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ATRAZINE TOXICITY AND REMEDIATION STRATEGIES

Kolekar Parag Dnyandeo[1]*, Pawar Ganesh Babaso[2], Jadhav Jyoti Prafullaa[1]

1.Department of Biotechnology, Shivaji University, Kolhapur, India.

2.Department of Botany, Shivaji University, Kolhapur, India

ABSTRACT: Increasing human population is putting enormous pressure on food supply and agriculture. To increase crop production, pesticides are used intensively. Atrazine (herbicide) which has proved economical in case of maize and sorghum for yield increase and price reduction for farmers. However, extensive use of atrazine in agriculture resulted in pollution and adverse effects on the environment.There is need to tackle atrazine contamination. This review explores the way for atrazine remediation.

Atrazine, atrazine toxicity, remediation, bioremediation, bacteria.

***Corresponding Author: Dr Kolekar Parag Dnyandeo** Ph. D.

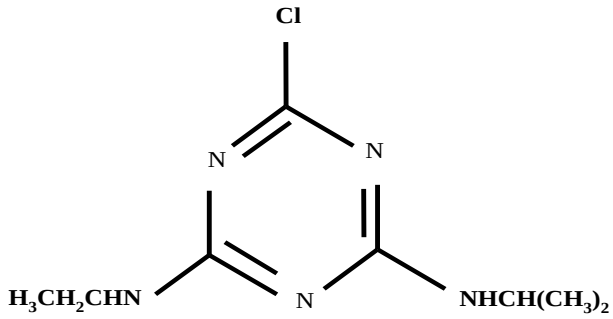
Department of Biotechnology, Shivaji University, Kolhapur, India

*Email Address: parag.kolekar@gmail.com

1. INTRODUCTION

At the beginning of 1950s Giegy Laboratories (Basel, Switzerland) Atrazine and other members of triazines were synthesized and tested for the first time (Gast, 1970). It is the most broadly used as a selective herbicide in maize farms.

Table 1: Atrazine (PubChem CID: 2256) characteristics (Kidd and James 1991, Tomlin, 1994)

Structure	
Synonyms	**6-Chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazin-2,4diamine, 2-chloro-4-ethylamino-6-isopropylamino-s-triazin,**
Common name	**Atrazine**
Chemical name	**2-Chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine**
Appearance	**colorless, crystalline solid**
Empirical formula:	**$C_8H_{14}ClN_5$**
Rel. molecular mass	**215.69**
Density:	**1.2 g/cm^3**
Boiling point:	**not distillable**
Melting point:	**173-175°C**
Vapor pressure:	**4 x 10^{-5} Pa**
Solubility	**Water: approx.33 mg/l; in ether: 12 g/l (20°C); in methanol: 18 g/l (at 27°C); in n-pentane: 36 mg/l (27°C);in chloroform: 52 g/l (27°C)**

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Atrazine weed control spectrum is the same as that of symmetric 2-chloro-4, 6-bis (alkylamino)-s-triazines. Strong action on dicotyledonous weeds as well as high efficiency against monocotyledonous perennial weed is characteristic of atrazine. This makes it favorable herbicide. Another attribute of atrazine is, it gets readily absorbed through leaves. Therefore, it can be used for the post-emergence application. Mineral oils as adjuvants help to increase post-emergence activity (Tomlin, 1994). For atrazine, there are no natural sources. It is chemically synthesized from cyanuric acid chloride with the addition of ethylamine and isopropyl amine. The reaction takes place successively in tetrachloromethane.

Atrazine fate

Atrazine is commonly used for pre and post-emergence control of grass as well as weeds. In dry conditions, it is highly persistent in the soil for the period up to 1 year. The half-life of atrazine is in the range of 60-100 days (Cohen et al., 1984). While in plants it gets absorbed through roots and foliage. It accumulates in the meristem parts and new leaves (Tomlin, 1994). It inhibits photosynthesis in absorbed plants. In some plants, it is metabolized (Zhang et al., 2014). Physical and chemical properties of atrazine like low vapor pressure (table 1); moderate solubility in water, enhances its leaching property. Distribution coefficient K_d in soil is (Hydroxyatrazine >> Atrazine > Deisopropylatrazine > Desethylatrazine). Hydroxyatrazine (HAT) has a strong affinity for soil while atrazine, hence Deisopropylatrazine (DIA) and Desethylatrazine (DEA) are more expected to desorb from the ground than HAT (Mersie and Seybold, 1996; Shipitalo and Owens, 2003). This is the prime reason for detection DEA and DIA than HAT in surface runoff and water body contamination (Lerch et al., 1999; Meyer and Elsner, 2013). In soil, both biotic, as well as abiotic processes, are responsible for atrazine degradation (Armstrong et al., 1967; Erickson et al., 1989). Abiotic factors like photocatalysis convert atrazine into hydroxyl atrazine (Jones et al., 1982). However, a biotic process like biodegradation by microorganisms forms predominantly dealkylated atrazine metabolites DIA and DEA which also acts as herbicides (Aelion and Mathur, 2001; Sene et al., 2010). Chemical hydrolysis and subsequent biodegradation by soil microorganisms are responsible for most of the atrazine degradation in the ground (Abdelhafid et al., 2000; Fang et al., 2001). Frequently soil-mediated chemical hydrolysis results in dechlorination (Armstrong et al., 1967; Erickson et al., 1989) which could be microbial-mediated, leading to the synthesis of primary hydrolytic product HAT (Behki and Khan, 1986; Mandelbaum et al., 1995; Struthers et al., 1998).

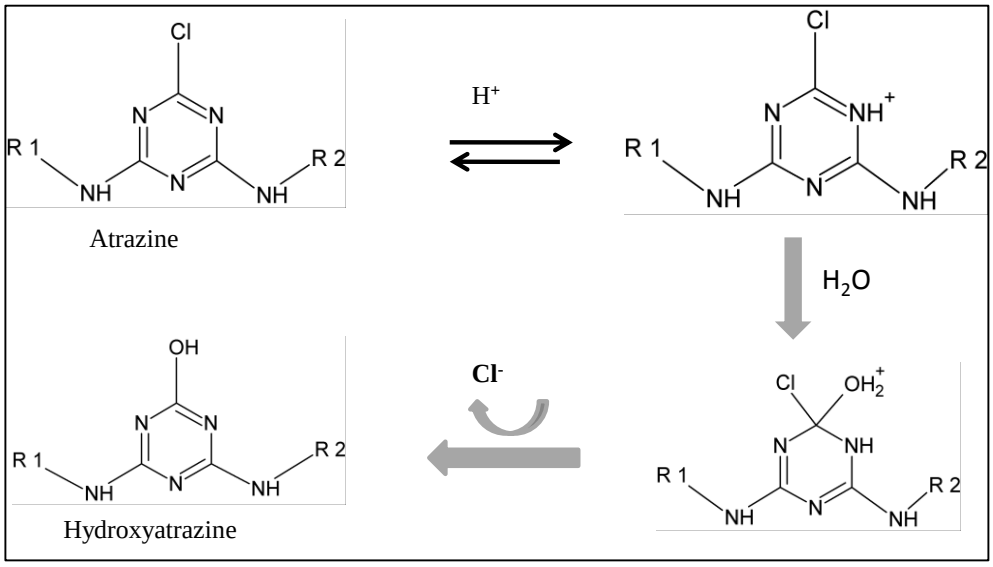

Fig. 1a: Atrazine transformation in acidic conditions

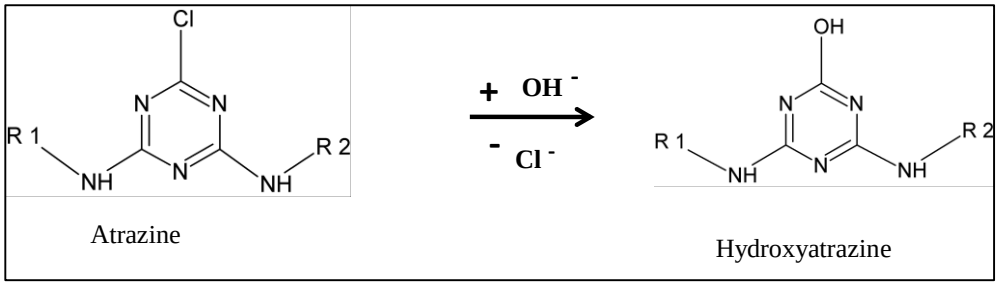

Fig. 1 b: Atrazine transformation basic conditions

Acidic or basic environments (Fig. 1) as well as high temperature, high organic content, and low moisture favor hydrolysis of atrazine in soil (Burkhard and Guth, 1981; Gamble and Khan, 1988; Smith and Walker, 1989). Hydrolysis rates may get accelerated in sterile residues or the presence of dissolved or humic or fulvic acids (Sparling and Aislabie, 1996).

Environmental concern

It is the second highest herbicide used in the world, and in India. Annual consumption of atrazine in India (Fig. 2) is 124MT (Kadian et al. 2008; Ministry of chemicals and fertilizers, GOI 2014). According to a survey, the biggest problems created by pesticide manufacturing and storage are in India, with affected populations of nearby residents. The major risk from pesticide manufacturing and stockpiling is caused by outdated and improper storage facilities can result in leakage into the environment (Harris and McCartor, 2011).

