DOI : [http://doi.org/10.22438/jeb/40/3\(SI\)/Sp-22](http://doi.org/10.22438/jeb/40/3(SI)/Sp-22)

Uptake of 2,4-bis(Isopropylamino)-6-methylthio-s-triazine by *Canna indica*

Paper received: 17. 04. 2018

Revised received: 14. 11. 2018

Accepted: 03.01. 2019

Authors Info

S. Sun¹, P. Lv², R. Datta³, J. Ni¹,
Y. Su², D. Sarkar⁴ and Y. Zheng^{1,5*}

¹National Plateau Wetlands Research Center/Wetlands College, Southwest Forestry University, Kunming-650224, P. R. China

²Yunnan Entry-Exit Inspection and Quarantine Bureau, Kunming- 650228, P. R. China

³Biological Sciences Department, Michigan Technological University, Houghton M- 49931, USA

⁴Civil, Environmental, and Ocean Engineering Department Stevens Institute of Technology, Hoboken, NJ-07030, USA

⁵Yunnan Agricultural University, Kunming-650201, P.R. China

*Corresponding Author Email : zhengyi@swfu.edu.cn

Abstract

Aim: The aim of this study was to the removal potential of 2,4-bis (Isopropylamino)-6-methylthio-s-triazine by *Canna* from hydroponic media.

Methodology: Green house study was carried out to investigate the potential of *Canna* as phytoremediation of prometryn. Liquid-liquid extraction was used for extracting prometryn from nutrition solution. Solvent ultrasonic extraction was used for extracting prometryn from the plant and Gas Chromatography-Mass Spectrometer was used for determination.

Results: The results showed that. *Canna* could grow well in the prometryn spiked (0.5mg.l⁻¹) nutrient solution. *Canna* uptake and removal of prometryn from the media were monitored for a period of first order kinetics, $C_t = 0.06023e^{0.042t}$, $R_2 = 0.9078$ (without *Canna*), $C_t = 1.219e^{-0.232t}$ (with *Canna*), $R^2 = 0.8191$. The dissipation half-life ($T_{1/2}$) was shortened by 17days, the dissipation time required for 99% ($T_{0.99}$) prometryn was shortened from 109 days (without *Canna*) to 20 days (with *Canna*). Combined analysis with kinetic of prometryn in the solution and in the plants, the possible prometryn degradation by *Canna* occurred between day10 and day16.

Interpretation: The preliminary result was encouraging and selected ways of prometryn removal.

Key words: *Canna indica*, Herbicides, Hydroponic media, Phytoremediation, Prometryn

Edited by

Prof. Ahmed Jalal Khan Chowdhury

Reviewed by

Dr. Hongbo Zhang
Dr. Xiaopeng Jia

Citation: Sun, S., P. Lv, R. Datta, J. Ni, Y. Su, D. Sarkar and Y. Zheng: Uptake of 2,4-bis(Isopropylamino)-6-methylthio-s-triazine by *Canna indica*. *J. Environ. Biol.*, **40**, 577-583 (2019).

Introduction

2,4-bis (isopropylamino)-6-methylthio-1,3,5-triazine, with trade name prometryn, is a s-triazine herbicide. Prometryn is used as a pre-and post-emergent agricultural herbicide for the control of annual gramineous, broad-leaved and perennial terrestrial weeds in crops, such as corn, dill, carrots, parsley, peanuts, cotton sugarcane, tea, soybean, fruit, potato, pigeon peas, alfalfa, rice, forestry and gardens of nursery (Dikic 2014; Jiang et al., 2011). In addition, prometryn is also used to remove filamentous algae, aquatic weeds, and other harmful algae from fish, shrimp, carb, shellfish and sea cucumber aquaculture industries (Zhou et al., 2011; Wang et al., 2014; Akerblom, 2004; Jamal et al., 2018). Significant evidence showed that s-triazine is also a groundwater pollution with genotoxic, endocrine disrupting and immune-toxic. Prometryn has been reported as an endocrine disrupting chemical by the United States. Japan and other countries on mammals and humans, which also elicits significant ecological impacts by affecting the survival of aquatic plants and phytoplankton due to its chemical stability and biological toxicity (Schumer et al., 2015; Shi et al., 2014; Veliske et al., 2014; Zhang et al., 2014; Velisek et al., 2014; Zhao and Shu-Min, 2018). Due to toxic nature, persistence and high mobility prometryn has been banned in 2004 by the European Union and prohibited to directly use in water and intertidal areas, in sandy and sandy loam soils with low organic matter content in the US (Li et al., 2014; USEPA, 2015). However, it is still widely used in many countries like China, the United States, Canada, New Zealand and South Africa (Chou et al., 2009; Zou et al., 2012; Kegley, 2010; Khan et al., 2018). Because of its high chemical stability, prometryn persists in the aquatic environment and has the potential of harming non-target organisms, even pose potential risk to human beings through food chain. Prometryn has been detected above the prescribed limit in the sea products from China (Li et al., 2014). Previous studies have reported that prometryn residues widespread aquatic pollution (Zhang et al., 2014; Stara et al., 2012; Stara et al., 2014; Vryzas et al., 2011; Ren et al., 2013), and the estimated risk quotient has over the acceptable values, 0.05–9 mg kg⁻¹ in vegetables in United States, 0.05–0.2 mg kg⁻¹ in food products from animals and plants, 0.14–0.19 mg kg⁻¹ in marine food products in Japan, for human and eco-toxicological risk (Wang et al., 2014; Stara et al., 2012; Papadakis et al., 2015 b).

