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Broad spectrum screening of 463 organic contaminants in rivers in Macedonia

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ABSTRACT

Target screening of 463 organic contaminants in surface water using ultra high performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) with direct injection was performed in spring of 2015 in northern Macedonia, at six sampling sites in four rivers belonging to Vardar basin: Kriva, Zletovska, Bregalnica and Vardar. The aim of the study was to differentiate between various types of organic contamination characteristic for different types of anthropogenic activities, such as mining, agriculture, and urbanization. Depending on the studied river, 9–16% of analyzed compounds were detected. The highest total levels of organic contaminants were recorded in agriculturally impacted Bregalnica River (1839–1962 ng L^{-1}) and Vardar River downstream from the city of Skopje (1945 ng L^{-1}), whereas the lowest level was found in the mining impacted Zletovska River (989 ng L^{-1}). The principal organic contaminants of the Bregalnica River were herbicides (45–55% of all detected compounds; 838–1094 ng L^{-1}), with the highest concentrations of bentazone (407–530 ng L^{-1}) and molinate (84–549 ng L^{-1}), common herbicides in rice cultivation. The main organic contaminants in the other rivers were drugs (70–80% of all detected compounds), with antibiotics as a predominant drug class. The highest drug concentrations were measured in the Vardar River, downstream from Skopje (1544 ng L^{-1}). Screening of surface water by UHPLC-QTOF-MS was proven as a practical tool for fast collection of comprehensive preliminary information on organic contamination of natural waters, which can present a significant contribution in the monitoring and preservation of good ecological status of freshwater ecosystems.

1. Introduction

Natural waters are in a great danger of getting more and more contaminated with numerous organic and inorganic contaminants, because of a use of surface waters as recipients for wastewaters; most municipal and industrial effluents, containing a large variety of contaminants (such as pharmaceuticals, surfactants, biocides, personal care products, and sweeteners), as well as their transformation products even after their treatment, end up in rivers, streams or lakes (Rodriguez-Mozaz et al., 2004). To protect the freshwater ecosystems, European Union has issued a set of environmental quality standards (EQS) in its Water Framework Directive (WFD), which for now comprises 45 priority substances in surface aquatic bodies (EPCEU, 2013). About one third of the priority substances covered by this Directive are pesticides, and although drugs are not yet properly covered by environmental regulations, they are considered as emerging contaminants, due to their toxicity in very low concentrations and continuous discharge into urban rivers (Zhou et al., 2014). The exposure to pesticides from water not only can have serious consequences for humans, but also may have ecotoxicological effects for aquatic flora and fauna (De Gerónimo et al., 2014). Similarly, water contamination with some pharmaceuticals has become a major subject of worldwide concern (Rodriguez-Mozaz et al., 2004) because they contribute to development of antibiotic-resistant bacteria (Guardabassi et al., 1998) and compromise the long-term survival of many species (Fuhrman et al., 2015). For example, intake of compounds with estrogenic properties via food or drinking water probably can cause a decrease of sperm counts and the increasing incidence of testicular cancer and other disorders regarding male infertility (Sharpe and Skakkebaek, 1993).

Currently, at European legislative level, only analytical methods focused on the target analysis of a limited number of pre-selected compounds included in the pesticide residue definition are taken into account (Villaverde et al., 2016). However, it is important to point out that monitoring of only those target compounds, which

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are listed in WFD, misses important site-specific and potentially ecotoxicologically relevant compounds that are not covered by the Directive. Therefore, information on the actual levels of wide range of organic contaminants, including pesticides and drugs, in the aquatic environment is fundamental for proper risk assessment and planning of risk reduction measures (De Gerónimo et al., 2014). As some of these chemicals have been shown to provoke toxic effects in fish, e.g. endocrine disruption, already at sub-nanogram levels (Hansen et al., 1998; Purdom et al., 1994), their determination requires high-sensitivity analytical methods (Rodriguez-Mozaz et al., 2004). Commonly, when assessing organic substances, numerous analytical methods may have to be used to cover a large number of known compounds (Gómez et al., 2011), which can be rather costly and time-consuming. Therefore, to overcome those difficulties, new strategies and instrumentations are needed, which will be focused primarily on fast and simple preliminary screening of samples (Allinson et al., 2015), with the general aim to identify the most problematic contaminants, which will be further on individually monitored.

