

Degradation of selected triazole fungicides in constructed wetlands with application of biopreparation

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ABSTRACT

The purpose of this study was to determine the feasibility of using a constructed wetlands to degrade selected pesticides from wastewater and to evaluate their removal efficiency. In addition, a biopreparation was used to increase the efficiency of the process, the rate of degradation and reduce the half-life of the compounds in wastewater. Pesticides from the triazole group of fungicides, which are commonly used in agriculture, were selected for the experiments. The study was conducted on a pilot scale, over 3 y, in two identical constructed wetlands systems supported and not supported by microorganisms. At the same time, in order to identify fungicides, an analytical method based on liquid chromatography-tandem mass spectrometry was optimized, enabling the determination of applied pesticides in wastewater with satisfactory sensitivity, accuracy and precision. The results obtained indicate that the applied constructed wetlands bed method for the treatment of pesticides from wastewater using *Phragmites australis* had a high average efficiency (88.1%). In the fungicide group, on day 2, the removal efficiency ranged from 21% to 25% in the constructed wetlands bed, while with biopreparation support it ranged from 22% to 26.6%. The removal efficiency on day 14 was already 84%. The kinetics of fungicide decomposition in the constructed wetlands bed was determined on the basis of mathematical models and equations of the dynamics of pesticide disappearance in wastewater. The parameters of DT_{50} half-life of pesticides in wastewater and the parameters of theoretical time to reach the concentration level of 0.01 mg/L were determined. The microorganisms used increased pesticide removal efficiency by up to 7 d.

Keywords: Pesticide; Fungicide; Constructed wetlands bed; Pesticide decomposition

1. Introduction

Pesticides are one of the many groups of chemical pollutants in wastewater [1–4]. Pesticides are mainly used in agriculture to protect crops. In recent years, there has been an increase in the use of pesticides in agriculture. Therefore, it can be assumed that the problem of contamination of water, soils and wastewater with them will also grow [5–8].

The adverse effects of contaminated wastewater resulting from the rapidly expanding agriculture on the soil and water environment are becoming an increasing problem [9,10]. The greatest threat is posed by sites that do not have access to sanitary sewers [10]. Removing pesticide contaminants from wastewater at the farm level is a huge challenge. The treatment of small-scale domestic wastewater has specific characteristics. Therefore, small treatment plants can be characterized by individual solutions. The problem of

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water contamination from local sources and the need for safe management of working fluid after crop protection treatments, especially contaminated water after filling and washing sprayers, has been recognized and communicated in the wider agricultural community [3,7,9,10]. There is currently a growing interest in the biological degradation of liquid residues from crop protection treatments. Their incompetent management, such as direct introduction into sewage systems, is associated with the risk of local pollution, which is the primary source of contamination of soils and waters with crop protection products.

There are many methods for treating wastewater containing pesticides, but these are procedures dedicated to single compounds [11–15]. The main methods used are sorption or oxidation, membrane filtration and coagulation. Among biological methods, we can distinguish the activated sludge method and hydrobotanical wastewater treatment, which involves the use of certain plant species with high bioremediation capacity [16]. Hydrophytic treatment plants are becoming increasingly popular [7,10,17,18]. Such solutions with the participation of plants are proven worldwide. A high treatment effect is achieved with low expenditures for construction and operation, and plants populating the beds take up nitrogen and phosphorus compounds. In the course of wastewater treatment in constructed wetlands beds, such processes as filtration, sorption, precipitation, chemical and biological transformations take place, the efficiency of which decreases slightly in the autumn–winter period. An extremely important element is that chemicals commonly used in conventional treatment plants are not used, as well as the lack of waste typical of the activated sludge method, and the systems easily blend into the landscape.

The purpose of this study was to determine the feasibility of using a technology of constructed wetlands to degrade selected pesticides from wastewater and to evaluate their removal efficiency. In addition, a biopreparation

was used to increase the efficiency of the process, the rate of degradation and to reduce the half-life of the compounds in the wastewater.

