# Degradation of Atrazine by Plants and Microbes

**S.N. Singh and Nitanshi Jauhari**

## 1 Introduction

Application of herbicides (or pesticides) is inevitable in the management practices of crop production in modern agriculture. Synthetic chemicals are designed to target eradication of weeds through specific mechanisms to minimize their competition with crop plants for use of nutrients and water from agricultural fields. Despite of all precautions, herbicides also adversely affect crops, microbes and animals. The use of herbicides not only affects the crop growth and development, but also the quality of crops. Thus, the herbicide pollutes the food chain, threatening the human health at large. Hence, there is an impending need to develop strategies to minimize the soil pesticide residues, water contamination and the toxicity of herbicides.

Among several chemical herbicides, atrazine (2-chloro-4-ethyl amino-y-6-isopropylamino-s-triazine, $C_8H_{14}ClN_5$) is one of the most widely used herbicides to eradicate many weeds in order to enhance crop production. The chemical structure of atrazine has been shown in Fig. 1.

It was discovered in 1950s and became most popular due to its high effectiveness against a wide spectrum of weeds. It is moderately soluble in water (33 mg $L^{-1}$ at 22 °C) (Tomlin 2000). An ideal herbicide should kill only targeted weeds and be easily biodegradable. Neither it should leach to the groundwater nor spread in the surroundings. However, this is likely to be rare quality of an herbicide or pesticide. Atrazine is rapidly degraded in soil with a half-life of 32–128 days (Krutz et al. 2008). However, its half-life becomes longer in sub-surface environments. Atrazine degradation varies from rapid (half-life 38 days) to no degradation in groundwater. However, atrazine half-life varied between 430 and 829 days in the anaerobic

S.N. Singh (✉) · N. Jauhari
Environmental Science Division, CSIR-National Botanical Research Institute, Lucknow 226001, India
e-mail: drsn06@gmail.com



S.N. Singh (ed.), *Microbe-Induced Degradation of Pesticides*, Environmental Science and Engineering, DOI 10.1007/978-3-319-45156-5_10

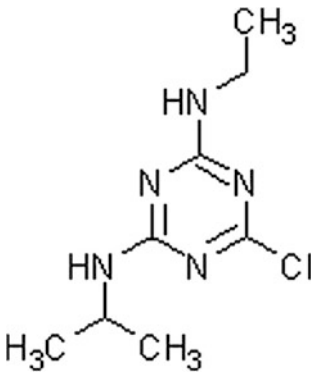


**Fig. 1** Chemical structure of atrazine (formula: $C_8H_{14}ClN_5$)

conditions (Talja et al. 2008). It has been recognized as an endocrine disrupting agent and moderately toxic to humans and animals. This is the reason that use of atrazine was banned in 1992 by the European Union for its persistence in groundwater. However, it is widely used as a herbicide in other countries outside the European Union, such as Brazil, China, India and Russia. About $3.4 \times 10^4$ tons of atrazine is applied in USA every year (Sadler et al. 2014), while China has usage of atrazine as much as 10820 tons in 2020. In Finland, Vuorimaa et al. (2007) found atrazine and its metabolites e.g. desethylatrazine (DEA), deisopropylatrazine (DIA) and desethyldeisopropylatrazine (DEDIA) in groundwater.

Atrazine is highly persistent in soil and gets accumulated over the period, as its microbial degradation rate is very slow as compared to its application rate (Fuscaldo et al. 1999). As a consequence, it becomes a serious threat to soil health which hampers plant growth and development. Further, atrazine moves to water sources through migration and leaching and thus becomes a potential threat to both wildlife and human beings. As of today, atrazine and its metabolites have been reported to be an alarming threat to ground and surface water exceeding threshold of 3 μg $L^{-1}$ as decided by US EPA. However, World Health Organization (WHO) restricted the permissible limit to only 2 μg $L^{-1}$. Thus, atrazine is a cause for high environmental threat due to its low biodegradability and its potential to contaminate both surface and ground water (Chan and Chu 2007).

Among the pesticides, atrazine is one of the most widely used herbicides throughout the world. It is extensively used on crops, like sugarcane, corn, sorghum, pineapple, conifers, forestry, grassland, macadamia nuts etc. (Luciane et al. 2010).

Atrazine (2 chloro-4 ethylamine-6-isopropyamine-5-triazine) is a chlorinated systemic selective herbicide used widely to eradicate weeds globally (Fig. 1). Atrazine is highly persistent in soil with average half-life ranging between 13 and 261 days (US EPA 2003). However, in river water, atrazine can persist for more than 100 days (Seiler et al. 1992). However, in sea water, it can stay for only 10 days (Armbrust and Crosby 1991) and about 660 days in the anaerobic conditions. No degradation was observed after incubation with adapted activated sludge.

Thus, due to its high mobility and persistence, residence of atrazine and its derivatives were detected in soil, surface water and groundwater after year's application (Schiavon 1988). Atrazine even at ppb level disrupts sexual development in amphibians, thus posing a serious ecological risk (Rhine et al. 2003).

Although many countries avoided using of atrazine due to its high toxicity, but it is still most popular herbicide in many countries (Jin and Ke 2002). In India, atrazine is still extensively used; hence there is high possibility of contamination of soil and water with atrazine in many parts. Therefore, there is a need to develop effective clean up technology for removal of atrazine. Due to its low biodegradability and high toxicity, it is still an environmental threat and has potential to contaminate surface and ground water (Chan and Chu 2007).

