Environmental Pollution 213 (2016) 76–83

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Sterol ratios as a tool for sewage pollution assessment of river sediments in Serbia☆

Ivana Matić Bujagić, Svetlana Grujić*, Zorica Jauković, Mila Laušević

University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade, Serbia

ARTICLE INFO

Article history:
Received 28 October 2015
Received in revised form
16 December 2015
Accepted 17 December 2015
Available online xxx

Keywords:
Hormones
Sterols
Sediments
LC-MS
The Danube
Fecal
Contamination

ABSTRACT

In this work, source pollution tracing of the sediments of the Danube River and its tributaries in Serbia was performed using sterol ratios. Improved liquid chromatography-tandem mass spectrometry method, which enabled complete chromatographic separation of four analytes with identical fragmentation reactions (epicoprostanol, coprostanol, epicholestanol and cholestanol), was applied for the determination of steroid compounds (hormones, human/animal and plant sterols). A widespread occurrence of sterols was identified in all analyzed samples, whereas the only detected hormones were mestranol and 17α-estradiol. A human-sourced sewage marker coprostanol was detected at the highest concentration (up to 1939 ng g^{-1}). The ratios between the key sterol biomarkers, as well as the percentage of coprostanol relative to the total sterol amount, were applied with the aim of selecting the most reliable for distinction between human-sourced pollution and the sterols originated from the natural sources in river sediments. The coprostanol/(cholesterol + cholestanol) and coprostanol/epicoprostanol ratios do not distinguish between human and natural sources of sterols in the river sediments in Serbia. The most reliable sterol ratios for the sewage pollution assessment of river sediments in the studied area were found to be coprostanol/(coprostanol + cholestanol), coprostanol/cholesterol and epicoprostanol/coprostanol. For the majority of sediments, human-derived pollution was determined. Two sediment samples were identified as influenced by a combination of human and natural biogenic sources.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Various point and non-point pollution sources, such as discharges of treated or untreated sewage as well as urban and agricultural run-off, contribute significantly to the deterioration of the water and the sediment quality. Coliform and enterococci bacteria were conventionally used for the assessment of fecal contamination of the environment (Savichtcheva and Okabe, 2006; Field and Samadpour, 2007). However, there are a number of shortcomings of bacterial indicators such as environmental instability, susceptibility to disinfection and the lack of source specificity. Therefore, chemical (*i.e.* molecular, organic) markers were proposed as alternative for identification of fecal pollution sources and evaluation of the sewage inputs. The chemical markers include steroid compounds (sterols and hormones), fluorescent whitening agents, bile acids, caffeine, etc (Devane et al., 2006; Tyagi et al., 2007; Martins et al., 2014b; Harwood, 2014). Obtaining the information about the origin of fecal contamination is necessary for devising measures for effective control and reduction of environmental pollution.

Human/animal and plant sterols are steroid biomarkers specific to its source which retain their chemical structure throughout different processes in the environment. These compounds can be used as indicators of anthropogenic input of organic matter as well as for differentiation between sources of the fecal matter. In addition, sterols can indicate the cumulative load of the fecal pollution and point to long-term contamination of the environment (Goodfellow et al., 1977; Takada and Eganhouse, 1998; Carreira et al., 2004; Machado et al., 2014). It was also determined that some steroid compounds act as endocrine and metabolic disruptors in aquatic environment, such as natural and synthetic hormones and plant sterols (Nieminen et al., 2002; Jobling et al., 2006) and even coprostanol (Gagné et al., 2001).

Tracking of pollution sources is based on the fact that sterol composition in feces of humans and animals is determined by diet, endogenous biosynthesis and bacterial biohydrogenation of sterols to stanols (Leeming et al., 1996). Coprostanol (5β-cholestan-3β-ol)

☆ This paper has been recommended for acceptance by Klaus Kummerer.

* Corresponding author.
E-mail address: cekili@tmf.bg.ac.rs (S. Grujić).

http://dx.doi.org/10.1016/j.envpol.2015.12.036
0269-7491/© 2016 Elsevier Ltd. All rights reserved.

is a sterol produced in the digestive tract of humans and higher vertebrates by hydrogenation of cholesterol (cholest-5-en-3β-ol) and it comprises 40–60% of the total sterols excreted in human feces (Leeming et al., 1996; Daughton, 2012). As a result of *in situ* production, coprostanol can also be present in anoxic sediments in small quantities (Grimalt et al., 1990). Due to its ubiquitous occurrence, cholesterol is not a specific indicator of fecal pollution as coprostanol (Dutka et al., 1974; Volkman, 1986). It is also important to highlight that cholesterol is predominantly reduced to coprostanol in human organism, whereas in the environment its reduction to cholestanol (5α-cholestan-3β-ol) is preferential (Jeng and Han, 1994; Martins et al., 2007). Epicoprostanol (5β-cholestan-3α-ol), isomer of coprostanol, can be found in human feces at very low concentrations and also can be formed during the anaerobic sewage treatment process (McCalley et al., 1981). Therefore, it can be used as an indicator of the sewage treatment level (Mudge et al., 1999) or the age of the fecal matter (Froehner et al., 2009).

Due to their predominance in vascular higher plants, plant sterols such as β-sitosterol (24-ethylcholest-5-en-3β-ol), campesterol (24-methylcholest-5-en-3β-ol) and stigmasterol (24-ethylcholest-5,22-dien-3β-ol) are commonly used as indicators of terrigenous organic matter (Martins et al., 2011). Also, campesterol and β-sitosterol are highly abundant in herbivorous diet, so their reduction products can be used for identification of fecal material from ruminant organisms such as cows and sheep (Bull et al., 2002).

