Pol. J. Environ. Stud. Vol. 21, No. 3 (2012), 697-702

Original Research

Phytoremediation Potential of Three Wetland Plant Species Toward Atrazine in Environmentally Relevant Concentrations

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Received: 28 May 2011
Accepted: 15 November 2011

Abstract

This study focused on assessing the phytoremediation potential of wetland plants toward atrazine in an aquatic environment. Changes in plant biomass and atrazine content were investigated for three plant species: sweet flag, broadleaf cattail, and narrow-leaf cattail. Atrazine removal and shifts in plant biomass were assessed. Two mathematical models were built to describe atrazine toxicity toward the studied plant species and fate of atrazine during long-term phytoremediation. Sweet flag exhibited the highest tolerance toward atrazine as well as the most efficient atrazine removal rate. The average atrazine half-life was significantly reduced from about 400 days to 5 days. The highest studied initial concentration of atrazine (20 mg/l) was reduced by more than 99% after 40 days.

Keywords: atrazine, cattail, phytoremediation, sweet flag, toxicity

Introduction

The common use of herbicides, apart from the desired action, contributes to soil, water, and subsequent food contamination. The application of pesticides is, therefore, beside the positive effect, tightly bound with negative and, a difficult-to-predict environmental impact.

Atrazine [2-chloro-4-(ethylamino)-6-isopropylamino-strizine] is considered to be a classic example of such pesticides. Atrazine is among the most widely distributed environmental pollutants in countries with agriculture-based economies, including Canada, China, India, and Switzerland [1]. In Europe it was recently observed that atrazine levels in water often exceed the maximum permissible level for drinking water (0.1 μg/l) [2]. Investigations concerning its effect on the human organisms indicate that chronic exposure to small doses of atrazine may pose a serious threat to human health [3-5]. Additionally, numerous studies have confirmed that food and drinking water containing atrazine contribute to an increased incidence of cancer diseases. As a result, atrazine has been listed in group C (possible human carcinogen) [4, 6] and withdrawn from the European market; however, it is still in use in Australia, and South and North America [7].

Because of its high solubility and mobility, atrazine has been detected in surface as well as ground waters [8, 9]. Simultaneously, it easily penetrates into deeper layers of the soil, where the degradation rate slows down [10]. This causes atrazine-containing runoff from agricultural lands to easily enter aquatic environments such as wetlands; there-

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fore the influence of atrazine on such systems has been extensively studied [11, 12]. As a consequence, atrazine has been classified as one of the major anthropogenic pollutants, which requires immediate attention and effective development of methods for its decontamination [13, 14].

Phytoremediation has been recognized as a potentially efficient and economically justified way to deal with atrazine contamination. Throughout numerous experiments, several atrazine-tolerant marsh plants, such as common club-rush (*Schoenoplectus lacustris*), bulrush (*Typha latifolia*), yellow iris (*Iris pseudacorus*), and common reed (*Phragmites australis*), as well as semiaquatic herbaceous perennial plants such as canna (*Canna generalis*), pickerel (*Pontaderia cordata*), and iris (*Iris x Charjoys Jan*) have been found and reported [15, 16]. High biomass production and resistance to the contamination are needed and crucial for the progress of a phytoremediation process [17]. However, in order to efficiently decontaminate, the plants should not only exhibit significant tolerance toward the pollutant, but also be capable of removing it from the environment and to transforming it into non-toxic endproducts [18]. Such plants may potentially be useful for enhancing atrazine removal, either actively, by direct uptake, or by improving the microbial activity of herbicide-degrading microorganisms [19]. Notable differences among the ability of various plant species to decontaminate a particular pollutant have been observed, which suggests that the natural biodiversity should be better explored and exploited in order to improve screening for the most appropriate plant species [18].

To explore the utility of these species for bioremediation of atrazine, the objective of this research was to investigate the phytoremediation potential of three aquatic plant species: sweet flag (*Acorus calamus* L.), broadleaf cattail (*Typha latifolia* L.), and narrow-leaf cattail (*Typha angustifolia* L.) in terms of atrazine tolerance and phytoremediation. The purpose of this study was to find a wetland plant species capable of effective atrazine removal and growth in heavily atrazine-contaminated areas, which may be readily applied in wetland phytoremediation processes.

