AQUATIC PLANTS AS A POSSIBLE WAY FOR PSYCHOACTIVE PHARMACEUTICALS AND DRUGS REMOVAL FROM WASTEWATER

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ABSTRACT

In this study, we analyzed 30 selected psychoactive drugs and metabolites in two WWTPs in Slovak republic. Consequently, we were interested in a possibility of alternative tertiary post-treatment of effluent water with aquatic plants. Our results indicate that compounds such as THC-COOH, benzoylecgonine, cocaine and amphetamine are considerably degraded in the mechanical and biological stage of WWTP. Both examined WWTPs are not able to effectively eliminate compounds like tramadol, oxazepam or venlafaxine (removal efficiency is about 30%). WWTPs influent was pretreated in batch tests with aquatic plants. Significant cleaning efficiency was observed after treatment with *Iris pseudacorus, Pistia stratiotes, Limnophila sessiliflora* and *Cabomba caroliniana*. All aquatic plants except *Pistia stratiotes* were able to eliminate most of the analyzed compounds with more than 50% effectiveness. Consequently, we have analyzed different parts of plants. The plant *Pistia stratiotes* contained in the leaves methamphetamine in concentration of 3666 ng/g, *Cabomba caroliniana* (289 ng/g) and *Limnophila sessiliflora* (1242 ng/g). In a leaf from *Cabomba caroliniana,* tramadol and venlafaxine were also identified (1128 a 367 ng/g). In addition to laboratory tests, we have also analyzed aquatic plant *Lemna minor* growing directly at the effluent of two WWTPs after a period of three months. Analyses of leaves of this plant, however, did not confirm the occurrence of the studied compounds.

Keywords: WWTP, wastewater, LC-MS/MS, aquatic plants, degradation, biosorption

1. Introduction

The compounds such as legal and illegal drugs, hormones, antibiotics, and antihistamines are degraded only to limited degree at the WWTPs and may penetrate into the surface water, which may impact the aquatic ecosystem. These compounds can be classified as metabolites of drugs, and psychoactive pharmaceuticals such as cotinine, carbamazepine, tramadol, venlafaxine, citalopram, or oxazepam (Dordio *et al*., 2011; Kotyza *et al*., 2009; Li *et al*., 2014; Mackuľak *et al*., 2014).

1.1. The possibilities of removal of psychoactive pharmaceuticals and drugs from wastewater

Recent studies suggest a potential use of aquatic plants in removing biologically inert pharmaceuticals from wastewater (Li *et al*., 2014; Mackuľak *et al*., 2015). The manner in which aquatic plants are capable of removing drugs from water is affected by various factors. The most important is the diffusion process of these substances, which is dependent on the physicochemical properties of the compounds. Hydrophobia of a substance plays an important role; it is expressed by a logarithm of the octanol/water - the coefficient Log Kow. During the penetration of pharmaceuticals into the structure of plants, accumulation or metabolic process may occur – degradation or incorporation of the compound directly into the cell wall (Li *et al*., 2014) and a subsequent degradation through a wide range of oxidation and hydrolytic enzymes

(Li *et al*., 2014). Plants applied in root treatment plants degrade organic compounds by the present specific bacterial communities concentrated on the root system. The year period significantly affects the activity of plants and microorganisms, thus, the efficiency of degradation (temperature, intensity of solar radiation, and presence of nutrients). Aquatic plants are also capable of using the root system to effectively release oxygen into water, and potentially also in oxidation of the present pharmaceuticals (Dordio *et al*., 2011; Kotyza *et al*., 2009; Li *et al*., 2014).

2. Materials and methods

2.1. Sampling and analysis

The sample used for the analysis of influent and effluent from WWTP was collected by an automatic sampling device (24-hour decanted samples) in 15-minute intervals. The samples were collected in plastic bottles and were frozen (-20°C) two hours after the sampling. The transported sewage samples were thawed at room temperature. Prior to analysis, isotope labelled internal standards were added to 10 ml of homogenized and filtered (filter type GFC, 0.45 μm) water. The in line SPE liquid chromatography coupled with hybrid quadrupole - Orbitrap high resolution mass analyser was used as method for the quantitative analysis of the tested drug residues (Mackuľak *et al*., 2014). Detail description of the method and its performance is given in paper of Fedorova *et al*. (2013). The pooled water samples were analysed in triplicate, whole degradation experiments were performed in pentaplicates. Means and standard error of the means were calculated.