Remediation strategies for soils and water contaminated with pesticides can be physical, chemical, biological or a combination of some or all of these approaches (Pascal-Lorber and Laurent, 2011; Nazihah et al., 2018). Bioremediation is an emerging technology. Plants possess pesticide-degrading capacities, which can be applied in the successful remediation of contaminated fields and water (Eevers et al., 2017). As a cost-effective and eco-friendly technique, phytoremediation has a high public acceptance and can often be carried out on site, to remove, transform or degrade contamination with herbicides (Dosnon-

Olette et al., 2010; Rahman and Hasegawa, 2011; Marcacciet al., 2006; Niti et al., 2013). *Canna indica*, commonly known as Indian shot, belongs to family Cannaceae. It is a perennial herbaceous plant, commonly used as ornamental plants in coastal zone, constructed wetland. Besides its ornamental function, it has varied ecological functions, such as treatment of industrial waste waters through constructed wetlands, removal of high organic load, color and chlorinated organic compounds from paper mill wastewater, as phytoremediation plant of mining substrate, pesticides (atrazine), triazophos, BTEX (benzene, toluene, ethylbenzene and xylenes), heavy metals. *Canna* plants not only remove pollutants from the environment and store them in their roots and rhizome, but also transform some of the pollutants into nontoxic forms (Choudhary, 2011; Macci et al., 2015; Dong et al., 2016; Xiao et al., 2010; Boonsaner et al., 2011; Cule N, 2012). This plant has experimentally been removed to produce a large amount of biomass in the eutrophic water system, develops a very thick strong fibrous root system in hydric media, it can be used as phytoremediation plants for improving the water quality of aquaculture (Ojoawo et al., 2015; Chyan et al., 2016).

In view of the above, the present study was carried out to identify the removal potential of prometryn by *Canna indica* from hydroponic media

Materials and Methods

Canna plants were purchased from the Expro. Plant and Bird Market in Kunming, P.R.China. Plants were washed with tap water and acclimatized in 20% modified Hoagland nutrient solution in a greenhouse in Southwest Forest University for one week. 20°C under 16/8 hr light and dark photoperiod. After one week of acclimatization in greenhouse, plants of uniform size were selected and washed with distilled water. Weighted and then placed in 20% Hoagland solution supplemented with 0.5 mg l⁻¹ of prometryn. Plant-free media containers with prometryn and prometryn-free media containers with plants were maintained as controls. All treatments were conducted in triplicate. The pH of the solution was measured each time when the plant samples were collected, to ensure that the pH of solution was within the range of 5.5–8.5. Electrical conductivity was measured periodically in the growth media. Nutrient solution was periodically added to maintain the initial volume. Plant samples were collected on day 0, 6, 12, 18, 24 and 30. The root and shoot samples were washed using deionized water and dried using filter paper. The samples were cut into 2–3 mm pieces, weighted and used for extraction of prometryn. Two milligram of plant tissue was treated twice using 40 ml acetonitrile for 40 min. The two extracts were combined, filtered and concentrated using a rotary evaporator equipped with water-bath (RE 5298A, Shanghai, P. R. China). The concentrated extract was dissolved in 3 ml n-hexane and then filtered through 0.45 µm nylon membrane and used for further analysis.

Samples of nutrient solution were collected at same time as plant samples. An aliquot of 50 ml from each container was

filtered by cotton, extracted by 50 ml ethyl acetate twice, the extracts were combined, filtered and concentrated as described above. The concentrated extract was dissolved in 3.0 ml n-hexane and then filtered through 0.045 µm nylon membrane for analysis (Sun et al., 2016). An equal volume of nutrient solution was added in the containers to make up the volume.

