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Thermal and Biological Degradation of Herbicides to Treat Wastewater and Soil Pollution

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Abstract: Since their discovery in 1958, atrazine and other members of s-triazine family have been extensively applied in order to control grassy and broadleaf weeds in crops. Although, it was proved by many researchers that atrazine was slow and partially degradable material. These pesticides were widely used to enhance crop quality and yield. Atrazine degradation can be achieved by the effects of air, sun, water, microorganisms and temperature. Thermal degradation plays an important role in agrochemicals elimination; so a better understanding of the role of thermal degradation of atrazine is essential. Quantitative determination of pesticide residues was studied by calculating the amount of herbicide residues left after decomposition using a simple thermo analytical technique (TG, DSC). In this study, we found that all samples undergo the process of melting, evaporation, decomposition and oxidation at a temperature higher than the maximum registered climate temperature which made us conclude that 80–90%wt) of the pesticides stay in the environment without change under 100 °C on different parts of plants like fruits, leaves, and some penetrate into the ground to reach the roots or continue to the groundwater causes toxic problems to their consumers and environmental pollution. This result gave the motivation to find a more effective approach to reduce the harmful effect of these pollutants. In this regard, biological degradation of atrazine by using two legumes roots rhizobium bacteria was investigated. It is found that both rhizobium are able to treat atrazine at moderate temperature (30 °C). In comparison to chickpea rhizobia, bean rhizobia showed a higher performance with a removal efficiency of 43.21% and 57.42% at mild and high atrazine concentrations (5 mg/L and 10 mg/L) respectively. The results from this research offer an elegant way to remove the harmful effect of herbicides through degradation and to bring the safe use of herbicides a step closer to applications.

Key words: Atrazine, biological degradation, herbicides, pesticides toxicity, thermal decomposition.

Introduction

Environmental contamination is predominant nowadays due to various harmful chemicals and other causes; among them, synthetic herbicides are one of the main sources of heavy water and soil pollution (Rashid et al., 2019; Shaker & Alhameed, 2016; Sultan et al., 2018). For many years, societies have enjoyed the benefits of using synthetic pesticides, herbicides fungicides, and insecticides to control weed, insect, fungus, parasitic, and rodent pests (Remucal, 2014).

Among the herbicides, atrazine, a chemical compound, is used to stop pre- and post-emergence broadleaf and grassy weeds in major crops such as sugarcane, sorghum, wheat, nuts, conifers, and corn. It is the most widely used herbicide due to its effectiveness and inexpensiveness (Iriel et al., 2014; Kumar & Singh, 2016; Singh et al., 2018; Wu et al., 2018). Atrazine is

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the second most commonly used conventional pesticide all over the world. In the United States, a high level of applied atrazine (over 24,000 tons per year) was recorded (Mudhoo & Garg, 2011; Zhai et al., 2020) to be present in the ecosystem.

It belongs to the family of s-triazines (atrazine and simazine), both of which were patented in 1958 under the names (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine) and (2-chloro-4,6-bis(ethylamino)-s-triazine). Based on the chemical formula of atrazine and simazine shown in Figure 1, we can conclude that they consist of an aromatic 6-carbon ring composed of three nitrogen atoms and three carbon atoms (Cheng et al., 2016; Sene et al., 2010; Udikovi-Koli et al., 2012; Ye et al., 2017; Zhang et al., 2015).

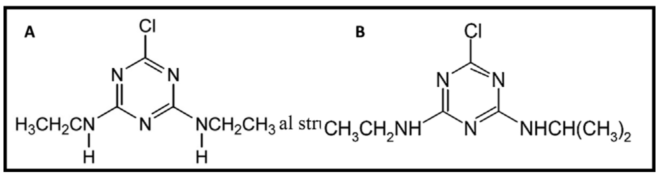

Figure 1: A. Simazine chemical structure. B. Atrazine chemical structure.

Atriazine has numerous trade names, such as Aatrex, Aktikon, Alazine, Atred, Atranex, Atrataf, Atratol, Azinotox, Crisazina, Farmco Atrazine, G-30027, Gesaprim, Giffex 4L, Malermais, Primatol, Simazat, and Zeapos (Aelion & Mathur, 2001; Cook, 1987).

