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Uptake, Translocation and Metabolization of Tramadol, Amitriptyline, Orphenadrine and Lidocaine by Garden Cress, Pea and Water Hyacinth

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Research Article

Keywords: Environmental analysis, plant metabolism, pharmaceuticals, drift-tube ion-mobility mass spectrometry, plant uptake.

Posted Date: March 9th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2466773/v1

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Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on February 16th, 2024. See the published version at https://doi.org/10.1007/s11356-024-32379-x.

Abstract

In the present work water hyacinth plants (*Eichhornia crassipes*) collected from two South African rivers were analyzed with respect to the presence of pharmaceuticals and their major metabolites. Thereby a number of drugs including amitriptyline, orphenadrine, lidocaine, and tramadol could be detected. Particularly for the latter substance relatively high concentrations (more than 5000 ng g^{-1} dry plant material) were detected in the water plants. Subsequently the uptake, translocation and metabolization of these four substances was investigated in a laboratory study employing plant garden cress *(Lepidium sativum*) and pea (*Pisum sativum*). These model plants were grown hydroponically using a growing medium containing the selected pharmaceuticals at concentrations ranging from 10 µg L^{-1} -10 mg L^{-1}. Time-resolved uptake studies revealed that all four pharmaceuticals were taken up by the roots and further relocated to plant stem and leaves. Investigations on the metabolization of the parent drugs within the plant led to the detection of a series of (mainly) phase I metabolites.

1 Introduction

Increasing knowledge in medicine coupled with the ongoing development and release of new pharmaceuticals results in a continuous increase in the consumption of drugs for human and veterinary use. This trend is magnified by the fact that more and more people populate this world and (luckily) an increasing percentage of the population gained access to medical treatment. Furthermore, the aging population (particularly in countries of the so called “western world”) even intensifies this process. Regrettably, this is affected with a negative side effect, namely the increasing release of pharmaceutically active substances (API´s) into the (primarily aquatic) environment (aus der Beck et al. 2016). One common route for contamination of surface waters with pharmaceuticals and personal care products (PPCP´s) is their introduction via the municipal wastewater system. Despite the fact that water is treated in wastewater treatment plants (WWTP), analysis of their effluents revealed that still a range of substances is not fully removed from the wastewater and is subsequently released into rivers or lakes. In addition, sewage sludge from WWTP or manure from treated animals may be in use as fertilizers in agriculture thereby representing another source of contamination (Heberer 2002). Also, improper discharge from industrial sites manufacturing API´s needs to be considered in regions with a density of pharmaceutical industry (Wang et al 2021). For this reason, within the last four decades, a large number of studies on the presence of PPCP ´s (and their metabolites) in environmental waters has been published (for exemplary reviews see: Adeleye et al. 2022; Anand et al. 2022; Bavumiragira et al. 2022; Gani et al. 2021); Hernández-Tenorio et al. 2022; Madikizela et al. 2021; Rathi 2021; Rodriguez dos Santos 2022).

In addition to the contamination of water, also its effect on plants and wild life should be considered. It is a well-known fact, that plants, when brought into contact with water containing PPCP´s, can take-up these substances and translocate them from roots into stem, leaves and fruit (if available) (for relevant reviews see: Bartons et a. 2017; Fu et al. 2019; Keerthanan et al. 2021; Madikizela et al. 2018; Pico et al. 2017; Reddy Pullagurala et al. 2018). After uptake, the parent drugs may subsequently be transformed into metabolites by the plant (for relevant reviews see: Klampfl 2018; Klampfl et al. 2021; Sauvetre et al. 2021). Phase I metabolites mostly formed by hydroxylation of the parent drug may be further bio-transformed by conjugation with for example sugars, small organic acids, or amino acids (for exemplary studies see: Emhofer et al. 2018; Emhofer et al. 2019; Fu et al. 2017; Kodešová et al. 2019; Riemenschneider et al. 2017). This issue is commonly studied by either experiments employing cell cultures of the respective plant (for an example see: Wu et al. 2016), growing plants hydroponically in drug containing growing medium (Madikizela et al. 2018) or by actual field studies whereby plants are cultivated under real farming conditions and irrigated with contaminated water (either prepared in the laboratory or water from an WWTP) (Wu et al. 2014). After harvesting, plants are extracted and extracts analyzed commonly by HLPC or GC with mass spectrometric (MS) detection. As for most of the potential metabolites mentioned above standard substances for their unambiguous identification are not available, all information that can be gained from chromatography and spectrometry should be combined to allow a tentative identification of metabolites with at least acceptable certainty according to the scheme of Schymanski (Schymanski et al. 2014). Besides retention time from chromatography, accurate mass measurement (from high-resolution MS instruments) and MS/MS experiments,

determination of collision cross section (CCS) values via drift-tube ion-mobility mass spectrometry has been added recently as another parameter for the description of potential metabolites detected in the plant extracts (Mlynek et al. 2020).