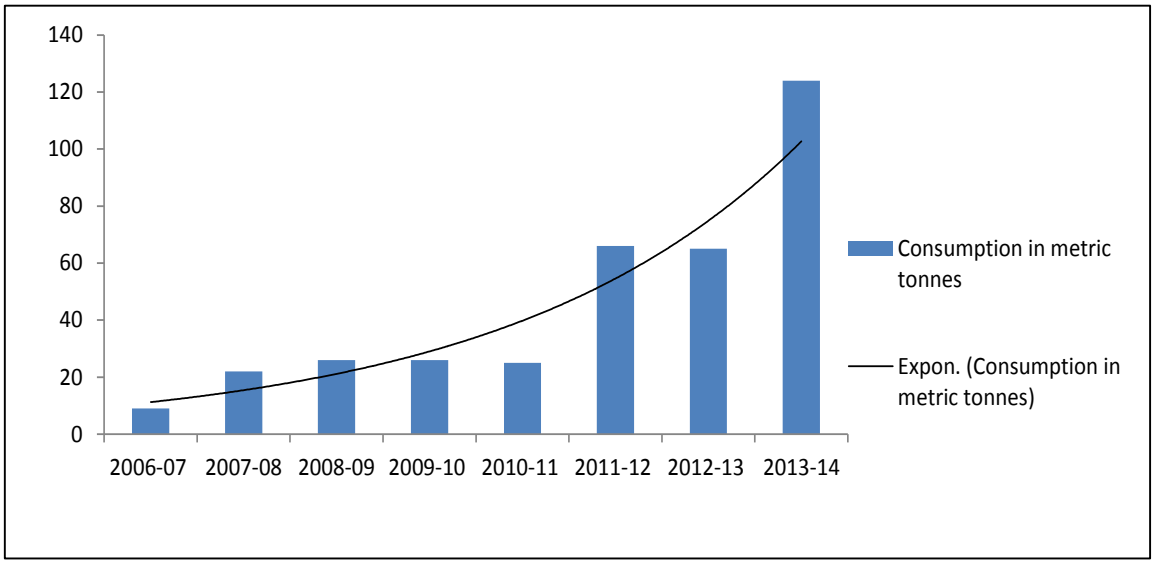

Fig. 2: Atrazine consumption in India (Ministry of chemicals and fertilizers, GOI 2014)

Environmental pollution due to extensive use of pesticides has turned out to be an immense concern to environmental regulatory bodies. Over 95% of samples collected from water bodies detected at least one pesticide. Furthermore, atrazine and its degradation product desethylatrazine were among the most frequently detected pesticides in agricultural areas (Squillace et al., 1993). Environmental investigations during last three decades in the USA have observed that atrazine concentrations are lower (U.S. EPA, 1999) but in some cases between 100 to over 600 mg L^{-1} have also been detected in natural water resources within areas of high agricultural use (Gaynor et al., 2002; Wu et al., 2009). Point sources of atrazine pollutions are pesticide manufacturing industry, handling and distribution activities, dumping site of pesticide wastes, wood treatment facilities, warehouse fires, and farms. The reasons for lesser reports on the presence of atrazine in domestic and industrial wastewater could be inconsistency in regular monitoring programs in India. Negligence in handling remaining atrazine containers by both domestic and industrial people results in the contamination of existing sewer systems after washing pesticide container (Monteith et al., 1995). By this way also atrazine can get mixed with domestic wastewater (Kearney and Roberts, 1998). Atrazine manufacturing plant effluent contains higher dissolved organic matter as well as many folds of atrazine concentration. Hence, various regulatory bodies like The United States Environmental Protection Agency (US EPA) classified atrazine as a natural water body contaminant. As per World Health Organization (WHO), the permissible limit in drinking water is lower to 2 μgL^{-1}; for US EPA 3 $\mu g\ L^{-1}$; for European Economic Council (EEC) 0.1 $\mu g\ L^{-1}$. There is no clear specification for pesticide detection limits in

India for atrazine (Council of the European Communities, 1980, Environmental Protection Rule of India, 1986, WHO, 1991).

Atrazine in soil is a severe risk to the environment because of its long persistence in a neutral environment. There are various reasons for atrazine pollution like excessive use, accidental spill, warehouse fires. This leads to the augmentation of atrazine and its metabolites in soil. Hence, atrazine remediation is necessary. According to a survey in the USA, near 20 commercial agrochemical applicators, highest pesticide concentrations were found. Herbicides such as atrazine, metolachlor, alachlor, and cyanazine were the most common. Recent studies also coherent with water contamination by atrazine (Habecker, 1989; Wu et al., 2010).

Ecotoxicity

Though atrazine is reported less toxic towards a mammal, the triazine pesticides are categorized by the EPA as possible human carcinogens (U. S. EPA, 1999). In 1991 Germany banned all atrazine-content herbicides from the German market followed by other nations like France, Norway. Atrazine and its metabolites affect aquatic as well as soil ecosystem. Generally, at higher doses atrazine toxicity effect was detected. However, in many aquatic organisms, detrimental effects were observed at low levels.

Fig. 3: Atrazine Pollution adapted from (Dalton, 2002)

These aquatic organisms' comprised of numerous species of algae (Bartell et al., 2013), fishes and frogs are affected by atrazine (Hayes et al., 2002; Oropesa et al., 2009; Zaya et al., 2011; Zhu et al., 2011). Species of algae are *Isochrysis galbana*, *Dunaliella tertiolecta, Phaeodactylum tricornutum, Pseudokirchneriella subcapitata and Synechococcus* sp. (Weiner et al., 2004). Low levels atrazine in water has been found to disrupt the sexual development of frogs by sex hormone disruption (Hayes et al. 2002). It also changes immune responses in frogs (Christin et al., 2004). These studies intensify the problems posed by atrazine and its metabolites in the environment.

Atrazine contamination is not limited to water resources (Newcombe and Crowley, 1999), it was also reported for soil contamination by various researchers (Chelinho et al., 2010; Ibrahim et al., 2013).

Earthworm is very well known as an important fauna in the soil ecosystem. Atrazine contamination affects soil communities like soil microorganisms and earthworm (Domsch et al., 1983; Moreno et al., 2007; Song et al., 2009). It was observed that soil microbial community was affected due to frequent applications of atrazine. In earthworm, atrazine caused DNA aberrations (Song et al., 2009) as well as increased lipid peroxidation and antioxidative enzymes like superoxide dismutase (SOD E.C. 1.15.1.1) and catalase (CAT, E.C. 1.11.1.6). There is very scarce information available on the toxicity of atrazine in soil communities. In Sprague-Dawley (SD) rats Atrazine, simazine, and cyanazine and atrazine metabolites HA, DEA, DIA, diaminochlorotriazine (DACT) produce mammary tumors in (Eldridge et al., 1999; Stevens et al., 1994), this can be linked to the atrazine disruption of hypothalamic regulation of prolactin and luteinizing hormone production (Bogdanffy et al., 2000; Cooper et al., 2000). It is associated with the development of male reproductive system hence atrazine can affect puberty in male rats. Atrazine metabolites (DEA, DIA, and DACT) also can affect puberty (Stoker et al., 2002) and thyroid function in rats (Hase et al., 2008). Atrazine can influence steroidogenesis by varying the hypothalamo-pituitary functions. Recent studies in male rats indicated that hypothalamo-pituitary-adrenal axis could be turned on by atrazine and its metabolites. This results in a rise in adrenocorticotropic hormone release, and it is responsible for elevated adrenal steroidogenesis (Fraites et al., 2009; Laws et al., 2009; Pogrmic-Majkic et al., 2010). There are various reports on atrazine toxicity which concludes that at higher concentrations it affects, but some cases it does not follow. At lower concentrations, it affects aquatic animals like fish, frog. Hence, due to lack of concluding data, regulatory authorities have decided that continuous monitoring programs should be implemented (US EPA atrazine updates, 2013).

Acute toxicity

Acute toxicity is the capability of a chemical to cause adverse effects resulted after a single dose or any severe toxic effect arising from a single or multiple short-term exposures (within 24 hours) to toxic compounds. LD_{50} is the dose of any substance tested required to kill half the number (50%) of test animals (Lorke, 1983). LD_{50} test includes feeding the substance by mouth, injecting it into veins, muscle tissue or the body cavity and applying it to the skin. The oral LD_{50} was observed for rats 1869-3080 mg, for mice 1750-3992 mg and rabbits 750 mg of technical grade atrazine Kg^{-1}. The 1 hour and 4-hour inhalation LC_{50} (Lethal Concentration) was detected to be more than 0.7 mg l^{-1} and 5.2 mg L^{-1} in rats. After consumption of a high oral dose, rat muscular weakness, convulsions, prostration, difficulty in breathing and death was observed in the rat (Ghosh and Philip, 2006).

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In acute toxicity characteristically the effect is toxicity (LC_{50}) in fish or mobility loss (EC_{50}) in aquatic invertebrates. Half maximal effective concentration (EC_{50}) is the concentration of a toxicant which stimulates a response in between the baseline and maximum after a particular contact period. LC_{50} is Lethal Concentration; the values typically denote to the concentration of a chemical which eradicates 50% of the test animals during the scrutiny time. Acute toxicity data shows atrazine is moderately toxic to freshwater fish at higher concentrations. Algae data indicated the EC_{50} value ranged from 22 to 460 ppb (US EPA, 2003). Various studies reported that lower values on algae, with values as low as 3.7 ppb (Phyu et al., 2013).

Chronic toxicity

It is the ability of a toxicant to cause critical health effects for a longer time. Several research data on various test organisms indicated the chronic toxicity of atrazine. In 6 months studies 40% rats were dead subsequently due to respiratory distress and paralysis of the limbs. Atrazine was given orally; dose concentration was 20 mg Kg^{-1} day^{-1}. Brain, kidney, liver, ovaries were affected structurally and chemically. Growth retardation was also observed in the case of dogs, for the period of 2 years 7.5 mg Kg^{-1} day^{-1} atrazine dose cause intake capacity was decreased food while heart and liver weights were increased. Additional effects like dropped blood cell counts and rigidity in the rear limbs or occasional tremors were noted at a dose of 75 mg Kg^{-1} day^{-1} (CDFA, 1990). Rats and dogs toxicity studies showed reduced weight which considered being symptoms of chronic toxicity (Kidd and James, 1991). Chronic toxicity values ranged with NOEC's from 60 ppb to 250 ppb for freshwater invertebrates and fish (US EPA, 2003).

