Chapter 2
Biodegradation and Bioremediation of *S*-Triazine Herbicides

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Contents

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K. M. Gothandam et al. (eds.), *Environmental Biotechnology Vol. 3*,
Environmental Chemistry for a Sustainable World 50,
https://doi.org/10.1007/978-3-030-48973-1_2

Abstract Pesticides have emerged as an integral tool of the farming activities and are used extensively to meet the increasing demand for food and feed. About 99% of the applied pesticides get accumulated in the nontarget organisms and environment. S-triazine herbicides have been classified as possible human carcinogens. Of these, atrazine was mostly used as it increases yield up to 50% based on crop and the most studied for toxicity and degradation. These pesticides are slowly degraded, and persistence leads to accumulation in soil or migrate to water bodies posing a severe threat to human and environment. Atrazine and its metabolites are frequently detected in surface water and ground water at concentrations exceeding the safety levels. We reviewed the biodegradation of s-triazine herbicides by microorganism, plants, and their degradation pathways. It was noted that atrazine degrading genes are widely distributed among the bacteria, but most of the bacterial strains do not contain all the genes required for atrazine mineralisation. Atrazine mineralisation appears to be more common in soils by microbial consortia than individual species. Certain bacteria including *Arthrobacter* sp. SC-JAK2 can degrade atrazine of above 1 g L^{-1} concentration which is far above the reported atrazine contaminant concentration in soil and water. Several reports concluded that excellent atrazine degraders in laboratory media, fail to do that in the complex natural environmental conditions that are suboptimal for growth or repress the synthesis of enzymes involved in the degradation pathway. Biostimulation and bioaugmentation studies showed rapid biodegradation of atrazine in contaminated sites. Major advances in the biodegradation of *s*-triazine-contaminated sites include the usage of genetically modified or engineered microorganisms, enzymatic bioremediation, and use of nanomaterials. With the help of advanced molecular and physiological approaches, it is possible to monitor the bioremediation and microbial community development in the atrazine-contaminated soil.

Keywords Atrazine · *S*-Triazine · Bioaugmentation · Biodegradation · Bioremediation · Biostimulation · Contaminant · Environment · Herbicides · Pesticides

2.1 Introduction

To meet the global requirement of food and fuel to some extent, pesticides are being used extensively in agriculture. Less than 0.1% of the applied pesticides reach the target organism, and the remainder gets deposited in soil and nontarget organisms or move into nearby water streams and lakes by leaching and agricultural runoff (Pimentel and Levitan 1986). These pesticides cause contamination of the environment and adversely affect the nontarget organisms and plants because of their persistence in the soil and water bodies. The persistence of pesticides in the soil and water mainly depend on chemical stability, solubility in water, soil physicochemical properties, climatic conditions, soil microbial activity, and leaching. Pollution of soil and water with pesticides and their toxic metabolites have become a major

environmental concern in the twenty-first century. Hence, the obliteration of persistent pesticides is essential to their sustained use.

2.1.1 S-*Triazine Herbicides*

Symmetrical triazine (*s*-triazine) relates to a large family of herbicides widely used worldwide to control broadleaf weeds and annual grasses in various plantations, residential lawns, and golf courses. The first triazine, chlorazine, was discovered in 1952 at J.R. Geigy Ltd. in Switzerland. Later in 1956, atrazine and simazine were discovered. The general structure of *s*-triazine herbicides is shown in Fig. 2.1. Side chains of triazine ring (X, R_1, and R_2) of commonly used triazine pesticides and their half-life in soil, applications in crops, as well as WHO classification are given in Table 2.1. Examples of *s*-triazines herbicides are chloro-*s*-triazines (atrazine, simazine, propazine, and cyanazine), the thiomethyl-*s*-triazines (ametryn, prometryn, terbutryn), and the methoxy-*s*-triazine (prometon). Cyanuric chloride (trichloro-1, 3, 5-triazine) is the basic for the production of several *s*-triazine herbicides including atrazine and simazine. Triazines are taken up into the plant roots, distributed throughout the plant via xylem, and act by interrupting photosynthesis in leaves specifically inhibiting the photosystem II. The effectiveness of triazines is dependent on several parameters including soil structure, moisture content, organic matter content, particle size distribution, and mode of application. Major advantages of using triazines are that it offers application flexibility and facility to mix with other herbicides for broad-spectrum weed control. They provide exceptional residual preemergence as well as early postemergence weed control. This enable farmers to use no-till and conservation tillage systems that minimise soil erosion by more than 50%. Triazine herbicides played a significant role in the adoption of conservation tillage, which significantly reduced fuel usage since fewer tillage trips are made across the field. Conservation tillage systems conserve soil moisture, increase the soil organic matter, and also dramatically decrease the water runoff and increase water infiltration. Minimizing soil erosion and water runoff will benefit the aquatic ecosystem. Further, these triazine herbicides paved way for the increased yield of food and feed in lesser space.

Only a fraction like less than 1% of the applied herbicide reaches the site of action within the plants. The loss is due to volatilisation, adsorption to soil, leaching

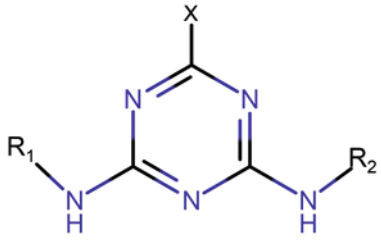

Fig. 2.1 General chemical structure of *s*-triazine pesticides (X, R_1, and R_2 are the side chains of triazine ring)

Table 2.1 List of *s*-triazine pesticides

s-Triazine herbicides	X	R_1	R_2	Soil half-life (days)	Applications	WHO classification
Atrazine	-Cl	$-NHC_2H_5$	$-NHC_3H_7$ (iso)	14–150	Corn, sorghum, sugarcane, pineapple, conifers, forestry	Slightly hazardous
Atratone	$-OCH_3$	$-NHC_2H_5$	$-NHC_3H_7$ (iso)	30	Non-agricultural areas, sugarcane, corn	Obsolete substance
Ametryn	$-SCH_3$	$-NHC_2H_5$	$-NHC_3H_7$ (iso)	37–250	Sugarcane, corn, pineapple	Moderately hazardous
Cyanazine	-Cl	$-NHC(CN)(CH_3)_2$	$-NHC_2H_5$	12–25	Vegetables, onions, potatoes, sweetcorn	Moderately hazardous
Desmetryn	$-SCH_3$	$-NH_2CH_3$	$-NHC_3H_7$ (iso)	9–50	Brassicas, onions and leeks, fodder rape	Slightly hazardous
Dimethametryn	$-SCH_3$	$-NHC_2H_5$	$-NHCH(CH_3)C_3H_7$ (iso)	37–250	Sugarcane, corn, pineapple	Slightly hazardous
Prometryn	$-SCH_3$	$-NHC_3H_7$ (iso)	$-NHC_3H_7$ (iso)	41–60	Cotton, celery, dill, potatoes, sunflowers, carrots, peanuts	Slightly hazardous
Prometone	$-OCH_3$	$-NHC_3H_7$ (iso)	$-NHC_3H_7$ (iso)	500	Non-cropland	Slightly hazardous
Propazine	-Cl	$-NHC_3H_7$ (iso)	$-NHC_3H_7$ (iso)	45–131	Corn, sorghum, carrots, fennel, ornamentals, greenhouse use	Unlikely to present an acute hazard
Simazine	-Cl	$-NHC_2H_5$	$-NHC_2H_5$	60–102	Corn, fruit and nut crops	Unlikely to present an acute hazard
Simetryn	$-SCH_3$	$-NHC_2H_5$	$-NHC_2H_5$	60	Rice, corn, bean, pea, cereals, cotton	Slightly hazardous
Terbutryn	$-SCH_3$	$-NHC_2H_5$	$-NHC(CH_3)_3$	14–74	Sugarcane, cereal, sorghum, sunflowers, peas, potatoes	Slightly hazardous

(continued)

Table 2.1 (continued)

s-Triazine herbicides	X	R_1	R_2	Soil half-life (days)	Applications	WHO classification
Trietazine	-Cl	$-N(C_2H_5)_2$	$-NHC_2H_5$	60	Potatoes, legumes, bananas, citrus, coffee, maize, sugarcane, tea, tobacco	Slightly hazardous
Terbuthylazine	-Cl	$-NHC_2H_5$	$-NHC(CH_3)_3$	22–60	Corn, sorghum, grape	Slightly hazardous

Where X, R_1 and R_2 = side chains of triazine ring (shown in Fig. 2.1)

by rainfall, photochemical degradation by sunlight, microbiological degradation by soil microorganisms, chemical degradation by soil constituents, and thermal degradation. *S*-triazines are toxic compounds and have been classified as possible human carcinogens. Simetryn, one of the major methylthio-*s*-triazine herbicides used in paddy fields, inhibits algal growth. Chlorinated triazine class of pesticides show common neuroendocrine mechanism of toxicity resulting in both reproductive and developmental concerns. The toxicity of these compounds has promoted research for their degradation. Atrazine is the most studied chlorinated triazine herbicide for toxicity and degradation.

