Behaviour of pesticides during the denitrification process

Kristina Panikova[1], Zuzana Bilkova[2], Jitka Mala[1]
[1]Institute of Chemistry
Brno University of Technology
Žižkova 17, 602 00 Brno
[2]RECETOX
Masaryk University
Kamenice 753/5, Pavilion A29, 625 00 Brno
CZECH REPUBLIC

kristina.panikova@vutbr.cz

Abstract: The presence of pesticides has been proven in many groundwater bodies worldwide. However, information about their behaviour and fate in anoxic conditions is lacking. The aim of this paper is to study the behaviour of selected pesticides during the process of denitrification. Laboratory batch tests were performed with terbuthylazine, atrazine, and tebuconazole. Poplar wood shavings were used as a carbon source for denitrification. The effect of pesticides on the denitrification process was evaluated from their inhibition of the denitrification rate. Abiotic and biotic losses were measured with high-performance liquid chromatography. No toxic effect on denitrification was observed. Biotic loss was found with terbuthylazine and atrazine. The highest abiotic loss was observed with tebuconazole.

Key Words: biodegradation, adsorption, wood shavings, terbuthylazine, atrazine, tebuconazole

INTRODUCTION

Pesticides rank among the most hazardous environmental pollutants due to their stability, mobility, high accumulative and persistent nature, and long-term effects on living organisms (Nasiri et al. 2020). The presence of pesticides in ecosystems has adverse effects which vary with the contaminant concentration, amount, and exposure time (Rice et al. 2007). However, current systems of agricultural management rely on the use of pesticides, meaning that about 3.6×10^6 t/y are applied worldwide (Food and Agriculture Organization of the United Nations. 2020). It was estimated that 10% of all pesticides applied to the soil reach non-target areas (Schulz 2004). In water, these compounds may metamorphose to produce substances with even greater toxicity (Nasiri et al. 2020). In 2018, metabolites of chloridazon, alachlor, metazachlor, metolachlor, acetochlor, atrazine, and dimethachlor were found in more than 5% of samples of groundwater in the Czech Republic (Kodeš 2019).

Pesticides from agricultural areas can be transported to groundwater together with nitrates, which are other typical pollutants found in agricultural run-offs. Nitrates undergo denitrification under anoxic conditions in subsurface regions. Similar conditions also prevail in denitrifying woodchip bioreactors, a passive treatment technology for the removal of nitrates from agricultural outflows (Weigelhofer and Hein 2015).

Atrazine (ATR) is a herbicide and its metabolites can persist in water and soil for decades (Jablonowski et al. 2011).

Due to the prohibition of atrazine in the European Union, terbuthylazine (TER) has gradually come to be employed as a replacement herbicide. It is considered to be the most persistent triazine herbicide in surface environments (Gikas et al. 2018).

Tebuconazole (TEB) is a systemic fungicide used against a variety of diseases affecting cereals and maize, and which controls numerous pathogens. It is highly adsorbed by soils and mainly concentrated in the topsoil layer (Herrero-Hernández et al. 2011).

Unfortunately, there is no standardized test available to predict the fate of chemical substances during denitrification. The aim of this work was to investigate the behaviour of three selected pesticides during the process of denitrification using the authors' own laboratory incubation test developed for this purpose (Pániková and Malá 2019).

MATERIALS AND METHODS

Chemicals and organic carrier

All of the analytical standards (atrazine, terbuthylazine and tebuconazole from the PESTANAL® product line) were obtained from Sigma-Aldrich (Germany) at ≥ 98% purity. Each stock solution was prepared in analytical grade methanol at a concentration of 1000 mg/l. The analytical standards were stored in the dark at 4 °C.

With respect to the employed denitrifying woodchip bioreactors, wood shavings were used as a bacterial carrier and a source of organic carbon. The wood shavings were from poplar trees; they were sieved at the 1.0–1.5 cm fraction. Argon gas (purity 99.996%) was purchased from Linde Gas (Czech Republic).

