The geochemistry of cocaine and ecological impacts in the Hawkesbury-Nepean river

Reynolds JK[1], Russom B[1], Wright IA[1] and Williams M.[1]

1 School of Science, Western Sydney University, NSW, 2753. Email: j.reynolds@westernsydney.edu.au

Key Points

- Sydney is commonly referred to as the cocaine capital of Australia in the National Wastewater Drug Monitoring Program. Our research question was to understand the potential for the treated wastewater to contain cocaine and/or its metabolites upon discharge to waterways.
- The research program analysed water from wastewater treatment plants that discharge into the Hawkesbury-Nepean river system.
- The outcomes of this work highlight that cocaine is discharged at levels that are comparable to other densely populated cities internationally.
- The implications of this work are that environmental waters in rapidly urbanising catchment areas such as the Sydney region are increasingly under pressure from trace amounts of illicit compounds.

Abstract

The presence of cocaine in environmental waters is of increasing concern in urbanizing catchment areas. Here we report on three years of cocaine seasonal data for the Hawkesbury-Nepean river in NSW. We demonstrate that cocaine concentrations are of ecological concern and demonstrate that cocaine and associated metabolites will persist in freshwater environments for up to several weeks? . This research shows that discharge from wastewater treatment facilities to freshwater environments provides a pathway for the exposure of illicit compounds to organisms and may have deleterious effects on the environment.

Keywords

Cocaine, Hawkesbury-Nepean, wastewater

Introduction

The appetite for the illicit drug cocaine is ever increasing, with the Federal government reporting that 1.6 tonnes of cocaine was seized at Australian borders in 2018-19 alone (Australian Criminal Intelligence Commission 2020). Cocaine is also considered the most commonly abused illicit drug in the Sydney region (Australian Criminal Intelligence Commission 2019) with the detection of illicit drugs and their metabolites in influent wastewater and extrapolation to catchment population since 2017 (Australian Criminal Intelligence Commission 2017). The major excretion metabolites of cocaine are benzoylecgonine and ecgonine-methyl-ester with ecgonine present as both a precursor and metabolite, followed by a range of other environmental degradation products reported in the literature (Bijlsma *et al.* 2013). The focus of wastewater research has been towards understanding influent composition, therefore only limited attention has been given to factors affecting cocaine persistence, accumulation and degradation in environmental waters (Yadav *et al.* 2019).

The presence of cocaine has potential to cause detrimental effects to organisms both close to wastewater discharge locations and further downstream. Ecotoxicological studies have shown the impacts of cocaine at just 20ng/L, with endocrine disruption, altered gill morphology, damaged skeletal muscle, altered skin and intestine histology and accumulate in assorted tissues of the European eel (Capaldo *et al.* 2019; Gay *et al.* 2016) and oxidative stress in zebra mussels at 40ng/L (Binelli *et al.* 2012; Parolini *et al.* 2016). There is little information regarding the presence and persistence of cocaine and its metabolites in Australian freshwater settings.

As the discharge of wastewater is continuous, contaminants are also constantly being emitted into receiving surface waters. In order to understand the potential risks associated with cocaine being discharged into the environment, the quantity of cocaine, the metabolites, and their persistence needs to be better understood. Here we explore three years of cocaine data and provide information to its persistence and fate in the Hawkesbury-Nepean River. Our aims were to explore and understand the persistence of cocaine and its metabolites in a natural environmental water and compare that to real-world concentrations.