## 2 Atrazine Degradation

### *2.1 Physico-Chemical Degradation*

Several physico-chemical techniques are in place for cleanup of atrazine from water, wastewater and contaminated soils, e.g. incineration, thermal absorption, UV, peroxides, metal oxides, reverse osmosis and electrodylysis (Rodrigo et al. 2014). These technologies are generally expensive and also cause formation of toxic by-products. In some cases, end products need to be further treated.

The natural dissipation of atrazine from soil is regulated by both biotic and abiotic processes. The clay and organic matter of the soil determine the extent of adsorption of atrazine to particles of soil and sediment (Nemeth-Konda et al. 2002). Adsorption of atrazine increases the accumulation of atrazine by reducing its availability. In this situation, soil microbes play an important role in the degradation of atrazine. Biodegradation of atrazine in soil is more in surface soil than in sub-surface zone. Low temperature and lack of degrading microbes are primary factors which limit atrazine degradation in the sub-surface aquifer conditions and vadose zone (Radosevich et al. 1989). Atrazine was considered to be moderately persistent in soil. However, in past several years, many bacterial strains were isolated which could completely mineralize atrazine (Sadowsky and Wackett 2001).

### *2.2 Degradation of Atrazine by Plants*

A few plants, which were found tolerant to organic xenobiotics, could remove pesticides by taking up through roots and degrading them via putative metabolic pathways (Schröder et al. 2002; Cui and Yang 2011; Zhang et al. 2011). Besides, some plants could clean up xenobiotics in conjugation with microbes (Jin et al. 2012; Li and Yang 2013). However, in this case, microbes play an important role in

degradation of herbicides, as rapid degradation of herbicides was caused by rhizospheric bacteria through specific enzymes (Tesar et al. 2002; Merini et al. 2009; Jin et al. 2012). However, for faster degradation of toxic compounds, special plant species have to be selected or genetically engineered (Richard 2000). Several dicots and monocots show high tolerance to toxic herbicides and some genotypes of these plants were found to degrade herbicides in the soil. Usually plant species with fibrous root system have high ability to absorb the pollutants through broad root surface and interact with soil microbes for biodegradation of herbicides. These microbes thrive mainly on the root exudates.

*Lolium multiflorum* is a plant species which thrives well even in the adverse environmental conditions. However, the process of atrazine accumulation and degradation mediated by ryegrass is yet not fully understood. Sui and Yang (2013) studied the accumulation and degradation of atrazine in several rye grass genotypes. Out of these, three genotypes of rye grass had potential to accumulate and degrade atrazine. They could translocate the root-mediated uptake of atrazine to above ground. Thus, these genotypes may be used for the phytoremediation of atrazine—contaminated soil on a large scale.

Plant root exudates also influence the degradation of atrazine through their impact on microbial activity. Fang et al. (2001) observed faster degradation of atrazine in contaminated soil planted with *Pennisetum clandestinum* than in unplanted soil (Singh et al. 2004a, b).

## 2.3 Bacterial Degradation

Many other bacteria are currently known to degrade atrazine which include the members of different genera, such as *Acinetobacter* (Singh et al. 2004a, b), *Agrobacterium* (Struthers et al. 1998), *Arthobacter* (Zhang et al. 2011), *Chelatobacter* (Cheyns et al. 2012), *Dehftia* (Vargha et al. 2005).

Many bacteria were isolated and studied for degradation of atrazine by members of genera *Pseudomonas, Acinetobacter, Agrobacterium, Arthrobacter, Rastonia* and *Norcardioides* etc. (Bouquard et al. 1997a, b; Struthers et al. 1998; Strong et al. 2002).

Atrazine biodegradation occurs via different pathways that funnel into cyanuric acid metabolism. The gene region of *Pseudomonas* sp. strain ADP encoding atrazine degradation enzymes has been cloned and characterized. Initially, atrazine is degraded to hydroxyatrazine by hydrolytic dechlorination (de Souza et al. 1995). Later on, two enzymes of aminohydrolase protein catalyzed the sequential removal of ethylamine and isopropylamine (Boundy-Mills et al. 1997; Sadowski et al. 1998).

*Nocardioides* (Omotayo et al. 2012), *Pseudaminobacter* (Topp et al. 2000), *Pseudomonas* (Hernandez et al. 2008), *Rastonia* (Stamper et al. 2002), *Rhizobium* (Bouquard et al. 1997a, b) and *Rodococcus* (Behki et al. 1993) have been reported to degrade atrazine. Among these bacteria, *Pseudomonas* sp. strain ADP reported