Materials and Methods

Hydroponic Cultures

The experiments were carried out using sweet flag (*Acorus calamus* L.), broadleaf cattail (*Typha latifolia* L.) and narrow-leaf cattail (*Typha angustifolia* L.) plant species obtained from the commercial market. Plants were taken out of pots and the soil was carefully removed by washing them under running water several times. Afterward, the plants were transferred to hydroponic containers (0.75 l) with 300 ml of Hoagland's medium and left to grow for 14 days at 26±1ºC, light intensity of 20,000 luxes, and 16:8 hour photoperiod. The average wet biomass of control plants was: 39.4 g for sweet flag, 24.2 g for broadleaf cattail and 10.6 g for narrow-leaf cattail.

Screening for Atrazine Tolerance

The plants were grown for a week in samples containing different atrazine concentrations at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, and 40 mg/l. Upon finishing the experiments, the plants were collected, carefully rinsed with distilled water, and weighed. The obtained wet biomass values were compared to control samples. Based on the results, a mathematical model describing atrazine toxicity toward the studied plant species was built in the form of an equation:

$$Y = a + \left(\frac{b}{2^{(\frac{x}{c})^d}}\right)$$

...where:

a – the value of the lower theoretical asymptote

b – the value of the upper theoretical asymptote

c – the value indicating the point of inflection (the concentration of atrazine, which causes a 50% reduction of the wet biomass increase)

d – the value determining the slope

Kinetic parameters for all figures are presented in Table 1.

Plant Cultures in the Presence of Atrazine

Only the plants exhibiting satisfactory growth and a well developed root system were selected for the experiments. The plants were weighed and placed in Erlenmeyer flasks (0.5 l) and filled with 300 ml of Hoagland's medium containing an appropriate amount of atrazine. The concentration of atrazine used during this experiment was determined based on the toxicity model and differed depending on plant species. The concentrations were 3.5, 5, 7, 12, and 20 mg/l for *A. calamus*; 3.5, 5, 7, 10, and 12 mg/l for *T. lat-*

Table 1. Parameters for mathematical models used throughout the studies: SF – sweet flag, BC – broad leaf cattail, NC – narrow-leaf cattail; numbers refer to atrazine concentrations [mg·l^{-1}].

Fig. 1	a_1=0.2510	b_1=92.4953	c_1=24.3543	d_1=6.2776
	a_1=0.1680	b_1=94.8202	c_1=8.76013	d_1=4.2688
	a_1=0.0.014	b_1=92.0886	c_1=5.23709	d_1=5.1134
Fig. 2	a_1=102.251	b_1=0.025	k_1=0.1526	-
	a_1=102.480	b_1=0.103	k_1=0.1416	-
	a_1=102.657	b_1=0.414	k_1=0.1242	-
Fig. 3	a_1=103.251	b_1=0.025	k_1=0.0550	-
	a_1=98.1901	b_1=1.2358	k_1=0.0398	-
	a_1=90.8840	b_1=11.2560	k_1=0.0274	-
Fig. 4	a_1=76.603	b_1=21.9820	k_1=0.0714	-
	a_1=97.025	b_1=2.2918	k_1=0.0324	-
	a_1=77.918	b_1=54.5100	k_1=0.1032	-

ifolia and 3.5, 5, and 7 mg/l for *T. angustifolia*, respectively. For the elimination of microorganisms' influence on atrazine removal, 10 ml/l of plant preservative mixture (PPM) (Plant Technology Inc.) was added. The experimental plants were grown for 180 days at the same conditions as presented above. During the experiments, samples (1 ml) were collected directly from the cultivation broth in order to determine the concentration of atrazine by high performance liquid chromatography (HPLC) analysis. At the end of the experiments, all plants were weighed again and compared with control cultures without atrazine.

HPLC Analysis

Determination of atrazine was carried out using a MERCK-HITACHI system consisting of an autosampler (model L-7250), pump (model L-7100), and DAD (model L-7455) set at 220 nm. Analyses were performed isocratically at a flow rate of 0.60 ml/min, at 30ºC on a Lichrospher® RP-18 250 x 4.60 mm column (MERCK). Acetonitrile and 1 mM sodium acetate (35:65) were used as a mobile phase. Samples were filtered prior to injection (0.22 µm, Millex-GS, Millipore). A standard was used as reference. This was accomplished via computer integration (Chromatography Data Station Software, MERCK-HITACHI) operated in the mode of external standard. A standard calibration solution was prepared within the range 0.10-5.00 mg/l and 0.10-20.0 mg/l and depended on the variant of the experiment.

