



## Evaluation of different smoking habits during music festivals through wastewater analysis

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### ABSTRACT

Wastewater analysis is a powerful method that can provide useful information about the abuse of legal and illicit drugs. The aim of our study was to determine nicotine consumption during four different music festivals and to find a connection between smoking and preferences for specific music styles using wastewater analysis. The amount of the nicotine metabolite cotinine was monitored in wastewater at the influent of three waste water treatment plants WWTPs in the Czech Republic and Slovakia, where the festivals took place. Urinary bio-markers of nicotine utilization were analyzed by LC–HRMS. More than 80,000 festival participants were monitored during our study from June to September 2014. A significant increase of nicotine consumption was observed in wastewaters during music festivals. The nicotine ingestion level was back-calculated and expressed as mass of pure drug consumed per day and per 1000 inhabitants for selected cities of both countries. The highest differences between typical levels of cotinine in wastewaters and the levels during music festivals were detected in Piešťany: 4 g/L/1000 inhabitants during non-festival days compared to 8 g/L/1000 inhabitants during the Topfest pop-rock festival and 6 g/L/1000 inhabitants during the Grape dance festival. No significant increase of the amounts of cotinine in wastewater was recorded for the Country and Folk festivals.

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### 1. Introduction

Nowadays, more than 1 billion people worldwide smoke cigarettes that contain nicotine. This number is approximately seven times greater than the number of people that use all illicit drugs combined. Tobacco utilization, especially smoking, is one of the most important factors affecting population mortality. According to the World Health Organization, ca. 5.4 million people each year die from nicotine addiction consequences, cardiovascular diseases, and cancer, and smoking cigarettes is a risk factor for six of the eight leading causes of death (WHO, 2009). In industrialized countries, smoking and tobaccoism is the main cause among ten

others that are responsible for various serious diseases (Prabhat et al., 2006). According to Nutt et al. (2007) the use of tobacco is more dangerous for the human body and society than illicit drugs, such as ecstasy, LSD, or marijuana. The EU has recently updated and revised the Tobacco Products Directive, and this is valid from 2016. The declared aim of the revision is to improve the functioning of the EU internal market for tobacco and related products while ensuring a high level of health protection for European citizens and to decrease the number of smokers, especially young smokers. The directive allows member states to implement strict regulations if necessary. From the view of public health, the proposal reflects the data on tobacco as the most frequent avoidable cause of death. Tobacco use leads to approximately 700,000 avoidable deaths in the EU each year. Most smokers begin smoking at an early age—70% begin smoking before 18, and 94% begin smoking before 25 years of age (Rughinis and Rughinis, 2014). The Slovak Public

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Health Office statistics say that every fifth person (20%) is an active smoker. Their number is falling by two percent every year. However, the trend in the younger generation is opposite. Data from 2014 showed an increase in smokers amongst children in the second level of elementary schools. In the case of secondary schools, the trend is stable, but there are 40 to 50% of students with smoking experiences.

In recent years, the abuse of both legal and illicit drugs has been connected with certain music styles or social groups. Several studies based on questionnaire surveys have dealt with the consumption frequency of drugs in society and have found an association between music style preferences and use of legal or illegal drugs (De Alemida and Silva, 2005; Forsyth et al., 1997; Pedersen and Skrondal, 1999). The main music styles such as dance, rave, house, or trance have been associated with increased frequency of tobacco, alcohol, ecstasy, cannabis, cocaine, LSD, benzodiazepines, ketamine, 'rave and hard drugs', and polydrug use (De Alemida and Silva, 2005; Forsyth et al., 1997; Pedersen and Skrondal, 1999). Pop music has been shown to have an association mainly with alcohol and cannabis use (Dent et al., 1992); 'Seattle grunge' has been associated with cannabis and amphetamine use (De Alemida and Silva, 2005); rap music has been associated with crack cocaine use (Dent et al., 1992); and metal has been associated mainly with alcohol, tobacco, and cannabis use (Martin et al., 1993).

