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The Inhibition of Bean Plant Metabolism by Cd Metal and Atrazine II: The Inhibition of Bioremediation of Atrazine in Heavy Metal Environment and it's Effect on Mineral Nutrients of Bean Plant

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Abstract: This recent research has pronounced effect of heavy toxic metal cadmium (Cd) on inhibition of bioremediation of atrazine herbicide. Results reflect that low population structure of beneficial microorganism due to toxic Cd metal attributed to the inhibition of biodegradation of atrazine. Due to which up taking of essential minerals ions from soil significantly reduced in seedling of bean plant. Morphological toxicity symptoms were appeared as yellow leaves of plant, which may results in, decrease in photosynthesis and impaired mineral nutrition.

Key words: Cd, atrazine, bioremediation, mineral ions, inhibition

INTRODUCTION

Herbicides are increasingly being used to enhance crop protection by controlling competing vegetation. The herbicide atrazine is used through out the world. Between 1980 and 1990 the United States used approximately 800 million pounds of atrazine. The processes of finding microorganism that digest harmful contaminates found in our environment, better known as bioremediation and utilizing them to clean up specific contamination sites shows a great deal of promise[1,2].

The alkyl side chain of atrazine [2-Chloro-4-ethylamino-6-isopropylamino-s-triazine which means triazine ring is symmterical] contains the only carbons capable of providing energy to microorganism through oxidation on the basis of the accumulation of dealkylated degradation products in soil treated with atrazine[3]. It seems that side chain carbon is susceptible to microbial attack. Atrazine concentration greater than 0.05 μm inhibited the protoplast O_2 evolution. The triazine herbicides affect the plant growth by inhibition of photosynthesis, it might change the performance of the crop and its nutrition. The primary mechanism for the dissipation of atrazine from the environment is through biological degredation[4]. Many vegetable crops are sensitive to atrazine, which is available as wettable powder. Plant takes herbicide after germinating until the seedling emerges from the soil. Because movement within the plant is limited, herbicide injury is confined primarily to roots and shoots[5].

Various studies[6-10] have been focused on the effect of heavy metal on bacterial community structure and found that beneficial microorganism, which are responsible for the biological functioning of soil and thus regulates the soil fertility have been found to be highly sensitive to toxicity of heavy metals[11]. In particular bacteria of the genus Rhizobium, which are able to fix atmospheric nitrogen in symbiosis with legumes die out in soil subject to only moderate contamination with heavy metals[12,13]

This research has been designed to establish base line concentration of Cd with atrazine herbicide and analyzed the effect of Cd metal on growth that are inhibitory or lethal to microbes found in roots of seedling of bean plant and their effect on mineral nutrient ions and morphology of plants. Results will also discuss the inhibition of bioremedation of atrazine due to low population of microbiota. This research will thus develop the understanding of the effects of pollutants and anthropogenic interference on microbial life and ecosystem functioning.

MATERIALS AND METHODS

The growth of the bean plants with varying concentration of atrazine viz., 5, 10, 50 and 100 ppm with constant concentration of Cd like 20, 100 and 200 ppm were observed in growth chamber in September 2004 in six pots containing half strength Hoagland solution for 2-8 weeks. Ten to fifteen seeds of bean, soaked in

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water for 4-6 h were surfaced sterilized with 0.3% calcium hypochlorite for five minutes and rinsed with deionised water. They were introduced into different pots containing 0 to 200 ppm Cd based Hoagland solution. Plants were analyzed after two weeks. Na, K, Mn, Mg, Ca and Fe were estimated through dry ash method by flame photometer and atomic absorption Spectrophotometer. For phosphate contents the extracts prepared by dry ashing method, was shaken with ammonium molybdate and stannous chloride. A blue colored complex with phosphate was obtained. Absorbance was measured at 660 nm by spectrophotometer[14].

Test for microbial community: Roots of bean plants have been removed for the microbial analysis after 8 weeks. Roots were rinsed with sterile saline, crushed and suspended in saline for over night then plated on nutrient agar. After 24 h Colony Forming Units (CFU) were counted and organisms were identified by biochemical tests. The isolated colonies were taken and identified by gm negative staining on TSI agar slant, Indol test, nitrate reduction test and growth on EMB agar plate were noted