The laboratory batch testing of denitrification

The denitrification process was simulated in 2-litre bottles as a laboratory batch test. The principle behind the test is clear from Table 1.

Table 1 Overview of the sample types

Sample	Composition of liquid medium, solution in deionised water (DIW)	Description
1	KNO_3 (30 mg/l NO_3-N), $NaHCO_3$ (3 g/l for ATR and TER, 0.5 g/l for TEB), methanol (0.1 ml/l)	Denitrification in progress.
2	KNO_3 (30 mg/l NO_3-N), $NaHCO_3$ (3 g/l for ATR and TER, 0.5 g/l for TEB), the tested pesticide (100 µg/l; solution in 0.1 ml/l of methanol)	Denitrification and biodegradation in progress.
3	KNO_3 (30 mg/l NO_3-N), $NaHCO_3$ (3 g/l for ATR and TER, 0.5 g/l for TEB), the tested pesticide (100 µg/l; solution in 0.1 ml/l of methanol), $HgCl_2$ (6.5 mg/l, inhibitor)	Biological processes are stopped.

At the beginning of the test, each bottle contained 25 g of poplar wood shavings and 2000 ml of liquid phase containing $NaHCO_3$ and KNO_3 (see Table 1). The bottles were aerated with argon until the concentration of dissolved oxygen (DO) dropped below 0.5 mg/l. The bottles were closed and incubated at $T = 20 \pm 0.5$ °C in the dark. After 48 hours, 25 ml samples of liquid phase were taken from every bottle and concentrations of NO_X-N, pH, and DO were analysed. The bottles were divided into three groups (see Table 1) and the reagents were added. After mixing, 10 ml of liquid phase was taken from samples 2 and 3. These solutions were analysed for the initial concentration of pesticide. The bottles were closed and incubated at $T = 20 \pm 0.5$ °C in the dark. Each sample had two replicates.

The test was terminated after seven days. Immediately after opening, liquid phase from samples 2 and 3 was collected for analysis of the tested pesticide. The remaining liquid phase was filtered through filter paper for qualitative analysis KA2, and concentrations of NO_X-N, pH, DO and COD were measured in the supernatant.

Analytical methods

The laboratory analyses were performed as follows: DO and pH with a Hach HQ40D multi meter, COD via the semi-micro method with potassium dichromate and photometric evaluation, and NO_X-N (NO_3-N+ NO_2-N) via the UV absorption method with a Hach optical Nitratax plus sc Sensor.

Pesticides were extracted from the water samples using solid phase extraction (SPE) cartridges. The cartridges (Oasis HLB, 6 ml, 0.5 g HLB sorbent material) (Waters, Milford, MA, USA) were activated with 7.5 ml of methanol:acetone (3:2)(Sigma-Aldrich, Steinheim, Germany) and washed with 7.5 ml of mili-q water. A 10 ml water sample and 100 µl of internal standard (IS) metolachlor (c= 1 ug/ml) were passed through the SPE cartridge. Afterwards, the SPE columns were washed with 7.5 ml of deionised water. The cartridges were then air-dried for 5 minutes and the adsorbed pesticide was eluted with 10 ml of methanol:acetone (3:2).

High-performance liquid chromatography (HPLC) analysis was performed using an Agilent 1200 chromatographic system (Agilent, Santa Clara, CA, USA) equipped with an Agilent Triple Quad 6410 mass spectrometer (Agilent, Santa Clara, CA, USA). The LC conditions were as follows: ACE 3 C18 chromatographic column of 150 mm length x 2.2 mm internal diameter and 3 µm particle size, with integrated guard column of 20 mm length x 2.2 mm internal diameter and 3 µm particle size (ACE, Scotland, UK). The column temperature was maintained at 30 °C. The mobile phase consisted of 0.1% formic acid (98% Sigma-Aldrich, Germany) in water and acetonitrile. The flow of the mobile phase was 0.3 ml/min during all the analyses. The resulting retention period was 10.4. The mean water recoveries of pesticides were 98%, and the limit of quantification (LOQ) achieved in the water samples was 1 µg/l.

