

# Presence of Illicit Drugs in the Sarno River (Campania Region, Italy)

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## Abstract

The presence of illicit drugs and their metabolites in surface waters has to be considered a new type of hazard, still unknown, for both the human health and the aquatic ecosystem, due to the potent pharmacological activities of all the illicit drugs. Our research was aimed at evaluating the presence of illicit drugs in the Sarno River (Campania region, Italy), crossing a densely populated area, the basin of the Sarno River, one of the largest and most important economic areas in Campania region, famous for the presence of zones with high landscape-environmental value. The drugs selected for this study were the most used in Campania region. The presence of illicit drugs in surface water was analyzed by a selective multi-residue assay based on liquid chromatography-tandem mass spectrometry. The analysis showed the presence of all the illicit drugs investigated: cocaine and its main metabolites (benzoylecgonine, nor-benzoylecgonine), morphine, THC-COOH and codeine; cocaine was the most abundant illicit drug. The presence of illicit drugs and their metabolites in the Sarno River suggests new potential risk for the basin's inhabitants, using water in the food chain, via field irrigation and animal feed, and for the health of the aquatic fauna.

## Keywords

Campania Region, Cocaine Pollution, Environmental Illicit Drugs, Sarno River Pollution, Surface Water Pollution

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## 1. Introduction

Recent studies showed the presence of illicit drugs, and their main metabolites, in surface waters [1]-[8]. Indeed,

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the increasing use of illicit drugs in enormous quantities worldwide, causes these drugs, and their metabolites, to enter into the sewage networks, from which they are often only partially removed by sewage treatment plants [1] [3] [4] [9]. Therefore, drugs and their metabolites get to contaminate the receiving surface waters, and can now be considered common organic contaminants of the aquatic environment [8]. The presence of illicit drugs in surface waters has to be considered a new type of hazard, still unknown, for both the human health, through the food chain, and the aquatic ecosystem. Indeed, all the illicit drugs have potent pharmacological activities [10] [11] and are present in surface waters as complex mixtures. Therefore, though the environmental concentrations of illicit drugs are generally low [1]-[7], their presence in surface waters may lead to unforeseen pharmacological interactions and cause toxic effects to human health and aquatic organisms.

In Campania region (Figure 1(a)), the basin of the Sarno River (Figure 1(b)), with a population of over 1193.000 inhabitants, and an urban average density of about 1300 inhabitants per km<sup>2</sup> [12], is one of the most densely populated regions of southern Italy. The basin is one of the largest and most important economic areas in Campania region, famous for the presence of zones with high landscape-environmental value, such as the Vesuvius National Park and the Picentini Mts. Regional Park, Sites of Community Importance like the Lattari