Statistical Analysis

The analysis of variance (ANOVA) was performed to test significant differences between the treated groups and control. Standard deviation and standard error were also calculated. The probability of α (type I error) was 5.00% ($P<0.05$).

During the initial experiments several calculations were carried out in order to determine the most suitable model. Based on the obtained data, it was concluded that the first-order kinetic equation was most fit for further modeling. The initial studies suggested that atrazine half-life value does not depend on its concentration in the studied range of concentrations. Therefore, the rate of atrazine removal was described by the one-phase exponential decay equation:

$$y = a \cdot \exp(-k \cdot t) + b$$

...where y is the concentration of atrazine [mg/l], t is time [days], a is the coefficient that represents the distance from the starting point to the bottom plateau, b is the bottom plateau value, and k is the rate constant [days^{-1}]. For each experiment, the half-life time ($t_{1/2}$) of atrazine was also calculated. The half-life time is the time required for half of the atrazine concentration to decay.

The half-life time and decay rate constant are related by the equation:

$$t_{1/2} = \frac{\ln(2)}{k} = \frac{0.693}{k}$$

Statistical analysis was carried out using STATISTICA (data analysis software system), version 6.0, Statsoft, Inc. (2004).

Results

Toxicity

The data obtained after plant growth experiments in the presence of atrazine was used to build a mathematical model. The model was used for determination of the 50% plant growth inhibition value (50% reduction of plant biomass increase) caused by atrazine. The results suggested that different species have different detoxification mechanisms (Fig. 1). The lowest susceptibility to pesticide was observed for sweet flag, which exhibited a biomass increase even in the presence of 30 mg/l of atrazine. For sweet flag, a 50% reduction of the biomass increase was observed at 24.3 mg/l of atrazine. The resistance of both cattail species was considerably lower. After the cultivation of broadleaf cattail, a 99% phytomass reduction could be observed for samples containing 14 mg/l of atrazine (compared to the control cultures), while the narrow-leaf cattail cultures exhibited a 99% reduction at only 8 mg/l of the xenobiotic (Fig. 1). The herbicide concentration, which caused a 50% reduction of the cattail biomass increase, was 5.24 mg/l for the narrow-leaf species and 8.76 mg/l for the broadleaf species, accordingly. During the cultivation of these plants in a medium containing a high level of atrazine, numerous morphological changes occurred (chlorosis, necrosis), which lead to withering and consequently resulted in a significantly lower biomass. Only the atrazine concentrations causing up to 90% reduction of plant biomass gain were used for further removal studies.

Atrazine Removal

Atrazine removal was monitored for 180 days days with subsequent mathematical modeling of further atrazine decrease for the next 180 days. The obtained results suggest that each of the studied species participated in the atrazine

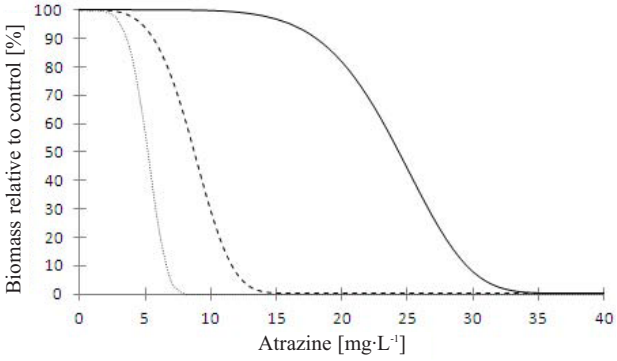

Fig. 1. Mathematical model describing atrazine toxicity toward the studied plant species: — sweet flag (*Acorus calamus* L.), - - - broadleaf cattail (*Typha latifolia*), and ••• narrow-leaf cattail (*Typha angustifolia*).

removal processes. The removal efficiency was different, depending on the plant species and atrazine concentration.

The most rapid atrazine removal occurred during hydroponic cultivation of sweet flag (*Acorus calamus* L.). When sweet flag grew in the presence of atrazine (3.5 mg/l), over 57% of the initial herbicide amount was reduced after 6 days with an average removal rate k = 0.1526 mg/day. Such a high removal rate resulted in a considerably low atrazine half-life time ($t_{1/2}$), equal to only 5 days (Fig. 2). After 21 days, a 97% reduction of the total herbicide content in the medium was observed (Fig. 2). Increasing the atrazine concentration had little effect. At the same time, approximately 90% of the herbicide was removed from the medium when atrazine was applied at the highest concentration of 20 mg/l. Overall, the removal pattern for all tested atrazine concentrations was similar.

