

# Bioremediation Strategies to Mitigate **21** the Impact of Atrazine on the Environment: **Recent Advances and Prospects**

Noelia Urseler, Romina Bachetti, Carolina Morgante, and Elizabeth Agostini

#### Abstract

Atrazine is an s-triazine herbicide widely used for the control of weeds, primarily in corn, sorghum and sugarcane crops. It is relatively persistent in the environment, moderately soluble in water and toxic to different organisms and humans. Its mobility through soil by leaching and runoff events frequently lead to contamination of sediments and water resources. Thus, atrazine has become a compound of public concern because it is frequently detected in surface, groundwater and rainfall samples in quantities exceeding the limit values set by regulatory agencies (the EU and the USA) for drinking water. In addition, several studies have shown its impact on the ecosystem and human health. For this reason, bioremediation strategies have been described to allow the removal of atrazine and avoid its dispersion in the environment. This chapter provides information on the behaviour and impact of atrazine in soil, aquatic ecosystems and non-target organisms and summarised current knowledge about bioremediation strategies for the clean-up of sites polluted with this herbicide. Recently, material-microbial-integrated technologies have been investigated in order to degrade atrazine, which will be also described. Finally, the bioremediation strategies are evaluated under laboratory and field conditions. Future advances

E. Agostini (🖂)

N. Urseler · R. Bachetti · C. Morgante

Instituto Multidisciplinario de Investigación y Transferencia Agro-alimentaria y Biotecnológica, IMITAB-CONICET, Instituto Académico Pedagógico de Ciencias Básicas y Aplicadas (IAPCByA), Universidad Nacional de Villa María, Villa María, Córdoba, Argentina

Departamento de Biología Molecular, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Instituto de Biotecnología Ambiental y Salud, INBIAS-CONICET, Río Cuarto, Córdoba, Argentina e-mail: eagostini@exa.unrc.edu.ar

related to atrazine degradation need to focus on an efficient removal and low environmental impact.

#### Keywords

 $A trazine \cdot Bioremediation \cdot Phytoremediation \cdot Organic \ pollutants \cdot Environment$ 

# 21.1 Introduction

Atrazine is the most widely used s-triazine herbicides to control broadleaf weeds for many crops in the world. However, the benefit of weed elimination in crops is offset by the negative impact generated by the application of herbicides on the environment and on living beings. In fact, the atrazine contamination has become a growing public concern because it is one of the most commonly detected pesticides in soil, surface water and groundwater, representing a serious risk for the environment and public health (Nguyen et al. 2014). To minimise the damage caused by this herbicide, it is necessary to find and apply processes that allow its removal from contaminated sites. A great number of technologies are developed, such as adsorpphotochemical catalysis. Among the different tion, biodegradation and methodologies proposed, bioremediation appears as a promising alternative that takes advantage of the metabolic potential of microorganisms to degrade contaminants and it can be carried out in different media such as sediments, soils, surface and groundwater, and biological sludges. In addition, both microbialassisted plant remediation and the use of immobilised microorganisms have been also proposed for atrazine degradation. In the present chapter information on the behaviour and impact of atrazine on soil and aquatic ecosystems will be discussed. Besides, the progresses of researches based on the abovementioned biological treatment technologies for atrazine removal will be reviewed and summarised as well as the future prospects of these approaches.

# 21.2 Atrazine: Main Characteristics, Behavior and Environmental Impacts

#### 21.2.1 Atrazine: Properties and Uses

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is an important *s*-triazine herbicide. Its molecular formula is  $C_8H_{14}ClN_5$ ; it is a solid crystalline powder and unstable at high temperature. Atrazine has a melting point of 175.8 °C, a water solubility of 33 mg L<sup>-1</sup> (20 °C) and is readily soluble in organic solvents (Lewis et al. 2016). Atrazine is highly persistent with a half-life of 32–128 days in soil and of 100 days in water (Krutz et al. 2008). However, their half-life is increased in subsurface environments due to the low natural microbial degradation potential (Singh and Jauhari 2017).

Atrazine has been commercially available for more than 50 years. It was registered in Switzerland in 1958 and is widely used since the early 1960s in the USA, Europe, Africa, Asia and South America to control broadleaf weeds and some grasses weeds that affect mainly on corn, sorghum and sugarcane crops (Viegas et al. 2012). Nowadays, atrazine is the second most extensively used herbicide globally with an annual consumption of about 70,000–90,000 tons (Cao et al. 2021). The United States (USA) applied about 33,560 tons every year (Gaffar et al. 2021), while China has consumed 23,000 tons (Liu et al. 2021). South America is one of the areas of the world where atrazine is massively applied in agriculture. In Argentina, about 10,000 tons of atrazine are consumed annually (Alonso et al. 2018). Atrazine can increase yields by 6–50% depending on the crop (Rajendran et al. 2021). Commercial atrazine formulations are different and depend on each country: flowable, wettable powder, water-dispersible granules and soluble concentrate (Viegas et al. 2012). The main advantages of using atrazine are the versatility of its application and facility to mix it with other herbicides, such as S-metolachlor, alachlor, paraquat and linuron for broad-spectrum weed control (Rajendran et al. 2021).

Atrazine is used as a non-selective herbicide on both fallow and non-farmland land (He et al. 2019). It acts on the target weeds by blocking electron transport in photosystem II. This blocking occurs because atrazine inhibits the plastoquinone binding site (QB) of the D1 protein in chloroplasts and consequently suppressing the electron flow between photosystems (de Albuquerque et al. 2020). Therefore, electrons are not stored as chemical energy and chlorophyll molecules are heavily loaded with energy leading to lipid peroxidation in the membranes, inhibition of carbohydrate synthesis, decrease of the carbon (C) stock and accumulation of carbon dioxide  $(CO_2)$  within plant cells and damage of leaf chlorophyll (Marchi et al. 2008). In the pre-emergence application, atrazine is first absorbed by the roots and then transported to the leaves, where its action produces chlorosis, necrosis and death. In post-emergence application, this herbicide is absorbed by the leaves (Souza et al. 2012; de Albuquerque et al. 2020). The action time of atrazine varies between 2 and 6 months, due to its stability in neutral and slightly alkaline or acid soil conditions (CASAFE, Cámara de Sanidad Agropecuaria y Fertilizantes 2013). The accumulation of atrazine in soil is prone to phytotoxicity to sensitive crops, such as soybeans, rice, oat and wheat (Chen et al. 2019).

The application of atrazine has been discussed due to its persistence and mobility in the environment, and consequently it is detected in the soil, surface water, groundwater, pastures, streams, lakes, sediments, foods and even glaciers in remote areas (Barchanska et al. 2012; Hansen et al. 2013; Sun et al. 2017; Pan et al. 2019; Wang et al. 2020). Consequently, the European Union (EU) banned its application in the year 2004. The USA and Canada have adopted restriction policies to minimise its potential environmental impact. However, it is still used extensively in agricultural practices in numerous countries, highlighting Argentina, China, Brazil and India (Sun et al. 2017; Montoya et al. 2019; de Albuquerque et al. 2020).

Massive application, mainly coinciding with rainy seasons, the high persistence and mobility are the main reasons for the atrazine detection in soil, surface water and groundwater at concentrations exceeding the limit established by normative values (Bachetti et al. 2021; Rajendran et al. 2021). The US Environmental Protection Agency (US EPA) has established the maximum atrazine concentration limit values in drinking water at 3  $\mu$ g L<sup>-1</sup> (US EPA 2007). However, the EU (EU 2004) and the World Health Organization (WHO 2011) have established the permissible limit to 0.1 and 2  $\mu$ g L<sup>-1</sup>, respectively. The persistence of atrazine on environmental compartments poses a serious threat to human health. Furthermore, the US EPA has classified atrazine in toxicity class III and as an endocrine disruptor herbicide (Morales-Pérez et al. 2016; Singh and Jauhari 2017). The International Agency for Research on Cancer (IARC) has categorised atrazine in the list of carcinogenic herbicide (Mahler et al. 2017).

#### 21.2.2 Behaviour and Impact of Atrazine on the Environment

After being applied, only 0.1% of the herbicide reaches the target organisms (weeds). The remainder can interact with the different environmental compartments through complexes biological, physical and chemical reactions. The environmental behaviour of atrazine depends upon several factors, including retention, transformation and transport processes, as well as by the interaction between them (Fig. 21.1) (Sun et al. 2019). These interactions are complex, being controlled simultaneously by biological, physical and chemical reactions. After being applied and before it reaches the soil, the herbicide may undergo photolysis, volatilisation and/or may be adsorbed or absorbed by the plant or by the stubble on the surface. Once in the soil, the xenobiotic is partitioned into solid, gas and liquid phases; in the latter, chemical and microbial degradation occurs, these processes being the most important for the dissipation of most herbicides. It is well known that the natural attenuation and fate of atrazine in soil environments are strongly related to adsorption, desorption and mineralisation processes (Liu et al. 2021).