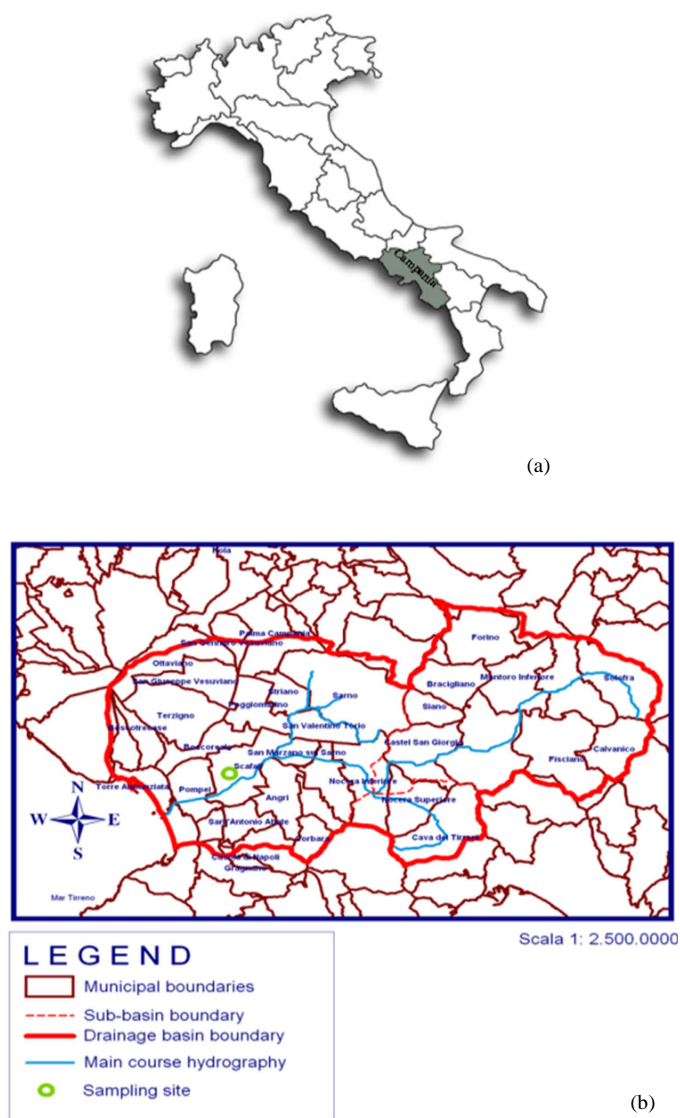


Figure 1. Map of Italy showing: (a) The location of Campania region; (b) The basin of the Sarno River, showing the sampling site (circle) at Scafati, Salerno.

Mts., Wildlife Reserves run by the WWF and other environmental associations, as well as important archaeological sites such as those of Pompeii, Herculaneum and Stabiae [12]. However, in 1992, the Council of Ministers proclaimed the Sarno basin an area at high risk of environmental crisis and proposed a reclamation project (Ministry Council's Decree 1992). Indeed, the Sarno River is a highly polluted river, affected by multiple pollution sources of domestic, agricultural and industrial origin [12]–[17]. Public health conditions are precarious as the basin's inhabitants consume low-quality water directly, but are also exposed to a cocktail of other pollutants due to the use of water in the food chain, via field irrigation and animal feed. Moreover, polluted basin waters flowing into the bay of Naples increase the contamination of sea water, damaging tourism and health and degrading local litoral quality [12]. Therefore, monitoring the pollution of the Sarno River water is very informative from a human health point of view, and may give information that could be used as a reference for future clean-up interventions.

Since recent data [18], indicated a wide illicit drug use in Campania region by its inhabitants, we hypothesized the presence of illicit drugs and their metabolites in the Sarno River, due to the high density of population of the area crossed by this river, including three provinces (Avellino, Naples and Salerno), and no fewer than thirty-nine municipalities. To verify our hypothesis, we selected the most used illicit drugs in Campania region [18]: cocaine and its main metabolites; morphine; a cannabis derivative, 11-nor-9-carboxy-delta<sup>9</sup>-tetrahydrocannabinol (THC-COOH), and an opioid pharmaceutical, codeine.

