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Toxic influence of key organic soil pollutants on the total flavonoid content in wheat leaves

Florina Copaciu,

University of Agricultural Sciences and Veterinary Medicine, Department of Chemistry and Biochemistry, 3-5 M n t ur, Cluj-Napoca 400372, Romania

Ocsana Opri ,

National Institute of Research and Development for Isotopic and Molecular Technologies, Cluj-Napoca 400293, Romania

Ülo Niinemets, and

Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, 1 Kreutzwaldi, Tartu 51014, Estonia; Estonian Academy of Sciences, Kohtu 6, 10130 Tallinn, Estonia

Lucian Copolovici

Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, 1 Kreutzwaldi, Tartu 51014, Estonia; Research Center of Technical and Natural Sciences, Faculty of Food Engineering, Tourism and Environmental Protection, "Aurel Vlaicu" University, Romania, 2 Elena Dragoi, Arad 310330, Romania

Abstract

Textile dyes and antibiotics are two main classes of environmental pollutants which could be found in soil and water. Those persistent pollutants can have a negative influence on plant growth and development and affect the level of secondary metabolites. In the present work we studied the effect of textile dyes and antibiotics on total leaf flavonoid contents in wheat (*Triticum aestivum* L.). Contaminant solutions were applied daily using concentrations of 0.5 mg L⁻¹ (lower) and 1.5 mg L⁻¹ (higher dose) for either one or two weeks. We observed that exposure to the higher concentration of textile dyes resulted in a reduction in flavonoid content while antibiotics enhanced flavonoid contents at lower doses of exposure, and reduced at higher doses of exposure. These results suggest that diffuse chronic pollution by artificial organic contaminants can importantly alter antioxidative capacity of plants.

Keywords

Antibiotics residues; Textile dyes; Abiotic stress; Environmental pollutants; Flavonoids; *Triticum aestivum*

1 Introduction

Plant growth environment in urbanized and managed areas is usually contaminated with numerous artificial organic compounds. Residues of textile dyes and antibiotics are two main classes of environmental pollutants found in significant concentrations in soil and waterbodies (Wojnárovits and Takács 2008; Martinez 2009; Li et al. 2009; Copaciu et al. 2013a; Opri et al. 2013a).

Textile industry is worldwide an essential economic sector employing a plethora of synthetic azo and anthraquinone dyes (Lee and Pavlostathis 2004; Epolito et al. 2005). In particular, metal-complex dyes are widely employed in textiles that require high color resistance when repeatedly exposed to sunlight and water. Such textile dyes are typically very stable (half-lives of 2-13 years in environment), and they have been detected in a number of rivers, lakes and terrestrial soils, causing toxicological problems to the aquatic environment (Moawad et al. 2003; Edwards et al. 2004; Umbuzeiro et al. 2005; Copaciu et al. 2013a; Imran et al. 2015). Use of such contaminated waters has increased in agriculture in many countries due to population growth and enhanced demand for food. However, intensive irrigation of agricultural lands with polluted water from various industries can negatively affect soil fertility and plant growth (Field et al. 2008).

So far, the information of overall effects and action mechanisms of textile dyes on plants is very limited. Studies have demonstrated that plants treated with textile industry effluents containing dyes had reduced concentrations of carbohydrates, proteins and chlorophyll pigments (Puvaneswari et al. 2006; Nilratnisakorn et al. 2007; Kaya et al. 2012, Copaciu et al. 2013b WSA). Kaushik et al. (2005) reported that the toxicity of some textile effluents reduced seed germination, plant shoot length, root length, plant biomass, chlorophyll and carotenoid content. By studying two varieties of sorghum (*Sorghum vulgare* Pers.), 'Pioneer jowar' and 'Desi jowar', a similar effect of textile mill effluents has been observed (Garg and Kaushik 2008). Furthermore, in a recent study, Copaciu et al. (2013b) demonstrated that textile dyes importantly induce abiotic stress responses in wheat. Application of two anthraquinone dyes and four azo dyes at different concentrations negatively influenced foliage photosynthesis rate, and chlorophyll contents and increased photoprotective carotenoid content and resulted in induction of emissions of lipoxygenase pathway products and monoterpenes in a dose-dependent manner (Copaciu et al. 2013b).