The transformation of atrazine can be due to photochemical or biochemical processes, producing simpler molecules with different properties from that of the original compound (Mudhoo and Garg 2011). In fact, atrazine can be degraded in more than 15 metabolites, and each transformation product varies in its persistence (months to decades) and toxicity (Xue et al. 2021). The major atrazine degradation products are hydroxyatrazine (HA), deethylatrazine (DEA) and deisopropylatrazine (DIA). Due to their mobility, they have been frequently detected in many surface and groundwater of the world (Jablonowski et al. 2011; Nödler et al. 2013). Formation of HA occurs through different (biotic or abiotic) degradation mechanisms leading to the hydrolysis of atrazine molecule, and the microbial mediated process of atrazine N-dealkylation produces the mobiles metabolites DEA and DIA (Mudhoo and Garg 2011). The HA is the most important metabolite, with a higher retention in soils compared to other products (Chokejaroenrat et al. 2020).

A comprehensive understanding of the herbicide behaviour in the environmental compartments is extremely important to implement appropriate environmental management strategies to reduce its impact on human and animal health in the vulnerable areas.



Fig. 21.1 Environmental behaviour of atrazine

## 21.2.3 Soil Contamination and Effects in Terrestrial Ecosystems

Soil is an important life-supporting system and plays a critical role for primary production, the regulation of biogenic gases, biogeochemical and hydrological cycles as well as the biodiversity preservation (Sun et al. 2017). However, soil pollution has become a worldwide concern because it acts as an important reservoir for numerous organic pollutants such as herbicides (Ali et al. 2019). In the soil, atrazine is distributed between aqueous and solid phases. Two different but coexisting processes are proposed for the atrazine movement in soils. One of them, is the rapid movement of the herbicide corresponding to a preferential flow through the soil macropores and the other one is a slow transport due to the sorption and degradation processes in the soil matrix (Mudhoo and Garg 2011).

The main processes that determine the persistence of atrazine in the soil environment are the physicochemical and microbiological properties (organic matter content, pH, texture, cation exchange capacity, microbial abundance and metabolic activity) of the soil, climatic characteristics (temperature, humidity, precipitation) and other parameters (mode and rate of application, prior history use, plant cover, topography) (Hernández et al. 2008; Prado et al. 2014). Atrazine breakdown in soil occurs mainly by chemical and microbial aerobic degradation (Viegas et al. 2012). Chemical degradation can occur by hydrolysis or by photodegradation. Atrazine is stable at room temperature, in the dark, at neutral pH and in the absence of microorganisms and organic matter (Prosen and Zupančič-Kralj 2005). This herbicide atrazine can be extremely persistent in soil environment due to its ability to bind to soil colloids (organic matter and clays) and become non-extractable residues (Martins et al. 2018). Recent studies have been dedicated to evaluate the behaviour and fate of atrazine in soils (Salazar-Ledesma et al. 2018; Sun et al. 2019; Liu et al. 2021), especially the adsorption and desorption processes between this herbicide and soil which influence its mobility and availability for weed control (Martins et al. 2018; Piratoba et al. 2021). Adsorption of atrazine seems to be positively correlated with organic matter and clay content and negatively with pH (Aparicio et al. 2015; Yue et al. 2017). It is reported that soil organic matter contains a variety of functional groups such as hydrophobic, hydrophilic and free radicals that can strongly entrap atrazine (Barriuso and Houot 1996). The pH is a factor affecting the adsorption of atrazine in soils, because when the pH increases, the soil surface tends to be negatively charged and organic molecules also tend to be ionised and negatively charged (Huang et al. 2013). This generates a repulsion of same-sign charges, which will be detrimental to the adsorption of organic molecules on the soil surface (Wang et al. 1999).

The accumulation of atrazine in soil (either dissolved or bound to colloids) is considered a long-term source of the compound leading to its possible occurrence to surface or groundwater. Numerous studies also reveal an accelerated degradation of atrazine due to the prolonged exposition of emergent native microbial populations capable to utilise the herbicide as a C (carbon) or N (nitrogen) source (Jablonowski et al. 2011; Sun et al. 2017). The exposure to some pesticides may change the resources that soil microorganisms use to obtain energy and nutrients, especially in soils with low levels of organic matter (Fernandes et al. 2020). Specific microorganisms are able to detoxify atrazine by N-dealkylation or dehalogenation reactions, and this may imply the development of microbial communities that can utilise the N in the triazine ring (Cycoń et al. 2017; Esquirol et al. 2020). Atrazine microbial degradation will be explained in Sect. 21.3 of this chapter. Soil macrofauna can also directly or indirectly affect the degradation and ultimately the fate of atrazine. In point of fact, the presence of earthworms in the soil may also affect the transport of atrazine. This is due to earthworms, which are keystone organisms that can ingest and transport the atrazine residues to deeper soil layers, enhancing the formation of non-extractable residues, thus reducing the leaching potential of this herbicide (Mudhoo and Garg 2011; Viegas et al. 2012).

Several studies have reported atrazine residues in soils and sediments from all over the world. Atrazine residues (19.5 g  $ha^{-1}$ ) and their degradate products were still found in agricultural soils a long time after the last herbicide application (more than 20 years), as well as in soils with no history of atrazine application (Jablonowski et al. 2010). Sun et al. (2017) showed that atrazine concentrations ranged from 1.0 to 113 ng  $g^{-1}$  dry weight, with a frequency of detection of 57.7% in soil samples of China (n = 241) and reported a close association between contamination and land use type. In Pakistan, Ali et al. (2019) evaluated the concentrations of 30 endocrine disrupting pesticides in soil and vegetable samples. Atrazine concentrations ranged from 1.7 to 120  $\mu$ g kg<sup>-1</sup> in soil samples, while no residues were detected in the vegetables studied. Alonso et al. (2018) showed atrazine residues (4–66  $\mu$ g kg<sup>-1</sup>) in soil samples (n = 58) from the provinces of Córdoba and Buenos Aires (Argentina), an important corn producing area of Argentina. In addition, Mac Loughlin et al. (2017) detected atrazine in sediments of Carnaval creek (n = 10) (Buenos Aires, Argentina) at concentrations ranging from 5.1 to 32.7  $\mu$ g kg<sup>-1</sup>, causing lethal and sublethal effects on benthic fauna.

The most susceptible group to the deposition of atrazine residues in the soil environment are non-target crops that may receive atrazine by spray drift, accidental spills or carryover. As a consequence of atrazine exposition, target and non-target plants often undergo oxidative stress because of an enhanced reactive oxygen species (ROS) production. Beker Akbulut and Yigit (2010) determined that ROS caused negative effects on peroxidase, ascorbate peroxidase and lipid peroxidation in Z. mays plants with postemergence atrazine application. Gao et al. (2011) reported that exposure to 10  $\mu$ g L<sup>-1</sup> atrazine significantly reduces plant fresh weight and total chlorophyll concentration. The authors also revealed a high plant mortality (up to 86.7%) at 100  $\mu$ g L<sup>-1</sup> concentration. Çanakci-Gülengül and Karabulut (2020) investigated the biochemical effects of atrazine concentrations (0, 200, 500 and 1000  $\mu$ M) on wheat (*Triticum aestivum* L.) seedlings. The results indicated a decrease in reduced glutathione/oxidised glutathione (GSH:GSSG) ratio and catalase activity (CAT) in leaf and root and an increase in superoxide dismutase (SOD) activity. Gao et al. (2019) showed that seagrass Zostera marina L. exposed to high concentrations of atrazine (1, 3 and 10  $\mu$ g L<sup>-1</sup>) significantly inhibited photosynthetic efficiency and reduced shoot sugar levels.

Atrazine also affected soil invertebrates, especially earthworm, mites, nematodes and collembolans species (Singh and Jauhari 2017). Lammertyn et al. (2021) exposed earthworms (*Eisenia fetida*) to different concentrations of atrazine to evaluate possible sublethal harmful effects. The results showed that atrazine (2 mg kg<sup>-1</sup>) affected the rate of cocoon production and increased lactate dehydrogenase and, especially, acetylcholinesterase activity. On the other hand, Dani et al. (2018) showed that earthworms exposed to sublethal concentrations of atrazine (362.4, 181.2, 90.6 and 45.3 ng cm<sup>-2</sup>) caused a general suppression in their metabolism, reduced ATP synthesis and had a negative impact on general health.

