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Understanding the Removal and Fate of Selected Drugs of Abuse in Sludge and Biosolids from Australian Wastewater Treatment Operations

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ABSTRACT

Illicit and pharmaceutical drugs are considered to be emerging contaminants of concern, and much research effort has gone into assessing their occurrence in wastewater. However, little information exists on their presence in treated sludge or biosolids. In this study, we examined sludge and biosolids from a large metropolitan wastewater treatment plant (WWTP) in Australia to determine the occurrence of five drugs of abuse, including benzoylecgonine as indicator of cocaine consumption, methamphetamine and 3,4-methylenedioxy methamphetamine (MDMA) as representative illicit stimulants, and codeine and morphine as pharmaceuticals with potential environmental risk. The samples were solid-phase extracted and analyzed by liquid chromatography–tandem mass spectrometry (LC–MS/MS). Benzoylecgonine and MDMA were present in raw sludge but were notably degraded during solids treatment processes, and were not detected in the dewatered sludge (after treatment) or in biosolids. Methamphetamine, codeine, and morphine were detected in all biosolids samples at mean concentrations of 20–50 $\mu g \cdot kg^{-1}$. The presence of these three drugs in biosolids shows that these compounds are relatively stable in the solids and in soil, and can persist in biosolids for at least several years. A simple environmental risk assessment based on estimated risk quotients (RQs) for these compounds indicated that the potential environmental risks associated with the land application of biosolids are very low at typical Australian biosolids application rates.

1. Introduction

Illicit drugs are considered to be emerging contaminants of concern due to their toxic effects on aquatic biota and ecosystems. Their presence has been reported in various environmental sources including surface water [1–4], drinking water [5–9], groundwater [10–12], aquatic biota (fish or other) [13–15], and sewage sludge [16–20]; however, little information is available regarding their possible presence in biosolids [21,22]. Here, the term *biosolids* refers to the treated sludge product of sewage treatment at wastewater treatment plants (WWTPs) [23].

Increasing global rates of secondary-level wastewater treatment using activated-sludge-type processes have led to increased volumes of sludge and biosolids production. Recent global biosolids production rate estimates are on the order of 2.5×10^7–6.0×10^7 t of dry solids per year [24], with much of this being applied to soil [25]. According to the Australian and New Zealand Biosolids Partnership (ANZBP) [26] report, about 3.3×10^5 t of dry biosolids were generated in Australia in 2017, of which 75% was applied in agriculture—a comparatively high amount in comparison with agricultural application in the United States and European Union (about 45%) [25]. Since biosolids are rich in nutrients, their land application as fertilizer is an attractive option for sustainable soil nutrient management and carbon sequestration [27,28]. In addition to agricultural nutrients and other soil-improving constituents, biosolids contain numerous contaminants of concern—in particular, persistent organic pollutants that include pharmaceuticals [11,21,29], pesticides, polychlorinated biphenyls (PCBs) or polycyclic aromatic hydrocarbons (PAHs) [30–33], and

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(potentially) residual amounts of illicit drugs. There is evidence that some drugs of abuse may be toxic to aquatic biota, but there are limited studies on their potential effect on terrestrial biota. For example, Parolini et al. [34] examined the oxidative status of the zebra mussel after exposure to a mixture of drugs of abuse (cocainics, amphetamines, and morphine) and reported a significant increase in antioxidant activities posing a potential threat to mussel health.

Hydrophobic compounds having high (> 4) to moderate (2.5–4) octanol–water partition coefficient logK_{OW} values have relatively high adsorption potential and are therefore more likely to accumulate in solids and sludge fractions [35,36]. Conversely, compounds with lower logK_{OW} value (< 2.5) have relatively low sorption potential and may be more likely to remain in the aqueous phase [35]. Compounds that are not eliminated effectively during wastewater solids treatment processes may be transported into the environment through biosolids-reuse programs, where they may enter the food chain through uptake by plants and crops. For example, past research has revealed the uptake of certain antibiotics by crops (e.g., cabbage, lettuce, and spinach) grown in biosolids-enriched soil [37–39]. Therefore, it is important to characterize and understand the levels of other pharmaceutical contaminants in biosolids, including drugs of abuse.