Antibiotics residues constitute another class of pollutants often encountered in environment at significant concentrations. Many antibiotics species have been used for decades as chemotherapeutic agents in human and veterinary medicine due to their antibacterial properties (Tamtam et al. 2008). Antibiotics are considered to be persistent contaminants due to their slow rate of metabolism (Seifrtová et al. 2009). These compounds are metabolized by humans and animals only to a minor degree and from excretions they can reach to natural waters even if wastewater is passed through the sewage treatment plants. Furthermore, antibiotics can directly enter into the soil through manure from animal husbandry, and be taken up by plants (Jjemba 2002; Dolliver et al. 2007). Antibiotics can be further carried to initially non-impacted areas by runoff water, and part of the antibiotics can

infiltrate deeper and affect groundwater, strongly impacting human and environmental health (Martínez-Carballo et al. 2007).

So far, few studies on the effects of antibiotics residues on plants have been carried out. Studies of some antibiotics such as tetracycline have shown hormetic effects with low concentrations stimulating germination and early plant development, but higher concentrations or chronic exposure resulting in toxic effects suppressing plant performance (Xie et al. 2011). At concentrations typically found in environment, streptomycin inhibited chlorophyll synthesis in *Hordeum vulgare* (Yaronskaya et al. 2007), whereas enrofloxacin (Migliore et al. 2003) and sulfadimethoxine (Migliore et al. 1996) reduced significantly plant growth, and ciprofloxacin reduced both photosynthesis and growth (Aristilde et al. 2010) in several plant species. Opri et al. (2013b) used antibiotics from five different classes (penicillins, cephalosporins, tetracyclines, fluoroquinolones and macrolides) at environmental concentrations to demonstrate dose-dependent reductions in foliage photosynthesis, and chlorophyll and increases in photoprotective carotenoid content and induction of emissions of lipoxygenase pathway products and monoterpenes in wheat.

Although there is increasing evidence that exposure to resistant organic pollutants constitutes a significant abiotic stress to plants, negatively affecting photosynthesis and growth (Copaciu et al. 2013b; Opri et al. 2013b), and resulting in enhanced stress volatile emissions and greater pools of photoprotective carotenoids (Brunetti et al. 2015; Esteban et al. 2015), there is a limited understanding of overall changes in secondary chemistry in impacted plants. In particular, flavonoids, a secondary class of plant metabolites have suggested to fulfill the highest number of key functions in plant growth and development (Nakabayashi & Saito 2015; Pedras & Yaya 2015). Flavonoids are polyphenolics formed through shikimate pathway in chloroplasts (Samanta et al. 2011; Di Ferdinando et al. 2012). Due to their metal-chelating and antioxidant capacities they protect plants against various biotic and abiotic stresses (Samanta et al. 2011; Di Ferdinando et al. 2012; Agati & Tattini 2010; Saewan & Jimtaisong 2013; Sisa et al. 2010; Neil et al. 2002; Mierziak et al. 2014). In addition, flavonoids are classically known for their capacity to absorb ultraviolet radiation, and thus, plants often respond to UV light by activation of flavonoid biosynthetic genes and by increased flavonoid contents (Liu et al. 1995; Lavola 1998; Fischbach et al. 1999; Andersen and Markham 2006). There is also evidence that flavonoids absorbing visible light, anthocyanins, can serve as screening pigments reducing absorption of photosynthetically active light (Pietrini et al. 2002; Sisa et al. 2010). Such reduction of light absorption can be beneficial under stress conditions that reduce photosynthetic activity and could lead to photoinhibition (Havaux and Kloppstech 2001; Pietrini et al. 2002). Due to their multiple defense properties, these pigments often play an important role in reproduction and early plant development by protecting reproductive tissue and seeds (Loreti et al. 2008; Takahashi et al. 1991; Saniewski et al. 1998).

