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EFFECT OF HEAVY METALS ON GROWTH OF SOME PATHOGENIC AND NON-PATHOGENIC SOIL MICROFLORA

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The heavy metals (Cd, Cr, Pb, Ni and Zn) inhibited the radial growth of soil microflora, including two soil-borne plant pathogens namely *Fusarium oxysporum* f. sp. ciceri and *F. oxysporum* f. sp. lini isolated from sewage irrigated field. None of the heavy metals caused complete inhibition on radial growth of any microorganism at test concentrations. However, a gradual increase in the inhibition of growth was recorded with increase in the concentration of heavy metals in the culture media. Cadmium was found to be most effective against both the pathogens whereas Zn was found to be least effective. The effectiveness of the heavy metals against the pathogens, recorded at 200 ppm, was in the following order : Cd > Ni > Cr > Pb > Zn and Cd > Cr > Ni > Pb > Zn for *Fusarium oxysporum* f. sp. *ciceri* and *F. oxysporum* f. sp. *lini* respectively.

Key Words : Heavy metals, growth inhibition, soil fungi.

Several scientists have reported the accumulation of heavy metals in soils as a result of sewage irrigation (Ikram and Naheed, 1986; Elliot and Linn, 1987; Sharpley, 1990). These heavy metals are highly toxic to the plants and animals. However, toxicity of heavy metals depends on the physico-chemical properties of the recipient environment (Babich and Stotzky. 1980, 1983; Wo *et al.*, 1980).

A review on interaction between heavy metals and microorganisms have been ignored or bareley mentioned (Ashida, 1965 and Ross, 1975). Hence, an attempt has been made to study the effect of some heavy metals on growth of pathogens and other microflora.

MATERIALS AND METHODS

Two fields situated at Bhagwanpur village adjacent to B.H.U. Campus, Varanasi, one irrigated with sewage released from Bhagwanpur Sewage Treatment Plant (hereafter called as treated field), and the other irrigated with tube well water (hereafter called as control field) were selected for the present study.

The soil samples of both the fields were collected in sterilized polythene bags and sewage in sterilized plastic bottles (non-transparent) in ice boxes from the Sewage Treatment Plant at monthly interval. The samples were brought into laboratory for the study.

Heavy metals analysis of sewage : Heavy metals were detected by Atomic Absorption Spectrophotometer (AAS) Model IL 751 with Air-Acetylene Flame. The pre-treatment of the samples were done according to the method specified in the Standard Methods for the examination of water and waste water and the concentration of the heavy metals was determined. Samples which were collected in 400 ml polythene bottles were acidified with conc. HNO_3 on the spot lowering the pH of the samples below 2.0. Heavy metals Cd, Cr, Pb, Ni and Zn) in the soils were detected by Atomic Absorption Spectrophotometer.

Effect of heavy metals on growth of some pathogenic and non-pathogenic soil microflora : Five heavy metals viz., Cadmium (Cd), Chromium (Cr), Lead (Pb), Nickel (Ni) and Zinc (Zn) in the form of their salts *i.e.* $CdCl_2H_2O$, $CrCl_2$, $Pb(NO_3)_2$, $NiCl_2.6H_2O$ and $ZnSO_4.7H_2O$ were selected and their effects against some dominant soil microflora viz., *Acrophialophora fusispora, Aspergillus flavus, A. luchuensis, A. niger, A. terreus, Penicillium citrinum. P. frequentans, P. rugulosum* and *Trichoderma harzianum*, two soil-borne plant pathogens namely *Fusarium oxysporum* f. sp. ciceri and *F. oxysporum* f. sp. *lini*, two unidentified bacteria (colourless and yellow colour bacteria) and two actinomycetes namely *Streptomyces rimosus* and *S. rochi* (SR_1) were evaluated by poisoned food technique.