2.2. The removal of psychoactive pharmaceuticals and drugs using aquatic plants

In our study, we examined four different types of aquatic plants (*Iris pseudacorus*, *Limnophila sessiliflora*, *Cabomba caroliniana* and *Pistia stratiotes*) obtained from the company Akvaria my Expert s.r.o., Slovakia. We defined the plants using the organic and inorganic composition (Tab 1). Single batch tests were carried out with a real effluent from the WWTP Malacky and Bratislava (Tab 2 and 3).

Table 1: Characteristics of the aquatic plants

	Lemna minor (leaf)	*Pistia stratiotes* (leaf)	*Cabomba caroliniana* (leaf)	*Iris pseudacorus* (root)	*Limnophila sessiliflora* (leaf)
total solids [g/kg]	64.5	45.3	75.5	90.3	48.3
ash [g/kg]	13.2	14.8	13.2	7.1	16.8
volatile solids [g/kg]	51.3	30.5	62.3	83.2	31.5

The first test was implemented using *Cabomba caroliniana*, *Limnophila sessiliflora* and *Pistia stratiotes* plants, weighing 9.02 g (leaf - 5.64 g), 7.60 g (leaf - 4.80 g) and 3.58 g (leaf – 2.02 g). 0.1 L of wastewater was added into a reactor of a total volume of 1 L. The tests were conducted simultaneously for 48 hours. The second test was implemented using *Iris pseudacorus*. In comparison with the plants in the first test, *Iris pseudacorus* differs in the density of the root system. 0.5 L of wastewater was added into a reactor of a total volume of 1 L. Weight of the plants was 152 g. The test was conducted for 96 hours. Changes in the composition of wastewater before and after the tests were measured by LC-MS/MS, and also the composition of the different parts of the plants (Tab 3).

After the batch tests, the individual plant parts (roots, leaves) were ground and extracted for 30 minutes in 30 ml of methanol (HPLC Grade, 99.8% Merck, Germany) in the presence of 10 mg of an inert sea sand, prepared according to the study Zhang *et al*., 2013. Afterwards the extract was analyzed on LC-MS/MS. The aqueous plant *Lemna minor* obtained directly from sewage treatment plants was prepared likewise. The weight of the leaves used in the extraction was 7.54 g (WWTP Malina) and 7.49 g (WWTP Bratislava). Thus obtained concentration of the identified substances in the extract (ng/1ml) was converted through the total dry weight of the plant and the amount of the plant part used in the experiment to a value corresponding to the quantity of the substance in ng per g of dry weight of the selected plant (ng/g). The results are evaluated in Table 3.

Table 2: Concentration of the selected compounds in different parts of WWTP Bratislava (20.3.2014) and Malacky (5.3.2014)

	WWTP Bratislava			**WWTP Malacky**	
	influent	**sewage water**	**effluent**	**influent**	**effluent**
		ng/L		**ng/L**	
Caffeine	20.0	184	46.1	< 12	< 5.4
Cotinine	13346	5477	56	ND	123
Codeine	195	85	121	170	168
Amphetamine	72	100	< 6.2	105	< 4.8
MDA	< 6.3	36	7.9	< 6.8	< 4.6
Methamphetamine	683	1114	328	928	112
MDMA	23.0	153	29.7	8.6	< 5.2
Mephedrone	< 6.9	< 18	< 14	< 7.4	< 12
Benzoylecgonine	162	59.0	11	13	6.8
Tramadol	985	840	1108	1240	1293
Cocaine	62.0	131	< 6.9	< 11	< 4.4
Venlafaxine	231	589	352	424	569
Oxazepam	93	78	105	60	63
Citalopram	155	200	116	107	171
EDDP	35	96	22	< 4	< 4.5
Methadone	20	41	14	< 5	< 4.7
THC-COOH	165	16	< 2.8	157	5.1

Table 3: Removal of psychoactive compounds from the WWTPs influents by aquatic plants and the incidence of psychoactive substances in different parts of plants