Methods

The Hawkesbury-Nepean River (HNR) stretches 470km with a catchment area of 21,400km^2.The Greater Sydney region is a rapidly urbanising landscape with estimations of population growth exceeding 7.5 million people by 2050. The Western Sydney district will be the centre for future population growth with the construction of a new international airport paving the way for further residential and industrial development (Greater Sydney Commission 2018). The entire Western Sydney region is serviced by the HNR. The entire river spans 12 local government areas and several overarching state government agencies. The main HNR wastewater network consists of 16 wastewater treatment plants (WWTP) which treat to a tertiary standard and 14 water recycling plants (WRP) which include additional treatments in preparation for water re-use programs. Catchment population data was sourced from Australian Bureau of Statistics census data (Australian Bureau of Statistics 2016)

The five studied water treatment facilities are situated in north-west Sydney (Figure 1). These facilities service residential, commercial and industrial dwellings. The five studied facilities cover 9% of Sydney's population. The amount of wastewater treated at these facilities varies. Each of the chosen sample locations discharge into freshwater creeks that flow to the HNR. A summary of each sampled WWTP is in Table 1.

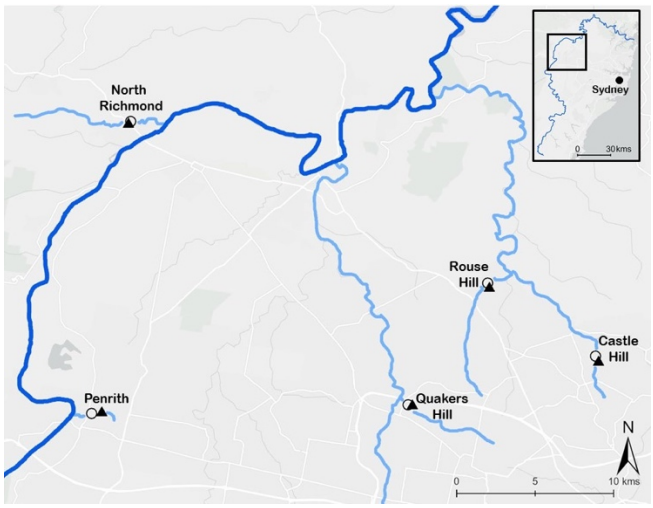

Figure 1. Map of the HNR and WWTPs. Triangles indicate wastewater treatment facility and circles represent sampled environment discharge outfalls. Dark blue represents the HNR and light blue represents primary and secondary tributaries relevant to the study sites.

Water sampling

In total 11 samples from WWTP discharge points were collected over three different years. Three times in 2020, on five occasions in 2019 and on three occasions in 2017. All samples were collected on weekdays. At each of the five sites, grab samples were collected in glass Schott bottles at the WWTP discharge point and 100 metres upstream to serve as site blanks. Water samples were acidified to pH 2 and stored on-ice and in the dark during transportation. Environmental metrics were recorded at each sample collection point. These included dissolved oxygen, water temperature, pH and conductivity. Within 16 hours of collection, samples were filtered to 6µm

with cellulose filter paper to remove particulate matter and debris from the water. Following filtration, samples were stored at 4°C in darkness until extraction.

Degradation study

Three replicate one litre grab samples of surface water were collected from the HNR (downstream of North Richmond) where previous work had shown no determinable impact from wastewater discharge. Samples were filtered to to 0.45 µm using a nylon membrane filter. A 250mL volume of filtered surface water was added to a 500mL glass jar and spiked to achieve a concentration of 100µg/L of cocaine in the sample jar. The pH was then buffered to pH 8 using 0.01M potassium phosphate buffer. This was repeated for each treatment for three replicates. Treatments were kept at 20°C and were kept in darkness. Sampling of treatments 1-5 occurred at 14 time points (0, 9hrs, 1, 2, 3, 4, 7, 10, 14, 18, 22, 28, 35 and 42 days).

Analytical approach

Solid Phase Extraction (SPE) was completed for both environmental and laboratory degradation water samples. Water sample were passed through Oasis MCX cartridges (500mg) and eluted with a 60:40 acetonitrile:methanol solution. LCMS analysis utilised the Water Acquity UPLC with autosampler coupled to a Waters Xevo Triple-Quadrupole Mass Spectrometer (Milford, MA, USA). A Waters Acquity HSS T3 column was attached (2.1 x 50mm, 1.8µm) and the LCMS was operated in positive electro-spray ionization (ESI) mode. Multiple Reaction Monitoring (MRM) mode was used to detect two target analytes and internal standards.