250 ml Erlenmeyer flasks containing 100 ml sterilized and cooled (40°C) Czapek dox agar medium, Jensen's medium and Thronton's medium for fungi, actinomycetes and bacteria respectively, were amended with calculated quantity of each heavy metal to make

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Table 1: Monthly variation in temperature and pH of sewage (July 1989 to June 1990)

Months	Temperature (°C)		pH	
	Untreated	Treated	Untreated	Treated
July	34.0	33.4	7.97	7.8
August	31.2	30.2	7.76	7.72
September	29.4	28.6	8.0	7.62
October	28.6	28.0	8.1	8.1
November	26.2	25.4	8.15	7.75
December	24.2	24.2	7.8	7.86
January	23.0	22.8	7.74	7.71
February	23.4	23.2	7.75	7.8
March	23.6	23.4	7.84	7.71
April	27./6	27.2	7.68	7.61
May	31.2	30.6	7.96	7.96
June	34.2	34.0	7.63	7.82

Untreated : Refers to raw sewage without any processing in treatment plant.
Treated : The raw sewage after processing in treatment plant.
The above explanations implies for all other tables wherever mentioned.

the final concentration as 20, 50, 100 and 200 ppm for Cd, Cr, Pb and Ni whereas 200, 400, 800, 1200 ppm for Zn. Twenty ml of supplemented medium was poured in each of sterilized 9 cm diameter Petri dishes in three replicates for each treatment and were inoculated at the centre with a 5 mm agar block, cut from the margins of actively growing 5 day old cultures of fungi and bacteria and 10 day old cultures of actinomycetes. The media without heavy metals were also inoculated and treated as control.

All the Petri dishes were incubated at 25±2, 30±2 and 35±2 °C for fungi, actinomycetes and bacteria, respectively and radial growth of the microorganisms were measured after 7 days of incubation. The per cent inhibition of growth of pathogens and other microorganisms was calculated by comparing the results of the treated plate with that of control and per cent inhibition or stimulation of microorganisms was calculated.

RESULTS AND DISCUSSION

Sewage samples were analysed for presence of some heavy metals like Cd, Cr, Pb and Zn in treated and untreated sewage (Table 1). Zn was found in greater quantity as compared others. The maximum quantity of Zn (1975 $\mu g l^{-1}$ and 1345 $\mu g l^{-1}$) and Cr (103.8 $\mu g l^{-1}$ and 91.8 $\mu g l^{-1}$) in untreated and treated sewage samples, respectively was recorded in June whereas the minimum quantity of Zn in untreated and treated sewage (758 $\mu g l^{-1}$ and 502 $\mu g l^{-1}$, respectively) was recorded in September. The presence of heavy metals in sewage was due to release of various chemical substances into the municipal sewage through factory effluent.

Five heavy metals i.e. Cd, Cr, Pb, Ni and Zn were detected in treated soil and the control soil samples (Table 2). Zinc was found to be present in high concentration as compared to the others. Overall, the concentration of heavy metals detected in treated soil was more than the control soil. The concentration of Zn in treated soil and control one was 35.1 $\mu g g^{-1}$ and 29.8 $\mu g g^{-1}$ respectively. The concentration recorded for Cd was 0.42 $\mu g g^{-1}$. Several scientists have also reported the accumulation of heavy metals in soils due to sewage irrigation (Meshef, *et al.*, 1989; Smith and Sharpley 1990).

