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Uptake and transformation of ciprofloxacin by vetiver grass (*Chrysopogon zizanioides*)

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ABSTRACT

Ciprofloxacin (CIP) is a synthetically produced and widely prescribed antibiotic. Due to incomplete metabolism and gut absorption, a significant portion of the consumed CIP is excreted and released into the environment through wastewater. Vetiver grass has been reported to tolerate many organic and inorganic pollutants. Our objectives were to evaluate the potential of vetiver grass to remove CIP from aquatic media with the ultimate goal of developing a plant-based method for wastewater treatment. We also examined the potential degradation/transformation of CIP in the plant and the metabolic pathways impacted by CIP. Results show that vetiver grass removed more than 80% CIP within 30 days. Ciprofloxacin elicited a stress response by inducing antioxidant enzymes, and metabolic profiling indicated an impact on key metabolic pathways. Transformation products of CIP in vetiver tissue indicate the potential role of root-associated microorganisms as well as plant metabolism in CIP degradation.

1. Introduction

Antibiotics are microbial byproducts widely used as medicines to control or prevent diseases (Yi et al., 2017). Municipal wastewater treatment plants (WWTPs) are not designed to remove antibiotics and act as a significant point source contributors of these emerging contaminants in the environment (He et al., 2015). Several studies have detected antibiotic-resistant bacteria in raw sewage and treated sewage in wastewater treatment plants (Everage et al., 2014), as well as raw and treated drinking water (Bergeron et al., 2015). United States Environmental Protection Agency (USEPA) has recently initiated regulatory actions against several hospitals to ensure they manage their wastewater containing hazardous substances such as active pharmaceutical compounds (He et al., 2015). Antimicrobial compounds are also artificially synthesized by the pharmaceutical industry (Sarmah et al., 2006). Apart from medicinal use, antibiotics are used as prophylactics and growth stimulators in concentrated animal feeding operations (CAFOs) such as dairy farms, poultry, and fisheries, etc. (Liu et al., 2013; Migliore et al., 2003). During the past few decades, the overuse of antibiotics has resulted in widespread groundwater and soil contamination (Gomes et al., 2017) that have contributed to severe problems that threaten the ecosystem and human health.

In the past decade, the medical and non-medical application of synthetic drugs such as ciprofloxacin (CIP) has increased significantly all over the world (Sarmah et al., 2006). CIP belongs to a group of synthetic drugs known as fluoroquinolones, which is a highly prescribed antibiotic due to its broad-spectrum activity (Brown, 1996). World Health Organization (WHO) recognized fluoroquinolones as a critically important class of antibiotic for human use (Eggen et al., 2011; WHO, 2007). CIP belongs to the third generation of fluoroquinolone antibiotics that is frequently detected in the environment due to its prolonged persistence (Hirsch et al., 1999; Maul et al., 2006; Pan et al., 2018; Picó and Andreu, 2007). CIP inhibits the enzymatic activities that play a key role in DNA replication (Pan et al., 2018). Unlike other pharmaceutical compounds, synthetic drugs such as fluoroquinolones are partially metabolized in the animal physiological system, and the majority of them are excreted as parent compounds.

Environmental remediation using a number of physicochemical treatments including advanced oxidation, membrane separation, and adsorption have been reported in the literature. One of the major drawbacks of these treatments is the formation of toxic daughter compounds. In addition, most of these treatment methods are not economically or environmentally sustainable due to their high cost, a requirement for major infrastructure development and disposal issues. Biosorption of CIP in wastewater using activated sludge and aerobic granular sludge was attempted, and a concentration and pH-dependent

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biosorption of approximately 30–60% was reported (Ferreira et al., 2016). Phytoremediation provides a safe and inexpensive alternative compared to the physical and chemical treatment methods and has become accepted as one of the best management practices for several environmental management services.

In this study, we investigated the performance of vetiver grass (*Chrysopogon zizanioides* (L.) Roberty, var. Sunshine) in removing CIP from aqueous media. The primary advantages of the vetiver system (VS) include its massive fibrous root system (Cheng et al., 2003) and fast biomass production rate (up to 354 kg/ha within 6 months). Vetiver grass showed effectiveness for both environmental protection, such as slope stabilization and revegetation of mine-impacted sites (Hill, 1992; Zhao et al., 2014) and environmental remediation, such as wastewater treatment, treatment of acid mine drainage, and phytoextraction of various metals and organics (Kiiskila et al., 2019; RoyChowdhury et al., 2015, 2018). The United States Department of Agriculture (USDA) declared vetiver grass as a non-invasive plant species and therefore the application of VS pose no threat to ecological systems (Robert, 2009). Previously, VS was reported to remove antibiotics such as tetracycline from the soil and hydroponic media (Datta et al., 2013; Panja et al., 2014; Sengupta et al., 2016), as well as CIP from saline shrimp pond water (Hoang et al., 2012). *Phragmites australis*, a common wetland plant, was also reported to remove CIP in a hydroponic setup, but due to the invasive nature, its application is not environmentally sustainable. We hypothesized that vetiver grass could uptake CIP from hydroponic media and transform and/or degrade the antibiotic in its physiological system. Based on different environmental concentrations reported in the literature, we chose four concentrations of CIP (0.05, 0.1, 1, and 10 mg/L). Our objectives for this study were to evaluate the potential of vetiver grass to remove CIP from aquatic media, and to observe the physiological response of vetiver grass due to exposure to CIP.

2. Materials and methods

2.1. Reagent and materials

Ciprofloxacin powder (HPLC grade, ≥ 98%), β-mercaptoethanol, Tris-HCl buffer and phenylmethyl sulfonyl fluoride (PMSF) were obtained from Sigma-Aldrich (Millipore-Sigma, St. Louis, MO) chemical. Ethylenediaminetetraacetic acid (EDTA), trace metal grade orthophosphoric acid (CAS 7664-38-2), HPLC grade methanol (CAS 67-56-1), and acetonitrile (CAS 75-05-8) were purchased from Fisher Scientific (Fairlawn, NJ).

2.2. Experimental design

Vetiver slips (approximately 8–10 inches long) were obtained from Agriflora Tropicals, PR, USA. The slips were acclimated in the greenhouse for 1.5 months (Datta et al., 2013). Plants were then uprooted and thoroughly washed to remove all the soil from the root zone. The plants were then transferred to half-strength Hoagland's nutrient media (Hoagland and Arnon, 1950) and allowed to acclimatize for two weeks.

2.3. Batch experiment

In this experiment, acclimatized plants were placed in 1L HDPE bottles (4% w/v) containing 800 mL of Hoagland's nutrient solution (Hoagland and Arnon, 1950) spiked with CIP at concentrations of 0.05, 0.1, 1 and, 10 mg/L. These concentrations cover the total range of reported values of CIP in surface water, municipal, and industrial wastewater streams (Kolpin et al., 2002). The plants were placed in a Thermo Scientific Precision plant growth chamber (25 ± 3 °C, 14 h photoperiod) allowed to grow for 30 days. Two types of controls were used. (1) Vetiver plants were grown in CIP-free Hoagland's solution and (2) unplanted CIP spiked Hoagland's solution was kept as negative control. All the treatments (both experimental and control) had three replicates. Periodical water samples (5 mL) were collected from each replicate for CIP analysis. Periodical volume adjustments were performed in both control and treatment to compensate for water loss due to evapotranspiration.

2.4. Plant sample analysis

Plant tissue samples (leaf and root) were collected after the 30-day experimental period for detection of CIP and its degradation products. Plant samples were rinsed multiple times with deionized water and allowed to dry at room temperature. Seven grams of leaf and root tissue were crushed separately in sterilized mortar and pestle using liquid nitrogen. The crushed tissue was weighed and prepared for further analysis as described below.

2.4.1. Sample preparation for high-performance liquid chromatography (HPLC) and electrospray ionization mass spectrometry (ESI-MS)

HPLC grade acetonitrile (5 mL) was added to 0.5 g of crushed plant tissue and vortexed for 10 min. The sample was then centrifuged for 10 min at 10,000 g, and the supernatant was filtered through a 0.45 μM nylon filter. The filtered sample was stored in −20 °C freezer for ESI-MS analysis.

2.4.2. Sample preparation for chlorophyll analysis

The sample for chlorophyll (Chl) determination was prepared according to (Liu et al., 2013) by homogenizing 100 mg of shoot tissue and extracting it in 5 mL of 80% (v/v) aqueous acetone. The absorbance of the solution was measured using Citation 3, Biotek® microplate reader at 663 and 645 nm for Chl a and Chl b respectively. Chl a and Chl b content was determined by using the following equation described by Arnon (1949).

$$Chl\ a = 0.0127Abs_{663} - 0.00269Abs_{645}$$

$$Chl\ b = 0.0229Abs_{645} - 0.00468Abs_{645}$$

2.4.3. Sample preparation for stress enzyme activity analysis

Plant protein was extracted according to Farkas et al. (2007) using extraction buffer composed of 50 mM Tris-HCL (pH 7.2), 1 mM ethylenediaminetetraacetic acid, 10 mM β-mercaptoethanol, and 1 μL of 20 mg/mL PMSF. The reaction mixture was sonicated for 30 min and centrifuged (10,000 g for 15 min) thereafter. The supernatant containing crude plant protein was collected in a microcentrifuge tube and stored at −80 °C freezer. Protein quantification was performed using Pierce™ bicinchoinic acid (BCA) protein assay kit (Thermo Scientific).