### 21.2.4 Water Contamination and Effects in Aquatic Ecosystems

Atrazine properties, such as low vapour pressures, moderate water solubility and low soil adsorption coefficient ( $K_{OC} = 100 \text{ cm}^3 \text{ g}^{-1}$ ), indicate its high leaching potential, particularly in well-structured soil profiles (de Albuquerque et al. 2020). Most atrazine movement occurs in the soil aqueous phase. Therefore, precipitation contributes to its dispersion in aquatic systems near the application zone (Jablonowski et al. 2011; Bachetti et al. 2021). Another, but less common, transport mechanism is adsorption of atrazine to eroded soil particles (Alonso et al. 2018). Atrazine is a frequently detected pesticides in groundwater and surface runoff from around the world (Table 21.1). Peng et al. (2018) identified the diversity and complexity of organic pollutants at 28 sampling sites in the Yangtze River Delta, finding an atrazine concentration of 1726 ng L<sup>-1</sup>. The authors concluded that this concentration exceeded the annual average environmental quality standards of Europe. In fact, the value was 9.4 times higher than the concentration reported by Battaglin et al. (2016) in seven US states for atrazine in surface water (183 ng  $L^{-1}$ ). These authors analysed a total of 86 water samples and atrazine was one of the most frequently detected herbicide (18% of the samples). In Europe, researchers collected 314 groundwater samples from the Júcar River European Union Pilot Basin (Spain), and the study showed that atrazine was frequently detected after terbuthylazine and bromacil (Menchen et al. 2017). Fingler et al. (2017) obtained samples from different surface and groundwater resources in Croatia, finding atrazine residues in all of them in concentrations of around 68 ng  $L^{-1}$ . Almasi et al. (2020) founded atrazine concentrations from 0 to 2,175,800 ng L<sup>-1</sup> in aquifers from Iran. In South America, atrazine is a common herbicide detected in various surface water and groundwater (De Gerónimo et al. 2014; Montagner et al. 2019; de Albuquerque et al. 2020). Particularly in Argentina, Montoya et al. (2019) reported its occurrence in 26% of groundwater samples (n = 95) with concentrations between 0.3 and 16.1 ng  $L^{-1}$ . The values detected in this study were lower than those informed by Mas et al. (2020) in aquifers from Santiago del Estero (Argentina), where concentrations ranged from 1 to 7921 ng L<sup>-1</sup>. Recent studies performed in the Ctalamochita river basin (Córdoba, Argentina) showed the high ubiquity and persistence of the herbicide in surface water courses and at concentrations reaching 5000 ng L<sup>-1</sup>. The results revealed that atrazine residues in surface waters increased

				Concenti	ation (ng L	-1)	
Continent	Country	Sites	Water resource	Min	Max	Mean	Reference
Europe	Croatia	Zagreb	Surface water	8.0	18.0	I	Fingler et al. (2017)
			Lakes	5.0	6.0		
			Groundwater	5.0	61.0		
			Drinking water	5.0	68.0		
	France		Bottled waters	2.0	4.0	I	Le Coadou et al. (2017)
	France	South	Groundwater	1.0	109.0	14.0	Sassine et al. (2017)
	Greece	Epirus	Surface water	75.0	77.0	13.0	Kapsi et al. (2019)
	Portugal	South	Surface water	2.0	3.0	I	Gonzalez-Rey et al. (2015)
	Spain	Catalonia	Sea water	0.0	3.8	1.1	Köck-Schulmeyer et al. (2019)
	Spain	Mancha Oriental	Groundwater	1.1	380.0	I	Menchen et al. (2017)
America	Argentina	Córdoba	Surface water	I	5000.0	I	Bachetti et al. (2021)
	Argentina	Santiago del Estero	Groundwater	1.0	7921.0	260.0	Mas et al. (2020)
	Argentina	Pampean plain	Rainwater	500.0	6.7E <sup>4</sup>	5490.0	Alonso et al. (2018)
	Argentina	La Pampa	Groundwater	0.3	16.1		Montoya et al. (2019)
	Brazil	São Paulo	Surface water	1.0	611.0	30.0	Montagner et al. (2019)
			Tap water	1.0	687.0	36.0	
			Groundwater	2.0	5.0	3.0	
	Brazil	Rio Grande do Sul	Tap water	5.0	37.0	16.0	Caldas et al. (2019)
			Surface water	5.0	49.0	19.0	
	Brazil	São Paulo	Surface water	5.2	516.0	I	Acayaba et al. (2021)
			Groundwater	<1.7	<1.7		
	Canada	Quebec	Tap water	30.0	195.0	69.0	Montiel-León et al.
							(2019b)
							(continued)

 Table 21.1
 Atrazine residues detected in water resources around the world

Table 21.1	(continued)						
				Concent	ration (ng L	()	
Continent	Country	Sites	Water resource	Min	Max	Mean	Reference
	Canada	Trois-Riviéres	Surface water	4.0	666.0	29.2	Montiel-León et al.
							(2019a)
	Canada	Ontario	Lakes	I	754.0	I	Metcalfe et al. (2019)
	Chile	Central	Surface water	I		45	Climent et al. (2019)
	USA	Rocky Mountain Nacional Park	Surface water	1	12.0	1	Battaglin et al. (2018)
	USA	Florida	Sea and surface water	5.0	21.0	1	Fernandez and Gardinali (2016)
	USA	Tifton	Surface water	1	1650.0	1	Glinski et al. (2018)
	USA	Ohio	Tap water	0.0	$1.6 \times 10^4$	I	Almberg et al. (2018)
Asia	China		Tap water	10.0	1441.0	1.7	Wang et al. (2020)
	China	Baima River	Surface water	10.0	1120.0	1	Sun et al. (2019)
	China	Guangxi	Tap water, groundwater and surface water	70.0	585.0	I	Li et al. (2018)
	China	Jiaozhou Bay	Sea water	20.3	174.0	I	Ouyang et al. (2019)
	China	Liaodong	Surface water	21.3	1726.1	191.4	Peng et al. (2018)
	China	Liaodong	Surface water	8.7	64.8	23.3	Xie et al. (2019)
	Iran	Shadegan Wetland	Surface water	0.0	$\frac{2.2}{10^6}$ ×	I	Almasi et al. (2020)
Africa	Egypt		Groundwater	0.0	5000.0	2000.0	Masoud et al. (2018)
	South Africa	Gauteng	Surface water Groundwater	< 5.0 100.0	1570.0 180.0	I	Rimayi et al. (2018)

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during the warm and rainy season as a consequence of atrazine application but also due to differences in textural and compositional characteristics of soil (Bachetti et al. 2021). Besides, atrazine has also been detected in rainwater from the Pampean plain of Argentina with detection frequency >80%, at concentrations from 500 to 67,280 ng L<sup>-1</sup> (Alonso et al. 2018). The detection of atrazine in water resources, in concentrations above the maximum acceptable levels for drinking water, is of concern as it represents a direct risk to human health through drinking water consumption. To minimise the damage caused by this herbicide, it is necessary to apply processes that allow its removal from *s*-triazine-contaminated sites.

Aquatic ecosystems are complex environments as they contain a great diversity of organisms (algae, bacteria, fungi and protozoa) that play important roles in primary productivity, decomposition of organic compounds and nutrient cycling (Mauffret et al. 2017). Aquatic environments receive direct and indirect inputs of different compounds such as herbicides, causing qualitative and quantitative changes on microbial communities. These effects can impact on higher trophic levels and on processes that contribute to overall water quality (Verrhiest et al. 2002; Ensz et al. 2003). In this context, environmental impact on aquatic organisms associated with the application of atrazine has been widely reported (Bai et al. 2015; Baxter et al. 2016; Singh and Jauhari 2017). In addition, atrazine can be absorbed by algae and aquatic plants through cell walls, exerting toxic action mainly through inhibition of photosynthesis (DeLorenzo et al. 2001). For instance, Esperanza et al. (2017) evaluated the impact and action mode of the atrazine on the cellular senescence process of Chlamydomonas reinhardtii. The results indicated an increase in intracellular calcium levels, alterations in nuclear and cell morphology, as well as in the activity of biochemical and molecular markers, suggesting that short-term exposure to atrazine can promote death of microalgae, which are the basis of aquatic food webs. Zhao et al. (2018) showed that atrazine inhibited the growth of the microalga Selenastrum capricornutum. Sun et al. (2020a) observed acute toxicity of atrazine in the microalga *Chlorella* sp. because atrazine damaged the reaction centre of photosystem II. Religia et al. (2019) demonstrated that phytoplankton (Raphidocelis subcapitata) exposed to sublethal doses of atrazine affect the population dynamics of its predator, *Daphnia magna*, due to the production of non-viable broods. Simultaneously, atrazine toxicity can change water quality, due to increased concentrations of C, N and/or inorganic phosphorus (P), pH modifications and increased electrical conductivity (Viegas et al. 2012; de Albuquerque et al. 2020) and decreased dissolved oxygen  $(O_2)$  concentration due to reduced primary productivity (C fixation) by photosynthetic organisms (DeLorenzo et al. 2001).