All extracted samples were analyzed using GC-MS (Agilent 7890B-5977AMSD). The samples were introduced into the machine using an Agilent automatic sample injector (Agilent 7693A, G4513A, China). The capillary column was 30m×0.32mm ID × 0.25µm HP-5MS(Agilent Technology 19019J-413, USA). Injection temperature was maintained at 260°C, injection volume was 1.0 µl with splitless. Helium at a flow rate of 1.0 ml min⁻¹ was used as a carrier gas by raising the temperature to 180°C at 25°C min⁻¹ from 70 °C and maintaining it for 1 min. This was followed by raising the temperature to 220°C at 5°C min⁻¹, followed by raising the temperature to 280°C at 20°C min⁻¹ and maintaining it for 3 min. Chromato Solution Light Chemstation software was employed to acquire and process chromatographic data obtained from GC. Mass spectrometry conditions, the ion source was electron bombardment ion (EI.), electron energy was 70eV, the temperature of ion source was 230°C, interface temperature was 280°C, temperature of quadrupole was 150 °C. Solvent delay time was 3.75 min; Full scan mode (SCAN) with selected ion monitoring mode (SIM) simultaneously with scan range of (m/z) 50-350, selected ion monitoring (SIM) was m/z 241, 184, 226, 199; Quantitative ion was m/z 184. The structure identifications were based on the interpretation of fragmentation pathways.

Statistical analyses: The data of this study were calculated, and the first-order equation was fitted by Microsoft Excel 2003. The significance were tested and analyzed by SPSS 17.0.

Results and Discussion

The method of prometryn extraction from the solution and *Canna* was same with that of *Vetiver* grass(Sun et al., 2016 a). As compared with the selective ion chromatogram of prometryn technical (Fig. 1 A), it was found that the selective chromatogram of prometryn from solution sample Fig.1 (B) was basically not affected by the complex matrix, some other impure peaks in the full scanning (Fig. 1 C), compared with the chromatogram full scanning of prometryn technical (Fig. 1 D), the characteristic ion was not interfered by the matrix of roots of *Canna* (Fig. 1E). The calibration curve of prometryn obtained by this method were $Y = 29177 X + 91.334$ (Y, represent the concentration of prometryn, X represent the area of peak), $R^2 = .9953$ for quantitative sample's analysis at the range of 0.01 to 1.0 mg l⁻¹ and $Y = 44966X - 17131$ $R^2 = .9989$ for quantitative sample analysis at the range of 1.0-10 mg l⁻¹. The limit of detection was 0.002 mg l⁻¹ and the method was to meet the demand of the experiment. The limit of detection was lower than that of using gas chromatography-nitrogen chemiluminescence detection (Sun et al., 2016a).

Canna indica can survive and grow normally in the prometryn spiked nutrient solution(0.5 mg l⁻¹) without eligible poison symptoms, the total biomass of each treatment increased at a mean of 60 g deducted total sampling biomass (3-5 g each time), no eligible symptoms of nutrient deficiency were observed. The conductivities of solution decreased slightly with the passage of time, possibly induced due to the uptake of nutrient ions by plant. It was reported that the growth and physiological metabolism of *Canna indica* was not affected by the low concentration(<1mg.l⁻¹) of triazophos, which confirmed that *Canna indica* have the potential resistance to those kind of organic pesticide with the spiked concentration. The pH values of the nutrient solutions increased slightly from 6.1±0.23 to 8.2±0.19 from initial time to day 20. This result was similar with the result of pH values change in the nutrient solution planted with marsh pennywort. Chlorophyll SPAD value of *Canna* leaves varied among different leaves, some labeled point was measured at each sampling time, the kinetic value was relatively stable between +2 and -2 from day 0 to day 20. Therefore, the spiked prometryn played no effect on the SPAD value. Previous studies have reported that chlorophyll of *Canna* is sensitive to triazophos. The photosynthetic of *Canna* was severely damaged system ≥ 10 mg l⁻¹ triazophos concentration in 7 days (Xiao et al., 2008). The stress tolerance of plant is influenced by multi-factors, including plant, concentration of contaminations, stressed time and plant growth surrounding.