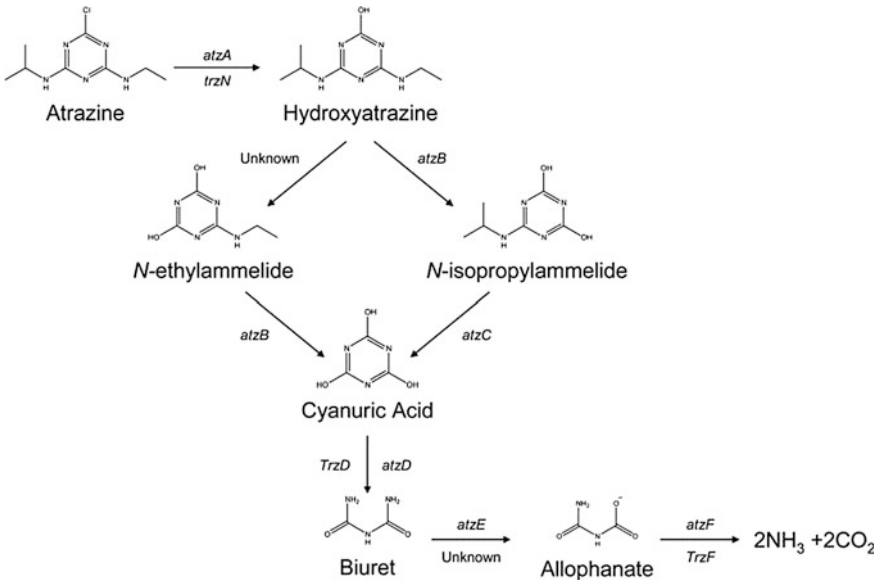


**Fig. 2** Atrazine degradation by bacteria (Krutz et al. 2008)

by Mandelbaum et al. (1995), was used to elucidate the sequences of catabolic enzymes involved in aerobic degradation pathway and to develop probes for the genes encoding these enzymes. Krutz et al. (2008) elucidated the bacterial metabolic pathway of atrazine degradation (Fig. 2).

They suggested that a unique operon of genes encoding s-triazine degradation was evolved in areas in which this herbicide was extensively applied. The gene regions encoding the first three enzymes responsible for atrazine degradation have been isolated and characterized from *Pseudomonas* sp. strain ADP (Boundy-Mills et al. 1997; de Souza et al. 1995, 1996; Sadowski et al. 1998). This bacterium could mineralize a very high concentration of 500 mg $L^{-1}$ under both growth and non-growth conditions, using the atrazine as the sole nitrogen source (Mandelbaum et al. 1995). The atzA gene, which encodes atrazine chlorohydrolase, dechlorinates atrazine to nonphytotoxic metabolite hydroxy atrazine (Fig. 3). The next step in the degradation pathway is hydrolytic removal of aminoethyl group from hydroxyatrazine by the hydroxyatrazine ethyl amidohydrolase which is *atz*B gene product. Finally, the *atz*C gene encodes for another aminohydrolase that converts N-isopropylammelide to cyanuric acid. Besides, Martinez et al. (2001) have sequenced the complete catabolic plasmid from *Pseudomonas* sp. strain ADP and identified three additional genes *atz*D, *atz*E and *atz*F encoding for cyanuric acid amidohydrolase, biurel-hydrolase and allophanate hydrolase. Thus, total genetic basis for complete atrazine metabolism in *Pseudomonas* sp. strain ADP was worked out.