#### 21.2.5 Effects on Higher Organisms

Toxicity effects of atrazine on other non-photosynthetic organisms (honeybees, birds and mammals) is lower in comparison to plants and algae. However, several works have demonstrated the effect of atrazine exposure on different higher organisms (Hirano et al. 2019; Soltanian 2016). Atrazine mainly affects the endocrine system

(Mukherjee et al. 2019; Graceli et al. 2020), and it also causes oxidative stress due to the formation of reactive oxygen species, leading to reduced semen quality and infertility in fish, crustaceans and mammals (Gely-Pernot et al. 2015; Owolabi and Omotosho 2017; Stara et al. 2018). In aquatic species, the exposure to atrazine (<5 $\mu g L^{-1}$ ) resulted in transgenerational reproductive dysregulation in *Oryzias latipes* (Cleary et al. 2019), disrupted immunity in Cyprinus carpio (Wang et al. 2019) and induced oxidative stress, reproductive dysfunction and neuroendocrine impairments in Danio rerio (Adeyemi et al. 2015). In addition, Hedayatirad et al. (2020) exposed Danio rerio to 0, 5 and 50  $\mu$ g L<sup>-1</sup> atrazine and observed that it increased cortisol level and decreased total immunoglobulin and lysozyme, affecting reproduction, thyroid function, stress reactivity and immunity of mature female zebrafish and subsequently their offspring. Abdulelah et al. (2020) demonstrated that exposure to atrazine (>10 ppb) causes significant DNA damage in crayfish lateral antennal cells, including olfactory sensory neurons, leading to impaired chemosensory abilities. Because crayfish rely on chemoreception for survival, changes in their ability to perceive odours following exposure to atrazine may have detrimental effects on their population size. Blahova et al. (2020) showed that common carp (Cyprinus carpio L.) chronically exposed to a range of atrazine concentrations (0.3; 300; 1000 and 3000  $\mu$ g L<sup>-1</sup>) for 12 weeks negatively influenced many health status such as oxidative stress indices, immune system response, indicators. haematological and biochemical profile and organ histopathology.

In mammals, Komsky-Elbaz and Roth (2017) indicated that bovine spermatozoa exposed to atrazine  $(0.1-3 \ \mu g \ L^{-1})$  negatively affected sperm membranes, sperm viability, acrosome reaction and mitochondrial function. In females it produces imbalances in sex hormones and interferes with androgen or oestrogen receptors, altering instinctive abortion, ovarian cycles and defect in birth development (Bohn et al. 2011). It was suggested that the negative effects of atrazine on the neuroendocrine system are caused by altered hormone levels, mainly follicle-stimulating hormone (FSH) and luteinising hormone (LH) (Song et al. 2014). Altered LH levels contribute to prolonged prolactin secretion and subsequent stimulation of mammary gland changes and increased incidence of mammary fibroadenomas and adenocarcinomas (Jowa and Howd 2011; Simpkins et al. 2011). Foradori et al. (2009) exposed ovariectomised female rats to several atrazine doses (0 and 200 mg kg<sup>-1</sup> day<sup>-1</sup>) for 4 days and reported that this herbicide reduced the number of activated gonadotropin-releasing hormone (GnRH) neurons. However, after 4 days of atrazine withdrawal, LH levels and GnRH activation markers returned to normal levels in treated animals. Although the negative effects could be reversed, it is unknown what may occur after longer exposure with the herbicide. Finally, Foradori et al. (2018) concluded that atrazine activates the hypothalamic-pituitary adrenal axis centrally and requires corticotropin-releasing hormone receptor activation. Atrazine also causes liver damage, the main metabolising organ of atrazine in mammals (Xing et al. 2015; Sagarkar et al. 2016). Cardiovascular system functioning is also affected by atrazine exposure (Cosselman et al. 2015). Besides, a possible association between atrazine contamination and a greater effect of several types of cancer in human cells, leukaemia and lymphoma has also been proposed (Thueson et al. 2015; Kirsten et al. 2017; Li et al. 2017; Brasil et al. 2018). There is limited epidemiological evidence on the adverse effects of prenatal atrazine exposure in humans. Consumption of drinking water with atrazine residues has been associated with an increased risk of preterm birth in Kentucky (Rinsky et al. 2012) and in four Midwestern states from the USA, where <10% of the population uses private well water (Stayner et al. 2017). In France, Chevrier et al. (2011) demonstrated that atrazine residues in maternal urine were associated with alterations in the babies, such as lower birth weight, head circumference and height. Xie et al. (2021) exposed human SH-SY5Y neuronal cells to 0.3, 3 and 30 µg L<sup>-1</sup> atrazine, showing alterations in neurite outgrowth and SNCA pathology, which leads to epigenome changes and an increased risk of Parkinson's disease. Previous epidemiological studies on the effect of atrazine on newborns are based on ecological estimates obtained from environmental monitoring data. Thus, a more reliable and accurate presence of atrazine is essential to ensure the safety of biota, human health and the environment.

## 21.3 Bioremediation of Atrazine-Contaminated Environments

Bioremediation involves the utilisation of microorganisms, plants or their enzymes for the partial or complete transformation of organic pollutants present in environments, in order to protect the natural ecosystem and prevent further pollution (Viegas et al. 2019). This biotechnological tool has several advantages compared to the physicochemical treatments, such as lower operational costs, in situ application, efficient elimination and minimum disturbance of the treated site (Hernández et al. 2008). Due to this intensive method which must be adapted to site-specific conditions, small-scale pilot experiments are necessary before they can be carried out in the contaminated field. However, bioremediation sometimes has limitations, which will affect the efficiency of microbial degradation. Therefore, it is necessary to search microorganisms with better performance and environmental tolerance. Besides, the use of genetic technologies to improve the degradation properties of microorganisms is also receiving increasing attention (He et al. 2019).

Biostimulation and bioaugmentation are natural attenuation methods that allow in situ microbiological remediation of atrazine. Natural attenuation involves physical, chemical or biological processes in the environment to dissipate the contaminant, being a very slow process. Biostimulation involves treating the contaminated soils to increase the pollutant bioavailability or adding a co-substrate or nutritional compound to increase the population of indigenous (or introduced) bacteria that degrade contaminants (Tyagi et al. 2011). In this case, the elimination of atrazine depends on its initial concentration, the pH of the medium, the inoculation time and the type of stimulant (Rajendran et al. 2021). On the other hand, bioaugmentation involves the inoculation with microbial strains or consortia (indigenous or not) to improve the system's biodegradation capacity of a specific organic pollutant in contaminated soils or water (Philp and Atlas 2005; Hernández et al. 2008). Furthermore, bioaugmentation may be required when indigenous degraders cannot degrade the pollutant rapidly or the degrading microorganisms are not present (Gentry et al.

2004). Both biostimulation and bioaugmentation are the most efficient methods of converting a pesticide into a less-harmful end product.

## 21.3.1 Bacterial Remediation

Bacteria are able to remove, degrade or breakdown xenobiotic compounds in less toxic or non-toxic ones by a process usually called bioremediation. This is a promising technology that includes different processes such as (a) pollutant transformation, (b) degradation to simple molecules, (c) mineralisation into inorganic compounds (such as  $CO_2$ ,  $H_2O$ ,  $H_2$ ,  $NH_3$ , etc.), (d) cell surface sorption and (e) intracellular accumulation, among others (Krastanov et al. 2013). It is a profitable and increasingly popular technology to restore the environment quality (Lyon and Vogel 2013). In this process, microorganisms employ the contaminants as a source of nutrients or energy for their growth (Benimeli et al. 2008). However, at the time of its application, it is important to consider the susceptibility of the contaminant to microbiological transformation, the biological activity (bioactivity) to promote microbial growth and activity and the affordability of the contaminants to microorganisms (bioavailability) (Niti et al. 2013).

