dried at 60°C for 16 h and the dry weight was estimated and subjected to acid digestion. The metal concentration was estimated using atomic absorption spectrophotometer by following the procedure described by Van Loon[7].

The data on differential growth behaviour of mycelium/spores/sclerotia of *T. harzianum* and *R. solani* and the respective levels of heavy metal are given in tables 1 and 2. In both *T. harzianum* and *R. solani* mycelial inoculum showed poor growth and uptake ability in comparison with inoculum provided as spores and sclerotia. The dry biomass of *T. harzianum* and *R. solani* was more at 10 mg/l and at higher concentrations the dry weight decreased.

Table 1 *Variation in inoculum potency for the growth (mg) and metal uptake (mg/l/g fungal mat) by T. harzianum*

		Metal concentration in mg/l			
Metal		Control	10	50	100
Dry biomass (mg)	1	130.00	156.00	132.00	123.00
	2	152.00	161.00	142.00	148.00
Zinc	1	0.08	6.12	18.15	17.12
	2	0.09	8.32	23.82	23.24
Nickel	1	0.01	0.83	1.21	3.29
	2	0.01	3.18	4.98	10.22
Cobalt	1	0.00	0.63	0.93	1.02
	2	0.01	2.42	2.81	1.93
Copper	1	0.08	0.91	3.13	6.90
	2	0.12	5.23	7.20	9.21
Lead	1	0.12	8.02	23.19	26.21
	2	0.13	12.39	26.02	31.28
Cadmium	1	0.00	0.01	0.09	0.08
	2	0.00	0.21	1.02	1.12

1. Mycelial inocula; 2. Spore/sclerotial inocula.

Table 2 *Variation in inoculum potency for the growth (mg) and metal uptake (mg/l/g fungal mat) by R. solani*

		Metal concentration in mg/l			
Metal		Control	10	50	100
Dry biomass (mg)	1	182.00	188.00	162.00	143.00
	2	190.00	196.00	164.00	148.00
Zinc	1	0 05	3.91	20.15	43.34
	2	0.06	5.83	21.81	52.61
Nickel	1	0.01	0.12	5.23	9.38
	2	0.01	2.14	8.14	10.32
Cobalt	1	0.00	0.01	2.13	2.13
	2	0.00	1.02	2.60	2.83
Copper	1	0.06	2.68	8.30	11.21
	2	0.06	3.80	10.22	16.11
Lead	1	0.13	6.21	24.81	36.33
	2	0.13	6.83	29.31	62.62
Cadmium	1	0.00	0.00	1.98	2.02
	2	0.00	0.12	2.01	2.82

1. Mycelial inocula; 2. Spore/sclerotial inocula.

In comparison with the control the fungal mat obtained from treated cultures showed higher amounts of heavy metals. Zinc and lead were the only metals found to be accumulated by *T. harzianum* and *R. solani* in large quantities. Even though there was reduction in the biomass with increased concentration of metals, the uptake potential appeared to be enhanced.

Susceptibility of mycelium on heavy metal medium may be due to the sudden exposure to adverse condition. Spores/sclerotia are known to perpetuate even under the adverse conditions and thereby tolerated elevated levels of metal ions.

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ADDITIONS TO THE FUNGI OF INDIA

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DURING the study of dematiaceous hyphomycetes colonizing diversified plant litter from some forest localities of Andhra Pradesh, India, two rare and interesting hyphomycetes were collected on the dead and decaying wood and were identified as *Berkleasmium caribense* Holubova-Jechova & Mercado Sierra[1] and *Rhinocladium pulchrum* S. Hughes and Holubova-Jechova[2]. These two species are not reported earlier from India and are briefly described here.

Berkleasmium caribense, figure 1

Colonies punctiform, black, sporodochial. Mycelium immersed. Conidiophores macronematous, pale brown, smooth, branched, 1.5–2.5 μm thick. Conidiogenous cells integrated, terminal, monoblastic and determinate. Conidia solitary, dry, acrogenous, simple, broadly ellipsoidal, flattened, constricted at the septa, muriform, septa dark brown to blackish brown, 28–38 μm long and 20–26 μm wide at the broadest region.