Results and Discussion

At all the sampling locations and time periods both cocaine and benzoylecgonine were detected in the surface water samples in the ng L^{-1} range (Table 1). No other metabolites of cocaine were detected in any of the surface water samples during the three year study period. The absence of ecgonine-methyl-ester (EME) is to be expected as it is formed through the enzymatic hydrolysis of cocaine and is therefore unlikely to be formed during cocaine degradation in water (Chen et al., 2013). Previous research has found EME to be relatively unstable in wastewater and therefore difficult to detect (Gheorghe et al., 2008). Importantly, no sampled effluent discharge location resulted in a non-detection. Four of the five studies facilities are classed as a WRP whilst North Richmond is classed as a WWTP. Given that WRPs have additional treatment steps for reclaimed water, it is possible that North Richmond WWTP water has a lower removal efficiency than WRP study sites. In this work, the highest concentrations of both cocaine and benzoylecgonine (BZE) were recorded at the North Richmond WWTP discharge point. The North Richmond WWTP is currently servicing a population that is growing rapidly through urbanisation. The consistently high reported values at North Richmond WWTP may be attributed to differences in treatment processes between the five studies water treatment facilities.

Table 1. Summary of the studied water treatment facilities and their discharge locations for all sampling periods * Discharge data from Sydney Water. ^ Three year mean values.

	Castle Hill	Rouse Hill	North Richmond	Penrith	Quakers Hill
Catchment population	27,020	114,200	4,670	109,300	162,550
Mean effluent discharge (ML day^{-1})*	4.3	18.5	0.9	8.3	24.9
pH^	7.2 ± 0.7	7.4 ± 0.8	7.5 ± 0.8	7.5 ± 0.5	7.4 ± 0.2
EC (µS/cm)^	863 ± 50	765 ± 72	717 ± 36	289 ± 19	863 ± 21
Benzo (ng/L)^	55 ± 19	78 ± 11	148 ± 18	45 ± 14	47 ± 7
Cocaine (ng/L)^	2.7 ± 1.4	6.9 ± 2.8	7.3 ± 2.1	6.8 ± 1.3	6.6 ± 2.8

Degradation study

In this experiment we compared the degradation of cocaine in environmental waters of the HNR system under laboratory conditions. For the experiments performed in MilliQ water alone, cocaine had completely degraded within 21 days (Figure 2A). An increase in the degradation compound ecgonine (ECG) occurred after 14 days in the MilliQ experiment with the relative abundance greater than both cocaine and BZE by day 21. The decrease in the concentration of BZE at pH 8 is attributed with the rapid increase in ECG abundance. However, when using the HNR environmental water no ECG was detected (Figure 2B) with BZE the only cocaine transformation produce detected. The non-detection of the ECG degradation product in these experiments supports the results of the field study where no ECG was found (Table 1).

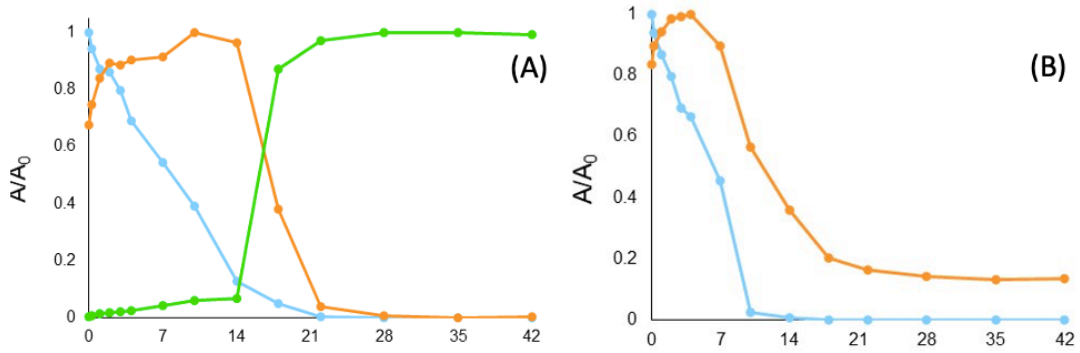

Figure 2. Relative abundance of cocaine and BZE in: (A) in pH-buffered MilliQ at pH 8, and (B) in HNR water filtered at 0.45µm. Blue = cocaine, orange = BZE, Green = ECG. Note: ECG only presents in MilliQ experiment and does not appear in HNR surface water and EME was not detected.