2.1.2 Atrazine

Atrazine is one of the most widely used herbicides. Atrazine is very effective against a wide range of weeds and less expensive when compared to the alternative products. Atrazine inhibits D-1 quinone binding involved in photosystem II. Atrazine increases yield of about 6–50% based on crop. Despite the high agricultural yield, there is a huge concern for the continued use of atrazine in several parts of the world. For three decades from its discovery, microbial degradation was not observed as the triazine ring contains no available electrons for aerobic biodegradation. For microbial degradation, the only available energy source in atrazine is the ethyl and isopropyl side chains attached to the triazine ring. However, atrazine was converted by nonspecific monooxygenases to desethylatrazine and desisopropylatrazine. Small amount of hydroxyatrazine was formed by chemical processes. Atrazine and its metabolites are frequently detected in surface water and ground water at concentrations exceeding the safety levels. Atrazine is believed to cause endocrine disruption; carcinogenic effects including non-Hodgkin's lymphoma, ovarian cancer, colon cancer, leukaemia, multiple myeloma, reduced sperm quality in humans (IARC 1999; Rusiecki et al. 2004), cancer, delayed reproductive development in

rats, and male hermaphroditism in amphibians; and negative effect on aquatic organisms particularly in combination with other pesticides.

In anaerobic aquatic environment, atrazine's overall half-life, water half-life, and sediment half-life were given as 608, 578, and 330 days, respectively. While in terrestrial environment, half-life of atrazine may range from 13–261 days (US-EPA 2006). Atrazine dealkylation metabolites, such as deethylatrazine and deisopropylatrazine, are also regulated compounds and may pose health risks. Massive application, high mobility, and persistence are the major reasons for the frequent detection of atrazine and its metabolites in surface and ground water at concentrations well above the legal limits globally. European Union banned atrazine use in October 2003 but still in use in many parts of the world including the United States and India. However, Environmental Protection Agency has set the maximum containment level for atrazine in drinking water at 3 ppb. Triazine herbicides are persisted in the soil for 3–12 months and are slowly degraded by biological, chemical, and physical processes. This persistence period leads to accumulation in soil and water bodies posing a serious threat to human and environment. Therefore, utmost priority should be given to develop effective technologies for detoxification and/or removal of triazine pesticides and their metabolites. However, the metabolites of atrazine including hydroxyatrazine is less acutely toxic than the parent atrazine.

2.2 Biodegradation

2.2.1 Biodegradation of S-*Triazine Herbicides by Bacteria*

Pseudomonas sp. strain ADP was the first isolated atrazine-mineralizing strain. Many other bacteria are found to degrade atrazine as shown in Table 2.2. Atrazine mineralisation by microbial consortia appears to be more common in soils than individual species as most of the bacterial strains do not contain all the genes required for atrazine mineralisation (Billet et al. 2019; Kolic et al. 2007; Smith et al. 2005). Bacteria use atrazine primarily as a nitrogen source. Satsuma (2010) reported that a newly isolated *Nocardioides* species strain DN36 not only mineralised simetryn, atrazine, and simazine but also transformed propazine, ametryn, prometryn, dimethametryn, atraton, simeton, and prometon.

Degradation Pathway

Triazine mineralisation is more or less similar to atrazine mineralisation and is achieved in two stages. In the first stage, atrazine is converted to cyanuric acid (2,4,6-trihydroxy-1,3,5-triazine) by dehalogenation and dealkylation of side chains. Conversion of atrazine to cyanuric acid takes place via one of the three pathways as shown in Fig. 2.2. P-1 is the hydrolytic pathway commonly found in many bacteria

Table 2.2 List of bacterial genera capable of degrading various triazine herbicides

Pesticide	Bacteria	References
Atrazine	*Acinetobacter* sp.	Singh et al. (2004a)
	Aerobacterium sp.	Vargha et al. (2005)
	Agrobacterium sp.	Devers et al. (2007)
	Arthrobacter sp.	Shapir et al. (2005a)
	Bacillus sp.	Vargha et al. (2005)
	Chelatobacter sp.	Rousseaux et al. (2001)
	Citricoccus sp.	Yang et al. (2018)
	Deinococcus sp.	Vargha et al. (2005)
	Clavibacter sp.	De Souza et al. (1998)
	Delftia sp.	Vargha et al. (2005)
	Microbacterium sp.	Vargha et al. (2005)
	Micrococcus sp.	Vargha et al. (2005)
	Nocardioides sp.	Piutti et al. (2003)
	Polaromonas sp.	Devers et al. (2007)
	Pseudaminobacter sp.	Topp et al. (2000)
	Pseudomonas sp.	Mandelbaum et al. (1995)
	Ralstonia sp.	Radosevich et al. (1995)
	Rhizobium sp.	Bouquard et al. (1997)
	Rhodococcus sp.	Behki et al. (1993)
	Sinorhizobium sp.	Devers et al. (2007)
Ametryn	*Agrobacterium* sp.	Moscinski et al. (1996)
Cyanazine	*Agrobacterium* sp.	Moscinski et al. (1996)
	Rhodococcus sp.	Behki (1993)
Prometon	*Agrobacterium* sp.	Moscinski et al. (1996)
Prometryn	*Bacillus* sp.	Mizrachi (1994)
	Leucobacter sp.	Liu et al. (2018)
	Pseudomonas sp.	Grossenbacher (1986)
Propazine	*Rhodococcus* sp.	Behki (1993)
Simazine	*Acinetobacter* sp.	Feakin et al. (1995)
	Agrobacterium sp.	Liao and Xie (2008)
	Klebsiella sp.	Sánchez et al. (2005)
	Rhodococcus sp.	Behki (1993)
	Moraxella (Branhamella) sp.	Kodama et al. (2001)
	Pseudomonas sp.	Hernandez et al. (2008)
Simetryn	*Bacillus* sp.	Mizrachi (1994)

Note: Gram-positive bacteria have the ability to degrade more than one *s*-triazine pesticide

catalysed by atrazine chlorohydrolase (AtzA) or triazine hydrolase (TrzN), hydroxyatrazine N-ethylaminohydrolase (AtzB) (BoundyMills et al. 1997), and N-isopropylammelide aminohydrolase (AtzC) (Sadowsky et al. 1998). In Gram-negative bacteria, AtzA is responsible for dechlorination of atrazine (deSouza et al. 1996). TrzN of Gram-positive bacteria showed broad-spectrum activity, not only dehalogenation but also dislodges azido, cyano, S-alkyl, and O-alkyl substituents of

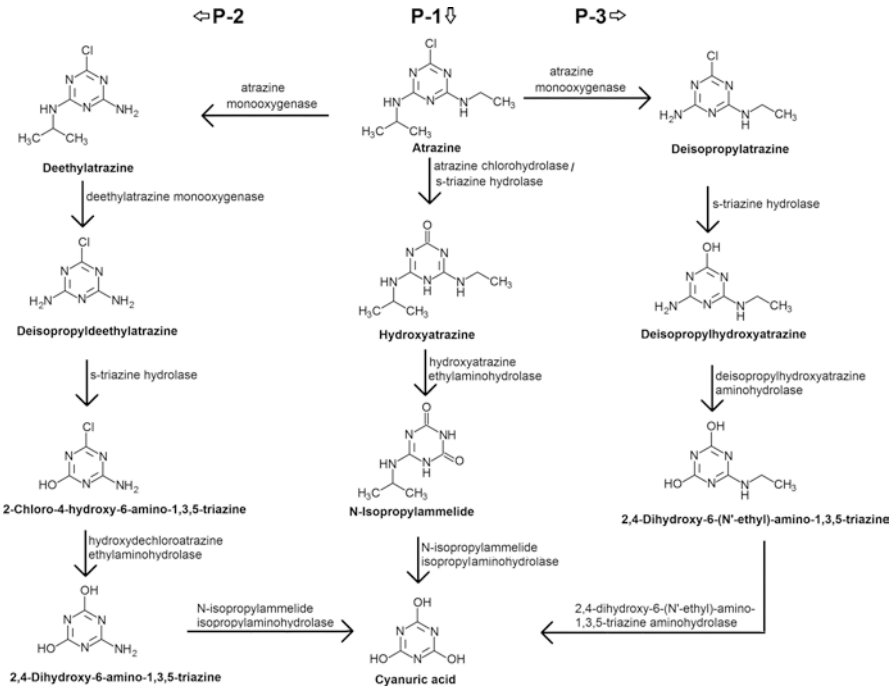

Fig. 2.2 Atrazine degradative pathways (P1, P2, and P3) showing conversion of atrazine to cyanuric acid

s-triazine herbicides (Topp et al. 2000). P-2 and P-3 are oxidative-hydrolytic pathways involving initial oxidative N-dealkylation of atrazine to deethylatrazine or deisopropylatrazine respectively by nonspecific monooxygenases (Devers et al. 2004). These products are dealkylated again to deisopropyldeethylatrazine, which is converted to cyanuric acid by hydrolytic dechlorination, deamination, and/or dealkylation (Govantes et al. 2009). Atrazine degradation via these routes is mostly reported by a consortium rather than individual bacteria and is less common. In the second stage, hydrolytic cleavage of the s-triazine ring of cyanuric acid and subsequently hydrolysis of biuret and allophanate occur to yield ammonia and carbon dioxide (Fig. 2.3). In most of the atrazine mineralising bacteria, these enzymes are encoded by the *atzDEF* operon (Fruchey et al. 2003; Karns 1999; Shapir et al. 2005b). Homologues to AtzD (TrzD) and AtzF (TrzF) perform the equivalent reactions in other bacteria with small differences in substrate affinity and specificity (Rousseaux et al. 2001; Shapir et al. 2006). Atrazine will be biodegraded to cyanuric acid by one of the above three pathways.