These chemical compounds can be noticed mainly near manufacturing sites and crops farms where they are applied as an herbicide. It is found that the s-triazine ring of atrazine is fairly resistant to natural degradation. In recent times, there has been a doubt that most of the biodegradation methods used for water treatment are not completely successful to remove atrazine from water due to its high chemical stability, low volatility and slow hydrolysis. As a result, atrazine normally accumulates in soil ecosystems (Desitti et al., 2016; Ji et al., 2015; Luo et al., 2016; Luo et al., 2021; Wu et al., 2017). Atrazine and its metabolites have been frequently reported on surfaces and in groundwater by many researchers (Claver et al., 2006; Loos et al., 2010; Luo et al., 2014; Yang et al., 2010).

Many authors classify atrazine and its metabolites as carcinogenic and endocrine disrupting agents, causing loss in the ability to conceive and decreasing prolactin release during the lactation period, as well as increasing the risk of breast and ovarian cancer (Luo et al., 2021; Wu et al., 2018).

The melting point and LD50 for atrazine and some degradation products are listed in Tables 1 and 2 (Pugh, 1994).

Table 1: Melting points of atrazine and its degradation product

Name	*Melting point, °C*
Atrazine 2-chloro-4-ethylamino-6-isopropylamino-s-triazine	175 - 177
Cyanuric Acid (OOOT) 2,4,6-trihydroxy-s-triazi	>360
Atrazine desisopropyl(CEAT) 2-amino-4-chloro-6-ethylamino-s-triazine	164-166
Atrazine desethyl (CIAT) 2-amino-4-chloro-6-isopropylamino-s-triazine	132-134
Atraton (MEIT) 2-methoxyatrazine	95-96

Table 2: Lethal dose for 50% of targets (LD50) of atrazine and some degradation products

Name	**LD50**	*LD50*	
	Oral Mouse	*Rat*	*Dermal Rabbit*
Atrazine 2-chloro-4-ethylamino-6-isopropylamino-s-triazine	1750	–	3000
Cyanuric Acid 2,4,6-trihydroxy-s-triazine	3400	7700 (tumorigenic agent)	7900
Atrazine desisopropyl 2-amino-4-chloro-6-ethylamino-s-triazine	Unknown		
Atrazine desethyl 2-amino-4-chloro-6-isopropylamino-s-triazine	Unknown		
Atraton 2-methoxy atrazine	1465	–	–

Generally, pesticides degradation depends on the soil features and different environmental elements. It was indicated that the effect of temperature on pesticides degradation can be considered as one of the most important factor that plays an essential role in the degradation of pesticides (Humans, 1991). In this regard, a good understanding of the thermal behaviour of pesticides can be achieved by studying the pesticides' thermal analysis (Kiss & Virág, 2009). Recently, the biological approach for pesticide treatment gained great importance regarding their role in biological degradation. It is found that several microorganisms have the ability to reduce the toxic effect of pesticides, herbicides and fungicides to acceptable levels (Hamed et al., 2020, 2021; Lian et al., 2021). These results gave the motivation to find an environmentally friendly approach for atrazine biodegradation before it can reach the food webs. Several strains of bacteria are known to degrade atrazine, including Acinetobacter spp., Bacillus spp., Micrococcus species, Rhodococcus species, Agrobacterium species, Microbacterium species and D. spp. Providencia, Acidovorans, Ochrobactrum, Ensife, and Pseudomonas spp. (Fang et al., 2015; Fernandes et al., 2018; Ma et al., 2017; Mandelbaum et al., 1995; Santos, 2020; Singh & Singh, 2016; Vargha et al., 2005; Wang et al., 2014). Simipattanakul et al. (2008) in their study showed that four bacteria types had been introduced as atrazine biodegradation strains: *Alcaligenes faecalis*, *Klebsiella ornithinolytica*, *Bacillus megaterium*, and *Agrobacterium tumefaciens*. Bacteria that degrade atrazine normally utilize it as a nitrogen source. According to various reports, the atrazine metabolite in bacteria is inactivated through three steps involving three catalytic enzymes; hydroxyatrazine ethylaminohydrolase (AtzB), isopropyl ammelide isopropyl aminohydrolase (AtzC) and the enzymatic degradation of N-isopropyl ammelide (Hess & Warren, 2002; Topp et al., 2000; Wackett et al., 2002).

