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Prasannarani Tanneru

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EFFECT OF LEAD ON GROWTH AND TOTAL CARBOHYDRATES PRODUCED IN THE CULTURE FILTRATE OF DIFFERENT FUNGI

Prasannarani Tanneru

Department of Botany, Lal Bahadur Shastri Mahavidyalaya Dharmabad Dist Nanded Maharashtra (India)

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ABSTRACT

Five test fungi *Aspergillus flavus, Aspergillus niger, Cladosporium herbarum, Curvularia lunata* and *Trichoderma viridae* were subjected to growth in lead containing basal medium and level of tolerance was determined at 10, 20, 30, 40 and 50ppm Pb concentrations. The growth parameters adopted to study toxicity and physiological responses is the dry weight of fungi and total carbohydrate content in their culture filtrate. It was observed that Pb is highly toxic to the fungi it causes inhibition of growth. The fungi which are very much sensitive and shown less growth are *Aspergillus flavus* and *Curvularia lunata* where as *Trichoderma viridae* and *Aspergillus niger* was found to be resistant as compared to other fungi. Sporulation was decreased in presence of lead. The total carbohydrate content at different concentrations was studied. Maximum decrease in carbohydrate content of culture filtrate of *Aspergillus flavus* and *Curvularia lunata* was observed at 40ppm concentration of Pb.

INTRODUCTION

The contamination of agricultural lands caused by heavy metals in and around industrial areas is a serious problem. Such Contamination is due to largely injudicious anthropogenic activities such as indiscriminate use of pesticides containing heavy metals in agriculture, discharge of untreated industrial wastes and effluents, faulty waste disposal,, high rate of burning of fossil fuels, mining etc. (Foy *et al*, 1978, Mehera *et al*, 1994, Vangronsveld and Clijsters, 1994, Prasad, 1997) Lead persists in the environment by its rapid uptake and accumulation, by food chain organisms and contribute potential environmental hazard.

Hence Biomonitoring is often needed as a support to chemical monitoring because toxicity cannot be tested without living organisms and biological responses. In the present investigation an attempt has been made to observe the effect of different concentrations of Lead on growth and total carbohydrate content in the culture filtrate of different fungi to evaluate the efficacy of organisms for accumulation of pollutants.

MATERIALS AND METHODS

Sterilization

All the media used in the study were sterilized at 15 lb pressure for 20 min in an autoclave (Metzer optiik) before use. All the glassware were thoroughly cleaned with acid dichromate cleaning mixture first, then with hot tap water, rinsed with distilled water and sterilized in oven (yorco) at 160^0C for 2 hours before use.

Preparation of inoculums

Spore suspension was prepared by adding sufficient sterile distilled water to 8 days old culture and 5 ml of it was used for each flask containing 50ml medium as an inoculums in all experiments. In every case optical density of spore suspension was standardized (0.4 O.D) by calorimeter (model AEII). All treatments were in triplicate and results were presented after repeating experiments.

Estimation of carbohydrates

Estimation of Total carbohydrates was done by Anthrone method as modified by Fairbairn (1953). The reagent consists of 0.1% solution of Anthrone in 72% H_2SO_4. 5 ml of this

**Corresponding author: Prasannarani Tanneru*
Department of Botany, Lal Bahadur Shastri Mahavidyalaya Dharmabad Dist Nanded Maharashtra (India)

reagent was added to 1 ml samples. Maximal color was obtained of glucose as well as the sample by placing the tubes in a boiling water bath for 9 minutes. After cooling in running water, the fine quartz particles resulting from the homogenization were spun off and color measures at maximum absorption (620μ).

Observations

Table 1 Effect of lead on growth of *Aspergillus flavus* Link

Sr. No.	Lead (ppm)	Dry weight of *Aspergillus flavus* in mg after days.			
		2	4	6	8
1	Control	150	245	338	430
2	10	50	140	200	250
3	20	25	80	110	180
4	30	25	70	110	160
5	40	20	65	100	150
6	50	18	50	98	130

Table 2 Effect of Lead on growth of *Aspergillus niger* van Tiegh.

Sr. No.	Lead (ppm)	Dry weight of *Aspergillus niger* in mg after days.			
		2	4	6	8
1	Control	60	100	180	270
2	10	50	110	170	260
3	20	50	100	160	260
4	30	50	100	160	260
5	40	40	110	150	250
6	50	30	100	140	230

Table 3 Effect of lead on growth of *Cladosporium herbarum* Link.