Rhodococcus sp. strain FJ1117YT degrades the methylthio-*s*-triazines such as simetryn, ametryn, desmetryn, dimethametryn, and prometryn when supplied as the sole sulphur source. The biodegradation pathway of simetryn involves the formation of methylsulfinyl analogue as the first metabolite followed by methylsulfonyl

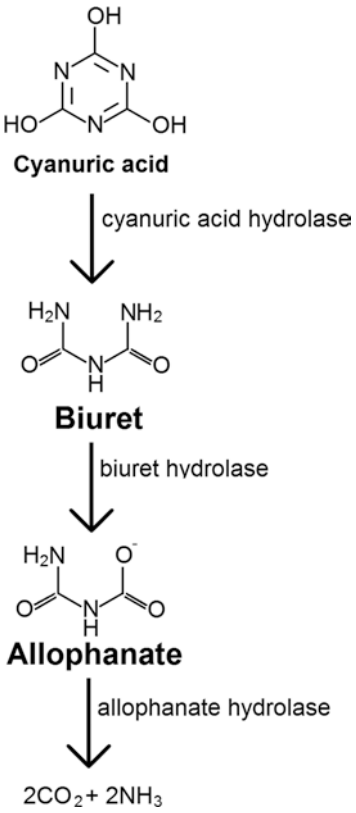

Fig. 2.3 Cyanuric acid degradative pathway showing conversion of cyanuric acid to carbon dioxide and ammonia

intermediate and the hydroxy analogues. The methylthio group of methylthio-*s*-triazines was progressively oxidised and hydrolysed (Fujii et al. 2007). Simazine degradation occurs via two pathways yielding either 2-hydroxysimazine or desethylsimazine. Organic or inorganic nitrogen sources stimulated N5C cell growth, but had little effect on the simazine degradation rate. Some of the bacteria use prometryn or ametryn as the sole source of sulphur for growth (Cook and Hütter 1982). Methylthio-*s*-triazines were transformed to their hydroxy compounds by whole cells and cell extracts of *Nocardioides* sp. strain C190 (Topp et al. 2000) and *Clavibacter michiganensis* strain ATZ1 (Seffernick et al. 2000). Recombinant TrzN from *Arthrobacter aurescens* strain TC1 rapidly hydrolyses ametryn and methylsufinyl ametryn to hydroxyametryn (Shapir et al. 2005a).

S-Triazine-Degrading Proteins

AtzA is a homohexamer of the amidohydrolase superfamily which contains one essential Fe^{2+} per monomer. AtzA hexamer is a trimer of dimers with a molecular weight of 315 kDa. AtzA gene has been proposed to be evolved from the TriA gene

(from *Pseudomonas* sp. strain NRRL B-12227) with only nine amino acid substitutions in response to atrazine induction. Despite of 98% sequence similarity, they are functionally different. AtzA is a dechlorinase with no deaminase activity, while TriA is a deaminase with low dechlorinase activity. TrzN is a zinc-dependent amidohydrolase which is ~25% identical to atzA (Mulbry et al. 2002). TrzN is a dimer containing a single Zn^{2+} bound in each active site. Both AtzB and AtzC (N-isopropylammelide aminohydrolase) have a zinc metal centre in the active site. Shapir et al. (2002) reported that molecular weight of AtzC holoenzyme is 174,000 and has a subunit size of 44,938 kDa. The activity of metal-depleted AtzC can be restored with Zn(II), Fe(II), Co(II), Mn(II), and Ni(II) salts. AtzD enzyme is a member of a family of ring-opening amidases. Apart from those enzymes discussed earlier, some other enzymes are reported to involve in the mineralisation of atrazine in few organisms. These include *Rhodococcus* sp. N186/21 cytochrome P450 (Nagy et al. 1995). Smith et al. (2005) reported that *Nocardia* converted hydroxyatrazine to N-ethylammelide via an unidentified gene product.

S-Triazine-Degrading Genes

Triazine-degrading genes may be located on large plasmids or on the bacterial chromosome (Devers et al. 2007). The atzABCDEF gene composition was found only in few bacterial strains including *Pseudomonas* sp. ADP and *Agrobacterium* sp. NEA-D and is located on a unique plasmid of 110 kb for ADP (pADP1 plasmid) and 137 kb for NEA-D. Atrazine mineralisation was well studied using *Pseudomonas* sp. ADP. AtzABC genes are dispersed in an unstable region and flanked by insertion elements with high homology to the known transposable DNA elements, IS1071 and IS801. The rearrangements result in the stochastic loss of one, two, or all three atz genes. In the absence of atrazine selection pressure, atzB can be easily lost as in *Aminobacter ciceronei* strain C147 formerly *Pseudaminobacter* sp. (Topp et al. 2000). The genes encoding atzDEF are clustered in the atzDEF operon, which is located in a stable region of pADP-1. Adaptation of soil microflora to atrazine degradation or mineralization may rely on horizontal gene transfer and repeated exposure. Atrazine mineralization greatly depends on regulatory phenomena in response to nitrogen limitation and transcriptional activation by LysR-transcriptional regulators. Devers et al. (2007) reported the presence of TrzN gene in Gram-negative bacteria such as *Sinorhizobium* sp. and *Polaromonas* sp.

Recombinants and Formulations

Genetically engineered microorganisms overexpressing catabolic genes considerably amplify the degradation in heavily atrazine-contaminated soils. Strong et al. (2000) employed transgenic AtzA-expressing *E. coli* to remove residual atrazine contamination in situ of soil contaminated with 29 g L^{-1} atrazine. Benson et al. (2018) observed superior biodegradation of atrazine by recombinant *E. coli*-expressing atrazine

chlorohydrolase encapsulated in organically modified silica gel. They reported that atrazine biodegradation is highly dependent on the adsorption.

2.2.2 *Biodegradation of* S-*Triazine Herbicides by Fungus*

Several soil fungi including *Aspergillus fumigatus*, *A. flavipes*, *A. ustus*, *Fusarium oxysporum*, *F. roseum*, *F. moniliforme*, *Rhizopus stolonifer*, *Penicillium decumbens*, *P. luteum*, *P. janthinellum*, *P. rugulosum*, and *Trichoderma viride* are reported to degrade atrazine by N-dealkylation of either alkylamino groups. They were unable to cleave the triazine ring. Although dealkylation is a pathway in majority of the fungal strains, formation of hydroxyatrazine was also observed in few fungal species such as *P. luteum* (Kaufman and Blake 1970). Other atrazine-degrading fungal strains include white rot fungus *Phanerochaete chrysosporium* (Mougin et al. 1994) and *Pleurotus pulmonarius* (Masaphy et al. 1993). Donnelly et al. (1993) studied the atrazine degradation efficiency of *Hymenoscyphus ericae*, *Oidiodendron griseum*, *Trappea darkeri*, and *Rhizopogon vinicolor* and reported that with increase in nitrogen concentration results in increased herbicide degradation. *Penicillium steckii* DS6F is the first simazine-degrading fungus ever reported (Kodama et al. 2001). Szewczyk et al. (2018) reported the degradation of the ametryn by entomopathogenic fungi. *Metarhizium brunneum* leads to formation of 2-hydroxy atrazine, ethyl hydroxylated ametryn, S-demethylated ametryn, and deethylametryn.