2.5. Analytical methods

2.5.1. HPLC

HPLC analysis of the extract was performed according to (Rakshit et al., 2013). The HPLC system was calibrated using known standards of CIP, and linear calibration (concentration vs. Peak area) was used for quantification.

2.5.2. ESI-MS

Unit mass resolution Collision Induced Dissociation (CID) spectra were recorded on a Waters Quattro Ultima Triple Quadrupole Mass Spectrometer equipped with an Electrospray Ionization (ESI) source using nitrogen as the desolvation gas. The samples were introduced to the source at a flowrate of 20 μL/min. The source and the desolvation gas temperature were held at 100 and 250 °C, respectively. The collision energy was varied between 4 and 30 eV. The capillary and cone voltage was set at 3 kV and 15 kV, respectively. The desolvation gas flow was maintained at 222 L/hr.

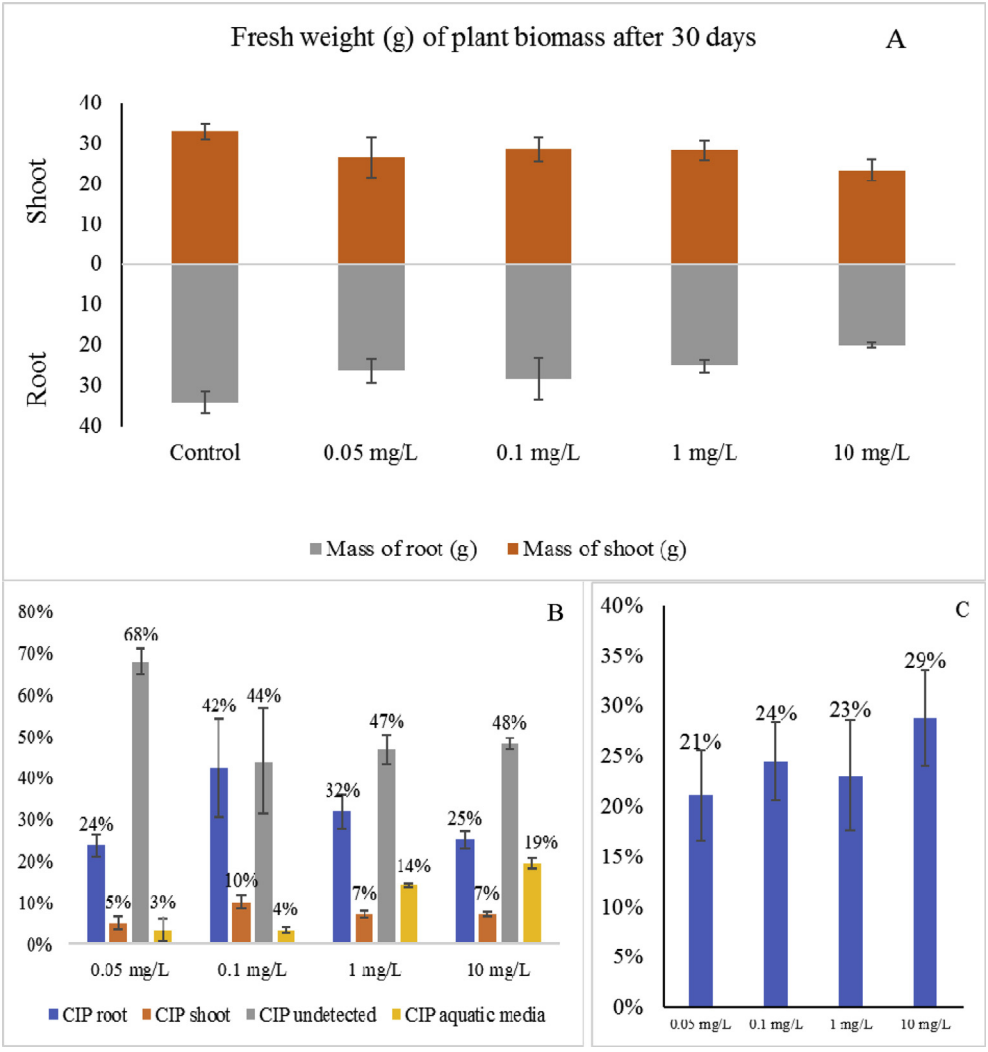


Fig. 1. A) Effect of CIP on the biomass accumulation by the end of 30-day experimental period. Data expressed as the mean (n = 3) ± one standard deviation. B) Mass distribution of CIP in different fraction (root, shoot, and aquatic media. C) Root to shoot translocation factor for each CIP treatment.

2.6. Stress enzyme analysis

Glutathione-s-transferase (GST) assay kit (Cayman Chemicals, Ann Arbor, MI) was used to determine the enzyme activity in plant leaf and root tissue according to Sengupta et al. (2016).

Guaiacol peroxidase (GPO) activity was measured according to Verma and Dubey (2003). The assay mixture was made up to 200 µL consisting of 40 mM potassium phosphate buffer (pH 6), 2 mM H_2O_2, 9 mM guaiacol, and 50 µL plant protein extract. Superoxide dismutase (SOD) assay kit (Cayman chemicals, Ann Arbor, MI) was used for determining SOD activity (Andra et al., 2011). 50 mM Tris-HCl (pH 8) containing 0.1 mM diethylenetriaminepentaacetic acid (DPTA), and 0.1 mM hypoxanthine was used as assay buffer. Catalase (CAT) assay kit (Cayman chemicals, Ann Arbor, MI) was used to determine the CAT activity in vetiver leaf and root according to Andra et al. (2011). The assay mixture was comprised of 100 µL of phosphate buffer (100 mM potassium phosphate, pH 7), 30 µL of methanol, 20 µL of plant protein extract, and 20 µL of H_2O_2.

2.7. Metabolomic analysis

The composite samples (100 mg) of lyophilized root and shoot tissue were prepared for the analysis. To validate our metabolomics results, another set of hydroponic experiment was performed using a higher concentration (30 mg/L) of CIP. The metabolomic analysis was performed as we described by (Pidatala et al., 2016). Briefly, the metabolites were extracted in 4 volume of extraction buffer containing methanol: acetonitrile (50: 50, v/v) and 0.125% formic acid prechilled at −20 °C. The metabolic profiling was conducted on an ACQUITY H-class UPLC system (Waters, MA) coupled with a Qtrap 6500 hybrid triple quadrupole mass spectrometer (Sciex, MA). The chromatographic separation of the metabolites was achieved on a Luna NH_2 amino propyl column (Phenomenex, CA). A total of 325 metabolites covering all the major metabolic pathways in plants were scanned in multiple reaction mode (MRM). Two injections were performed, one for negative mode (ESI−) and the other one for positive mode (ESI+). The dwell time was set at 3 ms. The compound-specific MS/MS parameters including retention time and MRMs were optimized using the purified standards. All the samples were analyzed in triplicates (n = 3) and metabolites detected in all replicates were considered for statistical analysis. Metaboanalyst 4.0 (https://www.metaboanalyst.ca) was used for the statistical analysis of all identified metabolites and metabolism pathways. Partial Least squares discriminant analysis (PLS-DA) and VIP score analysis were performed to predict the values of components considering continuous and discrete variables. VIP > 1.5 was considered as statistically significant. Enrichment analysis was also performed to check the metabolic pathways which increased or decreased due to upregulated or downregulation of metabolites. Rice (*Oryza*

sativa) was used as the reference.

2.8. Statistical analysis

Data obtained from the experimental study except metabolomic analysis were analyzed using JMP IN version 11.0 (Sall et al., 2005). Q tests were performed on all data to eliminate possible outliers at a 95% confidence interval. Mean values with one standard deviation were reported. Tukey Kramer Honest Significant Difference (HSD) test was performed to determine significant differences between the treatment means.

3. Results and discussion

3.1. Batch experiment

During the first week of the study, the plants exposed to CIP spiked nutrient solution did not show any visible physical stress symptoms. Eventually, the plants exposed to the higher doses of CIP (1 mg/L and 10 mg/L), developed stress symptoms like mild chlorosis, drying leaf tips, and lowering of biomass. CIP also had a negative effect on the growth of vetiver grass. The fresh weight of vetiver root exposed to 10 mg/L CIP was 44% lower than the control root (Fig. 1A).