Several reports have been published detailing the presence of drugs of abuse in aqueous wastewater environments [40–42]; however, to date, limited international data is available on their presence in biosolids and almost none has been published for Australia. For example, methamphetamine was detected in sludge (2 μg·kg^{-1}) from the Australian WWTP by Govindarasu [43], while Jones-Lepp and Stevens [21] reported comparable methamphetamine levels (4 μg·kg^{-1}) in biosolids from the United States. Several studies have reported the presence of codeine in biosolids from the United States (not detected–328 μg·kg^{-1}) [23,44,45] and Canada (2.9–110 μg·kg^{-1}) [46–48]. The limited data availability for biosolids is most likely a result of the emerging nature of wastewater epidemiology as a research field and also a reflection of the fact that much of this research has focused on raw sewage analyses to estimate population drug-consumption rates [40], rather than on understanding drug removal/partitioning during WWTP processing.

Accordingly, the aim of this study was to investigate the presence of several drugs of abuse in Australian sludge/biosolids. Methamphetamine and 3,4-methylenedioxy methamphetamine (MDMA) were chosen as examples of illicit stimulants, codeine and morphine were included as pharmaceuticals with potential environmental risk, and benzoylecgonine was chosen as an indicator (i.e., metabolite) of cocaine consumption (see Fig. 1 for structure). The compounds targeted in this study were chosen based on previous work in Australia, which had indicated relatively high concentrations of these drugs in wastewater (influent and effluent) and reported that some of these compounds have the potential to pose an environmental risk [40,49]. Thus, the objectives of this research were: ① to develop a method to determine the occurrence of specific drugs of abuse in biosolids, and ② to investigate the levels of these drugs at different stages during sludge/biosolids processing.

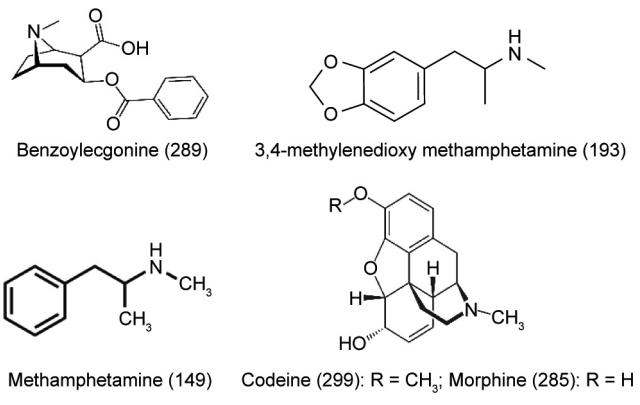

Fig. 1. Structures of targeted compounds including molecular weight (g·mol^{-1}), obtained from the PubChem Compound Database.

2. Material and methods

2.1. Chemicals and reagents

The reference standards and deuterated internal standards of each drug were purchased from Cerilliant (Cerilliant Corp., USA). Methanol, formic acid, dichloromethane, and isopropanol were acquired from Merck (Merck Pty. Ltd., Australia), while acetic acid was obtained from Spectrum® (Spectrum Chemical Mfg. Corp., USA) and ammonia from Optigen (Optigen Ingredients, Australia). Ultra-pure water was obtained from arium® pro VF water purification unit (Sartorius Stedim Biotech GmbH, Germany). Solid-phase extraction (SPE) cartridges (UCTTM XRDAH; 500 mg per 6 mL) were purchased from PM Separations (Australia).

2.2. Study site and sample collection

The sludge samples were collected from a treatment plant located in Australia (Fig. 2 provides the sampling locations). The treatment plant serves about 7 × 10^5 people and receives an average sewage flow of 150 ML·d^{-1}. The plant operates with a conventional activated-sludge reactor process comprising anaerobic, aerobic, and anoxic zones in series. The primary sludge is gravity thickened and waste-activated sludge (WAS) is thickened by dissolved air flotation, after which the combined sludge undergoes mesophilic anaerobic digestion with a solids retention time of 18 d. Part of the digested sludge volume further undergoes mechanical dewatering by centrifuge, while the majority is dewatered in sludge lagoons. Sludge lagoons are filled over a period of 1.5–3 years; the lagooned solids are then dried and stockpiled for a further minimum period of three years (i.e., the sampled three-year-old stockpiled biosolids in this study have their origins in wastewater entering the WWTP during the 2012–2014 period). The Grade A quality biosolids product is then used in broadacre agriculture as a soil supplement.