The aim of this study was to investigate the influence of abiotic stress resulting from exposure to diverse textile dyes and antibiotics on total leaf flavonoid contents in wheat (*Triticum aestivum* L.). The stress application consisted of watering the wheat plants with solutions of six textile dyes and nine antibiotics. The chemicals were applied at two moderately high concentrations found in the environment (0.5 mg L^{-1} or 1.5 mg L^{-1}) for

two different time periods, and thus, the study consisted of four different treatments for each pollutant, and two control treatments. The effects of two anthraquinone dyes (Optilan Blue (OB) and Lanasy Blue (LB)) and four azo dyes (Lanasy Red (LR), Nylosan Red (NR), Nylosan Dark Brown (NDB) and Lanasy Dark Brown (LDB)) were studied (Table 1). The antibiotics were from five different classes: penicillins (Amoxicillin (AMOX), Ampicillin (AMP) and Penicillin G (PENG)), cephalosporins (Ceftazidime (CFZ) and Ceftriaxone (CFX)), tetracyclines (Tetracycline (TET) and Doxycycline (DOXY)), fluoroquinolones (Ciprofloxacin (CIP)) and macrolides (Erythromycin (ERY)) (Table 2). We predicted that abiotic stress resulting from organic pollutant uptake results in enhanced flavonoid contents and that the increase occurs in a dose-dependent manner. *Triticum aestivum* is a major food crop worldwide that requires high water availabilities for optimal productivity, and thus, could be of particular risk for irrigation with industrial wastewaters in certain areas. We expect the results obtained with this model species to be further generalizable to other water-demanding crops.

2 Material and methods

2.1 Plant material and growth conditions

Wheat (*Triticum aestivum* L. cv. "Lovrin", Fundulea, Romania) seeds were sown in 1 L (10 × 10 × 10 cm) plastic pots filled with commercial garden soil with slow release NPK fertilizer including microelements (Biolan, Finland). The sowing depth was 1 cm. The plants were cultivated in a Percival growth chamber (model LT36VL, Percival, IA, USA) under a light intensity of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the plant level provided for 12 h light period and maintaining day/night temperatures at 25/18°C and air humidity at 60%.

2.2 Stress application

The experiment was started in 14 days after sowing the seeds when the second emerged leaf had reached at least 50% of its final length (Zadoks growth stage of 1.2; Zadoks et al. 1974). Since the start of the treatment, the plants were daily watered with aqueous solution of the given chemical at either 0.5 mg L⁻¹ or 1.5 mg L⁻¹ concentration, while the control treatment received distilled water. The textile dyes (OB, LB, LR, NR, NDB and LDB) were obtained from Clariant Produkte (Muttens, Switzerland) and antibiotics (AMOX, AMP, PENG, CFX, and TET) from Antibiotice (Iași, Romania), CFZ from GlaxoSmithKline (Brașov, Romania), DOXY and ERY from Sandoz (Târgu-Mureș, Romania), and CIP from Ranbaxy (Cluj-Napoca, Romania).

The effects of treatment with these environmental pollutants were analyzed after the plants had received 0.5 L of the pollutant solutions (after being treated with the given pollutant solution for one week) and after they had received 1 L of the solution (after 2 weeks). All treatments (six textile dyes and nine antibiotics in two concentrations and the control treatment) were replicated three times (for further details of the experimental treatments see Copaciu et al. 2013b; Opri et al. 2013b).