In this work we present the analysis of water hyacinth samples collected from two South African rivers with respect to the presence of pharmaceuticals and their major metabolites. Based on the findings from these analyses, investigations on the uptake, translocation and metabolization of four drugs namely amitriptyline (AMT), orphenadrine (ORP), tramadol (TRM), and lidocaine (LDO) was studied using two model plants (cress and pea). Furthermore, to study the kinetics involved in the uptake and translocation of these substances, a set of time-resolved experiments was conducted.

2 Experimental

2.1 Chemicals and materials

Amitriptyline Hydrochloride (AMT) (Lundbeck AG, Opficon, Switzerland), Atenolol (1A Pharma GmbH, Vienna, Austria), Citalopram (G.L. Pharma GmbH, Lannach, Austria) Orphenadrine citrate (ORP) (Meda Pharma GmbH, Vienna, Austria), Telmisartan (Ratiopharm GmbH, Ulm, Germany) and Tramadol Hydrochloride (TRM) (Ratiopharm GmbH, Ulm, Germany) were obtained as pharmaceutical preparation. Lidocaine Hydrochloride (LDO) was purchased at a local Pharmacy (IRIS Apotheke Kronstorf). Chemical structures are shown in Figure S1. Tablets were homogenized with mortar and pestle and individual stock solutions with a concentration of 1000 mg L^{-1} were prepared in methanol. Further dilutions of lower concentration were prepared by diluting the stock solution with Milli-Q water. For plant treatment, the solutions were further diluted with tap water.

Methanol and acetonitrile were delivered by VWR (Vienna, Austria). Formic acid (eluent additive for LC-MS, 98%) was purchased from Sigma-Aldrich (Steinheim, Germany). Hydrochloric acid 37%, analytical reagent grade, was obtained from Merck (Darmstadt, Germany). Purified water was produced by a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2 Plant cultivation and treatment

The cress seeds (*Lepidium sativum L.*) were supplied by Sperli (Everswinkel, Germany), and the pea seeds (*Karacter unpicled*) from Raiffeisen Ware Austria AG (Austria). All plants were cultivated under hydroponic conditions without any addition of nutrients. Approximately 5 g of cress seeds were distributed on the grid of the cultivation set, which was subsequently filled with the respective medium (tap water or API solution). One dish carried approximately 300 mL. The API solutions (10 mg L^{-1}, 1 mg L^{-1} or 10 µg L^{-1}) were prepared by diluting the standard stock solutions with tap water. The plants were then grown on the laboratory bench. Pea seeds were left in darkness on wet paper towels for two days to germinate. This was followed by a growing period of 7 days in a bed of wetted iron-on beads. Afterwards growing was continued in approximately 50 mL of tap water or an API solution.

For an investigation regarding uptake, translocation and possible metabolization of APIs in plants, time-study experiments were conducted with garden cress and peas. The plants were grown first hydroponically in tap water for a week, which was followed by the exposure with drug-containing water over the course of another 16 days. Three replicate samples were taken 1, 2, 4, 8 and 16 days after start of exposure. The samples were stored at -80°C until extraction. For each plant species and API, 2 experiments were conducted. In the first experiment, the plants were exposed to a 1 mg L^{-1} API solution in tap water. During the experiment, the cultivation container was repeatedly refilled with tap water at each sampling date. This experiment should simulate a single exposition of the drug followed by a depletion of bioavailable fraction. In the second experiment, a 10 µg L^{-1} API solution in tap water was used, which was exchanged with a freshly prepared one at each sampling date. This experiment should represent a continuous release of the drug into the environment.

2.3 Preparation of plant extracts for tentative identification of metabolites

Several sample preparation methods and extraction solvents were tested for the most efficient extraction of APIs and their metabolites. For this, garden cress was cultivated in a 10 mg L^{-1} API solution for 7 days. During harvesting, the plants were removed from the cultivation container and separated into roots and leaves. The individual plant materials were then washed thoroughly with tap water and subsequently with deionized water. After drying with kitchen paper, 500 mg ± 1% of the individual plant parts were weighed into an Eppendorf tube. Solid-liquid extraction was performed by adding 1 mL of an extraction solution to the samples. The extraction of analytes was further optimized by variation of the extraction methods and agents as described in Table S1 and S2.

The samples were homogenized using a swing mill (“Star Beater”, VWR, Vienna Austria) for 15 minutes at 25 Hz. The homogenized samples were centrifuged for 16 minutes at 4200 g. The supernatant was attained with a syringe and subsequently filtered into 1.5 mL HPLC glass vials using a 0.45 µm syringe filter. The samples were stored at -80° C until analysis.