Sr. No.	Lead (ppm)	Dry weight of *C. herbarum* in mg after days.			
		2	4	6	8
1	Control	250	390	790	1250
2	10	100	210	610	990
3	20	80	150	185	250
4	30	40	80	100	120
5	40	30	60	90	90
6	50	20	50	80	80

Table 4 Effect of lead on growth of *Curvularia lunata* (Wakker) Boedijn

Sr. No.	Lead (ppm)	Dry weight of *Curvularia lunata* in mg after days.			
		2	4	6	8
1	Control	200	600	1800	2560
2	10	130	220	280	300
3	20	80	100	100	120
4	30	30	60	80	84
5	40	20	60	62	65
6	50	20	40	50	55

Table 5 Effect of Lead on growth of *Trichoderma viride* Pers exS. F. Gray.

Sr. No.	Lead (ppm)	Dry weight of *Trichoderma viride* in mg after days.			
		2	**4**	**6**	**8**
1	Control	80	214	360	555
2	10	72	168	296	444
3	20	56	137	296	416
4	30	48	137	254	396
5	40	40	91	211	360
6	50	8	30	84	222

RESULTS AND DISCUSSION

It is clear from the results recorded in table 1, maximum reduction (430-250=180mg) in dry weight of *Aspergillus flavus* was observed at 10ppm Pb. Where as in *Aspergillus niger* there is not much reduction in dry weight at the end of 8 days maximum dry weight 260mg and minimum dry weight 230 mg was recorded at 50ppm. *Cladosporum herbarum* showed maximum decrease (990-250=740mg) at 20 ppm Pb and very less growth (80mg) recorded at 50ppm. Maximum effect is seen on growth of *Curvularia lunata*, 2560mg in control where as in presence of Pb 300mg at 10 ppm at the end of 8 days. It is evident from the results presented in table 5 maximum decrease (360-222=138mg) in dry weight of *Trichoderma viridae* was at 50 ppm Pb. In the present investigation out of the five fungi tested, *Aspergillus niger*, *Trichoderma viridae* were tolerable to Pb concentrations and *Curvularia lunata* and *Aspergillus flavus* are most sensitive showing much reduction in growth and sporulation. The relative toxicity of heavy metals has been reported by a number of researchers. (Eichorn, 1974, Monohan 1976).

Table 6 Effect of Lead on Total carbohydrate content in culture filtrate of different fungi.

Sr. no.	*Fungi*	Total carbohydrate content in mg at different concentrations of Lead (ppm) after 8 days.					
		Control	10	20	30	40	50
1	*A. flavus* Link.	0.299	0.286	0.240	0.223	0.208	0.208
2	*A. niger* van Tiegh.	0.280	0.250	0.232	0.218	0.201	0.201
3	*C. herbarum* Link..	0.278	0.252	0.241	0.218	0.212	0.212
4	*C. lunata* (Wakker) Boedijn.	0.240	0.221	0.198	0.163	0.142	0.142
5	*T. viride* Pers. exS. F. Gray.	0.254	0.244	0.230	0.216	0.198	0.198

Effect of lead on the Biochemical content such as Total carbohydrates produced in the culture filtrate was studied and presented in table no 6. Maximum reduction in total carbohydrate concentration (0.299-0.208=0.091mg) in *Aspergillus flavus* and 0.240-0.142=0.098mg in culture filtrate of *Curvularia lunata* was noted up to 40 ppm Pb concentrations where as less reduction (0.254-0.198=0.056mg) is noted in culture filtrate of *Trichoderma viridae*. It can be concluded that *T. viridae* and *A. niger* can tolerate the heavy metal Pb up to 40 ppm concentration. Results indicate that the tolerance limits for the test fungi was proved to be 40 ppm Pb, because at higher concentration 50 ppm the fungi cannot utilize carbon source. Hence the concentration of carbohydrates was recorded to be the same. There was no decrease in total carbohydrates as reported by author earlier (Nazareth and mavinkurve, 1987). In nature due to continuous exposure of microorganisms, many of them have developed detoxification mechanisms to overcome the detrimental effects of metals. Generally pollutants act on a number of cellular and biochemical processes. Critical to growth and reproduction have been found to comprise of two mechanisms one is passive uptake and the other is an active process (Volesky, 1994). In present study lead was effective on fungi at higher 50ppm concentration. Similar findings were reported by (Berland *et al*, 1976, Hessler, 1974, Kirchmann and Bonott 1974, Zavodnik, 1977). Hence microorganism like *Aspergillus flavus*, *Aspergillus niger*, *cladosporium herbarum*, *curvularia lunata* and *Trichoderma viridae* can be used as pollution indicator for sewage as well as Industrial effluents.

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