The results obtained with regards to the effects of Cd, Cr, Pb, Ni and Zn against the pathogens and test

Table 2: Analysis of heavy metals from sewage (µg/1)

Months	Cadmium (Cd)		Chromium (Cr)		Lead (Pb)		Zinc (Zn)	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
July	28.4	19.8	51.5	41.2	48.6	38.2	1950	1332
August	25.2	19.0	50.2	39.7	40.2	31.6	770	511
September	21.0	16.1	60.1	50.8	51.8	41.2	758	502
October	24.2	17.2	65.2	52.8	55.2	45.3	762	510
November	26.1	19.5	68.8	56.2	57.5	48.2	900	565
December	30.2	22.3	68.2	51.1	63.5	52.5	1070	795
January	31.3	23.1	70.1	62.8	65.2	53.4	1380	830
February	32.5	25.2	78.9	67.2	70.1	58.1	1403	935
March	37.1	31.1	80.0	68.4	74.4	70.5	1395	885
April	39.2	32.2	82.3	75.0	78.5	65.2	1502	995
May	40.5	32.6	89.5	78.9	84.6	70.3	1905	1312
June	44.8	34.8	03.8	91.8	96.5	84.2	1975	1345

Table 3: Effect of heavy metals (Cd and Cr) on per cent growth inhibition of some dominant microflora and the test pathogens

Name of Species	Concentration (ppm) Cd				Concentration (ppm) Cr			
	20	50	100	200	20	50	100	200
Acrophialophora fusispora	26.58	32.91	40.51	63.29	18.5	22.91	29.96	44.93
Aspergillus flavus	13.89	43.65	65.48	84.13	11.11	36.29	53.73	63.33
A. luchuensis	28.88	54.82	69.63	83.7	18.51	39.25	57.40	64.81
A. niger	16.0	34.0	40.00	62.5	11.11	29.62	40.79	60.37
A. terreus	24.67	50.0	68.67	84.0	12.50	34.37	40.63	53.13
Curvularia lunata	30.56	48.02	57.14	64.29	21.13	40.65	49.59	60.98
Fusarium oxysporum. f. sp. *ciceri*	14.29	34.28	67.14	81.82	10.94	19.53	25.56	39.38
F. oxysporum f. sp. *lini*	23.33	49.63	68.52	86.3	13.45	18.6	26.35	42.86
Penicillium citrinum	33.67	43.88	55.1	78.57	20.69	31.03	34.48	46.55
P. frequentans	23.6	41.57	56.18	67.42	11.43	14.29	25.71	42.86
P. rugulosum	26.96	46.09	66.09	77.39	5.3	18.18	31.81	47.72
Trichoderma harzianum	3.7	7.41	21.11	49.26	26.0	33.2	42.0	48.4
Colourless bacteria	8.97	13.46	32.69	45.51	18.7	33.55	47.1	54.83
Yellow colour bacteria	23.39	27.42	42.47	67.74	12.5	22.96	30.74	43.33
Streptomyces rimosus	34.61	53.85	67.31	69.23	18.52	22.22	31.48	40.74
S. rochi (S. R_1)	28.81	45.76	62.71	72.88	26.08	39.13	45.65	56.52

Cadmium and chromium were added in the culture medium in chloride form

Table 4: Effect of Lead and Nickel on per cent growth inhibition of some dominant microflora and the tet pathogens

Name of pecie	Concentration (ppm) Lead				Concentration (ppm) Nickel			
	20	50	100	200	20	50	100	200
Acrophialophora fusispora	11.11	22.22	33.33	41.67	12.35	28.09	43.82	66.29
Aspergillus flavus	8.89	14.44	26.67	35.56	14.86	29.71	42.86	54.29
A. luchuenis	9.58	22.24	31.28	42.13	10.74	23.7	37.78	49.29
A. niger	10.05	17.11	24.16	37.74	14.5	35.5	54.0	61.0
A. terreus	9.23	118.46	27.69	46.62	17.5	31.25	42.5	55.0
Curvularia lunata	6.17	17.28	23.46	28.4	14.55	30.45	50.0	66.36
Fuarium oxysporum. f. sp. *ciceri*	8.24	11.77	17.65	27.06	7.92	20.0	33.33	43.75
F. oxysporum f. sp. *lini*	12.22	16.67	21.11	32.22	8.14	117.4	29.26	40.0
Penicillium citrinum	15.54	27.46	39.38	49.74	7.5	13.95	27.91	44.19
P. frequentans	9.52	23.8	38.1	47.62	16.67	33.33	48.48	57.58
P. rugulosum	12.72	24.74	36.4	45.74	20.45	34.09	53.4	62.5
Trichoderma harzianum	-	7.78	16.67	21.11	12.96	26.67	38.52	47.04
Colourless bacteria	5.56	20.37	29.63	42.59	13.79	27.59	35.17	46.9
Yellow colour bacteria	14.87	27.03	44.6	54.04	9.68	20.56	25.0	33.47
Streptomyces rimosus	8.7	30.44	43.48	56.52	13.55	32.2	45.76	54.24
S. rochi (S. R_1)	14.29	33.33	47.62	56.19	9.52	17.39	34.78	43.48