Carcinogenicity

International Agency for Research on Cancer (IARC) had a conclusion; there was insufficient evidence in a human and as well as in the case of experimental animals (Group 2B). Drinking water contamination limit for atrazine was calculated which was of 2 μg L^{-1}. For this Tolerance Daily Intake (TDI) approach was found to be useful. Atrazine was acknowledged by US EPA as Group C, i.e., possible human carcinogen (Donna et al., 1989). Mcelroy study suggests that a tumor was developed in the woman due to exposure of triazines (Mcelroy et al., 2007; Simpkins et al., 2011). In 2000 US EPA classified atrazine as a likely human carcinogen instead of possible human carcinogen (US EPA, 2000). This was based on research carried out during a few years before 2000. However, later on, IARC removed atrazine from its list of suspected carcinogens (Jowa and Howd, 2011). Regardless of the various reports of atrazine effects on animals, the effects on humans are

still inconclusive, partly because data collected on animals cannot be directly extrapolated to humans (Stewart, 2012).

Results are mottled, with both definitive correlations in some cases and lack of tendency in others. This is particularly in finding causal linkage between atrazine exposure and chances of cancer and endocrine (hormonal) disruptions (hermaphroditic effects on African clawed frogs) (Hayes et al., 2002). Tumor formation in female SD rats was linked to exposure to atrazine (Jowa and Howd, 2011). However according to results of Agricultural Health Survey (AHS) are contradictory. A survey conducted (Boffetta et al., 2013) among farm workers regularly exposed to atrazine found no link between atrazine exposure (Weichenthal et al., 2010) and occurrence of cancer (Waggoner et al., 2011).

Genotoxicity

Genotoxicity or mutagenicity is the ability of a chemical to cause DNA damage and consequently DNA mutations which can guide to transmissible genetic faults like cancer. The genotoxicity database on atrazine is summarized in Table 2 (Hauswirth and Wetzel, 1998). Another study showed that due to a large oral dose of atrazine resulted in DNA lesions in the stomach, kidney, and liver of the rat (Pino et al., 1988). Atrazine also affects soil organism like earthworm (Song et al., 2009).

Table 2: Genotoxicity data on atrazine (Hauswirth and Wetzel, 1998)

Test	Reported responses for atrazine
Mammalian cells	**In vitro studies**
Human lymphocytes	1neg
Cho cells	1neg
Chromosomal aberrations	
Human lymphocytes	1neg
Cho cells	1neg
Uds/DNA damage	
Rat hepatocytes	1neg
Human fibroblasts	1neg
Mouse lymphoma	1neg
HGPRT in v79 cells	1neg
Bacterial cells and other	
E. Coli rec assay	1neg

Ames salmonella	4neg
Salmonella	2neg
Yeast	1neg
E. Coli	1neg
Mammalian	**In vivo studies**
Mouse micronucleus	1neg
Dominant lethal—mouse	1pos/3neg
Chinese hamster bone	
Marrow chromos aberrations	1neg
Sce chinese hamster	1neg
Mouse sperm abnormalities	1neg
Alkaline elution—rats	1neg
Mouse spot test	1neg
Mouse bone marrow	
Metaphase analysis	1pos/ 3neg
Chromosomal aberrations mouse	
Germ cells	2neg

pos stand for positive, and neg is negative

Reproductive effects and endocrine disruption

The most severe reproductive abnormalities are noted in rats, birds (de la Casa-Resino et al., 2012), amphibians, and fish at comparatively at low exposure concentrations (2–25 μg L^{-1}) (Ackerman, 2007; Suzawa and Ingraham, 2008). At a concentration of 0.5 μg L^{-1}, atrazine exposure causes considerable contrary reproductive effects on fish (Suzawa and Ingraham, 2008). This is below the threshold levels previously defined for fish toxicity which was found in the surface water of agricultural areas. Furthermore, this was substantiated by researchers by showing the effects on egg production and spawning in fathead minnow. It suggests that there are reproductive risks to wild fish populations who are exposed to atrazine. Therefore agricultural areas should not be undervalued by existing assessments (Tillitt et al., 2010; Wu et al., 2010). Atrazine disrupts hormones was substantiated by epidemiological studies which found links between atrazine exposure and

reproductive effects including low birth weight, increased risk of miscarriage, increased the frequency of congenital disability, and reduced male fertility (Swan et al., 2003; Winchester et al., 2009). At low concentrations as 0.1 to 1.0 µg L^{-1} incidences of hermaphroditic effects and feminization of *Xenopus laevis* (male African clawed frogs) have been reported (Hayes et al., 2002; Ralston-Hooper et al., 2009).

Atrazine removal technologies

Table 3: Atrazine removal strategies adapted from (Chris Frazar, 2000)

Atrazine remediation strategy	Technology	Cost per m^3	Duration in days	Treatment medium	Removal efficiency
Physical Methods	Low-temperature thermal desorption	100$ - $400	22 -23	Soil, sludge, and sediment	82% to >98%
	Incineration	$300 - $1000	30	Soil, sludge, and sediment	>99.99%
Biological methods	Bioremediation	$8.4 - $197	33	(*ex situ*) soil, sludge sediment, and groundwater	Up to 99.8%
	Phytoremediation	$60k - $100k/ acre	Na	Soil, sludge, sediment, and groundwater	Up to >80%

There is an immense risk imposed by atrazine due to contamination of water bodies, soil and consequently, it is affecting non-target organisms. Various toxicity studies, surveys put atrazine in focus for pollution problems. Hence, atrazine removal from environment gained attention globally. Much consideration and research need to be done so that it can be used in the policymaking process for an efficient remediation of polluted sites. Various technologies come into play for atrazine removal. It can be enlisted as chemical methods, physical methods, and biological methods.

Chemical degradation

For remediation of atrazine waste, chemical methods used are photolysis, oxygenation, hydrolysis, and dehalogenation. Breakdown of atrazine naturally occurs in the soil mostly by chemical hydrolysis of atrazine at 2-position (Armstrong et al., 1967). In strongly acidic or basic conditions HAT is synthesized as a result of chemical hydrolysis of atrazine (Erickson et al., 1989). In Alkaline hydrolysis, OH^- displaces the Cl^- from 2-position of atrazine by the direct nucleophilic attack. Hydrolysis in acidic conditions is possibly due to protonation of a ring or chain nitrogen atom and subsequent cleavage of the C-Cl bond by hydrolysis. Atrazine is rapidly hydrolyzed in alkaline conditions than in acidic conditions (Ghosh and Philip, 2006). Organic matter is also reported for chemical degradation of atrazine (Kadian et al., 2008). Sodium azide reported for its inhibition to hydroxy analogs of atrazine formation in soils. Rapid degradation of atrazine occurs at a higher temperature like 95°C. However; it contradicts aqueous solution without inorganic soil content. It proved the significance of inorganic soil contents for abiotic atrazine degradation (Skipper et al., 1967). Differing to these reports atrazine degraded chemically in the moderate acidic condition in the ground (Blumhorst and Weber, 1994). In the company of humic substances as well as in acidic or basic conditions favors the chemical hydrolysis (Kadian et al., 2008). For chemical degradation of atrazine, many new techniques have been explored. These include photocatalytic degradation (Minero et al., 1996), advanced oxidation (Arántegui et al., 1995), Electrocatalytic dechlorination (Monson et al., 1998; Stock and Bunce, 2002), and reductive degradation by the use of zero-valent iron (Dombek et al., 2001), and Fenton reactions (Arnold et al., 1995). Chemical methods for atrazine breakdown was done with the help of Fenton's reagent (Larson et al., 1991). Fenton's reagent degrades atrazine by dealkylation of atrazine (Plimmer et al., 1971). Modification of Fenton's reaction resulted in 10% of atrazine residues at a pH of 5 to 7.5. Within 30 seconds complete degradation of ^{14}C atrazine was carried out by Fenton's reagent resulted in the synthesis of 27 % of DACT, 28% of 2-acetoamido-4-amino-6-chloro-s-triazine (AMCT). Electrochemical generation of iron-catalyzed this reaction. Atrazine degradation was reduced at lower pH like three from 99% to 37% at a pH of 9 (Ghosh and Philip, 2006). Hapeman (1998) have studied the effects of pretreatment by chemical approaches followed by biological treatment enhances mineralization of several pesticides. Even though chemical pretreatment enhanced the biodegradation and mineralization of several other pesticides, however, it failed to enhance the mineralization capacity

of triazines (Hapeman et al., 1998). Hydrogen peroxide and UV treatment could not mineralize atrazine, but cyanuric acid was formed as an end product (Chan et al., 1992).

Physical methods

For atrazine removal from the environment, three methods are frequently used Incineration is used for solid waste and absorption is employed in the event of sewage.

Incineration

For treatment of most toxic waste stream, the US Environmental Protection Agency (US EPA) has selected incineration as the best demonstrated available technology. It is a demonstrated technology that has regularly used for remediation of pesticide-polluted locations of soil, sludge or sediments with organic contaminants (US EPA, 1994). An incinerator can destruct a waste or soil contaminant within a short period (generally minutes) simultaneously waste reduction at the noteworthy volumes. Oxidation, as well as volatilization of the organic compound, is achieved in the first step. The first phase of incineration includes heating the contaminated material at temperatures between 550°C and 900°C. In the second step; the temperature was maintained at 800°C to 1200°C. The second phase caused the complete destruction of organics (US EPA, 1994). The degraded end products are further dumped in landfills. Industries use this method for highly recalcitrant and high volume wastes. In optimum condition, 99.9% destruction of organic pesticide can be accomplished by this process (Ghosh and Philip, 2006). Heat and oxygen are efficiently used for the destruction of pollutants. The polluted soil and the organics are consequently oxidized. However major drawback of this procedure is the production of toxic and corrosive gasses. It leads to the additional pollution in the nearby area. For example, hydrochloric acid was generated during incineration of chlorinated pesticides, and NO, NO_2 were produced from nitrogen containing pesticides. These gasses are acidic and corrosive. Anti-corrosive material for the construction of incinerators increases the cost of the treatment. Furthermore, the toxic gasses generated during process needed to be treated before mixing out to the environment. Such causes make incineration method unfriendly for pesticide waste treatment (Ghosh and Philip, 2006).