## 2. Materials and Methods

### 2.1. Sample Collection

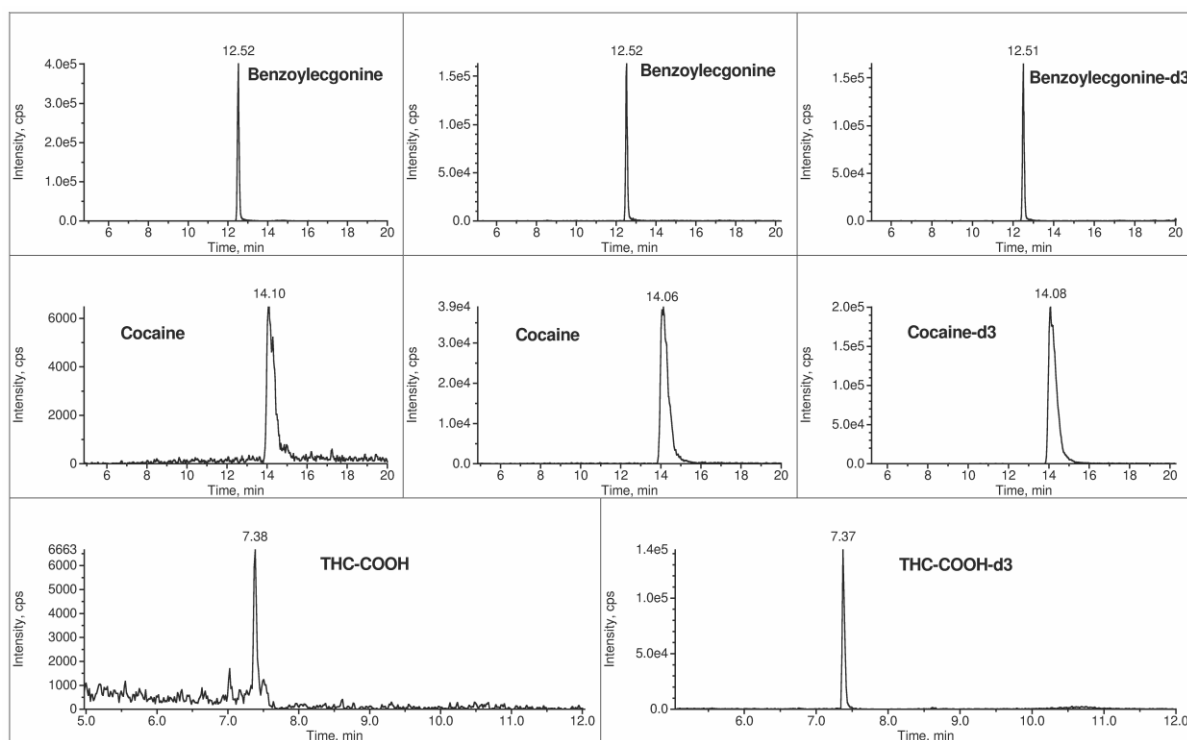
The sampling site was the hydrographic station of Scafati (Naples, Campania region, Italy) (Figure 1(b)), along the Sarno River [12]. This site is situated afterwards the confluence of the common Channel in which the streams Cavaiola and Solofrana, the main affluents of the Sarno River, flow. Composite water sample (pool of six 500 ml samples collected every 20 min during a period of 24 h) by a portable automatic sampler (Sigma 900 Standard, Hack Company, USA) was frozen immediately after the collection and was stored at  $-20^{\circ}\text{C}$ , until analysis [2] [3].

### 2.2. Chemicals and Materials

Reference standard compounds were obtained for cocaine and its metabolites benzoylecgonine (BE), nor-benzoylecgonine and nor-cocaine; morphine; THC-COOH; codeine, as previously described [3] [4]. The following deuterated analogues were used as internal standards (IS): cocaine-D<sub>3</sub>, benzoylecgonine-D<sub>3</sub>, norcocaine-D<sub>3</sub>, morphine-D<sub>3</sub>, THC-COOH-D<sub>3</sub>, acquired from Cambridge Isotope Laboratories (Andover, MA). All solvents and reagents were analytical grade. HPLC grade Milli-Q water was obtained with a MILLI-RO PLUS 90 apparatus (Millipore, Molsheim, France). Cartridges for solidphase extraction were 3 mL disposable Oasis MCX (60 mg, Waters Corp., Milford, MA).

### 2.3. Analytical Method

Illicit drugs, codeine and their metabolites were analysed in surface water, as previously described [3] [4]. Briefly, water sample was solid-phase extracted by mixed reversed-phase, cation exchange cartridges (Oasis-MCX). Before extraction, sample (500 ml) was spiked with IS, and the pH was adjusted to 2.0 with 37% HCl. The Oasis MCX cartridges were conditioned before use by washing with 6 ml methanol, 3 ml MilliQ water and 3 ml water acidified to pH 2. Sample was then passed through the cartridges under vacuum, at a flow rate of 10 ml/min. Cartridges were vacuum-dried for 5 min and eluted with 3 ml of methanol and 3 ml of a 2% ammonia solution in methanol. The eluates were pooled and dried under a nitrogen stream. Dried samples were redissolved in 200 ml of MilliQ water, centrifuged, transferred into glass vials and analysed by high-pressure liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) using an API 3000 triple quadrupole mass spectrometer, equipped with a turbo ion spray source (Applied Biosystems-Sciex, Thornhill, Ontario, Canada) and interfaced to LC Series 200 pumps and autosampler (Perkin-Elmer, Norwalk, CT). Drugs were analysed using an XTerra MS C18,  $100 \times 2.1$  mm,  $3.5 \mu\text{m}$  column (Waters Corp., Milford, MA), at a flow rate of 200  $\mu\text{l}/\text{min}$ . Typical chromatograms of the main substances in the Sarno River are provided in Figure 2. Information about the method performance in surface water and the instrumental repeatability and precision, the instrumental limits