2. Material and methods

2.1. Technological procedure

The research facility consisted of a retention tank and two parallel systems of constructed wetlands bed with planting of common reed *Phragmites australis*. The constructed wetlands bed, with the dimensions of a cylinder with a diameter of 0.50 m and a depth of 0.70 m (Table 1), were placed in a 120 L tank made of a special type of PE plastic, which is characterized by durability, equipped with a drain tap, drainage and ventilation. A cross-section of the test facility's bed is shown in Fig. 1. The constructed wetlands beds were constructed in a system with subsurface vertical flow of wastewater. All beds had three layers of fill from the top, with varying granulometric parameters:

Vertical flow beds were designed and built based on domestic and foreign experience, including, among others: Dąbrowski et al. [17], Cooper [18], Brix and Arias [18], Langergraber [17,18], Gajewska and Obarska-Pempkowiak [18]. The assumed filling thickness of vertical flow deposits was aimed at good oxygenation. Based on the grain size curve, an average grain diameter of $d_{50} = 0.45$ mm and a grain size uniformity factor of $d_{60}/d_{10} = 2.6$ were determined. Material with such parameters is recommended as a substrate for reed planting [17,18]. For the laid gravel layer, the average grain diameter was $d_{50} = 3.5$, while the coefficient of uniformity of grain size was $d_{60}/d_{20} = 2.2$. The fill was exclusively inorganic material.

During the first research period of deposit working, according to the guidelines given by Dąbrowski et al. [17] and Gajewska & H. Obarska-Pempkowiak [18], in order to acclimatize the plants well, the deposit was fed with

| | | | |
|---|---------|-----------------|--------|
| 1 | Layer A | gravel 2÷8 mm | 0.15 m |
| 2 | Layer B | gravel 8÷20 mm | 0.40 m |
| 3 | Layer C | stones 20÷80 mm | 0.15 m |

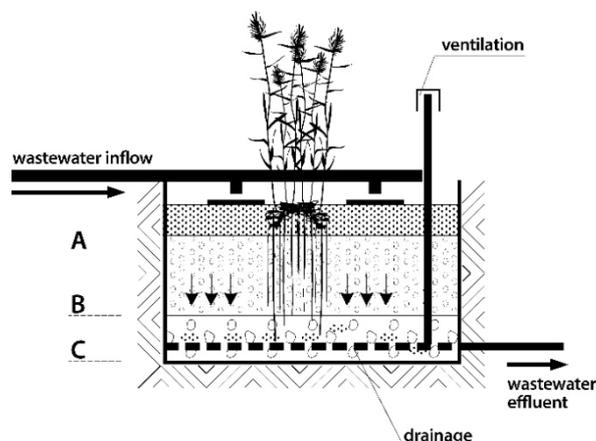


Fig. 1. Cross-section of a vertical flow constructed wetlands bed at a semi-technical scale research facility.

treated wastewater, followed by raw wastewater. During the period of deposit development, the biopreparation BIO AQUA PUR KOD 106 from EKOB-TBA was dosed into treated domestic wastewater. It enriches the microorganisms of biological deposits to improve their degradation properties. This preparation is formed by selected microorganisms immobilized on mineral supports, particularly active against compounds resistant to natural biological degradation in wastewater treatment plants. They cause a significant reduction in chemical oxygen demand. The formulation was applied once a week. Maintaining a high level of flooding of the bed, made possible by regulated drainage, as well as an increase in air temperature, allowed the intensive development of reeds and the emergence of new shoots, and the addition of biopreparations resulted in the formation of a characteristic microflora, which was considered the beginning of the research experiment's proper phase.

Wastewater was dosed in a constructed wetlands bed at a bed load of 0.1 m³/m²-d. To evaluate pesticide removal efficiency, domestic wastewater enriched with pesticide mixtures was used for the study.

The pesticides most commonly used in Polish agriculture in the fungicide group were selected for the study. The structural formulas of the active substances contained in the fungicides are shown in Table 2.

After the beds had been worked for a year, a previously prepared mixture of domestic wastewater and fungicides was dosed into the retention tank. The wastewater was then dosed at a rate of 20 L once a day, respectively, for each bed. BIO AQUA PUR 106 biopreparation was dosed once a week.

