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The Inhibition of Bean Plant Metabolism by Cd Metal and Atrazine: I. The Effect of Atrazine with Cd Metal on Growth, Photosynthesis, Nutritional Level and Rhizosphere of Soil

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Abstract: Sublethal atrazine concentration with toxic Cd (cadmium) metal induced, general inhibition on growth, photosynthesis and nutritional level of bean plant. Progressive reduction in protein, amino acid and carbohydrate synthesis were associated with increasing herbicide concentration at all experimental periods. Nodulation failed to occur in roots of leguminous plant. The rhizosphere was found to be affected and colonies of helpful organism were observed in which *Rhizobium* species were not isolated from the roots. The results of these investigations allowed me to draw a conclusion that soil enriched with heavy metal kill the microbial life. Therefore they cannot degradate the atrazine herbicide, which is highly toxic to photosynthetic processes of the plant and translocated to the tips of leaves. But soil enriched with toxic metal was effected on the microbial life. This causes the lowering of the soil ability in mitigation of the dangerous herbicide activity.

Key words: Atrazine, Cd, growth, rhizosphere, degradation

INTRODUCTION

Soil applied with atrazine is taken by plant roots and moves upwards with in the water transport system of the plant I-e xylem to be concentrated in the leaves. Atrazine herbicide is photosynthesis inhibitor, which shut down the photosynthesis (food producing) process in susceptible plants by binding to specific sites with in the plants chloroplasts[1,2]. The inhibition of photosynthesis could results in a slow starvation of the plant. However, the plant experiences a more rapid death that is believed to be due to the production of secondary toxic substance[3,4]. Holoford *et al.*[5] reported the effect of atrazine herbicide on wheat plant and found that wheat growing on an alkaline black earth was severely damaged by atrazine residue where as wheat growing in normal soil was undamaged. The nitrate-reducing activity of the normal susceptible biotype of lambsquarters was strongly inhibited by atrazine. Percich and Lockwood[6] investigated that loam soil treated with atrazine resulted in increased population of actinomycetes, bacteria and fungi over those in non-treated soil. The increase was in proportion to the amount of atrazine and persisted for at least two months.

Radosevich *et al.*[7] reported that microorganism was capable of using atrazine under aerobic condition and found the effects of anthropogenic toxicants on the microbiota have shown that the toxicity of a pollutant is dependent on the physicochemical characteristics of the environment in to which the pollutant is deposited. For example with some gaseous pollutant (e.g. SO_2, NO_2, NH_3) deposition in a well-buffered environment elicit different effect on the biota than deposition in a poorly buffered environment[8,9]. The effect of Cd stress on nodulation, N_2-fixation of the root nodule, the change in ultra structure of the root nodule, soybean growth and the distribution of Cd in plant were studied by Chen *et al.*[10]. Result shared that nodulation of soy bean was greatly inhibited by the addition of Cd.

Cd is considered as major environmental pollutant and its phytotoxicity is well known and most studies on Cd in plants concern essentially on uptake and translocation mechanism. The present study examines the effect of Cd metal with atrazine herbicide on plant growth and rhizobial population. As water of irrigation contain high percentages of heavy metals and atrazine spray has been applied to the crops to control the weeds.

MATERIALS AND METHODS

Seeds of *Vigna radiata* were purchased from National Institute of Agriculture and Biology, Faisalabad

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and were surface sterilized with 1% mercuric chloride solution, then washed with deionised water. Experiment was conducted in plastic pots in growth chamber at 30 to 25°C for four weeks in August 2004. Three-concentrations of $CdCl_2$ solution viz., 0, 20, 100 and 200 ppm were prepared in half strength Hoagland solution. The various concentration of atrazine like 5, 10, 50, 100 ppm were taken keeping 20, 100 and 200 ppm as constant concentration of $CdCl_2$. Fifteen seeds of *Vigna radiata* were introduced into each pot of experiment. Germination of seeds was performed in sterilized net strainer placed on pot of Hoagland solution. A seed was considered germinated when the radical had attained a length at least 1.5 mm[11].