2.2.3 *Biodegradation of* S-*Triazine Herbicides by Plants*

In plants, three metabolic pathways are involved in atrazine transformation. The major pathway of atrazine detoxification in some resistant weeds is glutathione conjugation in which the glutathione *S*-transferase displaces chlorine atom at 2-carbon atom of atrazine (Lamoureux et al. 1970). The second mechanism is hydrolysis where the chlorine atom in atrazine is replaced with a hydroxyl group. Resistance of corn to atrazine and simazine was primarily attributed to 2- hydroxylation pathway (Hamilton and Moreland 1962). The third pathway is N-dealkylation, in which cytochrome P450 monooxygenases remove the ethylamino and isopropyl amino side chains. In pea and resistant sorghum, only the N-dealkylation pathway was performed in which atrazine is degraded to desethylatrazine and desisopropylatrazine. The first instance of atrazine uptake and degradation by aboveground plant biomass was shown in poplar trees (Burken and Schnoor 1997). In poplar trees, corn (*Zea mays* L.), and sorghum (*Sorghum vulgare* Pers.), atrazine metabolism occurs via 2-hydroxylation and N-dealkylation pathways (Shimabukuro 1967). Plant root exudates influence the atrazine degradation through the enhancement of microbial activity. Atrazine-contaminated soils planted with *Pennisetum clandestinum* showed faster atrazine degradation than in unplanted soil (Singh et al. 2004b). Rhizosphere

soils from *Kochia scoparia* and maize plants showed to accelerate mineralization of atrazine (Perkovich et al. 1996; Piutti et al. 2002). Wang et al. (2012) used a hydroponic system to evaluate the potential of three emergent hydrophytes, *Iris pseudacorus*, *Lythrum salicaria*, *and Acorus calamus* for atrazine removal and uptake. Schmidt et al. (2008) studied the biconversion of [^{14}C] atrazine to hydroxyatrazine and dealkylated products (de-ethyl-, deisopropyl- and de-ethyl-deisopropylatrazine) in heteroirophic cell-suspension cultures of soyabean (*Giycine max* L. Merr), carrot (*Daucus caroia*), purple foxglove (*Digitalis purpurea*), corn cockle (*Agrostemma githago*), wheat (*Tritician aestivum*), and thorn-apple (*Datura stramonium*).

2.2.4 Abiotic Degradation of S*-Triazine Herbicides*

Several physicochemical methods are proposed for cleaning of atrazine from contaminated soils, water, and wastewater. These techniques include incineration, reverse osmosis, electrodialysis, thermal absorption, ultraviolet, peroxides, and metal oxides. Various adsorbents including hypercrosslinked polymers (Streat and Horner 2000), zeolites, and organoclays (Bottero et al. 1994) have been studied for the removal of atrazine. Chemical methods used for atrazine degradation are photolysis, hydrolysis, dehalogenation, and oxygenation. Chemical hydrolysis of atrazine produces hydroxyatrazine in strongly acidic or basic solutions. These technologies are expensive and also release toxic by-products, which require further treatments. Atrazine degradation is negligible by sunlight, i.e. direct photolysis and result in the formation of hydroxyatrazine and dealkylated products of hydroxyatrazine. Photosensitisers such as dissolved organic carbon and nitrate absorb and transfer light energy (indirect photolysis) to catalyse the degradation of atrazine to form cyanuric acid (Cessna 2008). Corrosive and toxic gases are formed during the incineration process according to the component of the pesticide incinerated. For example, pesticides containing chlorine can produce hydrochloric acid, and nitrogen-containing pesticides can produce nitrogen oxide and nitrogen dioxide during incineration. All the above gases are acidic and corrosive. These toxic exhaust gases are to be treated before letting it out to the environment.

2.3 Bioremediation

Bioremediation refers to the process of detoxifying the contaminated environments using microorganisms, plants, or their enzymes. This includes partial or complete transformation (mineralisation) of the pollutant via biodegradation process. Bioremediation is carried out by adding an enriched microbial culture capable of degrading the pollutant or by stimulating the native xenobiotic degrading bacteria. The major advantages of bioremediation process are that it is environment friendly and cost-effective. Benoit et al. (1998) reported the immobilisation of atrazine by

fungal biomass in soils enriched with lignocellulosic materials where the density of fungal mycelia may be high. Immobilisation of microbial cells on solid porous structures is used for bioremediation of triazine pesticides in water (Yu et al. 2019).

2.3.1 Phytoremediation

Phytoremediation has been suggested as an alternative bioremediation technique to the microbial degradation of pesticide-contaminated sites. Phytoremediation involves the use of vegetation for the in situ treatment of contaminated soil. Though phytoremediation may take longer period for cleaning up the contaminated sites, it is extremely useful for the sites with higher pesticide concentration that will inhibit the microbial growth and activity. Phytoremediation helps in enhancing the organic carbon in soil which helps in microbial growth. Phytoremediation occurs via four mechanisms: (i) direct uptake and accumulation of pesticides and subsequent metabolism in plant tissues are efficient mechanism of pesticide removal, (ii) transpiration of volatile organic hydrocarbons through the leaves, (iii) release of exudates that stimulates microbial activity and biochemical transformations in the soil, and (iv) enhancement of mineralization at the root-soil interface by microorganisms (Schnoor et al. 1995). Phytoremediation can be a cost-effective and eco-friendly way of atrazine degradation. Pesticide-tolerant and nontarget plants can uptake and transform the pesticides to lesser toxic metabolites. Kawahigashi et al. (2006) proposed phytoremediation of atrazine using transgenic rice plants expressing human cytochrome P450 genes CYP1A1, CYP2B6, and CYP2C19. Sanchez et al. (2019) indicated that the atrazine removal from soils was improved by the electric field coupled to phytoremediation.

2.3.2 Biostimulation and Bioaugmentation

Biostimulation is the method of adding appropriate and limiting nutrient amendments to soils to enhance the rapid growth of indigenous bacteria, thereby increasing atrazine degradation rate (Getenga 2003; Qiu et al. 2009). Essential nutrients in limiting quantities usually control the growth of native microbial population. The atrazine removal varies significantly depending on the concentration of atrazine, stimulant type, pH of medium, and inoculation time (Dehghani et al. 2019). Biostimulation will not be effective when the bioavailability of the pesticide is low. Bioaugmentation is proposed for rapid and cost-effective cleaning of atrazine-contaminated sites (Zhao et al. 2019). The addition of layered double hydroxide bionanocomposites (Alekseeva et al. 2011) and carbon nanotubes (Zhang et al. 2015) has been reported to enhance the atrazine biodegradation rate. Bioaugmentation is not that much successful in field trials due to the poor environmental adaptability of the degraders, reduced bioavailability of atrazine, readily available carbon and

nitrogen sources, low utilization of additive substrates, and other complex environmental condition that affects the growth and metabolic activity of the atrazine degraders. The addition of poultry manure increased atrazine removal two-fold as compared to that of control (Gupta and Baummer 1996). Lin et al. (2018) studied the role of earthworm in microbial degradation of atrazine. Earthworms accelerated atrazine degradation by consuming soil humus, neutralizing soil pH, altering bacterial community structure, excreting the intestinal atrazine-degrading bacteria, and enriching indigenous atrazine degraders. Biostimulation and bioaugmentation helps to reduce the atrazine concentrations significantly in heavily contaminated soils.

2.3.3 Enzymatic Bioremediation

Enzymatic bioremediation will be a futuristic approach in resolving the pesticide-contaminated sites especially when the usage of genetically modified or engineered microorganisms is restricted by government regulations. Enzymes help to overcome the most disadvantages pertaining to the use of microbes and plants. Atrazine-degrading enzymes perform well in soil having high nitrogen content which supress the atrazine degradation pathway system in the microbial cells. Enzymes can reach the soil pores which are inaccessible to microbes and will be active in the presence of microbial predators or antagonists. The enzymes are highly selective in degrading the pollutants when the microorganisms prefer the more easily available carbon and nitrogen sources (Rajendran et al. 2018). *Aspergillus* laccase immobilised on biosorbents prepared with peanut shell and wheat straw has a strong potential for the effective removal of pesticides including atrazine and prometryn from water and soil by biosorption coupled with degradation (Chen et al. 2019). Enzymatic bioremediation also suffers from some drawbacks. The free enzymes may be degraded rapidly by the proteases released by the native soil microorganisms. Some enzymes require cofactors which have to be applied along with the enzymes. Further higher purity of the enzymes is much costlier compared to the use of microorganisms. They require optimal environmental conditions for maximum activity. Enzymes may reduce or lose their activity upon pesticide transformation and require repeated applications. Enzyme immobilisation offers long-term stability and can be reused or recovered. Enzymes can be immobilised on natural or synthetic supports through various immobilisation mechanisms. Immobilised enzymes have been reported to have higher stability and activity than the free enzymes.