Evaluation of the data

The effect of pesticides on the denitrification process was evaluated from their inhibition of the denitrification rate, which was calculated as the amount of NO_X-N removed per time unit. The effect of the tested substance was evaluated from the difference between the denitrification rates of samples 1 and 2.

The loss of the tested substance due to adsorption was assessed from the decrease in its concentration in sample 3. The decrease in the tested substance due to biodegradation was assessed by comparing the decrease in concentrations of the tested substance in samples 2 (biotic loss + abiotic loss) and 3 (abiotic loss).

RESULTS AND DISCUSSION

During the tests, organic substances of an acidic character were released from the wood shavings. The dose of 3 g/l of $NaHCO_3$ had a sufficient buffering capacity to preserve the pH at a level beneficial to the denitrification process. At the end of the test, the measured pH values were 7.97 ± 0.23 in the case of TER. When ATR was tested, the pH values increased to 8.97 ± 0.07. Therefore, in the case of TEB the dose of $NaHCO_3$ was reduced to 0.5 g/l, which caused pH to drop to 7.52 ± 0.11. pH values between 7.5 and 8.0 are more appropriate for denitrification than those measured during the ATR test (Paul 2007). The COD values at the end of the tests ranged from 160 to 393 mg/l. This amount of COD is sufficient to support denitrification (Lahdhiri et al. 2017). In the analysed samples without the added inhibitor (sample 1 and 2), DO values lower than 0.5 mg/l were measured after seven days.

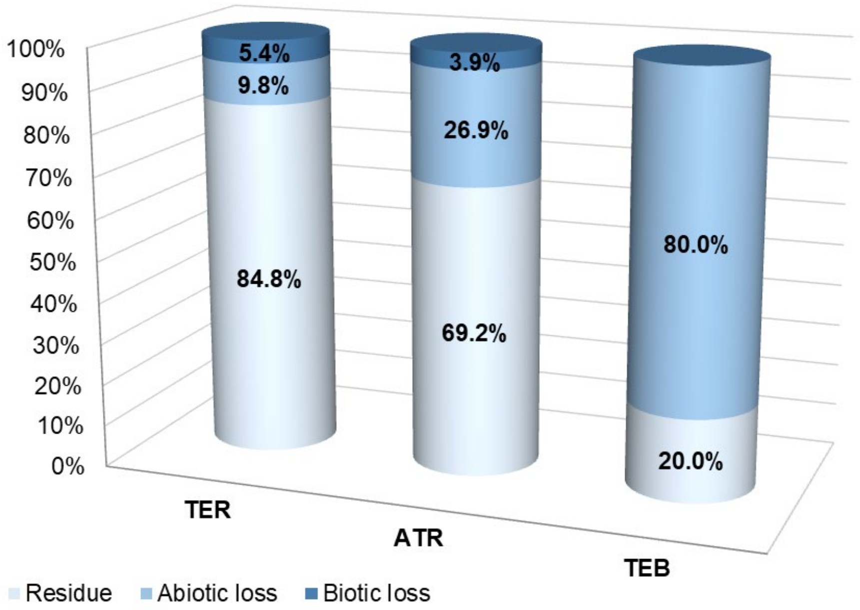
Table 2 Overview of average denitrification rates

Sample	Average denitrification rate mg/l/d		
	TER	ATR	TEB
1	2.71	2.44	1.30
2	2.72	2.33	1.54