Interestingly, the pattern of cocaine degradation in surface water filtered to 0.45um (Figure 2B Blue line) is similar to that observed in MilliQ water. (Figure 2A Blue line). The findings of this study suggest that the conditions present in the natural local surface water matrix influence the rate of cocaine transformation. This has significant implications for understanding potential ecological impacts in these environments. The removal of the surface water particulate matter which incorporates biological components alters cocaine transformation and degradation products (Chen et al. 2013). The findings of this experiment align with previous research which found that cocaine degrades faster in wastewater with less filtration.

The BZE metabolite was still present in the HNR degradation experiment after 42 days. This is consistent with previous research which determined that BZE is relatively stable in surface water (Gheorghe et al. 2008). Whilst ecgonine was identified only in the MilliQ experiment. As the laboratory experiments were kept in the dark and in the absence of chlorine, transformation products as a result of chlorination and photo-degradation are unlikely (Bijlsma et al. 2013). Previous research has found that cocaine in MilliQ at pH 8.3 resulted in BZE being the exclusive transformation product (González-Mariño et al. 2012) and this contrasts with the findings of this experiment where ECG was also detected.

Model

With the exception of metabolite BZE, no other transformation products of cocaine were observed in the filtered HNR surface water matrix used in the laboratory experiments or in the collected surface water samples that were directly analysed. BZE is the primary transformation product and is formed through the hydrolysis of cocaine by demethylation of the ester group. Research has shown that cocaine metabolites lacking an ester or those containing only a benzoyl ester appear stable in untreated wastewater (Bisceglia et al. 2014). The mean discharge concentrations into the Hawkesbury-Nepean river system determined in this study are at levels comparable to other densely populated cities internationally (Table 2).

Table 2. Reported concentrations of cocaine in and benzoylecgonine in surface waters (ng L^{-1}) in this study and similar studies worldwide.

Country	Cocaine	Benzoylecgonine	Source
China	0.42 ± 0.09	0.99 ± 0.12	Li *et al.* 2016
Spain	0.49 ± 0.36	7.44 ± 6.48	Vazquez-Roig *et al.* 2010
Italy	0.5 ± 0.2	3.7 ± 1.2	Zuccato *et al.* 2008
United Kingdom	0.64 ± 0.23	17.8 ± 5.51	Kasprzyk-Hordern *et al.* 2008
Australia	6.06 ± 1.04	74.65 ± 21.31	This Study
Belgium	7.32 ± 2.73	37.35 ± 12.91	van Nuijs *et al.* 2009b

This contrasts with ECG which is considered the final degradation product of cocaine. It can be formed through the cleavage of the methyl from the ester group of egonine-methyl-ester (EME) via chemical hydrolysis. In the absence of EME, ECG can be formed through the cleavage of the benzoyl ester group by carboxylesterase enzymes. The findings of this study suggest that ECG can be formed in the absence of enzymatic catalysts. The proposed pathway for a potential reaction is suggested to be through the chemical hydrolysis of BZE. This is due to the observation that formation of ECG matches the degradation of BZE at pH 8. The proposed potential pathway for this reaction is shown in Figure 3.