To meet arising requirements of developing science, industry and society, a rapid development in the field of pesticide and other organic compound analysis occurred, including the development of different mass spectrometry detectors (triple quadrupole, ion-trap, time-of-flight, quadrupole-time-of-flight), as well as development of "ambient-ionization" mass spectrometry techniques (Botitsi et al., 2011). New screening methods were created that combine a mass-structure database with gas or liquid chromatography coupled to mass spectrometry to create a system that can screen samples for large number of compounds and give reliable indication of the presence of specific trace organic chemicals in analyzed samples (Botitsi et al., 2011; Allinson et al., 2015; Guibal et al., 2015; Stipaničev et al., 2015). Such possibilities in the field of water analysis are provided by application of high resolution quadrupole time-of-flight mass spectrometry (QTOF-MS), which enables simultaneous quantitative determination of numerous target compounds, due to its sensitivity and selectivity in full scan analysis, as well as additional qualitative analysis of other compounds included in a mass spectral library (Guibal et al., 2015). It is especially useful for analysis of transformation/degradation products of organic compounds, such as pesticides (Sevilla-Morán et al., 2014). The sensitive full spectrum acquisition and the high mass resolution and mass accuracy provided by TOF-MS make this technique especially suited for wide-scope screening in the environment, where a large number and types of organic contaminants are present (Hernández et al., 2012), and, additionally, it allows the investigation of hundreds of compounds in the same run (Díaz et al., 2012). Screening approach was already proven as a useful basis for monitoring of natural waters in several field studies, such as assessment of groundwater quality in Netherlands (ter Laak et al., 2012) and screening of several German, Dutch, Swedish, French and UK rivers (Schwarzbauer and Ricking, 2010).

Accordingly, the main aim of our study was to perform wide-scope target screening of organic contamination of several rivers in northern Macedonia which belong to Vardar river basin by application of ultra high performance liquid chromatography (UHPLC) coupled to QTOF-MS (UHPLC–QTOF–MS). Since liquid chromatography-mass spectrometry (LC-MS) has been proven as an excellent analytical tool in the determination of pesticides (Masiá et al., 2014), the proposed instrumental system will be an ideal option for accomplishing our goal. It provides a possibility of simultaneous analysis of a large number of organic compounds, it enables fast, simple and reliable performance by direct injection of samples, it has a low detection limits (on the order of single ng L^{-1}), and it does not require sample preconcentration (e.g. solid phase extraction (SPE)) prior to analysis (Kowal et al., 2009; Yu et al., 2012). However, despite the strong potential, wide scope screening of hundreds of compounds by combined LC-TOF has been scarcely explored (Hernández et al., 2012). In our previous research, UHPLC–QTOF–MS has been already successfully applied to analysis of pharmaceuticals in raw and treated wastewater from Virovitica wastewater treatment plant in Croatia (Topić Popović et al., 2015) and to screening of water samples of the Danube River within Joint Danube Survey 3 (Stipaničev et al., 2015).

In northern Macedonia, there is a great need for characterization of ecological quality and contamination status of local rivers, since they are flowing through an area of active mining and developed agriculture, specifically rice cultivation (Andreevska et al., 2013; Ramani et al., 2014; Rebok, 2013; Stuhlberger, 2010). In addition, there are only three active wastewater treatment plants in that country: in the cities of Ohrid, Prespa and Dojran, but they are not fully completed and untreated sewage is discharged directly into rivers and/or lakes (Mitev and Mitanovska, 2011). So far, detailed analysis of physico-chemical and inorganic contamination of several rivers in north-eastern Macedonia has been performed (Ramani et al., 2014), but diffuse agricultural sources of pollution and their impact on quality of water resources were not thoroughly examined (Mitev and Mitanovska, 2011). Therefore, specific aim of our study was to analyze, by use of UHPLC–QTOF–MS, surface water samples of four rivers flowing through northern Macedonia, which are influenced by different sources of pollution, specifically by municipal and industrial wastewaters of large towns, mining effluents and agricultural runoff. Additionally, we aim to define organic contaminants which are representative for each of the studied pollution sources and areas, which will facilitate further monitoring of

Table 1
Characteristics of rivers and sampling sites chosen for screening of organic contaminants in surface water by UHPLC-Q-TOF-MS.

River	Sampling site	Coordinates	Location	Sources of pollution
Kriva	**Kriva Palanka**	N 42°11′39″ E 22°18′34″	The exit of town Kriva Palanka, 15 km downstream from active Pb/Zn mine Toranica	Urban and mining influence, mild agricultural runoff (orchards and gardens)
Zletovska	**Zletovo**	N 40°58′54″ E 22°14′10″	2.5 km downstream from town Zletovo, 6 km downstream from active Pb/Zn mine Zletovo, and 15 km downstream from town Probištip	Mining influence, battery factory
Bregalnica	**Teranci**	N 41°51′45″ E 22°20′58″	11 km downstream from town Kočani	Runoff from rice fields
	Kežovica	N 41°43′55″ E 22°10′27″	35 km downstream from the mouth of the Zletovska River into the Bregalnica River, and 3 km downstream from town Štip	Runoff from rice fields, urban and industrial influences (textile and meat industry, poultry and pig farms)
Vardar	**Upstream from Skopje**	N 42°00′22″ E 21°19′57″	0.5 km upstream from the city of Skopje	Industrial, municipal and traffic influences of the capital of Macedonia
	Downstream from Skopje	N 41°57′45″ E 21°32′42″	2 km downstream from the city of Skopje	