The denitrification process with TER (sample 2) ran at almost the same denitrification rate as in the sample without TER (sample 1), being 2.72 mg/l/d and 2.71 mg/l/d, respectively. During the test with ATR, the denitrification rate was 2.44 mg/l/d (sample 1). With the addition of ATR (sample 2), the rate was slightly reduced to 2.33 mg/l/d. This does not contradict the findings from the literature that denitrification rates were not inhibited by atrazine concentrations of 5 mg/l (Ilhan et al. 2011). In the case of TEB, the results were different from ATR and TER, i.e. rates of 1.30 mg/l/d (sample 1) and 1.54 mg/l/d (sample 2). The denitrification process can be promoted by a dose of tebuconazole. Cycoń et al. (2006) observed significant stimulation of the abundance of cultivable denitrifying bacteria in soil by tebuconazole at dose rates >2.7 mg/kg. The tests have shown that all tested pesticides had negligible effect on the denitrification rates in a seven-day test at the concentration of 0.1 mg/l.

Figure 1 presents individual processes occurring during the batch tests. The abiotic loss was dominant for all tested pesticides. The mean abiotic loss for TER was 9.8%, for ATR 26.9%, and for TEB 80.0%. It can be assumed that the prevailing mechanism of abiotic loss was the sorption of pesticides on the wood shavings (Ilhan et al. 2011). The sorption of triazine pesticides TER and ATR was similar, while the sorption of TEB was much higher. This difference may be due to the different chemical structure and properties of these compounds. These results were in accordance with reports by Fenner et al. (2013) and Čadková et al. (2013). According to Fenner et al. (2013), the degradation of pesticides involves both biotic and abiotic processes. The transformation processes a given pesticide undergoes are determined by its structural affinity to specific types of transformation, and the environmental conditions it is exposed to as a result of its distribution and transport behaviour. Čadková et al. (2013) observed high adsorption of tebuconazole in soils with high contents of organic matter.

Figure 1 Fate of pesticides during denitrification after seven days



The highest biotic loss was observed for TER, namely 5.4%. This value is slightly higher than would be expected from the results of Navarro et al. (2004), who indicated that the dissipation half-life of TER in groundwater lies in the range from 263 to 366 d. In the case of ATR, a biotic loss of 3.9% was measured in our experiments. This is in accordance with the results of Douglass et al. (2014), who observed a half-life of 35–40 d for ATR. With TEB in liquid solution, no biotic loss was measured in our experiments. This finding was surprising considering the results of Caldas et al. (2010), who reported a half-life of TEB in groundwater that was in the range from 7 to 28 d.

The highest value of residue was measured in the case of TER, namely 84.8%, whereas the lowest was for TEB, 20.0%. From the point of view of the removal of pesticides from water, e.g. from agricultural drainage, the low residue in TEB in water is very positive. These results also correspond with the half-lives of the pesticides: the half-life of TER in groundwater is 263 to 366 d, while for TEB it is 7–28 d. The higher the half-life, the higher the residue value, and vice versa.

When assessing these results, it is necessary to take into account the fact that pesticides are very stable substances and 7 days is a very short time for their decomposition. It is possible that for some substances, decomposition would continue at an increased rate after the initial lag phase. Therefore, after the necessary adaptation of the methodology, the experiments will continue with a longer reaction time.

CONCLUSION

Behaviour of three pesticides (terbuthylazine, atrazine, and tebuconazole) during the denitrification process was assessed using a seven-day laboratory batch test developed for this purpose. At a concentration of 0.1 mg/l, terbuthylazine did not affect the denitrification rate, atrazine caused its 4.5% inhibition, while tebuconazole its 15.6% stimulation. Tebuconazole did not undergo biochemical degradation. Atrazine and terbuthylazine showed biotic losses of 3.9% and 4.5% respectively. The dominant process involved in the decrease in concentration of the pesticides in aqueous phase was the abiotic loss: tebuconazole 80%, atrazine 26.9%, and terbuthylazine 9.8%.

In future, the methodology will be used to test other micropollutants and, moreover, will be further developed to allow longer test durations.

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