Low-temperature thermal desorption

It is regularly used for remediation purpose of pesticide-contaminated sites. This cleanup method is an *ex-situ* process. Even though it is not a novel method, its practical advantages are considerable. It is able of eliminating semi-volatile and volatile organic compounds (pesticides) from soils as well as from sludge and filter cakes. The contaminant material is heated to between 150°C and 500°C for

volatilization but does not the breakdown of organic compounds. The end product of this process is an organic contaminated gaseous stream. Either passing can treat this through burner or condenser. Such methods destroy contaminants. The remaining gas is converted into a liquid phase by the condenser for further treatment while the adsorption beds used for immobilization. Complete degradation of pollutants is not possible by this technique. A major limitation of this technique is a prerequisite of highly specialized machinery, materials which add maintenance which is comparably high. This technology is not suitable for remediation of heavy metals and to pollutant-containing more than 20% solids components (US EPA, 1994).

Adsorption

It is the accumulation of a contaminant at the line between two stages (air or water). By use of this technology, atrazine is removed from drinking water. For such adsorption (GAC) granular activated carbon (Jones et al., 1998) or (PAC) powdered activated carbon (Adams and Watson, 1996) has frequently been used. Adsorption is acknowledged as the best available technology. Though, the practicability of a specific system required to be reviewed. From adsorption isotherm values feasibility, the specific system can be evaluated. Most frequently used adsorption isotherms are developed by Langmuir, Freundlich and Brunauer, Emmet, and Teller (BET isotherm) (Ghosh and Philip, 2006). This adsorption isotherm ends in considerably different functional associations. Atrazine and other pesticides are removed by adsorption process reported by various studies. However, many of them failed. In the case of PAC adsorptive capability was depending on pH (max at pH 6)(Adams and Watson, 1996). More adsorbent required for removal metabolites of atrazine than atrazine. PAC was incompetent to reduce pesticide level to the European Community's (EC) maximum admissible concentration (0.1 μg L^{-1}). GAC was successful in the removal of effluent triazine levels below it. However, the major drawback is its regeneration frequency. It was exceeding levels required for taste and odor control (Croll et al., 1992). At Fremont GAC removed 47% of atrazine from wastewater. The mean value of atrazine concentration was 4.83 μg L^{-1} (Miltner et al., 1989). According to several studies reduction in adsorption capacity for chlorine, herbicides is because of DOC (Dissolved oxygen carbon) (Hapeman et al., 1998). The fate of atrazine in the environment is determined by adsorption of atrazine on the soil particles. Hence, adsorption is one of the crucial factors for remediation purpose. Adsorption or desorption of atrazine in the ground has reviewed by various research studies to find the fate of atrazine in the environment (Bakke et al., 1972; Celis et al., 1998). They observed that the adsorption of atrazine on clay apart from of

chemical nature, highly acidic adsorbent gave superior results as compared to near neutral adsorbent (Boivin et al., 2005). According to one study atrazine adsorption was most likely reversible and inversely proportional to surface charge density of the smectites (Mamy and Barriuso, 2007). Lower affinity was observed in the case of DEA for the same adsorbents however desorption followed the pattern of atrazine. HAT one of the primary products of atrazine degradation showed strong adsorption on the sediments (Chung et al., 1996). Even though high cost of activated carbon, it is quite economical when it comes to sole contaminant removal of micropollutant. However, it becomes expensive when it is present along with DOC. Atrazine removal hampered in the wastewater because of competition for adsorption by DOC. This, in turn, boosts the treatment maintenance as well as the frequency of carbon regeneration. Such troubles are responsible for finding out for a low-cost adsorbent which can remove atrazine reduce DOC. Studies on atrazine adsorption on materials (Clausen and Fabricius, 2001) showed that only kaolinite was suitable for the atrazine adsorption (Zolgharnein et al., 2011).

Biological methods

Inexpensive technologies have the greatest potential. Bioremediation technologies are among the less expensive remediation techniques (Cookson Jr, 1995). Additionally, they have an advantage over other methods is that they are based on processes occurring in the environment. Consequently, it minimizes disturbance of the site. Biological methods are classified according to the type of organism used for remediation purpose. Phytoremediation refers to the use of plants similarly microbial bioremediation use microorganism.

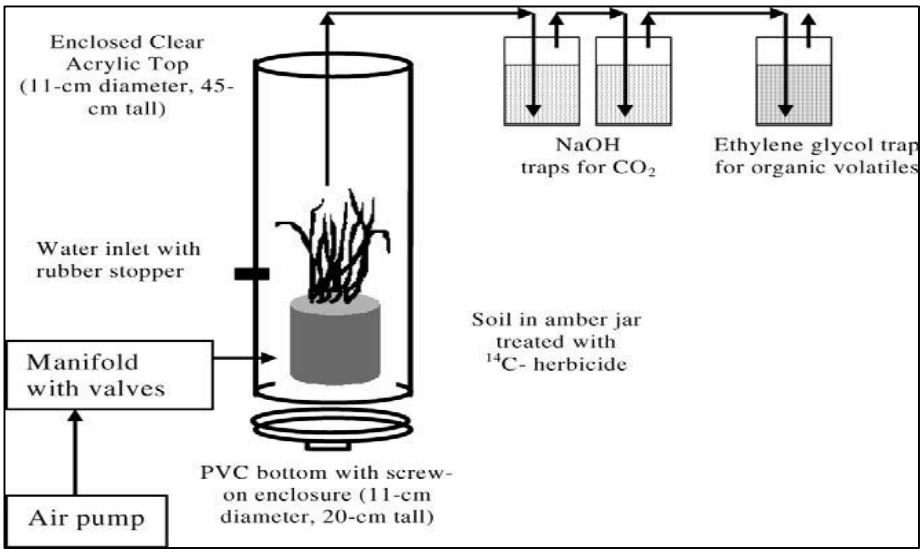

Fig. 4: Phytoremediation adapted from (Henderson et al., 2007)

Phytoremediation

There is a growing appreciation for phytoremediation because it is innovative technology and furthermore it is economical, aesthetically pleasing way of remediating polluted sites. The in situ use of plants for bioremediation is characterized as phytoremediation. It is a promising technology for the cleanup of polluted soil and water (Dec and Bollag, 1995). Several investigations have been carried out to establish the efficacy of remediating POP with different plant species (Fig. 4) (Henderson et al., 2007; Raveton et al., 1997). Pesticide pollution in soils is a severe problem at agrochemical manufacturing and storage sites because of unintentional spillage during mixing and loading of chemicals for the application. Costly remediation methods like chemical and physical methods may not be economically practicable for them. Additionally, natural bioremediation strategy may be hindered by the presence of mixtures of pollutants at high concentrations. At various sites, herbicide contaminants are frequently found as major contaminants due to its extensive use (Wu et al., 2010). Phytoremediation approach as an *in situ* solutions for cleanup of these soils is not easy. Because herbicides are intended to destroy plants and use of plants for remediation or removal of them can be a complex and problematical task (Kruger et al., 1997). A major research has been conducted to check the efficacy and capability of plants to remove heavy metal polluted soils. Various tolerant plants could be used at the contaminated sites for remediation. Plants are frequently able to uptake and storage of heavy metals and other toxicants at significant concentrations in their

roots, shoots, and leaves. This process is called as phytoextraction. Phytotransformation term is used when toxic organic pollutant transformed into less toxic and more stable form by plants. It comprises phytodegradation; phytovolatilization. Phytodegradation is degradation of the organic pollutants by the plant enzymes. Phytovolatilization is the volatilization of organic pollutants by passing it through the plant leaves. Phytostabilization is the process which immobilizes contaminants and hinders migration of contaminants through the soil by absorption of binding leachable components to the plant cells. This process efficiently reduces the bioavailability of the toxic pollutants. Nearly any plants present at polluted sites will be involved in the phytostabilization process (Arthur et al., 2005). The triumph of phytoremediation technique chiefly hinges upon the selection of proper tolerant plant and suitable soil (Arthur et al., 2005). Through roots plant uptake the main pollutant with other various nutrient components. This consequently alters the soil chemistry, and it can be seen increased rhizospheric bacterial activity. By symbiosis route plant and microbial populations provide each other considered necessary nutrients. In the rhizospheric milieu, bacterial populations are abundant as well as higher microbial metabolic activity. They are capable of boosting the rate of microbial degradation of pollutants. Often the plant is not directly involved in the biodegradation process. They are involved indirectly and act as a catalyst. This consequently ends in the heightened microbial growth and activity. Microbial biodegradation potential can easily increase by such type of symbiosis. This process is known as plant-assisted bioremediation or phytostimulation. Phytoremediation was found to be more suitable and efficient in case of rhizospheric soil than other soil types (Arthur et al., 2005). This suggests that rhizospheric interactions between the plant and microorganisms have led to the increased degradation of the pesticides (Marecik et al., 2008). However, the rhizospheric remediation potential is restricted, as it does not extend far from the root region. The plants that are capable of accumulating toxic pollutants in the root region are termed to as hyper accumulators. These include the mustard plant, alpine pennycress, broccoli and cabbage (Arthur et al., 2005). Maize showed to remove about 80% of atrazine from the soil in greenhouse study (Ibrahim et al., 2013). The submerged aquatic plant removes approximately 59% of atrazine (^{14}C)(Rice and Sikka, 1973) poplar trees were efficient in the fast assimilation of atrazine (90%) within nine days from sandy soil (Burken and Schnoor, 1996) while in the clayey soil the acclimatization was destitute. Plants can establish a hydraulic regulator to avoid the migration of polluted water outside of remediation site, and it accelerated biodegradation while performing this. For groundwater remediation deep-rooted and quick-growing poplar tree has been displayed to be

positive in lab studies. This has already recognized as efficacious for the remediation of atrazine-contaminated soil and groundwater (University of Washington, 2007). In this study Hybrid poplars removed large quantities of nitrate, atrazine from groundwater and this site was approximately 1.5 acres. Results were positive with reduction of both the nitrates and herbicides in the groundwater (US EPA, 2000). Kawahigashi (Kawahigashi et al., 2005; Kawahigashi et al., 2006) used advanced molecular biology techniques for remediation of atrazine. Some cytochrome P450 genes of human origin (CYP1A1, CYP2B6, and CYP2C19), were inserted into rice plants by recombinant DNA technology. These transformed Rice plants showed tolerance not only to atrazine but also to other herbicides. Therefore it was able to decrease some quantity of herbicides (Kawahigashi et al., 2006).