2.3 Analysis of total flavonoid content

The extraction procedure for the determination of total flavonoid content follows Opri et al. (2011). The plant material was powdered in liquid nitrogen and 5 mL 80:20 ethanol:water mixture (v/v) was added. For maceration, the extracts were kept for 10 min in an oven (Model UNE 200, Memmert, Schwabach, Germany) at 35°C, and then they were sonicated with Bandelin Sonorex (Berlin, Germany) for 30 min at the same temperature. Each extract was filtered through a 0.45 µm PTFE membrane filter and brought to a final volume of 5 mL with the same solvent mixture.

For flavonoid determination, we used the reaction of total flavonoids with aluminum chloride that results in formation of a yellow complex (Petry et al. 2001) following the protocol of Romanian Pharmacopoeia (1993). The absorbance of the solution was measured spectrophotometrically at 420 nm, and the assay was calibrated relative to rutin hydrate (Sigma-Aldrich, Steinheim, Germany).

2.4 Data Analysis

Means $\pm$ standard error (SE) for three independent replicate plants are reported for each treatment. The means were statistically compared with Student ANOVA followed by post hoc Tukey's tests using Origin 8 (OriginLab Corporation, Massachusetts, USA). All statistical tests were considered significant at $P < 0.05$.

3 Results

3.1 Influence of textile dyes on flavonoid content

Application of textile dyes at a concentration of 0.5 mg L⁻¹ did not affect leaf flavonoid concentrations when the treatment continued for one week (altogether 0.5 L solution applied) (Fig. 1a). However, after two weeks (1 L solution applied), treatments with 0.5 mg L⁻¹ LB, NDB and LDB solutions resulted in significantly reduced total flavonoid content (Fig. 1b). In the case of treatments with 1.5 mg L⁻¹, total flavonoid content was reduced in all cases after both 0.5 L and 1 L application, whereas the decrease was more severe for NR, NDB, LDB azo textile dyes (Fig. 1a, b).

Total flavonoid content of control leaves was increased after two weeks since the start of the treatment compared with the values after one week since the start of the treatment (Fig. 1a,b). Despite the overall increase in flavonoid content found in controls, the relative reduction in flavonoid content in the treatments with 1.5 mg L⁻¹ dye solutions was greater after 1 L than after 0.5 L solution application (Fig. 1a, b) in most cases, except LDB.

3.2 Effects of treatments with antibiotics on flavonoid content

The treatment with 0.5 L antibiotics solution at the concentration of 0.5 mg L⁻¹ and 1.5 mg L⁻¹ increased the total flavonoid content for PENG, TET, DOXY, CIP and ERY treatments (Fig. 2a). The flavonoid content also increased in the case of the plants treated with AMOX and CFX, but just for the concentration of 0.5 mg L⁻¹ antibiotic solution. However, no effect of the treatment with 1 L antibiotic solution at the concentration of 0.5 mg L⁻¹ was observed

for any of the antibiotics (Fig. 2b), and the flavonoid content decreased significantly for the treatments with 1.5 mg L⁻¹ for AMOX, AMP, PENG, CFZ, and CFX antibiotics (Fig. 2b).

4 Discussion

4.1 Textile dyes and antibiotics treatment applied

In the present study, we used textile dyes and antibiotics in environmentally realistic concentrations of 0.5 mg L⁻¹ and 1.5 mg L⁻¹ that are commonly found in wastewater samples, and examined the effects on plants by using chronic exposure for a period of one week (watering with 0.5 L of solution of given textile dyes and given antibiotics) and for two weeks (watering with 1 L of solution). Thus, the total pollutant exposures applied were 0.25 mg (0.5 mg L⁻¹, 0.5 L), 0.5 mg (0.5 mg L⁻¹, 1 L), 0.75 mg (1.5 mg L⁻¹, 0.5 L) and 1.5 mg (1.5 mg L⁻¹, 1 L). The concentrations applied in our study were somewhat lower, but exposure times longer than in other studies (Mittler 2006; Pan et al. 2008; Kholodova et al. 2011; Xie et al. 2011). Given that the studied organic pollutants are persistent in soil and water, we consider chronic exposures as used in this study appropriate for gaining an insight into plant responses under realistic pollution scenarios.