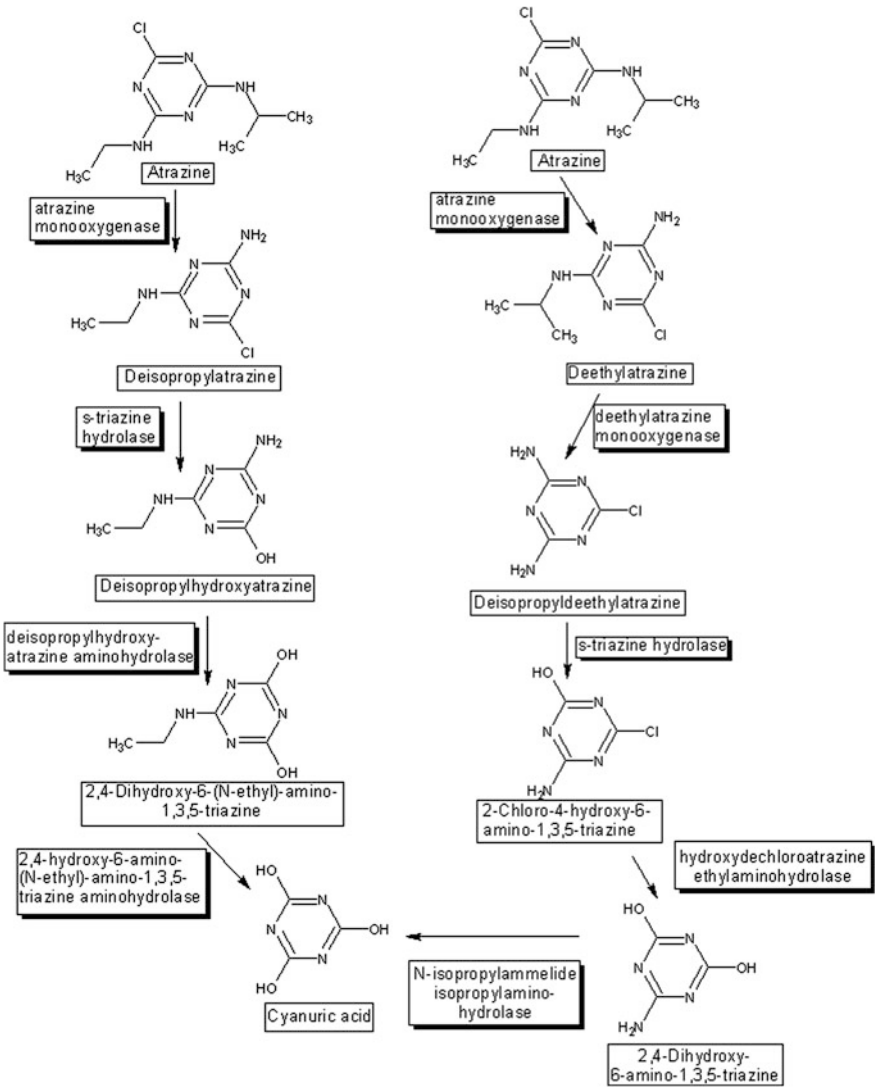


**Fig. 3** Bacterial degradation pathway of atrazine (Wackett et al. 2002)

Degradation of atrazine occurs through different pathways. One pathway involves dealkylation of amino groups to give 2-chloro-4-hydroxy-6-amino-1,3,5-triazine. The another pathway, which is well established, involves hydrolysis of C-$C_1$ bond, ethyl and isopropyl groups leading to formation of cyanuric acid which is further utilized to release $CO_2$ (Sagarkar et al. 2013). Similar catabolic pathway of atrazine degradation was reported by Wackett et al. (2002) and Fazlurrahman et al. (2009). Surprisingly, there was no difference in the

intermediates formed during atrazine degradation in the presence and absence of surfactants like rhamnolipids and triton X-100. However, addition of surfactant in soil enhanced the degradation of atrazine by increasing desorption of atrazine from soil and making it to more available for degradation by bacteria. However, glycolipid-type surfactant like rhammolipids exhibited higher potential of enhanced degradation than synthetic surfactant like triton X-100.

The characterization of atrazine-degrading bacterial strains revealed the presence of *atz*ABCDEF genes in plasmid and encoding enzymes were found in the oxygen-dependent degradation of atrazine (de Souza et al. 1996; Martinez et al. 2001). Further, investigation revealed that genes are highly conserved in different microbial genera. Their worldwide spread indicates a potential molecular mechanism for the dispersion of the *atz*ABC genes to other soil bacteria (de Souza et al. 1998). Besides, two other atrazine-degrading genes encoding the enzymes atrazine chlorohydrolase and cyanuric acid hydrolase, were characterized as *trz*N and *trz*D, respectively (Karns 1999; Mulbry et al. 2002).

### 2.3.1 Atrazine Degradation by Microbial Consortia

Microbial degradation of atrazine follows different metabolic pathways, involving stepwise transformations carried out by individual species or microbial consortia. For this purpose, several atrazine degrading bacteria were isolated and their separate metabolic pathways for atrazine degradation were thoroughly studied, but their synergistic action in atrazine metabolism is still not well investigated.

In a study of atrazine degradation by microbial consortia, it was first observed that atrazine was dechlorinated to hydroxyatrazine in two ways. In one pathway, hydroxyatrazine was transformed by *Nocardia* sp. to N-ethylammelide via unidentified product, whereas in another pathway, hydroxyatrazine was hydrolyzed to N-isopropylammelide by *Rhizobium* sp. having the gene *atz*B. However, all the consortium members contained *atz*C responsible for cleavage of the ring, besides the gene *trz*D. However, Smith et al. (2005) reported that none of the microbe carried all three genes i.e. *atz*D, *atz*E, and *atz*F.

Subsequently, Kolic et al. (2007) found that a four member microbial community enriched from an agrochemical factory was able to mineralize atrazine very rapidly to $CO_2$ up to 78 % within a week. When the genetic potential of community members was studied individually, it was observed that *Arthrobacter* strain ATZ1 with *trz*N and *atz*C genes and ATZ2 strain having *trz*N, *atz*B and *atz*C genes, could be involved in the upper pathway, producing cyanuric acid, while others *Ochrobacterium* sp. CA1 and *Pseudomonas* sp. CA2, both with *trz*D gene, were found involved in cyanuric acid metabolism.

Microbial community members in biofilms interact at the genetic level among intra and inter species and are also exposed to genetic events like transformation, transduction and conjugation at the contaminated site. Hence, they acquired improved capability to degrade hazardous substances (Stoodley et al. 2002). It was

observed that microbes modified the biofilms through natural transformation to improve their degradation ability (Perumbakkam et al. 2006). They transformed biofilm communities with the gene *atzA* which encoded atrazine chlorohydrolase. Both pure and soil-borne culture may be transferred with *atzA* gene cloned in plasmid PBBR1NCSS, to have the ability to degrade atrazine. Thus natural transformation may be used as a tool to enhance atrazine biodegradation performed by biofilms.

## 2.4 Fungal Degradation of Atrazine

Several fungi like *Aspergillus fumigates, A. flavipes, Fusarium moniliforme*, *Penicillium decumbens*, *Rhizopus stolonifer, Trichoderma viride* etc. are reported to degrade atrazine (Kaufman and Blake 1970; Mougin et al. 1994). Fungal degradation begins with N-dealkylation leading to formation of deethylatrazine and deisopropylatraine as the degradation products (Kaufman and Blake 1970). A white rot fungus i.e. *Phanerochaete chrysosporium*, could metabolize 48 % of atrazine in the growth medium after 4 days of incubation. In this process, 25 % of mineralization of ethyl group of herbicide occurred with the formation of hydroxylated and/or N-dealkylated metabolites. However, mineralization of 14C-labeled atrazine was not reported (Mougin et al. 1994). Besides, *Pleurotus pulmonarius* is another fungus which degraded atrazine in liquid medium and formed N-dealkylated metabolites, such as deethylatrazine, deisopropylatrazine, deethyliecthyatrazine and also hydroxyisopropyl atrazine as the hydroxypropyl metabolites (Masaphy et al. 1996a). Atrazine transformation was enhanced in the presence of 300 μM of maganese by this fungus with the accumulation of both N-dealkylated and propylhydroxylated metabolites (Masaphy et al. 1996b).