RESULTS AND DISCUSSION

The trizaine ring found in atrazine is well known for being resistant to degradation under aerobic condition. The extensive use of this herbicide along with the triazines resistance to degrade greatly increases the chance for long-term environmental contamination. Results showed that the roots of bean plant were more effected as compared to the shoots (Table 1) and therefore mineral up taking of the roots (as compared to control plants Table 2) markedly suppressed and regular decline in concentration of mineral ion were observed with the increase in concentration of atrazine along with Cd (Table 3 and 4). Investigation suggests that Cd is one of the most harmful metal which can constrain the plant growth and development. Nodulation of bean plant was greatly inhibited in presences of Cd and atrazine[11-13]. The weight ratio of bean plant root/ leaf decrease, which might explain, the reason for nodulation decrease. High dose of Cd were also associated with the changes in the structure of root nodule in which effective N_2-fixing area was reduced and N_2-fixing cells in the area also reduced[10]. This may be related with the low community[15] of the soil beneficial microorganism due to Cd (Table 5). Delay in seed germination may also be related to the nitrogenase activity of nodules and reduced root length. These results show that atrazine damage and reduce the activity of the immune system. Morphological toxicity symptoms were observed, especially at high dose of Cd in the leaves, leaves turns yellow and plant die in seedlings stage. It was observed that plant was unable to stand in erect position. High concentration of atrazine is known to reduce germination percentage. Growth reduction due to Cd may result from decreased photosynthesis and impaired mineral nutrition[16]. K stimulated growth rates of apical and intercalary segments of plant and also responsible for ionic balance, opening of stomatas and other plant movements as well as enzyme and protein synthesis. Cd exposure of *Vigna radiata* led to substantial changes in nutrients mineral ions composition (Table 3 and 4). In fact leaves of plant grown in the presence of Cd contained less Fe then control one (Table 2). Decrease of this nutrients could be the major origin of necrosis and chlorosis appeared in leaves. Iron deficiency was reported in several plant species treated by cadmium. Mg^{2+} was also reduced by Cd application in the leaves. Cd inhibits Mg^{2+} transport to the shoot of sugar beet and could be the cause of leaf area decrease and loss of chlorophyll. Atrazine degradating microorganism, a solution to the clean up of atrazine pollution. But microorganism is sensitive towards heavy metal contaminated environment and low population structures of organism in soil unable to degradate the atrazine. Therefore significant reduction in seed germination, plant growth, physiological processes and

Table 1: Effect of Cd metal with atrazine on growth of seedlings of bean plant

Atrazine (ppm)	Average root length (cm) (Cd 20 ppm)	Average shoot length (cm) (Cd 20 ppm)	Average root length (cm) (Cd 100 ppm)	Average shoot length (cm) (Cd 100 ppm)	Average root length (cm) (Cd 200 ppm)	Average shoot length (cm) (Cd 200 ppm)
0	4.50±0.01	20.0±0.02	4.54±0.01	21±0.10	4.38±0.04	17.40±0.02
5	4.18±0.05	19.0±0.04	4.41±0.02	20±0.10	3.82±0.01	15.10±0.01
10	4.00±0.02	18.8±0.01	3.90±0.04	19±0.01	3.90±0.01	11.04±0.03
50	3.50±0.01	18.6±0.01	3.50±0.01	18±0.02	3.90±0.01	1.04±0.01
100	3.20±0.03	17.8±0.01	3.00±0.10	19±0.02	1.82±0.01	1.42±0.02

Table 2: Analysis of mineral ions of *vigna radiation* in control plant

Control	K (%)	Na (%)	Ca (%)	Mg (%)	Mn (%)	Fe (%)	PO_4 (%)
Roots	2.90±0.03	3.2±0.04	0.51±0.01	0.44±0.02	0.05±0.02	0.015±0.01	4.1±0.01
Shoots	3.41±0.02	3.6±0.04	0.61±0.02	0.35±0.02	0.31±0.01	0.044±0.02	4.7±0.10

Table 3: Effect of Cd in conjunction with atrazine on mineral ions contents of roots of bean plant

	(Cd) 20 ppm						
Atrazine (ppm)	K (%)	Na (%)	Ca (%)	Mg (%)	Mn (%)	Fe (%)	PO_4x10^{-3} (%)
0	2.610±0.01	2.91±0.03	0.41±0.00	0.310±0.05	0.027±0.02	0.022±0.01	2.2±0.01
5	2.030±0.01	2.70±0.00	0.35±0.01	0.290±0.02	0.025±0.01	0.019±0.02	2.1±0.10
10	1.820±0.02	2.10±0.01	0.30±0.01	0.250±0.01	0.021±0.02	0.014±0.02	2.0±0.10
50	0.052±0.02	1.80±0.02	0.29±0.02	0.210±0.02	0.019±0.01	0.014±0.01	1.5±0.05
100	0.050±0.02	1.50±0.02	0.27±0.03	0.200±0.02	0.150±0.05	0.011±0.04	105±0.02
(Cd) 100 ppm							
0	2.070±0.02	1.90±0.06	0.39±0.05	0.025±0.20	0.025±0.20	0.019±0.03	1.8±0.10
5	2.010±0.02	1.70±0.01	0.37±0.11	0.024±0.30	0.023±0.02	0.019±0.02	1.6±0.20
10	1.960±0.02	1.40±0.02	0.25±0.02	0.019±0.02	0.019±0.01	0.015±0.02	1.5±0.10
50	1.700±0.02	1.00±0.02	0.19±0.02	0.010±0.01	0.018±0.01	0.013±0.02	1.1±0.02
100	1.500±0.01	0.99±0.20	0.12±0.02	0.110±0.02	0.011±0.01	0.012±0.01	1.0±0.02
(Cd) 200 ppm							
0	2.040±0.01	2.00±0.01	0.36±0.01	0.025±0.01	0.024±0.01	0.015±0.03	1.7±0.02
5	2.030±0.02	1.91±0.21	0.35±0.01	0.171±0.01	0.024±0.01	0.140±0.01	1.4±0.02
10	2.010±0.01	1.81±0.02	0.32±0.02	0.108±0.01	0.021±0.02	0.013±0.01	1.3±0.02
50	2.020±0.02	1.20±0.02	0.25±0.02	0.108±0.02	0.018±0.01	0.012±0.02	1.2±0.02
100	1.900±0.02	1.00±0.01	0.19±0.02	0.100±0.01	0.018±0.02	0.011±0.05	1.0±0.02

