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Transport and dissipation study of the herbicide terbuthylazine and its major metabolites in wetland sediment substrates planted with *Typha latifolia* L.

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ABSTRACT

It is widely recognized that the organic micropollutants, coming from the intensive agricultural use of land, are the major thread against surface and ground water. However, they are an environmental engineering challenge in order to encounter the pollution by the use of constructed wetlands. The aim of this work is the study of the potential transport and dissipation of the herbicide terbuthylazine (TER) and its major hydroxy and dealkylated metabolites at the vertical profile of a constructed wetland sediment substrate, planted with *Typha latifolia* L., in order to determine the processes and study the possible remediation mechanisms for wetland ecosystems contaminated by the aforementioned substances. The results show that the dissipation of TER exhibits a gradient behavior through depth of the sediment substrate of wetlands and its major degradation products follow the effect of biotic and abiotic mechanisms of degradation in the bioreactor substrate. Moreover, the greater recovery of the herbicide appears in the sediments substrate with zeolite content.

Keywords: Remediation; Biodegradation; Constructed wetland; *Typha latifolia* L.; Sediment; Terbuthylazine metabolites; Zeolite

1. Introduction

s-Triazines are used worldwide as selective pre- and post-emergence herbicides for the control of both grasses and broadleaf weeds in many agricultural crops like corn, wheat, maize, barley, sorghum, grape, peaches, apple, and asparagus as well as for non-agricultural purposes such as soil sterilization and road maintenance [1]. During and after the herbicide application to the farming land, triazines may be transported to both ground and surface water but also into the atmosphere [2]. Atrazine (AT) is the most commonly used and the main representative of s-triazines. Due to environmental pollution, the commercial use of atrazine has been forbidden in the European Union [3] and has been gradually replaced by terbuthylazine (TER). In water and soil, TER is subjected to various biotic and abiotic degradation processes such as photolysis, oxidation, hydrolysis, and

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biodegradation, leading to dealkylation of the amine groups, dechlorination, and subsequent hydroxylation. The main degradation products in ground and surface waters via biotic mechanism are the dealkylated chloro metabolites, such as desethylterbuthylazine (DET) and desisopropylatrazine (DIA) [4–6]. Hydroxyterbuthylazine (HT) is the major abiotic degradation product in water and soil [7]. Other major metabolites of TER are desethylhydroxyterbuthylazine (DEHT) and desisopropylhydroxyatrazine (DIHA) [8–10] (Fig. 1).

Constructed wetlands are commonly used for the treatment of agricultural, municipal, industrial, and storm water waste mainly for inorganic pollutants. There has been little evaluation of their treatment of pesticides by constructed wetlands due to the fact that these organic compounds and their transformation products are difficult to analyze so the research is limited [11,12]. On the other hand, other contaminants such as metals, does not form degradation products and are easier to measure [13,14]. The use of constructed wetlands is an environmental friendly, low cost method of waste treatment, based on wetland plants and soil substrate texture [15–20].

Understanding of sorption is essential to predict the persistence and transport of herbicides through the wetland soil. Sorption is the major process that controls the degradation (both biotic and abiotic) and the mobility of a herbicide in soil [21]. If the herbicide is irreversibly bound to a soil or its desorption is very slow, its mobility or its release back to solution is negligible. In addition, transformations of organic compounds in a constructed wetland are carried out by sediment-borne microorganisms associated with the wetland plant community. Moreover, if an organic contaminant is sorbed by the sediment substrate, the bioavailability of compound to the microorganisms community, associated with the wetland plant in soil solution will be decreased. As a result the biodegradation of the pollutant is limited. Thus it is crucial to determine the potential transport and dissipation of an organic pollutant and its degradation products in order to make out further useful points concerning the biodegradation and sorption mechanisms. Binding of a pollutant and its degradates to the wetland sediment may also be an important mechanism of loss from the water column. There are studies providing evidence that the wetland sediment is an important sink for triazine residues [21,22]. In the aforementioned studies, the sediment substrate was tested as a whole homogeneous unit, without taking into account the probable gradient dissipation of the pollutant in the vertical profile of the substrate. Moreover in our study is attempted to determine the potential core of rhizosphere bioreactor of *Typha latifolia* L. in the vertical profile of substrate.