4.2 Reduction in total flavonoid content in response to stress induced by textile dyes

As denoted in the introduction section, an enhancement of flavonoid content is a ubiquitous stress response induced by several stresses such as chilling (Havaux & Kloppstech 2001), enhanced UV (Lavola 1998), and visible radiation (Havaux & Kloppstech 2001). The role of enhanced flavonoid contents in stressed plants is not fully understood, but flavonoids can protect leaves directly by serving as antioxidants, and indirectly by serving as pigments screening excess visible and ultraviolet radiation and thereby reducing excess radiation damage Havaux & Kloppstech 2001; Neill 2002).

However, contrary to expectations, our data demonstrated that wheat (*T. aestivum*) exposed to textile dye solutions exhibited a significant decrease in flavonoid levels (Fig. 1). In particular, total flavonoid content significantly decreased compared to the control, in the case of all dyes at the concentration of 1.5 mg L⁻¹ at both volumes of dye solutions applied during the treatments (Fig. 1a and 1b). In the case of LB, NDB and LDB textile dyes (1 L), a decrease in total flavonoid content was registered even at the lower concentration applied (Fig. 1b). The reduced content of flavonoids can be either associated with a reduced rate of flavonoid biosynthesis or with a reduction of flavonoid content as the result of antioxidative reactions involving flavonoids under abiotic stress induced by textile dyes. Due to flavonoid ability to chelate metal ions in the cytosol or in the vacuole (Kondo et al. 1992), flavonoids also can reduce the adverse effects of metal toxicity due to their direct chelating properties (Landi et al 2015). Apart from direct chelating capacity, the detoxification systems of the plants against exogenous phytotoxic chemicals also involve the conjugation to glutathione (GSH) by glutathione S-transferase, followed by the conjugate removal from the cytosol by membrane-associated transport proteins Clemens et al. 2002; Jozefczak et al. 2012. There is evidence that flavonoids can increase synthesis of GSH, thereby enhancing indirectly the phytochelating activity of cells (Myhrstad et al. 2002).

Flavonoids are synthesized in chloroplasts through the shikimate pathway, activity of which is typically upregulated when photosynthetic carbon metabolism is inhibited (Lavola et al. 1994; Nogués and Baker 2000; Hamada 2001). However, there is evidence of inhibition of shikimate pathway upon air pollution (Kato et al. 1989). To our knowledge, direct effects of compounds with azo or anthraquinone functional groups on shikimate pathway activity have not been demonstrated.

4.3 Enhanced flavonoid contents in antibiotics' exposed plants

Differently from textile dyes, solutions of antibiotics generally enhanced flavonoid content in agreement with the past observations of upregulation of flavonoid biosynthesis under abiotic stress. Thus, the increase of flavonoid content after application of 0.5 L antibiotics solution for PENG, TET, DOXY, CIP, and ERY treatments (Fig. 2a) may indicate enhanced antioxidative capacity (Agati et al., 2012; Petrusa et al., 2013) under these treatments. Also, this trend was observed in the case of AMOX and CFX treatments, but only for 0.5 mg L⁻¹ concentration. In contrast, the reduction after the 1 L (Fig. 2b) antibiotics solution applications at the concentration of 1.5 mg L⁻¹ suggests that a sustained influence of antibiotics can reduce foliage antioxidative capacity indicating that the foliage defense capacity becomes exhausted (Neil et al. 2002; Mierziak et al., 2014). These decreases in flavonoid content were statistically significant for the plants treated with AMOX, AMP, PENG, CFZ, and CFX.

5 Conclusions

This study demonstrates important effects of two types of organic pollutants, textile dyes and antibiotics, on flavonoids, a biologically important compound class. While exposure to textile dyes resulted in a reduction in flavonoid content, antibiotics enhanced flavonoid contents at lower doses of exposure, and reduced at higher doses of exposure. These results suggest that diffuse chronic pollution by artificial organic contaminants can importantly alter antioxidative capacity of plants. From a human perspective, the important implication is that the presence of these environmental pollutants in the environment can significantly alter forage and food quality.