**Figure 2.** Typical HPLC chromatograms of benzoylecgonine, cocaine and THC-COOH and their correspondent labelled internal standards used for quantification.

of detection and quantification, and the linearity of the analytical response has been published elsewhere [3] [4].

## 2.4. Statistical Analysis

The concentrations of illicit drugs (cocaine, morphine, THC-COOH and Codeine) were tested together for significance using one-way analysis of variance (ANOVA), followed by Duncan's test for multigroup comparison and Student's *t* test for between group comparison. The same type of test was applied to the cocaine metabolites. Differences were considered significant when  $P < 0.05$ .

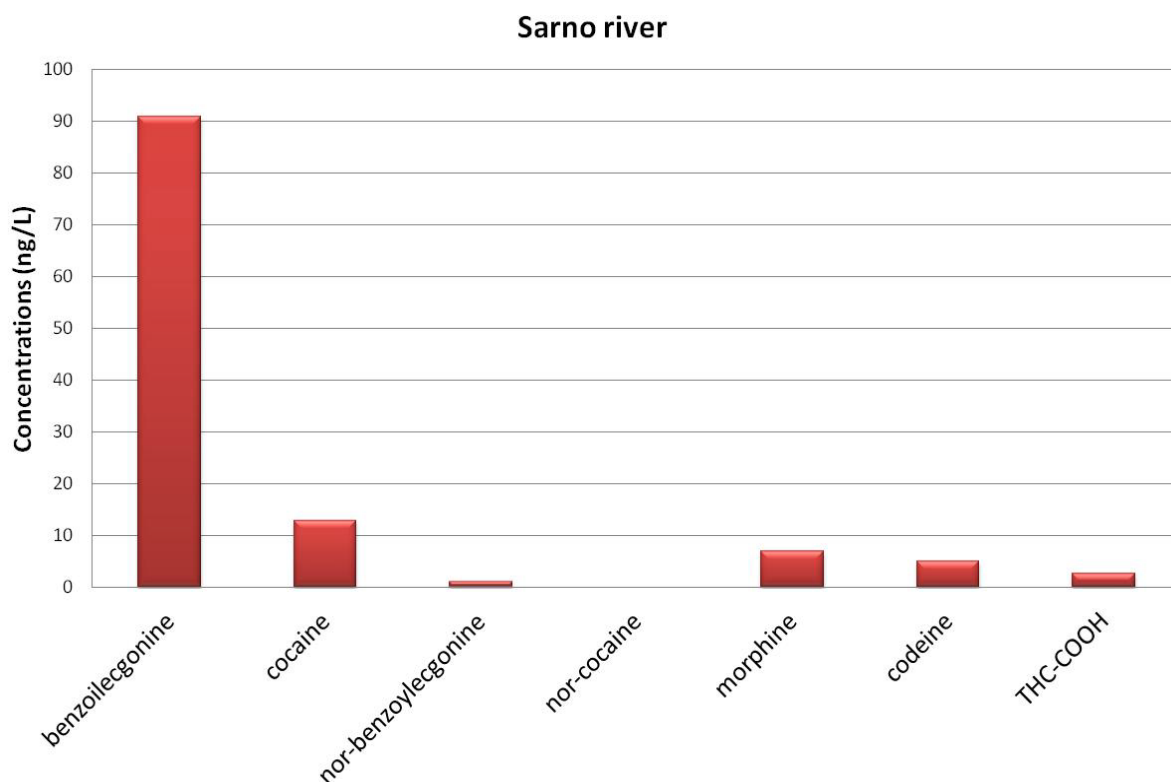
## 3. Results

All the drugs selected were found in the Sarno River. Concentrations of cocaine and its metabolites, morphine, THC-COOH and codeine ranged from 1 to 91 ng/l; cocaine concentration was significantly ( $P < 0.001$ ) higher than morphine, THC-COOH and codeine. Among the metabolites of cocaine, benzoylecgonine was the most abundant ( $P < 0.001$ ); nor-cocaine was undetectable (**Figure 3**).

