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RESEARCH ARTICLE

Phytoremediation of carbamazepine and its metabolite 10,11-epoxycarbamazepine by C_3 and C_4 plants

Helena Ryšlavá[1] · Alice Pomeislová[1] · Šárka Pšondrová[2] · Veronika Hýsková[1] · Stanislav Smrček[2]

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Abstract The anticonvulsant drug carbamazepine is considered as an indicator of sewage water pollution: however, its uptake by plants and effect on metabolism have not been sufficiently documented, let alone its metabolite (10,11-epoxycarbamazepine). In a model system of sterile, hydroponically cultivated *Zea mays* (as C_4 plant) and *Helianthus annuus* (as C_3 plant), the uptake and effect of carbamazepine and 10,11-epoxycarbamazepine were studied in comparison with those of acetaminophen and ibuprofen. Ibuprofen and acetaminophen were effectively extracted from drug-supplemented media by both plants, while the uptake of more hydrophobic carbamazepine was much lower. On the other hand, the carbamazepine metabolite, 10,11-epoxycarbamazepine, was, unlike sunflower, willingly taken up by maize plants (after 96 h 88 % of the initial concentration) and effectively stored in maize tissues. In addition, the effect of the studied pharmaceuticals on the plant metabolism (enzymes of Hatch-Slack cycle, peroxidases) was followed. The activity of bound peroxidases, which could cause xylem vessel lignification and reduction of xenobiotic uptake, was at the level of control plants in maize leaves contrary to sunflower. Therefore, our results indicate that maize has the potential to remove 10,11-epoxycarbamazepine from contaminated soils.

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✉ Helena Ryšlavá
helena.ryslava@natur.cuni.cz

[1] Department of Biochemistry, Faculty of Science, Charles University, Hlavova 2030, Prague 2 128 40, Czech Republic

[2] Department of Organic Chemistry, Faculty of Science, Charles University, Hlavova 2030, Prague 2 128 40, Czech Republic

Keywords Acetaminophen · Carbamazepine · 10,11-Epoxycarbamazepine · Ibuprofen · NADP-ME · PEPC · Peroxidases · Phytoremediation

Abbreviations

NADP-ME	NADP-malic enzyme
PEPC	Phosphoenolpyruvate carboxylase
PPDK	Pyruvate, phosphate dikinase

Introduction

Over the last two decades, the release of pharmaceuticals from sewage effluents to the environment has become an issue of concern (Larsson et al. 2007; Loffler et al. 2005). The worldwide consumption of active substances is estimated to exceed 100,000 tons per year (Randak and Li 2009). Antibiotics, hormones, steroids, non-steroidal anti-inflammatory drugs, beta-blockers, and cancer therapeutics represent the most common classes of drugs found in effluents (Iori et al. 2012). After their use, pharmaceuticals are excreted with urine and feces either unmodified or metabolized, and thus get to the wastewater, which can further contaminate soils, surface waters, and groundwater (Loffler et al. 2005). The metabolism of drugs introduces hydrophilic functionalities into the original structure, which provides these compounds with new biological properties (Loffler et al. 2005; Makhijani et al. 2014). Manufacturers, landfill leachates, and animal farms also constitute an important source of pharmaceuticals and their metabolites in the environment (Nikolaou et al. 2007).

Once they enter ecosystems, pharmaceuticals and their metabolites can influence the same metabolic pathways of animals with identical or similar target organs, tissues, cells, and biomolecules to humans (Fent et al. 2006). The presence of

these compounds in the environment may lead to abnormal physiological processes and reproductive impairment, increased incidences of cancer, and development of antibiotic-resistant bacteria (Kolpin et al. 2002). There is an increasing incidence of endocrine-related diseases and physiological effects on animals. It is well known that changes in the reproductive health of humans, including declining male fertility, birth defects, and breast and testicular cancer, could be linked to exposure to endocrine-disrupting chemicals, including pharmaceuticals and their metabolites (Nikolaou et al. 2007; Campion et al. 2012).

As the awareness of residual pharmaceuticals in ecosystems was growing due to improved analytical detection techniques, the investigation of the fate of these substances and their potential detrimental effects on the environment became more important (Iori et al. 2012; Randak and Li 2009). There has been an effort to develop efficient, cheap, and ecologically responsible methods, which would remove these xenobiotics from the environment (Meagher 2000, Loffler et al. 2005).

Phytoremediation is a newly developing method using higher plants to extract, sequester, and/or detoxify environmental pollutants. Generally, plants are an appropriate tool for remediation of soil, sediment, groundwater, and surface water due to their physiological, genetic, and biochemical properties (Blaylock 2000; Iori et al. 2012; Makhijani et al. 2014; Marmiroli et al. 2006; Meagher 2000; Newman and Reynolds 2004). Moreover, plants exhibit a great ability to bind organic compounds from air (Macek et al. 2000). Phytoremediation represents a cheap, simple, and ecologically responsible alternative to destructive physical remediation methods, which are currently used (Meagher 2000). Furthermore, phytoremediation can be carried out in situ and thus limit human and environmental exposure to pollutants (Stephenson and Black 2014).

Plants respond to nutrition availability and other physiological conditions such as temperature, light intensity, or pathogen infection by changes in metabolic pathways. The presence of xenobiotics in soil, which are taken up by plant roots, can also act as a stressor and affect the plant metabolism in roots and shoots. The most important is the formation of reactive oxygen species that participate in signal transduction pathways and activate the antioxidant system, which consists of antioxidant compounds and antioxidant enzymes. Among others, peroxidases participate in the reduction of H_2O_2 content and catalyze the oxidation of various metabolites including lignin in cell walls. Recently, in C_3 plants, the participation of enzymes of the Hatch-Slack cycle in response to some types of stress was found out (Doubnerova et al. 2009; Doubnerova and Ryslava 2011; Hyskova Doubnerova et al. 2014; Muller et al. 2009; Ryslava and Doubnerova 2010; Ryslava et al. 2003). First of all, this cycle is known as a mechanism of concentration of CO_2 in C_4 plants; phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) catalyzes the primary fixation of CO_2 into a four-carbon compound in mesophyll cells, NADP-malic enzyme (NADP-ME, EC 1.1.1.40) catalyzes the release of CO_2 in bundle sheath cells, and pyruvate, phosphate dikinase (PPDK EC 2.7.9.1) catalyzes the regeneration of phosphoenolpyruvate. These reactions, or at least some of them, can be also beneficial for tissues, in which these enzymes do not have any photosynthetic function (roots of C_4 and whole C_3 plants) (Doubnerova and Ryslava 2011).