## 2.5 Strategies for Enhanced Atrazine Degradation by Microbes

In addition to natural occurring microbes, genetically engineered microbes have been used for boost up degradation of xerobiotic compounds. As reported by Rousseaux et al. (2001), *Chelatobacter heintzii* Cit1 could mineralize atrazine in soil 3 folds more with the inoculum of $10^4$ $UFCg^{-1}$ than the control. Gupta and Baummer (1996) investigated the effect of poultry on the biodegradation of atrazine and found that atrazine removal was enhanced by 2 folds as compared to soil without poultry manure. However, mineral nutrients, like nitrates and phosphates, played no role in atrazine degradation.

In a field study of soil contaminated with atrazine spillover (approx. 29000 ppm), bioaugmentation was performed using a killed and stabilized whole

cell suspension of recombinant *E. coli* to overproduce atrazine chlorohydralase, *atz*A. It was observed that there was a decline of 52 % in atrazine level in plots having killed recombinant *E. coli* cells. In another investigation, cell-free crude extracts from *Pseudomonas* sp. ADP containing the enzymes that catalyzed atrazine degradation, were entrapped in sol-gel glass. In this situation, there was a significant loss of enzyme activity as compared to non-entrapped crude extract (Kauffmann and Mandelbaum 1996). This strategy seems to be more promising than use of transgenic bacterial cells to field application. Silva et al. (2004) observed that bioaugmentation of *Pseudomonas* sp. ADP in combination with biostimulation with citrate or succinate, markedly enhanced atrazine mineralization. In comparison to other carbon sources, cellulose enhanced dealkylation of atrazine side-chain by soil microbes as compared to other carbon substrates. Glucose, as an end-product of cellulose depolymerization, may be responsible of inhibition of dealkylaion enzyme, resulting in a decrease in atrazine side chain mineralization (Yassir et al. 1998). Atrazine degradation by an anaerobic mixed culture was found higher in co-metabolic process than in absence of external C and N sources.

Most of atrazine degradation bacteria use herbicides as a source of nitrogen. Hence, presence of another N source decreased atrazine degradation by *Pseudomonas* sp. ADP (Clausen et al. 2002). Nitrogen is found detrimental to atrazine degradation, but stimulated the primary growth of bacteria. Thus microbial processes and C uptake used to influence the herbicide degradation.

## 3 Effect of Atrazine on Microbial Communities

Microbes play an important role in the functions of natural ecosystems, such as organic matter decomposition, nutrient cycling and natural attenuation of toxic compounds. Thus, they provide invaluable service to soil and water purification processes by their metabolic activities.

Abiotic and biotic factors both influence the structural composition and diversity of microbial community. These factors may be classified as natural and anthropogenic factors. The natural factors are vegetation, temperature, moisture, pH of the soil, while anthropogenic activities are management of soil through tillage and application of pesticides and fertilizers (Zhou et al. 2008). Soil microbes are adversely affected by the application of pesticides including fungicides, insecticides and herbicides.

Hu et al. (2005) studied the effect of atrazine application at the rate of 50 mg $kg^{-1}$ to soil and found that soil respiration rate was enhanced, but microbial diversity was reduced. Atrazine application significantly shifted the microbial community structure and function (Seghers et al. 2003). Besides, Chen et al. (2015) found an inhibitory effect on the soil nitrification process and microbial community in microcosm incubation.

## 4 Conclusion

Microbial degradation of atrazine is an eco-friendly and low cost technology. Better understanding of microbial mineralization of atrazine by indigenous or transgenic microbes can boost up elimination of atrazine from agricultural soils in a cost-effective manner. Microbes have the inherent ability to utilize herbicides as carbon and nitrogen sources. Our understanding about atrazine degradation can lead to development of an effective bioremediation process for other recalcitrant herbicides, used in agriculture.

## References

Armbrust, D. L., & Crosby, D. G. (1991). Fate of carbaryl 1-naphthol and atrazine in seawater. *Pacific Science, 45*, 314–320.

Behki, R., Topp, E., Dick, W., & Germon, P. (1993). Metabolism of the herbicide atrazine by *Rhodococcus* strains. *Applied and Environment Microbiology, 59*, 1955–1959.

Boundy-Mills, K. L., de Souza, M. L., Mandelbaum, R. T., Wackett, L. P., & Sadowsky, M. J. (1997). The *atz*B gene of *Pseudomonas* sp. strain ADP encodes the second enzyme of a novel atrazine degradation pathway. *Applied and Environment Microbiology, 63*, 916–923.

Bouquard, C., Ouazzani, J., Prome, J., Michel-Briand, Y., & Plesiat, P. (1997a). Dechlorination of atrazine by a *Rhizobium* sp. Isolate. *Applied and Environment Microbiology, 63*, 862–866.

Bouquard, C., Ouzzani, J., Prome, J. C., Michel Briand, Y., & Plesiat, P. (1997b). Dechlorination of atrazine by a *Rhizobium* isolate. *Applied and Environment Microbiology, 63*, 862–866.

Chan, K. H., & Chu, W. (2007). Effect of humic acid on the photolysis of the pesticide atrazine in a surfactant-aided soil-washing system in acidic condition. *Water Research, 39*, 2154–2166.