Numerous bacterial strains (Gram-positive and Gram-negative) have been described with the capability to use atrazine as a substrate (C and/or N source) for their growth (Udiković-Kolić et al. 2012). Among them, Pseudomonas sp. ADP has been the bacteria used for the study of the metabolic pathway of s-triazine degradation and its regulation (Cao et al. 2021). The two catabolic pathways of atrazine degradation, one upper and one lower, involve the atzABCDEF genes located in the pADP-1 plasmid (Martinez et al. 2001). The enzymes encoded by these genes catalyse six successive hydrolysis: one dechlorination, two dealkylations, biuret deamination, ring cleavage and an allophanate hydrolysis (Fig. 21.2). Thus, the upper catabolic pathway transforms atrazine to cyanuric acid, and the enzymes responsible for these transformations are coded by the *atz*A, *atz*B and *atz*C genes (Mandelbaum et al. 1995; De Souza et al. 1996, 1998; Sadowsky et al. 1998). An initial hydrolase different from atzA, but with identical function, called trzN, has also been described, mainly in Gram-positive bacteria, such as Arthrobacter aurescens TC1, Nocardioides sp. C190 and Nocardioides sp. SP12 (Topp et al. 2000a; Piutti et al. 2003; Sajjaphan et al. 2004; Smith et al. 2005). However, its presence has also been demonstrated in Gram-negative genera such as Sinorhizobium and Polaromonas (Devers et al. 2007). Thus, atrazine dechlorination results from the activity of *atzA* (atrazine chlorohydrolase) or *trzN* (triazine hydrolase), both aminohydrolases, which produce HA. It is known that atzA and trzN possess different substrate ranges: atzA hydrolyses s-triazine compounds whereas trzN hydrolyses radical groups (-OCH<sub>3</sub>, -SCH<sub>3</sub>, -CN, -F, -Cl) of both s-triazines and pyrimidines (De Souza et al. 1996; Seffernick et al. 2000, 2002; Strong et al. 2002; Shapir et al. 2006). Subsequently, HA is transformed into N-ethylammelide or N-isopropylammelide by hydrolysis of the N-ethyl or N-isopropyl side groups. Then, the transformation to N-isopropylammelide is encoded by *atzB* 



Fig. 21.2 Pathways of atrazine mineralisation

(hydroxyatrazine hydrolase) (Boundy-Mills et al. 1997) which is capable of catalysing the hydrolytic deamination of N-ethylammelide to cyanuric acid (Smith et al. 2005), an intermediate in the catabolism of *s*-triazines (Cook 1987). However, N-isopropylammelide is transformed to N-isopropylamine and cyanuric acid by the enzyme *atz*C (N-isopropylammelide hydrolase) (Sadowsky et al. 1998). These intermediates freed from the *s*-triazine ring by *atz*B and *atz*C can be used as sources of C, N and/or energy for the growth of the microorganism itself or others present in the environment (Strong et al. 2002; Kolić et al. 2007).

The lower pathway is carried out by three enzymes coded by the atzD, atzE and atzF genes (Cao et al. 2021) and leads to the final mineralisation of cyanuric acid to CO<sub>2</sub> and NH<sub>3</sub>. It begins with ring cleavage in cyanuric acid catalysed by the enzyme cyanuric acid hydrolase (atzD) resulting in the production of the intermediate biuret carboxylate, which rapidly decomposes to biuret and CO<sub>2</sub> (Seffernick et al. 2012). Alternatively, the trzD gene, with homologous function to the atzD gene, which encodes an enzyme involved in the cleavage of the *s*-triazine ring of cyanuric acid, has been identified in several *s*-triazine-degrading bacterial genera such as *Pseudomonas*, *Paenarthrobacter*, *Arthrobacter*, *Aminobacter*, *Nocardioides*, *Klebsiella*, *Alcaligenes* and *Ralstonia* (Cheng et al. 2005; Arbeli and Fuentes 2010; Yang

et al. 2010; Fernández et al. 2013; Li et al. 2020). The hydrolysis of biuret to allophanate is mediated by biuret hydrolase (*atz*E) (Martinez et al. 2001; Cheng et al. 2005), and this deamination releases ammonium, which can be used by bacteria as a N source for growth. Finally, allophanate hydrolase (*atz*F) produces  $CO_2$  and NH<sub>4</sub> from allophanate (Martinez et al. 2001; Cheng et al. 2005; Shapir et al. 2005, 2006). Comparisons among known degradative strains reveal substantial heterogeneity in the organisation and location of these catabolic genes in the genome. Thus, they may be located in (a) a single plasmid (Piutti et al. 2003; Aislabie et al. 2005; Devers et al. 2007), (b) several plasmids of varying size in the same host (Topp et al. 2000b; Rousseaux et al. 2002; Devers et al. 2007) and (c) occasionally in the microbial chromosome (Cai et al. 2003; Devers et al. 2007; Vaishampayan et al. 2007).

Bacterial strains that use partially degraded s-triazine as a N source were obtained from wastewater and soil from Switzerland and identified as *Klebsiella pneumoniae* strain 90 and 99 and Pseudomonas sp. (strains A, D and F) (Cook and Hütter 1981). Pseudomonas sp. YAYA6 was the first pure strain capable of mineralising atrazine and using it as a C source (Yanze-Kontchou and Gschwind 1994). A year later, Mandelbaum et al. (1995) identified from the US soils with a previous s-triazine herbicide application the bacterium *Pseudomonas* sp. ADP capable of growing with atrazine as C and N source, which was used for the characterisation of the enzymatic mechanism of atrazine mineralisation, as was previously described (De Souza et al. 1995, 1996; Mandelbaum et al. 1995; Boundy-Mills et al. 1997; Sadowsky et al. 1998; Martinez et al. 2001). Since then, numerous bacterial isolates capable of degrading s-triazines, either totally or partially, belonging to phylogenetically diverse groups have been isolated worldwide (Table 21.2). There are several parameters that influence the efficiency of the degradation process. The effect of N sources on the regulation of the atrazine catabolic pathway has been the subject of numerous studies (García-González et al. 2005). The external addition of N has shown a negative effect on atrazine biodegradation in most of the bacteria studied (García-González et al. 2003). High water salinity affected atrazine degradation in an industrial wastewater bacterial community (Udiković et al. 2003). Atrazine degradation efficiency both in the presence of  $O_2$  and in anaerobiosis showed no differences in both conditions, so that  $O_2$  would not influence the metabolism of this herbicide (Mandelbaum et al. 1995). Atrazine residues have a very ubiquitous distribution and can be found in different compartments of the environment, so these contaminated sites are the most appropriate for the isolation of tolerant microorganisms with the capacity to degrade them (Ortiz-Hernández et al. 2001). Although there is a great diversity of atrazine-degrading isolates obtained worldwide, most of them have been isolated from agricultural soils with a previous history of atrazine application (Fernández et al. 2013; Li et al. 2020; Cao et al. 2021) and only a few from effluents of agrochemical manufacturing industries (Li et al. 2008; Yang et al. 2010).

New investigations are being carried out to improve biodegradation of atrazine, including the use of bacterial consortia, immobilised cells on different natural or synthetic materials, among others, as will be described later.