The aim of this study was to compare the uptake of more hydrophobic carbamazepine, which is also considered as an indicator of pollution by pharmaceuticals, and its metabolite 10,11-epoxycarbamazepine on one side with more hydrophilic ibuprofen and acetaminophen on the other side by two plant species, sunflower (C_3) and maize (C_4). Since carbamazepine can run off into surface water, which is used for the watering of agricultural plants, the effect of the above-mentioned xenobiotics on the metabolism of maize and sunflower was followed. Activities of PEPC, NADP-ME, and PPDK that could participate in the response to plant stress, were determined in plant roots and leaves. Moreover, the activity of peroxidases, including individual isoenzymes, was studied.

Material and methods

Synthesis and identification of 10,11-epoxycarbamazepine

Carbamazepine (1.00 g, 4.23 mmol, Sigma-Aldrich, USA) was dissolved in chloroform (20 mL), and a solution of *m*-chloroperoxybenzoic acid (2.24 g, 8.46 mmol) in chloroform (20 mL) was added at room temperature. The resulting mixture was stirred for 5 min. The stirring was then stopped, and the mixture was allowed at room temperature overnight. The mixture was washed three times with 60 mL of 10 % aqueous solution of $NaHCO_3$. The organic layer was dried with $MgSO_4$ and then concentrated in vacuo. The residue was separated on silica gel (diethyl ether-acetone 1:1). The yield was 0.594 g (59.4 %) of 10,11-epoxycarbamazepine as a yellow amorphous solid. ESI MS, *m/z* 275.2 $[M+Na]^+$ (22). ^1H and ^{13}C NMR spectra were identical with ref. (Vogna et al. 2004).

Plant material

Seeds of experimental plants *Zea mays* L. G1 and *Helianthus annuus* L cv. BELEM (Monsanto, Czech Republic) were sterilized in 75 % (*v*/*v*) ethanol for 30 s and in 10 and 5 % solutions of commercial bleacher (Savo), 0.2 % NaOH solution for 10 and 20 min, respectively. After that, six seeds were sown in 15 mL of sterilized growth medium pH 5.8 in a sterile 0.5-L Erlenmeyer flask containing Murashige-Skoog medium supplemented with saccharose (3 g/L) and myo-inositol (1 g/L). pH was adjusted to 5.8 prior to the autoclaving. Germination

and growth proceeded at 26 °C and a 16-h photoperiod with additional irradiance with fluorescent tubes (cool white 36 W/m^2, distance 40 cm) for 2 weeks in the case of maize and 3 weeks in the case of sunflower.

Supplementation of the cultivation medium with xenobiotics and sample preparation

Each of the studied xenobiotics (both acetaminophen and carbamazepine from Sigma-Aldrich, USA; ibuprofen from Zentiva, Czech Republic; and 10,11-epoxycarbamazepine synthesized as mentioned above) was dissolved in dimethyl sulfoxide (PCC tested, Sigma-Aldrich, USA), and the solution was added to a fresh cultivation medium to its final concentration of 15 mg/L. Totally, 10–15 plants were grown separately in 15 mL of cultivation medium enriched with a particular xenobiotic, and 3 plants were grown in a control cultivation medium in which no xenobiotic was used. Every 24 h, 0.5 mL of the cultivation medium was collected for the determination of the xenobiotic content and stored at −20 °C. On the fifth day of the experiment, the plants were harvested, the weight of each plant was determined, and fresh samples of leaves and roots were immediately frozen in liquid nitrogen and stored at −80 °C until the enzyme activity measurements.

Determination of xenobiotic content by HPLC

Analysis of the cultivation medium collected during the plant cultivation phase was done using the high-performance liquid chromatograph INCOS, Czech Republic, equipped with a Reprosil 100 C-18 column (4.4×250 mm). The mobile phase contained methanol/acetonitrile/1 % acetic acid in water in the ratio 1/1/0.7 (*v*/*v*/*v*) for ibuprofen; methanol/water in the ratio 1/2 (*v*/*v*) adjusted to pH 3 with H_3PO_4 for acetaminophen; and methanol/water in the ratio 8/2 and 6/4 with 0.1 % acetic acid for carbamazepine and 10,11-epoxycarbamazepine, respectively. Wavelengths for a particular xenobiotic were 230, 240, 285, and 225 nm for ibuprofen, acetaminophen, carbamazepine, and 10,11-epoxycarbamazepine, respectively. Chromatography was performed with isocratic flow 0.8 mL/min in all measurements. Experimental data were analyzed using the Clarity (DataApex) program with an automatic calculation of the compound amount according to the measured calibration curve with a detection limit 0.05 mg/L.

Enzyme activity assays

The preparation of plant extracts for enzyme activity assays (homogenization, extraction, centrifugation), similarly as the determination of PEPC, NADP-ME, and PPDK, was done according to our previous study (Ryslava et al. 2003). In the case of maize NADP-ME, the Tris-HCl buffer, pH 8.1, was used.

Peroxidase activity was determined in both the cytosolic soluble fraction (supernatant) and the membrane-bound fraction (sediment). The sediment was washed with an extraction buffer (100 mM Tris-HCl pH 7.8; 1 mM EDTA; 1 mM dithiothreitol; 5 mM $MgCl_2$; 5 % (*v*/*v*) glycerol) and then resuspended with 1 M NaCl in the extraction buffer and centrifuged at 16,600×*g* at 4 °C for 15 min. The reaction mixture contained 50 mM phosphate buffer pH 7.0, 1 mM of 3,3′-diaminobenzidine (dissolved in ethanol), 20 mM H_2O_2, and 0.1 mL of the plant extract. The increase of absorbance at 430 nm after 5 min was proportional to the increase of the amount of the peroxidase product (oxidized 3,3′-diaminobenzidine) and thus to the peroxidase activity.

Native polyacrylamide gel electrophoresis and detection of NADP-ME and peroxidases

The native electrophoretic separation of samples prepared from leaves and roots of phytoremediation plants and detection of NADP-ME activity in polyacrylamide gradient 6–12 % gel was done according to our previous study (Doubnerova et al. 2009). The peroxidase activity was detected in gels using 3,3′-diaminobenzidine as a substrate in the reaction mixture described above.

Statistical analysis

In general, at least 10 samples were used for the determination of the xenobiotic content and the plant weight and at least 3 measured values for the enzyme activity assay were compared using *t* test ($p \leq 0.05$). Statistical analyses were performed with the program SigmaPlot 9.0.

