STUDY OF PHARMACEUTICALS IN A MODEL URBAN RIVER AS POTENTIAL MOLECULAR MARKERS OF WASTEWATER EFFLUENTS, THEIR SOURCES AND SOCIO-ECONOMIC CORRELATES (THE CITY OF KHARKIV, UKRAINE)

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ABSTRACT
In recent decades the amount of pharmaceutical residues have significantly increased in water bodies located near and within urbanized areas, as a result of extensive use of different medical drugs. Pharmaceutical residuals are suspected to enter rivers, streams and surface waters through the effluent of sewage treatment plants. After intake, pharmaceuticals are excreted with urine or faeces to raw sewage in both an unchanged form and as metabolites. The presence and behaviour of these emerging pollutants in the aquatic environment are still poorly known, and continuous research is demanded. The goal of our study was to identify the targeted pharmaceuticals and their potential sources in a model river, Lopan, in the city of Kharkiv, Ukraine. The Polar Organic Chemical Integrative Samplers (POCIS) have been applied for the detection of pharmaceuticals in sites upstream and downstream of wastewater treatment plants from which treated wastewaters are discharged to the river. The sampling has been done during May 2009 and December 2009. Totally, 19 of 21 targeted substances were detected in sites upstream and downstream of the urban areas and treated wastewater discharge. Such compounds as, diclofenac, carbamazepine, caffeine were found in the highest contents in all installed passive sensors. The carbamazepine can be used for tracing pathways of sewage water, even the treated one. In contrast, the caffeine is a labile and easy degradable compound so it can be used for the identification of effluents of untreated wastewaters. It was also found that the presence of some pharmaceuticals exhibits seasonal variations.. In spite of steady increase of pharmaceutical residues in water bodies, the elimination of these substances is not provided on a high level within the conventional sewage treatment technologies.

KEYWORDS: Pharmaceuticals; urban waters; detection methods; Kharkiv; Ukraine

INTRODUCTION

In recent decades the amount of pharmaceutical residues have significantly increased in water bodies located near and within urbanized areas, as a result of extensive use of different medical drugs. Pharmaceutical production and usage have increased worldwide as a result of improvement of the human life quality, easier access for medicines, insufficient regulation of drugs market, «nation’s senescence», etc. More than 12 thousand drugs are known and used nowadays.

The metabolites of medicines can accumulate in and affect the ecosystems being involved into food chains and acting as disruptors of biological cycles of aquatic organisms. Pharmaceutical residuals are suspected to enter rivers, streams and surface waters through the effluent of sewage treatment plants, untreated waters from hospitals and, possibly, with runoff. Conventional water treatment technologies are not able to remove such residues. Other pathway the pharmaceuticals enter the rivers is through interflow and unregulated wastewaters discharge. Pharmaceuticals occurrence in groundwater is a result of infiltration (Fig. 1). These substances can also be an important pollution source for soils if primary and secondary sludge (to which they are adsorbed) is applied on land [1, 2, 6, 9, 11, 13].

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The other reason for detection of these pollutants in natural waters is uncertainty of transformation processes of chemical molecules and their metabolites because of natural and anthropogenic factors.

The goal of our study was to identify the targeted pharmaceuticals and their potential sources in a model river of large urbanized area in Ukraine.

EXPERIMENTAL DESIGN

Site selection and sampling

The Lopan River was chosen as a model to identify principal pharmaceutical contaminants, their distribution and sources of origin. This small river (length – 96 km, catchment area – 2000 km^2) is one of four that flow through the city of Kharkiv, Ukraine. The river is used for recreation, industrial water supply, irrigation and sport fishing. The Lopan River is a sink for treated mixed municipal and industrial wastewaters (about 600,000 m^3 per day) from the city and its suburbs [7].

Water sampling was done in May 2009 and December with passive samplers installed at three sites on the Lopan River: 1) upstream of the Kharkiv city boundaries and approximately 2 km downstream of discharges from the Kharkiv Zootechnic and Veterinary Academy in the Mala Danylivka village; 2) in the city, upstream of the wastewater discharges from the 'Dykanivka' Municipal Sewage Works (MSW); 3) approximately 0.7 km downstream of the wastewater discharge from the 'Dykanivka' MSW [5,6].