The cattail species appeared to be more vulnerable. In both cases atrazine was degraded at a considerably lower rate (Figs. 3 and 4).

It is noticeable that in the case of broadleaf cattail it took approximately 50 days to reduce the initial atrazine concentrations (3.5 and 5 mg/l) by 90%. At the same time, less than 70% of atrazine supplemented at a concentration of 12 mg/l was removed. Additionally, at a concentration of 12 mg/l the herbicide appeared to be removable only up to 90%, as 10% of the initial concentration was detectable even after 180 days. The mathematical model also suggested a long persistence of atrazine at a concentration of 12 mg/l. At the same time, it took 21 days to decrease the lowest atrazine concentration (3.5 mg/l).

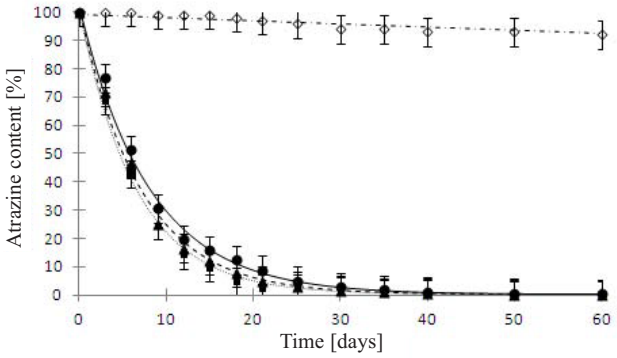

Fig. 2. Atrazine degradation in the presence of sweet flag (*Acorus calamus* L.). Points represent experimental data and lines represent the mathematical model. Atrazine concentration at: ● 3.5 mg·l^{-1} (—), ▲ 7 mg·l^{-1} (- - -), and ■ 20 mg·l^{-1} (•••).

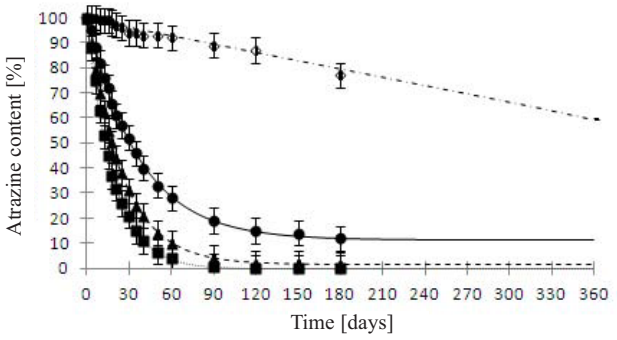

Fig. 3. Atrazine degradation in the presence of broad leaf cattail (*Typha latifolia*). Points represent experimental data and lines represent the mathematical model. Atrazine concentration at: ● 3.5 mg·l^{-1} (—), ▲ 7 mg·l^{-1} (- - -), and ■ 12 mg·l^{-1} (•••).

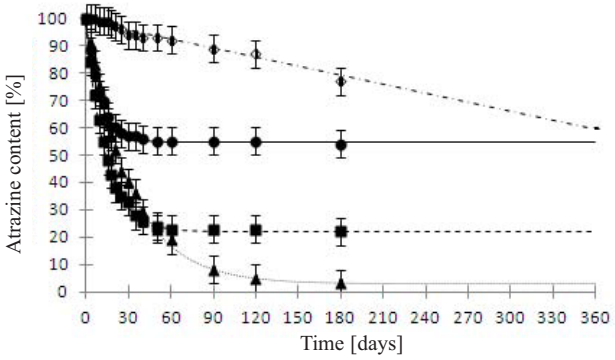

Fig. 4. Atrazine degradation in the presence of narrow-leaf cattail (*Typha angustifolia*). Points represent experimental data and lines represent the mathematical model. Atrazine concentration at: ● 3.5 mg·l^{-1} (—), ▲ 5 mg·l^{-1} (- - -), and ■ 7 mg·l^{-1} (•••).

Broadleaf cattail was generally more resistant than narrow-leaf cattail. Under the same conditions (3.5 mg/l) only 61% of the herbicide was degraded for the narrow-leaf cattail experiments. A further reduction to only 30% of the initial herbicide dosage was observed when atrazine was supplemented at a 5 mg/l concentration. However, the narrow-leaf cattail presented a very limited survival ability in the presence of atrazine at such a concentration.