2.4 Extraction of water hyacinth samples from South African rivers

300 mg of the freeze dried plant sample were weighed into 15 mL falcon tubes and 5 ml of extraction solvent (50% 0.1 M HCl / 50% MeOH) was added. The samples were vortexed until the plant material was soaked with the extraction media and put into an ultrasonic bath (Elma Ultrasonic, Elmasonic S60 H) for 10 minutes, followed by centrifugation at 4200 g for 16 minutes using a VWR Mega Star 1.6R Centrifuge. The supernatants were separated from the solid residues, filtered through a 0.45 µm nylon filter into an HPLC glass vial and stored at 80°C until analysis.

2.5 HPLC / DTIM-QTOF-MS and HPLC / QqQ-MS/MS

The samples were separated by RP-HPLC using an Agilent 1260 HPLC system from Agilent Technologies (Waldbronn, Germany) equipped with a degasser, a quaternary pump and an autosampler. The separation was performed on a Poroshell 120 EC-C18 column (3 x 75 mm, particle size 2.7 µm, Agilent) that was protected with a C18 guard column (4 × 3 mm, particle size 3 µm) from Phenomenex (Aschaffenburg, Germany).

For HPLC separation, a water/acetonitrile gradient was applied. Starting conditions were set to 95% solvent A (water with 0.1% formic acid) and 5% solvent B (acetonitrile with 0.1% formic acid). From minute 0 to 5 solvent B was increased to 15%, from minute 5 to 10 solvent B was increased to 30%, from minute 10 to 15 solvent B was increased to 50%, followed by 5 min 100% solvent B and 5 minutes equilibration with starting conditions, resulting in a total run time of 25 min. The flow rate was set to 0.6 mL min^{-1}, the temperature of the column heater was 30°C and an injection volume of 20 µL was used.

For the targeted analysis of APIs in water hyacinth samples, an HPLC was coupled to an Agilent 6420 triple quadrupole QqQ-MS/MS (Agilent Technologies, Waldbronn, Germany) equipped with an ESI source. The QqQ-MS/MS system was operated in the positive ionization mode. Applied parameters were as follows: capillary voltage 4000 V, drying gas flow rate 11 L min^{-1}, drying gas temperature 350°C, nebulizer pressure 55 psi. The optimized parameters (selected transitions, fragmentor voltages and collision energies) for the multiple reaction monitoring (MRM) mode are given in Table S3.

For the tentative identity confirmation of metabolites, the HPLC system was hyphenated with an Agilent 6560 DTIM-QTOF LC-MS/MS (operated either in the “QTOF only” or ion-mobility MS mode) equipped with a Dual AJS ESI source (Agilent Technologies, Waldbronn, Germany).

The DTIM-QTOF-MS was tuned in the “fragile ion” mode and operated in the positive ionization mode with the following source parameters: drying gas temperature 300°C, drying gas flow rate 10 L min^{-1}, nebulizer pressure 50 psi, sheath gas

temperature 300°C, sheath gas flow rate 10 L min^{-1}, capillary voltage 3500 V, nozzle voltage 1000 V and fragmentor 425 V. For MS/MS experiments nitrogen was used as collision gas and collision energies of 20 V and 30 V were applied.

The $^{DT}CCS_{N2}$ values were obtained using nitrogen as drift gas and the following parameters for the DTIM device: 4-bit multiplexing, frame rate 0.9 frames s^{-1}, IM transient rate 18 transients $frame^{-1}$, max drift time 60 ms, trap fill time 3900 µs and trap release time 250 µs. Calibration was performed according to a “single-field” approach, allowing the determination of the $^{DT}CCS_{N2}$ values. In order to relate the measured drift times to known and standardized $^{DT}CCS_{N2}$ values of the calibrant analytes, a tune mix calibrant was measured (applying the same conditions as for the samples) before analysing the actual sample. Drift tube parameters (for “single-field” measurements) were the following: drift tube entrance 1567 V, drift tube exit 217 V, rear funnel entrance 210.5 V and rear funnel exit 38 V.

2.6 Data processing

For data evaluation, Agilent MassHunter Qualitative Analysis B.07.00, MassHunter Quantitative Analysis B.10.1, MassHunter PCDL Manager B.08.00, PNNL PreProcessor (2020.03.23) and the IM-MS Browser B.10.00 were used.

A database (PCDL) was created, similar as described in a previous study (Mlynek et al. 2020). Therefore, APIs or hydroxylated APIs were in-silico combined with common building blocks of phase II metabolites like glucose or malonic acid in every combination and any number possible. This database is used for screening the MS spectra of treated plant samples. The results were verified by a targeted MS/MS looking at the accurate masses and fragmentation pattern. With the fragmentation pattern, possible sum formulas for metabolites were reconstructed from mass losses.

Ion-mobilty (IM) data files were first demultiplexed using the PNNL PreProcessor software. Afterwards, the data were calibrated with the recorded single field tune using IM-MS browser. The drift times and the $^{DT}CCS_{N2}$ were determined by first performing a feature extraction (Find features IMFE). Parameters were: processing chromatographic, isotope model common organic molecules, limit charge state z < = 1, ion intensity > = 1. The drift times and the collision cross sections of all features were automatically determined by the software. Within all the features found by the software, the analytes of interest were identified according to their m/z values and their retention times.