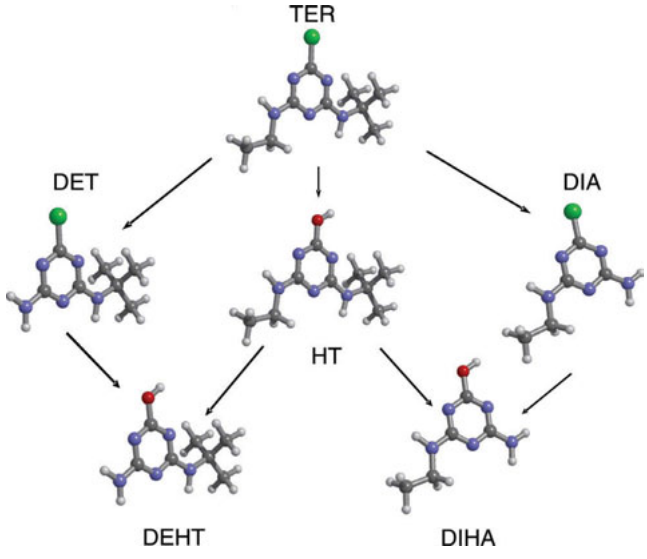

Fig. 1. The major TER degradation pathway.

The aim of this work is the study of the transport and dissipation of the herbicide TER together with its major hydroxy and dealkylated metabolites at the vertical profile (10, 20 and 30 cm) of a constructed wetland sediment substrate, planted with *Typha latifolia* L., in the context of an extensive remediation program that takes place in our laboratory.

2. Materials and methods

2.1. Wetland microcosms

Eight commercial plastic containers are appropriately transformed. In order to resemble to the free surface of wetlands, the upper layer of the container has been removed. The dimensions of wetland cells were 0.28 m width, 0.45 m height and 0.70 m length with a total area of 0.196 m^2. The wetlands were filled with 60 l of substrate. Two types of substrate were used: (1) sandyloam soil for four wetlands, (2) a mixture of sandyloam soil and zeolite (clinoptilolite) for the other four. The proportion of soil/zeolite was 4/1 per volume. The sandyloam soil was excavated from 30 to 40 cm depth, from an adjacent area of the Gallikos River in the municipality of Sindos, Thessaloniki, Greece. Prior the construction of wetlands, the soil substrate was analyzed for potential residues of TER and its metabolites. The soil was classified as a sandyloam (68% sand, 23% silt and 9% clay) with an organic matter content of 0.46% The cation exchange capacity (CEC) was 14 meq/100 g for sandyloam soil (S) and 226 meq/100 g for the 20% zeolite substrate (SZ). Four wetlands were planted with six (6P) *Typha latifolia* L. rhizomes and the other four with two (2P) rhizomes. Wetland microcosms were kept under cover preventing them from direct sunlight and rain exposure. Studies were conducted

from May to September in Northern Greece. The level of water was maintained to 10 cm. Seven liters of a TER solution (1.5 mg/l) was applied in the first day of the experiment. In order to prepare the applied 1.5 mg/l TER solution 10.5 mg of analytical-grade TER were initially dissolved in 200 ml acetone and then diluted with the appropriate volume of pure water. For the rest of the days (2nd–44th) an appropriate amount of pure tap water was loaded in the entrance of each wetland daily in order to maintain the free water level at 10 cm (30 l water totally in every constructed wetland), counterbalancing the losses from evaporation and evapotranspiration.