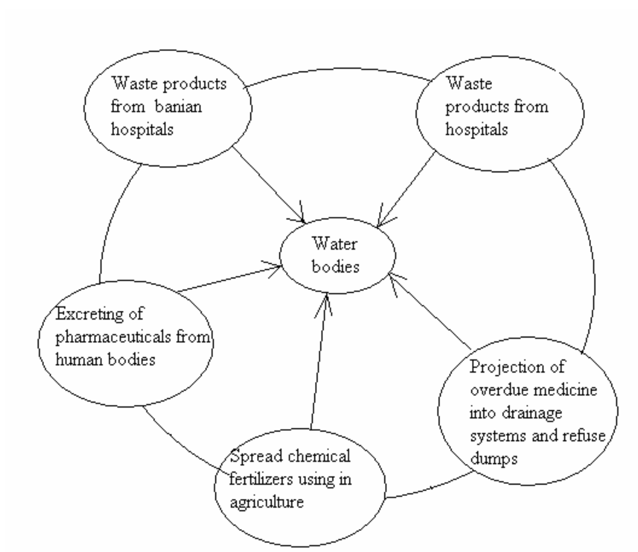

Figure 1. Sources of pharmaceuticals in water bodies.

MATERIALS AND METHODS

The Polar Organic Chemical Integrative Samplers (POCIS) have been applied for the detection of pharmaceuticals in sites upstream and downstream of wastewater treatment plants from which treated wastewaters are discharged to the river. The POCIS consists of a solid sequestration medium enclosed within a microporous membrane for the integrative sampling of hydrophilic organic chemicals [1,12]. The sampler enables estimation of the cumulative aqueous exposure to hydrophilic organic chemicals and permits to determine the biologically relevant time-weighted average (TWA) concentration in water [10].

The extraction procedures for POCIS were based on previously developed methods [12]

The time of POCIS exposure in water was 3 weeks. The surface membrane was detached from the stainless steel rings and rinsed with ultra-pure water. The phase from two membranes of each POCIS has been eluted with 5 mL of ultra-pure water per each membrane, in cartridges cleaned by methanol teflon and dried under vacuum for 1 h. The sorbent was eluted using methanol, dichloromethane and methanol/dichloromethane mixture (50:50) with 10 mL of each solution per sample and being spiked with internal standards. The extracts obtained from the sorbent were finally evaporated to dryness using a nitrogen stream and transferred into injection vials 50 μL of acetonitrile.

The mass of sorbent has been measured with gravimetry method for each POCIS after drying. Blanks were performed in the laboratory concurrently with water samples to monitor possible contamination. Recovery rates of the POCIS samples were determined by the spike samples [3].

RESULTS AND DISCUSSION

Presence of pharmaceuticals in the river

Totally 21 pharmaceuticals of different therapeutic classes (psychiatric drugs: alprazolam, amitriptyline, diazepam, doxepin, fluoxetine, imipramine; nordiazepam, carbamazepine, bromazepam; analgesics: aspirin, paracetamol; broncholidator: clenbuterol, salbutamol, terbutaline; non-steroidal anti-inflammatory drug: diclofenac, ibuprofen, ketofen, naproxen; lipid regulator: gemfibrozil; stimulants: caffeine, theophylline) were targeted for detection in the Lopan River, of which 15 were found in samples. The highest concentrations were peculiar to such compoundss as diclofenac, carbamazepine, caffeine, ketoprofen (Table 1).

The contents of carbamazepine and diclofenac in water were much higher (about 30-100 times) downstream the Dykanivka MSW than upstream. Caffeine and theophylline were detected in rather the same quantity both upstream and downstream of the MSW except higher contents of caffeine were detected in winter samples downstream of the MSW as a result of slower temperature-dependent rate of metabolism in an aquatic ecosystem. Paracetamol, diazepam and ketoprofen were also found at the sites located upstream MSW. Ketoprofen contents in the river upstream the MSW were 8 times higher in summer than in winter whilst at the site located downstream of the MSW it was not detected in summer and was detected in winter at concentration 15 times higher than upstream. Such differences may relate to a broad use of ketoprofen in the veterinary [11] with possible discharge to the Lopan River from the Veterinary Academy located upstream of the sampling site 1 and/or with the run-off from adjacent farms combined with lower metabolism rate in aquatic environment at low temperatures

Doxepin and imipramine were not been detected. Terbutaline, salbutamol, clenbuterol and alprazolam were measured at trace levels (up to 10 ng g^{-1} of the POCIS sorbent) [4,8].

