Int. Res. J. of Science & Engineering, 2013, Vol.1 (1): 5- 9 ISSN:2322-0015

ORIGINAL ARTICLE

Phytoremediation potential of *Brassica juncea* L. with reference to Atrazine

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ABSTRACT

Atrazine is one of the most widely used herbicide in the agriculture today. It is a triazine herbicide that is used for control of broad leaf weeds, mainly in corn and sorghum and also many other crops. Atrazine is highly persistent in soil and is leached directly from the soil into groundwater, surface water, and drinking water. Contamination may pose a significant threat to humans, wildlife, and the environment.

Greenhouse experiments were carried out to determine potential capability of *Brassica juncea* L. plant to remediate atrazine contaminated soil. Persistence of atrazine in the treated soil and its residue were estimated by High performance liquid chromatography (HPLC). Data obtained with soil treated at 1000ppm of atrazine showed that residues of atrazine were less in soil planted with *Brassica juncea* L. compared with unplanted soil. Considerable concentration of atrazine i.e. 20.5µg/ml and 18.5µg/ml were detected in sterilized unplanted soil and soil planted with *Brassica juncea* L. respectively after 30 days of sowing. While these values were 17.5µg/ml and 14.0µg/ml in non-sterilized unplanted and planted soil respectively. This study demonstrated that residues of atrazine were reduced at faster rate in treated soil which was planted with *Brassica juncea* L. than the unplanted soil indicating that *Brassica juncea* L. was useful for phytoremediation of soils contaminated with atrazine.

KEYWORDS

Atrazine, *Brassica juncea* L., Phytoremediation, HPLC

INTRODUCTION

High concentration of pesticides in the soils is a serious problem at agrochemical dealer sites where contaminations has resulted from inadvertent spillage during mixing and loading the chemicals for application to crops. Expensive remediation technologies may not be economically feasible for such dealerships. In addition, biological approaches (bioremediation) may be inhibited by the presence of mixtures of contaminants at high concentrations.

Plants can have a positive influence on removal of organic wastes by taking up contaminants into the plant tissue, with the possibility of further metabolism into innocuous compounds. Plants also influence the degradation of contaminants as a result of increased microbial activity associated with the roots (Anderson *et al*., 1993).

A phenomenon that can occur in soil that has had long-term exposure to chemicals is enhanced degradation. When the chemical is applied to soil that has previously been exposed to that chemical, accelerated decomposition can occur. This enhanced selective pressure will dominate and be poised to more rapid degradation of the compound upon the next application (Roeth, 1986).

Atrazine- Atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine) is a commonly used triazine-class herbicide. The major atrazine

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consuming states are Punjab, Haryana, Uttar Pradesh, Bihar, and Southern states of India. Atrazine is used against broadleaf weeds and grassy weeds in monocot (primarily corn) production (Humburg *et al*., 1989). It has wide range of half lives reported in the literature, ranging from two weeks Winklemann and Klaine, 1991 to four months (Weed *et al*., 1995) after field application. The average half-life value is around two months (Weed *et al*., 1995). Atrazine is not very soluble in water, which limits its mobility in soil. Despite this moderate mobility atrazine is frequently found contaminating groundwater resources, especially in the midwest (Hallberg, 1989). It is not volatile, so little is lost to the atmosphere. The major mechanisms of atrazine disappearance from a point of deposition are chemical and biological degradation. Phytoremediation has the potential to be a cost-effective alternative for remediation of soils contaminated with atrazine.

Atrazine has been the most important herbicide used over the last 30 years for non selective weed control on industrial and non cropped land (Radosevich, 1994). It is also used as pre-emergence herbicide for selective control of weeds mainly in maize, sorghum and sugarcane cultures, as well as in asparagus, vines, coffee, oil palms, roses, grassland, forestry, fruit orchard such as citrus, bananas, pineapples, guavas, and macadamia (Tomlin, 1994), and in irrigation channels for cotton production (Van Zwieten and Kennedy, 1995).

MATERIALS AND METHODS

1. Seed Testing:

Seeds of *Brassica juncea* L. (PM-1) were purchased from M.P Krishi vidyapeeth (Rahuri, Ahmednagar). Effect of various concentrations of atrazine (20ppm, 100ppm, 500ppm, 1000ppm,) on seed germination of *Brassica juncea* were examined in petridish by wet filter paper method.100ppm, 500ppm and 1000ppm were found suitable, hence chosen for further work.

2. Degradation Studies:

Garden soil was used for the experiments. Soils were treated by atrazine solution at different concentrations (100ppm, 500ppm and 1000ppm). Before the treatment half of the soil was sterilized by autoclaving. Sterilized and Non-sterilized soil was kept aside for testing/ analysis. 42 pots were filled with soil. Half of the pots with autoclaved soil were sown with seeds and remaining half were used without growing the plants. Similarly, half of the Non-autoclaved soil pots were sown with seeds and remaining half Non-autoclaved soil pots were without sown by seeds. All the pots with Autoclaved/ Non-autoclaved, planted/Unplanted soil treated by atrazine at different concentrations were kept under observation up to 30 days. Controls were kept without adding any solutions. After 10 days 12 pots of different concentrations from autoclaved and Non-autoclaved soils and 2 control pots were removed. All plant and soil samples were collected from each pot dried, ground to a fine powder and subjected to further analysis after extraction. Similar procedure was followed after 20 and 30 days.