Biochemical test: Sample of roots and shoots were collected randomly then washed thoroughly with deionised water. The contents of chlorophyll a and b, carbohydrate, protein and amino acid were analyzed after four weeks of germination in roots and shoots of plant. The extract of chlorophyll a and b, proteins, carbohydrates and amino acid has been prepared in acetone, water and ethanol, respectively whereas optical density were measured at 663, 645 and 620 nm for chlorophyll a, b, proteins and carbohydrate, respectively by visible spectrophotometrically[12-15]. The measurement of length of roots and shoots were performed after passing two days and compared with control. Magnesium ion were determined by atomic absorption spectrophotometery

Analysis of rhizosphere: Roots of *Vigna radiata* were collected in the flowering season (after 6 weeks), then rinsed with saline. The roots were sterile saline, crushed and suspended in saline for 24 h then plated on nutrient agar. After passing one day microbial colonies were obtained in which Colony Forming Units (CFU) were counted. Gram staining identified the microbial flora. The gram-negative organism was streaked on EMB agar plates for isolation of pure culture. Further identification was performed on Indol Test, nitrate reduction and growth on TSI agar slant.

RESULTS AND DISCUSSION

Atrazine is a selective triazine herbicide used to control broad leaf and grassy weeds in corn, sorghum, sugarcane, pineapple, chistmas trees and other crops and in conifor reforestation plantings. Plants mainly through the roots, but also through the folige absorb atrazine[16]. Once absorbed atrazine is translocated upward and accumulates in the growing tip and new leaves of the plants. Atrazine blocks the plants ability to carry on photosynthesis and also increases the uptake of arsenic

Table 1: Growth of microbial flora in presence of atrazine combine with Cd

Conc. of atrazine (ppm)	Dilution	No. of colonies	No. of colonies x DF	Average (CFU)
Cd (20 ppm)				
0	10^{-3}	200	0.2 $2x10^1$	$7.09x10^2$
	10^{-4}	120	$1.2x10^2$	
	10^{-5}	80	$8.0x10^4$	
5	10^{-3}	150	$1.5x10^1$	$7.61x10^2$
	10^{-4}	100	$1.0x10^2$	
	10^{-5}	90	$9.0x10^4$	
10	10^{-3}	130	$1.3x10^1$	$4.791x10^2$
	10^{-4}	95	$9.5 x10^3$	
	10^{-5}	90	$9.0x10^4$	
50	10^{-3}	90	$9.0x10^2$	$3.35x10^2$
	10^{-4}	75	$7.5x10^2$	
	10^{-5}	50	$5.0x10^4$	
100	10^{-3}	50	$5.0x10^2$	$1.79x10^2$
	10^{-4}	35	$3.5x10^3$	
	10^{-5}	25	$2.5x10^4$	
Cd (100 ppm)				
0	10^{-3}	170	$1.7x10^1$	$6.11x10^2$
	10^{-4}	130	$1.3x10^2$	
	10^{-5}	50	$5.0x10^4$	
5	10^{-3}	150	$1.5x10^1$	$5.3x10^2$
	10^{-4}	80	$8.0x10^2$	
	10^{-5}	40	$4.0x10^4$	
10	10^{-3}	132	$1.32x10^1$	$4.68x10^2$
	10^{-4}	100	$1.10 x10^3$	
	10^{-5}	93	$9.3x10^4$	
50	10^{-3}	90	$9.0x10^2$	$3.28x10^2$
	10^{-4}	80	$8.0x10^3$	
	10^{-5}	50	$5.0x10^4$	
100	10^{-3}	40	$4.0x10^2$	$1.45x10^2$
	10^{-4}	35	$3.5x10^3$	
	10^{-5}	20	$2.x10^4$	
Cd (200 ppm)				
0	10^{-3}	150	$1.5x10^1$	$5.42x10^2$
	10^{-4}	120	$1.2x10^2$	
	10^{-5}	80	$8.0x10^4$	
5	10^{-3}	140	$1.4x10^1$	$5.3x10^2$
	10^{-4}	105	$1.0x10^2$	
	10^{-5}	95	$9.5x10^4$	
10	10^{-3}	120	$1.2x10^1$	$4.31x10^2$
	10^{-4}	90	$9.0 x10^3$	
	10^{-5}	40	$4.0x10^4$	
50	10^{-3}	98	$9.8x10^2$	$3.2x10^2$
	10^{-4}	75	7.510^3	
	10^{-5}	50	$5.0x10^4$	
100	10^{-3}	40	$4.0x10^2$	$1.40x10^2$
	10^{-4}	20	$2.0x10^3$	
	10^{-5}	11	$1.1.x10^4$	