2.3.4 Factors Affecting Atrazine Biodegradation

Environmental and soil conditions such as temperature, soil pH, structure, type, moisture content, nutrient availability, cation exchange capacity, fertility, organic matter, oxygen, and bioavailability of *s*-triazine pesticide greatly vary and affect the

biodegradation process. Atrazine was found to adsorb to humic acids and clays and to the various interrelated physical and chemical mechanisms of soil (Moreau-Kervévan and Mouvet 1998). Nitrogen compounds have been shown to have negative effect on atrazine degradation by numerous bacterial strains tested in pure cultures and in soil (Alvey and Crowley 1995; Entry et al. 1993; Garces et al. 2007). However, *Agrobacterium radiobacter* J14a (Bichat et al. 1999) and *Arthrobacter* sp. SC-JAK2 (Rajendran et al. 2018, 2019) are not influenced by the simultaneous presence of ammonium, nitrate, and urea in the growth medium. Atrazine-degrading enzymes are inducible in resting cells, if cells are acclimated in media containing growth-limiting nitrogen source, atrazine, or a pathway metabolite. However, their presence in media containing other nitrogen sources did not stimulate the atrazine degradation indicating that these microorganisms prefer the other nitrogen sources for their growth and metabolism. Low atrazine biodegradation is mainly attributed to its low water solubility and migration to soil pores inaccessible to microorganisms. Although addition of surfactants enhances their solubility, they inhibit the microbial activity. Atrazine mineralization rate increases with the increase of water content up to 40% of field capacity. Mineralization was proportional to the organic matter content of the soils and oxygen content. Atrazine mineralization was found to be much slower under denitrifying conditions (Nair and Schnoor 1994). In spite of the presence of significant populations of native atrazine-degrading microorganisms, their ability to significantly degrade the atrazine under complex environmental conditions appears to be limited.

2.4 Nanotechnology in Removal of *S*-Triazine Pesticides

Nanoscale materials are of significant research interest over the past several years because of their improved properties when compared to their bulk form. Nanomaterials including silver, titanium dioxide, and zinc oxide were used as photocatalysts for the heterogeneous degradation of pesticides. Zero-valent metals have been extensively researched for their usage in environmental remediation due to the strong reductive activity. Iron-based nanomaterials have obtained considerable attention in environmental remediation due to their high specific surface area, superparamagnetism, non-toxic and economic characteristics, and abundance. There is a concern on the usage of most nanomaterials intended for environmental application due to their toxicological effects on different biological systems. At present, only iron nanoparticles are considered to be safe for the environmental usage and bioremediation purpose. Some of the nanoparticles developed for *s*-triazine pesticide degradation or removal are presented in Table 2.3.

Table 2.3 Nanomaterials used for *s*-triazine pesticide degradation or removal

S-triazine herbicide	Nanoparticle/nanocomposite	Process	References
Ametryn	Iron – functionalised with 1-butyl-3-methylimidazolidium bromide	Adsorption	Ali et al. (2016)
	Er^{3+}:$Y_3Al_5O_{12}$/Pt-(TiO_2-Ta_2O_5)	Sonocatalysis	Li et al. (2017)
Atrazine	*Penicillium* sp. doped with nano Fe_3O_4 in polyvinyl alcohol-sodium alginate gel beads	Biodegradation	Yu et al. (2018)
	Alginate-stabilised silver nanoparticle	Adsorption	Pal et al. (2015)
	Zinc oxide	Ozonation	Yuan et al. (2017)
	Zero-valent copper	Hydroxyl radical-induced degradation	Hong et al. (2017)
	Pd, PdO, and Ag-Pd on hierarchical carbon structures	Degradation	Vijwani et al. (2018)
Simazine	Au–TiO_2	Sonophotocatalysis	Sathishkumar et al. (2014)
	Diatomite-supported Zero-valent iron	Degradation	Sun et al. (2013)
	Al_2O_3 and Fe_2O_3	Sorption	Addorisio et al. (2011)
Propazine	Titanium dioxide	Photocatalysis	Konstantinou et al. (2001)
Cyanazine	Titanium dioxide	Photocatalysis	Konstantinou et al. (2001)
Prometryne	Titanium dioxide	Photocatalysis	Konstantinou et al. (2001)

2.5 Isolation of *S*-Triazine-Degrading Microorganisms

Several triazine-degrading bacteria were isolated from agricultural and other contaminated sites in various geographical regions. Minimal salt media containing varying concentrations of *s*-triazine herbicides as the sole source of carbon and/or nitrogen were used to enrich and isolate the efficient degraders. Enrichment can be carried out by transferring the initial culture to fresh media containing herbicide every week (up to 5–6 weeks). When the herbicide is first applied, a lag period is observed before degradation proceeds. On subsequent exposures, immediate rapid degradation of atrazine takes place. The addition of carbon source enhances the microbial growth which in turn stimulate the biodegradation process. Nitrogen amendment to the media inhibits the atrazine degradation in many bacterial strains (Entry et al. 1993; Garces et al. 2007). Repeated exposure to atrazine increases the degradation efficiency of the microorganism. After enrichment, potential atrazine-degrading isolates can be identified by clearance zone around the colony on minimal media agar plates containing triazine pesticide. Very limited reports are available on anaerobic degradation of triazines compared to aerobic condition, perhaps due to

the difficulty in working with anaerobic cultures and slow growth of anaerobes. Under anaerobic conditions, soil samples can be enriched using either sulphate (20 mM of sodium sulphate) or nitrate (20 mM of potassium nitrate) as electron acceptors.

2.6 Analysis of *S*-Triazine Herbicide Degradation

In the early 1970s, triazine herbicides were analysed spectrophotometrically in the visible and UV regions and by paper- and thin-layer chromatography. Until the last decade, radiolabeled atrazine was used to study the uptake and detoxification of atrazine followed by quantification with thin-layer chromatography or high-performance liquid chromatography (HPLC). Advanced chromatographic approaches such as GC and HPLC have been developed for the detection of triazine herbicides. Abbas et al. (2015) reviewed the application of gas chromatography and high-performance liquid chromatography for analysis of triazine herbicide residues in various samples. Li et al. (2008) extracted atrazine with dichloromethane from soil and liquid media and analysed with gas chromatography system equipped with a flame ionization detector. Alkali flame detector and electrolytic conductivity detector were used together with a flame photometric detector which is specific for methylthiotriazines and microcoulometric detector specific for chlorotriazines for analysis of specific triazine herbicides. ^{63}Ni electron capture detector was used to analyse halogenated compounds including atrazine.

HPLC is often the method of choice after extraction process. The major advantage of *s*-triazine analysis with HPLC is that it does not require chemical derivatization normally required for gas chromatography analysis. HPLC also provides accurate analysis even in the presence of interfering compounds with GC such as n-alkanes which do not absorb UV light at the wavelength chosen for triazine quantification. Pacáková et al. (1988) separated 18 *s*-triazine derivatives using reversed-phase C18 columns with both UV and amperometric detection by HPLC. UV detection was good for detection of all triazines, while amperometric is useful for hydroxyl derivatives of triazine compounds. Further confirmation can be done with gas chromatography- or liquid chromatography- mass spectrometry in conjunction with thermospray coupling using either a high-resolution or a quadrupole mass spectrometer. There are several studies of HPLC being used as the preferred technique for triazine analysis.

2.7 Conclusion

Farmers have concern over the ban of atrazine. Atrazine ban will have substantial financial impacts on farmer as well as nation economy. An estimated 2 billion dollars and as much as 343 million dollar were estimated to be the revenue loss

annually by corn and sugarcane industries, respectively, in the USA due to elimination of atrazine (US-EPA 2006). Furthermore, there will be much more loss when all the atrazine-dependent crops are included. Postemergence application of other herbicides involves several risk including (i) crop injury as it is applied directly to the emerged crop and weeds, (ii) greater competition between crop and weeds until herbicide application, and (iii) fewer or lack of emergency remedies for weed control if the application of herbicide is missed due to the bad weather or other factors. Physical and chemical methods have not been effective in detoxifying the herbicides under field conditions. Biological methods are the most practical. Research on phytoremediation for *s*-triazine-contaminated soil is limited. Microorganisms have inherent ability to degrade triazine pesticides by utilising them as carbon and nitrogen source. Several reports concluded that excellent atrazine degraders in laboratory media fail to do that in the complex natural environmental conditions that are suboptimal for growth or repress the synthesis of enzymes involved in the degradation pathway. In these cases, enzymatic bioremediation is the excellent solution available now. Since these herbicides are often used in combination with other pesticides, the remediation approaches must able to cope up and degrade or remove these multi-pesticides. The limiting factor in atrazine biodegradation is the lack of efficient atrazine-mineralising microorganisms that can cleave the triazine ring. Much research has to be focussed on biostimulation, bioaugmentaion, and developing recombinant strains to cope up these conditions. With the help of advanced molecular and physiological approaches, such as fluorescent in situ hybridization, denaturing- and temperature-gradient gel electrophoresis and phospholipid fatty acid analysis, and community-level physiological profiling, it is possible to monitor the bioremediation and microbial community development in the atrazine-contaminated soil. The degradative potential of atrazine compromised sites can be established using the primers for the atzABC enzymes.

