Environmental Pollution 234 (2018) 39–47

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Pharmaceutical and personal care products-induced stress symptoms and detoxification mechanisms in cucumber plants☆

Chengliang Sun [a, *], Stacia Dudley [a], John Trumble [b], Jay Gan [a]

[a] Department of Environmental Science, University of California, Riverside, CA 92521, USA
[b] Department of Entomology, University of California, Riverside, CA 92521, USA

ARTICLE INFO

Article history:
Received 25 July 2017
Received in revised form
6 October 2017
Accepted 9 November 2017

Keywords:
Chemicals of emerging concern
Oxidative stress
Reactive oxygen species
Antioxidant enzymes
Conjugation

ABSTRACT

Contamination of agricultural soils by pharmaceutical and personal care products (PPCPs) resulting from the application of treated wastewater, biosolids and animal wastes constitutes a potential environmental risk in many countries. To date a handful of studies have considered the phytotoxicity of individual PPCPs in crop plants, however, little is known about the effect of PPCPs as mixtures at environmentally relevant levels. This study investigated the uptake and transport, physiological responses and detoxification of a mixture of 17 PPCPs in cucumber seedlings. All PPCPs were detected at higher concentrations in roots compared to leaves, with root activity inhibited in a dose-dependent manner. At 5–50 μg/L, the mature leaves exhibited burnt edges as well as a reduction in photosynthesis pigments. Reactive oxygen species (ROS) production and lipid peroxidation increased with increasing PPCP concentrations; and their contents were greater in roots than in leaves for all PPCP treatments. Enzymes involved in various functions, including oxidative stress (superoxide dismutase and ascorbate peroxidase) and xenobiotic metabolism (peroxidase and glutathione S-transferase), were elevated to different levels depending on the PPCP concentration. Glutathione content gradually increased in leaves, while a maxima occurred at 0.5 μg L^{-1} PPCPs in roots, followed by a decrease thereafter. This study illustrated the complexity of phytotoxicity after exposure to PPCP mixtures, and provided insights into the molecular mechanisms likely responsible for the detoxification of PPCPs in higher plants.

1. Introduction

The use of reclaimed wastewater for irrigation and biosolids or animal wastes as fertilizers in agriculture is on the rise worldwide (Kinney et al., 2006; Malchi et al., 2014). There are many benefits from reuse of these waste materials, such as augmenting water supply, increasing soil nutrient content and improving crop yields (Carter et al., 2015). However, concerns remain about the safety of such practices (Boxall et al., 2012), as they introduce a multitude of trace contaminants, including pharmaceuticals and personal care products (PPCPs), into the agroecosystems (Bartrons and Peñuelas, 2017; Wu et al., 2013). In recent years, the fate of PPCPs in the soil-plant continuum has been extensively studied (Dalkmann et al., 2014; Grossberger et al., 2014; Xu et al., 2009). Furthermore, several studies have considered uptake and accumulation of subsets of PPCPs by different crop species (Bartrons and Peñuelas, 2017; Wu et al., 2015). Although many PPCPs are inherently bioactive substances, their toxicity to plants is comparatively less understood.

A few studies showed that exposure to PPCPs affected plant development and physiological functions (Bartrons and Peñuelas, 2017; Carter et al., 2015; Christou et al., 2016). For example, root growth and development were markedly reduced when pinto beans were exposed to chlortetracycline antibiotics (Batchelder, 1981). Tetracyclines and sulfonamides were shown to negatively affect seed germination (Liu et al., 2009a), and the influence varied among plant species and the different PPCPs considered in the study (Carvalho et al., 2014). Although such studies have shown various phytotoxic effects from PPCPs, the corresponding physiological and molecular mechanisms contributing to the toxicity were not adequately explored.

Once taken up by the plant root, PPCPs may be metabolized, leading to their detoxification, inactivation and sequestration (He et al., 2017; Wu et al., 2016). Another nodal point in the response of plant cells to xenobiotics is reactive oxygen species (ROS)

☆ This paper has been recommended for acceptance by Dr. Chen Da.

* Corresponding author.
E-mail address: chengliang.sun@ucr.edu (C. Sun).

https://doi.org/10.1016/j.envpol.2017.11.041
0269-7491/

generation, ultimately imposing oxidative stress to fundamental plant biomolecules (Ahammed et al., 2012; Liu et al., 2009b). Recent studies suggested that ROS overproduction-triggered oxidative damage may be the cause of the longer-term visual phytotoxic responses (Mittler, 2002), such as root growth inhibition and seed germination reduction. For instance, phenanthrene-induced oxidative stress in *Arabidopsis* was responsible for the observed reductions in germination and root growth and the damage to organelle structures (Liu et al., 2009b). On the other hand, plants have developed sophisticated antioxidant mechanisms to protect their cellular components from oxidative damage (Mittler, 2002). However, to our knowledge, so far little information is available on the potential impacts of PPCPs on ROS metabolism in higher plants, such as ROS production, oxidative damage and antioxidant system responses.

When exposed to single compounds of PPCPs, the observed toxic effects to plants were generally low (Migliore et al., 2003). However, PPCPs always enter agroecosystems as mixtures of many compounds. Comparison between individual and mixtures of PPCPs in aquatic organisms suggested that exposure to single PPCPs underestimated the actual environmental effect, and did not allow prediction of the risk of mixtures at environmentally relevant doses (Fernández et al., 2013).

This study was designed to evaluate PPCP accumulation and potential effects on ROS production and oxidative damage. Cucumber seedlings were exposed hydroponically to a mixture of 17 PPCPs at incremental levels (0, 0.5, 5, 50 μg L^{-1}) covering environmentally relevant occurrence (Christou et al., 2016; Meffe and de Bustamante, 2014; Wu et al., 2013). The molecular mechanisms involved in the detoxification of PPCPs were also explored.