CFU = Colony Forming Units, DF= Dilution Factor

by treated plants. Soil beneficial microorganism degredate the atrazine and population structure of soil bacteria improved in soil containing atrazine and becomes less toxic to the plant growth. But heavy metal contamination in aquatic resources is harmful to colonies of bacteria. Soil beneficial microorganisms have been found sensitive towards toxicity of heavy metals[17]. These microorganisms are responsible for the biological functioning of soil and thus regulate soil fertility. Investigation shows that bacterial colonies were effected specially the bacteria of the genus *Rhizobium* die out in soils containing moderate contamination of heavy metals (Table 1). In absence of beneficial microorganism results shows that Cd seems to be highly toxic to the growth of plant, its accumulation in

Table 2: Effect of Cd with atrazine herbicides on nutritional value in roots of bean plants

Conc. of atrazine (ppm)	Growth of roots (cm)	(%) of carbohydrate in 10^{-3}	(%) of protein	(%) of amino acids 10^{-3}
Cd (20 ppm)				
0	4.0±.02	2.65±0.02	1.16±0.01	1.475±0.02
5	2.0±0.1	4.09±0.03	1.75±0.01	_
10	1.5±0.2	4.22±0.02	1.29±0.02	_
50	1.0±0.1	4.04±0.02	1.25±0.02	_
100	1.0±0.1	2.95±0.01	1.16±0.02	_
Cd (100 ppm)				
0	3.0.±.1	2.47±0.04	1.16±0.01	_
5	2.6±0.2	4.17±0.05	1.54±0.02	_
10	2.2±0.1	4.00±0.02	1.45±0.03	_
50	2.1±0.1	3.68±0.01	1.37±0.10	_
100	2.0±0.2	3.50±0.01	1.20±0.01	_
Cd (200 ppm)				
0	2.0±0.1	1.32±0.05	1.14±0.03	_
5	1.5±0.1	2.70±0.02	0.91±0.02	_
10	1.1±0.2	2.48±0.01	0.85±0.01	_
50	1.0±0.1	1.84±0.04	0.82±0.03	_
100	1.0±0.2	1.26±0.01	0.77±0.02	_

Table 3: Effect of Cd with atrazine herbicides on nutritional value in shoots of bean plants

Conc. of atrazine (ppm)	Growth of shoots (cm)	(%) of carbohydrate in 10^{-3}	(%) of protein	(%) of amino acids 10^{-3}
Cd (20 ppm)				
0	21.0±0.3	1.56±0.01	0.83±0.01	3.94±0.01
5	20.0±0.5	4.8±0.02	1.24±0.01	2.92±0.01
10	19.0±0.6	4.77±0.04	0.72±0.05	3.94±0.01
50	17.0±0.2	2.11±0.02	0.61±0.01	3.36±0.01
100	10.0±0.1	1.47±0.02	0.53±0.01	4.18±0.01
Cd (100 ppm)				
0	16.0±0.2	1.42±0.02	0.71±0.01	_
5	1.0±0.2	1.71±0.01	0.86±0.01	_
10	15.0±0.2	1.60±0.01	0.75±0.02	_
50	14.0±0.1	1.05±0.01	0.63±0.02	_
100	13.0±0.1	0.78±0.01	0.61±0.06	_
Cd (200 ppm)				
0	11.0±±0.5	0.91±0.01	0.55±0.01	
5	11.0±0.3	2.12±0.02	0.83±0.02	
10	10.0±0.2	1.97±0.02	0.81±0.01	
50	9.0±.0.6	1.61±0.01	0.70±0.02	
100	9.0±0.2	1.35±0.02	0.70±0.02	