Acknowledgements Dr. Kumar extend heartfelt thanks to Prof. Alisa S. Vangnai, Prof. Jittra Piapukiew, and Graduate School, Chulalongkorn University, Bangkok, for their support. Authors thank the authorities of Alagappa University for sponsoring Postdoctoral fellowship under RUSA 2.0. The authors are thankful for the RUSA Scheme Phase 2.0 grant [F-24-51/2014–U, Policy (TNMulti-Gen), Department of Education, Govt. of India. Dt.09.10.2018].

References

Abbas HH, Elbashir AA, Aboul-Enein HY (2015) Chromatographic methods for analysis of triazine herbicides. Crit Rev Anal Chem 45(3):226–240. https://doi.org/10.1080/10408347.2014.927731

Addorisio V, Pirozzi D, Esposito S, Sannino F (2011) Decontamination of waters polluted with simazine by sorption on mesoporous metal oxides. J Hazard Mater 196:242–247. https://doi.org/10.1016/j.jhazmat.2011.09.022

Alekseeva T, Prevot V, Sancelme M, Forano C, Besse-Hoggan P (2011) Enhancing atrazine biodegradation by *Pseudomonas* sp. strain ADP adsorption to Layered Double Hydroxide bionanocomposites. J Hazard Mater 191(1–3):126–135. https://doi.org/10.1016/j.jhazmat.2011.04.050

Ali I, Al-Othman ZA, Alwarthan A (2016) Green synthesis of functionalized iron nano particles and molecular liquid phase adsorption of ametryn from water. J Mol Liq 221:1168–1174. https://doi.org/10.1016/j.molliq.2016.06.089

Alvey S, Crowley DE (1995) Influence of organic amendments on biodegradation of atrazine as a nitrogen-source. J Environ Qual 24(6):1156–1162. https://doi.org/10.2134/jeq1995.00472425002400060015x

Behki R, Topp E, Dick W, Germon P (1993) Metabolism of the herbicide atrazine by *Rhodococcus* strains. Appl Environ Microbiol 59(6):1955–1959

Benoit P, Barriuso E, Calvet R (1998) Biosorption characterization of herbicides, 2,4-D and atrazine, and two chlorophenols on fungal mycelium. Chemosphere 37(7):1271–1282. https://doi.org/10.1016/S0045-6535(98)00125-8

Benson JJ, Sakkos JK, Radian A, Wackett LP, Aksan A (2018) Enhanced biodegradation of atrazine by bacteria encapsulated in organically modified silica gels. J Colloid Interface Sci 510:57–68. https://doi.org/10.1016/j.jcis.2017.09.044

Bichat F, Sims GK, Mulvaney RL (1999) Microbial utilization of heterocyclic nitrogen from atrazine. Soil Sci Soc Am J 63(1):100–110. https://doi.org/10.2136/sssaj1999.03615995006300010016x

Billet L, Devers M, Rouard N, Martin-Laurent F, Spor A (2019) Labour sharing promotes coexistence in atrazine degrading bacterial communities. Sci Rep 9:18363. https://doi.org/10.1038/s41598-019-54978-2

Bottero JY, Khatib K, Thomas F, Jucker K, Bersillon JL, Mallevialle J (1994) Adsorption of atrazine onto zeolites and organoclays, in the presence of background organics. Water Res 28(2):483–490. https://doi.org/10.1016/0043-1354(94)90286-0

Bouquard C, Ouazzani J, Prome JC, MichelBriand Y, Plesiat P (1997) Dechlorination of atrazine by a Rhizobium sp. isolate. Appl Environ Microb 63(3):862–866

BoundyMills KL, deSouza ML, Mandelbaum RT, Wackett LP, Sadowsky MJ (1997) The atzB gene of *Pseudomonas* sp strain ADP encodes the second enzyme of a novel atrazine degradation pathway. Appl Environ Microbiol 63(3):916–923

Burken JG, Schnoor JL (1997) Uptake and metabolism of atrazine by poplar trees. Environ Sci Technol 31(5):1399–1406. https://doi.org/10.1021/es960629v

Cessna A (2008) Nonbiological degradation of triazine herbicides: photolysis and hydrolysis. In: LeBaron H, McFarland J, Burnside O (eds) The triazine herbicides. Elsevier, San Diego, pp 329–353

Chen XC, Zhou QZ, Liu FM, Peng QR, Teng PP (2019) Removal of nine pesticide residues from water and soil by biosorption coupled with degradation on biosorbent immobilized laccase. Chemosphere 233:49–56. https://doi.org/10.1016/j.chemosphere.2019.05.144

Cook AM, Hütter R (1982) Ametryne and prometryne as sulfur sources for bacteria. Appl Environ Microbiol 43(4):781–786

Dehghani M, Nozari M, Samaei MR, Shiri MA (2019) Biostimulation and bioaugmentation for the enhanced atrazine degradation in semi-saline medium. Desalin Water Treat 142:364–370. https://doi.org/10.5004/dwt.2019.23498

deSouza ML, Sadowsky MJ, Wackett LP (1996) Atrazine chlorohydrolase from *Pseudomonas* sp strain ADP: gene sequence, enzyme purification, and protein characterization. J Bacteriol 178(16):4894–4900. https://doi.org/10.1128/jb.178.16.4894-4900.1996

De Souza ML et al (1998) Molecular basis of a bacterial consortium: interspecies catabolism of atrazine. Appl Environ Microb 64(1):178–184

Devers M, Soulas G, Martin-Laurent F (2004) Real-time reverse transcription PCR analysis of expression of atrazine catabolism genes in two bacterial strains isolated from soil. J Microbiol Methods 56(1):3–15. https://doi.org/10.1016/j.mimet.2003.08.015

Devers M, El Azhari N, Kolic NU, Martin-Laurent F (2007) Detection and organization of atrazine-degrading genetic potential of seventeen bacterial isolates belonging to divergent taxa indicate a recent common origin of their catabolic functions. FEMS Microbiol Lett 273(1):78–86. https://doi.org/10.1111/j.1574-6968.2007.00792.x

Donnelly PK, Entry JA, Crawford DL (1993) Degradation of atrazine and 2,4-Dichlorophenoxyacetic acid by mycorrhizal fungi at 3 nitrogen concentrations in-vitro. Appl Environ Microbiol 59(8):2642–2647

Entry JA, Mattson KG, Emmingham WH (1993) The influence of nitrogen on atrazine and 2,4-dichlorophenoxyacetic acid mineralization in grassland soils. Biol Fertil Soils 16(3):179–182. https://doi.org/10.1007/Bf00361404

Feakin S, Blackburn E, Burns R (1995) Inoculation of granular activated carbon in a fixed bed with *S*-triazine-degrading bacteria as a water treatment process. Water Res 29(3):819–825. https://doi.org/10.1016/0043-1354(94)00209-P

Fruchey I, Shapir N, Sadowsky MJ, Wackett LP (2003) On the origins of cyanuric acid hydrolase: purification, substrates, and prevalence of AtzD from *Pseudomonas* sp. strain ADP. Appl Environ Microbiol 69(6):3653–3657. https://doi.org/10.1128/Aem.69.6.3653-3657.2003

Fujii K, Takagi K, Hiradate S, Iwasaki A, Harada N (2007) Biodegradation of methylthio-*s*-triazines by *Rhodococcus* sp. strain FJ1117YT, and production of the corresponding methylsulfinyl, methylsulfonyl and hydroxy analogues. Pest Manag Sci 63(3):254–260. https://doi.org/10.1002/ps.1331

Garces RA, Hansen AM, van Afferden M (2007) Mineralization of atrazine in agricultural soil: inhibition by nitrogen. Environ Toxicol Chem 26(5):844–850. https://doi.org/10.1897/06-328r.1

Getenga ZM (2003) Enhanced mineralization of atrazine in compost-amended soil in laboratory studies. Bull Environ Contam Toxicol 71(5):933–941. https://doi.org/10.1007/s00128-003-8952-4

Govantes F, Porrua O, Garcia-Gonzalez V, Santero E (2009) Atrazine biodegradation in the lab and in the field: enzymatic activities and gene regulation. Microb Biotechnol 2(2):178–185. https://doi.org/10.1111/j.1751-7915.2008.00073.x

Grossenbacher H (1986) Biodegradation von s-Triazin-Herbizid-Derivaten. PhD thesis, ETH Nr. 8156, Zürich

Gupta G, Baummer JI (1996) Biodegradation of atrazine in soil using poultry litter. J Hazard Mater 45(2-3):185–192. https://doi.org/10.1016/0304-3894(95)00090-9

Hamilton RH, Moreland DE (1962) Simazine: degradation by corn seedlings. Science 135(3501):373–374. https://doi.org/10.1126/science.135.3501.373