## 4. Discussion

The present results show, for the first time, the presence of illicit drugs in the Sarno River: cocaine and some metabolites; morphine, a common metabolic residue of heroin, codeine and morphine itself; THC-COOH, the main active compound of cannabis; codeine, an opioid analgesic that can be metabolized and excreted as morphine [19] [20]. These results show therefore that also the Sarno River is exposed to the same type of illicit drug contamination as many others Italian and European rivers [1]-[7] and confirm that the illicit drugs can be considered common contaminants of the aquatic environment [3] [8].

Cocaine concentration was higher than morphine, THC-COOH and codeine, in agreement with data showing a higher use of this drug in Campania region, compared with other illicit drugs [18]. Among the metabolites of cocaine, BE, the major metabolite, was the most abundant in the Sarno River, as was found in other rivers [1]-[7].



**Figure 3.** Illicit drug residues and main cocaine metabolites (ng/l) in the Sarno River, at the hydrographic station of Scafati (Naples, Italy). Cocaine was the most abundant illicit drug. Among cocaine metabolites, benzoylecgonine was the most abundant; nor-cocaine was undetectable. <sup>a</sup>Values significantly ( $P < 0.001$ ) different from morphine, THC-COOH and codeine values; <sup>b</sup>Values significantly ( $P < 0.001$ ) different from nor-benzoylecgonine and nor-cocaine values.

Cocaine ( $13 \pm 2$  ng/l) and BE ( $91 \pm 10$  ng/l) levels, found in the Sarno River, were included in the range of levels found in other rivers, as the Po (cocaine:  $0.5 \pm 0.2$  ng/l; BE:  $3.7 \pm 1.2$  ng/l), the Arno (cocaine:  $1.7 \pm 1.2$  ng/l; BE:  $21.8 \pm 11.9$  ng/l), the Olona (cocaine: 44 ng/l; BE: 183 ng/l), the Lambro (cocaine: 15 ng/l; BE: 50 ng/l) rivers (Italy) and the Thames River (England) (cocaine:  $4 \pm 0.1$  ng/l; BE:  $13 \pm 0.6$  ng/l) [3]. These data confirm the high degree of pollution of the Sarno River, at the present one of the most polluted rivers in Europe. Indeed, chemical (mainly heavy metals and nitrogen), organic and microbial pollutants [12] [13] [15]-[17] [21] [22] were found in this river.

The presence, in the Sarno River, of illicit drugs, indicate a new, further hazard for the health of the basin's inhabitants and aquatic ecosystem. It is well known that illicit drugs influence the central nervous system and synaptic transmission, the circulatory system, the peripheral tissues, and regulate gene expression with epigenetic mechanisms [10] [23]-[27]. Therefore, unforeseen effects on humans, that use water in the food chain, via field irrigation and animal feed [12], could be assumed. Moreover, toxic effects on the aquatic fauna, still unknown, are predictable. Indeed, it is possible that illicit drugs behave like pharmaceuticals widespread in the aquatic environment, affecting the behavior of exposed organisms [28] [29] and accumulating in fish tissues [30] [31].

## 5. Conclusion

In conclusions, we have demonstrated that the Sarno River is polluted by many illicit drugs, and their metabolites. This suggests a new, potential risk for humans, that use water in the food chain, via field irrigation and animal feed, and for the health of the aquatic fauna.

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## Abbreviation List

11-nor-9-carboxy-delta9-tetrahydrocannabinol (THC-COOH); benzoylecgonine (BE); internal standards (IS); high-pressure liquid chromatography-tandem mass spectrometry (HPLC-MS/MS); one-way analysis of variance (ANOVA)

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