



## Full length article

# Illicit drug consumption in school populations measured by wastewater analysis



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## ARTICLE INFO

## Keywords:

School students  
Italy  
Drug consumption  
Wastewater analysis

## ABSTRACT

**Background:** Analysis of student consumption of illicit drugs (ID) by school population surveys (SPS) provides information useful for prevention, but the results may be influenced by subjective factors. We explored wastewater (WW) analysis to improve the information.

**Methods:** We used WW analysis to measure ID consumption in eight secondary schools in Italy in 2010–13 (students aged 15–19). Samples were collected from the sewage pipes of the schools during lessons for one week each year. Samples were analysed by mass spectrometry to measure ID and consumption by students was compared to that of the general population.

**Results:** We found THC–COOH (human metabolite of THC) concentrations in 2010 indicating significant consumption of cannabis in all the schools and benzoylecgonine (human metabolite of cocaine) suggesting a limited consumption of cocaine in all but one school. Morphine was only found in traces, and amphetamine, methamphetamine, ecstasy, ketamine and mephedrone were not detectable. Repeated analysis showed cannabis stable until 2012 with increases in 2013, low cocaine and morphine levels, and none of the other ID.

**Discussion:** WW analysis suggested that students used amounts of cannabis comparable to the general population, with low, sporadic use of cocaine and opioids, but excluded the use of significant amounts of amphetamine, methamphetamine, ecstasy, ketamine and mephedrone. WW analysis was useful to confirm SPS figures and provides complementary findings for effective prevention strategies. This is the first time WW analysis has been used to investigate consumption of a large number of ID and new psychoactive substances (NPS) in schools.

## 1. Introduction

Monitoring consumption is useful for preventing the spread of illicit drugs (ID) in the population and is particularly important for young people. In Italy, a school population survey (SPS-DAP) on drug use started at the beginning of the 2000 s (DPA, 2014) and is being repeated yearly (DPA, 2012). It permits analysis of the school population habits regarding the consumption of legal and illegal psychoactive substances and gives essential information for planning prevention strategies and enforcement action.

However, population surveys can be influenced by subjective factors, namely, whether the respondents answer questions truthfully about the substance abused, as investigation of a sensitive and stigmatized behavior may generate inaccurate reporting (Harrison, 1997), and the bias can hamper the targeting and efficacy of preventive measures. It is still harder to study consumption of new psychoactive

substances (NPS), as consumers frequently do not know which they are really taking. In this case WW analysis provides findings that could not be obtained by any other method, including population surveys. In this study, we explored the utility of wastewater (WW) analysis for complementing school population surveys, with the aim of generating detailed and reliable figures of ID use by students, useful for prevention and enforcement.

WW analysis is a recently proposed approach to population studies to measure ID consumption in communities (Zuccato et al., 2008; Zuccato et al., 2005). It is based on chemical analysis and quantification of the metabolic residues of the drugs excreted by consumers in WW with urine (urinary biomarkers) (Castiglioni et al., 2006). Its application to study ID consumption has given results that complement figures from population surveys (Banta-Green et al., 2009; Reid et al., 2012; Zuccato et al., 2016; Zuccato et al., 2011).

WW analysis has been applied to large communities, and smaller

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ones, like prisons (Postigo et al., 2011) or schools (Panawennage et al., 2011), have also been investigated. Here we present the findings of a systematic investigation of ID consumption by secondary school students in Italy (aged 15–19 years). To our knowledge, this is the first investigation of ID consumption in schools conducted by WW analysis. Panawennage et al. conducted a pilot study in collaboration with our group (Panawennage et al., 2011), while Burgard et al. (Burgard et al., 2013) reported results on amphetamine during an investigation on ADHD-drugs abuse in 2013. Our study has investigated ID consumption within several schools more extensively (all the common drugs and some NPS) and over a greater period of time than previous studies. The results might be useful to complement school population surveys, and to provide an integrated assessment of ID consumption by students.

We collected WW samples from sewage pipes of eight secondary schools in Italy, attended by more than 6000 students. Samples were collected daily during school time, for five or six consecutive days, and analyzed for biomarkers of consumption of cocaine, opioids (morphine/heroin), cannabis, amphetamine, methamphetamine, ecstasy, ketamine and mephedrone by the people attending the schools. Sampling was done in March 2010 and repeated in three schools in October 2011, March 2012, October 2012 and November 2013.

The study was conducted in collaboration with the National Agency for Drug Policy (Dipartimento Politiche Antidroga). Samplings were done with the informed consent of the school authorities but to avoid biases students were not aware of the study. For privacy reasons, results are reported anonymously. Only the location of the schools will be disclosed, and any other information blinded, as established by the ethical guideline recently developed for wastewater analysis studies (Prichard et al., 2014).