Chen, Q., Yang, B., Wang, H., He, F., Gao, Y., & Scheel, R. (2015). Soil microbial community toxic response to atrazine and its residues under atrazine and lead contamination. *Environmental Science and Pollution Research, 22*, 996–1007.

Cheyns, K., Calcoen, J., Martin-Laurent, F., Bru, D., Smolders, E., & Springael, D. (2012). Effects of dissolved organic matter (DOM) at environmentally relevant carbon concentrations on atrazine degradation by *Chelatobacter heintzii* SalB. *Applied Microbiology and Biotechnology, 95*, 1333–1341.

Clausen, G. B., Larsen, L., Johnsen, K., de Lipthay, J. R., & Aamand, J. (2002). Quantification of the atrazine degrading *Pseudomonas* sp. strain ADP in aquifer sediment by quantitative competitive polymerase chain reaction. *FEMS Microbiology Ecology, 41*, 211–229.

Cui, L. E., & Yang, H. (2011). Accumulation and residue of napropamide in alfalfa (*Medicago sativa*) and soil involved in toxic response. *Journal of Hazardous Materials, 190*, 81–86.

De Souza, M. L., Newcombe, D., Alvey, S., Crowley, D. E., Hay, A., Sadowsky, M. J., et al. (1998). Molecular basis of a bacterial consortium interspecies catabolism of atrazine. *Applied and Environment Microbiology, 64*, 178–184.

De Souza, M. L., Sadowsky, M. J., & Wackett, L. P. (1996). Atrazine chlorohydrolase from *Pseudomonas* sp. strain ADP: Gene sequence, enzyme purification, and protein characterization. *Journal of Bacteriology, 178*, 4894–4900.

De Souza, M. L., Wackett, L. P., Boundy-Mills, K. L., Mandelbaum, R. T., & Sadowsky, M. J. (1995). Cloning, characterization, and expression of a gene region from *Pseudomonas* sp. strain ADP involved in the dechlorination of atrazine. *Applied and Environment Microbiology, 61*, 3373–3378.

Fang, C., Radosevich, M., & Fuhrmann, J. J. (2001). Atrazine and phenanthrene degradation in grass rhizosphere soil. *Soil Biology and Biochemistry, 3*, 671–678.

Fazlurrahman, B. M., Pandey, J., Suri, C. R., & Jain, R. K. (2009). Isolation and characterization of an atrazine-degrading Rhodococcussp. strain MB-P1 from contaminated soil. *Letters in Applied Microbiology, 49*, 721–729.

Fuscaldo, F., Bedmar, F., & Monterubbianesi, G. (1999). Persistence of atrazine, metribuzin and simazine herbicides in two soils. *Pesquisa Agropecuária Brasileira, 34*, 2037–2044.

Gupta, G., & Baummer, J, I. I. I. (1996). Biodegradation of atrazine in soil using poultry litter. *Journal of Hazardous Materials, 45*, 185–192.

Hernandez, M., Morgante, V., Avila, M., Villalobos, P., Miralles, P., Gonzalez, M., & Seeger, M. (2008). Novel s-triazine-degrading bacteria isolated from agricultural soils of central Chile for herbicide bioremediation. *Electronic Journal of Biotechnology 11*(5 Special Issue), 1–7.

Hu, J., Dai, X. Z., & Li, S. P. (2005). Effects of atrazine and its degrader *Exiguobaterium* sp. BTAH1 on soil microbial community. *Chinese Journal of Applied Ecology, 8*, 138–143.

Jin, R., & Ke, J. (2002). Impact of atrazine disposal on the water resources of the Yang river in Zhangjiakou area in China. *Bulletin of Environment Contamination and Toxicology, 68*, 893–900.

Jin, Z. P., Luo, K., Zhang, S., Zheng, Q., & Yang, H. (2012). Bioaccumulation and catabolism of prometryne in green algae. *Chemosphere, 87*, 278–284.

Karns, J. S. (1999). Gene sequence and properties of an s-triazine ring-cleavage enzyme from *Pseudomonas* sp. strain NRRLB-12227. *Applied and Environment Microbiology, 65*, 3512–3517.

Kauffmann, C. G., & Mandelbaum, R. T. (1996). Entrapment of atrazine-degrading enzymes in sol-gel glass. *Journal of Biotechnology, 51*, 219–225.

Kaufman, D. D., & Blake, J. (1970). *Soil Biology and Biochemistry, 2*, 73–80.

Kolic, N. U., Hrsak, D., Kolar, A. B., Petric, I., Stipicevic, S., Soulas, G., et al. (2007). Combined metabolic activity with an atrazine-mineralizing community enriched from agrochemical factory soil. *International Biodeterioration and Biodegradation, 60*, 299–307.

Krutz, L. J., Shaner, D. L., Accinelli, C., Zablotowicz, R. M., & Henry, W. B. (2008). Atrazine dissipation in-triazine–adapted and nonadapted soil from Colorado and Mississippi: Implications of enhanced degradation on atrazine fate and transport parameters. *Journal of Environmental Quality, 37*, 848–857.

Li, Y. Y., & Yang, H. (2013). Bioaccumulation and degradation of pentachloronitrobenzene in *Medicago sativa*. *Journal of Environmental Management, 119*, 143–150.