2. Materials and methods

2.1. Chemicals and plant treatments

A total of 17 PPCPs were selected based on their occurrence in treated wastewater and biosolids. These compounds included 15 pharmaceutical compounds, i.e., acetaminophen, caffeine, meprobamate, atenolol, trimethoprim, carbamazepine, diazepam, gemfibrozil, primidone, sulfamethoxazole, dilantin, diclofenac, naproxen, ibuprofen and atorvastatin; and 2 personal care products, i.e., triclosan, triclocarban. The standards of these PPCPs were purchased from Alfa Aesar (Ward Hill, MA) and TCI America (Portland, OR), and their chemical purity was >98%. The corresponding deuterated standards, including their sources, are given in Text S1 in Supporting Information (SI). Stock solutions of each PPCP at 5, 50, 500 mg/L was prepared in methanol and stored at −20 °C before use. Solvents, including acetonitrile, methanol, and formic acid were Ultima grade (Fisher Scientific, Fair Lawn, NJ). All other chemicals and reagents used in enzyme activity measurement were of analytical grade or better. Purified water was obtained from a Milli-Q system (Millipore, Carrigtwohill, Cork, Ireland).

Cucumber (*Cucumis sativus* L.) seeds were obtained from Fisher Scientific (Fair Lawn, NJ). Seeds were germinated in a growth medium filled with a mixture of vermiculite and perlite (3/1, v/v) in a 72-hole plate. When the cotyledons were fully expanded, seedlings were transplanted to 500-mL glass jars filled with aerated, full-strength Hoagland nutrient solution and cultivated under controlled conditions (12 h/25 °C day and 12 h/25 °C night cycle; relative humidity of 75–80%). The nutrient solutions were renewed every 2 d. After 7 d, uniform seedlings were exposed to 0, 0.5, 5, or 50 μg L^{-1} PPCP mixture by spiking 50 μL of the stock solutions to the aerated, fresh hydroponic solution. In order to monitor the effect of different PPCP concentrations on the plant response, the solution was not changed during the short exposure experiment. Blank controls (with plant but no PPCPs) were set up simultaneously. The jars were wrapped with aluminum foil to prevent direct sunlight on the nutrient solution. Cucumber seedlings were sampled 7 d after the treatment, and the roots were gently dried with a paper towel, the cucumber plants were separated into roots and shoots (including stems and leaves). The content of reactive oxygen species (ROS), level of lipid peroxidation and activities of antioxidant enzymes in both roots and shoots were immediately analyzed after sampling. The remaining plant samples were frozen in liquid nitrogen and stored at −80 °C until processing. Each experiment was repeated at least three times.

2.2. Determination of chlorophyll content

The chlorophyll content in leaves was determined spectrophotometrically after chlorophyll extraction with 80% acetone (Liu et al., 2009b). Briefly, fresh leaves (0.2 g) were thoroughly homogenized with 15 mL of 80% acetone containing 0.2 g calcium carbonate and quartz sand until the tissue turned white. The homogenate was kept undisturbed for 5 min and was then filtered through a filter paper into a flask. The absorbance at 663, 646 and 470 nm was measured on a Cary 50 UV-Visible spectrophotometer (Varian, Palo Alto, CA). Chlorophyll *a* and *b* contents were calculated according to the following equations:

$$\text{Chlorophyll } a \text{ (mg g}^{-1}\text{ FW)} = (12.21A_{663}\text{-}2.81A_{645})/\text{FW} \quad (1)$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1}\text{ FW)} = 20.13A_{645}\text{-}5.03A_{645}/\text{FW} \quad (2)$$

where A_{663} and A_{645} are absorbance at 663 nm and 645 nm for chlorophyll *a* and *b*, respectively, A_{470} is the absorbance at 470 nm for carotene, and FW is the fresh weight of leaves.

2.3. Determination of root activity

Root activity was evaluated according to the triphenyltetrazoliumchloride (TTC) method (Wang et al., 2010b). Roots were excised and incubated in freshly prepared 5 mL of 0.4% TTC and 5 mL potassium phosphate buffer (PBS; 60 mM; pH 7.0) at 37 °C for 2 h. The reaction was stopped by the addition of 2 mL of 1 M sulfuric acid. Blank controls were included using the same procedure, but sulfuric acid was added at the beginning of incubation. After incubation, the roots were extracted with 10 mL ethyl acetate and absorbance was measured at 485 nm on a Cary 50 UV-Visible spectrophotometer (Varian, Palo Alto, CA).

2.4. Determination of PPCPs in cucumber tissues

The freeze-dried plant tissue samples were extracted and analyzed following the same method as in Wu et al. (2012). Briefly, plant materials were ground to a fine powder and placed in 50 mL centrifuge tubes, spiked with deuterated PPCPs as recovery surrogates and mixed with 20 mL methyl *tert*-butyl ether (MTBE). The mixture was then extracted in an ultrasonic water bath (50/60 Hz, Fisher) for 20 min prior to centrifugation at 8000*g* for 20 min. The supernatant was collected in 40 mL glass vials. Pellets were subsequently extracted with 20 mL acetonitrile. The pooled supernatant from MTBE and acetonitrile extractions was concentrated to near dryness under nitrogen. The residue was then re-dissolved in 1 mL methanol, followed by addition of 20 mL deionized water. The extract was loaded onto an Oasis™ HLB cartridge (150 mg, Waters, Milford, MA) that was preconditioned with 6 mL methanol and 12 mL deionized water. The cartridge was eluted with 15 mL methanol under gravity and the eluate was evaporated to dryness under nitrogen. The residue was recovered in 1.5 mL