Results

In this study, we wanted to find out how pharmaceutically important compounds (ibuprofen, acetaminophen, and carbamazepine) are taken up by crops commonly used in agriculture and differing in type of photosynthesis and whether the plant metabolism would be affected or not. For this purpose, sunflower was used as a model C_3 plant and maize as C_4. Moreover, a human metabolite of carbamazepine, 10,11-epoxycarbamazepine, was synthesized using *m*-chloroperoxybenzoic acid and tested as a xenobiotic compound in further experiments. The yield of the synthesis was 59.4 %, and formation of the product was confirmed by MS ESI and by NMR spectroscopy (results not shown).

High concentrations of 15 mg/L of pharmaceuticals were used in sterile hydroponically grown plants to find out if plants are able to survive under these conditions and if they have a potential to eliminate these xenobiotics from the cultivation medium. Figure 1 demonstrated the ability of experimental

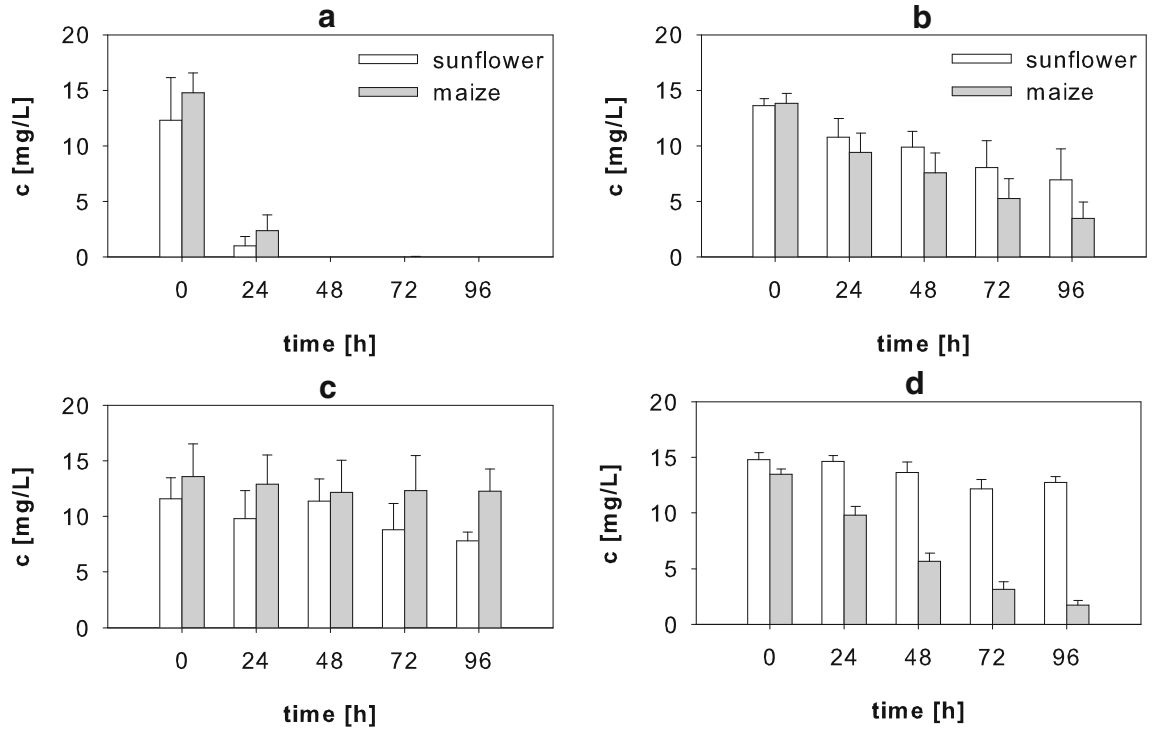
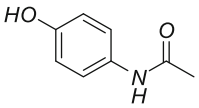
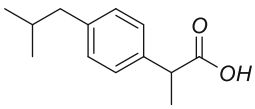
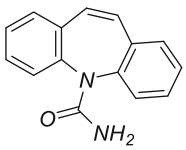
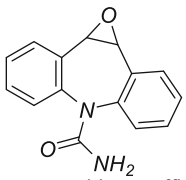


Fig. 1 Time dependence of the average concentration of ibuprofen (**a**), acetaminophen (**b**), carbamazepine (**c**), and 10,11-epoxycarbamazepine (**d**) in cultivation media of sunflower (*white columns*) and maize (*grey columns*). Initial concentration (time 0) is immediately affected by interactions with root surface and therefore is lower than applied 15 mg/L

plants to remove ibuprofen (a), acetaminophen (b), carbamazepine (c), and 10,11-epoxycarbamazepine (d) from the cultivation medium and their capacity to metabolize or store these compounds in their tissues. Simultaneously, control experiments with containers without plants were carried out and the concentration of drugs in the medium remained at the initial value of 15 mg/L±standard deviation of the determination (results not shown). Therefore, adsorption on the surface of the containers, photodegradation, and precipitation in the cultivation medium can be excluded. The phytoremediation experiment showed that ibuprofen was very effectively removed from the cultivation medium by both plants. After 48 h, the concentration of this compound in the cultivation medium was not detectable. Acetaminophen was also removed by both plant species quite readily. However, after 96 h, acetaminophen was still present in the cultivation medium of sunflower and maize; 50 and 25 % from the initial amount remained in the medium, respectively. On the other hand, carbamazepine was removed from the growth medium unwillingly by both plants. The carbamazepine concentration in the cultivation medium of sunflower decreased after 96 h to 70 % and maize only to 90 % from the initial content. The ability of maize and sunflower to remove 10,11-epoxycarbamazepine from the medium was significantly different. While maize removed this compound quite efficiently and the concentration of 10,11-epoxycarbamazepine in the growth medium decreased almost linearly, sunflower did not exhibit such properties. After 96 h, sunflower drew only 15 % of the initial 10,11-epoxycarbamazepine content from the medium, while maize did 88 %. The uptake of xenobiotics is dependent on physicochemical properties of their molecules. Relative molecular mass, solubility in water, vapor pressure, Henry's law constant, octanol/water partition coefficient K_{ow}, and pK_a are summarized in Table 1.