Table 4: Effect of Cd in conjunction with atrazine on uptake of mineral ions content of shoots of bean plant

	(Cd) 20 ppm						
Atrazine (ppm)	K (%)	Na (%)	Ca (%)	Mg (%)	Fe (%)	Mn (%)	PO_4 (%)
0	3.23±0.01	3.60±0.01	0.45±0.02	0.25±0.01	0.045±0.01	0.0251±0.02	0.025±0.02
5	2.52±0.01	2.90±0.01	0.31±0.02	0.23±0.02	0.041±0.02	0.0241±0.02	0.024±0.01
10	2.52±0.01	2.60±0.02	0.29±0.02	0.19±0.02	0.390±0.02	0.0210±0.02	0.023±0.01
50	2.41±0.01	2.10±0.01	0.21±0.01	0.15±0.02	0.028±0.02	0.0070±0.02	0.0019±0.02
100	2.81±0.02	1.90±0.01	0.18±0.02	0.08±0.05	0.002±0.02	0.0060±0.02	0.0018±0.03
(Cd) 100 ppm							
0	3.69±0.01	4.01±0.02	0.39±0.02	0.30±0.01	0.060±0.01	0.0240±0.01	0.021±0.01
5	2.85±0.01	3.90±0.01	0.31±0.01	0.29±0.01	0.040±0.01	0.0230±0.01	0.019±0.01
10	1.82±0.01	3.10±0.02	0.30±0.02	0.28±0.01	0.030±0.01	0.0100±0.01	0.017±0.01
50	1.91±0.02	2.80±0.01	0.29±0.01	0.19±0.02	0.025±0.01	0.0130±0.01	0.120±0.05
100	1.95±0.02	1.70±0.02	0.20±0.01	0.17±0.02	0.025±0.01	0.0090±0.01	0.011±0.01
(Cd) 200 ppm							
0	3.13±0.02	3.20±0.01	0.31±0.01	0.28±0.01	0.030±0.01	0.2000±0.01	0.09±0.02
5	2.02±0.02	2.90±0.01	0.29±0.01	0.25±0.01	0.020±0.02	0.1900±0.01	0.016±0.01
10	2.01±0.01	2.50±0.01	0.25±0.01	0.21±0.01	0.010±0.01	0.1600±0.01	0.014±0.01
50	1.40±0.01	2.30±0.01	0.19±0.03	0.17±0.02	0.009±0.02	0.1300±0.01	0.013±0.01
100	1.30±0.01	1.60±0.01	0.15±0.01	0.12±0.01	0.008±0.02	0.1000±0.01	0.011±0.01

Table 5: Microbes found in roots of bean plant in presence of Cd and atrazine

Sample category	Gram reaction	Arrangement	Shape	Growth on EMB	Growth on TSI	Indol	Organism
Cd 0 ppm	Gram-ve	Scattered	Rods	LF	A/A gas +ve H_2S-ve	+ve	Klebsiella
Cd 20 ppm	Gram-ve	Scattered	Rods	LF	A/A gas +ve H_2S-ve	+ve	Entreobacter
Cd 100 ppm	Gram-ve	Scattered	Rods	LF	A/A gas +ve H_2S-ve	+ve	Klebsiella
Cd 200 ppm	Gram-ve	Scattered	Rods	LF	A/A gas +ve H_2S-ve	+ve	Klebsiella

EMB=Eosin Methylene blue, TSI=Triple Sugar Iron, LF= Lactose Fermenter

mineral up taking were observed. It may be related that atrazine were not degradated in presence of heavy metal contaminated environment due to low bacterial community which ultimately effect the processes of photosynthesis and translocation into tips of leaves[17-20] reduced the chlorophyll content by lowering the concentration of Mg^{2+} in the leaves of the seedling.