Concentration of prometryn in the solution decreased with time. Compared to control, *Canna* expedited the uptake significantly; the removal rate increased by 10% from day 5 to day 10, extra 53.06% removal was found at day 15 and 41.74% at day 20, respectively. The kinetic residue of prometryn in the solution and in the plants were analyzed, the first removal period of prometryn from the media was from day 0 to day 10, the main removed rate of prometryn was due to the uptake of *Canna*, 8.6% of spiked initial prometryn was removed by the uptaking of *Canna*. The second removal period of prometryn from the media was from day 10 to day 16, the prometryn was possibly degraded in this stage apart from the uptaking by *Canna*. It was reported that 41.6 % triazophos was removed by *Canna Indica* L. at day 21, and the main mechanism should be the phosphatase induced by *Canna* (Cheng et al., 2008). The contributions of *Canna indica* on the removal of triazophos were 65.5%, was the largest part of contribution compared with the natural hydrolysis of 8.7% and microbial of 25.8% (Xiao, 2006).

The removal of prometryn from nutrient solution matched with the first equation of first order kinetics, $Y = 0.06023e^{-0.042x}$, $R^2 = 0.9078$ (CK), $Y = 1.219e^{-0.232x}$, $R^2 = 0.8191$, the degradation half-life ($T_{1/2}$) was reduced from 20 days to 3 days, the time required for removal of 99% prometryn ($T_{0.99}$) reduced from 109 days (without plant) to 20 days (with *Canna*). Prometryn with initial concentration of 2.5 mg l⁻¹ reduced half-life by *Vetiver* grass with 11.5 days. It has been reported that *Vetiver* grass and marsh

Fig. 1 : GC-MS chromatogram of Prometryn (A) extracted ion chromatogram of prometryn technical with SIM sanning; (B) extracted ion chromatograms of prometryn from spiked solution with SIM sanning; (C) Extracted ion chromatogram of prometryn from roots of *Canna*; (D) full scanning chromatogram of prometryn technical € full scanning chromatogram of prometryn from the roots of *Canna*.

pennywort can remove prometryn from hydroponic media (Ni et al., 2018, Sun et al., 2016b), Compared with the efficiency of Vetiver grass and marsh pennywort for removal of prometryn from hydroponic media, *Canna indica* is also a good potential candidate for removal of prometryn. Possibly, these plants can be

used for phytoremediation (Sun et al., 2016). The efficiency of several plants combined prospectively be focused in the following research.

As shown in Fig. 4. the faster uptake by roots occurred

Fig. 2 : Prometryne dynamic concentration in water

Fig. 3 : First order kinetic equation of concentration change of prometryn in aqueous solution.

Fig. 4 : Dynamic apparent residue of prometryn in roots and leaves of Canna.

from day 0 to day 10, and the transfer peak from roots to leaves occurred between day 10 to day 15, the apparent residue decreased both in roots and leaves from day 15, combined with

the kinetic concentration in the solution, the major degradation occurred during this period.

Pesticides and its eco-toxicological risk in environment has raised certain concern (Zhang *et al.*, 2014; Stara *et al.*, 2014; Vryzaset *et al.*, 2011; Ren *et al.*, 2013; Papadakis *et al.*, 2015a). However, most researches still focus on the determination method in the food and environment (Bernardiet *et al.*, 2016; Da *et al.*, 2018; Qin *et al.*, 2016; Xiao *et al.*, 2008). Phytoremediation also was used as the removal of pesticide from the soils (Eevers *et al.*, 2017), for wetland vegetations, most researches were focused on the nitrogen and phosphorus removal from hydroponic media (Eevers *et al.*, 2017; Hongxia *et al.*, 2017), as a kind of wetland plant, canna was used as some organic pollutants and heavy metal's removal (Liu *et al.*, 2015; Ojoawo *et al.*, 2015, Subhashini and Swamy, 2014, Xiao *et al.*, 2010). This study confirmed that Canna was tolerant to 0.5 mg.l⁻¹prometryn, which was a potential wetland plant for pollution remediation induced by herbicide.

The dissipation half-life ($T_{1/2}$) reduced from 20 days to 3 days, the dissipation time required for 99% ($T_{0.99}$) prometryn was shortened significantly. The first removal period during day 0 to day 10 was contributed by uptake of Canna, the second faster removal period was from day 10 to day 16, the possible degradation happened at this stage. The mechanism of degradation and metabolites was investigated in the following step.

Acknowledgments

This work was supported by the National Natural Science Fund of China (No. NSFC41563014, NSFC31460551, NSFC41461059) and the Center of Postdoctoral Studies of Landscape Architecture, Southwest Forestry University, Kunming, the People's Republic of China.

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