Hernandez M et al (2008) Isolation and characterization of a novel simazine-degrading bacterium from agricultural soil of Central Chile, *Pseudomonas* sp. MHP41. FEMS Microbiol Lett 286(2):184–190. https://doi.org/10.1111/j.1574-6968.2008.01274.x

Hong R, Guo Z, Gao J, Gu C (2017) Rapid degradation of atrazine by hydroxyl radical induced from montmorillonite templated subnano-sized zero-valent copper. Chemosphere 180:335–342. https://doi.org/10.1016/j.chemosphere.2017.04.025

IARC (1999) Monographs of the evaluation of carcinogenic risks to humans – Atrazine, vol 73. International Agency for Research on Cancer, Lyon, pp 59–113

Karns JS (1999) Gene sequence and properties of an s-triazine ring-cleavage enzyme from *Pseudomonas* sp strain NRRLB-12227. Appl Environ Microbiol 65(8):3512–3517

Kaufman DD, Blake J (1970) Degradation of atrazine by soil fungi. Soil Biol Biochem 2(2):73–80. https://doi.org/10.1016/0038-0717(70)90010-6

Kawahigashi H, Hirose S, Ohkawa H, Ohkawa Y (2006) Phytoremediation of the herbicides atrazine and metolachlor by transgenic rice plants expressing human CYP1A1, CYP2B6, and CYP2C19. J Agric Food Chem 54(8):2985–2991. https://doi.org/10.1021/jf052610u

Kodama T, Ding LX, Yoshida M, Yajima M (2001) Biodegradation of an *s*-triazine herbicide, simazine. J Mol Catal B-Enzym 11(4-6):1073–1078. https://doi.org/10.1016/S1381-1177(00)00169-7

Kolic NU et al (2007) Combined metabolic activity within an atrazine-mineralizing community enriched from agrochemical factory soil. Int Biodeterior Biodegradation 60(4):299–307. https://doi.org/10.1016/j.ibiod.2007.05.004

Konstantinou IK, Sakellarides TM, Sakkas VA, Albanis TA (2001) Photocatalytic degradation of selected s-triazine herbicides and organophosphorus insecticides over aqueous TiO_2 suspensions. Environ Sci Technol 35(2):398–405. https://doi.org/10.1021/es001271c

Lamoureux GL, Shimabukuro RH, Swanson HR, Frear DS (1970) Metabolism of 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (atrazine) in excised sorghum leaf sections. J Agric Food Chem 18(1):81–86. https://doi.org/10.1021/jf60167a029

Li QY, Li Y, Zhu XK, Cai BL (2008) Isolation and characterization of atrazine-degrading *Arthrobacter* sp AD26 and use of this strain in bioremediation of contaminated soil. J Environ Sci-China 20(10):1226–1230. https://doi.org/10.1016/S1001-0742(08)62213-5

Li G et al (2017) Preparation of (5.0%) Er^{3+}:$Y_3Al_5O_{12}$/Pt-(TiO_2-Ta_2O_5) nanocatalysts and application in sonocatalytic decomposition of ametryn in aqueous solution. Ultrason Sonochem 34:763–773. https://doi.org/10.1016/j.ultsonch.2016.07.009

Liao M, Xie X (2008) Effects of combination of plant and microorganism on degradation of simazine in soil. J Environ Sci-China 20:195–198

Lin Z et al (2018) Effects of two ecological earthworm species on atrazine degradation performance and bacterial community structure in red soil. Chemosphere 196:467–475. https://doi.org/10.1016/j.chemosphere.2017.12.177

Liu J et al (2018) Enhanced degradation of prometryn and other s-triazine herbicides in pure cultures and wastewater by polyvinyl alcohol-sodium alginate immobilized *Leucobacter* sp. JW-1. Sci Total Environ 615:78–86. https://doi.org/10.1016/j.scitotenv.2017.09.208

Mandelbaum RT, Allan DL, Wackett LP (1995) Isolation and characterization of a *Pseudomonas* sp that mineralizes the s-triazine herbicide atrazine. Appl Environ Microbiol 61(4):1451–1457

Masaphy S, Levanon D, Vaya J, Henis Y (1993) Isolation and characterization of a novel atrazine metabolite produced by the fungus *Pleurotus pulmonarius*, 2-Chloro-4-Ethylamino-6-(1-Hydroxyisopropyl)Amino-1,3,5-Triazine. Appl Environ Microbiol 59(12):4342–4346

Mizrachi R (1994) Degradation of the s-triazine herbicides prometryn and simetryn by strain of *Bacillus cereus* and their possible use in soil bioremediation. PhD thesis. Hebrew University of Jerusalem

Moreau-Kervévan C, Mouvet C (1998) Adsorption and desorption of Atrazine, Deethylatrazine, and Hydroxyatrazine by soil components. J Environ Qual 27(1):46–53. https://doi.org/10.2134/jeq1998.00472425002700010008x

Moscinski J, Jayachandran K, Moorman T (1996) Mineralization of the herbicide atrazine by *Agrobacterium radiobacter*. American Society for Microbiology, Washington, DC, p 458

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*. Appl Environ Microbiol 60(2):705–708

Mulbry WW, Zhu H, Nour SM, Topp E (2002) The triazine hydrolase gene trzN from *Nocardioides* sp strain C190: cloning and construction of gene-specific primers. FEMS Microbiol Lett 206(1):75–79. https://doi.org/10.1016/S0378-1097(01)00517-1

Nagy I, Compernolle F, Ghys K, Vanderleyden J, Demot R (1995) A single cytochrome-P-450 system is involved in degradation of the herbicides Eptc (S-ethyl Dipropylthiocarbamate) and atrazine by *Rhodococcus* sp. strain Ni86/21. Appl Environ Microbiol 61(5):2056–2060

Nair D, Schnoor J (1994) Effect of soil conditions on model parameters and atrazine mineralization rates. Water Res 28(5):1199–1205. https://doi.org/10.1016/0043-1354(94)90208-9

Pacáková V, Štulík K, Příhoda M (1988) High-performance liquid chromatography of s-triazines and their degradation products using ultraviolet photometric and amperometric detection. J Chromatogr A 442:147–156. https://doi.org/10.1016/S0021-9673(00)94464-9

Pal J, Deb M, Sircar J, Agnihotri P (2015) Microwave green synthesis of biopolymer-stabilized silver nanoparticles and their adsorption behavior for atrazine. Appl Water Sci 5:181–190. https://doi.org/10.1007/s13201-014-0179-5

Perkovich BS, Anderson TA, Kruger EL, Coats JR (1996) Enhanced mineralization of [C-14]atrazine in Kochia scoparia rhizospheric soil from a pesticide-contaminated site.

Pestic Sci 46(4):391–396. https://doi.org/10.1002/(Sici)1096-9063(199604)46:4<391::Aid-Ps374>3.0.Co;2-L

Pimentel D, Levitan L (1986) Pesticides: amounts applied and amounts reaching pests. Bioscience 36(2):86–91. https://doi.org/10.2307/1310108

Piutti S, Hallet S, Rousseaux S, Philippot L, Soulas G, Martin-Laurent F (2002) Accelerated mineralisation of atrazine in maize rhizosphere soil. Biol Fertil Soils 36(6):434–441. https://doi.org/10.1007/s00374-002-0545-6

Piutti S et al (2003) Isolation and characterisation of *Nocardioides* sp SP12, an atrazine-degrading bacterial strain possessing the gene trzN from bulk- and maize rhizosphere soil. FEMS Microbiol Lett 221(1):111–117. https://doi.org/10.1016/S0378-1097(03)00168-X

Qiu YP, Pang H, Zhou ZL, Zhang P, Feng YC, Sheng GD (2009) Competitive biodegradation of dichlobenil and atrazine coexisting in soil amended with a char and citrate. Environ Pollut 157(11):2964–2969. https://doi.org/10.1016/j.envpol.2009.06.003

Radosevich M, Traina SJ, Hao YL, Tuovinen OH (1995) Degradation and mineralization of atrazine by a soil bacterial isolate. Appl Environ Microbiol 61(1):297–302

Rajendran K, Vangnai AS, Piapukiew J (2018) Recipe for enzyme production, bacterial extracellular enzyme formulation for herbicide (triazine) biodegradation and storage conditions of extracellular enzyme. Thailand Application No. 1801007046; filed 16th November 2018. Thailand Patent

Rajendran K, Vangnai AS, Piapukiew J (2019) Maize – *Arthrobacter* sp. based biological model for bioremediation of atrazine contaminated soils. In: Jeyakanthan J, Basu MJ (eds) International conference on innovative and emerging trends in botany – 2019, Alagappa University, Karaikudi, Tamil Nadu, India, 2019, vol 1. Shanlax Publications, Madurai, p 52

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 Microbiol Ecol 36(2–3):211–222. https://doi.org/10.1016/S0168-6496(01)00135-0