Luciane, S., Attilio, C., Geslaine, A. R. S., & Rita, D. C. G. S. (2010). New aspects on atrazine biodegradation. *Brazilian Archives of Biology and Technology, 53*, 487–496.

Mandelbaum, R. T., Allan, D. L., & Wackett, L. P. (1995). Isolation and characterization of a *Pseudomonas* sp. that mineralizes the s-triazine herbicide atrazine. *Applied and Environment Microbiology, 61*, 1451–1457.

Martinez, B., Tomkins, J., Wackett, R., Wing, R., & Sadowsky, M. J. (2001). Complete nucleotide sequence and organization of the atrazine catabolic plasmid pADP-1 from *Pseudomonas* sp. strain ADP. *Journal of Bacteriology, 183*, 5684–5697.

Masaphy, S., Henis, Y., & Levanon, D. (1996a). Manganese-enhanced biotransformation of atrazine by the white rot fungus *Pleurotus pulmonarius* and its correlation with oxidation activity. *Applied and Environment Microbiology, 2*, 3587–3593.

Masaphy, S., Levanon, D., & Henis, Y. (1996b). Degradation of atrazine by the lignocellulolytic fungus *Pleurotus pulmonarius* during solid—state fermentation. *Bioresource Technology, 56*, 207–214.

Merini, L. J., Bobillo, C., Cuadrado, V., Corach, D., & Giulietti, A. M. (2009). Phytoremediation potential of the novel atrazine tolerant *Lolium multiflorum* and studies on the mechanisms involved. *Environmental Pollution, 157*, 3059–3063.

Mougin, C., Laugero, C., Asther, M., Dubroca, J., Frasse, P., & Asther, M. (1994). Biotransformation of the herbicide atrazine by the white rot fungus *Phanerochaete chrysosporium*. *Applied and Environment Microbiology, 60*, 705–708.

Mulbry, W. W., Zhu, H., Nour, S. M., & Topp, E. (2002). The triazine hydrolase gene *trz*N from *Nocardioides* sp. strain C190: Cloning and construction of gene-specific primers. *FEMS Microbiology Letters, 206*, 75–79.

Nemeth-Konda, L., Füleky, G., Morovjan, G., & Csokan, P. (2002). Sorption behaviour of acetochlor, atrazine, carbendazim, diazinon, imidacloprid and isoproturon on Hungarian agricultural soil. *Chemosphere, 48*, 545–552.

Omotayo, A. E., Ilori, M. O., Radosevich, M., & Amund, O. O. (2012). Metabolism of atrazine in liquid cultures and soil microcosms by Nocardioides strains isolated from a contaminated nigerian agricultural soil. *Soil and Sediment Contamination: An International Journal, 22*, 365–375.

Perumbakkam, S., Hess, T. F., & Crawford, R. L. (2006). A bioremediation approach using natural transformation in pure-culture and mixed-population biofilms. *Biodegradation, 17*, 545–557.

Radosevich, M., Traina, S. J., & Tuovinen, O. H. (1989). Biodegradation of atrazine in surface soils and subsurface sediments collected from an agricultural research farm. *Biodegradatin, 7*, 137–149.

Rhine, E. D., Fuhrmann, J. J., & Radisevich, M. (2003). Microbial community response to atrazine exposure and nutrient availability: Linking degradation capacity to community structure. *Microbial Ecology, 46*, 145–160.

Richard, B. M. (2000). Phytoremediation of toxic elemental and organic pollutants. *Current Opinion in Plant Biology, 3*, 153–162.

Rodrigo, M. A., Oturan, N., & Oturan, M. A. (2014). Electrochemically assisted remediation of pesticides in soils and water: A review. *Chemical Reviews, 114*, 8720–8745.

Rousseaux, S., Hartmann, A., & Soulas, G. (2001). Isolation and characterisation of new Gram-negative and Gram-positive atrazine degrading bacteria from different French soils. *FEMS Microbiology Ecology, 36*, 211–222.

Sadler, E. J., Sudduth, K. A., Lerch, R. N., Baffaut, C., & Kitchen, N. R. (2014). A simple index explains annual atrazine transport from surface runoff-prone watersheds in the north-central USA. *Hydrological Processes, 28*, 210–217.

Sadowski, M. J., Tong, Z., Souza, M. L. D., & Wackett, L. P. (1998). AtzC is a new member of the aminohydrolase protein superfamily and is homologous to other atrazine-metabolizing enzymes. *Journal of Bacteriology, 180*, 152–158.

Sadowsky, M. J., & Wackett, L. P. (2001). Genetics of atrazine ands-triazine degradation by *Psedomonas* sp. strain ADP and other bacteria. In J. C. Hall, R. E. Hoagland & R. M. Zablotowicz (Eds.), *Pesticide biotransformation in plants and microorganisms: Similarities and divergences*. ACS Symposium Series 777 (pp. 268–282). Washington, DC: American Chemical Society.

Sagarkar, S., Mukherjee, S., Nousiainen, A., Bjorklof, K., Purohit, H. J., Jorgensen, K. S., et al. (2013). Monitoring bioremediation of atrazine in soil microcosms using molecular tools. *Environmental Pollution, 172*, 108–115.

Schiavon, M. (1988). Studies of the leaching of atrazine, of its chlorinated derivatives, and of hydroxyatrazine from soil using 14C ring-labeled compounds under outdoor conditions. *Ecotoxicology and Environmental Safety, 15*, 46–54.