Table 21.2         Main characterised bac	cterial isolates with atrazine-degrading cap	ability described to	o date	
Strain	Final products of degradation	Catabolic genes	Origin	References
Gram-negative bacteria				
Agrobacterium radiobacter J14a	CO <sub>2</sub> + NH <sub>3</sub>	atzABCDEF	Soil (USA)	Struthers et al. (1998) De Souza et al. (1998)
Agrobacterium sp. NEA-D	$CO_2 + NH_3$	atzABCDEF	Soil (France)	Devers et al. (2007)
Alcaligenes sp. SG1	CO <sub>2</sub> + NH <sub>3</sub>	atzABC, trzD	Industrial waste (USA)	De Souza et al. (1998)
Aminobacter aminovorans	CO <sub>2</sub>	atzC, trzD	Soil (France)	Rousseaux et al. (2001)
Ancylobacter sp. T10A11	ND	atzABCDEF	Soil (Colombia)	Arbeli and Fuentes (2010)
Chelatobacter heintzi Cit1	$CO_2 + NH_3$	atzABC, trzD	Soil (France)	Rousseaux et al. (2001)
Chelatobacter heintzi Sal1	Hydroxyatrazine	atzA	Soil (France)	Rousseaux et al. (2001)
Ensifer sp. CX-T	$CO_2 + NH_3$	atzABCDEF	Soil (China)	Ma et al. (2017)
Klebsiella ornithinolytica ND2	<b>UD</b>	atzA	Soil (USA)	Siripattanakul et al. (2009)
Polaromonas sp. NEA-C	Cyanuric acid	trzN, atzBC	Soil (France)	Devers et al. (2007)
Pseudaminobacter sp. C147	CO <sub>2</sub>	atzABC	Soil (Canada)	Topp et al. (2000b)
Pseudaminobacter sp. C150	Hydroxyatrazine	atzAC	Soil (Canada)	Topp et al. (2000b)
Pseudomonas sp. ADP	CO <sub>2</sub> + NH <sub>3</sub>	atzABCDEF	Soil (USA)	Mandelbaum et al. (1995) De Souza et al. (1996)
Pseudomonas sp. MHP41	$CO_2 + NH_3$	atzABCDEF	Soil (Chile)	Hernández et al. (2008)
Pseudomonas sp. YAYA6	CO <sub>2</sub>	atzA	Soil (Switzerland)	Yanze-Kontchou and Gschwind (1994)
Ralstonia brasiliensis M91-3	CO <sub>2</sub> + NH <sub>3</sub>	atzABC, trzD	Soil (USA)	Radosevich et al. (1995) De Souza et al. (1998)
Rhizobium sp. PATR	Hidroxyatrazine	atzA	Soil (France)	Bouquard et al. (1997)
Schlesneria spp.	$CO_2 + NH_3$	atzBC	Soil (USA)	Douglass et al. (2017)
Shewanella sp. YJY4	Cyanuric acid	atzABC	Soil (China)	Ye et al. (2016)
Sinorhizobium sp. NEA-B	Cyanuric acid	trzN, atzBC	Soil (France)	Devers et al. (2007)
				(continued)

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		Catabolic		
Strain	Final products of degradation	genes	Origin	References
Stenotrophomonas maltophilia	Hydroxyatrazine	atzA	Soil (France)	Rousseaux et al. (2001)
Variovorax sp.	ND	atzBC	Soil (USA)	Douglass et al. (2016)
Gram-positive bacteria				
Arthrobacter aurescens TC1	Cyanuric acid	trzN, atzBC	Soil (USA)	Strong et al. (2002)
Arthrobacter crystalopoietes Cit2	Cyanuric acid	trzN, atzBC	Soil (France)	Rousseaux et al. (2001)
Arthrobacter nicotinovorans HIM	Cyanuric acid	atzABC	Soil (New Zealand)	Aislabie et al. (2005)
Arthrobacter sp. FD; MD; SD	Cyanuric acid	trzN, atzBC	Soil (Argentina)	Fernández et al. (2013)
Arthrobacter sp. 3A; 2B	Hydroxyatrazine	<i>tr</i> ZN	Soil (Croatia)	Devers et al. (2007)
Arthrobacter sp. AD1	ND	atzA	Waste water (China)	Cai et al. (2003)
Arthrobacter sp. AD26	<b>DN</b>	trzN, atzBC	Waste water (China)	Li et al. (2008)
Arthrobacter sp. AG1	Cyanuric acid	trzN, atzBC	Soil (China)	Xian-Zhu et al. (2007)
Arthrobacter sp. AK-YN10	Cyanuric acid	trzN, atzBC	Soil (India)	Sagarkar et al. (2016)
Arthrobacter sp. C2	Cyanuric acid	trzN, atzBC	Soil (China)	Cao et al. (2021)
Arthrobacter sp. C3	Hydroxyatrazine	<i>trz</i> N	Soil (China)	Wang et al. (2016)
Arthrobacter sp. CMU6	Cyanuric acid	trzN, atzC	Soil (USA)	Vibber et al. (2007)
Arthrobacter sp. DNS10	Cyanuric acid	trzN, atzBC	Soil (China)	Zhang et al. (2011)
Arthrobacter sp. FM326	Cyanuric acid	trzN, atzBC	Soil (China)	Li et al. (2020)
Arthrobacter sp. MCM B-436	Biuret	trzN,	Soil (India)	Vaishampayan et al. (2007)
		atzABCD		
Arthrobacter sp. T12B12	ND	trzN, atzBC	Soil (Colombia)	Arbeli and Fuentes (2010)
Arthrobacter sp. T3AB1	Cyanuric acid	trzN, atzBC	Soil (China)	Yang et al. (2021)
Arthrobacter sp. TES6	Cyanuric acid	trzN, atzBC	Soil (Egypt)	El Sebaï et al. (2011)
Arthrobacter sp. ZXY-2	Cyanuric acid	trzN, atzBC	Pesticide plant	Zhao et al. (2017)
			(China)	
Arthrobacter spp.	ND	trzN, atzBC	Soil (USA)	Douglass et al. (2017)

Table 21.2 (continued)

Bacillus licheniformis ATLJ-5	Hydroxyatrazine and N-isopropylammelide	ND	Soil (China)	Zhu et al. (2019)
Bacillus megaterium ATLJ-11	Hydroxyatrazine and N-isopropylammelide	DN	Soil (China)	Zhu et al. (2019)
Clavibacter michiganese ATZ1	N-ethylammelide	atzAB	Soil (USA)	De Souza et al. (1998)
Nocardia sp.	Cyanuric acid	trzN, atzC	Soil (USA)	Smith et al. (2005)
Nocardioides kribbensis CMU5	Cyanuric acid	trzN, atzBC	Soil (USA)	Vibber et al. (2007)
Nocardioides panacihumi	Cyanuric acid	trzN, atzC	Soil (USA)	Vibber et al. (2007)
Nocardioides sp. 1D	Hydroxyatrazine	trzN	Soil (Croatia)	Devers et al. (2007)
Nocardioides sp. C190	N-ethylammelide	trzN	Soil (Canada)	Topp et al. (2000a)
Nocardioides sp. C1S	ND	<i>tr</i> ZN, <i>at</i> ZCDEF	Soil (Colombia)	Arbeli and Fuentes (2010)
Nocardioides sp. NEA-A	Cyanuric acid	trzN, atzBC	Soil (France)	Devers et al. (2007)
Nocardioides sp. SP12	Cyanuric acid	trzN, atzBC	Soil (France)	Piutti et al. (2003)
Nocardioides sp. V3A16	ND	trzN, atzBC	Soil (Colombia)	Arbeli and Fuentes (2010)
Paenarthrobacter ureafaciens AAC22	Cyanuric acid	<i>tr</i> zN, <i>atz</i> BC	Surface water (Argentina)	Bachetti et al. pers. communication
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#### 21.3.2 Phytoremediation

The use of plant species for in situ treatment of contaminated sites is called phytoremediation. Pesticides can be transported across plant cell membranes and removed from the environment. Phytoremediation of pesticides involves several processes: pesticides in the soil can be absorbed by plant roots (rhizofiltration) or adsorbed by plant tissues (phytoextraction); pesticides in plant tissues can be transformed by plant enzymes (phytotransformation) or volatilised into the atmosphere (phytovolatilisation); and pesticides in the soil can be degraded by microorganisms in the rhizosphere (rhizoremediation) (Morillo and Villaverde 2017). Afterwards, incineration removes the compounds sequestered in the plant tissues (Gerhardt et al. 2009). Several studies have shown that root exudates of some plants significantly increase the desorption of organic pesticides in contaminated soils, increasing their bioavailability (Kidd et al. 2008; Muratova et al. 2009). Physical-chemical properties of the compounds and environmental and plant species characteristics are some of the causes that determine the rate of pesticide uptake (Singh and Jauhari 2017). Phytoremediation technology has many advantages: reduced costs compared to other remediation technologies; reduced erosion rate; improved physical, chemical and biological properties of the soil; aesthetic improvement; and environmentally friendly. However, it also presents some inconveniences such as longer restoration time of the contaminated site, extent and depth of the contaminated area, high dependence on climatic conditions, concentration and bioavailability of the contaminant and plant tolerance to contaminants (Morillo and Villaverde 2017; He et al. 2019). In addition, phytoremediation species can act as environmental filters at strategic water recharge points. For example, tree species capable of enhancing pesticide degradation in agricultural fields can be planted in alternating rows or as riparian forests to reduce or prevent their transport to rivers or groundwater (de Araújo et al. 2019). Inga striata and Caesalpinia ferrea are species that have shown their high tolerance to the atrazine herbicide (de Araújo et al. 2019; Aguiar et al. 2020).