3 Results And Discussion

3.1 Analysis of water hyacinth samples from South African rivers

It is known from a range of experiments that plants grown hydroponically tend to take up contaminants such as pharmaceuticals and personal care products (PPCPs) from the growing solution (for exemplary references see Madikizela et al. 2018). Therefore, water plants growing in potentially contaminated surface waters (rivers, lakes) might be a useful tool for evaluating the medium/long-time exposition of these waters. Thus, water hyacinths (*Eichhornia crassipes*) were collected at different positions in two South African rivers (Mbokodweni River and Mdloti River), dissected into root, leaves and stem, freeze-dried, and finally the extracts prepared from these materials were analyzed with respect to the presence of PPCPs (and their potential metabolites) in the different plant parts. Screening of the corresponding extracts allowed the detection and quantification of seven APIs, namely citalopram, telmisartan, amitriptyline (AMT), orphenadrine (ORP), tramadol (TRM), atenolol and lidocaine (LDO). Detailed results from the analyses of different plant parts collected in the two rivers are provided in Table 1. Highest concentrations, compared to the other pharmaceuticals, lying in the µg/g (dried matter) range, were found for TRM. This is also reflected in the LC-MS chromatograms obtained for extracts from stem of water hyacinths collected in the estuary of Mdloti river, showing quite prominent peaks for TRM and three of its main metabolites, depicted in Fig. 1. This might be explained by the growing problem of opoid use disorder in African countries (but also in other regions of the world) (Kurth et al. 2018; Salm-Reifferscheidt 2018). Focusing on the difference between the points of collection, no clear trend with respect to the vicinity of WWTPs could be observed – so a variety of sources for

introduction of TRM must be considered. One explanation might be the fact that TRM for example is not only used in human medicine, but also employed for the treatment of livestock (Kusari et al. 2014), leading to additional contamination from this side.

Table 1

Comparison of average concentrations (in ng g^{-1} dry plant material) of the parent drugs in root, stem and leaves from water hyacinth collected from the Mbokodweni River and from the Mdloti River

	Mbokodweni River						**Mdloti River**					
	Collection point 1			Collection point 2			Collection point 1			Collection point 2		
	roots	stem	leaves	roots	stem	leaves	roots	stem	leaves	roots	stem	leaves
Citalopram	6	n.d.*	n.d.	81	78	278	22	65	25	14	84	n.d.
Telmisartan	47	n.d.	n.d.	400	51	26	413	20	14	533	29	48
Amitriptylin	51	61	10	208	536	162	30	20	84	25	197	122
Orphenadrin	137	468	74	132	1285	472	28	142	2320	17	413	1717
Tramadol	1263	3102	680	195	2652	1223	319	5456	2056	270	4727	1356
Atenolol	1230	180	111	239	81	42	344	28	16	982	892	172
Lidocaine	8	50	92	3	30	40	8	125	415	6	90	206
*n.d. not detected												

These findings were regarded as a starting point for conducting further studies on the uptake and the subsequential fate (translocation, metabolization) of these APIs in the model plants pea (*Pisum sativum*) and garden cress (*Lepidium sativum*) cultivated in our laboratories. As investigations on citalopram, atenolol (Reichl et al. 2018) and telmisartan (Lang et al. 2021) had already been published by our group, further experiments were restricted to the remaining APIs (AMT,ORP,TRM, and LDO).

3.2 Evaluation of extraction methods for fresh plant material

First, a protocol for the preparation of extracts from fresh plant material had to be developed. The starting point for studying this issue was experience from research within this field conducted in our lab throughout the last years (Emhofer et al. 2018; Emhofer et al. 2019; Reichl et al. 2018). Thereby, garden cress was cultivated hydroponically in solutions containing the respective API at a level of 1 mg L^{-1}. After 21 days the plant material was harvested, dried, roots and upper part (leaves) of the plant were separated and extracts were prepared employing different extraction protocols comprising four procedures and six extraction solvents/solvent mixtures. More details regarding the procedures and solvent mixtures tested can be obtained from Tables S1 and S2 respectively (included in the Supporting Information). Comparing the peak areas for the parent compounds from LC-MS runs of the resulting extracts from roots and leaves, best results were obtained with extraction procedure E2 and solvent mixture SM3 – a combination that was subsequently employed for all further studies.

3.3 Studies on the metabolization of the four APIs

Three sets of experiments each (differing in the concentration of the APIs added to the growing solution) were prepared for hydroponically cultivating garden cress and pea. Two of them namely the plants grown in solutions containing 10 mg L^{-1} and 1 mg L^{-1} of the selected pharmaceutical were employed to study the uptake and translocation of the parent drugs and to search for potential metabolites formed within the plant (the third, containing 0.01 mg L^{-1} was employed for the time studies described in section 3.4). To differentiate between metabolites already formed within the growing solution and

those actually formed in the plant, comparative analysis of a “window solution” i.e. a solution of the respective substance objected to the same environmental conditions (including sunlight) as the plants was performed.