Schröder, P., Harvey, P. J., & Schwitzguébel, J. P. (2002). Prospects for the phytoremediation of organic pollutants in Europe. *Environmental Science and Pollution Research, 9*, 1–3.

Seghers, D., Verthé, K., Reheul, D., Bulcke, R., Siciliano, S. D., Verstraete, W., et al. (2003). Effect of long-term herbicide applications on the bacterial community structure and function in an agricultural soil. *FEMS Microbiology Ecology, 46*, 139–146.

Seiler, A., Brenneisen, P., & Green, D. H. (1992). Benefits and risks of plant protection products possibilities of protecting drinking water: Case atrazine. *Water Supply, 10*, 31–42.

Silva, E,. Fialho, A.M., Sa-Correia I., Burns, R.G., Shaw, L.J. (2004). Combined bioaugmentation and biostimulation to cleanup soil contaminated with high concentrations of atrazine. *Environmental Science & Technology, 38*, 632–637.

Singh, N., Megharaj, M., Kookana, R. S., Naidu, R., & Sethunathan, N. (2004a). Atrazine and simazine degradation in *Pennisetum rhrizosphere*. *Chemosphere, 56*, 257–263.

Singh, P., Suri, C. R., & Cameotra, S. S. (2004b). Isolation of a member of *Acinetobacter* species involved in atrazine degradation. *Biochemical and Biophysical Research Communications, 317*, 697–702.

Smith, D., Alvey, S., & Crowley, D. E. (2005). Cooperative catabolic pathways within an atrazine degrading enrichment culture isolated from soil. *FEMS Microbiology Ecology, 51*, 265–273.

Stamper, D. M., Radosevich, M., Hallberg, K. B., Traina, S. J., & Tuovinen, O. H. (2002). *Ralstonia basilensis* M91-3, a denitrifying soil bacterium capable of using s-triazines as nitrogen sources. *Canadian Journal of Microbiology, 48*(12), 1089–1098.

Stoodley, P., Sauer, K., Davis, D. G., & Costerton, J. W. (2002). Biofilms as acomplex differentiated communities. *Annual Reviews in Microbiology, 56*, 187–209.

Strong, L. C., Rosendahl, C., Johnson, G., Sadowsky, M. J., & Wackett, L. P. (2002). *Arthrobacter auresens* TC1 metabolizes diverse s-triazine ring compounds. *Applied and Environment Microbiology, 68*, 5973–5980.

Struthers, J. K., Jayachandran, K., & Moorman, T. B. (1998). Biodegradation of atrazine by *Agrobacterium radiobacter* J14a and use of this strain in bioremediation of contaminated soil. *Applied and Environmental Microbiology, 64*, 3368–3375.

Sui, Y., & Yang, H. (2013). Bioaccumulation and degradation of atrazine in several Chinese ryegrass genotypes. *Environmental Science: Processes and Impacts, 15*, 2338–2344.

Talja, K. M., Kaukonen, S., Kilpi-Koski, J., Malin, I., Kairesalo, T., Romantschuk, M., et al. (2008). Atrazine and terbutryn degradation in deposits from groundwater environment within the boreal region in Lahti, Finland. *Journal of Agriculture and Food Chemistry, 56*, 11962–11968.

Tesar, M., Reichenauer, T. G., & Sessitsch, A. (2002). Bacterial rhizosphere populations of black poplar and herbal plants to be used for phytoremediation of diesel fuel. *Soil Biology and Biochemistry, 34*, 1883–1892.

Tomlin, C. D. S. (2000). *The pesticide manual* (12th ed.). Surrey, UK: The British Crop Protection Council.

Topp, E., Zhu, H., Nour, S. M., Houot, S., Lewis, M., & Cuppels, D. (2000). Characterization of an atrazine degrading *Pseudaminobacter* sp. isolated from Canadian and French agricultural soils. *Applied and Environmental Microbiology, 66*, 2773–2782.

US EPA. (2003). Interim reregistration eligibility decision for atrazine. US: EPA.

Vargha, M., Takats, Z., & Marialigeti, K. (2005). Degradation of atrazine in a laboratory scale model system with Danube river sediment. *Water Research, 39*, 1560–1568.

Vuorimaa, P., Kontro, M., Rapala, J., Gustafsson, J. (2007) *Torjunta-aineiden esiintyminen pohjavedessä* [Occurrence of pesticides in groundwater]. Helsinki: The Finnish Environment 42, Finnish Environment Institute (in Finnish).

Wackett, L. P., Sadowsky, M. J., Martinez, B., & Shapir, N. (2002). Biodegradation of atrazine and related s-triazine compounds: From enzymes to field studies. *Applied Microbiology and Biotechnology 58*, 39–45.

Yassir, A., Rieu, C., & Soulas, G. (1998). Microbial Ndealkylation of atrazine: Effect of exogeneous organic substrates and behaviour of the soil microflora. *Pesticide Science, 54*, 75–82.

Zhang, Y., Jiang, Z., Cao, B., Hu, M., Wang, Z., & Dong, X. (2011). Metabolic ability and gene characteristics of *Arthrobacter* sp. strain DNS10, the sole atrazine-degrading strain in a consortium isolated from black soil. *International Biodeterioration and Biodegradation, 65*, 1140–1144.

Zhou, Z. S., Wang, S. J., & Yang, Z. M. (2008). Biological detection and analysis of mercury toxicity to alfalfa (*Medicago sativa*) plants. *Chemosphere, 70*, 1500–1509.