3. Extraction of atrazine from soil

Atrazine was extracted from the soils by weighing 0.5gm of soil moistened with 5ml distilled water into a 15ml conical tube and vortexing the soil-water suspension twice for 10seconds. The soil-water suspension was transferred to 1.5ml microcentrifuge tubes and the tube was centrifuged at 20,000×g for 15min. the supernatant was transferred to a 0.22µm Teflon filter inserted in a microfuge tube and filtered by centrifuging at 4,000×g for 5min. the filtrate was transferred to 1.5ml HPLC vial and was analyzed with a HPLC instrument (Shaner *et al*., 2007.).

4. Extraction of atrazine from Plant Biomass

Plant Biomass Root and Shoot were removed from soil washed, dried completely, ground in a mortar and pestle to a fine powder and sieved. Shoot and Root sample were extracted by 4:1 (Methanol: Water) 0.2gm of dried plant material homogenized with 10ml of 80% CH_3OH (4:1 CH_3OH: Distilled water) for 1min using homogenizer at 10,000rpm. Followed by sonication for 20 minutes. Samples were centrifuged for 20 minutes at 12,000×g (7000rpm) at 0ºC. Supernatant was decanted. The extraction procedure repeated for 1hr. on residue remaining in the centrifuge tubes using 100ml of 80% CH_3OH. The filtrate was transferred to 1.5ml HPLC vial for analysis.

5. Preparation of standards:

Atrazine standard were purchased from Sigma Aldrich in the form of Atrazine Pestanal®. Three standard proportions were made in water at 0.3mg/ml, 0.2mg/ml and 0.1mg/ml Distilled water.

The other three standard proportions were made in methanol: water (4:1) at 0.3mg/ml, 0.2mg/ml and 0.1mg/ml.

6. HPLC Analysis:

The standards of Atrazine, soil and plant samples were analysed by Agilent HPLC instrument equipped with a UV-visible detector. Analytes were separated on an Eclipse plus-C18 (5µm 4.6×250mm) column. The filtrate of plant and soil samples were analysed using mobile phase as acetonitrile: 5Mm ammonium acetate adjusted pH 4.6 as (35:65vol/vol) and it was run isocratically at 40ºC at a flow of 1ml min^{-1}. The injection volume was 100µl. atrazine was detected at 223nm and the retention time was 8.9 min. The limit of detection was 1.2µg ml^{-1} (Shaner *et al*., 2007).

RESULTS AND DISCUSSION

Soil was spiked with 100ppm, 500ppm, and 1000ppm of atrazine and incubated for 10, 20, 30 days. Removal of atrazine from soil was estimated by HPLC. The results were observed on the retention time (Rt) 8.9 at 223nm. The concentration of atrazine in each samples were observed in µg/ml.

No peaks were observed at retention time 8.9 in Autoclaved and Non-Autoclaved soil before atrazine treatment indicating absence of Herbicide.

Table 1: Degradation of atrazine in planted and unplanted soils(Autoclaved/Non-autoclaved) at various time intervals.

Days	Concentrations (ppm) of atrazine used/ atrazine (µg/ml) detetected in samples.											
	100				500				1000			
	AUP	AP	NAUP	NAP	AUP	AP	NAUP	NAP	AUP	AP	NAUP	NAP
0	33.3	-	31.7	-	37.2	-	36.4	-	42.5	-	41.0	-
10	25.4	23.4	23.2	18.9	32.6	31.2	31.0	26.2	37.5	37.0	37.1	34.0
20	14.0	11.8	13.0	7.8	25.8	20.2	21.0	18.4	31.0	24.5	26.0	20.0
30	7.4	5.4	7.2	4.8	13.0	10.8	9.80	8.8	20.5	18.5	17.5	14.0

Table 2: Percentage reduction in concentration of atrazine at various time intervals.

Days	Concentrations (ppm) / Percentage reduction.											
	100				500				100			
	AUP	AP	NAUP	NAP	AUP	AP	NAUP	NAP	AUP	AP	NAUP	NAP
10	23.73	29.73	26.82	40.38	12.37	16.13	14.84	28.03	11.77	12.95	9.52	17.08
20	57.96	64.57	59.00	75.40	30.65	45.70	42.31	49.46	27.06	42.36	36.59	51.22
30	77.78	83.79	77.29	84.86	64.06	70.97	73.08	75.83	51.77	56.48	57.32	65.86

Table 3: Concentration of atrazine in plant biomass grown in Autoclaved/Non-autoclaved soil treated with atrazine at various time intervals.