In our work, we investigated two types of rhizobium; Pea rhizobia and bean rhizobia for their ability to metabolise atrazine. Rhizobium refers to root and life in Greek, "rhiza" and "bios". Gram-negative rhizobia are bacteria that can form symbiotic relationships with many legumes such as beans, peas, soybeans, alfalfa, and so on. Rhizobium was also known as promising fertilisers due to their ability to enhance plant growth and crop quality (Nagananda et al., 2010).

The significance of this study is enhanced by the fact that; a high percentage of atrazine and simazine residues cause serious toxic effects on the environment. In this study, we investigated the thermal degradation of two pesticides: atrazine and simazine and the biological degradation of atrazine and compare these two approaches to highlight the most successful procedure to reduce the effect of these pollutants on the environment.

Experimental Work

Materials

All chemicals were purchased from VWR Prolabo (Belgium) and were of analytical grade or higher. Sigma Chemical Co. (U.S.) provided atrazine and simazine (98% purity).

Thermal Degradation

Differential scanning calorimetric (DSC), and thermo-gravimetry (TG) analyses were applied to evaluate the extrapolated onset temperatures, and the mass loss percentage (weight loss %) values increases with rising temperature, melting, evaporation, crystallisation, and decomposition reactions are detected. About 2 mg of atrazine and simazine (98% purity) were used individually for thermal analysis. The most important properties of atrazine and its degradation products are provided by Sigma Chemical Co. (USA), and are represented in Table 3.

Table 3: Main properties of atrazine used in this work

Properties	*The value*
Molecular formula	$C_8H_{14}ClN_5$
Molar mass	215.68 g. mol^{-1}
Appearance	Crystal colourless solid
Density	1.187 gcm^{-3}
Melting point	175 °C, 448 K,
Boiling point	200 °C, 473 K,
Solubility in water	7 mg/100 m

DSC/TG was applied using Heraeus TA – 500 thermal analyser under static air atmospheres with heating rates of 5 ºC, the experimental error was within ± 3 ºC.

Biological Degradation

Rhizobia from Root Nodules

To obtain rhizobia cultures from legumes roots, the nodules were gently removed from the roots and washed using sterilised water to eliminate any sedimentations or soil. After washing, the nodules were soaked in mercuric chloride for 5 minutes to eliminate any microorganisms present on the surface of the nodule. Next, the roots were washed with ethanol and sterilised water many times and air dried. Aftert that we crushed the nodules with a glass rod and added a 50 ml solution of 0.1 M phosphate buffer for 30 minutes. We plated 100 L of the dilution on yeast mannitol agar (YMA) and incubated the plates under room temperature for 3 days, and finally storing at 4 °C.

Atrazine Biodegradation by Cell Culture

To inoculate 500 ml of yeast mannitol broth, we used one gram of mannitol, one gram of $CaCO_3$, 1 gram of K_2HPO_4, 2 grams of $MgSO_4$, 1 gram of yeast extract, and 1 gram of NaCl per liter of deionised water. After the pH was adjusted to 7.3, the bacteria were shaken at 30 °C for 3 days. A centrifuge was used to remove the cells for 30 minutes at 4 °C and the pellets were stored at –20 °C. The cell pellets were re-introduced into 300 ml of the Yeast Mannitol broth in a 1-L flask supplemented with mild and high concentrations of atrazine (5 and 10 mg/L), respectively. A control with no bacterial cultures was also applied. After 7 days of incubation at the growth condition, the cell pellets were collected by centrifugation and freeze-dried using liquid nitrogen, and then stored at –80 °C until the metabolic analysis was done.

Metabolite Analysis of Atrazine

The free atrazine concentration in the media after 7 days of exposure using a gas chromatograph with a mass selective detector (GC-MSD) was evaluated according to Siripattanakul et al. (2008). Briefly, the sample (1 µl) was injected into the GC manually. The injection port temperature was 220 °C. The GC oven temperature was changed depending on the following program: 50 °C (1 min) followed by intervals increasing by 20 °C per min until 270 °C, then the temperature was held for 1 min. Two quantification ions of atrazine were chosen at m/z = 200 and 187 under selective ion monitoring mode (bacterial degradation).

Results and Discussion

Thermogravimetric analysis (TGA) has been used to study the effects of temperature on the physical and chemical properties of materials.