leaves turns the colour from green to yellow which attributable for the inhibition of process of photosynthesis, however the plant experiences a most rapid death that is believed to be due to the production of secondary toxic substance in conjunction with atrazine. Investigation shows injury symptoms include yellowing (Chlorosis) of leaf tissue followed by death (necrosis) of the tissues. Cd combined with atrazine act as a seedling growth inhibitor and these are active at two main sites, the developing shoot and the root. The roots of bean plant were more effected (Table 2). Cd stops the cell division in roots, which ultimately effect on growth of shoot (Table 3) and inhibits shoot elongation and lateral root formation. The contents of chlorophyll a and b, carbohydrate, proteins and amino acids decline as the concentration of heavy metal combine with atrazine get enhanced. The amino acid were found to be absent at high concentration of toxic Cd metal (Table 2 and 3). Amino acid synthesis inhibitors act on a specific enzyme to prevent the production of specific amino acid, key building blocks for normal plant growth and development. Cadmium lowered the contents of chlorophyll a and b (Table 4) which may be attributed to the inhibition of protochlorophyllide reductase and inhibition of aminolevalinic acid synthesis. Effect of Cd on plants stops the production of amino acid at high dose. From Table 2 it was observed that amino acid contents at 20 ppm of Cd and high dose of atrazine were found to be increases in shoots of plants which may be due to the degradation of atrazine by soil microorganism. Because bioremedation of atrazine gives ammonia which ultimately converts into nitrate. Further more, the reduction of chlorophyll content seemed to be more intensive at higher concentration of cadmium, which can be explained by the high accumulation of metal in the plants. It has been suggested that Cd inhibits chlorophyll biosynthesis via inhibition of enzymes implicated in this biosynthesis[18]. The lower level of chlorophyll contents may relate with Mg^{+2} deficiency in seedling (Table 4).

Atrazine in presence of heavy metal contaminated water sewage sludge also act as a pigment inhibitors, prevent plants from forming photosynthesis pigments. As results shows that plant turns yellow to translucent and plants growth, was effected especially, as the cadmium concentration increased, with the deleterious effect observed for the root. The results also indicate that N_2-fixation of root nodule was stimulated to some extant at the low level of cadmium addition, but decreased sharply with further increase of the Cd concentration[10]. It was also found that high concentration of cadmium significantly inhibited the endogenous, IAA and FC induced growth of the maize simultaneously with growth measured acidification of the medium also inhibited by cadmium[19].

Analysis of microbial population in roots of plant: It was observed that total number of CFU were high in 20 ppm concentration as compared to 100 and 200 ppm of Cd as shown in Table 1. Atrazine is a toxic herbicide which is degredated by microorganism and convert into CO_2 and NH_3 which help in removing the harmful effect of herbicide but presence of heavy toxic metal effect the microbial life[20] results in the inhibition of bioremdiation of atrazine which attributed to the constrain of plant growth

Table 4: Effect of Cd with atrazine herbicides on Chlorophyll contents of bean plants

R Conc. of atrazine (ppm)	Chlorophyl (mg g^{-1})					
	Cd (20 ppm)		Cd (100 ppm)		Cd (200 ppm)	
	a	b	a	b	a	b
0	0.128±0.03	0.047±0.02	0.095±0.02	0.033±0.02	0.067±0.03	0.031±0.02
5	0.102±0.02	0.043±0.03	0.261±0.06	0.132±0.02	0.087±0.03	0.033±0.1
10	0.078±0.02	0.034±0.02	0.229±0.04	0.097±0.02	0.082±0.5	0.035±0.02
50	0.077±0.01	0.033±0.01	0.187±0.02	0.094±0.02	0.074±0.3	0.034±0.03
100	0.073±0.01	0.023±0.01	0.155±0.02	0.037±0.02	0.073±0.02	0.03±0.04

and lower population structure. A soil bacteria *Agarobacterium radiobacter* has ability to degredate atrazine under various cultural conditions[21]. Other bacteria can do mineralization of atrazine but they are sensitive towards toxic heavy metal therefore due to their lower population size (Table 1), they were not able to degredate the atrazine and therefore atrazine metabolism were effected.

CONCLUSION

The present investigation reveals that Cd is highly toxic to the seed germination, growth and nutritive value of plants and can be considered as major environmental pollutant. It induces growth inhibitory effect as well as physiological and metabolic disturbances. Its toxicity was prominent when atrazine herbicide spray used for controlling weeds, which also acts as growth inhibitor of photosynthesis process. Microbial life of soil found to be lowered due to the toxicity of Cd, which results in the inhibition of degredation of atrazine.

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