2.2. Analytical procedure

Analytical studies were carried out at the Plant Protection Institute – National Research Institute using a Waters liquid chromatograph apparatus and AB SCIEX mass spectrometer using validated test procedures for the determination of pesticide residues in wastewater and plant material [5,19]. Chromatographic analysis was performed using an Eksigent Ultra LC-100 liquid chromatograph coupled to a QTRAP 6500 mass spectrometer (AB Sciex Instruments, Foster City, CA). Separation of analytes was performed on a KINETEX C18 column (100 mm × 2.1 mm,

2.6 μm) maintained at 40°C. The volume of the injected sample was 10 μL. The mobile phase was 0.5% formic acid solution with 2 mmol ammonium formate in water (phase A) and in methanol (phase B). Analysis was carried out using gradient elution, with the following program: 0–1 min 1% phase B; 1–13 min 1% to 90% phase B; 13–23 min 90% phase B; 23–25 min 90% to 1% B; 25–30 min 1% phase B. Detection was carried out using electrospray ionization (ESI) in the positive ion formation mode characterized by the following parameters: voltage applied to the electrode (IS) 5,000 V; ion source temperature 400°C; sputtering assist gas pressure 60 psi, auxiliary gas 50 psi, shielding gas 30 psi. Nitrogen was used as sputtering gas, auxiliary gas and shielding gas. The optimized parameters of instrumental analysis in the mode of monitoring multiple fragmentation reactions in parallel (MRM) for each pesticide are given in Tables 3 and 4. To ensure the reliability of the results, validation was carried out based on the SANTE/11945/2015 guide. During the validation process, parameters such as linearity, recovery, precision, limit of detection (LOD), limit of quantification (LOQ), matrix effect (ME) and expanded uncertainty of the method (U) were determined. Precision calculated as relative standard deviation (RSD) was less than 22%. The effluent and plant matrix effect for most compounds did not significantly affect the attenuation or amplification of signals.

In the analyzed concentration range (0.0001–0.30 mg/L), satisfactory linearity of the method was achieved with a correlation coefficient of $R^2 > 0.99$. The limit of quantification (LOQ) was set equal to 0.001 mg/L, and the limit of detection (LOD) was set at 0.00003 mg/L. The expanded measurement uncertainty averaged between 8% and 22%.

Table 1
Parameters of constructed wetlands bed of the research facility

| Parameter | Value |
|---|----------|
| Diameter D, m | 0.5 |
| Surface area F, m ² | 0.2 |
| Depth H, m | 0.7 |
| Hydraulic load, m ³ /m ² -d | 0.1 |
| Organic compound load, g-BZT5/m ² /d | 57 ÷ 730 |

Table 2
Fungicides used in the study (structural formulas of the pesticide group used: <https://www.chemicalbook.com>)

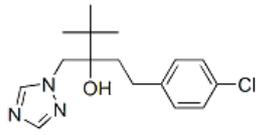
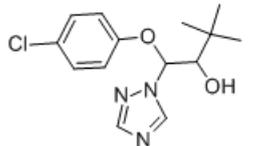
| Active substance | Chemical group | Structural formula | Molecular weight (g/mol) |
|------------------|----------------|--|--------------------------|
| Tebukonazol | Triazol |  | 307.82 |
| Triadimenol | Triazol |  | 295.76 |

Table 3
Optimized LC-MS/MS system operating parameters for analyzed pesticides

| Fungicides | Retention time (min) | Quantitative/Qualitative determination | | | Qualitative determination | | | DP (V) | EP (V) |
|-------------|----------------------|--|--------|---------|------------------------------|--------|---------|--------|--------|
| | | Fragmentation reaction (m/z) | CE (V) | CXP (V) | Fragmentation reaction (m/z) | CE (V) | CXP (V) | | |
| Tebukonazol | 10.28 | 308.1 > 70 | 57 | 8 | 308.1 > 125.1 | 59 | 8 | 41 | 10 |
| Triadimenol | 9.63 | 296 > 70 | 19 | 8 | 296 > 227 | 15 | 8 | 56 | 10 |

DP – cluster clustering potential; CE – collision energy; CXP – Ion exit potential from the collision chamber.

To describe the persistence of fungicides in wastewater, a mathematical model recommended by the European Union was used to assess the behavior of compounds in soil. Based on the obtained individual concentrations of fungicide active substances in runoff wastewater (C_{ODP}) collected over time, the half-life and rate of disappearance of active substances were calculated. The dynamics of pesticide disappearance in wastewater samples enriched with microorganisms (MIK) and without microorganisms were described by a first-order reaction kinetic equation [20]:

$$C_t = C_0 \exp(-kt) \quad (1)$$

where C_t – concentration at time t , (mg/L), C_0 – initial concentration at time $t = 0$, (mg/L), t – duration of experiment (d), k – process rate constant (1/d)

From the knowledge of the mathematical equation and the rate constant, the half-life was calculated DT_{50} (degradation time) defined as the decay/degradation time, which is the time required for 50% of the mass of a given compound to disappear. Disappearance time values (DT_{50}) for individual pesticides were calculated from a linear equation obtained from a regression between the natural logarithm of pesticide concentration to time:

$$DT_{50} = \frac{\ln(2)}{k} \quad (2)$$

From the knowledge of the mathematical equation, assuming a C_t of 0.05 mg/L and a C_t of 0.01 mg/L, the theoretical disappearance time of the substance to these $C_{0.05}$ and $C_{0.01}$ levels was calculated.