Samples were initially collected (three replicates) after anaerobic digestion for method development (extraction and analytical). Later, three more samples (45 samples in total: five treatment stages × three-time points × three replicates) were collected for analyses between Dec 2016 and Jun 2017. The samples were collected at different WWTP stages including primary sludge, mixed digested sludge, centrifuge-dewatered biosolids, lagoon-stabilized sludge, and biosolids from the three-year-old stockpiles. Primary sludge was collected as a 24 h composite and the remaining were grab samples. All samples were stored at −20 °C prior to sample preparation and analysis.

2.3. Sample preparation and extraction

All samples were freeze-dried (Lyph-Lock 6, Labconco Corp., USA) followed by homogenization. First, two solution mixtures were tested for the extraction of the targeted drugs: ① 8 mL of a solution mixture of methanol and 0.1 mol·L^{-1} acetic acid (1:1 v/v) after Monsalvo [50]; and ② 20 mL of 50 mmol·L^{-1} formic acid and methanol (80:20 v/v), based on the methods of Kaleta et al.

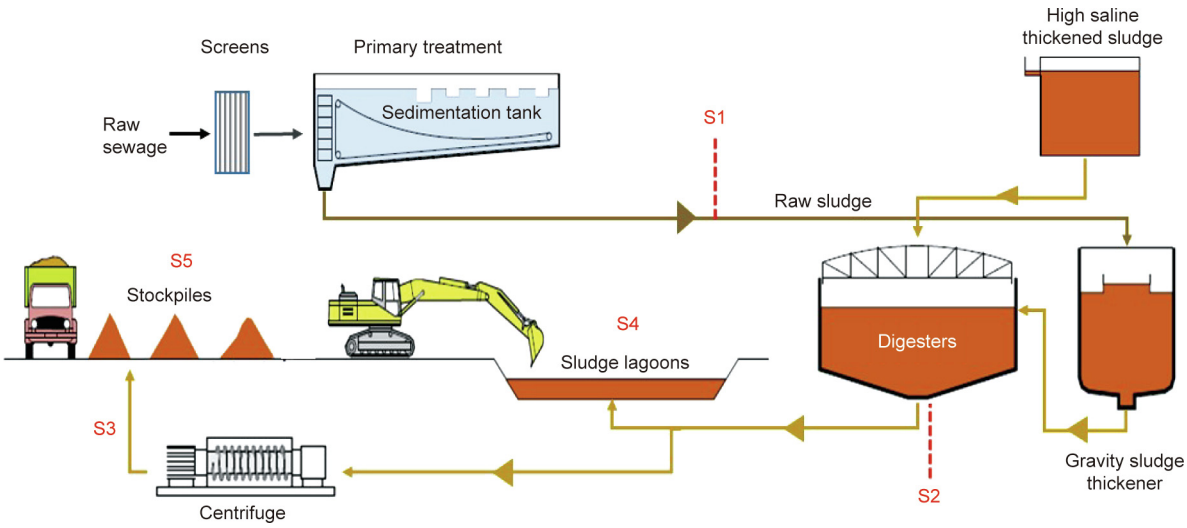

Fig. 2. Schematic diagram highlighting the sludge treatment processes at the selected WWTP and sampling points. S1: primary sludge; S2: mixed digested sludge; S3: centrifuge-dewatered biosolids; S4: sludge lagoons; S5: biosolids three-year-old stockpiles.

[20]. Fig. 3 illustrates the detailed extraction procedures. In summary, 1 g of freeze-dried sample was weighed and spiked with a 200 μL mixture of the deuterated internal standards of each drug. The sludge sample with the added solution mixtures was ultrasonicated for 15 min, followed by rotary mixing for 1 h. After mixing, the sample was centrifuged at 3500 $r \cdot min^{-1}$ (2851 *g*) for 10 min (Allegra X-12R Beckman Coulter Australia Pty. Ltd., Australia). The supernatant was then collected into a glass bottle, and the extraction was repeated three times and combined. Following that, one sample was directly evaporated to dryness under nitrogen at 40 °C and another sample was extracted using a SPE protocol previously published for wastewater samples [51]. For this