As shown in Figures 2 and 3 for atrazine and simazine, the compounds undergo a phase transition from solid to liquid in the first step without any change in their composition.

The results obtained from thermogravimetric and differential scanning calometric curves can be summarised in Table 4.

The TG curve of atrazine indicated that the weight loss for atrazine was 27%, 36% and 95% at 470K (197 °C), 505K (232 °C) and 515K (242 °C), respectively. It can be seen that this weight loss begins with varying thermal fusion when the temperature rises higher than 100 °C, also it was observed that the the maximum degree of decomposition was at 480 K (207 °C) (Figure 2). The loss in weight reached 95% and sludge remaining after the decomposition rate was 5%.

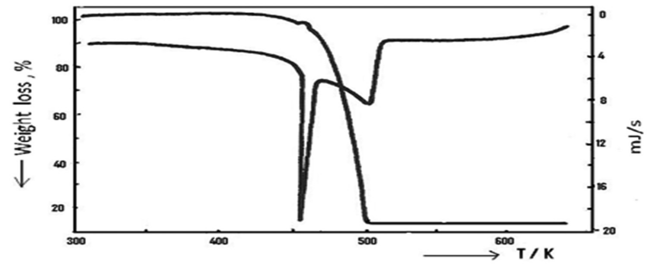

Figure 2: The DSC and TG curves of atrazine.

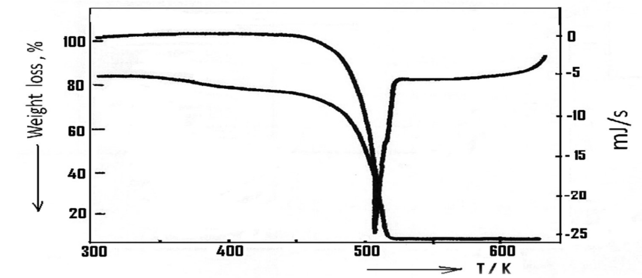

Figure 3: The DSC and TG curves of simazine.

Table 4: Thermoanalytical data of some atrazine and simazine herbicides

Pesticide type	*Induction Period, K*	*Melting temp, K*	*Decomposition. temp, K*	*Weight loss wt %*
Atrazine	Up to 380 (107Co)	420 (174 Co)	480 (207 Co)	94
Simazine	UP to 430 (157Co)	452 (179 Co)	505 (232 Co)	84

It was found that simazine required a higher temperature than atrazine (505K/232 °C) to be decomposed. The TG-curve showed that the weight loss of simazine at 470K (197 °C), 505K (232 °C) and 515K (257 °C) was 0%, 15% and 89%, respectively, and the maximum degree of decomposition of simazine was at 505K (302 °C) (Figure 3). The loss in weight ratio reached 89% and sludge remaining after decomposition was 11%. A comparison between the thermal removal of atrazine and simazine after complete decomposition at different temperatures is shown in Figure 4.

Biodegradation of Atrazine

After 7 days of exposure to mild and high concentrations of atrazine (5 gm/L and 10 gm/L), it was found that the concentration of residual atrazine in the media decreased by (2.16) mg/L and (5.74) mg/L at mild and high atrazine doses, respectively in a media containing bean

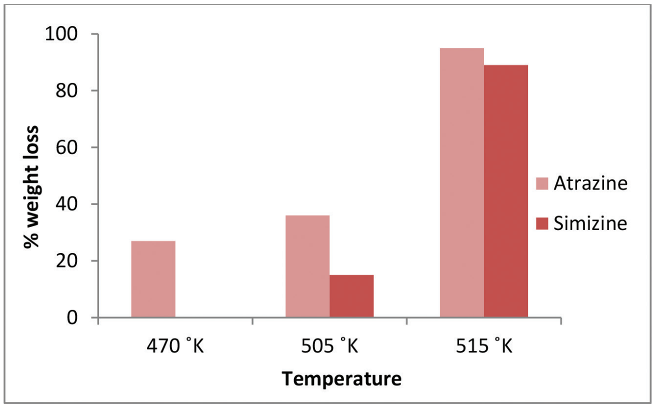

Figure 4: Weight loss % of Atrazine and Simazine herbicides against increase in temperature.