The next aim was to compare the effect of the studied xenobiotics on the plant growth. Fresh weights of all experimental plants after 96 h of the phytoremediation experiment were compared. All tested xenobiotics had either stimulating or no significant effect on the maize growth. The presence of carbamazepine and 10,11-epoxycarbamazepine in media did not have almost any impact on the weight of both maize and sunflower plants (Fig. 2a). While the presence of ibuprofen increased the weight of both experimental plants by approximately 30 %, acetaminophen increased only the weight of maize plants. On the contrary, acetaminophen decreased the weight of sunflower plants by almost 20 % (Fig. 2a). Considering the amount of xenobiotics, which experimental plants were able to sequestrate in their tissues after 96 h, it was found out that both maize and sunflower plants stored or metabolized ibuprofen and acetaminophen very willingly (Fig. 2b). No difference was observed between the ability of maize and sunflower in ibuprofen and acetaminophen sequestering. In contrast, the carbamazepine storage in both plants was limited and sunflower was a bit better than maize at this

Table 1 Physicochemical constants of studied xenobiotics. Data are taken from refs (Calderon-Preciado et al. 2012) and PubChem compound database

Compound	Structure	MW	Log K_{ow}	pK_a	Vapor pressure, mm Hg (25°C)	Solubility in water, mg/L (25°C)	Henry's law constant, atm . m^3/mol (25°C)
Acetaminophen *N*-(4-Hydroxyphenyl)-acetamide		151.16	0.46	9.38	6.29×10^{-5}	14000	6.42×10^{-13}
Ibuprofen 2-[4-(2-Methylpropyl)-phenyl]propanoic acid		206.28	3.97	4.91	4.74×10^{-5}	21	6.20×10^{-6}
Carbamazepine *5H*-Dibenzo*[b,f]*azepine-5-carboxamide		236.27	2.45	13.9	1.84×10^{-7}	18	1.08×10^{-7}
10,11-Epoxycarbamazepine 1a,10b-Dihydro-6*H*-dibenzo[*b*,*f*]oxireno[2,3-*d*]azepine-6-carboxamide		252.27	ND	ND	ND	ND	ND

MW relative molecular weight, K_{ow} octanol/water partition coefficient, K_a dissociation constant, *ND* not determined

process. 10,11-Epoxycarbamazepine was stored in maize tissues even better than ibuprofen and acetaminophen, but the effectiveness of metabolite sequestering in sunflower tissues was low (Fig. 2b).

Next, we wanted to know whether the tested xenobiotics would affect the activity of enzymes of the Hatch-Slack cycle, namely PEPC, NADP-ME, and PPDK. The most significant differences between the control plants and the plants cultivated in the presence of xenobiotics were found in roots of maize (Fig. 3b, d, f). All tested xenobiotics caused a three- to sixfold statistically significant increase in PEPC and NADP-ME activity in maize roots compared to control plants (Fig. 3b, d). PPDK in maize roots was increased only by 10,11-epoxycarbamazepine, namely 2.6-fold (Fig. 3f). Therefore, it seems that Hatch-Slack cycle enzymes participate on the response to the presence of xenobiotics in maize roots. On the other hand, the activity of PEPC and PPDK in sunflower roots was not significantly changed by xenobiotics in comparison to control samples (Fig. 3d, f). Only the NADP-ME activity in sunflower roots was induced by carbamazepine and 10,11-epoxycarbamazepine (Fig. 3d). The more pronounced differences in PEPC, NADP-ME, and PPDK activity were found in roots than in leaves of maize (Fig. 3). All tested xenobiotics caused a decrease of the activity of the photosynthetic PEPC

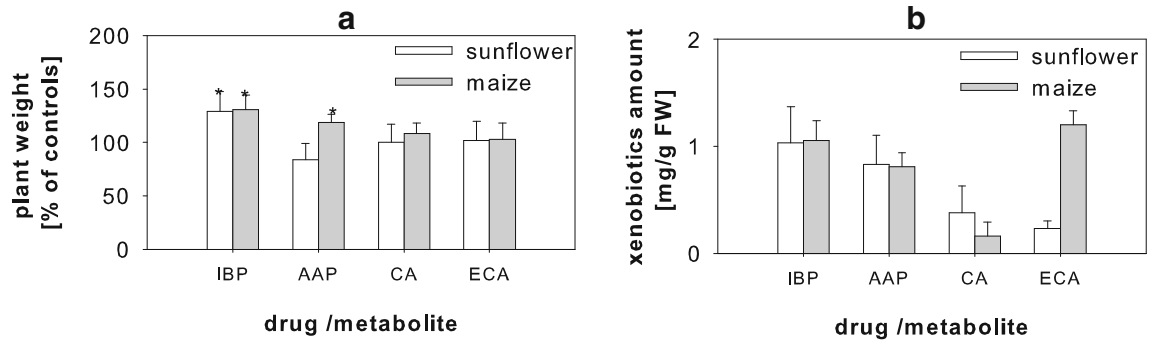


Fig. 2 Fresh weight of plants exposed to xenobiotics for 96 h (expressed in %) compared with non-treated controls (**a**) and an average weight of xenobiotics, which sunflower (*white color*) or maize (*grey color*) can store in their tissues, calculated per 1 g of the fresh plant weight (**b**). At least three samples were processed; significant differences from non-treated controls evaluated by *t* test with $P \leq 0.05$ are labeled with *. *IBP* ibuprofen, *AAP* acetaminophen, *CA* carbamazepine, *ECA* 10,11-epoxycarbamazepine

in maize leaves; 10,11-epoxycarbamazepine decreased the PEPC activity in this tissue up to 60 % of the control. Photosynthesis in maize is probably negatively affected by tested pharmaceuticals; however, the plant mass was not decreased (Fig. 2). Acetaminophen and carbamazepine also decreased the non-photosynthetic PEPC activity in sunflower