Nevertheless, phytoremediation is a likely choice for remediation of a variety of contaminants like heavy metals and many pesticides. However, its application is limited to surface soils and subsurface soils. Plants uptake pollutants from the ground and carry it to its various parts without biodegrading it to innocuous forms. Upon consumption of such plants by herbivores could be troublesome (Ghosh and Philip, 2006). For effective remediation, phytoremediation requires a long time as compared with other techniques. Phytoextraction and phytostabilization accumulate or immobilize the contaminant; they do not result in the degradation of contaminant directly. Irrespective of such positive results in phytoremediation technology, destruction of the pollutant is necessary. This technology is still comparatively less proven, and the potential of phytoremediation is still being discovered.

Bioremediation

Various cutting-edge technologies have arisen in the environmental biotechnology for tackling problems cleanup problems of polluted sites. A perfect example could be bioremediation with the help of bacteria. It exploits the high catabolic versatility of bacteria for degradation of pollutants to less toxic compounds. Bacterial biodegradation decontaminates and transforms wastes. Bioremediation defined as usage and regulation of biological organisms for remediation of the polluted environment (land, air, water) (Stenuit et al., 2008). It is an innovative technology that is regularly employed for the remediation of polluted sites (US EPA, 1996). This process accelerates the natural process of the bacterial degradation of pollutants by augmenting bacteria with nutrients, carbon sources or electron donors. Remediation of a contaminated site can be achieved by using indigenous bacteria or by adding an enriched microbial culture involved in contaminant biodegradation at a faster rate. Ideally, results of microbial bioremediation are the complete mineralization contaminant without the forming intermediates. Mineralization is nothing but the total

degradation of the parent chemical to CO_2 and H_2 or CO_2 and CH_4 depending upon aerobic and anaerobic conditions respectively. Biodegradation is the process of the modification of the toxicant or chemical by natural means. It is not imperative to every bioremediation to form CO_2, H_2O or CH_4 (Donnelly et al., 1993; Ghosh and Philip, 2006). The terms 'biotransformation', biocatalysis' and 'biodegradation' have been used almost interchangeably, depending on the interest of the researcher studying the reaction (Handelsman and Wackett, 2002). Though several methods are accessible for the controlling atrazine pollution, the only natural method can mineralize atrazine. For the bioremediation of polluted grounds, water land farming, composting, above–floor bioreactors and several in situ treatment techniques were employed (Sadowsky, 1999). Biostimulation is the process where the adding nutrients or chemicals substances which accelerate the growth of indigenous bacterial community involved in the degrading pollutant in contaminated environments (Dec and Bollag, 1995). Even though biostimulation and bioaugmentation are more costly than natural attenuation, they remain a valuable alternative. Under natural conditions, the time required for removal by the indigenous microorganism is more. Sometimes the biodegradation potential of some bacterial communities is not suitable for efficient removal. However frequently this found to be successful (Eyers et al., 2008). Over a period of 30 years, various microorganisms were used in bioremediation processes. For herbicide-contaminated soils, land farming has been one of the most important bioremediation sites which involve biostimulation (Felsot et al., 2003). In bioremediation of groundwater study, atrazine is readily biodegraded under controlled conditions (Shapir et al., 2007). It was found that biodegrading capacity is inhibited in the presence of external nitrogen and carbon sources (Govantes et al., 2009). On the other study of *Agrobacterium radiobactor* strain J14a, the degradation rate of atrazine was enhanced in Sequential Batch Biofilm Reactor (SBBR) after the additional supply of carbon source (Protzman et al., 1999). Bioaugmentation of *Pseudomonas* strain was found to be an encouraging indigenous treatment for atrazine spilled at high concentrations in soil (Lima et al., 2009). Rousseaux et al. (2003) observed a 3-fold increase in biodegradation capacity after the bioaugmentation of 10^4 CFU g^{-1} of *Chelatobacter heintzii* Cit1 in the soil. Hydrolysis of atrazine was dominant in (85%) Kappa soil than in (48%) Molokai soil (Obien and Green, 1969). Improved degradation and mineralization of atrazine were probably due to rich organic content and a largely active bacterial population of the Kappa soil. In aerobic condition and in the absence of biological modifications it was found that 73 % of the atrazine was mineralized within 11 weeks by enrichment culture method (Alvey and Crowley, 1995). A recent study explored the possibility of

bioremediation by enzyme catalysis called as free-enzyme bioremediant which was able to reduce 90 % of atrazine (Scott et al., 2010). They used triazine hydrolase enzyme, Trz N (Mulbry et al., 2002). In another novel approach, *atz*A gene was overexpressed in *Escherichia coli*. Then whole-cell suspensions of this dead recombinant *E. coli* was used for bioremediation of 26 m^3 of soil in heavily polluted by an atrazine spill (around 29 000 ppm) (Strong et al., 2000). Bioremediation was successful in atrazine removal. It was the first field-scale study of atrazine remediation study with the help of killed, recombinant bacteria (Wackett et al., 2002). There are few reports on reactor development for the treatment of atrazine-containing wastewaters. Batch and sequential batch reactors were employed for the cleanup of atrazine wastewater with the help of *Pseudomonas* strain yaya6 for (Yanze-Kontchou and Gschwind, 1994). Atrazine concentrations observed in this study were higher than previously recorded values in surface and subsurface waters around the pesticide handling and distribution sites. Most of such studies were performed in aerobic suspended growth batch process (Behki et al., 1993; Mandelbaum et al., 1993; Struthers et al., 1998). Aerobic and anoxic batch bioreactor system was applied for mineralizing atrazine in soil microcosm (Giardina et al., 1982; Nair and Schnoor, 1992). The hybrid reactor was used with GAC and pure bacterial culture for atrazine removal from drinking water (Feakin et al., 1994). For studying of the atrazine degradation reaction kinetics by microorganism's continuous flow, aerobic biofilm was found to be useful (Galluzzo et al., 1999). Atrazine biodegradation in anoxic environments was executed in Fixed Bed Reactor (FBR) (Stucki et al., 1995), Anaerobic Sediment Suspended Growth Reactor (Chung et al., 1996) and Anaerobic Fixed Film Reactor (FFR) (Crawford et al., 1998). Ghosh and Philip (2004) and Ghosh et al. (2006) reported mixed bacterial consortium for atrazine biodegradation. For biodegradation anaerobic sequential batch reactor was employed. Various other reactors, like soil slurry reactor (Gupta and Baummer III, 1996), soil microcosm (Alvey and Crowley, 1995; Mandelbaum et al., 1993) and soil perfusion system (Armstrong et al., 1967; Giardina et al., 1982) were used for determining the status of atrazine in the aerobic condition in the ground. Activated Sludge Process (ASP) can influence the fate of various pesticides including atrazine (Monteith et al.1995).

Biodegradation of atrazine

Organohalides were initially thought anthropogenic pollutants with restricted or no natural corresponding chemicals and hence intrinsically problematic, this way of philosophy is divergent to the scientific facts. Various natural biogenic and geogenic sources have now been discovered which supported these facts. Biogenic sources of organohalides are bacteria, fungi, sponges, insects, plants,

and mammals. However, geogenic sources of organohalides are forest fires and volcanic releases. The organochlorine compound is produced during combustion of organic matter having inorganic chloride. These are polychlorinated phenols, dibenzo-p-dioxins, and dibenzofurans. Given the fact that microbes are exposed to abundant natural products and a large number of synthetic chemicals which makes the massive number of potential substrates for microbial systems (Häggblom and Bossert, 2003). The halogen and N-alkyl substitutes on the s-triazine ring of atrazine hinder the bacterial metabolism (Wackett et al., 2002). As initially atrazine was considered to be recalcitrant, but few microorganisms reported for degradation till mid-1990. However, now various microorganisms capable of degrading atrazine were reported across the globe due to extensive substrate ranges discovered in bacteria (Shapir et al., 2007; Vaishampayan et al., 2007). Not only had this but the reports of intermediate metabolites of atrazine degradation also increased. Usually they were dealkylated forms, however now hydroxylated forms of atrazine reported. Such observations are also seen in new metabolic pathways of atrazine biodegradation which suggests that atrazine metabolism has evolved in the last three decades (Shapir et al., 2007). Various research studies have pointed out that several s-triazine degradation pathways usually converge to cyanuric acid, subsequently by hydrolytic ring cleavage (Eaton and Karns, 1991a; Eaton and Karns, 1991b). However, the general mechanisms comprised of the molecular and biochemical processes involved in cyanuric acid degradation came out by Eaton and Karns (1991a, b). Initially, the research on atrazine-degrading microorganisms was focused on the culture-dependent methods. This involves isolation and characterization of naturally occurring bacteria in pesticide contaminated environments. The bacterial degradation of s-triazines is a catabolic process where two most important factors limit the rate of atrazine biodegradation (carbon and nitrogen limiting conditions). Even with limitations of atrazine as an energy source, because of completely oxidized carbon rings (Radosevich et al., 1995), its susceptibility for catabolism is enhanced in C and N-limited conditions by the both carbon and nitrogen sources in the atrazine. They are sources of a carbon substrate (energy) and as a nitrogen source for bacteria. The triazines can act as both a C and N source (Erickson et al., 1989). Under various C and N-limiting conditions atrazine biodegradation rate can be manipulated by addition (or in limiting) external C and N sources. N-based fertilizers could be used as a nitrogen source, whereas external C sources would be the glucose, lactate, and glycerol. The additional C sources have a marked accelerating effect on the biodegradation rate by the co-metabolism process (i.e., C limiting conditions). For mineralization by microorganisms, C, and N