To start with, all four parent drugs (ORP, AMT, TRM, and LDO) were detected in roots, leaves and stem of the model plants after exposure for 7, 14, and 21 days. Furthermore, a number of primary metabolites listed in Table 2 could be tentatively identified. Information extracted from the HPLC / DTIM-QTOF-MS/MS runs leading to the proposal of tentative structures included: some rough estimation of polarity (by comparing retention times on RP-columns), accurate mass (allowing proposing molecular formulas), and MS/MS spectra recorded at different collision energies (providing some structural information). Additionally, the use of an MS instrument equipped with a drift-tube ion-mobility unit allowed the determination of $^{DT}CCS_{N2}$, giving some idea about similarities and dissimilarities in the three-dimensional structure of the analytes. For TRM several phase I metabolites were formed either by N-, and O-demethylation or by hydroxylation. These transformation products are also known from metabolism in humans. LDO showed N-deethylation also combined with hydroxylation as well as degradation to 2,6 xylidine whereby the latter substance was further modified by hydroxylation or oxidation of one methyl group to the corresponding benzoic acid. AMT underwent N-demethylation and hydroxylation. Finally for ORP only two primary metabolites namely N-demethyl-orphenadrine and N,N-didemethyl-orphenadrine were found. These transformation products are also known from metabolism of these drugs in humans. Subsequently LC-MS runs were checked via an in-silico database, previously developed in our lab (Mlynek et al. 2021), to search for potential phase II metabolites. Surprisingly no secondary metabolites were detected for TRM and LDO and only glycosylated species were found for the other two compounds.

Table 2
Data of four APIs and tentatively identified metabolites. CCS values were determined from South African real sample extracts. If no CCS values are provided, the ion abundance was too low for accurate CCS determination.

Name	Formula	RT	m/z $[M+H]^+$	CCS ($Å^2$)
Amitriptyline				
AMT	$C_{20}H_{23}N$	13.7	278.1903	167.6
AMT-OH	$C_{20}H_{23}NO$	10.0	294.1852	168.6
NTP	$C_{19}H_{21}N$	16.6	264.1747	165.8
NTP-OH	$C_{19}H_{21}NO$	9.8	280.1696	166.5
DMN	$C_{18}H_{19}N$	13.4	250.159	165.1
AMT-Glc	$C_{26}H_{33}NO_5$	12.5	440.2431	201.5
AMT-OH-Glc	$C_{26}H_{33}NO_6$	8.9	456.2381	205.1
Lidocaine				
LDO	$C_{14}H_{22}N_2O$	6.0	235.1805	156.6
LDO-OH	$C_{14}H_{22}N_2O_2$	3.1	251.1754	*
MEGX	$C_{12}H_{18}N_2O$	4.7	207.1492	*
Orphenadrine				
ORP	$C_{18}H_{23}NO$	13.0	270.1852	166.4
ORP-Glc	$C_{24}H_{33}NO_6$	12.0	432.2381	199.8
Tramadol				
TRM	$C_{16}H_{25}NO_2$	8.2	264.1958	160.7
ODT	$C_{15}H_{23}NO_2$	5.4	250.1802	159.7
NDT	$C_{15}H_{23}NO_2$	8.4	250.1802	156.7
TRM-OH	$C_{16}H_{25}NO_3$	8.9	280.1907	166.6
* concentrations too low for CCS determination				

Finally, also the extracts from water hyacinth collected in the two South-African rivers were re-investigated with respect to the presence of the metabolites found in cress and pea. Thereby O-desmethyltramadol (ODT), N-desmethyltramadol (NDT), hydroxylated tramadol (TRM-OH), monoethylglycinexylide (MEGX), and hydroxylated lidocaine (LDO-OH) could be detected.

3.4 Time-studies on the uptake and translocation of four APIs by garden cress and pea

Two different types of time studies investigating the uptake and translocation of AMT, ORP, TRM, and LDO by cress and pea were conducted. In one case plantlets were grown hydroponically in water spiked with the corresponding drug at a level