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
	ng/L	**ng/L**	**ng/g**	**ng/L**	**ng/L**	**ng/L**	**ng/g**	**ng/L**	**ng/g**
Cotinine	**13 369**	10 032	ND	**ND**	239	55	ND	134	ND
Codeine	**173**	160	ND	**75**	< 3	36	ND	21	ND
Amphetamine	**61**	< 5	ND	**56**	< 6	< 6	ND	< 5	ND
Methamphetamine	**683**	375	3 666	**406**	127	54	1 242	14	289
Benzoylecgonine	**183**	140	ND	**< 9**	< 7	< 6	ND	< 6	ND
Tramadol	**928**	622	ND	**1614**	578	849	ND	447	1 128
Venlafaxine	**299**	297	ND	**370**	112	157	ND	71	367
Oxazepam	**111**	110	ND	**99**	37	95	ND	101	ND
Citalopram	**149**	157	ND	**107**	< 22	30	ND	< 12	ND
EDDP	**42**	40	ND	**8**	< 5	< 4	ND	< 7	ND
Methadone	**25**	11	ND	**< 7**	< 6	< 8	ND	< 6	ND
THC-COOH	**88**	15	ND	**17**	<11	< 2	ND	< 2	ND

A - WWTP Bratislava influent (14.3.2014), *B - influent after Pistia stratiotes 48 hour, C - extract Pistia stratiotes leaf;* ***D - WWTP Malacky influent (7.3.2014),*** *E - influent after Iris pseudacorus, F - influent after Limnophila sessiliflora 48 hour, G - extract Limnophila sessiliflora leaf, H - influent after Cabomba caroliniana 48 hour, I - extract Cabomba caroliniana leaf*

3. Results

In our work we have focused on the analysis of 30 psychoactive pharmaceuticals, illegal drugs and metabolites (MDMA, MDA, cotinine, caffeine, methamphetamine, amphetamine, THC-COOH, benzoylecgonine, cathinone, codeine , oxycodone, heroin, 6-acetylmorphine, LSD, 2-Oxy-3-hydroxy-LSD (O-H-LSD), ketamine, norketamine, mephedrone, methylphenidate, tramadol, midazolam, venlafaxine, oxazepam, citalopram, buprenorphine, norbuprenorphine, MDEA, MBDB, EDDP and methadone) in wastewater from two WWTPs (Bratislava and Malacky). Of these selected compounds, only MDMA, cotinine, caffeine, methamphetamine, amphetamine, THC-COOH, benzoylecgonine, codeine, tramadol, venlafaxine, oxazepam, citalopram, EDDP and methadone reached the inlet concentration greater than 30 ng/L (Tab 2 and 3). However, both WWTPs are capable to remove only limited amounts of most of these compounds. In the case tramadol and venlafaxine, an increase in concentration was observed at the outlet from the WWTP Malacky. This may be due the influence of sludge management, since supernatant from anaerobic processes is used as a source of nitrogen and phosphorus for biological processes. The supernatant can contain significant amounts of pharmaceuticals and drugs, which may have the effect of an increase in concentration at the outlet. This fact corresponds with other work in this area (Golovko *et al*., 2014; Mackuľak *et al*., 2015).

In the next part of the study, aquatic plants were investigated as possible means for wastewater treatment. The obtained results described in Table 3 indicate, that different types of aquatic plants achieved significantly different removal efficiencies of psychoactive compounds. At the WWTP inlet, aquatic plants such as *Cabomba caroliniana* or *Limnophila sessiliflora* were able to effectively remove even poorly bio-degradable compounds such as tramadol, methadone, citalopram, methamphetamine, venlafaxine, with efficiency above 45%. Conversely, a low activity has been observed for oxazepam (below 10%); this fact corresponds with the study Mackuľak *et al*., 2015. Floating water plant *Pistia stratiotes* did not achieve such high efficiency, especially for tramadol, venlafaxine and citalopram (efficiency below 40%). Similar to *Cabomba caroliniana* or *Limnophila sessiliflora*, it was unable to remove oxazepam. Studies in this area also show a limited ability to remove substances such as monensin, narasin, salinomycin, diclofenac, ketoprofen, amoxicillin, clarithromycin, sotalol, clofibric acid, ampicillin, erythromycin, lincomycin and carbamazepine. On the other hand, efficiency of over 70% was observed for ciprofloxacin, oxytetracycline, nadolol, paracetamol, salicylic acid, tetracycline, and caffeine (Kotyza *et al*., 2009; Li *et al*., 2014). The last examined aquatic plant was *Iris pseudacorus*. These plants are often used in the construction of wetlands (Li *et al*., 2014). They have extensive root systems capable of removing different types of pharmaceuticals from wastewater. *Iris pseudacorus* was able to remove most of the compounds with efficiency above 60% after 96 hours at the WWTP inlet.