During the summer months from June to August, numerous music festivals of various genres take place in the Czech Republic and Slovakia. The most popular are rock, pop, and dance festivals. Usually more than 10,000 people participate in these events (Statistical Office of the Slovak Republic, 2014; Statistical Office of the Czech Republic, 2014). Direct monitoring of nicotine consumption is very difficult at these festivals. Most of the surveys are predominantly focused on the use of illicit drugs and are based on questionnaires. Polls that are focused on legal drug consumption among young people that participate at music festivals are less frequent in the Czech Republic and Slovakia (EMCDDA, 2012, 2013, 2014). The results obtained are usually not systematic, and, therefore, the comparison of drug incidence at different music festivals with different music styles is almost impossible. Analysis of drugs and their metabolites in wastewaters using modern analytical methods offers the possibility to determine and monitor the amount of drug use in the individual regions at the sub-microgram level (Karolak et al., 2010; Lopes et al., 2014; Mackul'ak et al., 2014; Thomas et al., 2012). Illegal and legal drugs appearing in the wastewater come from excreta of regular or irregular drug consumers or are excreted in sweat and subsequently washed away via showers into sewage (Thomas et al., 2012). The compounds in the sewage system are further degraded mainly via biological processes (Kuo et al., 2015). Nicotine or caffeine concentrations in wastewater are often very low and varied throughout a year (Lopes et al., 2014). The fluctuations are not due to the changes of the number of consumers, but rather they are due to the instability of parent substances in the sewage system. The level of cotinine is thus typically higher than 1500 ng/L in wastewater (Buerge et al., 2008; Chen et al., 2013; Lopes et al., 2014). Therefore, Lopes et al. (2014), in their study on nicotine, focused on its more stable metabolite: cotinine. Although, according to Chen et al. (2013), cotinine is a much more stable compound in the sewage system than nicotine, it also partially undergoes degradation processes, where its residence time is an important factor. An equation to calculate a stability correction factor was therefore derived, which helps to improve the quantification of the actual cotinine concentration in wastewater (Lopes et al., 2014).

The aim of our study was to monitor and determine the load of the metabolite cotinine in the wastewaters of three cities during music festivals and to study the association of smoking with various music style preferences. Four different music festivals with

different music genres and more than 80,000 participants were selected, and their derived effluents were analyzed at three wastewater treatment plants in the Czech Republic and Slovakia. Another aim of our study was to propose a complementary evaluation of nicotine consumption data for better quantification of further epidemiological studies. The results obtained were subsequently compared with the official statistical data on nicotine consumption.

## 2. Materials and methods

### 2.1. Standards and materials

LC-MS grade methanol and acetonitrile (LiChrosolv, Hypergrade) were purchased from Merck (Darmstadt, Germany). The formic acid used to acidify the mobile phases was purchased from Labicom (Olomouc, Czech Republic). Ultrapure water for HPLC analysis was obtained from an Aqua-MAX-Ultrasytem (Younglin, Kyounggi-do, Korea). Polypropylene bottles and syringes were used for collection, storage, and handling of the wastewater samples. Disposable regenerated cellulose filters with 0.45 µm pore size for removal of particles prior to the analysis were purchased from Labicom (Olomouc, Czech Republic). Standard 10 mL autosampler vials with PTFE/silicon septa (Labicom, Olomouc, Czech Republic) were used for sample analysis. Both native and <sup>13</sup>C labeled cotinine, which was used as an internal standard, were purchased from Cambridge Isotope Laboratories Inc. (Tewksbury, MA, USA) as 1 mg/mL standard solutions in methanol. Spiking mixtures were prepared by diluting stock solutions in methanol to the concentration of 1 µg/mL for each compound. Both stock and working solutions were stored at -20 °C.

### 2.2. Characterization of investigated locations

In our study, three towns and cities in the Czech Republic and Slovakia in which festivals took place were examined. The detailed characteristics of Piešťany can be found in the study of Mackul'ak et al. (2014).

Valašské Meziříčí lies at the confluence of two rivers: the Rožnovská Bečva and the Vsetinská Bečva in the eastern part of the Czech Republic. Its population is about 27,000, and there are approximately 40 restaurants and pubs, five high schools, and two faculties there. Every year the Gulasfest – a folk music festival – is organized near this town. The wastewater is treated in the Valašské Meziříčí WWTP, and the length of the sewage system ranges from two to 20 km (Table 1).

Zubří is situated on the right bank of the Rožnovská Bečva river. Its population is ca. 5700 with more than 20 restaurants. Every year, Vandaalfest – a metal music festival – is organized in the district of this town. The wastewater is treated in the Zubří WWTP, and the length of the sewage system ranges from one to 20 km (see Table 1) (Statistical Office of the Czech Republic, 2014).

### 2.3. Description of the music festivals included in the study

Four important Slovak and Czech music festivals were monitored (Table 1). All the festivals significantly increased the number of people connected to the respective WWTP. Therefore, the load of cotinine in the wastewater was recalculated according to the real number of people connected to the respective WWTP during festival days (Table 1).

The Grape Festival is a dance music festival organized at the Piešťany airport. From the 15<sup>th</sup> to the 16<sup>th</sup> of August 2014, approximately 13,000 people attended the festival. Wastewater from this cultural event was completely processed at the Piešťany WWTP.

The Topfest festival is the largest music festival in Slovakia. The main festival genres are rock and metal; however, some pop bands

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