Phytoremediation application is suitable in sites with low contamination and spread over large areas (Bini 2009). Several dicot and monocot species have been characterised as having high herbicide tolerance and some genotypes degrade herbicides in soil (Singh and Jauhari 2017). Herbicide removal is mainly attributed to the enzymes secreted by plant roots, such as peroxidases, polyphenol oxidases and invertases, followed by the uptake and transformation of the plants themselves (He et al. 2019). Merini et al. (2009) found that *Lolium multiflorum* had a higher atrazine removal capacity than natural attenuation in soil and water. Sui and Yang (2013) studied different rye grass genotypes, finding three genotypes capable of accumulating and degrading atrazine. Sánchez et al. (2017) investigated the phytoremediation of atrazine with *Lolium perenne*, *Festuca arundinacea*, *Hordeum vulgare* and *Zea mays*. The results showed that all plants had the ability to degrade atrazine, but *Z. mays* was the most efficient. Zhang et al. (2017) employed a genetically modified rice containing a metabolic enzyme glycosyltransferase 1 (ARGT1) capable of transforming atrazine. Cao et al. (2018) showed that the

interaction between Pennisetum americanum and atrazine-contaminated soil influenced microbial communities and enhanced rhizosphere bacterial diversity by reshaping some soil physicochemical properties (urease activity, catalase activity, water-soluble organic carbon content and pH). In addition, some specific bacteria that could facilitate the degradation of organic pollutants or soil nutrient cycling were only identified in the rhizosphere of *P. americanum*. Aguiar et al. (2020) evaluated the remediation potential of Inga striata and Eremanthus crotonoides in atrazine-contaminated soils. They found that atrazine modified the physiological variables of these plants (photosynthetic rate,  $CO_2$  consumption and transpiration) but without compromising their development. Eremanthus crotonoides and I. striata were able to reduce atrazine residues even in soils with high concentrations, allowing their use in polluted sites. More recently. the electrokinetic-assisted phytoremediation (EKPR) is also used in the atrazine removal for improving the effect of phytoremediation, in soil mesocosms using ryegrass (Lolium perenne L.) (Sánchez et al. 2020).

#### 21.3.3 Plant Microbial Remediation

Plant microbial remediation is a technology that uses plants and microorganisms to remove pollutants. Synergistic treatment between soil microorganisms and plant roots can promote the degradation of persistent organic pollutants in contaminated sites (Zhang et al. 2014; Asemoloye et al. 2017). Rhizodeposition and root exudation provide a source of nutrients for microorganisms present in the soil. Evidence suggests that organic acids, sugars, amino acids, tannins, phenolic compounds and vitamins found in root exudates have an important role in root-microbe communication (Tanimoto 2005). In addition, P solubilisation and N fixation occur in the rhizosphere, so bacterial populations benefit from increased availability of P and N (Shimp et al. 1993). Therefore, plants benefit from rhizosphere microorganisms through their metabolic detoxification of contaminants that can affect growth and these microorganisms, in turn, benefit from root exudates (Asemolove et al. 2019). The detoxification mechanism may include three aspects: plants absorb organic pollutants to metabolise or accumulate them in their tissues, enzymes produced by the elimination of pollutants and microorganisms favour plants favour mineralisation.

A wide variety of plant species have demonstrated efficient degradation of organic pollutants present in the rhizosphere (Abhilash et al. 2012). Dong et al. (2016) combined *Canna indica* with *Funnelliformis mosseae* (arbuscular mycorrhizal fungi) to remove atrazine. They found that *F. mosseae* could reduce the inhibition of atrazine on photosynthesis and growth of *Canna indica*, while the combination of *C. indica* with *F. mosseae* increased the degradation rate to 95.7% compared with phytoremediation alone (68.1%). Bazhanov et al. (2017) used *Arthrobacter ureafaciens* DnL1-1 in combination with alfalfa and wheat to degrade atrazine and the results showed that DnL1-1 strain could help crops from the negative effect of the herbicide. The degradation rates of atrazine by the DnL1-1

alfalfa and DnL1-1-wheat combinations were 75.6% and 99.8%, respectively. James et al. (2018) isolated *Pseudomonas* strains from the roots of *Typha latifolia*, Acorus calamus and Phragmites karka and employed them in combination to remove atrazine. The results showed that A. calamus and Pseudomonas strains presented the highest degradation rate of atrazine, and the combination of plants-microbes could enhance the herbicide removal as compared with the use of single microorganisms or plants. Qu et al. (2018) evaluated the atrazine degradation and its detoxification by Myriophyllum spicatum in combination with the bacterial community present in the lake sediments. The results of the study indicated that M. spicatum and possibly the predominant sediment bacteria (Nitrospirae and Acidobacteria) degraded atrazine to biuret over a 60-day incubation period. Jiang et al. (2020) investigated the effects of *Pseudomonas chlororaphis* strain PAS18, a type of plant growth-promoting bacterium (PGPB), on the growth and physiological responses of *Pennisetum americanum* (L.) K. Schum seedlings under three different levels of atrazine (0, 20 and 100 mg  $kg^{-1}$ ) in a pot experiment. The results suggest that strain PAS18 could alleviate atrazine-induced growth and stress in P. americanum by enhancing photosystem II repair and antioxidant defence capacity, as well as balancing  $Ca^{2+}$  influx. Yang et al. (2021) showed the influence of co-inoculation of Trichoderma harzianum LTR-2 and A. ureafaciens DnL1-1 on wheat treated with atrazine. Strains LTR-2 and DnL1-1 caused significant increases in shoot biomass, root biomass and root/shoot ratio and significantly decreased the amount of atrazine and its degradation products.

Plant microbial remediation presents a wide range of application and research prospects, due to the low cost of application, low energy consumption and possible large-scale application to remediate contaminated environments (He et al. 2019).

## 21.3.4 Material-Microbial Remediation

Microorganisms used in bioremediation processes constitute an attractive methodology for the recovery of contaminated environments. Some of the strategies used to achieve that goal include on-site introduction of nutrients and  $O_2$ , which stimulate native microbial strains (biostimulation) or on-site inoculation with competent microorganisms (bioaugmentation) (Nzila et al. 2016). However, an important factor to be considered in a bioaugmentation process is using the correct inoculation system ensure the successful adaptation of the inoculated microorganisms. to Immobilisation on biological or polymeric supports can be an appropriate inoculation strategy during bioaugmentation. The advantages of microbial immobilisation are (a) microbial protection from adverse environmental conditions or possible predators (protozoa, parasites, etc.) that threaten microbial survival, (b) nutritional stress prevention by allowing the exchange of nutrients and waste products, (c) the introduction of a higher density of degrading bacteria to the environment, (d) a major stability of microorganisms in sites with high concentrations of contaminants, (e) cell viability preservation in the long term by offering the possibility of repeated inoculation of cells, (f) allows microbial metabolism to remain relatively constant over time and (g) the easy recovery of the already decontaminated solution (Hsieh et al. 2008; Khondee et al. 2012; Angelim et al. 2013). In addition, immobilised cells are easier to handle, thus minimising the risks of contamination during transport, application and storage (Park and Chang 2000).

There is a wide range of substrates used as cell immobilisation matrices, which usually show an appropriate ability to be applied for removing organic pollutants. Synthetic polymers include polyacrylamide, polyethylene glycol, polyvinyl alcohol and polyurethane (Wang et al. 2012; Tong et al. 2014; Zhang et al. 2020) whereas natural substrates include alginate, carrageenan, agar, collagen, chitin, chitosan and biochar (Morgante et al. 2010; Banerjee and Ghoshal 2011; Lu et al. 2012; Sun et al. 2020b; Yu et al. 2020). Desitti et al. (2017) encapsulated *Pseudomonas* sp. strain ADP in core-shell electrospun microtubes and then used it for atrazine removal in a reactor. Besides, the sol-gel process was employed to immobilise ADP strains in thin silica layers, which were coated onto carrier materials (Pannier et al. 2014). Currently, there is an increasing focus of interest to find low-cost, more efficient and easier-to-handle support matrices. Khatoon and Rai (2018) investigated the potential of sugarcane bagasse as an immobilising support for *Bacillus badius* strain ABP6 for atrazine biodegradation. The results showed that the cells were strongly adsorbed and completely dispersed on the bagasse surface after immobilisation, removing 85.3% of atrazine at 14 days of assay.