Lead (Pb) was added to the culture medium in nitrate form and Nickel (Ni) in chloride form.

microorganisms are presented in Tables 3-5. A significant variation was recorded (p = 0.01/0.05) between per cent inhibition of microorganisms and concentration of heavy metals. All the heavy metals inhibited the radial growth of the pathogens and other test microorganisms. None of the heavy metals caused complete inhibition of any microorganism at any used concentration. However, a gradual increase in the inhibition of growth was recorded with increase in the concentration of heavy metals in the culture media. Huisingh and Huisingh (1974) have mentioned that all the elements essential or non essential, are potentially toxic depending on their form, concentration and route of exposure.

Cd was found to be most effective heavy metal against both the pathogens whereas Zn was least effective. Cd is potent component of a few fungicides to control various diseases of crops due to toxicity against several pathogenic fungi. Toxicity of Cd and effect of various factors on its toxicity against the microbes have been extensively studied by Babich and Stotzky (1978).

Table 5: Effect of Zinc on per cent growth inhibition of some dominant microflora and the test pathogens

Name of Species	Concentration (ppm) Zn			
	200	400	800	1200
Acrophialophora fusispora	22.12	36.28	49.33	62.83
Aspergillus flavus	14.71	32.35	47.06	60.78
A. luchuensis	15.56	31.48	47.03	58.51
A. niger	21.85	39.62	46.67	60.37
A. terreus	23.29	30.68	40.90	57.39
Curvularia lunata	11.06	31.91	52.76	65.11
Fusarium oxysporum. f. sp. *ciceri*	7.14	14.28	23.80	41.42
F. oxysporum f. sp. *lini*	8.90	14.11	28.89	41.11
Penicillium citrinum	23.29	39.72	52.05	61.64
P. frequentans	19.73	32.89	50.00	59.21
P. rugulosum	15.79	32.63	49.47	57.89
Trichoderma harzianum	11.11	29.25	41.11	53.33
Colourless bacteria	19.35	32.25	43.87	57.42
Yellow colour bacteria	13.30	23.38	37.50	66.94
Streptomyces rimosus	20.00	38.57	51.42	60.00
S. rochi (S. r_1)	24.07	40.74	51.85	64.06