Days	Concentrations (ppm) / Percentage reduction.											
	Biomass: Autoclaved soil						Biomass: Non-autoclaved soil					
	100ppm		500ppm		1000ppm		100ppm		500ppm		1000ppm	
	S	R	S	R	S	R	S	R	S	R	S	R
10	-	1.2	1.5	3.0	-	11.0	-	2.0	2.0	4.5	6.0	22.0
20	27.0	33.0	28.0	37.0	31.5	72.0	26.0	34.0	45.0	59.0	45.5	73.0
30	32.0	43.0	59.0	91.0	80.0	119.0	52.0	46.0	66.0	116.0	85.0	125.0

Abbreviations –

AS- Autoclaved soil immediately after adding solution; **AP**- Autoclaved planted.
NAS- Non-autoclaved soil immediately after adding solution; **NAP**- Non-autoclaved planted.
AUP- Autoclaved Unplanted; **NAUP-** Non-autoclaved unplanted; **S-** Shoot; **R-** Root.

Concentration of atrazine in Autoclaved soil (AS) immediately after addition of 100ppm, 500ppm, and 1000ppm solutions was 33.3µg/ml, 37.2 µg/ml and 42.5 µg/ml respectively **(*Table 1*)**. Concentration of atrazine in Non- Autoclaved soil (NAS) immediately after addition of 1000ppm, 500ppm, and 100ppm solutions was 31.7µg/ml, 36.4µg/ml and 41.0µg/ml respectively **(*Table 1*)**.

The result showed gradual decrease in concentration of atrazine in all the soil samples with passage of time. However this reduction was maximum in non-autoclaved planted soil (65.86%) and minimum in autoclaved unplanted soil (51.77%) **(*Table 2*)** at the end of 30days. A comparison of planted and unplanted soil for reduction of atrazine concentration (AUP/AP and NAUP/NAP) showed better results in planted soil for the same period of time. Previously results with respect to percentage we obtained with NAUP (57.32%) when compared to AUP (51.77%). Similarly better results were obtained with NAP (65.86%) when compared to AP (56.48%) (***Table 2***).

Though there was decrease in peak area of atrazine (Rt-8.9), with passage of time there was a corresponding increase in the number of other peaks, which probably indicated appearance of degradation of atrazine, this was more prominent in soils.

Better results obtained with planted soils indicated that *Brassica juncea* L. played an important role in degradation of the herbicide. This degradation was aided further by micro flora in Non autoclaved soils. Rate of degradation in Autoclaved soil was lesser in first few days due to lack of organisms but it caught- ups with time up to 30days.

The amount of degradation occurring can be affected by the concentration of atrazine in the soil before the addition of vegetation and by the length of time the plants are allowed to grow (Zhao *et al*., 2003). Promotion of degradation in the rhizosphere has been demonstrated using switchgrass, this study also showed that after 25 days, more than 80% of the atrazine in a soil planted with switchgrass is degraded to less toxic metabolites, and 47% of these residues are hydroxylated metabolites that are considerably less mobile (Lin *et al*., 2008). Further a combination of switchgrass and soil microbes proved to be most effective in the degradation of atrazine, with no atrazine being detectable in treated sand after seven days (Murphy and Coats, 2011).

Plant biomass viz. shoot (S) and Roots (R) of *Brassica juncea* L. grown in Autoclaved and Non-autoclaved soils spiked with 100, 500 and 1000ppm of atrazine were harvested at interval of 10,20,30 days.

The results indicated **(*Table 3*)** increase in accumulation of atrazine in biomass with increase in both concentration as well as time. Accumulation being more in Roots when compared to shoots. Atrazine could not be detected in shoots at 100ppm concentration at the end of 10 days both in Autoclaved and Non-autoclaved soils. Higher concentrations of atrazine were detected in Roots and shoots of Non-autoclaved when compared to Autoclaved soils.

Uptake and accumulation of soil contaminants by the plant biomass is a well reported phenomenon. Many contaminants are also known to be broken down by plant enzymes into metabolites of parent compounds and these metabolites are feverally, though not always less toxic one than the parent compound (Arthur *et al*., 2005). In the present investigation increase in number of peaks at different retention time with increase in time of harvest probably indicates appearance of degradation products of atrazine. Similar observations were made by Henderson *et al*. 2007 with switchgrass for this herbicide.

Further Murphy and Coats (2011) reported significantly higher rate of atrazine degradation in switchgrass system when compared to naturally occurring chemical degradation suggesting possibility of using switchgrass in phytoremediation technology.

Similar observations were made in present investigation where *Brassica juncea* L. a system gave higher atrazine degradation when compared to natural method of degradation, indicating that this fast growing plant can be used as a potential degrader for remediation of atrazine contaminated sites.

CONCLUSION

In present investigations where higher atrazine degradation was achieved in *Brassica juncea* L. system indicates that this plant can be used in

phytoremediation technology for remediation of atrazine contamination sites.

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Cite this article as: Khan Shahana J and Gaikwad Rupali S. Phytoremediation potential of *Brassica juncea* L. with reference to Atrazine, Int. Res. J. of Science & Engineering, 2013; 1 (1): 5-9

Source of support: None **Conflict of Interest:** None