Discussion

Phytoremediation is considered to be among the most popular strategies for atrazine removal [20-22]. Lin et al. have suggested that atrazine mostly persists in soil and only less than 15% of its initial content is lost prone to leaching [23]. However, several other studies have reported that atrazine has been commonly detected in surface and ground waters [24, 25]. Consequently, this contaminant is able to spread across a wide area, and flow into aquatic environments and drinking water sources [26]. The concentration of atrazine in runoff water is associated with its initial level during field treatment and may exceed 740 µg/l [27]. Furthermore, atrazine may be accumulated in some systems due to possible sorption [28], therefore finding appropriate plant species for decontamination of atrazine-polluted drainage water is of high priority.

As reported by Scott et al., collecting spent irrigation water for further reuse in farm activities may be very beneficial, providing time for pesticide residues to degrade [29]. Osborne and Kovacic have suggested that using wetlands as a discharge site for agricultural runoff may potentially be an effective approach [30]. It is commonly considered that wetlands play an important role in improving the quality of water [31]. Many wetland plants expansively take over the

water space, through a rapid development of rhizomes. The entangled rhizomes form a dense net that accumulates the organic material that flows in the water. Additionally, some plant species are able to grow in eutrophic conditions or even in an environment contaminated by waste water, which further contributes to their potential use in atrazine phytoremediation.

However, the tolerance of aquatic plants toward atrazine may significantly differ [32, 33]. Therefore, evaluating the susceptibility of a plant to atrazine is of highest priority when determining the usefulness of a given species in phytoremediation processes. Only the resistant species may be used for a direct or an indirect removal of xenobiotics, through the stimulation of indigenous soil microorganisms.

As clearly pointed out by Kawahigashi et al., plants for phytoremediation should be easily cultivated and maintained [34]. Additionally, they should also have a large biomass increase rate to cope with remediation of large amounts of chemicals in the field.

All of the tested plants are commonly found not only in wetlands but also in roadside and agricultural ditches. Under natural conditions, the chosen macrophytes grow in the muddy soil of a wetland environment, reaching a height of 1.5 m (sweet flag) to 2.5 m (cattails). They exhibit a fast growth rate and high biomass production. These features make them interesting for atrazine removal tests.

The results of the present studies confirmed that tolerance toward atrazine is species-dependent. Sweet flag exhibited the highest tolerance toward atrazine, with a 50% biomass growth inhibition value, over two times greater compared to broadleaf cattail and almost five times greater compared to the narrow-leaf cattail. This may confirm the hypothesis that the presence of atrazine may induce changes in the aquatic community composition. The diminishment of less tolerant species and as a response to cumulative atrazine contamination may be plausible.

Enhancing the atrazine removal rate is crucial for an effective phytoremediation process. Atrazine is characterized by a relatively slow removal rate. The disappearance rate of this herbicide in field conditions, referred to as its half-life period ($t_{1/2}$), is between four and 57 weeks [29]. As demonstrated by our study, the use of appropriate plant species is significant for a satisfactory reduction of atrazine content in a wetland environment. The use of less tolerant species may result in biomass growth deficiency at higher atrazine concentrations, which further contributes to an incomplete removal and a cumulative accumulation of the herbicide. However, using highly-resistant species, such as sweet flag, may result in a notable decrease of an atrazine half-life period (5 days).

Although the use of contaminant-tolerant species with a considerable phytoremediation potential appears to be a good bioremediation strategy, there are still several important environmental issues that must be considered before the application of wetlands for improving herbicide removal can be safely carried out. Future studies will focus on evaluating the long-term changes occurring in the aquatic community as a response to atrazine contamination and assessing the resistance mechanisms observed for sweet flag.

Conclusions

The present studies confirmed that both tolerance toward atrazine and its removal efficiency during phytoremediation processes carried out in an aquatic environment may significantly differ, depending on the plant species used. Out of the three studied wetland plant species, sweet flag (*Acorus calamus* L.) exhibited the most remarkable phytoremediation potential. It proved satisfactory in terms of both biomass growth and atrazine removal. The cattail species (*Typha angustifolia* and *T. latifolia*) were more susceptible to atrazine, resulting in a considerably decreased phytoremediation potential. Additionally, the subsequent mathematical modeling results obtained for the cattail species suggest that no changes in atrazine content may be observed, even after a period of 360 days.

Acknowledgements

This study was supported by Grant No. N N305 035434. Ł. C. and A. S. greatly appreciate Cooperation Grant PUT-PULS ICt 32-143/2010.

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