According to the number of authors (Shah et al., 2007; Tolosa et al., 2014; Martins et al., 2014b; Saeed et al., 2015) individual levels of sterols are not reliable enough as biomarkers of human fecal contamination, and therefore ratios of different selected sterols should be used to enhance reliability of the pollution assessment. Fecal sterols (human/animal and plant sterols), as well as their ratios, were used for assessing sewage pollution in different environmental compartments: water (e.g. Grimalt et al., 1990; Isobe et al., 2002; Devane et al., 2006; Furtula et al., 2012; Froehner and Sanez, 2013; Alsalahi et al., 2015), suspended particulate material (Cordeiro et al., 2008), and sediments (e.g. Fattore et al., 1996; Mudge et al., 1999; Marvin et al., 2001; Seguel et al., 2001; Reeves and Patton, 2005; Cordeiro et al., 2008; Froehner et al., 2009; Campos et al., 2012; Machado et al., 2014; Martins et al., 2014a; Tolosa et al., 2014; Saeed et al., 2015). Studies using fecal sterols were developed worldwide, including remote areas, such as Antarctica (Venkatesan et al., 1986; Venkatesan and Mirsadeghi, 1992; Martins et al., 2014a; Dauner et al., 2015; Leeming et al., 2015).

Taking into account the hydrophobic nature of the steroid compounds and their strong preference to bind to solid matrices, the aim of this paper was to use a range of sterol ratios for source pollution tracing of river sediments in Serbia. The idea was to critically assess and determine a set of ratios most reliable for differentiation between human-sourced pollution and the sterols originated from the natural sources. Commonly reported analytical method for trace analysis of sterols in environmental matrices is gas chromatography-mass spectrometry. In our previous paper (Matić et al., 2014), liquid chromatography-tandem mass spectrometry (LC–MS^2) method was developed for analysis of human/animal and plant sterols and hormones and operating parameters (LC mobile-phase gradient, MS parameters for data acquisition, analytes' fragmentation reactions for quantification and conformation purposes) were selected. Also, sediment sample preparation was developed, optimized and validated. In this study, LC–MS^2 method was improved and applied for analysis and sewage pollution assessment of sediments of the Danube River and its tributaries in Serbia. No previous studies using steroid compounds as indicators of sewage inputs or sterol ratios for differentiation between pollution sources have been conducted in Serbia.

2. Materials and methods

2.1. Sample collection and pretreatment

Sediment samples were collected from the middle courses of the rivers, using a stainless steel hand bucket. The surface layer of the sediments was sampled. Samples were air-dried at room temperature in the dark for several days. After drying, the water content in the samples was less than 0.05%. Samples were crushed, homogenized and sieved through 500 μm sieve to remove gravel, plant roots and other debris.

The collection of sediment samples was performed at 11 sampling sites in Serbia (S1–S11, Fig. 1). Since 92% of the country (81,374 km^2) lies within the Danube Basin, the majority of investigated samples were taken from the Danube River and its major tributaries the Tisa, the Sava, and the Morava. Sampling locations at the Danube were in the cities of Smederevo (before and after municipal wastewater discharge, samples S1 and S2, respectively) and Donji Milanovac (sample S3, Fig. 1). Samples from the Tisa River, the longest Danube tributary, were collected before and after the populated area (samples S4 and S5, respectively), the latter being about 1 km from the confluence with the Danube. One sediment sample was taken from the Sava River, about 5 km from the junction with the Danube (sample S6). Sampling sites at the Morava River were located before and after the populated area (samples S7 and S8, respectively), and the latter was taken about 1 km from the confluence with the Danube. The Morava River area is well-known for its agriculture and livestock farming. The average flows of the aforementioned rivers are: 6460 $m^3\ s^{-1}$ (the Danube), 794 $m^3\ s^{-1}$ (the Tisa), 1564 $m^3\ s^{-1}$ (the Sava) and 232 $m^3\ s^{-1}$ (the Morava) (ICPDR, 2012).

In addition to the Danube-related samples, sediments were also collected from two small rivers (the Topčiderka and the Veliki Lug) with known human-sourced pollution. The Topčiderka River is a tributary of the Sava with an average flow of less than 1 $m^3\ s^{-1}$ and high influence of untreated sewage from the heavily populated city of Belgrade. The first sample from the Topčiderka (sample S9) was collected near the river source, in the area still not affected by human activities, and is regarded as unpolluted. The second sample of the Topčiderka River (sample S10) was taken in the city of Belgrade downstream from a number of the wastewater discharges. The Veliki Lug is a small river (average flow 2.2 $m^3\ s^{-1}$) also considered to be heavily contaminated by municipal wastewaters of the city of Mladenovac. One sediment sample (sample S11) was taken downstream from all wastewater discharges of the city of Mladenovac.

Also, it should be noted that about 90% of the municipal wastewater in Serbia is not treated prior to the discharge and that there are no wastewater treatment plants (WWTPs) in the vicinity of the sampling locations where sediments were collected.

2.2. Sample preparation

Sediment samples were prepared for the analysis using previously optimized and validated procedure (Matić et al., 2014). Briefly, 2.0 g of the sediment sample was extracted using 5 ml of methanol in the ultrasonic bath for 10 min. The sample was then centrifuged (10 min, at 4000 rpm) and supernatant was separated. Ultrasonic extraction was repeated two more times. Obtained extract (15 ml) was evaporated to the volume of 1 ml and transferred onto silica gel/anhydrous sodium sulphate clean up cartridge. Prior to clean up, cartridge was preconditioned with 5 ml of methanol. Analytes were eluted using methanol (10 ml), eluate was evaporated under the gentle nitrogen stream and reconstituted with methanol to the volume of 0.5 ml. Obtained extract was

Download English Version:

https://daneshyari.com/en/article/6314817

Download Persian Version:

https://daneshyari.com/article/6314817

Daneshyari.com