Sediments samples were taken from each wetland after the end of experiment (45th day) at three depths, 10, 20 and 30 cm of the wetland substrate, in order to study the distribution of TER and its metabolites in the vertical profile of the wetlands. The sampling performed using a soil-coring device that was inserted to the bottom of wetland. The samples were air dried and maintained in air tight containers in –35°C until the analysis procedure.

2.2. Sample preparation

Regarding the sediments samples extraction procedure, the samples were air-dried for a week at room temperature and then are sieved trough a 500 μm sieve in order to remove potential solid residues such as stones, plant residues and dead insects. Ten grams of soil samples were extracted with 25 ml ultra pure water by shaking on an orbital shaker for 2 h. The supernatant liquid fraction was removed and vacuum filtered trough 0.2 μm Titan membranes in Buchner funnels. The extraction was repeated with another 25 ml ultra-pure water and the extract was filtered as above. Prior to the use of ultra pure water, various other extraction solvents have also been evaluated for monitoring the recoveries and the chromatographic behavior of analytes. Owing to the amine moieties of TER and its metabolites, they show significant affinity for the negatively charge clay minerals. The addition of an appropriate concentration of HCl, can potentially replace the adsorbed analytes from the soil matrix with H^+ with an ion exchange mechanism. Two different concentrations of aqueous HCl, namely 1 and 5 N have been used. The analyte recoveries were very low, with important interference in the chromatogram for all substances assayed for both HCl concentrations (1 and 5 N). Additionally, a mixture (1:1, v/v) of acetone: 0.1 N HCl was used for the cleanup step, resulting also in a high degree of interference. The use of ultra pure water in the aforementioned extraction step resulted to the optimum recovery values for all analytes (both alkylated and hydroxy) (91.8% TER, 89.3% DEHT, 95.3% DET, 96.5% DIA, 96.3% HT, 93.0 % DIHA).

Sample clean up was performed using Oasis® MCX SPE cartridges (60 mg, 3 ml). The cartridges were equilibrated initially with 2 ml methanol (MeOH) and subsequently rinsed with 2 ml HPLC-grade water. After the sample loading, the cartridges were washed with 2.0 ml 0.1 N HCl and subsequently with 4 ml MeOH, dried under vacuum and finally eluted using 3 ml of 4% ammonium hydroxide in acetonitrile (AcN). The extract was then dried under a gentle N_2 stream and reconstituted with 10% AcN in 0.1 N HCl . The limit of detection (LOD) of the method was found to be 3.3 ng g^{-1} for all substances analyzed [23].

2.3. HPLC analysis

A high performance liquid chromatography (HPLC) system comprising of a Spectra system P4000 quaternary pump (Finnigan, Riviera Beach, FL), equipped with a 7725i injector (Rheodyne, Rohnert Park, CA) coupled to a Finnigan Spectra system UV 6000LP diode array detector (DAD). The gradient elution program used for the separation of the six substances is described briefly as follows: Initial conditions were 90% ammonium acetate (AMA 0.01 M) and 10% AcN followed by a linear gradient to 100% AcN in 10 min and the flow rate was 1 ml/min. After that the above gradient maintained for 5 min and then followed decrease to 10% AcN in 2 min. At the end of each run, i.e. 17 min, the column was left to equilibrate at the starting mobile phase composition (i.e. 90% A–10% B) for an additional 3 min, giving a total chromatographic analysis time of 20 min. The flow rate was 1 ml min^{-1}. The UV spectral confirmation for all analytes was achieved acquiring spectra between 200 and 400 nm with the aid of the DAD system. The maximum absorbance (λ_{max}) for all substances was determined to be at 235 nm, thus the recording of the chromatograms at the aforementioned wavelength in order to gain maximum sensitivity.