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Figure 1.

Changes in total flavonoid content in *Triticum aestivum* leaves in response to stress induced by textile dyes. The measurements were performed after the plants were watered with a total volume of textile dye solutions of 0.5 L (a) and 1 L (b) in concentration of 0.5 mg L⁻¹ and 1.5 mg L⁻¹. The signs above the columns represent: * = statistical difference between the given textile dye treatment and the control.

Figure 2.

Changes in total flavonoid content in *Triticum aestivum* leaves in response to treatments by antibiotics. The measurements were performed after the plants were watered with a total volume of antibiotics solutions of 0.5 L (a) and 1 L (b) in concentration of 0.5 mg L⁻¹ and 1.5 mg L⁻¹. The signs above the columns as: * = statistical difference between the given antibiotics treatment and the control.

Table 1

Characteristics of textile dyes used in plant treatment.

Trade name/ CAS number/ Abbreviation	Chemical formula/ Molecular weight (g mol ⁻¹)	Chemical structure
Anthraquinone dye		
Optilan Blue MF-GL 125328-86-1 OB	C ₂₄ H ₂₁ N ₂ O ₅ SLi 456.44	
Lanasyn Blue F-L 150 4474-24-2 LB	C ₃₂ H ₂₈ N ₂ O ₈ S ₂ ·2Na 678.68	
Azo dyes		
Lanasyn Red M-GA 70209-87-9 LR	C ₃₂ H ₂₁ CrN ₁₀ O ₁₁ S·2Na 851.61	
Nylosan Red N-2RLB 71873-39-7 NR	C ₂₄ H ₂₁ ClN ₄ O ₆ S ₂ ·Na 584.02	
Nylosan Dark Brown S-MBL 52587-68-5 NDB	C ₃₈ H ₂₅ CrN ₈ O ₇ S·2Na 835.69	

Trade name/ CAS number/ Abbreviation	Chemical formula/ Molecular weight (g mol ⁻¹)	Chemical structure
Lanasyn Dark Brown M-GLN 70236-62-3 LDB	C ₃₈ H ₂₄ CrN ₉ O ₉ S·2Na 880.69

Table 2

Characteristics of antibiotics used in plant treatment.

Trade name/ CAS number/ Abbreviation	Chemical formula/ Molecular weight (g mol ⁻¹)	Chemical structure
Penicillins		
Amoxicillin 34642-77-8 AMOX	C ₁₆ H ₁₈ N ₃ O ₅ Na 387.39	
Ampicillin 69-52-3 AMP	C ₁₆ H ₁₈ N ₃ O ₄ Na 371.39	
Penicillin G 113-98-4 PENG	C ₁₆ H ₁₇ N ₂ O ₄ SK 372.48	
Cephalosporins		
Ceftazidime 72558-82-8 CFZ	C ₂₂ H ₂₂ N ₆ O ₇ S ₂ 546.58	
Ceftriaxone 104376-79-6 CFX	C ₁₈ H ₁₆ N ₈ O ₇ S ₃ Na ₂ 598.54	
Tetracyclines		
Tetracycline 64-75-5 TET	C ₂₂ H ₂₄ N ₂ O ₈ .HCl 480.89	
Doxycycline 10592-13-9 DOXY	C ₂₂ H ₂₄ N ₂ O ₈ .HCl 480.89	
Fluoroquinolones		
Ciprofloxacin 86393-32-0 CIP	C ₁₇ H ₁₈ FN ₃ O ₃ .HCl 367.80	

Trade name/ CAS number/ Abbreviation	Chemical formula/ Molecular weight (g mol ⁻¹)	Chemical structure
Macrolides		
Erythromycin 1264-62-6 ERY	C ₄₃ H ₇₅ NO ₁₆ 862.05	