of 1 mg L^{-1}. Whenever samples were taken (after 1, 2, 4, 8, and 16 days), the lowered water level (due to evaporation) was adjusted by addition of pure water. For the second type plants were grown in an aqueous solution containing 0.010 mg L^{-1} of the respective pharmaceutical. Whenever plants were removed for sampling, the complete solution was renewed. The first setup should mimic a situation where plants get in contact with water that is severely contaminated with drug residues; approach two resembled the scenario when for example plants are regularly irrigated with treated wastewater still containing the investigated drug residues at trace levels. Thereby two different trends could be observed. When plants were subjected to the higher concentration of the drug (1mg L^{-1}) for cress maximum peak areas for root extracts were already obtained after one day of exposure. Subsequently peak areas for the parent drug in roots decreased steadily, whereas those in leaves increased, showing the subsequent transport of the substances within the plant. For pea concentration in the roots increased till day eight–indicating a slower take up of the parent drug compared to cress. After day eight also in this plant a decrease in the drug concentrations in root extracts was observed. In leaves and stem of pea, peak areas (similar as in cress) showed a steady increase till the end of the experiment, hinting to the transport of the parent drug from the roots to the upper plant parts. When the second approach was used (solution containing 0.010 mg L^{-1} that was regularly replaced by a fresh one), peak areas for roots showed a steady increase from day one. This can be understood as evidence that the kinetics of drug uptake is substantially influenced by the availability of higher amounts of the drug in the growing solution. Concurrent an increase in the leaves was observed. Figure 2 shows these trends on the example of tramadol in cress and pea.

In addition, also some of the phase I metabolites originally detected in plants when a much higher concentration of the parent drug was applied (10 mg L^{-1}) could be found during these time-studies. Focusing on the experiments conducted with the 1 mg L^{-1} solutions, four AMT metabolites (AMT-OH, NTP, NTP-OH and DMN) were found in cress roots and leaves. For pea a similar result was obtained, but with much slower kinetics, meaning that only after day eight the metabolites were detected in roots stem and leaves. A more detailed picture of this behavior can be seen from Table S4 (Supplementary material). Switching to LDO and TRM, for the first LDO-OH and MEGX were found in cress and pea. In the case of TRM, ODT and NDT could be identified. In most cases a gradual increase of metabolite concentrations over time could be detected. As a typical example the time-profile of NDT and ODT in pea is presented in Fig. 3. Here substantially larger peak areas (factor of ten) were obtained for ODT. Whereas this metabolite showed a steady increase over time with largest peak areas obtained for leaf-extracts, NDT showed an increase in the roots till day eight followed by a significant decrease from this time on. Simultaneously the concentration in leaves showed a steep increase. Extracts from pea stem exhibited only marginally higher peak areas at the end of the observation period.

4 Conclusions

A number of pharmaceuticals was detected in water hyacinth samples collected from two South African rivers. In-laboratory studies employing garden cress and pea as model plants, led to the result that also these plants take up, translocate and metabolize the drugs found in the water hyacinths. Thereby a number of metabolites could be tentatively identified, and studies regarding the kinetics of uptake, translocation and metabolization were conducted. These findings suggested, that water plants might serve as a good indicator for estimating the long time pollution of lakes and rivers with PPCP´s. Whereas water analyses just provide information on the momentary status of the aquatic system, harvesting and analyzing water plants after a defined growing time might allow estimating the amount of PPCP´s introduced over a longer time period. Whereas the present study should just be seen as a proof of concept, follow up studies should include a wider range of plants. Additionally, more points of collection providing a finer resolution in space, allowing to define potential sources for introduction of PPCP´s might be needed.

Declarations

Data availability: All data and materials are included in this published article.

Authors contributions: AD and CG performed the experimental work; MH and FM supervised the experimental work and provided help in performing and interpretation of LC-MS/MS experiments; LM provided freeze-dried samples from water hyacinth and discussion on the manuscript; WB provided useful comments for writing up the manuscript; CWK wrote up the manuscript.

Compliance with ethical standards

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable

Competing interests: The authors declare that there are no competing interests concerning the research described in this work

Funding: No funding was received for this work.