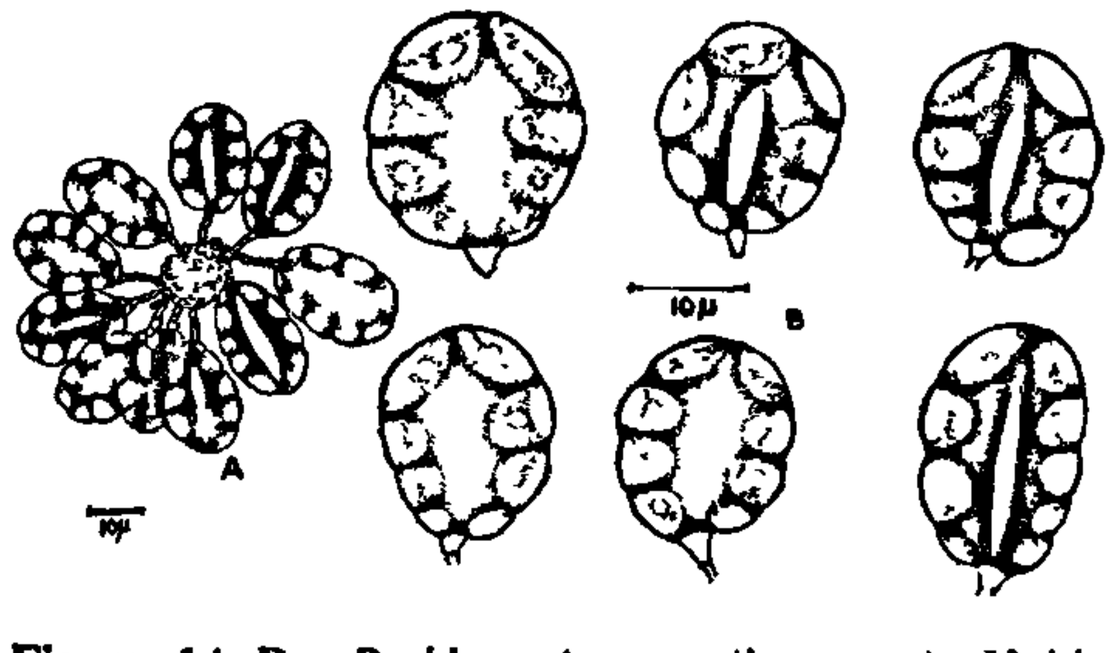

Figure 1A, B. *Berkleasmium caribense.* **A.** Habit; **B.** Detached conidia.

Collected on dead wood by N. Krishna Rao in a forest near Maredumilli, E. G. Dist., A.P., on 31 October 1984. Herbarium, OUMH/NKR/109 and IMI: 296856.

Rhinocladium pulchrum, figure 2

Colonies effuse, cottony, brownish black. Mycelium mostly immersed. Conidiophores macronematous,

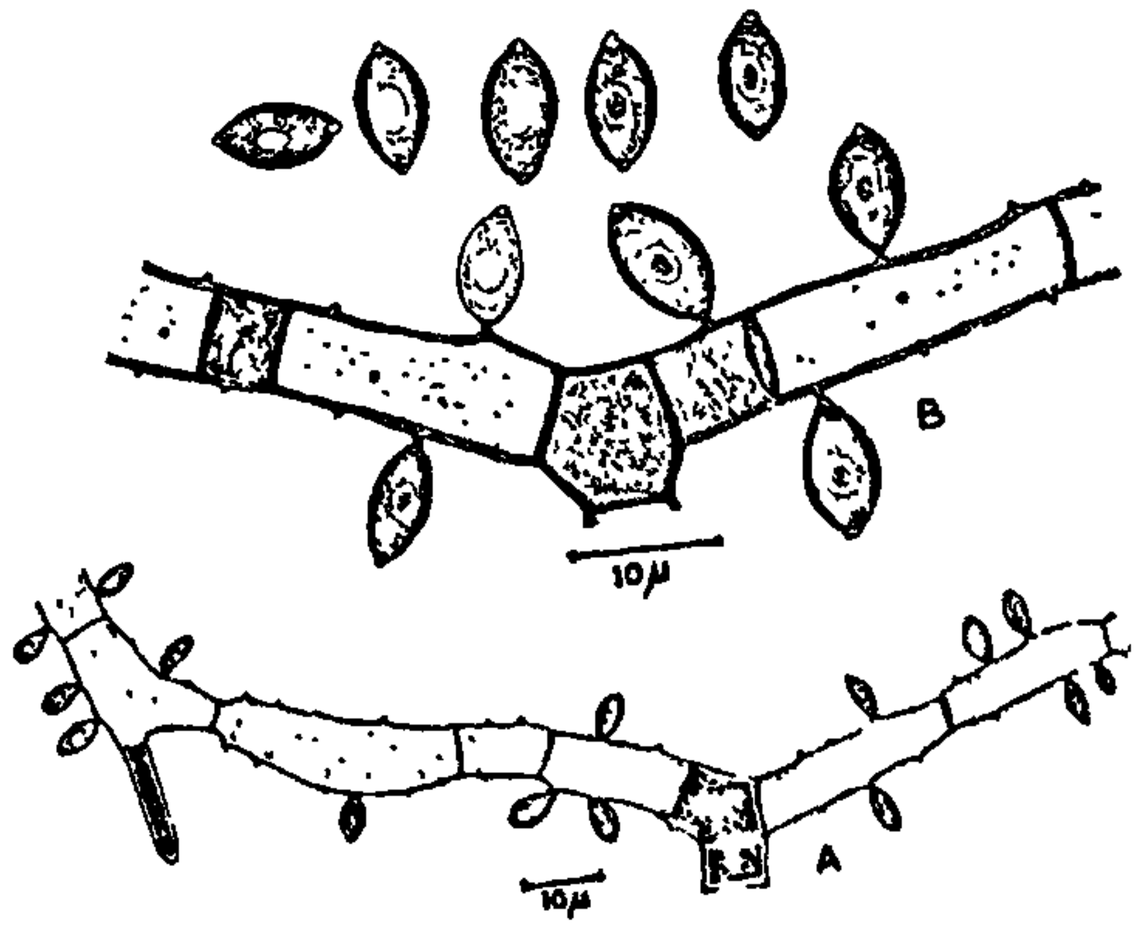

Figure 2A, B. *Rhinocladium pulchrum.* **A.** Habit; **B.** Conidiophores with attached conidia and free conidia.

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