limiting conditions define atrazine biodegradation pathway (Gebendinger and Radosevich, 1999; Grigg et al., 1997) In natural environment, i.e., soil, atrazine mineralization rate decreases inversely with depth, from surface zones > rhizosphere > vadose zone (Radosevich et al., 1995; Topp et al., 1995; Vryzas et al., 2012). Reasons are decreased total organic carbon (TOC), temperature (T), available N and phosphorous (P) as well as population size with depth in each zone (Blume et al., 2004; Vryzas et al., 2012). As a result of limited N availability, the repeated exposure to atrazine may increase biodegradation (Fang et al., 2001; Rhine et al., 2003). This was further substantiated by Silva et al. (2004). Atrazine mineralization was accelerated after 28 days of acclimatization period. There are few reports on microorganisms capable of using atrazine as a sole C source. Sing et al. (2004) reported an *Acinetobacter* genus bacteria which was able to degrade atrazine at high concentrations (250 ppm) using atrazine as a carbon source. Numerous microorganisms have been isolated (table 4) that can degrade atrazine partially or entirely. Under aerobic conditions in soils mineralization atrazine of is reported by many researchers (Obien and Green, 1969). *Nocardia* (soil bacterium) was capable of using atrazine as the sole carbon and nitrogen source. It transformed atrazine into dealkylated and deaminated metabolites (Giardina et al., 1982; Kuhn and Suflita, 1989; Vaishampayan et al., 2007). In various biodegradation studies, dealkylation of both side chains was frequently observed over dechlorination by hydroxylation. This lead to the conclusion that presence of both alkylated side chains inhibits microbial dechlorination. However, the *Pseudomonas* was involved in mono-dealkylation of atrazine. Adams and Thurman (1991) and Skipper (1967) reported that ethyl side chain mineralization of atrazine was much quicker than the isopropyl side chain. Successful mineralization of >94% of atrazine (50 μg ml^{-1}) by *Agrobacterium radiobacter* strain J14a using atrazine as a nitrogen source, was capable of dealkylation, dehalogenation, and mineralization of s-triazine ring (Struthers et al., 1998). Radosevich et al. (1995) reported an atrazine-degrading bacterium which was capable of partial mineralization of atrazine through ring cleavage. *Rhodococcus* strains are known as ubiquitous in soil with diverse biodegradative potential was screened for atrazine biodegradation (Behki et al., 1993). Identified *Rhodococcus* strain TE1 contained a 77 -kb plasmid with atrazine-degrading capacity. Other researchers reported microbial associations (Assaf and Turco, 1994; de Souza et al., 1998a; de Souza et al., 1998b; Ghosh and Philip, 2004; Smith et al., 2005) and fungal species (Donnelly et al., 1993) which mineralized atrazine. White-rot fungus *Phanerochaete chrysosporium* degraded a 48% atrazine within four days (Mougin et al., 1994). Another lignocellulolytic fungus *Pleurotuspulm onarius* was reported mainly

for producing the N-dealkylated metabolites like DEA, DIA, DDA and the deisopropylhydroxyatrazine (DEA-OH)(Masaphy et al., 1996). *Pseudomonas* sp. Strain Yaya rapidly mineralized atrazine from soil (Wenk et al., 1998). *Pseudomonas* strain ADP was isolated from an atrazine spill site. *Pseudomonas* strain ADP was one of the first organisms identified with the ability to completely mineralize atrazine (Mandelbaum et al., 1995; Yanze-Kontchou and Gschwind, 1994). Later on, *Arthrobacter* was reported by various researchers for complete degradation of atrazine (Sene et al., 2010). Most of the time bacteria follow the degradation by *Pseudomonas* or *Arthrobacter* routes. However, *Rhodococci* is exceptions (Nagy et al., 1995). Culture-dependent methods have ruled over last few decades in the area of bioremediation. However, the advent of DNA-based methods changed the perspectives of studying microorganisms. It was easier and efficient to study bacterial communities by genetic characteristics unlike morphological, biochemical methods used in culture-dependent methods. Straightforward sequencing of PCR-amplified 16S rDNA avoids time-consuming isolation, characterization methods. In microbial ecological studies, bacterial diversity can be explored with the help of molecular techniques like denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE). These molecular techniques have been demonstrated their importance in screening microbial communities as a tool (Davies et al., 2004). These qualities make them a powerful tool for monitoring community changes after exposure to pollutants and bioremediation of contaminants. In environmental microbiology studies often involves the sampling at various time points over an extended period of bioremediation. With the help of these advanced molecular tools, changes in the bacterial community can be studied meticulously over the time of degradation of toxic compounds (Muyzer and Smalla, 1998). DGGE was used to examine the effect of atrazine on the microbial population by many researchers (Goux et al., 2003; Ros et al., 2006). Furthermore, it has been used for application of consortium involved in atrazine degradation by various researchers (Siripattanakul et al., 2009). It was carried out with *Agrobacterium tumefaciens, Caulobacter crescentus, Pseudomonas putida, Sphingomonas yaniokuyae*, *Nocardia* sp. *Rhizobium* sp., *Flavobacterium oryzihabitans,* and *Variovorax paradoxus* (Smith et al. 2005).

Genetic basis of atrazine biodegradation

The study of single bacteria by their genes and enzymes helped to mark out the primary means of microbial interactions with atrazine in the complex and variable soil environment. The link between microorganism interactions in the ground with atrazine and fate of atrazine in soil is the subject of

intense research. Among the pesticides, atrazine stands for one of the most comprehensively scrutinized pollutants.

Table 5: Genes involved in biodegradation of atrazine

Gene	Enzyme	Source	Reference
atzA	Atrazine chlorohydrolase	*Pseudomonas* strain ADP	(de Souza et al., 1996)
TrzN	Triazine hydrolase	*Nocardioides* sp strain C190	(Mulbry et al., 2002)
trzA	s-triazine hydrolase	*Rhodococcus corallines*	(Shao et al., 1995)
atrA	Cytochrome P-450	*Rhodococcus* strain NI86/21	(Shao et al., 1995)
thcB	CytochromeP-450 Monooxygenase	*Rhodococcus* strain NI86/21	(Shao and Behki, 1996)
atzB	Hydroxyatrazineamidohydrolase	*Pseudomonas* strain ADP	(Boundy-Mills et al., 1997)
atzC	N-isopropylammelide-isopropylamino hydrolase	*Pseudomonas* strain ADP	(Sadowsky, 1999)
trzC	Ammelide aminohydrolase	*Klebsiella pneumonia* strain 99	(Karns and Eaton, 1997)
trzD	Cyanuric acid hydrolase	*Klebsiella pneumonia* strain 99	(Karns and Eaton, 1997)
trzE	Biuret aminohydrolase	*Klebsiella pneumonia* strain 99	(Karns and Eaton, 1997)

Initially, Erickson and Lee (1989) reported that some bacteria dealkylated side chains of atrazine at carbons 4 and 6 while some bacteria transformed atrazine by hydrolysis at carbon 2 forming hydroxyatrazine (HAT). Furthermore, some bacteria degraded atrazine by opening atrazine ring (Ralebitso et al., 2002). Recently there has been little progress in our understanding of the cellular and molecular mechanism by which bacteria degrade atrazine. Enzymes and genes (table 5) for the dealkylation, dechlorination, and subsequent mineralization of s-triazine herbicides to innocuous metabolites like CO_2 and NH_3 have been reported in great detail (Udiković-Kolić et al., 2012). *Pseudomonas* sp. strain ADP has become a model and has been used to reveal sequences of the

catabolic enzymes *atzA*, *atzB*, *atzC*, and *atzD*, which are involved in aerobic degradation pathway. To develop probes for the genes encoding these enzymes, *Pseudomonas* played a major role. Furthermore, sequencing of genes involved in biodegradation process has led to the findings that homologous genes are present on plasmids in various bacterial species. The complete nucleotide sequencing of pADP-1 (the atrazine gene-containing plasmid of *Pseudomonas* ADP) revealed the arrangement and composition of atrazine degradation genes (Martinez et al., 2001). Many studies indicated that many gram-negative (Topp et al., 2000; Wackett et al., 2002) as well as gram positive atrazine-degrading bacteria contain large molecular weight plasmids containing atrazine degradation genes (Behki et al., 1993; Cai et al., 2003; Rousseaux et al., 2001; Strong et al., 2002; Vaishampayan et al., 2007). The presence of atrazine degradation genes on different size plasmids, suggests wide-spread of atrazine degradation genes among unrelated bacteria was not exclusively due to direct plasmid transfer. Furthermore, this view was further confirmed by the finding of bacteria with different combinations of atrazine degradation genes such as including *trzN-atzBC*, *atzABC-trzD*, and *atzABCDEF* (Devers et al., 2005). Atrazine catabolism initiated by triazine hydrolase *TrzN*, in a *Norcardiodes* sp. strain as well as *A. aurescens* (Mulbry et al., 2002; Strong et al., 2002). However, in *Pseudomonas* sp. strain ADP it was catalyzed by enzyme atrazine chlorohydrolase. *AtzA*. *TrzN* has been now found in a variety of gram-positive bacteria, including (Shapir et al., 2006). *AtzA* and *TrzN* form hydroxyatrazine by removing the chlorine from atrazine hydrolytically. These two enzymes have different substrate range. *AtzA* has narrow substrate specificity and restricted chlorine and fluorine groups and transforms atrazine, simazine, desethylatrazine and terbutylazine (de Souza et al., 1996). On the other hand *TrzN*, a zinc amidohydrolase, act on 23s-triazine substrates (Shapir et al., 2006; Strong et al., 2002). While in *Rhodococcus* strains the first step was N-dealkylation forming DIA and DEA by *thcB* encoded cytochrome 450. Two accessory proteins, *thcC*, and *thcD* were identified as an associated electron-supply system for the cytochrome P450 system. Respective encoding genes (Table 5) have been reported (Nagy et al., 1995; Shao and Behki, 1996). *AtrA* gene in *Rhodococcus* strain TE1 involved in N-dealkylation of atrazine and forms DIA and DEA (Shao et al., 1995). *AtzB* (hydroxyatrazine ethylaminohydrolase) catalyzes hydroxyatrazine and form N-isopropylammelide which further catalyzed by *AtzC* (N-isopropylammelide isopropylamino hydrolase), forming a cyanuric acid (Martinez et al., 2001; Shapir et al., 2007). The lower atrazine degradation pathway in *Pseudomonas* ADP consists of three enzymes, *AtzDEF*, resulting in ring cleavage and a subsequent transformation. *AtzD* gene coding for cyanuric acid amidohydrolase

converts cyanuric acid into biurate. *AtzE* identified as a biuret amidohydrolase hydrolyze biuret to produce allophanate. *AtzF*, a protein involved in the last step in atrazine biodegradation converts allophanate to CO_2 and NH_4 (Martinez et al., 2001).The *trzB* gene has been identified as the ammeline aminohydrolase involved in the conversion of ammeline to ammelide. The gene encodes the ammelide aminohydrolase enzyme known as *trzC*. This enzyme converts ammelide to cyanuric acid. The cyanuric acid amidohydrolase encoded by *trzD* is responsible for the ring cleavage of cyanuric acid to form biuret (Eaton and Karns, 1991b). *trzCD* have also been found in another strain of *Pseudomonas* (strain 12228) as well as *Klebsiella pneumonia* 99 (Eaton and Karns, 1991a).