3. Results and discussion

The results show that TER exhibits a gradient distribution through the depth of sediment substrate of constructed wetlands. As the depth of substrate is increased the concentration of TER is decreased with the corresponding values ranging from 177.4 at 10 cm to 14.4 ng/g at 30 cm depth. At the bottom (30 cm depth) of the wetland, the lowest concentration of TER has been detected (Table 1). It has been pointed out by other studies that the amount of ATR that is absorbed by constructed wetland substrates reaches 38% of the initial amount of substance that has been applied in the wetlands, with the major amount 48.9% of the herbicide to be accumulated in the free water column of the wetland

Table 1
Vertical distribution of TER (ng/g) at the sediment substrate of constructed wetlands (**S** soil, **SZ** soil with 20% zeolite, **P** rhizomes of *Typha latifolia* L.)

TER (n = 2)					
Depth (cm)	**SZ-6P**	**S-6P**	**SZ-2P**	**S-2P**	**Mean**
10	157.5	96.8	177.4	113.6	136.3
20	139.9	82.4	145.4	75.5	110.8
30	41.0	14.4	47.1	17.7	30.0
t-test	t_{05} = 4.45		t_{05} = 4.31		

[14,21]. However, in the aforementioned studies the estimation of the herbicide distribution (mass balance) was performed without pointing out the possible gradient distribution of the herbicide in the substrate, the approach that has been followed in our study. After the mass balance evaluation of the initial amount (10.5 mg) of TER that has been applied in each wetland, the mean recovery for each layer of sediment substrate (10, 20 and 30 cm) has indicated that 25.4% of TER was accumulated in the upper layer of 10 cm, 20.6% in the middle 20 cm and only 5.5% in the last 30 cm supporting its gradient distribution at the sediment substrate.

Moreover, the results show that there are differences between the wetlands with different substrate. The wetlands with a zeolite content (SZ) in their substrate, show greater recovery of TER than those with a pure soil substrate without zeolite (S). The phenomenon appears both for wetlands with six and two rhizomes of *Typha latifolia* L. with zeolite substrate SZ-6P, SZ-2P. The aforementioned behavior of TER in the zeolite containing substrate (SZ) can be explained by the greater sorption of parent molecule to the zeolite absorbent compared to that of the pure soil substrate. This can be explained probably due to the fact that zeolite contains more available absorption moieties for TER than that of pure soil. As the TER molecule contains amine moieties that can be positively charged, it is possible that it is more susceptible to sorption by the zeolite available negative moieties than that of pure sandyloam substrate. It is important to point out the probability of bound residues in sediment substrate of wetlands that can't be recovered from the sediment matrix by the aforementioned analytical method due to strong absorption [14]. As sorption of triazines to soils and sediments is dependent largely on the quantity as well as the quality of the sediment organic matter, the low content of organic matter (0.46%) indicates that its role in sorption of TER in this substrate was negligible. In addition, the probable systematic error of non extractable residues of TER from organic matter is the same both for the pure soil and the soil–zeolite substrate. As it has been aforementioned the recovery values are acceptable for the specific sediment samples. The analytical method applied can extract the available analytes directly from the soil solution that are sorbed to the sediment matrix and can be desorbed to the soil solution, as the recoveries are above 89.3% for all analytes. The use of an organic solvent for the extraction of TER has demonstrated slightly higher recoveries from the matrix for TER. However, as mentioned in the sample preparation section, the use of ultra pure water shows the maximum recoveries for all analytes in one extraction step both for the parent molecule and for the hydroxy and *n*-dealkylated metabolites. Thus the use of ultra pure water demonstrates selective extraction for all aforementioned analytes from the specific soil matrix (68% sand, 23% silt and 9% clay), with optimum chromatographic behavior and minimum interferences from the matrix.

Regarding the vertical distribution of *n*-dealkylated TER metabolites DET and DIA in the sediment substrate of wetlands, the data show a tendency to be accumulated at the 20 cm at the middle depth of substrate and then at the depth of 10 cm (Table 2).