rhizobia. However, when chickpea rhizobia cells were used the remaining atrazine dropped by (1.52) mg/L and (4.02) mg/L at mild and high atrazine concentrations respectively as shown in Figure 5. Thus, the removal efficiency of atrazine by bean rhizobia was 43.21% (at mild concentration) and 57.42% (at high concentration) compared to 30.5% and 40.2% at mild and high concentrations of atrazine when chickpea rhizobia were used (Figure 6). This result is well compatible with the result published by Siripattanakul et al. (2008). In their research, they introduced two bacterial strains (*Kl. ornithinolytica* and *Ag. tumefaciens*) as atrazine degraders. The phylogenetic analysis of *Ag. tumefaciens* strain showed 100 % compatibility with rhizobium. In line with these results, it is found that *Achromobacter* and *Ensife* are capable to degrade atrazine into non-toxic compounds (Fernandes et al., 2018; Ma et al., 2017). In addition, complete atrazine mineralisation by using three strains (*Agrobacterium*, *Bacillus* and *Klebsiella)* was reported (Kumar & Singh, 2016; Wang et al., 2014).

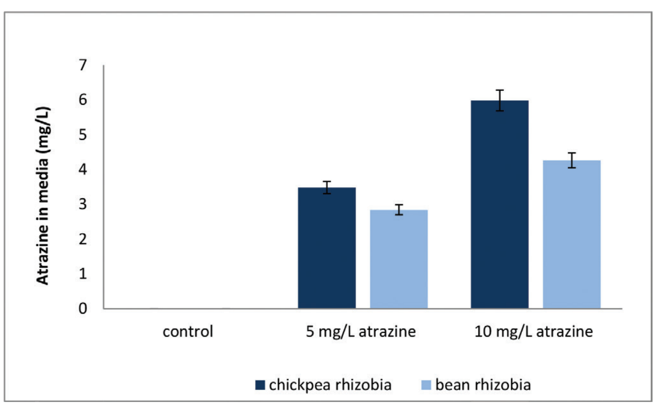

Figure 5: Residual atrazine in a media contain chickpea rhizobia and bean rhizobia. Different atrazine's concentrations were used; 0 mg/L (as a control), 5 mg/L and 10 mg/L. The duration time was 7-days. Each value was replicated 5 times to find the (± standard error (SE)).

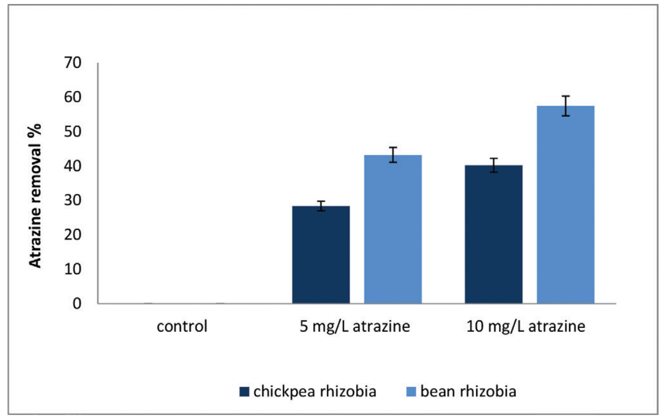

Figure 6: Removal efficiency of atrazine in a media contain chickpea rhizobia and bean rhizobia. Different atrazine concentrations were used; 0 mg/L (as a control), 5 mg/L and 10 mg/L. The duration time was 7-days. Each value was replicated 5 times to find the (± standard error (SE)).

Indeed, data of free atrazine contents were reflecting the high bioaccumulation ability of atrazine by both types of legumes (bean rhizobia and pea rhizobia) and recommended the use of bean rhizobia over chickpea rhizobia as a bioremediatory microorganism.

Conclusion

Atrazine and simazine are examples of herbicides, which are used to prevent weeds. They are frequently used and applied in a spraying manner and they are a pollution source for water, soil and crop. The present study indicated that a large proportion of these agrochemical components do not degrade at ambient temperature. The biological approach, when applied to rhizobium roots of legumes, showed that the initial concentrations of atrazine were significantly reduced. As a result, bean rhizobia are more efficient in removing atrazine. This finding is supported by the low percentage of the residual atrazine in a media containing bean rhizobia. Thus, we assume that bean rhizobia could be a superior species for atrazine bioremediation at ambient temperature.

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