Inhibition of bioremdiation of atrazine: The increase in ionizea radiation and pollution of our environment with herbicides, pesticides, heavy metal compounds and other toxic mutagenic and carcinogenic substances presents a real danger to living organism today and their progeny in the future. Heavy metal effects on microbial life[21], which may be attributed to the inhibition of atrazine degradation. The Fig. 1 mechanism showed the complete degradation of atrazine by biological system, which converts the atrazine into the NH_3 and CO_2[17], but effect of heavy metal on rhizosphere reflects that atrazine in conjunction with heavy metal become more toxic to the seedlings of plants.

In present study the low mineral ions concentration in shoots and roots of plant show relation with the persistent of atrazine[22]. Considering the soil pollution by water soluble heavy metal salts in the industrial regions

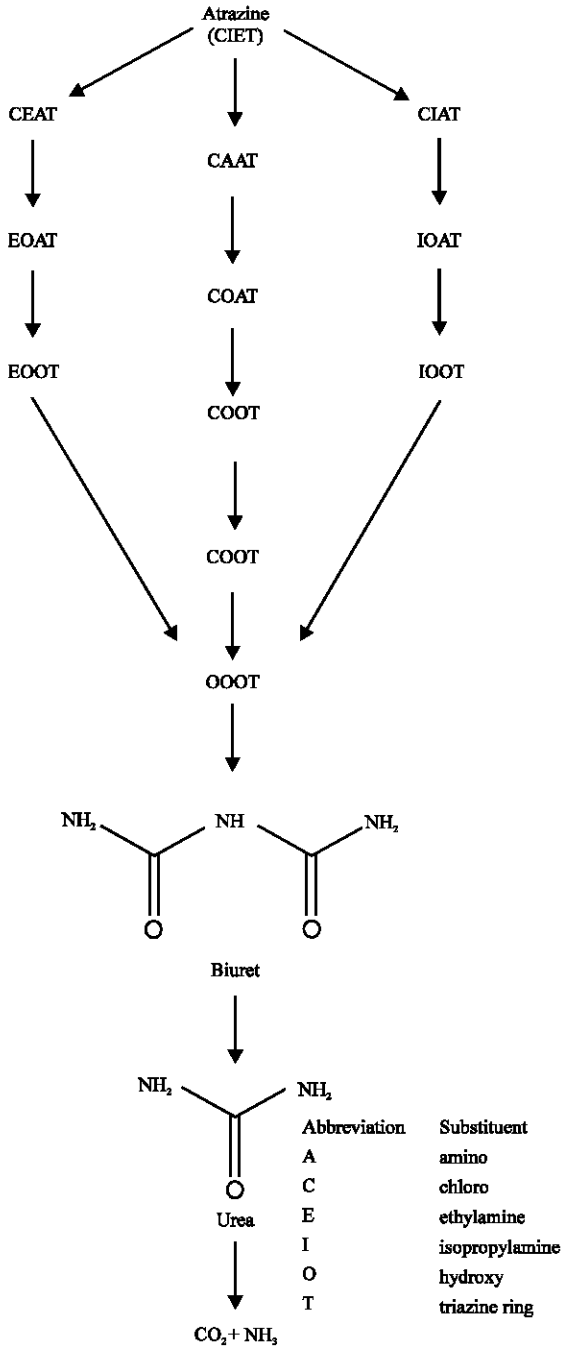

Fig. 1: Bioremediation of atrazine by soil microorganism

and the long term excessive use of mineral fertilizer, pesticides and herbicide in agricultural regions, the crops particularly vegetables and roots crops, accumulates excess amounts of harmful admixtures. All these toxic material lower the soil ability and cause the decrease in microbial life[23], which ultimately reduces the productivity of the crops. Herbicides have a considerable influence on soil microorganism and soil biochemistry. These influence are likely to be reflected in soil fertility[22] and plant growth. The microbial population of cellulose decomposers was very sensitive to herbicides. This restriction seems to depend on unfavorable food condition for these microorganisms in a soil without weeds, or it is due to enzyme inhibition by pesticides. Many studies have focused on the effects of heavy metal on bacterial community structure and have been found very sensitive towards toxic heavy metal[20-24].

CONCLUSIONS

The low population structure of microbial flora was mainly due to the heavy toxic metal which relates with the nodules inhibition in the roots of seedlings of leguminous plant. Thus absence of beneficial microorganism helps in persistent of atrazine. Atrazine combined toxic Cd metal act as photosynthesis inhibitor and retarded the growth of the plant and effects on up taking of essential mineral ions which were essential for the health of plant.

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