Biochar produced from biological sources is an important source of C and with high stability to chemical and microbial degradation (Kupryianchyk et al. 2016). The biochar has been studied for remediation of pesticide-contaminated sites, due to its multiple advantages including the possibility of trapping bioremediation bacteria in the biochar micropores (Morillo and Villaverde 2017). Yu et al. (2019) evaluated the immobilisation of Arthrobacter sp. strain ZXY-2 on mushroom pellet biochar (Aspergillus niger Y3). The self-immobilised biomixture was capable to remove  $50 \text{ mg L}^{-1}$  of atrazine in 1 h. Tao et al. (2020) evaluated the immobilisation of four phosphate-solubilising bacteria and one atrazine-degrading bacterium (Acinetobacter lwoffii DNS32) on BC550 straw-based biochar. The results indicated that the combined immobilisation showed 49% higher capacity to degrade 100 mg  $L^{-1}$  atrazine in 24 h and 27% higher capacity to degrade 20 mg kg<sup>-1</sup> atrazine after 3 days in liquid and soil, respectively.

# 21.4 Strategies for Bioremediation of Atrazine Under Laboratory and Field Conditions

As was previously highlighted, atrazine is a persistent herbicide frequently found in agricultural areas, surface water as well as groundwater (by infiltration through soils) (Alonso et al. 2018; Bachetti et al. 2021), and different alternatives have been proposed for its remediation. Among them, microbial degradation is one of the most efficient strategies from an economical and environmental point of view. However, increasing the efficiency of biodegradation processes is a challenge that researchers have to solve. Selection of suitable microorganisms, the increase of

pollutant uptake and degradation, the immobilisation of microorganisms in adequate matrices and/or performing an appropriate bio-formulation are some of the approaches used to improve tolerance and atrazine removal (Desitti et al. 2017; Chen et al. 2019; Zhu et al. 2019; Herrera-Gallardo et al. 2021). More recently, computational models have been used to make simulations in order to design proper media not only for growth optimisation but also for enhancing atrazine biodegradation (Ofaim et al. 2020). Other strategies include optimisation of certain agronomic factors; the management of soils and microbial communities, including rhizospheric bacteria, fungi and endophytic microorganisms; and their selection and improvement through different techniques to generate a beneficial effect on plants or modify atrazine bioavailability (Liu et al. 2021; James and Singh 2021). For instance, it was reported that an epiphytic root bacteria *Pseudomonas* spp. strains AACB and TTLB and Arthrobacter spp. strain PPKB isolated from emergent hydrophytes could decontaminate atrazine at different pH and temperatures. Due to these strains also exhibiting PGP properties, they could be successfully applied as bioinoculants, for the phyto/rhizoremediation of terrestrial and aquatic ecosystems (James and Singh 2021). In the same way, intracellular crude enzyme extracts from these *Pseudomo*nas strains were applied for atrazine detoxification and could be proposed as an alternative remediation technique (James and Singh 2021).

Despite several bacterial strains being able to display a great catabolic potential under laboratory conditions, they could fail to behave similarly in natural surroundings. This could be due to suboptimal growth environmental conditions, such as variations in pH, temperature and nutrient sources; the competition with welladapted indigenous organisms and, also, the possible presence of environmental traits that might repress the genes responsible for the catabolic activities. These aspects are often not taken into account and are causes of failure of strains in the fields because to date, a high number of available results of atrazine decontamination by different microorganisms have been carried out under controlled laboratory conditions. On the other hand, it is interesting to consider that the experimental design should not only include synthetic solutions but the results must also be validated using more complex matrices such as soil, sediments, surface waters, etc. since many times certain microorganisms can degrade atrazine with high efficiency in a simple matrix but not in more real environments. In this sense, although it is widely recognised that Pseudomonas sp. ADP is able to degrade atrazine with high efficiency in synthetic solutions, the same did not occur in bioaugmentation experiments carried out in a liquid phase of sediment slurries, as well as in water circulating in columns filled with sediments. Besides, concomitant biostimulation with Na-citrate did not affect atrazine degradation in these experimental conditions (Liu et al. 2020). This and many other examples have been described in the literature showing diverse experimental devices for bioremediation studies. Such preliminary tests are fundamental to address in an appropriate way this highly complex subject. However, assays at microcosm and mesocosm levels are also needed. These systems constitute experimental devices that are interesting to make an approximation to reality, since they allow evaluating bioavailability and the effects of temperature, radiation, etc., which may have a decisive role on the observed responses. In addition, they allow to study the optimal conditions for implementing a biotechnological process. Microcosms can also be used to analyse certain specific target genes, related with degradation activities, and these studies could serve to predict the results of certain bioremediation strategies (Sagarkar et al. 2013). The literature provides many examples of studies related to bioremediation of atrazine using microcosms and mesocosms as experimental systems that were performed in the last years (Liu et al. 2021; Sagarkar et al. 2014, 2013; Urseler et al. 2021). For instance, bioaugmentation with *Arthrobacter* sp. strain AAC22 improved atrazine removal avoiding its lixiviation, being almost complete (>99%) after 8 days of treatment in a microcosm system using an agricultural soil. A bioassay indicated that toxic by-products were not detected after this treatment, demonstrating that AAC22 could be an efficient biotechnological tool for remediating atrazine-polluted soils (Urseler et al. 2021). Other research works suggest the existence of complex regulatory pathways for atrazine degradation in agricultural soils, which may be affected by the presence of N (Govantes et al. 2009).

Simulating field conditions, several bioremediation strategies have been proved at mesocosm level, in order to scale-up the process. For instance, a bacterial consortium with atrazine-degrading capabilities was used and this process was monitored at biochemical and genetic level (Sagarkar et al. 2014). Nonetheless, it is very important to follow a gradient of work scales and finally to confirm the behaviour of these systems on a larger scale, for example, in contaminated sites, since atrazine bioavailability as well as climatic traits could generate greater discrepancies between laboratory and field conditions. As it can be easily deduced, one of the main challenges is still the utilisation of these technologies to larger extensions, in the field and/or in the aquifers. In this sense, an analysis of the actual situation shows that there is some experience at the level of basic studies but its implementation in the field is very limited. Thus, future studies should be focused on reducing the gaps between atrazine bioremediation at laboratory and at field scales to find a proper and adaptable strategy to remediate extensive polluted sites.

## 21.5 Conclusion and Perspectives

Pollution caused by herbicides, such as atrazine, is considered among the top ten environmental hazards, which require the contribution of several disciplines to find strategies that allow mitigating its effects on ecosystems and also on human/animal health. As it was highlighted in this chapter, bioremediation is a cost-effective, efficient and clean strategy as against other common detoxifying methods, which has to be continuously improved. In the last years, several microorganisms have demonstrated to be efficient for atrazine removal. However, microbial consortia frequently showed more advantages because they can better withstand different environmental conditions. Despite an extensive knowledge now available in this regard, the continuous selection of more suitable and effective microorganisms is still an area of great interest. Another key aspect is the understanding of the complex biodegradation processes of atrazine in natural environments. In this context, the use of new computational platforms together with the integration of different omic approaches (genomics, metagenomics, proteomics, metabolomics, etc.) is likely to strongly contribute to this aim and will promote the progress and the improvement of novel bioremediation strategies to enhance atrazine remediation applicability. Besides, the study of microbial communities by molecular tools could provide new knowledge related on catabolic potential and diversity of microorganisms. Thus, this information could improve treatments against pollution in addition to encouraging a better comprehension of these complex biological communities and opening new ways for biotechnological advances. Surely, some of these powerful approaches could help for the development of eco-friendly and efficient strategies for atrazine biodegradation.

On the other hand, a well-formulated and designed strategy is needed in order to implement an efficient bioremediation technology, by taking into consideration several aspects that influence the process. In this sense, the search and selection of new and low-cost matrices and nanomaterials would be useful to make this process more economical and environmentally viable. In fact, the development of new biomaterials, which possess good performance, is a new and promising research line in the area of pollutant remediation. However, they sometimes have certain disadvantages related to the entrapment material, the preparation of the biomaterial and the toxicity of certain components, among others. These and other aspects have to be solved previous to their large-scale application and constitute the aims of future research lines related to the use of such new technologies.

It is expected that atrazine bioremediation at different environments can be improved in the coming years not only by integrating recent findings but also by combining various strategies simultaneously in a safe way, by means of a synergistic action. This is one of the approaches that require more investigation in the future to test the efficiency. It should also deepen all those aspects related to the successful application of these technologies on a larger scale and with low environmental impact, which constitutes a challenge for the scientific community.

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