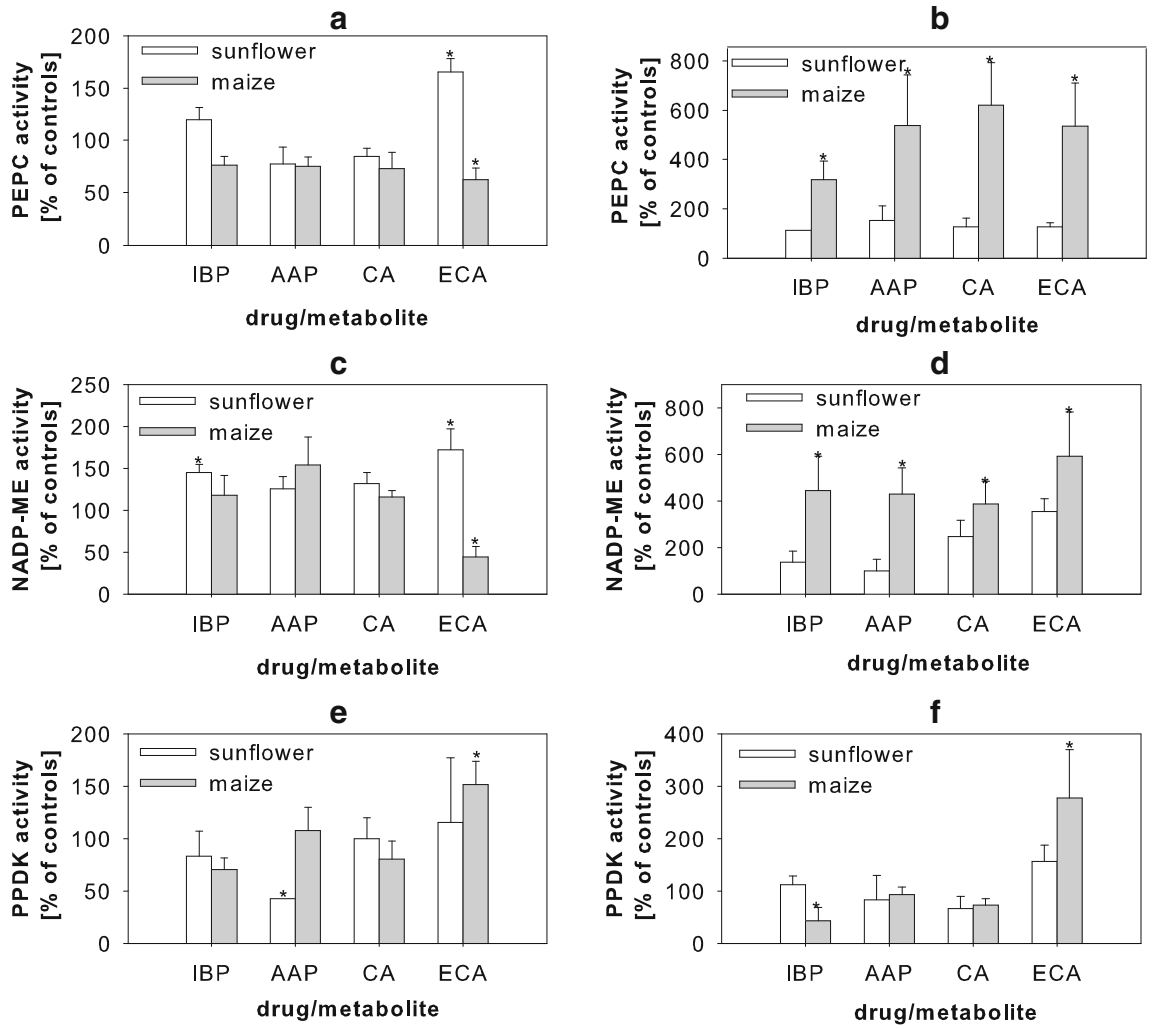


Fig. 3 Activity of PEPC (**a**, **b**), NADP-ME (**c**, **d**), and PPDK (**e**, **f**) in leaves (**a**, **c**, **e**) and roots (**b**, **d**, **f**) of sunflower (*white columns*) and maize (*grey columns*) plants. The activity is expressed in percent, where 100 % corresponds to the activity in control non-treated plants. At least three samples were processed; significant differences from non-treated controls evaluated by a *t* test with $P \leq 0.05$ are labeled with *. *IBP* ibuprofen, *AAP* acetaminophen, *CA* carbamazepine, *ECA* 10,11-epoxycarbamazepine

leaves, while ibuprofen and 10,11-epoxycarbamazepine induced the PEPC activity in this tissue (Fig. 3a). It means that also in leaves, the non-photosynthetic PEPC isoform can respond to the individual xenobiotics by increased activity. The activity of NADP-ME in leaves of both experimental plants was induced by the tested compounds except 10,11-epoxycarbamazepine, which reduced the NADP-ME activity in maize leaves (Fig. 3c). Acetaminophen decreased the PPDK activity in sunflower leaves, while 10,11-epoxycarbamazepine caused a moderate increase of activity in both plants (Fig.3e).

The activity of NADP-ME was detected in gels after separation by native electrophoresis, which enabled to compare the isoenzyme content. In the case of sunflower, only one isoenzyme was detected; in maize as a C_4 plant, the photosynthetic isoform of NADP-ME was predominant (Fig. 4).

The presence of xenobiotics in the cultivation medium can cause induction of the antioxidant system. Therefore, the activity of peroxidases was followed (Fig. 5). The effect of xenobiotics on the peroxidase activity was more pronounced in sunflower leaves than in maize leaves; only soluble peroxidases from maize leaves exhibited a slightly enhanced activity (Fig. 5a). The highest increase of activity was found for membrane-bound peroxidases from sunflower leaves (Fig. 5c), soluble peroxidases from sunflower roots (10,11-epoxycarbamazepine, Fig. 5b), and membrane-bound peroxidases from sunflower roots (ibuprofen, 10,11-epoxycarbamazepine, Fig. 5d).

Several isoforms of soluble peroxidases were separated by native electrophoresis followed by peroxidase detection (Fig. 6). Several isoforms of peroxidases in sunflower leaves were found; two isoforms with the highest mobility were more pronounced in extracts supplemented with carbamazepine and 10,11-epoxycarbamazepine, whereas isoforms with lower mobility were less abundant and were affected by acetaminophen (Fig. 6a). The highest number of peroxidase isoforms (two major and several minor) was found in roots of sunflower (Fig. 6b). On the contrary, maize leaves contained more soluble peroxidase isoforms than roots (Fig. 6c, d). The xenobiotics did not significantly affect the activity of isoforms of soluble peroxidases in maize plants (Fig. 6).

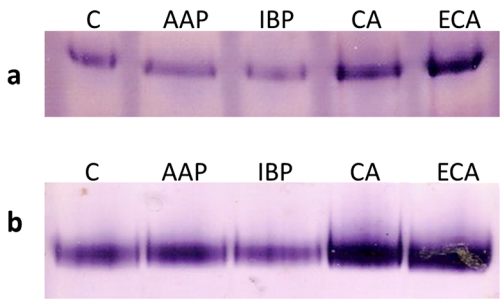


Fig. 4 Detection of NADP-ME activity in a gradient 6–12 % polyacrylamide gel after native electrophoretic separation of sunflower (**a**) and maize (**b**) extracts from leaves. *C* control non-treated plant, *AAP* acetaminophen, *IBP* ibuprofen, *CA* carbamazepine, *ECA* 10,11-epoxycarbamazepine