Zinc (Zn) was added to the culture medium in the form of sulphate

Cd, Ni, Cr, Pb and Zn exhibited 81.82, 43.75, 39.38, 27.06 and 7.14% inhibition of radial growth of *F. oxysporum* f. sp. *ciceri* respectively at 200 ppm concentration while 86.3, 42.86, 40, 32.22 and 8.9% inhibition was observed in case of *F. oxysporum* f. sp. *lini* with Cd, Cr, Ni, Pb and Zinc respectively at the same concentration. It was observed that the maximum inhibition in radial growth due to Cd was recorded in case of *A. flavus* (84.13%) followed by *A. terreus* (84.0%) and *A. luchuensis* (83.7%) whereas the minimum in case of colourless bacteria (Table 3). Several studies have shown varying degree of tolerance to fungi to cadmium (Ashida, 1965). Strains of *Fusarium oxysporum* isolated from soil heavily contaminated with Cd, Cu, Pb and Zn, had greater than normal tolerance to these heavy metals than isolates from uncontaminated soil. The strains tolerant to the heavy metal accumulate these metals in mycelium. The toxic effect of Cd on fungi may be exerted on several forms of fungal development on mycelial growth, fruiting body formation and spore germination. In general actinomycetes are more tolerant to Cd than the gram +ve bacteria (Babich and Stotzky, 1983a). Toxicity of Cd and effect of various factors on its toxicity against the microbes have been extensively studied by Babich and Stotzky (1978). They reported toxic effect of Cd on mycelial growth of *Amantia muscaria* and *Rhizopus roseolus.* Cadmium acts as growth inhibitor for some bacteria like *Bacillus cireus*, *E. coli* and *Agrobacterium tumefaciens* and actinomycetes (*Nocardia parafinae*). *A. luchuensis* showed maximum inhibition (64.81%) in radial growth due to Cr whereas minimum inhibition due to Cr was recorded in case of *S. rimosus* (40.74%) (Table 3). Pb was found to be very effective against *S. rimosus* (56.52%), *S. rochi* (56.19%) and yellow colour bacterium (54.04% inhibition) (Table 4). Babich and Stotzky (1983) have also observed the toxic effect of lead against the growth of *A. niger*, *Saprolegnia* sp. and *Achlya* sp. Thirty, 42.86, 40.0, 32.22 and 8.9% inhibition was observed in case of *F. oxysporum* f. sp. *lini* with Cd, Cr, Ni., Pb, and Zn respectively at the same concentration. The inhibitory effect of Cd, Pb, Ni, and Zn was reported by Dubey and Dwivedi (1988) and Bashar (1990) on *Macrophomina phaseolina* and *F. oxysporum* f. sp. *ciceri* respectively. Zinc and Cr caused considerable effect to chek the growth of the test pathogens as well as some dominant microorganisms. The maximum growth inhibition of *F. oxysporum f. sp. ciceri* (41.42%) and *F. oxysporum* f. sp. *lini* (41.11%) was caused due to Zn at 1200 ppm. Zn has been reported as an essential element for the growth of fungi at low concentration (Mc Han and Johnson. 1970; Ross, 1975). Bashar (1990) reported that Zn and Fe checked the growth of the pathogen at 1200 ppm. Murugeson (1990) observed that increasing concentration of Ba, Cu, Mn and Zn, individually or in combination, inhibited the radial growth and hyphal dry weight of *Rhizoctonia bataticola*. The chloride forms of Cd, Co, Ni and Hg have been found to inhibit mycelial growth and sclerotia formation in *Sclerotium rolfsii* and *Rhizoctonia solani* (Dwivedi *et al.*, 1986).

The maximum inhibition in radial growth in case of Ni was recorded for *A. terreus* and *P. frequentans* at 20 ppm concentration; *A. niger* and *P. rugulosum* at 50 and 100 ppm and *C. lunata* and *Acrophialophora fusispora* at 200 ppm concentrations whereas minimum inhibition was recorded in case of yellow colour bacteria (Table 4). Inhibitory effect of Ni was found against *Bacillus urevis*, *Psedomonas* sp., actinomycetes and filamentous fungi e.g. *Trichoderma viride*, *Rhizopus stolonifers*, *Aspergillus giganteous*, *A. niger* and *Penicillium vermiculatum* (Babich and Stotzky, 1986). Toxicity of Cu, Mg and Pb has been reported against some fungi by few workers (Babick and Stotzky. 1983b; Babich and Stotzky. 1986).

The growth of yellow colour bacteria was suppressed (66.36%) by Zn followed by *Curvularia lunata* (65.11%) and S. rochi (64.04%). Effect of Ni, Pb and Zn was found least inhibitory to the radial growth of *T. harzianum*. The toxicity may be due to permeability of fungal cells to metals (Ross and Old. 1973).

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