3.2. Mass distribution of CIP

Plant leaf and root tissue were analyzed for CIP content after the 30-day experimental period. Majority of the removed CIP was localized in vetiver root (Fig. 1B), which correlates with elevated stress enzyme activity in the root zone. The mass distribution shows that for each treatment, a significant ($p < 0.05$) percentage of CIP was undetected. The undetected CIP could be due to enzymatic degradation of CIP in the root zone by root-associated microorganisms or within the plant tissue. The highest percentage of undetected CIP (68%) was observed in the 0.05 mg/L treatment. At higher CIP concentrations, the percentage of undetected CIP ranged between 44 and 51%. The mass balance of CIP at the end of the experimental period is shown in Table S3. The root to shoot translocation factor ranged from 20 to 30% for all treatments (Fig. 1C).

3.3. CIP removal from wastewater

Vetiver at a fixed plant density of 4% was exposed to different concentrations of CIP (0.05, 0.1, 1, and 10 mg/L). Vetiver removed approximately 80% of the initial CIP content from 0.05 to 0.1 mg/L treatments within the first three days (Fig. 2A and B). For the other two concentrations, a biphasic pattern of CIP removal was observed, i.e. the removal kinetics was fast during the first week of treatment followed by a slower removal of CIP throughout the rest of the experimental period. For lower concentrations of CIP (0.05 and 0.1 mg/L), the removal was 0.36 and 1.31 mg CIP per kg of plant biomass. At the end of the experimental period, 10.09 mg and 98.94 mg CIP was removed per kg of plant biomass from 1 to 10 mg/L treatment respectively. Rydzyński et al. (2017) also observed maximum CIP uptake by yellow lupin (*Lupinus luteus*) within the first week of the hydroponic experiment. For higher concentrations of 1 and 10 mg/L, the initial CIP drop in the water accounted for approximately 75% of removal and continued until the days 6 and 7 (Fig. 2C and D). After the rapid removal phase, CIP removal slowed down in all the planted treatments. We also observed maximum stress enzyme activity in vetiver within the first week of the experimental period. The drop in the CIP removal efficiency by vetiver grass could be related to the increased stress in vetiver grass. At the end of the experimental period (30 days), approximately 97% CIP removal was obtained in plants exposed to 0.05 and 0.1 mg/L CIP, and 80% and 85% CIP respectively from 1 to 10 mg/L CIP treatments.

3.4. Chlorophyll content

In addition to the impact on biomass, yellowing of the leaves was observed as CIP concentration in treatments increased (Fig. S1A). At the end of the experimental period, the extent of chlorosis in the leaf tissue was directly proportional to the CIP content in the solution (Fig. S1). Chlorophyll content in the plant is one of the important parameters of photosynthetic and metabolic health, and its decline indicates physiological stress in plants (Gáborčík, 2003; Huang et al., 2004; Liu et al., 2013). Total Chl as well as Chl a and b content in vetiver leaf tissue were negatively affected by CIP exposure (fig. S1 B-D). Specifically, the plants exposed to high CIP doses (1 and 10 mg/L) exhibited significant ($p < 0.05$) decrease in the total Chl content (Fig. S1B). These results can also be visualized from Fig. S1A which shows the initial and final color of the leaves respective to different CIP exposures. For all treatments, the rate of Chl depletion was more rapid for Chl-b than Chl-a (Figs. S1C and S1D). Plant exposed to 10 mg/L CIP, showed three times less Chl-a content compared to the untreated control. The highest depletion of Chl-b was also observed in plants exposed to 10 mg/L CIP. Previous research has shown that the presence of fluoroquinolone antibiotic can inhibit enzymatic activity in the chloroplast. Specifically, the quinolone ring and the amino group present in the piperazine ring of CIP act as inhibitors of quinone site in photosystem-II (PS-II), which plays an important role in photosynthetic electron transport (Aristilde et al., 2010; Wall et al., 2004).

There were no significant changes observed in the Chl a, Chl b, and total Chl content in the untreated control.

3.5. Protein content

Protein content in vetiver root exposed to CIP dropped significantly ($p < 0.05$) with time. Over the experimental period, there was no significant difference in the root protein content in the untreated control plants (Fig. S2A). Continuous depletion of root protein content was observed in all CIP treated plants until the first week of the experimental period. A slight recovery of protein content in CIP treated vetiver root was observed during the rest of the experimental period, particularly for the 0.05 and 0.1 mg/L treatments. In vetiver shoot, depletion in the protein content was not as drastic as those of the roots, particularly at the lower CIP concentrations of 0.05 and 0.1 mg/L (Fig. S2B). The loss of shoot protein content was high in the plants exposed to higher CIP concentrations (1 and 10 mg/L). Previous research reported that fluoroquinolones could affect nucleic acid and protein synthesis in plants (Zhou et al., 2009). As CIP was primarily localized in the root zone, root protein content declined rapidly compared to the shoot. For all treatments, a slight recovery in the shoot protein content was observed between 3 and 5 days (Fig. S2B). The shoot of the untreated plant exhibited no significant differences in the protein content during the entire experimental period.

3.6. Enzymatic response due to CIP induced stress

Reactive oxygen species (ROS) are the toxic byproducts which are constitutively produced at basal levels due to various metabolic pathways (Luis et al., 2006; Navrot et al., 2007). The presence of xenobiotic compounds in plants can elevate the rate of ROS production which activates stress-response and antioxidant enzymes defense system (Dordio et al., 2009; Liu et al., 2013). The members of the antioxidant enzyme class include glutathione-s-transferase (GST), guaiacol peroxidase (GPOD), superoxide dismutase (SOD), and catalase (CAT). These enzymes play vital roles in scavenging the ROS and minimizing stress in plants (Xu et al., 2010). We observed that all stress enzyme levels in vetiver grass became significantly ($p < 0.05$) high within a week due to the exposure of CIP. In all CIP treatments, the stress enzyme activity was higher in the root zone compared to shoot. As CIP was localized mainly in the root zone, the stress level increased. Hence an elevated

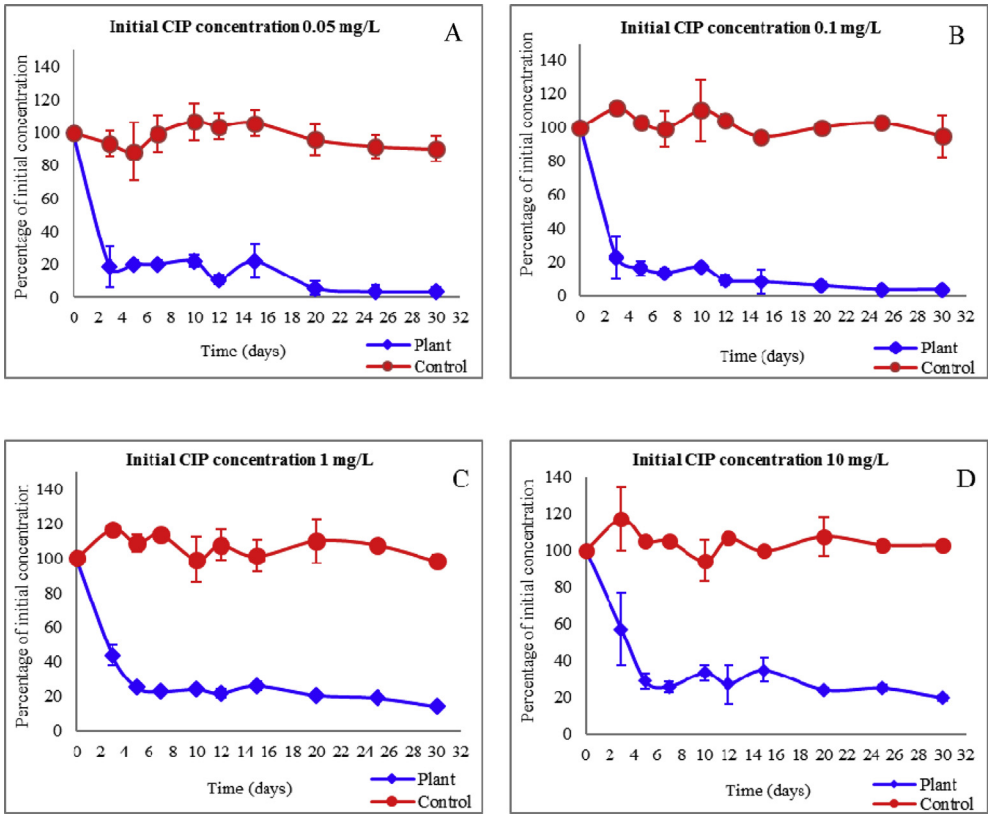


Fig. 2. Removal kinetics of CIP (1 A: 0.05 mg/L, 1 B: 0.1 mg/L, 1C: 1 mg/L, 1D: 10 mg/L) from hydroponic media by vetiver grass. Data expressed as the mean of three replicates (n = 3) ± one standard deviation.

stress enzyme activity was recorded.