Discussion

Phytoremediation is an ecological, effective, and non-expensive way to eliminate drugs and their metabolites from the environment. Certain pharmaceuticals, such as carbamazepine, can leak out from sewage water plants into the ecosystem, and thus, they could contaminate agricultural soils. Plants are able to take up xenobiotics by their roots, to transport them to shoots, and to metabolize them. This ability is not general for all plant species and properties of the xenobiotic compound are important. Moreover, plants can also take up volatilized xenobiotics from air by above-ground plant parts (Collins et al. 2011). In this study, maize and sunflower were tested, because they had been successfully used for remediation of heavy metals in the first place (Fassler et al. 2010) and for remediation of polychlorinated biphenyls as well (Kacalkova and Tlustos 2011). Furthermore, we wanted to know whether carbamazepine and its metabolite can get into the tissues of edible plants and thus enter the food chains. The next reason of the plant choice was a different type of photosynthesis and the possibility to compare enzymes of the Hatch-Slack cycle (PEPC, NADP-ME, and PPDK) in photosynthetic tissues of C_4 leaves and in non-photosynthetic tissues, i.e., C_4 roots and C_3 leaves and roots.

While acetaminophen and ibuprofen, which belong to the most commonly used pharmaceuticals worldwide, have been frequently used for various phytoremediation experiments (Carvalho et al. 2014), the uptake of carbamazepine by plants has not been sufficiently documented, let alone its metabolites. In this study, we tested and compared the ability of sunflower and maize to take up carbamazepine and its metabolite, 10,11-epoxycarbamazepine, from the growth medium, the impact of these xenobiotics on the plant growth, and the stress response of plants. Acetaminophen and ibuprofen were used as reference substances, as they are taken up by plants easily due to the presence of polar function groups. For instance, it was found that horseradish and flax very efficiently removed acetaminophen (50–100 %) and ibuprofen (40–94 %) from cultivation media, which contained these compounds in the range 0.2–1.2 mM (Carvalho et al. 2014; Kotyza et al. 2010). Furthermore, tests with pale yellow lupine (*Lupinus luteolus*), barley, and reed (*Phragmites australis*) performed at the same concentrations showed that acetaminophen was the most effectively removed by *Lupinus*, while ibuprofen was taken up the best by *P. australis*, followed by barley (Kotyza et al. 2010; Carvalho et al. 2014). In another study, which was carried out with *Salvinia molesta*, *Lemna minor*, *Ceratophyllum demersum*, and *Elodea canadensis* at a concentration 10 μg/L of pharmaceuticals in media, ibuprofen was removed the most

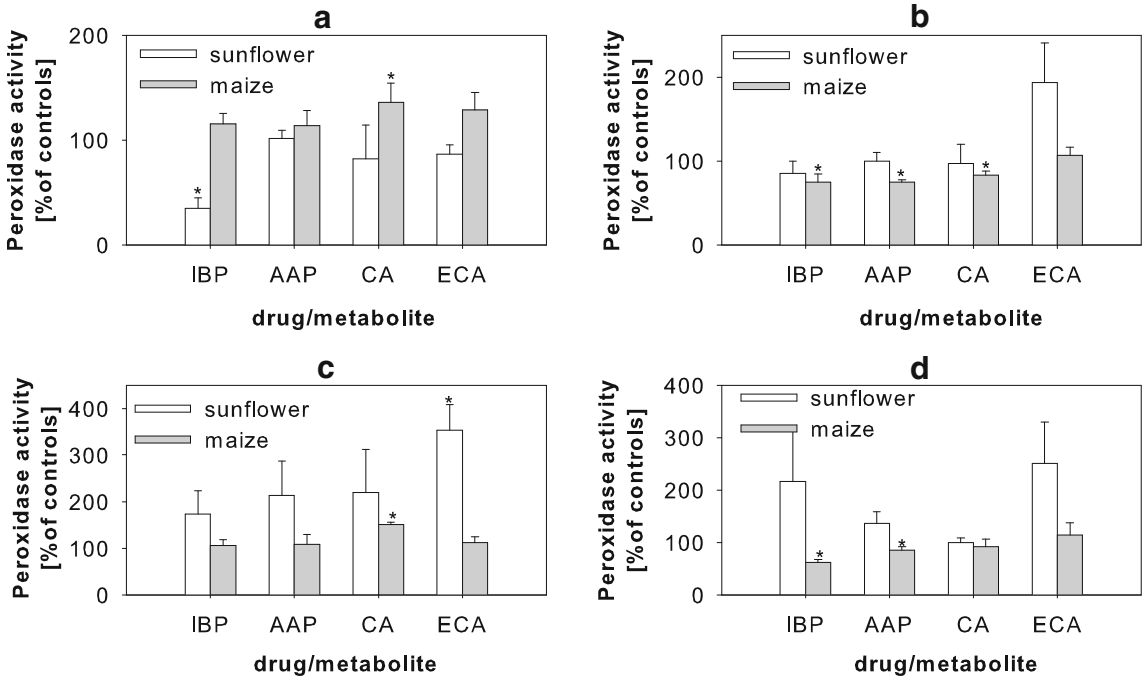


Fig. 5 Activity of soluble (**a**, **b**) and membrane-bound (**c**, **d**) peroxidases in leaves (**a**, **c**) and roots (**b**, **d**) of sunflower (*white columns*) and maize (*grey columns*). At least three samples were processed; significant differences from non-treated controls evaluated by a *t* test with $P \leq 0.05$ are labeled with *. *IBP* ibuprofen, *AAP* acetaminophen, *CA* carbamazepine, *ECA* 10,11-epoxycarbamazepine

in media of submerged plants *E. canadensis* (95 %) and *C. demersum* (83 %) (Carvalho et al. 2014; Matamoros et al. 2012).