Rusiecki JA et al (2004) Cancer incidence among pesticide applicators exposed to atrazine in the agricultural health study. J Natl Cancer Inst 96(18):1375–1382. https://doi.org/10.1093/jnci/djh264

Sadowsky MJ, Tong ZK, de Souza M, Wackett LP (1998) AtzC is a new member of the amidohydrolase protein superfamily and is homologous to other atrazine-metabolizing enzymes. J Bacteriol 180(1):152–158

Sanchez M, Garbi C, Martinez-Alvarez R, Ortiz LT, Allende JL, Martin M (2005) *Klebsiella planticola* strain DSZ mineralizes simazine: physiological adaptations involved in the process. Appl Microbiol Biotechnol 66(5):589–596. https://doi.org/10.1007/s00253-004-1735-y

Sanchez V, Francisco L-BJ, Rodrigo MA, Rodriguez L (2019) Enhancing the removal of atrazine from soils by electrokinetic-assisted phytoremediation using ryegrass (*Lolium perenne* L.). Chemosphere 232:204–212. https://doi.org/10.1016/j.chemosphere.2019.05.216

Sathishkumar P, Mangalaraja R, Mansilla H, Gracia-Pinilla M, Anandan S (2014) Sonophotocatalytic (42 kHz) degradation of Simazine in the presence of Au–TiO_2 nanocatalysts. Appl Catal B Environ 160–161:692–700. https://doi.org/10.1016/j.apcatb.2014.06.027

Satsuma K (2010) Mineralization of s-triazine herbicides by a newly isolated *Nocardioides* species strain DN36. Appl Microbiol Biotechnol 86(5):1585–1592. https://doi.org/10.1007/s00253-010-2460-3

Schmidt B, Siever M, Thiede B, Breuer J, Malcherek K, Schuphan I (2008) Biotransformation of [ring -U-^{14}C]atrazine to dealkylated and hydroxylated metabolites in cell-suspension cultures. Weed Res 37(6):401–410. https://doi.org/10.1046/j.1365-3180.1997.d01-71.x

Schnoor JL, Licht LA, Mccutcheon SC, Wolfe NL, Carreira LH (1995) Phytoremediation of organic and nutrient contaminants. Environ Sci Technol 29(7):A318–A323. https://doi.org/10.1021/es00007a002

Seffernick JL, Johnson G, Sadowsky MJ, Wackett LP (2000) Substrate specificity of atrazine chlorohydrolase and atrazine-catabolizing bacteria. Appl Environ Microbiol 66(10):4247–4252. https://doi.org/10.1128/Aem.66.10.4247-4252.2000

Shapir N, Osborne JP, Johnson G, Sadowsky MJ, Wackett LP (2002) Purification, substrate range, and metal center of AtzC: the N-isopropylammelide aminohydrolase involved in bacterial atrazine metabolism. J Bacteriol 184(19):5376–5384. https://doi.org/10.1128/Jb.184.19.5376-5384.2002

Shapir N, Rosendahl C, Johnson G, Andreina M, Sadowsky MJ, Wackett LP (2005a) Substrate specificity and colorimetric assay for recombinant TrzN derived from *Arthrobacter aurescens* TC1. Appl Environ Microbiol 71(5):2214–2220. https://doi.org/10.1128/Aem.71.5.2214-2220.2005

Shapir N, Sadowsky MJ, Wackett LP (2005b) Purification and characterization of allophanate hydrolase (AtzF) from *Pseudomonas* sp strain ADP. J Bacteriol 187(11):3731–3738. https://doi.org/10.1128/Jb.187.11.3731-3738.2005

Shapir N, Cheng G, Sadowsky MJ, Wackett LP (2006) Purification and characterization of TrzF: biuret hydrolysis by allophanate hydrolase supports growth. Appl Environ Microbiol 72(4):2491–2495. https://doi.org/10.1128/Aem.72.4.2491-2495.2006

Shimabukuro RH (1967) Atrazine metabolism and herbicidal selectivity. Plant Physiol 42(9):1269–1276. https://doi.org/10.1104/pp.42.9.1269

Singh P, Suri CR, Cameotra SS (2004a) Isolation of a member of Acinetobacter species involved in atrazine degradation. Biochem Biophys Res Commun 317(3):697–702. https://doi.org/10.1016/j.bbrc.2004.03.112

Singh N, Megharaj M, Kookana RS, Naidu R, Sethunathan N (2004b) Atrazine and simazine degradation in Pennisetum rhizosphere. Chemosphere 56(3):257–263. https://doi.org/10.1016/j.chemosphere.2004.03.010

Smith D, Alvey S, Crowley DE (2005) Cooperative catabolic pathways within an atrazine-degrading enrichment culture isolated from soil. FEMS Microbiol Ecol 53(2):265–273. https://doi.org/10.1016/j.femsec.2004.12.011

Streat M, Horner DJ (2000) Adsorption of highly soluble herbicides from water using activated carbon and hypercrosslinked polymers. Process Saf Environ Prot 78(B5):363–382. https://doi.org/10.1205/095758200530880

Strong LC, McTavish H, Sadowsky MJ, Wackett LP (2000) Field-scale remediation of atrazine-contaminated soil using recombinant *Escherichia coli* expressing atrazine chlorohydrolase. Environ Microbiol 2(1):91–98. https://doi.org/10.1046/j.1462-2920.2000.00079.x

Sun Z, Zheng S, Ayoko GA, Frost RL, Xi Y (2013) Degradation of simazine from aqueous solutions by diatomite-supported nanosized zero-valent iron composite materials. J Hazard Mater 263(Pt 2):768–777. https://doi.org/10.1016/j.jhazmat.2013.10.045

Szewczyk R, Kusmierska A, Bernat P (2018) Ametryn removal by *Metarhizium brunneum*: biodegradation pathway proposal and metabolic background revealed. Chemosphere 190:174–183. https://doi.org/10.1016/j.chemosphere.2017.10.011

Topp E, Mulbry WM, Zhu H, Nour SM, Cuppels D (2000) Characterization of S-triazine herbicide metabolism by a *Nocardioides* sp isolated from agricultural soils. Appl Environ Microbiol 66(8):3134–3141. https://doi.org/10.1128/Aem.66.8.3134-3141.2000

US-EPA (2006) Atrazine: finalization of interim reregistration eligibility decision and completion of tolerance reassessment and reregistration eligibility process. In: EPA U (ed) United States Environmental Protection Agency, Washington, DC, p 91

Vargha M, Takats Z, Marialigeti K (2005) Degradation of atrazine in a laboratory scale model system with Danube river sediment. Water Res 39(8):1560–1568. https://doi.org/10.1016/j.watres.2004.10.013

Vijwani H, Nadagouda M, Mukhopadhyay S (2018) Robust nanocatalyst membranes for degradation of atrazine in water. J Process Eng 25:15–21. https://doi.org/10.1016/j.jwpe.2018.05.016

Wang QH, Zhang W, Li C, Xiao B (2012) Phytoremediation of atrazine by three emergent hydrophytes in a hydroponic system. Water Sci Technol 66(6):1282–1288. https://doi.org/10.2166/wst.2012.320

Yang XY, Wei HY, Zhu CX, Geng B (2018) Biodegradation of atrazine by the novel *Citricoccus* sp strain TT3. Ecotoxicol Environ Saf 147:144–150. https://doi.org/10.1016/j.ecoenv.2017.08.046

Yu J, He H, Yang WL, Yang C, Zeng G, Wu X (2018) Magnetic bionanoparticles of *Penicillium* sp. yz11-22N2 doped with Fe_3O_5 and encapsulated within PVA-SA gel beads for atrazine removal. Bioresour Technol 260:196–203. https://doi.org/10.1016/j.biortech.2018.03.103

Yu TM, Wang L, Ma F, Yang JX, Bai SS, You JY (2019) Self-immobilized biomixture with pellets of *Aspergillus niger* Y3 and *Arthrobacter*. sp ZXY-2 to remove atrazine in water: a bio-functions integration system. Sci Total Environ 689:875–882. https://doi.org/10.1016/j.scitotenv.2019.06.313

Yuan X et al (2017) Enhanced ozonation degradation of atrazine in the presence of nano-ZnO: performance, kinetics and effects. J Environ Sci (China) 61:3–13. https://doi.org/10.1016/j.jes.2017.04.037

Zhang CD, Li MZ, Xu X, Liu N (2015) Effects of carbon nanotubes on atrazine biodegradation by *Arthrobacter* sp. J Hazard Mater 287:1–6. https://doi.org/10.1016/j.jhazmat.2015.01.039

Zhao XY, Bai SW, Li CY, Yang JX, Ma F (2019) Bioaugmentation of atrazine removal in constructed wetland: performance, microbial dynamics, and environmental impacts. Bioresour Technol 289:121618. https://doi.org/10.1016/j.biortech.2019.121618