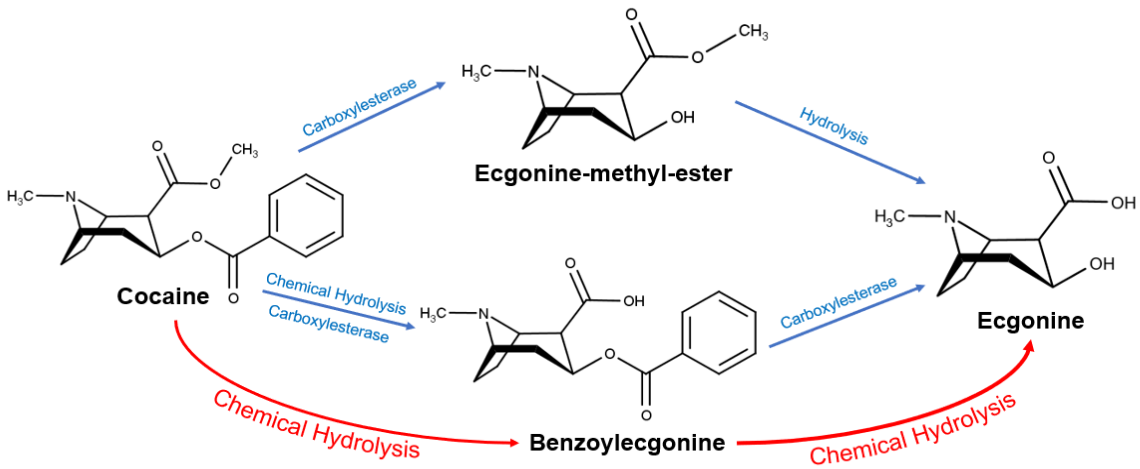

Figure 3. Known and proposed transformation pathway of cocaine to ecgonine-methyl-ester. Proposed alternate pathway indicated by red. Documented transformation pathways indicated by blue.

In the environment, cocaine degradation depends on the biotic and abiotic conditions. The primary degradation pathway of cocaine is hydrolysis, where O-demethylation occurs on the ester group to form BZE. Several studies have suggested that in the absence of biota, not all transformation reactions of cocaine occur therefore producing fewer transformation product (Bisceglia *et al.,* 2014). If we consider subsequent interactions between sunlight and chlorination Bijlsma *et al.,* 2013 reported a further sixteen degradation products of cocaine. Whilst these products may occur over time and in the presence of full sunlight and/or chlorination, they are of decreasing concentrations and are of likely less environmental concern.

Conclusions

The outcomes of this work highlight that cocaine is discharged into the Hawkesbury-Nepean river system at levels that are comparable to other densely populated cities internationally. The implications of this work are that surface water in rapidly urbanising catchment areas such as the Sydney region are increasingly under pressure from trace amounts of illicit compounds. The concentrations of cocaine and BZE detected in this study have are not at levels that previous ecotoxicology studies have shown an effects on vertebrate aquatic organisms. Impacts on invertebrates and broader environmental impacts are poorly understood. This is especially important when noting that this study shows the persistence of cocaine may last for time periods ranging to weeks. The specific risks associated to species in the HNR system need to be assessed and must include native species in addition to those which are farmed along the river. The results of the laboratory degradation study suggest that cocaine and BZE persist to six weeks at the measured environmental pH levels. The addition of an unfiltered surface water matrix among other environmental variables such as sunlight and temperature, are likely to accelerate the rate of degradation (Kasprzyk-Hordern et al. 2008). Both cocaine and BZE are likely to degrade completely within six weeks, however, the constant input from effluent discharge into receiving waters provides a continual source of contamination. Prior to complete degradation there is still opportunity for cocaine and metabolites to have an ecological impact.

Acknowledgments

This research was conducted using facilities and equipment of Western Sydney University's School of Science. We thank Sydney Water for water volume data and note that flow data may be subject to change following data calibration and validation. We thank WSU Mass Spectrometry Facility for their generous assistance.