Table 1. The concentrations of top pharmaceutical compounds in the Lopan River (May and December 2009)

Pharmaceuticals	Concentration, ng g^{-1}			
	Site 1 upstream MSW (summer)	Site 1 upstream MSW (winter)	Site 3 downstream MSW (summer)	Site 3 downstream MSW (winter)
Diclofenac	73	28	3560	2635
Caffeine	178	181	421	1536
Carbamazepine	65	20	7802	4017
Ketoprofen	420	53	<[1]	796

Pharmaceuticals transformation during purification at Municipal Sewage Works and in natural waters

The pharmaceuticals transformation is different in natural waters and during purification at MSW. Some pharmaceutical contaminants absolutely dissolve because of reagent action, another do not change their molecular structure at all. Starting from different properties of pharmaceuticals there exist different treatment methods: 1) filtration (using membranes, micro-strainers, ultra-filtration); 2) sorption (mineral and molecular polymers, active carbon); 3) oxidation; 4) biological treatment. But there is no universal method providing for a treatment of all parmaceuticals.

The properties and transformation level of compounds depend on many factors. For example, presence of nitrate in the aqueous solution enhances the rate of phototransformation for many pharmaceuticals. The observed effect may be ascribed to the formation of OH-radicals due to photolysis of nitrate.

When humic acids act mainly as inner filters, their addition will result in a decrease of the rate of photodegradation compared to the rate measured in bi-distilled water (as in the case of carbamazepine and diclofenac). On the other hand, when the promoting effect by humic acids prevails, an enhancement in the rate of phototransformation is observed [2].

Caffeine is absolutely dissolved if acidic elements present in the water during methylation process.

Such factors as seasonality, presence of aquatic macrophyte vegetation also have an influence on metabolism patterns of pharmaceuticals.

Carbamazepine and caffeine as markers of surface water pollution by wastewater

Organic compounds that are specific to certain pollution sources can be used as water-soluble molecular markers of wastewaters for the detection of sewage particles in natural waters [4,8].

Carbamazepine and caffeine are featuring different properties and different behaviour during purification and in natural waters (Table 2).

Caffeine as a labile indicator of untreated wastewater has previously been studied for coastal and freshwater systems. In some studies of sewage effluents caffeine was detected below the limits of detection, and its degradation most probably continues when it is discharged into the surface waters [9].

[1] < - present at lower concentration than limits of detection (1 ng g^{-1} of sorbent)

Table 2. Characteristics of caffeine and carbamazepine in the Lopan River, the city of Kharkiv, Ukraine.

Pharmaceuticals	Carbamazepine	Caffeine
Prescription	Established drug for the control of grand mal and psychomotor epilepsy; it is used in the treatment of trigeminal neuralgia and in bipolar depression.	A central nervous system and metabolic stimulant, it is used both recreationally and medically to reduce physical fatigue and restore mental alertness. It is also used on newborns to treat apnea and correct irregular heartbeats.
Molecular formula	$C_{15}H_{12}N_2O$	$C_8H_{10}N_4O_2$
Average concentration, ng g^{-1} , upstream MSW	42.5	180
Average concentration, ng g^{-1}, downstream MSW	5900	970
Reported treatment efficiency, % [2]	8	>99.9

Carbamazepine as a conservative marker of treated sewage has previously been studied for coastal and freshwater systems. This pharmaceutical was resistant to degradation and has a conservative behaviour in the natural environment. Carbamazepine can be useful for tracing pathways of sewage water, even the treated one. In contrast, caffeine is a labile compound and easily degraded during the water treatment processes [3]. Its presence in water may detect direct discharge of untreated wastewater effluents.

CONCLUSIONS

The POCIS passive sampling technique has been proved for effective detection of pharmaceuticals in urban waters. Concentrations of the most of targeted pharmaceuticals were many times higher downstream than upstream of discharge from Municipal Sewage Works, which do not secure appropriate treatment for these compounds. In this study were found that some compounds (in particular, carbamazepine and caffeine) can be potential molecular markers of wastewater effluents in natural water bodies. Carbamazepine can be used for tracing pathways of both treated and untreated sewage water, whilst caffeine, as a labile and easily degraded compound can be used for the identification of effluents of untreated wastewaters.

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