GST plays a vital role in protecting plants against several xenobiotics and ROS induced damages (Foyer et al., 1997; Nemat Alla, 1995; Nie et al., 2013). It detoxifies the electrophilic xenobiotic compounds via conjugation with reduced glutathione (GSH) (Alla and Hassan, 2006). GST activity increased in all CIP exposed plants during the first five days of the experiment (Fig. 3A and B). The cytochrome P450 mediated metabolism of CIP generates electrophilic metabolites and might be responsible for the elevated level of GST in plant tissue (Gürbay et al., 2001; Ilgin et al., 2015; Talla and Veerareddy, 2011). The plant roots exposed to 0.1, 1, and 10 mg/L showed 2–3 times higher GST activity compared to the untreated control root (Fig. 3A). After the 5th day, the GST activity began to drop in the treated roots but remained significantly ($p < 0.05$) higher than the control plants (Fig. 3A). This result agrees with the GST activity observed by Melato et al. (2016) in stressed vetiver leaves and roots. All CIP exposed shoots showed a similar trend in GST activity, but the magnitude of GST activity was lower than the observed level in roots (Fig. 3B). The controls (both root and shoot) showed no significant changes in GST activity during the experimental period.

GPOD protects the plant against invading pathogens, helps in healing of physical damage and acts as antioxidant defense system during the growth and senescence processes (Asada, 1992). Until the 5th day, GPOD activity increased in vetiver root exposed to 0.05 and 0.1 mg/L CIP. Thereafter, the enzyme activity for these two treatments decreased, but remained above the control plants (Fig. 3C). On the other hand, a rapid increase in the GPOD activity was observed in vetiver root exposed to 1 and 10 mg/L CIP. With reference to the control, the GPOD activity of 1 and 10 mg/L CIP treatments was 12 and 15-fold higher by the end of the experimental period. The increased GPOD activity in vetiver root indicates the overproduction of hydrogen peroxide along with other ROS in the plant physiological system. Similarly, a rapid increase in the GPOD activity was observed in vetiver shoot on the very first day and continued until 5–10 days depending on the treatment (Fig. 3D). Thereafter, a gradual decline in GPOD activity was observed in all treatments. In leaves, the peroxidase is found in different parts like vacuole, cytosol, and extra-cellular spaces (Liu et al., 2013). Due to the vast spatial distribution of peroxidase in leaves might be related to the gradual decrease of GPOD activity in CIP treated vetiver shoot. On day 30, vetiver shoots exposed to 1 and 10 mg/L showed approximately 6 and 10-fold higher GPOD activity respectively compared to the control shoot.

SOD acts as a first line of defense against most oxidative stress inducers in the plant physiological system (Davies et al., 2009). In our study, SOD activity was not as strongly induced compared to other stress enzymes (Fig. 3E and F). Increased SOD activity was observed in vetiver root exposed to CIP amended nutrient solution. The SOD activity in CIP treated root reached the maximum level within the 24 h of the experiment and remained high until the 5th day (Fig. 3E). Vetiver roots exposed to 10 mg/L CIP showed 25% higher GST activity compared to the control root. However, the SOD activity started to decrease significantly ($p < 0.05$) after the 5th day and at the end of the experimental period, the plants exposed to higher CIP concentrations (1 and 10 mg/L) showed the least enzyme activity. In leaves, the difference in SOD activity between treated and untreated plants was not as pronounced as in the roots. Shoot tissue of CIP treated plants showed only slightly elevated SOD activity during the first five days (Fig. 3F). The SOD activity in both root and shoot showed an initial increase within five days and then decreased gradually. This observation was consistent with Liu et al. (2013) who showed similar trends of SOD activity in *Phragmites australis* exposed to different doses of CIP. The drop in the enzyme activity might be related to the overall depletion of protein content in plant tissue. Plants exposed to 0.05 mg/L CIP showed a significant decrease in the SOD activity at the end of the experimental period. There were no significant changes in the SOD activity observed in the root and shoot of the control plants.

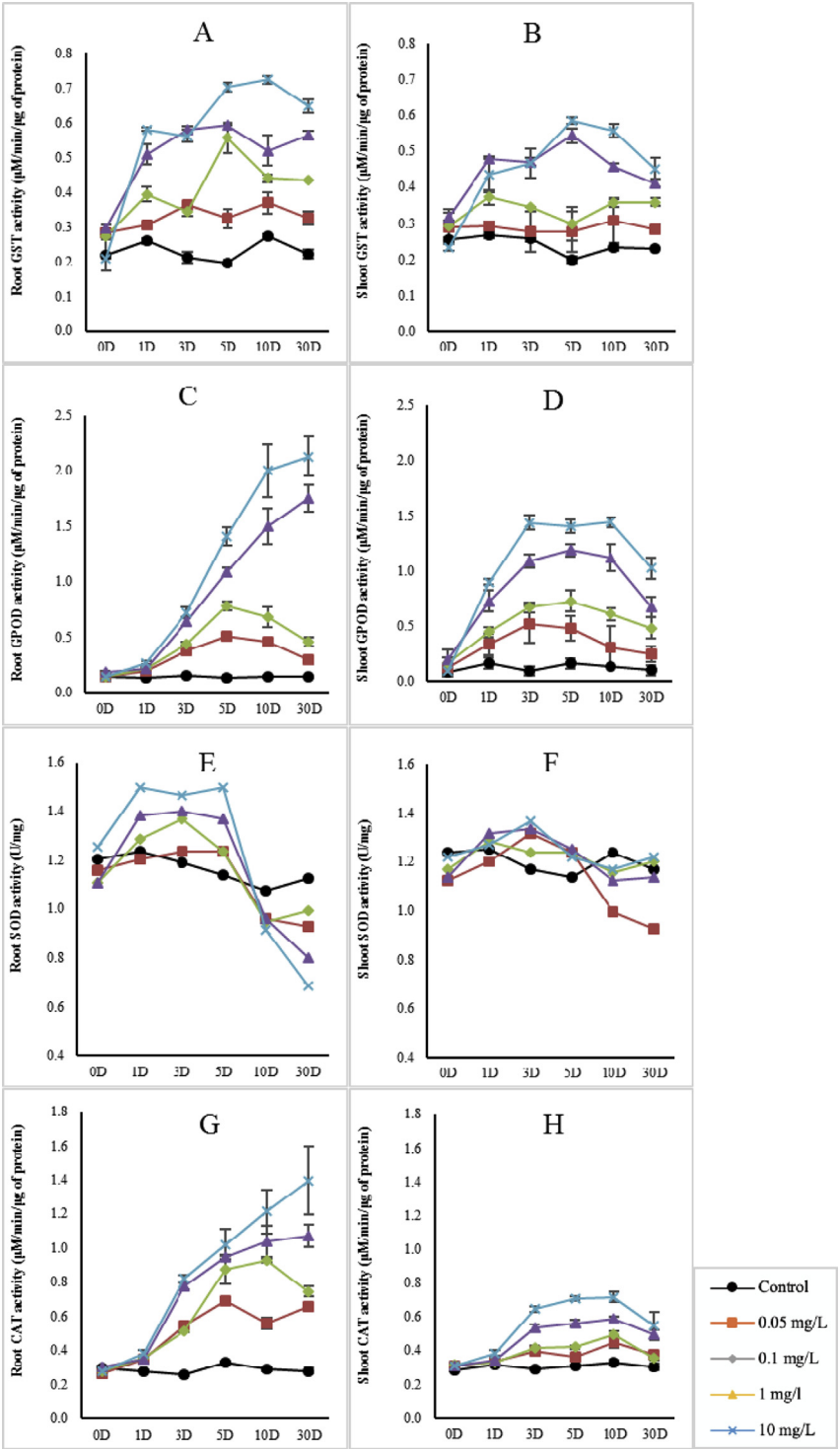


Fig. 3. Stress enzymes (GST, GPOD, SOD, and CAT) activities in vetiver root and shoot over the entire experimental period. GST activity (µM/min/µg of protein) in vetiver root (A) and shoot (B). GPOD activity (µM/min/µg of protein) in vetiver root (C) and shoot (D). SOD activity in vetiver root (E) and shoot (F) and CAT activity in vetiver root (G) and shoot (H) All data were expressed as means (n = 3) ± standard deviation.

Catalase is well-known for its efficiency to remove toxic peroxides (Lin and Kao, 2000). A rapid increase in CAT activity was observed in all plants exposed to CIP. In CIP treated vetiver root, the CAT activity increased significantly ($p < 0.05$) with time (Fig. 5G). The root exposed to 1 and 10 mg/L CIP showed highest CAT activity compared to control roots (5 and 7 times respectively). On the other hand, roots exposed to 0.05 and 0.1 mg/L CIP showed an initial increase in the CAT activity until the 5th day and eventually become stable during the rest of the experimental period. The increase in CAT activity in vetiver root was dose-dependent, but the activity did not decrease with time like the other stress enzymes. A similar trend of increasing CAT activity was also observed in vetiver shoot exposed to CIP. The shoot exposed to 10 mg/L CIP showed 2-fold higher CAT activity compared to the control during the first week of the experiment (Fig. 3H). After 10 days, the CAT activity eventually dropped in all CIP treated plants. The decline in the CAT activity might occur due to acute stress resulting in

malfunctioning of the cellular defense system in plants (Guecheva et al., 2003; Lichtenthaler, 1996). Xu et al. (2010) observed similar patterns of CAT activity in the leaves of *Phragmites australis* exposed to organic pollutants. The CAT activity in control plants showed no significant changes during the experimental period (Fig. 3G and H).