Atrazine biodegradation pathways

Understanding the mechanism of biodegradation pathway of contaminant under a particular condition is valuable for superior remediation process. Degradation of can occurs via biotic and abiotic processes. Biodegradation process for atrazine had been studied comprehensively by various researchers (Kaufman and Kearney 1970, Erickson and Lee 1989) whereas; the abiotic degradation had been reviewed by Jordan et al. (1970). There are various metabolites of atrazine formed after degradation (biotic and abiotic). The structure of various transformed metabolites in the biodegradation process is described in Fig. 5 and table 6.

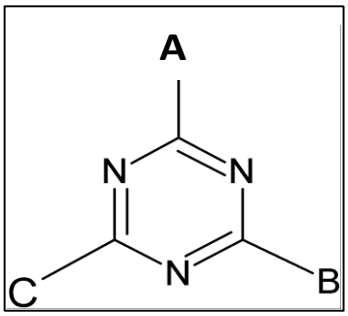

Fig. 5: Atrazine and its transformed metabolites

Biodegradation of atrazine is initiated by two mechanisms dealkylation and hydroxylation. Dealkylation occurs by removing the alkylamino (generally secondary amines, NHR, where R is an alkyl chain) groups at positions 4 and 6 into primary amine (NH_2). Hydroxylation replaces (Cl) at position 2 with the hydroxyl group (OH). The mechanism of degradation defines the nomenclature of degraded or altered metabolites as well, in the form of suitable prefixes added to the base name

atrazine. For example, the prefixes desethyl or deisopropyl is added to the name atrazine in dealkylation reaction depending on the type of group changed or removed.

Likewise, for hydroxylation, the prefix hydroxyl is added to the name atrazine. These further transformations are all indicated by the nomenclature system, with multiple prefixes being added in arrangement to the base name atrazine. For abbreviations, some of the metabolites occurring in middle and lower pathway have their names abbreviated, for example for two dealkylations; the prefix didealkyl- is preferred over the deethyldeisopropyl-prefix. In cases of ammeline, ammelide, and cyanuric acid overlap occur with other disciplines. Shorter names are preferentially used. There are many pathways of atrazine biodegradation. Various researchers have proposed as well as reported atrazine biodegradation pathway. Proposed a general degradation pathway for the s-triazine herbicides by literature is described Fig. 6 (de Souza et al., 1998a; Erickson et al., 1989; Ghosh and Philip, 2006; Ralebitso et al., 2002).

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Table 6: The chemical structure, names, and abbreviations used for atrazine and its metabolites (Erickson and Lee 1989, Govantes et al. 2009).

Common Name	Abbreviations	A	B	C
Atrazine	ATR	Cl	C_2H_5NH	CH_3CHCH_3NH
Desethylatrazine	DEA	Cl	NH_2	CH_3CHCH_3NH
Deisopropylatrazine	DIA	Cl	C_2H_5NH	NH_2
Diaminochlorotriazine/ Didealkylatrazine	DDA/DACT	Cl	NH_2	NH_2
Hydroxyatrazine	HAT	OH	C_2H_5NH	CH_3CHCH_3NH
Deisopropylhydroxyatrazine	DIA-OH	OH	C_2H_5NH	NH_2
Desethyldeisopropylhydroxyatrazine /Ammeline	DDA-OH AMML	OH	NH_2	NH_2
N-isopropylammeline	DEA-OH	OH	NH_2	CH_3CHCH_3NH
N-ethlylammeline	DIA-OH	OH	C_2H_5NH	NH_2
N-isopropylammelide	DEAMMD	OH	OH	CH_3CHCH_3NH
N-ethlylammelide	DIAMMD	OH	C_2H_5NH	OH
Ammelide	AMMD	OH	OH	NH_2
Cyanuric acid	CYA	OH	OH	OH
Melamine	MA	NH_2	NH_2	NH_2

The major stages of atrazine degradation pathway are dealkylation, hydrolysis, and deamination. This pathway can be the result of abiotic as well as biotic processes occurring in the environment. Ring cleavage is the last step in the pathway as end products are CO_2, H_2O, and NH_3. Hydrolysis of atrazine

results in the formation of hydroxyatrazine (Fig. 6). Dealkylation of hydroxyatrazine forms N-isopropyllamilide and N-ethylammelide. Deisopropylatrazine, desethylatrazine, and didealkylatrazine or deisopropyladesethylatrazine are the results of dealkylation of atrazine. Deamination of dealkylated atrazine metabolites forms cyanuric acid. Hydroxylation of dealkylated atrazine metabolites results in the formation of N- isopropyllamilide and N-ethylammelide. However till this date pathway of atrazine biodegradation is not very clear because of differences in its initial steps biodegradation. As many microorganisms take different pathway for biodegradation of atrazine (Fig. 7). Gram positive and gram negative microorganisms take different routes (Fig. 7 and Fig. 9).

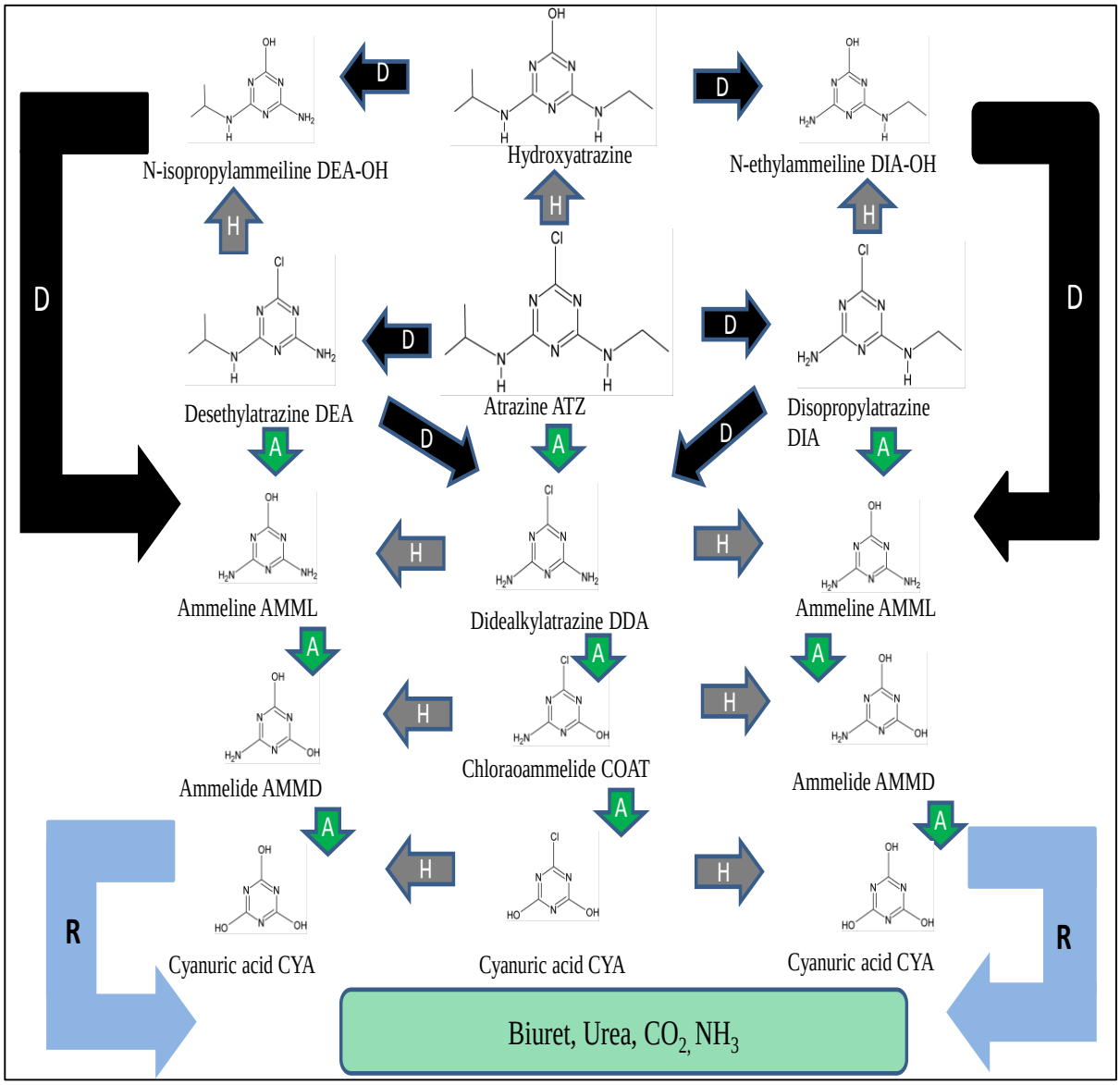

Fig. 6: General atrazine degradation pathway

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"D" labeled black arrows dealkylation reactions, "H" labeled gray arrows hydroxylation, "A" labeled green

arrows deamination and "R" labeled blue arrows denotes ring cleavage reactions. The reaction pathways

shown are combined from the various reaction pathways provided (Adams, 2014).

Most of the studies on atrazine degradation were focused on Gram-negative *Pseudomonas* sp. bacteria, Fig. 7 which displays a cascade of adaptive mechanisms used to mineralize atrazine at high concentration. This includes upper pathway and lower pathway.