3. Results and discussion

Tebukonazol (TEB) was dosed in August at a dose of 25.1 mg/L. The determined concentration of the active substance in the wastewater after thorough mixing was 23.142 mg/L, which confirms the correctness of the analytical method, and the difference is within the error of the method. The first sample was taken after 24 h and a concentration of 20.101 mg/L was recorded. On the second day, the concentration decreased by about 2 units, and on the next day by about 1 unit, to a value of 17.115 mg/L (Fig. 2). On the 7th day, the TEB value was 12.376 mg/L, and on the 14th day it reached 7.656 mg/L. After the 33rd day, the TEB concentration was at 0.457 mg/L and a value of 0.011 mg/L was

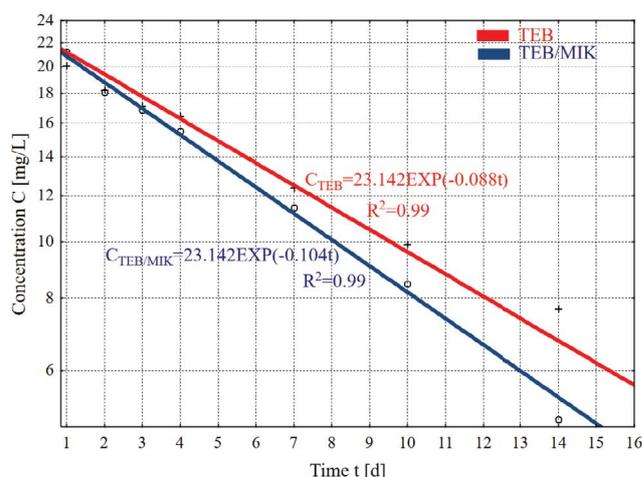


Fig. 2. Dynamics curve for the disappearance of tebuconazole in wastewater passed through a constructed wetlands bed.

recorded on the 56th day of the experiment. When microorganisms were used, the initial decrease in concentration was lower than TEB without microorganisms. After day 1, the concentration was 21.212 mg/L. On the second day, the concentration decreased by about 2 units, and on the fourth day by about 2.5 units, to a value of 15.472 mg/L (Fig. 2). On the 7th day, the TEB value was 11.421 mg/L, and on the 14th day it reached 4.945 mg/L. After the 33rd day, the TEB concentration was at 0.285 mg/L and a value of 0.009 mg/L was recorded on the 56th day of the experiment. The compound was not detected after 105 and 88 d, without and with the addition of MIK, respectively.

The mass deposition expressed as % of TEB mass in the wastewater passed through the bed without microorganisms (TEB) and enriched with microorganisms (TEB/MIK) is shown in Figs. 3 and 4. Tebuconazole after the first day of application was decomposed in the bed without MIK by 13.1%, while in the bed enriched with MIK and 8.3%. On day 2, the efficiency remained at a similar level of 21%–22%. On day 4, TEB concentration was reduced by 29% without MIK and 33% with MIK. After the 7th day, TEB/MIK efficiencies of 47/51% were recorded, and after the 10th day, 57% and 63%, in both cases. The largest difference in reduction efficiency was recorded on day 14: 67% and 79%. After the 33rd day, efficiency was at similar levels of 98% and 99% and day 52: 99.9%. After 56 d, a reduction of 100% was not achieved in both cases. These differences indicate, the

activity of microorganisms in the decomposition of tebuconazole. Agudelo et al. [11–13], studying chlorpyrifos removal in a constructed wetlands bed with subsurface flow of overgrown reeds (*Phragmites australis*), found an average removal efficiency of 96.2% with values ranging from 92.2% to 97.4%. Ignatowicz et al. [7] confirmed the high removal efficiency of 8 pesticides from industrial wastewater in a reed (*Phragmites*) overgrown treatment plant with subsurface horizontal flow: azoxystrobin, boscalid, epoxiconazole, fenarimol, fenazaquin, nicosulfuron, procymidone, pyraclostrobin, thiachloprid and trifloxystrobin at varying constructed wetlands bed loading: 0.01, 0.02 and 0.03 m²/m²/d. The average pesticide removal efficiency was 99.8%.