In the last part of the study, individual parts of aquatic plants were analyzed after degradation. It was found that the root and leaves of *Iris pseudacorus* do not contain any of the 30 analytes, similarly to the leaves of *Lemna minor*, which was cultivated directly in the wastewater at the outlet from WWTPs Bratislava and Malacky. Leaves of *Pistia stratiotes* contained the compound methamphetamine, in a concentration of dry matter of the leaf – 3 666 ng/g, as *Cabomba caroliniana* (289 ng/g) and *Limnophila sessiliflora* (1 242 ng/g). Subsequently, we identified in the leaf of *Cabomba caroliniana* the drug tramadol (1 128 ng/g) and venlafaxine (367 ng/g). This may be caused by hydrophobia of methamphetamine, tramadol and venlafaxine expressed by the coefficient Log Kow. Log Kow values of the most concentrated psychoactive compounds in wastewater ranged from 0.5 to 3.28: venlafaxine (3.28), tramadol (3.01) and methamphetamine (2.07) (Meylan and Howard, 1995). Some studies have pointed out the good degradation of compounds with log Kow values ranging from 0.5 to 3. Caffeine (log Kow < 1) was substantially taken up by the plant roots and then translocated to shoot tissues (110 – 6 110 ng/g in the roots and 6 390 – 13 650 ng/g in the shoots) (Zhang *et al*., 2013). Drugs that are transported to the plant can be metabolized or transformed to less toxic compounds and subsequently can be mineralized or stored in plant cell walls. Little is known about the metabolic transformation of pharmaceuticals in plants. A disadvantage in the use of aquatic plants is their gradual contamination during the purification process. The plants must therefore be subsequently removed by burning, to prevent release of the parent drug or the metabolized forms back to the environment.

4. Conclusions

In our study, we dealt with the analysis of 30 psychoactive substances in wastewater and their possible degradation using aquatic plants (*Iris pseudacorus*, *Pistia stratiotes*, *Cabomba* *caroliniana* a *Limnophila sessiliflora*). It was found that these aquatic plants are capable of effectively removing compounds such as venlafaxine, citalopram, methamphetamine or tramadol. The obtained results indicate that compounds such as venlafaxine, tramadol methamphetamine are able to accumulate mainly in the leaves of these plants. Leaves of *Pistia stratiotes* contained the compound methamphetamine of a concentration 3 666 ng/g, *Cabomba caroliniana* (289 ng/g) and *Limnophila sessiliflora* (1242 ng/g). In the leaf of *Cabomba caroliniana* was identified the drug tramadol (1128 ng/g) and venlafaxine (367 ng/g). We also studied the composition of aquatic plant *Lemna minor* growing directly at the outlet of selected treatment plants. The analysis did not confirm the presence of psychoactive substances in the leaves.

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REFERENCES

1. Mackuľak T., Škubák J., Grabic R., Ryba J., Birošová L., Fedorova G. *et al*. (2014), National study of illicit drug use in Slovakia based on wastewater analysis. Sci Total Environ., **494–495**, 158-165.
2. Kotyza J., Soudek P., Kafka Z and Vaněk T. (2009), Pharmaceuticals – New Environmental Pollutants. Chem listy.,**103**, 540-547.
3. Fedorova G., Randak T., Lindberg R.H and Grabic R. (2013), Comparison of the quantitative performance of a Q-Exactive high resolution mass spectrometer with that of a triple quadrupole tandem mass spectrometer for the analysis of illicit drugs in wastewater. Rapid Communications in Mass Spectrometry., **27**,1751-1762.
4. Li Y., Zhu G., Ng J.W and Tan K.S. (2014), A review on removing pharmaceutical contaminants from wastewater by constructed wetlands: Design, performance and mechanism. Sci Total Environ., **468-469**, 908-932.
5. Mackuľak T., Mosný M., Škubák J., Grabic R and Birošová L. (2015), Fate of psychoactive compounds in wastewater treatment plant and the possibility of their degradation using aquatic plants. Environ. Toxic. Pharm., in press doi:10.1016/j.etap.2015.02.018.
6. Golovko O., Kumar V., Fedorova G., Randak T and Grabic R. (2014), Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. Chemosphere, **111**, 418 – 426.
7. Meylan M.W and Howard H.P. (1995), Atom/fragment contribution method for estimating octanol-water partition coefficients. J. Pharm. Sci., **84**, 83–92.
8. Zhang D.Q., Hua T., Gersberg R.M., Zhu J., Ng W.J and Tan, S.K. (2013). Fate of caffeine in mesocosms wetland planted with *Scirpus validus*. Chemosphere, **90**, 1568 –1572.