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Figures

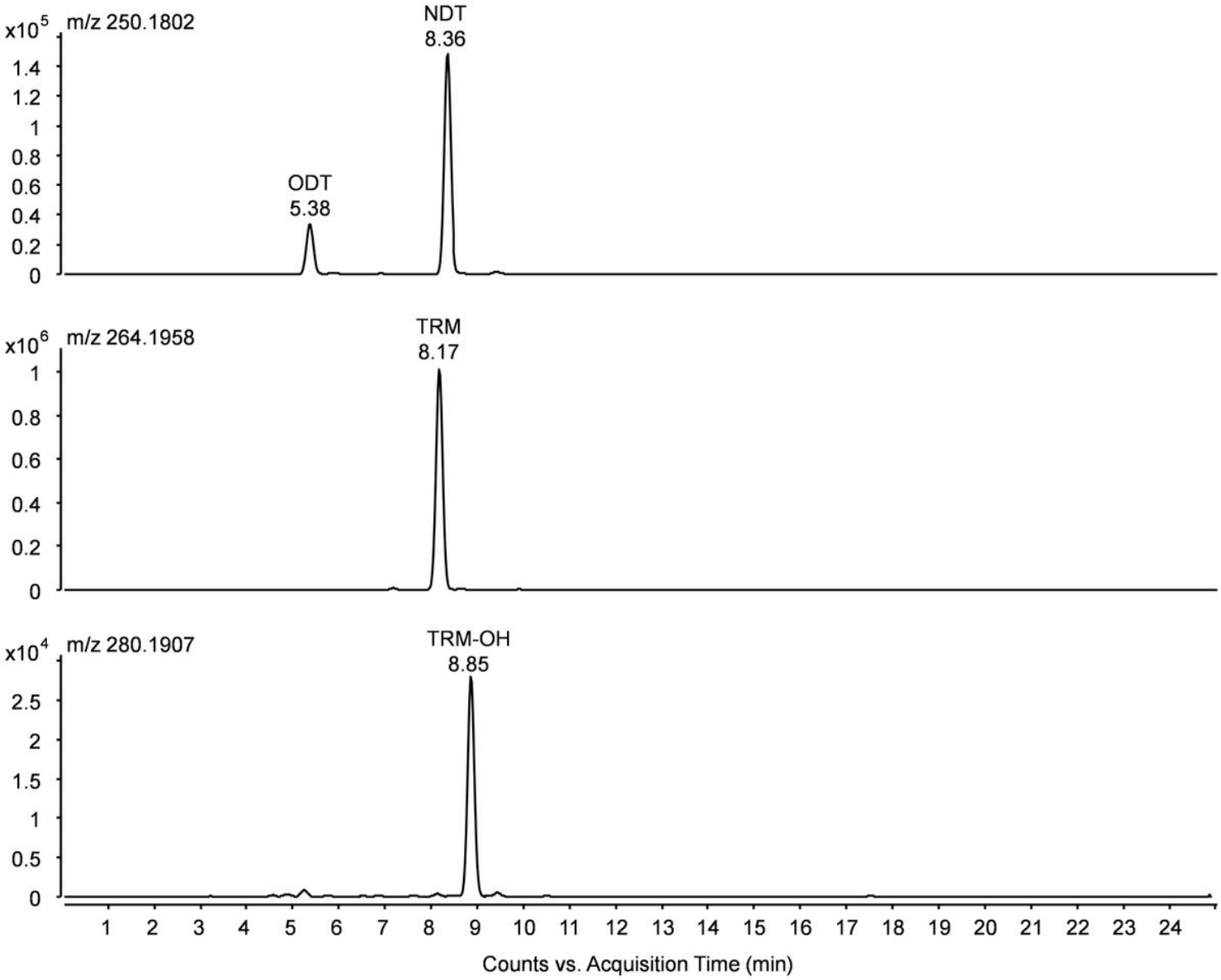


Figure 1

HPLC-MS chromatogram of tramadol an its major metabolites found in the extract from water hyacinth-stem collected from Mdloti river. Peaks ODT: O-demethylated-tramadol; NDT: N-demethylated-tramadol; TRM: tramadol; TRM-OH: hydroxylated tramadol. For conditions see Experimental Section.