The SFO model was used to determine the half-life of fungicides. The choice of this model was dictated by the available literature data describing pesticides as substances that degrade, which can be described by a first-order mathematical function, and due to the lack of data describing the degradation process with models of greater complexity that can be fitted to the data without significant limitations or high uncertainty. Based on the results of instrumental analyses, the relationships between the concentration of the active substance and the theoretical time of its degradation were determined. The correctness of the used model of decay curves described by first-order kinetic reaction

equations is confirmed by the high coefficient of determination (R^2). Such a choice of reaction order in their works is also described by other authors including Fenoll et al. [21], Gajbhiye et al. [22]. The trends of changes in effluent concentrations of active substances were used to determine their half-life (DT_{50}) [20]. The determined exponential equations were used to determine the theoretical time required to reach the concentration level of 0.01 mg/kg ($t_{0.01\text{teor}}$).

The dynamics curves of tebuconazole decay in wastewater without microorganisms added (TEB) and enriched with microorganisms (TEB/MIK) passed through a constructed wetlands beds are shown in Fig. 2. The dynamics of tebuconazole decay without microorganisms added is described by the equation calculated using Gauss–Newton nonlinear estimation with respect to the general notation of Eq. (1) $C_{\text{TEB}} = 23.142e^{-0.088t}$ ($R^2 = 0.99$), with MIK added $C_{\text{TEB/MIK}} = 23.142e^{-0.104t}$ ($R^2 = 0.99$). Therefore, the values of the process rate constant k are for TEB $k = 0.088 \text{ d}^{-1}$ and for TEB/MIK $k = 0.104 \text{ d}^{-1}$. After substitution into Eq. (2), the disappearance time values DT_{50} of the pesticide were obtained. After transformation of Eq. (1), $t_{0.05}$ and $t_{0.01}$ values were calculated. The parameters of the disappearance curves of tebuconazole in wastewater without microorganisms (TEB) and enriched with microorganisms (TEB/MIK) passed through the constructed wetlands beds are shown in Table 5.

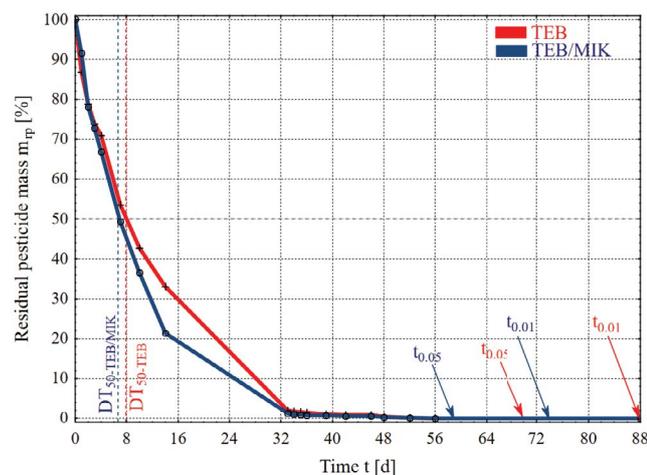


Fig. 3. Mass deposition (% of residual TRI mass) in wastewater without microorganisms (TRI) and enriched with microorganisms (TRI/MIK) passed through a constructed wetlands bed.

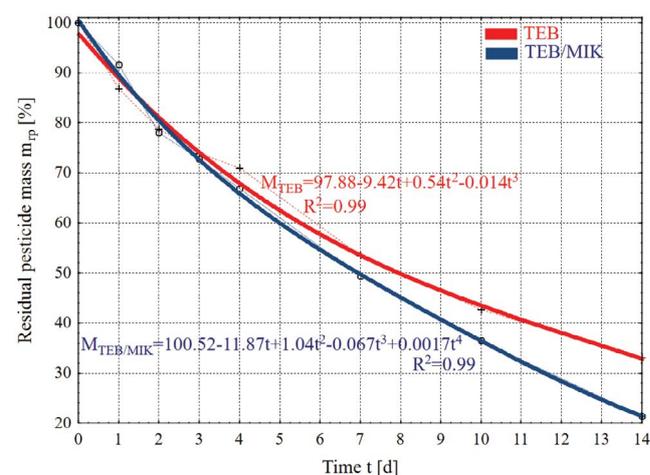


Fig. 4. Mass deposition (% of residual TRI mass) in wastewater without microorganisms (TRI) and enriched with microorganisms (TRI/MIK) passed through a constructed wetlands bed.