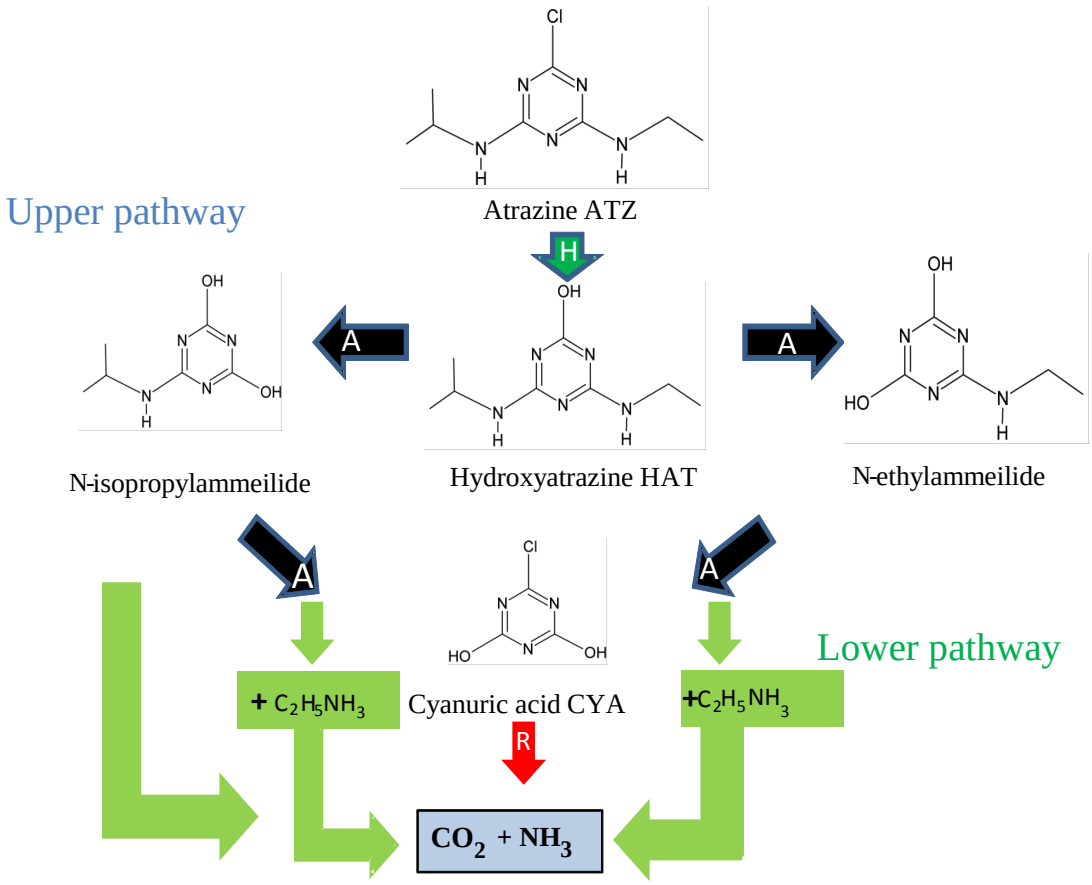

Fig. 7: Atrazine degradation pathway in *Pseudomonas* sp. ADP

"A" labeled black arrows deamination reactions, "H" labeled green arrow hydroxylation, "and "R"

labeled red arrow denotes ring cleavage reactions. The reaction pathways shown are combined from the various reaction pathways provided. (Desouza et al. 1998, Adams, 2014). Atrazine is converted into cyanuric acid in upper pathway while lower pathway includes mineralization of cyanuric acid. Two major mechanisms have been extensively described particularly in *Pseudomonas* sp. and *Arthrobacter* as standard models. The only difference in between two bacteria is the way they initiate the first step of biodegradation. Two different enzymes are performing same reaction *atzA* and *TrzN*.

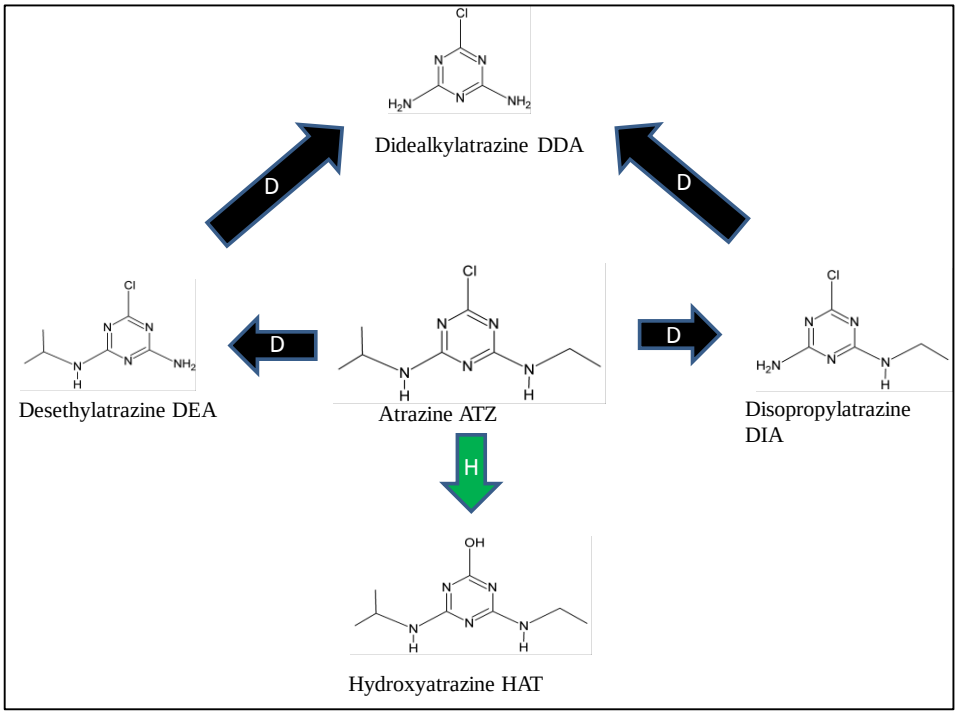

Fig. 8: General atrazine degradation pathway in *Rhodococcus*

"D" labeled black arrows dealkylation reactions, "H" labeled green arrows hydroxylation.

The reaction pathways shown are combined from the various reaction pathways provided (Behki et al., 1993; Nagy et al., 1995; Shao et al., 1995). Unlike both bacteria (*Pseudomonas* sp. and *Arthrobacter*), *Rhodococcus* cannot mineralize atrazine, but it has shown capabilities Fig. 8 over the period by transforming atrazine into various products occurring either of these pathways. *Bacillus subtlis* was reported for atrazine biodegradation to cyanuric acid (Wang et al. 2014). Wang et al. (2014) proposed biodegradation pathway for atrazine by *Bacillus subtlis* (Fig. 9).

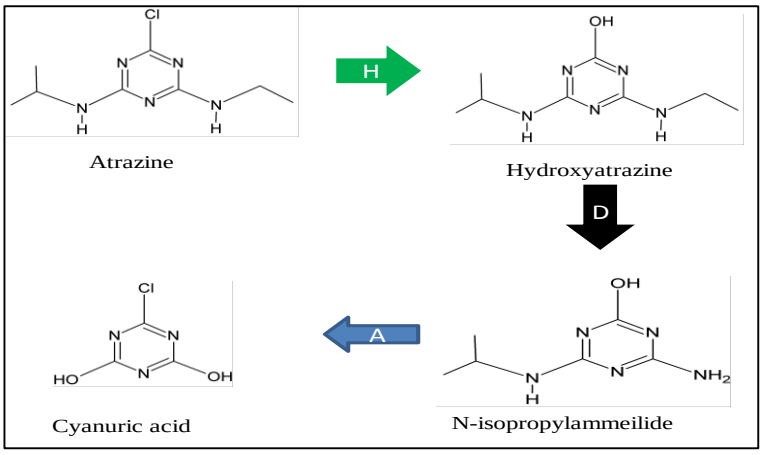

Fig. 9: Atrazine biodegradation pathway for *Bacillus subtilis*. "D" labeled black arrows dealkylation reactions; "H" labeled green arrows hydroxylation; "A" labeled blue arrows deamination

Quorum sensing and bioremediation

The phenomenon of intracellular communication between many bacterial species is called as quorum sensing (QS). This phenomenon is universal in bacteria and bacterial communities. Contact based chemical exchange, chemical signaling, and electric signaling are reported methods of QS (Yong et al., 2015). QS Bacteria secrete chemical molecules (autoinducers) which synchronize behavior of the group of bacteria. With the help of QS many types of tasks which are not possible for individual bacteria can be achieved. For communication, individual microorganism uses one or more than one type of chemical messengers from an enormous range of diverse classes of chemical signals. These multifaceted categorized regulatory circuits have evolved to integrate and process the sensory information to differentiate between species in associations (Miller and Bassler, 2001). Gram positive and gram negative bacteria use different molecules, various circuits for quorum sensing. Gram positive bacteria use oligopeptides while gram negative bacteria use N-acylated-L-homoserine lactones (AHLs). *Pseudomonas aeruginosa, Agrobacterium tumefaciens* have been reported for QS. For biofilm formation, *Pseudomonas aeruginosa* uses QS mechanisms (Yong et al., 2015). *Pseudomonas* is reported for various biodegradation of xenobiotics including atrazine. However, QS phenomenon of bacteria in bioremediation is not explored very well except phenol degradation (Yong and Zhong, 2010). In case of bioremediation waste water treatment *Pseudomonas*, *Aeromonas*, *Acinetobacter* reported for improving the stability of microbial community and biodegradation

capacity. This approach has not been used apart from phenol bioegradation. Recent *in the silico* study indicated that there is phylogenetic overlap between aromatics degrading bacteria and AHLs-based QS bacteria (Huang et al., 2013). In future for bioremediation applications, bacterial species which are harboring both QS and the xenobiotic degrading genetic system would be beneficial.

Conclusions and future perspectives

Atrazine bioremediation by bacteria remains viable and efficient. Use of genetic makeup of bacteria for remediation is explored very well in case of atrazine degrading bacteria. However, QS mechanism in all (atrazine biodegrading) bacteria is not covered yet. Use of QS is still limited in the case of bioremediation. The relationship between QS and biodegradation should be explored to achieve efficient bioremediation strategies.

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