## 2. Methods

### 2.1. Schools

Eight schools were selected, one each in Bologna, Florence, Milan, Naples, Palermo, Rome, Turin and Verona. They are all senior high schools (student age 15–19), for classical studies, science or artistic education (called “*Licei*” in the Italian education system) (three in Milan, Naples and Verona), or for vocational education (called “*Istituti tecnici e professionali*”) (five). There were 6126 students attending the schools in 2010 (388–1474 students/school). We did not know the exact number of staff employed but we were informed that they amounted to about 10% of the students.

In a preliminary phase, letters were sent to the mayors of the cities involved, to obtain consent to involve the local water board. The water board personnel, under our supervision, conducted samplings at the sewage pipes of the school buildings. Head teachers of the schools were contacted and informed of the aims and method of the project and provided consent. Sampling was carried out with the permission and cooperation of the school authorities. To prevent biases, students were not informed of the study.

### 2.2. Sampling

WW samples were collected with automatic sampling devices by city’s water board personnel from the main sewage pipe of each school building. Samples were daily composite, with one liter taken each hour during school time, therefore representative of the WW discharged throughout the whole school period. Five (from Monday to Friday) or six (from Monday to Saturday) consecutive daily samples were collected at each school. Samplings were all done in May 2010. In three schools (Rome, Turin, Verona) we repeated sampling in October 2011, March and October 2012 and November 2013. Samples were immediately frozen after collection and stored at  $-20^{\circ}\text{C}$  until analysis.

### 2.3. Analysis

Chemical analysis were carried out as previously described (Castiglioni et al., 2015; Castiglioni et al., 2006). Briefly, WW samples were filtered, extracted and purified on SPE columns, concentrated and analyzed by high-pressure liquid chromatography tandem mass spectrometry (HPLC–MS/MS).

The following substances were analyzed: benzoylecgonine (BE; human metabolite and major urinary excretion product of cocaine), cocaine, 11-nor-9-carboxy-delta9-tetrahydrocannabinol (THC–COOH; human metabolite and major urinary excretion product of THC, which is the active substance of cannabis), 6-acetylmorphine (human metabolite and urinary excretion product of heroin), morphine (major human urinary excretion product of heroin, codeine and morphine), amphetamine, methamphetamine, 3,4-methylenedioxy-N-methylamphetamine (ecstasy), ketamine (major urinary excretion products of the respective parent drugs), and mephedrone (only in samples collected in October 2012 and November 2013).

### 2.4. Consumptions

We used concentrations of the substances detected in WW (ng/L) to calculate loads (g/day). Concentrations were multiplied by the flow rate of the WW at the sewage pipe in each school (measured at the pipe by a flow meter or estimated from water consumption in the school building). We used flow meters in each school for a preliminary analysis of the flow dynamics. This preliminary analysis confirmed that the flow increased at the end of each lesson period/hour. Our sampling was therefore designed to collect samples at the end of each lesson period/hour, including breaks. The flows we used to estimate ID consumption were based on water consumption (difference at the counter from the beginning and end of the lessons). Daily loads of the substances in WW were then processed to measure consumption (g/day) of the parent drugs THC, cocaine, morphine, amphetamine, methamphetamine, ecstasy, ketamine and mephedrone by the people in each school, as previously described (Castiglioni et al., 2013; Zuccato et al., 2008). WW analysis has some uncertainties related to sampling, stability of target residues in wastewater, accuracy of analytical methods, reliability of back-calculation procedures and estimation of population sizes. Recently, the uncertainty related to all these factors has been assessed and this enabled us to identify some “best practice” requirements for each of the steps in estimating drug use (Castiglioni et al., 2013). We adopted this protocol in order to minimize uncertainty and make more reliable estimates.

Consumption in schools was compared with that in the general population in Milan, Rome and Verona, which were measured by analysis of WW samples collected from the respective urban WW treatment plants. The principal treatment plants of the three cities were sampled for seven consecutive days in May 2010 and samples were analysed as described elsewhere (Zuccato et al., 2016) to measure the daily load of the biomarkers and to estimate ID consumption in the general population served by the plants.

## 3. Results

In the WW samples collected from the sewage pipes of the schools in May 2010, we found significant concentrations of THC–COOH, indicating the consumption of THC, the active ingredient of cannabis, in all the schools investigated, and concentrations of BE and cocaine, suggesting the consumption of cocaine, in all but one school. We also found traces of morphine, indicative of therapeutic use of morphine or codeine or of heroin abuse, in two schools, and 6-acetyl morphine, a specific metabolite of heroin, in one school and only on one occasion. Amphetamine, methamphetamine, ecstasy, and ketamine were never detected.

Concentrations were used to calculate loads of the urinary

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