3.7. Effect of CIP on metabolic pathways

Plants undergo a series of changes in their metabolic pathways due to the stress imposed by xenobiotic compounds. The metabolic profile of vetiver exposed to CIP was studied. A total of 325 metabolites were targeted to include the major plant metabolic pathways. Multivariate analysis was performed to identify the discriminating metabolites in response to CIP in plant tissue, followed by metabolic network analysis. Since the individual root and shoot samples did not show consistent data in the initial metabolomic analysis, we chose to prepare composite samples for the metabolomics analysis. At lower CIP concentrations of 0.05 and 0.1 mg/L, negligible metabolic response in the plant samples was detected. Hence we included a higher concentration (30 mg/L) of CIP for the metabolomics study. PLS-DA analysis of composite metabolites from root and shoot tissue showed that the control tissue had significant variance from CIP treated plant tissue (Fig. 4). A clear separation between metabolites in CIP treated and untreated plant tissue was observed, in addition to a concentration effect. In plant tissue, 13.5% and 46.5% of the variation between control and treated groups could be explained by component 1 and component 2 respectively. Treatment with increasing concentrations of CIP resulted in delineation of separate clusters (Fig. 4).

Metabolite analysis showed that CIP impacts several metabolic pathways in the plant. The major upregulated pathways include taurine and hypotaurine metabolism, purine metabolism, phospholipid biosynthesis, citric acid cycle, sucrose, and mannose degradation. The pattern hunter analysis based on spearman correlation (Fig. 5A) showed metabolites like N-acetyl aspartyl glutamic acid, lysophosphatidylcholine, guanosine triphosphate (GTP), and deoxyadenosine monophosphate are amongst a few metabolites significantly ($p < 0.01$) upregulated ($R^2 > 0.6$) with increasing CIP doses. The variable importance in projection (VIP) based on PLS-DA results shows metabolites with significant ($p < 0.05$) variation between untreated control and increasing concentrations of CIP (Fig. 5B). Pathway analysis showed that taurine, hypotaurine, and purine metabolism were significantly ($p < 0.05$) upregulated due to the presence of CIP (Fig. 5C). The major downregulated pathways include homocysteine degradation, cardiolipin biosynthesis, phosphatidylcholine, phosphatidylethanol amine and catecholamine biosynthesis, carnitine biosynthesis, ubiquinone biosynthesis, etc. (Fig. 5D). Taurine and hypotaurine metabolism has been reported to be induced by salt stress (Zhang et al., 2017). These metabolites are known to be osmolytes and also act as antioxidants (Yancey, 2005). Lysophosphophatidylcholine, which also accumulated in CIP treated vetiver plants has been reported to accumulate in response to freezing, wounding, pathogen infection or the application of elicitors (Hou et al., 2016). Lysophospholipids can be hydrolyzed from membranes as a result of oxidative stress, which can initiate signaling cascades activating the H^+ transporting ATPase of the plasma membrane affecting auxin responses and the tonoplast H^+/Na^+ -antiporter activity, bringing about pH changes in plant cells (Hou et al., 2016). Pterin is a precursor of folate biosynthesis, which also requires GTP. Both these metabolites were upregulated (Fig. 5C). In *Arabidopsis*, upregulation of genes in folate biosynthesis was reported in response to oxidative stress (Baxter et al., 2007). However, downregulation of these genes was also reported in plants under salt stress (Storozhenko et al., 2007). Cardiolipin, which is downregulated (Fig. 5D) is an important component of the mitochondrial membrane and is involved in plant response to stresses that induce programmed cell death, such as heat and extended darkness (Pan et al., 2014).

When plants are exposed to a bioactive compound, metabolic pathways are impacted. Metabolomics provides an avenue to dissect these plant responses by measuring the changes in hundreds of endogenous metabolites (Pidatala et al., 2016). Metabolomic profiling provides a snap-shot of the physiological adaptations in plants and help in understanding the mode of action of the xenobiotic compound. Our group has previously reported on the effect of tetracycline on vetiver metabolic pathways (Sengupta et al., 2016), which impacted glyoxylate metabolism, TCA cycle, biosynthesis of secondary metabolites, tryptophan metabolism, and inositol phosphate metabolism in vetiver roots. Our current results show that the metabolic signature of CIP is distinct from those of tetracycline.

3.8. Degradants of CIP in plant tissue

ESI-MS scan detected the presence of ten CIP degradation products inside the plant tissue (Table 1). The m/z value of the identified compounds ranged from 206 to 362. Among the detected CIP degradation products, almost 50% were fluorinated aromatic hydrocarbons, and no aliphatic compounds were detected. Majority of the CIP degradation products were identified in vetiver shoot compared to the root. For all the identified products, chemical alterations were observed in the sidechains (7th and 8th carbon) and piperazine moiety, but the

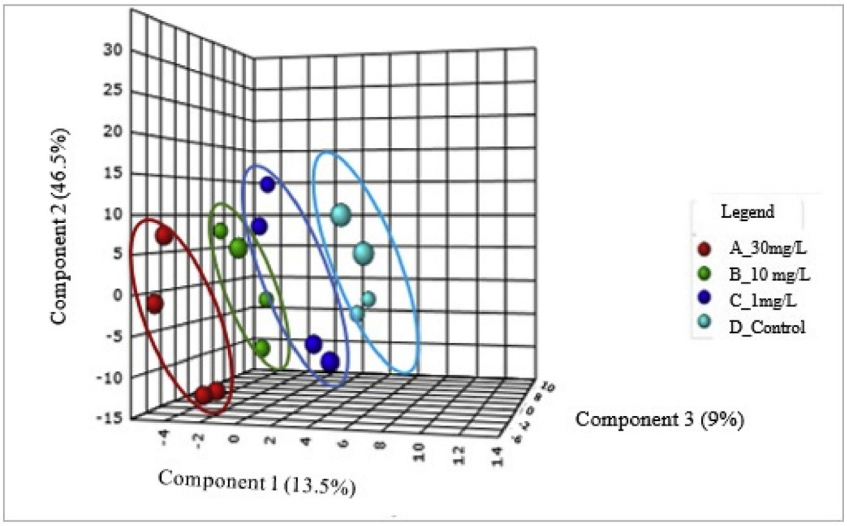


Fig. 4. PLS-DA analysis of metabolites in vetiver exposed to CIP. A) 30 mg/L, B) 10 mg/L, C) 1 mg/L and D) Control.

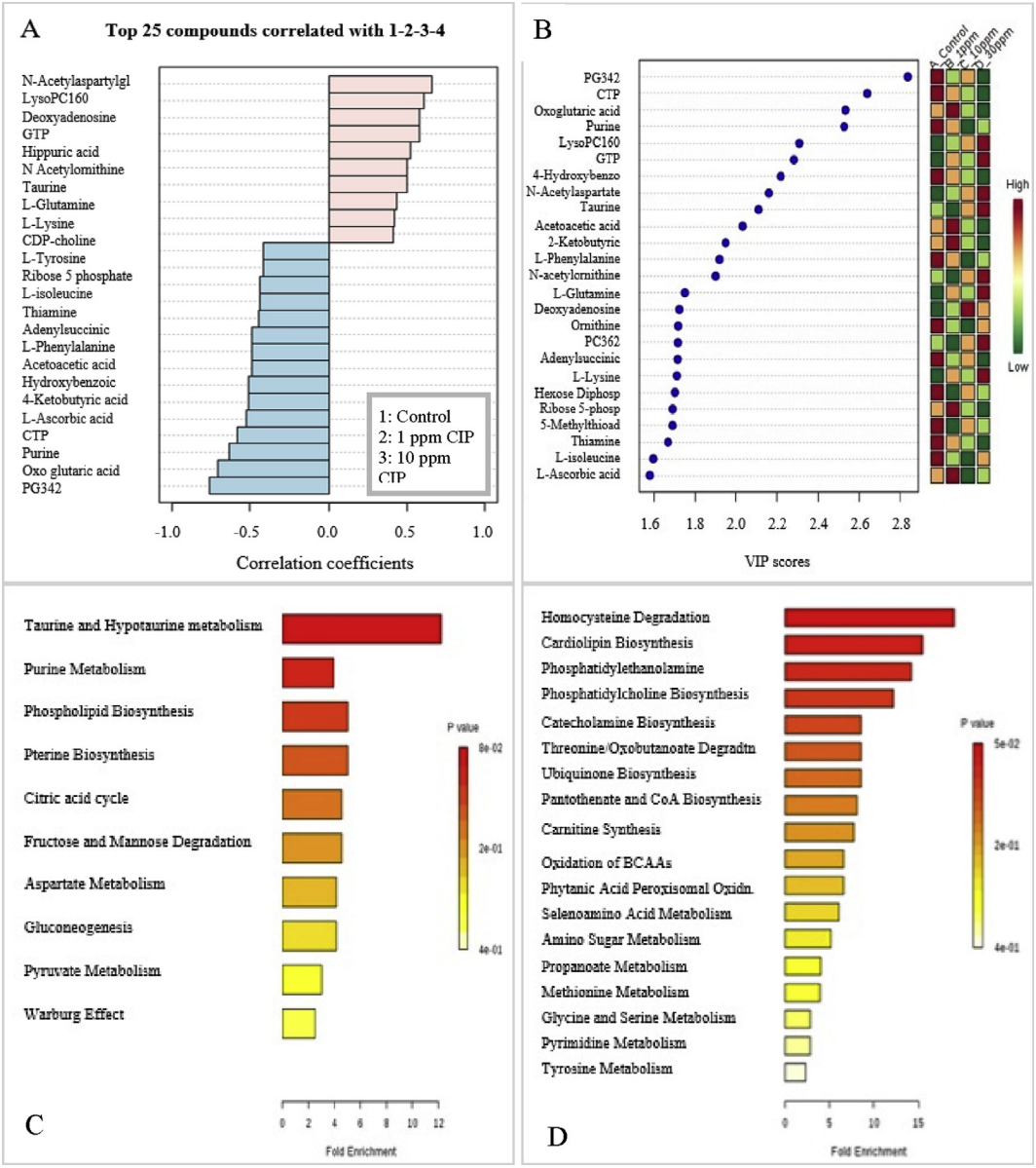
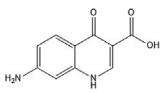
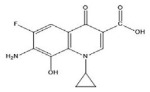
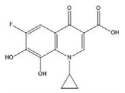
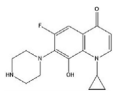
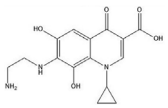
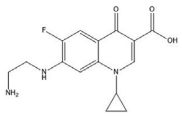
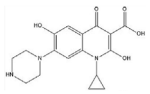
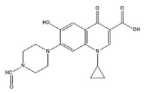
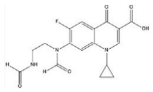
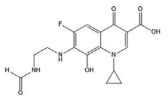