Table 4
Validation parameters of the method

| Pesticide | R^2 | mg/L | | | | | | | | | | U | ME |
|-------------|-------|-------|-----|------|-----|-----|-----|------|-----|-------|-----|----|-----|
| | | 0.001 | | 0.05 | | 0.5 | | 10.0 | | 100.0 | | | |
| | | R | RSD | R | RSD | R | RSD | R | RSD | R | RSD | | |
| Tebukonazol | 0.99 | 90 | 9 | 88 | 7 | 93 | 18 | 91 | 4 | 92 | 4 | 9 | 9 |
| Triadimenol | 0.99 | 102 | 11 | 103 | 8 | 110 | 12 | 105 | 14 | 110 | 13 | 14 | -13 |

U – Extended measurement uncertainty; ME – matrix effect; R – recovery; RSD – relative standard deviation.

The determined half-life is 7.88 d without MIK and 6.67 d after the addition of microorganisms, and the theoretical time for TEB to reach a concentration of 0.05 mg/L is shorter by about 11 d in MIK-enriched wastewater and is 59, and the concentration of 0.01 mg/L without MIK and with MIK: 88 and 74 d, respectively.

Triadimenol (TRI) was dosed in August at a dose of 6.5 mg/L. The determined concentration of the active substance in the wastewater after thorough mixing was 6.342 mg/L, which confirms the correctness of the analytical method, and the difference is within the error of the method.

The first sample was taken after 24 h and a concentration of 5.421 mg/L was recorded. On the second day, the concentration decreased by about 0.8 units, on the next day by about 0.5 units, to a value of 4.125 mg/L. On day 7, the TRI value was 2.412 mg/L, and on day 14 it reached 1.241 mg/L (Fig. 5). After the 33rd day, the TRI concentration was at 0.045 mg/L and a value of 0.01 mg/L was recorded on the 39th day of the experiment, 0.002 mg/L on the 46th day, and no more TRI was detected on day 48. When microorganisms were used, the initial decrease in concentration was lower than that of TRI without microorganisms (5.845 mg/L). After day 1, a TRI concentration of 4.754 mg/L was determined. On the second day, the concentration decreased by about 1 unit, and on the fourth day it reached a value of 3.542 mg/L. On day 7, the TRI value was 2.214 mg/L, and on day 14 it reached 1.065 mg/L (Fig. 5). After the 33rd day, the TRI concentration

Table 5
Parameters of tebuconazole disappearance curves in wastewater treated on constructed wetlands bed without microorganisms (TRI) and enriched with microorganisms (TRI/MIK)

| TRI | | | | | TRI/MIK | | | | |
|--------|-----------------|-----------|------------|------------|---------|-----------------|-----------|------------|------------|
| C_0 | k | DT_{50} | $t_{0.05}$ | $t_{0.01}$ | C_0 | k | DT_{50} | $t_{0.05}$ | $t_{0.01}$ |
| mg/L | d ⁻¹ | day | day | day | mg/L | d ⁻¹ | day | day | day |
| 23.142 | 0.088 | 7.88 | 70 | 88 | 23.142 | 0.104 | 6.67 | 59 | 74 |

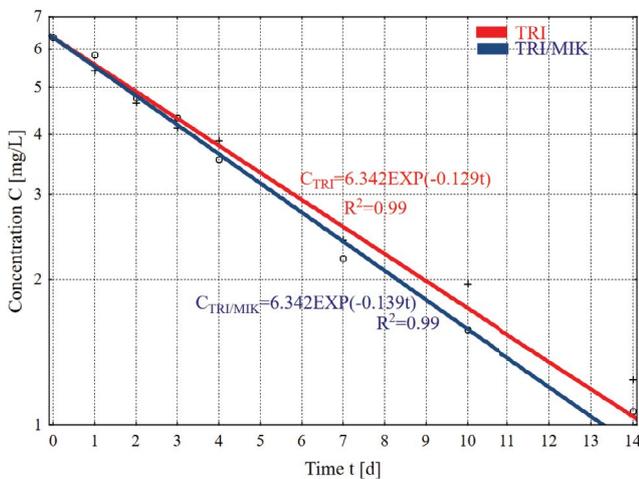


Fig. 5. Curve of the dynamics of the disappearance of triadimenol in wastewater passed through the constructed wetlands bed.

was at 0.024 mg/L and a value of 0.008 mg/L was recorded on the 39th day of the experiment, and no TRI/MIK concentration was determined on day 42.