References

Australian Bureau of Statistics (2016) 'Local Government Areas Database'. Available from: https://www.abs.gov.au/websitedbs/D3310114.nsf/Home/2016%20search%20by%20geography

Australian Criminal Intelligence Commission. *National Wastewater Drug Monitoring Program Report 1* [Internet]. (2017). Available from: https://www.acic.gov.au/sites/default/files/national_wastewater_drug_monitoring_program_report_1_0.pdf?acsf_files_redirect

Australian Criminal Intelligence Commission. *National Wastewater Drug Monitoring Program* [Internet]. (2019). Report No.: 8. Available from: https://www.acic.gov.au/sites/default/files/national_wastewater_drug_monitoring_program_report_8_2019_pdf.pdf?v=1571983781

Australian Criminal Intelligence Commission. *National Wastewater Drug Monitoring Program Report 9* [Internet]. (2020). Report No.: 9. Available from: https://www.acic.gov.au/sites/default/files/2020/03/nwdmp-r9-060220_ec_v8_small.pdf

Bijlsma, L., Boix, C., Niessen, W.M.A., Ibáñez, M., Sancho, J.V., & Hernández, F. (2013). Investigation of degradation products of cocaine and benzoylecgonine in the aquatic environment. *Science of The Total Environment* **443**:200–208.

Binelli, A., Pedriali, A., Riva, C., & Parolini, M. (2012). Illicit drugs as new environmental pollutants: Cyto-genotoxic effects of cocaine on the biological model Dreissena polymorpha. *Chemosphere* **86**:906–911.

Bisceglia, K.J., & Lippa, K.A. (2014). Stability of cocaine and its metabolites in municipal wastewater – the case for using metabolite consolidation to monitor cocaine utilization. Environmental Science and Pollution Research. Mar;21(6):4453–60

Capaldo, A., Gay, F., & Laforgia, V. (2019). *Changes in the gills of the European eel (Anguilla anguilla) after chronic exposure to environmental cocaine concentration*. Ecotoxicology and Environmental Safety. 2019 Mar;169:112–9.

Chen, C., Kostakis, C., Irvine, R.J., Felgate, P.D., & White, J.M. (2013). *Evaluation of pre-analysis loss of dependent drugs in wastewater*. Drug Test Analysis. 2013 Aug;5(8):716–21.

Gay, F., Ferrandino, I., Monaco, A., Cerulo, M., Capasso, G., & Capaldo, A. (2016). *Histological and hormonal*

changes in the European eel (Anguilla anguilla) after exposure to environmental cocaine concentration. Journal of Fish Diseases. Mar;39(3):295–308.

Gheorghe, A., van Nuijs, A., Pecceu, B., Bervoets, L., Jorens, P.G., & Blust, R. (2008). *Analysis of cocaine and its principal metabolites in waste and surface water using solid-phase extraction and liquid chromatography–ion trap tandem mass spectrometry*. Anal Bioanal Chem. Jun;391(4):1309–19.

González-Mariño, I., Quintana, J.B., Rodríguez, I., Sánchez-Méndez, N., & Cela, R. (2012) Transformation of cocaine during water chlorination. Anal Bioanal Chem. Dec;404(10):3135–44.

Greater Sydney Commission. *Western City District Plan* [Internet]. (2018). Available from: https://gsc-public-1.s3-ap-southeast-2.amazonaws.com/western-district-plan-0618.pdf

Kasprzyk-Hordern, B., Dinsdale, R.M., & Guwy, A.J. (2008) *The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK*. Water Research. Jul;42(13):3498–518.

Parolini, M., Magni, S., Castiglioni, S., & Binelli, A. (2016). *Genotoxic effects induced by the exposure to an environmental mixture of illicit drugs to the zebra mussel*. Ecotoxicology and Environmental Safety. Oct;132:26–30.

Yadav, M.K., Short, M.D., Gerber, C., Awad, J., van den Akke,r B., & Saint, C.P. (2019) *Removal of emerging drugs of addiction by wastewater treatment and water recycling processes and impacts on effluent-associated environmental risk*. *Science of The Total Environment*. 2019 Aug 25;680:13–22