Fig. 5. Upregulated and downregulated metabolites with correlation to CIP doses (A) and VIP score analysis (B) to show the significance of variables in PLS-DA with metabolites in vetiver exposed to CIP. Upregulated (C) and downregulated (D) metabolic pathways in vetiver grass on exposure to CIP.

chemical backbone of CIP remained unaltered. The "green liver model" is used to explain plant metabolism of various organic contaminants, where the degradation/transformation of organic contaminants is compared to similar detoxification function of the mammalian liver (McCutcheon and Schnoor, 2003). According to this model, three phases of plant metabolism of organic compounds include transformation (phase I) involving enzymes such as P450 and peroxidases, conjugation of the parent compound and/or the phase I metabolites (phase II), and sequestration of the phase II conjugates (phase III). A cytochrome P-450-mediated phase I transformation and GST-mediated phase II conjugation could be involved in CIP metabolism in vetiver. Previous research in the field of plant-mediated CIP biodegradation is sporadic and limited information is available on intermediate CIP degradation products. We detected several CIP degradation products in vetiver root and shoot. The results indicated the presence of both fluorinated and defluorinated aromatic compounds in vetiver biomass.

The CIP degradation products present in vetiver root were D1 (m/z 206) and D2 (m/z 263) (Table 1). Liao et al. (2016) proposed bacterial biotransformation pathways that produce D1 and D2 from CIP. The generation of D1 from CIP involves a two-step reaction, and D2 is an intermediate product of this process. D2 is formed due to the loss of piperazine moiety linked with the 7th carbon atom of CIP molecule. A further loss of the cyclopropyl group and fluorine atom produce D1 from D2. We detected both D1 and D2 intermediates in the root. We also detected D2 in vetiver leaves. The formation of D1 from D2 releases fluorine which is a highly reactive halogen species. The presence of fluorine in plants causes chlorosis, necrosis, and growth suppression (Jacobson et al., 1966) due to oxidative stress, which is indicated by the induction in antioxidative enzyme activities. This supports our observation of chlorosis and growth suppression in vetiver, which were directly proportional to CIP doses (Figs. 1 and S1). Since D1 and D2 were found in the root zone, these transformations could have been mediated by root-associated microorganisms. Several studies have also shown that the rhizosphere exerts selective pressure resulting in the emergence of antibiotic-resistant bacteria (ARBs) which could play a role in CIP transformations. Wetzstein et al. (1999) identified the compound D-3 (m/z 280), which was formed due to enzymatic degradation of CIP by Brown Rot Fungus (*Gloeophyllum striatum*).

Table 1
Chemical structures of different CIP degradation products found in the root and shoot of vetiver grass during ESI-MS analysis.

Location	Product	m/z	Proposed structure	IUPAC name	Reference
Root	D1	206		7-amino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid	Liao et al. (2016)
Root and shoot	D2	263		7-amino-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid	(Liao et al., 2016; Prieto et al., 2011)
Shoot	D3	280		1-cyclopropyl-6-fluoro-7,8-dihydroxy-4-oxo-1,4- dihydroquinoline-3-carboxylic acid	Wetzstein et al. (1999)
Shoot	D4	288		1-cyclopropyl-6-fluoro-8-hydroxy-7-(piperazin-1-yl)quinoline-4(1H)-one	Vasconcelos et al. (2009)
Shoot	D5	304		7-((2-aminoethyl)amino)-1-cyclopropyl-6,8-dihydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid	(Ji et al., 2014; Wetzstein et al., 1999)
Shoot	D6	306		7-((2-aminomethyl)amino)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihyroquinoline-3-carboxylic acid	Prieto et al. (2011)
Shoot	D7	346		1-cyclopropyl-2,6-dihydroxy-4-oxo-7-(piperazin-1-yl)-1,4- dihydroquinoline-3-carboxylic acid	Wetzstein et al. (1999)
Shoot	D8	346		1-cyclopropyl-7-(4-formylpiperazin1-yl)-6-hydroxy-4-oxo-1,4- dihydroquinoline-3-carboxylic acid	Babić et al. (2013)
Shoot	D9	362		1-cyclopropyl-6-fluoro-7-(N-(2-formamidoethyl)formamido)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid	Ji et al. (2014)
Shoot	D10	362		7-((2-Acetamidoethyl)amino)-1-cyclopropyl-6-fluoro-8-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid	Prieto et al. (2011)

According to Wetzstein et al. (1999), consecutive reduction in the 7th and 8th carbon of CIP molecule cleaves the amine moiety and generates D3. However, D3 was detected in vetiver leaves. Oxidoreductase class of enzymes can catalyze these reactions and could have transformed CIP to produce D3 in vetiver leaves. The decarboxylation of CIP molecule leads to the formation of D4 (m/z 288) (Vasconcelos et al., 2009). This transformation of CIP could be catalyzed by decarboxylase enzyme present in vetiver leaves. Previous studies indicated that vetiver root-associated microbial community could have mediated the decarboxylase reaction (Del Giudice et al., 2008). D5 (m/z 304) is formed in a two-step process that involves the substitution of fluorine atom with a hydroxyl group, and oxidation of piperazine moiety in the CIP molecule. Ji et al. (2014) detected D5 during the degradation of CIP by free sulfate radical derived from ferrous-activated persulfate. The formation of strong oxidants (H_2O_2, HO_2, $^1O_2$) like free sulfate radical can also occur in plant during stress and could be related to the formation of D5. Wetzstein et al. (1999) also detected D5, which was formed due to fungal degradation of CIP. Prieto et al. (2011) studied the degradation of CIP by white rot fungus (*Trametes versicolor*) and detected D6 (m/z 306) in the fungal cells. D6 is probably formed due to oxidation in the piperazinyl ring of CIP. D6 was not only detected during degradation of CIP by *Gloeophyllum striatum* (Wetzstein et al., 1999) but also detected during ozonation of aqueous solution of CIP (De Witte et al., 2009). Stress enzymes such as peroxidases in vetiver shoot could also have oxidized CIP molecule and formed D6.

D7 and D8 are two different molecules with the same m/z value. Substitution of fluorine with a hydroxyl group in CIP molecule forms D7 (m/z 346) and detected by Wetzstein et al. (1999) during fungal degradation of CIP. On the other hand, Babić et al. (2013) studied the photolysis of CIP and explained the formation of D8 due to the oxidation of piperazine moiety. We found the ESI-MS peak of m/z 346 in vetiver shoot; either it was formed due to enzymatic degradation or due to the photon mediated CIP degradation in vetiver shoot. D9 and D10 also had the same m/z value (362). The ESI-MS peak of m/z 362 was detected in vetiver shoot. D9 is formed due to the cleavage of piperazine moiety in an oxidative environment (Ji et al., 2014). Alternatively, D10 was formed due to fungal degradation of CIP which involves hydroxyl substitution of fluorine and subsequent breakdown of piperazine moiety.