This tendency can be explained by the fact that the *n*-dealkylated metabolites were primarily biotic degradation products [8]. Since the core of the bioreactor exist in the rhizosphere of *Typha latifolia* L. plants at the 20 cm of sediment substrate, the greater production of *n*-dealkylated metabolites in these area can lead to the greater detection of DIA and DET comparably with the rest of substrate, 10 and 30 cm. During the aforementioned mechanism of rhizosphere biodegradation, the plant

Table 2
Vertical distribution of DET, DIA and HT metabolites (ng/g) at the sediment substrate of constructed wetlands (**S** soil, **SZ** soil with 20% zeolite, **P** rhizomes of *Typha latifolia* L.)

Depth (cm)	**S-6P**	**S-2P**	**SZ-2P**	**SZ-6P**
DET (n = 2)				
10	23.2	28.9	30.8	15.7
20	30.4	33.5	34.3	19.3
30	5.0	3.7	2.1	nd
DIA (n = 2)				
10	nd	nd	2.2	nd
20	2.2	2.0	3.1	nd
30	nd	nd	nd	nd
HT (n = 2)				
10	30.1	34.8	33.4	31.7
20	26.4	27.6	11.3	29.6
30	5.6	7.1	4.2	7.3

releases natural substances through its roots that supply nutrients to micro-organisms in the soil. The microorganisms enhance biological degradation [24].

Moreover as have been reported from other studies of the herbicide ATR distribution and its metabolites thereof in soils and sediments from wetlands, the *n*-dealkylated metabolites of ATR like DIA and DEA, have greater solubility in water compared to ATR and show greater accumulation at the lower depths of the wetland substrate (water solubility of ATR, DIA and DEA is 0.5, 1.2 and 2.0 mM/l respectively). Moreover, the above metabolites have lower absorption capacity to the wetland substrate compared to ATR and the hydroxyatrazine (HA) metabolites [25–27]. Consequently, even if the *n*-dealkylated metabolites DIA and DET of TER result from both abiotic and biotic metabolism that takes place mainly at the surface water of wetlands, they can be transported to the lower depths of sediment substrate. In addition the K_{ow} of DET is lower (1.98) than that of TER (3.21), supporting the role of the lower lipophilicity of dealkylated products that can be transported at the deeper layers of the sediments substrate.

The hydroxy metabolite HT of herbicide TER shows a tendency to be detected to the first 10 cm depth of sediment substrate of all constructed wetlands, (Table 2). The lower water solubility of HT and the greater absorption to substrate compared to the other metabolites leads to slower mobility to the lower layers of sediment substrates of wetlands. The same behavior is reported by other studies for the HA metabolite of ATR [25–27]. In addition, the HT metabolite is produced both by biotic in *Typha latifolia* L. rhizosphere and by abiotic metabolism mainly at the surface water of wetland. This leads to the greater detection of HT at the 10 cm of sediment substrate. In another study has been reported that hydroxylated compounds have lower solubility than TER, they preferentially accumulate in the first 10 cm of the soil layer and therefore may be considered as less potentially polluting the ground water [7].

Regarding the DIHA and DEHT metabolites, they were not detected (<LOD), so their absorption to the sediment substrate has not been accounted for the fate of herbicide to the wetland system.

4. Conclusions

Wetland microcosms may be used to remove TER as up to 25.4% of the initial amount can be retarded in sediment due to the fact that the substrate is a significant compartment for herbicide fate in these wetlands. Moreover the gradient dissipation in the vertical profile of substrate can be useful for further remediation studies of herbicide TER because the sorption of both herbicide and its metabolites, potentially removes pesticides from contaminated water preventing contamination of surface and ground water by the run-off and leaching phenomena. In addition, the higher recoveries in the middle layer of substrate of *n*-dealkylated metabolites of TER that are the major biotic products of metabolism, indicate that the core of bioreactor exists in the rhizosphere of *Typha latifolia* L. wetland plant close to the associated microorganisms that biodegrade the organic pollutant.

Finally, the study of dissipation, persistent and transport of herbicide and its metabolites at the sediment substrate of constructed wetlands should be considered as a useful tool for the further study of wetland remediation management.

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