The mass deposition expressed as % of TRI mass in the wastewater passed through the bed without microorganisms (TRI) and enriched with microorganisms (TRI/MIK) is shown in Figs. 6 and 7. During the first day, the TRI removal efficiency was higher in the wastewater without microorganisms and was 14.5% and with TRI/MIK 7.8%. After the 2nd day of the experiment, it was at similar levels of 26.6% and 25%. After the 4th day, the decomposition efficiency of TRI was higher in wastewater with the addition of MIK and amounted to 44.1% and was maintained until the 14th day: 83.1%, and TRI 80.3%. Complete removal of TRI was achieved on day 46, and TRI/MIK on day 39. These differences confirm the activity of decomposition of the compound by the addition of microorganisms after the 4th that day of application. By the

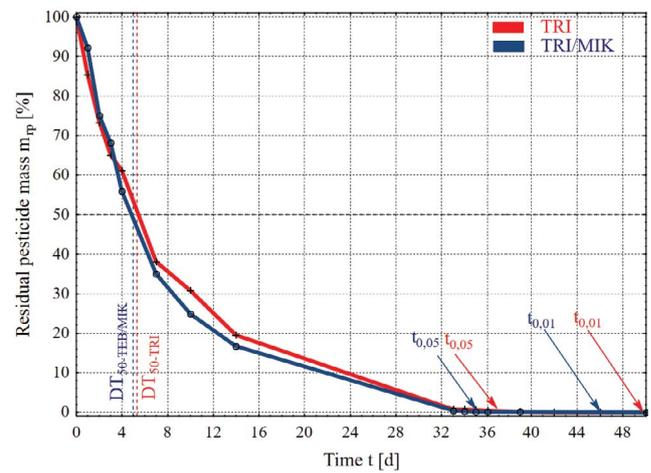


Fig. 6. Mass deposition (% residual TRI mass) in wastewater without microorganisms (TRI) and enriched with microorganisms (TRI/MIK) passed through a constructed wetlands bed.

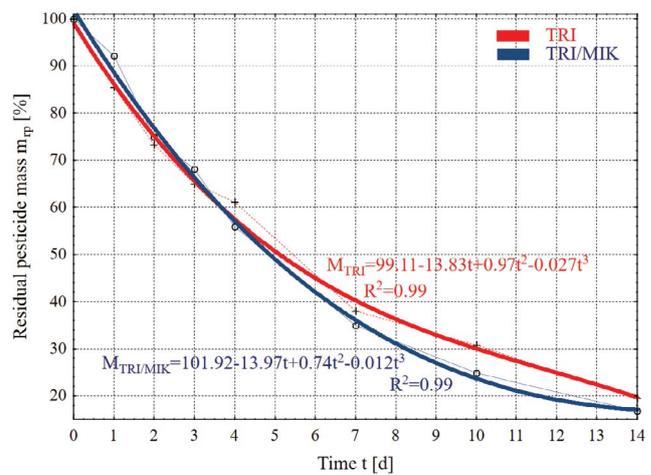


Fig. 7. Mass deposition (% TRI residual mass) in wastewater without microorganisms (TRI) and enriched with microorganisms (TRI/MIK) passed through a constructed wetlands bed.

Table 6

Parameters of triadimenol decay curves in wastewater treated in constructed wetlands bed without microorganisms (TRI) and enriched with microorganisms (TRI/MIK)

| TRI | | | | | TRI/MIK | | | | |
|-------|-----------------|-----------|------------|------------|---------|-----------------|-----------|------------|------------|
| C_0 | k | DT_{50} | $t_{0.05}$ | $t_{0.01}$ | C_0 | k | DT_{50} | $t_{0.05}$ | $t_{0.01}$ |
| mg/L | d ⁻¹ | day | | | mg/L | d ⁻¹ | day | | |
| 6.432 | 0.129 | 5.33 | 37 | 50 | 6.432 | 0.139 | 4.95 | 35 | 46 |

4th of that day, decomposition was inhibited in MIK. These observations were confirmed by other researchers. A study conducted by Ignatowicz [7], Puchlik [8] and Łozowicki et al. [23] using a constructed wetlands bed on a semi-technical scale to treat fruit and vegetable wastewater with a fungicide and insecticide showed very promising results in the removal of these compounds. Azoxystrobin was removed 93.1%, and ethyl chlorpyrifos 97.7%.