4. Conclusion

In this study, vetiver grass successfully removed CIP in a hydroponic setup. However, CIP caused oxidative stress, chlorosis and a moderate decline in plant growth. The CIP in the aqueous media was taken up and degraded either by vetiver root-associated microorganisms in the root zone, or by the plant. The metabolic response in CIP treated plants showed that CIP imposed significant ($p < 0.05$) stress on vetiver. Our results indicate that antioxidant enzymes play a major role in the transformation and detoxification of CIP in vetiver. Further studies are

needed to elucidate the role of root-associated microorganisms in CIP degradation. Our laboratory study is the first step toward understanding the feasibility of using the vetiver system for the removal pharmaceuticals from domestic and industrial wastewater.

Declarations of interest

None.

Competing Interest Statement:

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibiod.2019.05.023.

References

Alla, M.N., Hassan, N., 2006. Changes of antioxidants levels in two maize lines following atrazine treatments. Plant Physiol. Biochem. 44 (4), 202–210.
Andra, S., Datta, R., Reddy, R., Saminathan, K., Sarkar, D., 2011. Antioxidant enzymes response in vetiver grass: a greenhouse study for chelant-assisted phytoremediation of lead-contaminated residential soils. Clean Soil, Air, Water 39 (5), 428–436.
Aristilde, L., Melis, A., Sposito, G., 2010. Inhibition of photosynthesis by a fluoroquinolone antibiotic. Environ. Sci. Technol. 44 (4), 1444–1450.
Arnon, D., 1949. Estimation of total chlorophyll. Plant Physiol. 24 (1), 1–15.
Asada, K., 1992. Ascorbate peroxidase–a hydrogen peroxide-scavenging enzyme in plants. Physiol. Plantarum 85 (2), 235–241.
Babić, S., Periša, M., Škorić, I., 2013. Photolytic degradation of norfloxacin, enrofloxacin and ciprofloxacin in various aqueous media. Chemosphere 91 (11), 1635–1642.
Baxter, C.J., Redestig, H., Schauer, N., Repsilber, D., Patil, K.R., Nielsen, J., Selbig, J., Liu, J., Fernie, A.R., Sweetlove, L.J., 2007. The metabolic response of heterotrophic Arabidopsis cells to oxidative stress. Plant Physiol. 143 (1), 312–325.
Bergeron, S., Boopathy, R., Nathaniel, R., Corbin, A., LaFleur, G., 2015. Presence of antibiotic resistant bacteria and antibiotic resistance genes in raw source water and treated drinking water. Internatl. Biodeter. Biodegrad. 102, 370–374.
Brown, S., 1996. Fluoroquinolones in animal health. J. Vet. Pharmacol. Ther. 19 (1), 1–14.
Cheng, H., Yang, X., Liu, A., Fu, H., Wan, M., 2003. A study on the performance and mechanism of soil-reinforcement by herb root system. In: Proceedings of Third International Vetiver Conference, Guangzhou, China, pp. 390.
Datta, R., Das, P., Smith, S., Punamiya, P., Ramanathan, D.M., Reddy, R., Sarkar, D., 2013. Phytoremediation potential of vetiver grass [Chrysopogon zizanioides (L.)] for tetracycline. Int. J. Phytoremediation 15 (4), 343–351.
Davies, L.C., Cabrita, G., Ferreira, R., Carias, C., Novais, J., Martins-Dias, S., 2009. Integrated study of the role of Phragmites australis in azo-dye treatment in a constructed wetland: from pilot to molecular scale. Ecol. Eng. 35 (6), 961–970.
De Witte, B., Dewulf, J., Demeestere, K., Van Langenhove, H., 2009. Ozonation and advanced oxidation by the peroxone process of ciprofloxacin in water. J. Hazard Mater. 161 (2–3), 701–708.
Del Giudice, L., Massardo, D.R., Pontieri, P., Bertea, C.M., Mombello, D., Carata, E., Tredici, S.M., Talà, A., Mucciarelli, M., Groudeva, V.I., 2008. The microbial community of Vetiver root and its involvement into essential oil biogenesis. Environ. Microbiol. 10 (10), 2824–2841.
Dordio, A.V., Duarte, C., Barreiros, M., Carvalho, A.P., Pinto, A., da Costa, C.T., 2009. Toxicity and removal efficiency of pharmaceutical metabolite clofibric acid by Typha spp.–Potential use for phytoremediation? Bioresour. Technol. 100 (3), 1156–1161.
Eggen, T., Asp, T.N., Grave, K., Hormazabal, V., 2011. Uptake and translocation of metformin, ciprofloxacin and narasin in forage-and crop plants. Chemosphere 85 (1), 26–33.
Everage, T.J., Boopathy, R., Nathaniel, R., LaFleur, G., Doucet, J., 2014. A survey of antibiotic-resistant bacteria in a sewage treatment plant in Thibodaux, Louisiana, USA. Internatl. Biodeter. Biodegrad. 95, 2–10.
Farkas, M.H., Berry, J.O., Aga, D.S., 2007. Chlortetracycline detoxification in maize via induction of glutathione S-transferases after antibiotic exposure. Environ. Sci. Technol. 41 (4), 1450–1456.
Ferreira, V.R.A., Amorim, C.L., Cravo, S.M., Tiritana, M.E., Castro, P.M.L., Afonso, C.M.M., 2016. Fluoroquinolones biosorption onto microbial biomass: activated sludge and aerobic granular sludge. Internatl. Biodeter. Biodegrad. 110, 53–60.
Foyer, C.H., Lopez-Delgado, H., Dat, J.F., Scott, I.M., 1997. Hydrogen peroxide-and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. Physiol. Plantarum 100 (2), 241–254.
Gáborčík, N., 2003. Relationship between contents of chlorophyll (a+ b)(SPAD values) and nitrogen of some temperate grasses. Photosynthetica 41 (2), 285–287.
Gomes, M., Gonçalves, C., Moreirade, J., Miranda, B., Vieirada, S., Cruz, S., Bicalho, L., Figueredo, C., Garcia, Q., 2017. Ciprofloxacin induces oxidative stress in duckweed (*Lemna minor* L.): Implications for energy metabolism and antibiotic-uptake ability. J. Hazard. Mat. 328, 140–149.
Guecheva, T.N., Erdtmann, B., Benfato, M.S., Henriques, J.A., 2003. Stress protein response and catalase activity in freshwater planarian Dugesia (Girardia) schubarti exposed to copper. Ecotoxicol. Environ. Saf. 56 (3), 351–357.
Gürbay, A., Gonthier, B., Daveloose, D., Favier, A., Hincal, F., 2001. Microsomal metabolism of ciprofloxacin generates free radicals. Free Radic. Biol. Med. 30 (10), 1118–1121.
He, K., Soares, A.D., Adejumo, H., McDiarmid, M., Squibb, K., Blaney, L., 2015. Detection of a wide variety of human and veterinary fluoroquinolone antibiotics in municipal wastewater and wastewater-impacted surface water. J. Pharm. Biomed. Anal. 106, 136–143.
Hill, R., 1992. Calorimetric value of vetiver. Vet Newsl 8, 18.
Hirsch, R., Ternes, T., Haberer, K., Kratz, K.-L., 1999. Occurrence of antibiotics in the aquatic environment. Sci. Total Environ. 225 (1–2), 109–118.
Hoagland, D.R., Arnon, D.I., 1950. The Water-Culture Method for Growing Plants without Soil. Circular. California agricultural experiment station, pp. 347 (2nd edit).
Hoang, T., Tu, L., Le, N., Dao, W., Trinh, p., 2012. Fate of fluoroquinolone antibiotics in Vietnamese coastal wetland ecosystem. Wetlands Ecol. Manage. 20 (5), 399–408.
Hou, Q., Ufer, G., Bartel, D., 2016. Lipid signalling in plant responses to abiotic stress. Plant Cell Environ. 39 (5), 1029–1048.
Huang, X.-D., El-Alawi, Y., Penrose, D.M., Glick, B.R., Greenberg, B.M., 2004. Responses of three grass species to creosote during phytoremediation. Environ. Pollut. 130 (3), 453–463.
Ilgin, S., Can, O.D., Atli, O., Ucel, U.I., Sener, E., Guven, I., 2015. Ciprofloxacin-induced neurotoxicity: evaluation of possible underlying mechanisms. Toxicol. Mech. Methods 25 (5), 374–381.
Jacobson, J.S., Weinstein, L.H., McCune, D., Hitchcock, A., 1966. The accumulation of fluorine by plants. J. Air Pollut. Control Assoc. 16 (8), 412–417.
Ji, Y., Ferronato, C., Salvador, A., Yang, X., Chovelon, J.-M., 2014. Degradation of ciprofloxacin and sulfamethoxazole by ferrous-activated persulfate: implications for remediation of groundwater contaminated by antibiotics. Sci. Total Environ. 472, 800–808.
Kiiskila, J.D., Sarkar, D., Panja, S., Sahi, S.V., Datta, R., 2019. Remediation of acid mine drainage-impacted water by vetiver grass (Chrysopogon zizanioides): a multiscale long-term study. Ecol. Eng. 129, 97–108.
Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999− 2000: a national reconnaissance. Environ. Sci. Technol. 36 (6), 1202–1211.
Liao, X., Li, B., Zou, R., Dai, Y., Xie, S., Yuan, B., 2016. Biodegradation of antibiotic ciprofloxacin: pathways, influential factors, and bacterial community structure. Environ. Sci. Pollut. Control Ser. 23 (8), 7911–7918.
Lichtenthaler, H.K., 1996. Vegetation stress: an introduction to the stress concept in plants. J. Plant Physiol. 148 (1–2), 4–14.
Lin, C.C., Kao, C.H., 2000. Effect of NaCl stress on H2O2 metabolism in rice leaves. Plant Growth Regul. 30 (2), 151–155.
Liu, L., Liu, Y.-h., Liu, C.-x., Wang, Z., Dong, J., Zhu, G.-f., Huang, X., 2013. Potential effect and accumulation of veterinary antibiotics in Phragmites australis under hydroponic conditions. Ecol. Eng. 53, 138–143.
Luis, A., Sandalio, L.M., Corpas, F.J., Palma, J.M., Barroso, J.B., 2006. Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling. Plant Physiol. 141 (2), 330–335.
Maul, J.D., Schuler, L.J., Belden, J.B., Whiles, M.R., Lydy, M.J., 2006. Effects of the antibiotic ciprofloxacin on stream microbial communities and detritivorous macroinvertebrates. Environ. Toxicol. Chem. 25 (6), 1598–1606.
McCutcheon, S., Schnoor, J., 2003. In: Phytoremediation: Transformation and Control of Contaminants. John Wiley & Sons, Inc., New Jersey.
Melato, F., Mokgalaka, N., McCrindle, R., 2016. Adaptation and detoxification mechanisms of Vetiver grass (Chrysopogon zizanioides) growing on gold mine tailings. Int. J. Phytoremediation 18 (5), 509–520.
Migliore, L., Cozzolino, S., Fiori, M., 2003. Phytotoxicity to and uptake of enrofloxacin in crop plants. Chemosphere 52 (7), 1233–1244.
Navrot, N., Rouhier, N., Gelhaye, E., Jacquot, J.P., 2007. Reactive oxygen species generation and antioxidant systems in plant mitochondria. Physiol. Plantarum 129 (1), 185–195.
Nemat Alla, M., 1995. Glutathione regulation of glutathione S-transferase and peroxidase activity in herbicide-treated Zea mays. Plant Physiol. Biochem. 33 (2), 185–192.
Nie, X.-P., Liu, B.-Y., Yu, H.-J., Liu, W.-Q., Yang, Y.-F., 2013. Toxic effects of erythromycin, ciprofloxacin and sulfamethoxazole exposure to the antioxidant system in Pseudokirchneriella subcapitata. Environ. Pollut. 172, 23–32.
Pan, L.-j., Li, J., Li, C.-x., Yu, G.-w., Wang, Y., 2018. Study of ciprofloxacin biodegradation by a Thermus sp. isolated from pharmaceutical sludge. J. Hazard Mater. 343, 59–67.
Pan, R., Jones, A.D., Hu, J., 2014. Cardiolipin-mediated mitochondrial dynamics and