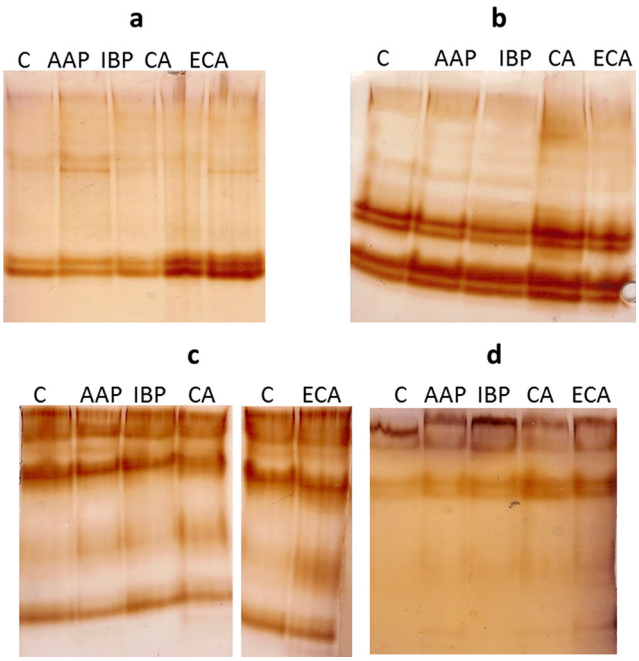


Fig. 6 Detection of soluble peroxidase activity in a gradient 6–12 % polyacrylamide gel after native electrophoretic separation of sunflower (**a**, **b**) and maize (**c**, **d**) extracts from leaves (**a**, **c**) and roots (**b**, **d**). *C* control non-treated plant, *AAP* acetaminophen, *IBP* ibuprofen, *CA* carbamazepine, *ECA* 10,11-epoxycarbamazepine

As it is difficult to remove carbamazepine, one of the commonly used antiepileptic drug, by sewage treatment plant, it serves as a marker of surface water pollution by pharmaceuticals (Clara et al. 2004). This compound is ubiquitous in nature and is supposed to be one of the most recalcitrant pharmaceuticals in the aquatic environment. It is frequently detected in wastewater treatment plant effluents and in natural waterbodies in concentrations ranging from micrograms per liter to nanograms per liter (Dordio et al. 2011; Fent et al. 2006; Leclercq et al. 2009; Zhang et al. 2008). Tests carried out on constructed wetlands showed in most cases rather low elimination of carbamazepine compared to elimination of other drugs. Particularly, the carbamazepine uptake in constructed wetlands with *Acorus* and *Typha* plants (Park et al. 2009) strongly depended on the season: it showed a 66 % uptake in May and no uptake in August. Another test carried out in mesocosm-scale constructed wetlands planted with *Typha* spp. and *P. australis* (Hijosa-Valsero et al. 2010) showed a moderate removal of carbamazepine—24–36 % in winter and 48 % in summer from the initial concentration.

Among recent in vitro studies, Dordio et al. (2011) tested the ability of hydroponically cultivated *Typha* spp. to remove carbamazepine (0.5–2.0 mg/L) from the cultivation media. Over the maximum period of 21 days, it showed that the removal of carbamazepine reached values of 56 % for the initial concentration of 2.0 mg/L and 82 % for the initial concentration of 0.5 mg/L. Furthermore, experiments with hydroponically grown *Scirpus validus*, which was exposed to carbamazepine at the same range of concentrations (0.5–2.0 mg/

L), and at the same maximum period of 21 days, showed that carbamazepine elimination from the nutrient solution reached 74 % (Zhang et al. 2013). Another test with *P. australis*, which was grown semi-hydroponically and was exposed to 5 mg/L of carbamazepine, showed a 90 % removal of the initial concentration of carbamazepine after 9 days of the experiment (Sauvetre and Schroder 2015). In our study, the carbamazepine concentration was reduced after 4 days to 70 and 90 % of the initial content in the cultivation medium of sunflower and maize, respectively (Fig. 1c). In the case of the carbamazepine metabolite, 10,11-epoxycarbamazepine, the results of uptake experiment in maize were different than with carbamazepine alone. Maize was able to extract 10,11-epoxycarbamazepine willingly; the initial concentration was reduced to 12 % (Fig. 1d). It could be caused by different lipophilicity of the oxygenated carbamazepine metabolite.

The uptake of xenobiotics by plants comprises a compound-specific active transport and passive processes (Gao and Zhu 2003). Apart from few compounds with a hormone-like structure, which can be taken up by active transport, xenobiotics are expected to enter the plant through passive diffusion and their further distribution in the plant is driven by the transpiration process (Calderon-Preciado et al. 2012). To predict the uptake by passive diffusion, it is useful to consider physicochemical properties of tested compounds like solubility in water, vapor pressure, Henry's law constant, octanol/water partition coefficient K_{ow}, molecular weight, and pK_a (Calderon-Preciado et al. 2012; Susarla et al. 2002); data for tested xenobiotics are summarized in Table 1. On the contrary, if the active process dominates, it is difficult to find a straight relationship between plant uptake and physicochemical parameters of the xenobiotic (Gao and Zhu 2003).

As mentioned above, the uptake of organic compounds by plants strongly depends on their molecular weight. It is supposed that the absorption of compounds with a molecular weight larger than 1000 by cellular membranes is impossible (Sanderson et al. 2004; Topp et al. 1986; Winker et al. 2010). Furthermore, the uptake of non-ionic molecules is assumed to be greatest for compounds with a log K_{ow} in the range of 1–4, because these compounds are hydrophobic enough to move through the lipid bilayer of membranes and water soluble enough to travel into the cell fluids (Calderon-Preciado et al. 2012).

According to these presumptions, ibuprofen seems to be the best xenobiotic candidate for the use of phytoremediation techniques, followed by carbamazepine, while acetaminophen should be taken up by plants poorly because of its low log K_{ow} value (Table 1). Nonetheless, our experiments showed that acetaminophen was taken up by tested plants efficiently and its concentration in media decreased linearly (Fig. 1b), while carbamazepine remained in large part in culture media of both plant species (Fig. 1c). Only ibuprofen was taken up by both plants completely in 48 h (Fig. 1a), and its physicochemical properties indeed corresponded with the ability of plants to remove this drug from media. The processes occurring during phytoremediation seem to be more complex, and they could not be predicted only according to physicochemical parameters. There are other processes which can also affect the uptake of pharmaceuticals. One of them is adsorption on the root surface, but this effect is limited by the size of roots. Considering the complexity of the plant cell wall, pharmaceuticals can bind to cell wall structures. Plant roots form a rhizosphere that can secrete various compounds including organic acids and enzymes, which alter the immediate surroundings of the plant and thus can change the properties of pharmaceuticals and the possibility of their uptake. Another aspect, which can influence natural systems, is the presence of symbiotic microorganisms. The uptake, partially degradation, and accumulation, depend on all constituents of the ecosystem. Therefore, it is important to carry out growth experiments in soils in the future, before assessing maize and sunflower to be a convenient plant species for phytoremediation of contaminated soils. This study served as a preliminary evaluation of chosen plant species to test their ability to take up xenobiotics and the effect of these xenobiotics on their metabolism.