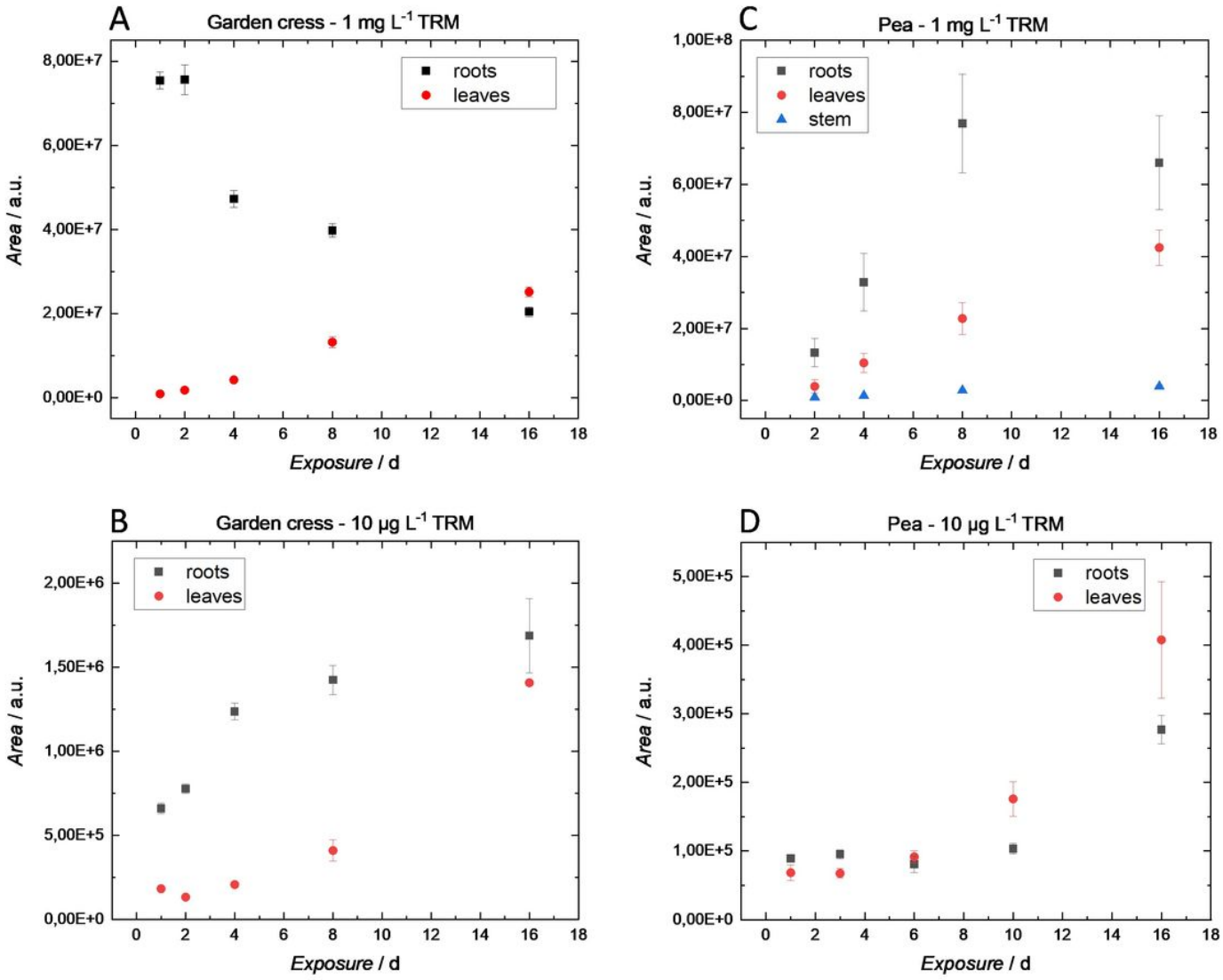


Figure 2

TRM time study in garden cress (A) and (B) and pea (C) and (D). Data points refer to peak areas (mean values from three replicates) obtained for TRM in extracts from roots, stem and leaves. Plants were cultivated for 16 days in solutions containing TRM at a level of 1 mg L^{-1} (A) and (C) and 10 mg L^{-1} (B) and (D). Error bars represent standard deviations.

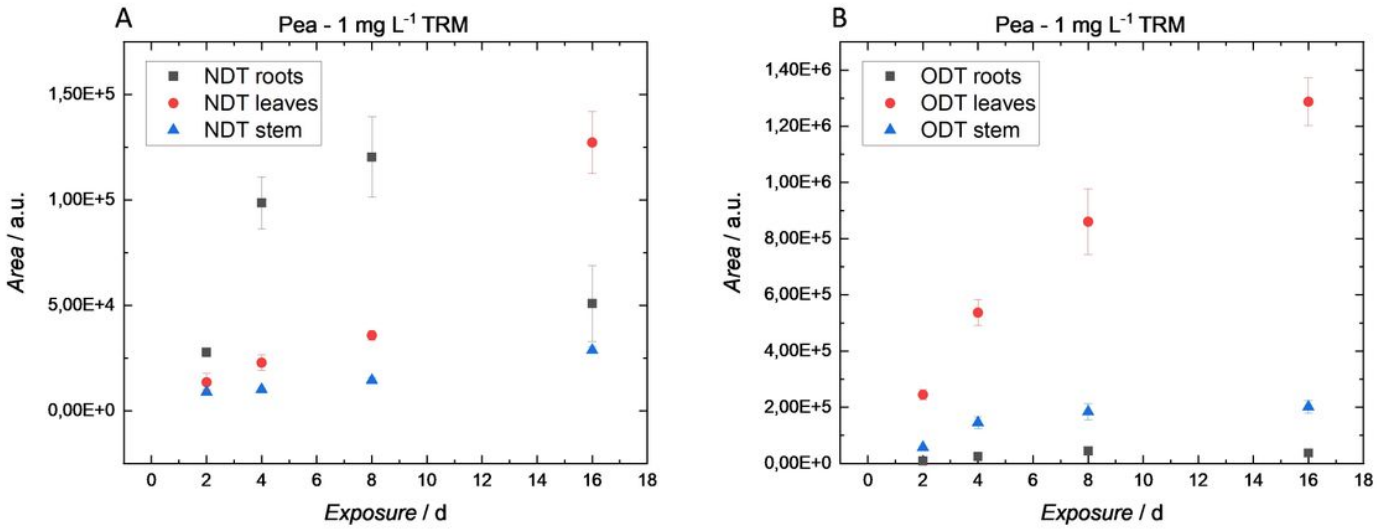


Figure 3

Time study of TRM metabolites NDT (A) and ODT (B) in peas after exposure to a 1 mg L^{-1} TRM solution. Data points refer to peak areas (mean values from three replicates). Error bars represent standard deviations.

Supplementary Files

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