The curves of the dynamics of the disappearance of triadimenol in wastewater without microorganisms (TRI) and enriched with microorganisms (TRI/MIK) passed through the constructed wetlands beds are shown in Fig. 5. The dynamics of the disappearance of triadimenol without microorganisms is described by the equation calculated using Gauss–Newton nonlinear estimation with respect to the general notation of Eq. (1): $C_{\text{TRI}} = 6.432e^{-0.129t}$ ($R^2 = 0.99$), with the addition of MIK $C_{\text{TRI/MIK}} = 6.432e^{-0.139t}$ ($R^2 = 0.99$). Therefore, the values of the process rate constant k are for TRI $k = 0.129 \text{ d}^{-1}$ and for TRI/MIK $k = 0.139 \text{ d}^{-1}$. After substitution into Eq. (2), the DT_{50} disappearance time values of the pesticide under consideration were obtained. After transformation of Eq. (1), the values of $t_{0.05}$ and $t_{0.01}$ were calculated. The parameters of the decay curves of triadimenol in wastewater without the addition of microorganisms (TRI) and enriched with microorganisms (TRI/MIK) passed through the constructed wetlands beds are shown in Table 6. The determined half-life is 5.33 d without biopreparation MIK and 4.95 d after the addition of microorganisms, and the theoretical time for TRI to reach a concentration of 0.05 mg/L is shorter by about 2 d in MIK-enriched wastewater and is 35, and the concentration of 0.01 mg/L without MIK and with MIK: 50 and 46 d, respectively.

Azole compounds, which include tebuconazole and triadimenol, can potentially affect the endocrine system of living organisms. The scientific literature lacks scientific studies on the effectiveness of removing triazoles from wastewater, although their presence in the environment should be a cause for increased concern. The occurrence and fate of nine agricultural azole fungicides, used as biocides and pharmaceuticals, were studied by Kahle et al. [24,25] in wastewater treatment plants (WWTPs) and lakes in Switzerland. Fluconazole and clotrimazole were detected in wastewater flowing into the WWTP at 10–110 ng/L, while propiconazole, tebuconazole VW concentrations of 1–30 ng/L. Tests of untreated and treated wastewater indicated that fluconazole, propiconazole and tebuconazole were not affected by wastewater treatment, but clotrimazole was effectively eliminated (>80%). Incubation studies with activated sludge showed that tebuconazole showed slow

degradation and sorption (DT_{50} 2–3 d). The presence of tebuconazole in the lakes confirms its origin from agricultural land or industrial wastewater.

Our own studies confirm the slow degradation and high sorption of tebuconazole and triadimenol. Their half-life was calculated at 5 to 8 d. The theoretical time for TEB and TRI to reach a concentration of 0.01 mg/L is estimated at 46 to 88 d. Removal of TEB and TRI of more than 50% of the initial mass was achieved after just 7 d, and after day 14 there was about 80% removal efficiency. In the case of triadimenol, its degradation to tradimefon was confirmed. Like triadimenol, more than 50% reduction of the initial mass of 53 mg/L was achieved after 7 d, and after 14 d there was about 80% removal efficiency (78.6% and 83.2%).

4. Conclusion

Based on the study, it can be concluded that:

- constructed wetlands beds can be used with great success for the decomposition of fungicides applicable when spraying agricultural crops.
- the use of three layers of granulometrically differentiated non-chemically inactive filter material influenced the high efficiency of treatment. During the entire experimental period, the wastewater feeding the bed was treated satisfactorily.
- the tested group of fungicides was characterized by a reduction rate of 88.1%.
- the determined parameters of half-life of pesticides in DT_{50} wastewater ranged from 4.95 (TRI/MIK) to 6.67 (TEB/MIK) with biopreparation and 5.33 (TRI) to 7.88 (TEB) without biopreparation.
- the determined parameters of the theoretical time to reach the concentration of 0.01 mg/L $t_{0.01}$ were 46 and 74 d for MIK and 50 and 80 d without MIK.
- the microorganisms used increased the efficiency of pesticide removal from wastewater, the DT_{50} decomposition time was reduced by up to 1.5 d, and the $t_{0.01}$ time was reduced by 14 d. This will allow future design of liquid disposal stations for fungicides used for spraying agricultural crops.

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