The xenobiotics present in a plant can act as toxic compounds inducing stress response. Besides synthesis of secondary metabolites, the primary metabolic pathways could be also changed. PEPC, NADP-ME, and PPDK possess various functions and can participate in a defense response against both biotic and abiotic stress (Doubnerova and Ryslava 2011). Our results indicate that the tested xenobiotics decreased activities of photosynthetic enzymes and thus probably function as abiotic stressors in maize leaves (Fig. 3). In this tissue, the PEPC activity was inhibited by all tested pharmaceuticals, more pronouncedly by the metabolite 10,11-epoxycarbamazepine. Similarly, the photosynthetic NADP-ME was strongly inhibited by this metabolite (Fig. 3a, c). The photosynthesis of maize plants is probably negatively affected by tested xenobiotics; the strongest effect was caused by 10,11-epoxycarbamazepine metabolite, although the uptake was worse than by more hydrophilic ibuprofen (Fig. 1). However, the toxic effect on the whole photosynthesis was not much significant as the total plant weight in the case of all xenobiotics was higher or equal compared to control plants (Fig. 2).

On the contrary, maize root non-photosynthetic PEPC, NADP-ME, and, in the case of 10,11-epoxycarbamazepine, also PPDK (Fig. 3b, d, f) were statistically significantly induced by used xenobiotic compounds. The PEPC and NADP-ME activity was statistically increased in sunflower leaves too, namely by the metabolite 10,11-epoxycarbamazepine (Fig. 3a, c). The reason why enhanced activities of non-photosynthetic PEPC and NADP-ME were found in plants grown in the presence of xenobiotics is not clear. These compounds act in the plant as a stressor, which could activate a response and change the metabolism of the cell. Some functions of PEPC, NADP-ME, and PPDK could be involved in

this response. A lot of various functions were suggested for non-photosynthetic isoforms. The role of PEPC is mainly anaplerotic; the product oxaloacetate can be supplied for the citric acid cycle. Moreover, this enzyme can connect the metabolism of saccharides with the metabolism of amino acids and proteins. In addition, PEPC with NADP-ME is involved in the regulation of pH and malate fermentation in the case of anaerobic conditions (Latzko and Kelly 1983; O'Leary et al. 2011). Similarly to PEPC, the non-photosynthetic PPDK is involved in the metabolism of nitrogen. It was proved that this enzyme accelerates the remobilization of nitrogen during the leaf senescence to prefer the seeds' growth (Taylor et al. 2010). Moreover, the reaction catalyzed by PPDK is reversible and, in the case of need, this enzyme can alternate the glycolytic enzyme pyruvate kinase, which is energetically more advantageous (Plaxton and Tran 2011). The main function of the non-photosynthetic NADP-ME is to provide the reducing equivalent NADPH for various biosynthetic processes and a role as a coenzyme (Doubnerova and Ryslava 2011). In plants exposed to xenobiotic compounds, the NADPH-dependent cytochrome P450 monooxygenase, which participates in phase I of biotransformation, can be provided with NADPH from this source. As a coenzyme, NADPH is important mainly for the antioxidant system, especially for the glutathione reductase, since the phase II reactions are associated with the glutathione-mediated metabolism of xenobiotics in plants. In the case of need, NADPH provided by NADP-ME can be re-oxidized by the respiration chain using a specific mitochondrial NADPH-dehydrogenase to yield ATP (Edwards et al. 1998).

Soil contamination with toxic compounds (e.g., heavy metals) generally evokes oxidative stress in plants (Jouili et al. 2011). The antioxidative system is in relation with the function of peroxidases, which participate in H_2O_2 elimination, oxidation of various compounds, hormonal catabolism, ethylene biosynthesis, and polymerization of phenolics to form lignin and suberin (Jouili et al. 2011; Passardi et al. 2005). In our experiments, the effect of xenobiotics in the cultivation medium enhanced the activity of peroxidases, but not in all studied cases; significant differences were between the experimental plants (Figs. 5 and 6). In sunflower leaves, the activity of bound peroxidase was statistically significantly enhanced, especially when 10,11-epoxycarbamazepine was used as a xenobiotic compound (Fig. 5c), and corresponded to its low uptake (Fig. 1d). As bound peroxidase functions in crosslinking of hydroxycinnamic alcohols to form lignin and suberin and in the linkage of ferulate to polysaccharides, peroxidases participate in cell wall strengthening (Herrero et al. 2013; Passardi et al. 2004). Thus, limited transport of toxic compounds could occur as a consequence of the reduction of the size of xylem vessels due to significant lignification as a part of the physical barrier against intoxication (Jouili et al. 2011; Barcelo et al. 1988). Therefore, plants with a high level of peroxidases participating in cell wall lignification are probably not suitable as phytoremediation plants. On the other hand, the function of peroxidases in the antioxidant protection (e.g., as a part of ascorbate-glutathione cycle) is indispensable (Caverzan et al. 2012). In maize leaves, the activity of soluble peroxidases was moderately increased (Fig. 5a), whereas the activity of bound peroxidases was similar to that of control plants (Fig. 5c). It is possible that this result points to one of many factors influencing a better uptake of 10,11-epoxycarbamazepine by maize than sunflower plants (Fig. 1d). In roots, the effect of xenobiotics was not visible; only 10,11-epoxycarbamazepine enhanced both soluble and bound types of peroxidases, respectively (Fig. 5b, d). It seems that xenobiotics caused oxidative stress in plant cells and tissues, but the ability to tolerate this stress was dependent on the plant species, the properties, and the structure of xenobiotics.

Conclusion

In summary, unlike polar xenobiotics ibuprofen and acetaminophen, more hydrophobic carbamazepine was taken up unwillingly by both maize and sunflower plants. Sunflower plants were somewhat better at carbamazepine uptake than maize. On the other hand, our results indicate that maize has a potential to eliminate the carbamazepine metabolite 10,11-epoxycarbamazepine from the cultivation medium. Firstly, maize removed 10,11-epoxycarbamazepine from the cultivation medium much more efficiently than sunflower and was able to store this metabolite in its tissues. In addition, the metabolism of maize plants seems to be better adjusted to the presence of xenobiotics in the medium. Even though the activity of photosynthetic PEPC was decreased in maize leaves, the non-photosynthetic Hatch-Slack enzymes, which can participate in the alleviation of stress consequences, were significantly induced in roots. Finally, the activity of bound peroxidase, which could cause xylem vessel lignification and reduction of contaminant uptake, was at a level of control plants in maize leaves, whereas the effect of xenobiotics on the peroxidase activity was more pronounced in sunflower. The results of this study can be important for crop production in fields containing contaminated soils, near rivers, or when contaminated surface water is used for crop watering. Before assessing the utility of maize and sunflower for phytoremediation of soils, further growth experiments in soils are necessary.

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