stress response in Arabidopsis. Plant Cell 113, 121095 tpc.
Panja, S., Das, P., Sarkar, D., Deng, Y., Datta, R., 2014. Potential of vetiver grass to remove oxytetracycline and ciprofloxacin from aquatic media: preliminary results from a hydroponic study. Geological Society of America Abstracts with Program 35–40.
Picó, Y., Andreu, V., 2007. Fluoroquinolones in soil—risks and challenges. Anal. Bioanal. Chem. 387 (4), 1287–1299.
Pidatala, V.R., Li, K., Sarkar, D., Ramakrishna, W., Datta, R., 2016. Identification of biochemical pathways associated with lead tolerance and detoxification in Chrysopogon zizanioides L. Nash (Vetiver) by metabolic profiling. Environ. Sci. Technol. 50 (5), 2530–2537.
Prieto, A., Möder, M., Rodil, R., Adrian, L., Marco-Urrea, E., 2011. Degradation of the antibiotics norfloxacin and ciprofloxacin by a white-rot fungus and identification of degradation products. Bioresour. Technol. 102 (23), 10987–10995.
Rakshit, S., Sarkar, D., Elzinga, E.J., Punamiya, P., Datta, R., 2013. Mechanisms of ciprofloxacin removal by nano-sized magnetite. J. Hazard Mater. 246, 221–226.
Robert, J.J., 2009. Plant Guide,'Sunshine'Vetiver Grass Chrysopogon Zizanioides (L.). USDA NRCS.
RoyChowdhury, A., Datta, R., Sarkar, D., 2018. Heavy metal pollution and remediation. In: Green Chemistry. Elsevier, pp. 359–373.
RoyChowdhury, A., Sarkar, D., Datta, R., 2015. Remediation of acid mine drainage-impacted water. Current Pollution Reports 1 (3), 131–141.
Rydzyński, D., Piotrowicz-Cieślak, A.I., Grajek, H., Michalczyk, D.J., 2017. Instability of chlorophyll in yellow lupin seedlings grown in soil contaminated with ciprofloxacin and tetracycline. Chemosphere 184, 62–73.
Sall, J., Creighton, L., Lehman, A., 2005. JMP Start Statistics: a Guide to Statistics and Data Analysis Using JMP and JMP IN Software. Thomson, SAS Inst. *Inc.*, Toronto, Ontario.
Sarmah, A.K., Meyer, M.T., Boxall, A.B.A., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65 (5), 725–759.
Sengupta, A., Sarkar, D., Das, P., Panja, S., Parikh, C., Ramanathan, D., Bagley, S., Datta, R., 2016. Tetracycline uptake and metabolism by vetiver grass (Chrysopogon zizanioides L. Nash). Environ. Sci. Pollut. Control Ser. 23 (24), 24880–24889.
Storozhenko, S., De Brouwer, V., Volckaert, M., Navarrete, O., Blancquaert, D., Zhang, G.-F., Lambert, W., Van Der Straeten, D., 2007. Folate fortification of rice by metabolic engineering. Nat. Biotechnol. 25 (11), 1277.
Talla, V., Veerareddy, P., 2011. Oxidative stress induced by fluoroquinolones on treatment for complicated urinary tract infections in Indian patients. J. Young Pharm. 3 (4), 304–309.
Vasconcelos, T.G., Henriques, D.M., König, A., Martins, A.F., Kümmerer, K., 2009. Photodegradation of the antimicrobial ciprofloxacin at high pH: identification and biodegradability assessment of the primary by-products. Chemosphere 76 (4), 487–493.
Verma, S., Dubey, R., 2003. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Sci. 164 (4), 645–655.
Wall, M.K., Mitchenall, L.A., Maxwell, A., 2004. Arabidopsis thaliana DNA gyrase is targeted to chloroplasts and mitochondria. Proc. Natl. Acad. Sci. U. S. A 101 (20), 7821–7826.
Wetzstein, H.-G., Stadler, M., Tichy, H.-V., Dalhoff, A., Karl, W., 1999. Degradation of ciprofloxacin by basidiomycetes and identification of metabolites generated by the brown rot FungusGloeophyllum striatum. Appl. Environ. Microbiol. 65 (4), 1556–1563.
WHO, 2007. In: Critically Important Antimicrobials for Human Medicine: Categorization for the Development of Risk Management Strategies to Contain Antimicrobial Resistance Due to Non-human Antimicrobial Use: Report of the Second WHO Expert Meeting, Copenhagen, pp. 29–31 May 2007.
Xu, J., Zhang, J., Xie, H., Li, C., Bao, N., Zhang, C., Shi, Q., 2010. Physiological responses of Phragmites australis to wastewater with different chemical oxygen demands. Ecol. Eng. 36 (10), 1341–1347.
Yancey, P.H., 2005. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. J. Exp. Biol. 208 (15), 2819–2830.
Yi, K., Wang, D., Li, X., Chen, H., Sun, J., An, H., Wang, L., Deng, Y., Liu, J., Zeng, G., 2017. Effect of ciprofloxacin on biological nitrogen and phosphorus removal from wastewater. Sci. Total Environ. 605, 368–375.
Zhang, Z., Mao, C., Shi, Z., Kou, X., 2017. The amino acid metabolic and carbohydrate metabolic pathway play important roles during salt-stress response in tomato. Front. Plant Sci. 8, 1231.
Zhao, F., Liu, C., Rafiq, M.T., Ding, Z., Zeng, Z., Aziz, R., Yang, X., 2014. Screening wetland plants for nutrient uptake and bioenergy feedstock production. Int. J. Agric. Biol. 16 (1).
Zhou, W., Qiao, X., Xiao, H., Wang, Y., Qu, L., 2009. Effects of antibiotics on chlorophyll-a level in marine microalga. Mar. Environ. Sci. 28 (3).