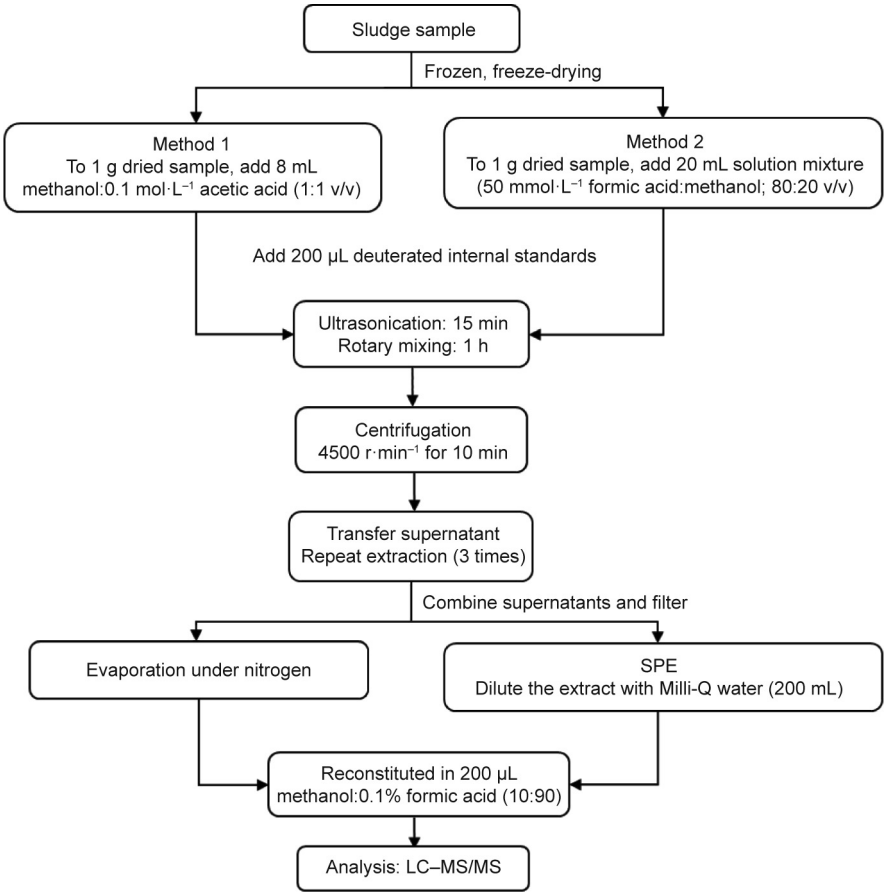

Fig. 3. Extraction protocols for sludge/biosolids samples.

purpose, the filtered supernatant was loaded onto mixed-mode SPE cartridges pre-conditioned with methanol (6 mL) and sodium acetate buffer (6 mL). The SPE cartridges were washed with sodium acetate buffer (6 mL), 0.1 mol·L^{-1} acetic acid (2 mL), and methanol (6 mL). Elution of the analytes was achieved with a mixture of 4% ammonia and 96% dichloromethane/isopropanol (80:20) and the samples were evaporated to dryness. Both dried samples (after direct evaporation and the SPE method) were reconstituted with 20 μL of 0.1% formic acid in methanol and 180 μL of 0.1% formic acid in Milli-Q water prior to analysis by liquid chromatography–tandem mass spectrometry (LC–MS/MS). The final extraction solution was selected based on the accuracy and recovery of the samples spiked with known concentrations of the drugs (Table 1).

2.4. Chromatography analysis

The analytical instrumentation consisted of a Shimadzu (Shimadzu Corp., Japan) high-performance liquid chromatograph (HPLC; LC-20AD), autosampler (SIL-20A/HT), pump system (LC-20AD), and degasser (DGU-20A), coupled to an API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Canada) equipped with an electrospray ionization (ESI) source.

Chromatographic separation was achieved using a Phenomenex™ (Phenomenex Inc., USA) Luna® pentafluorophenyl (PFP) column (100 mm × 4.6 mm, 3 μm) coupled to a PFP guard column (4 mm × 2.0 mm, 5 μm), at a total flow rate of 0.6 mL·min^{-1}, based on a previously published method used for wastewater samples with slight modification [40]. The mobile phase consisted of Solvent A (5% methanol + 0.1% formic acid) and Solvent B (95% methanol + 0.1% formic acid). The sample injection volume was 10 μL. The gradient started with 30% B with an immediate linear increase to 100% B until 4 min, followed by a 4 min isocratic period and then a linear decrease to 30% over 0.1 min. The gradient was then maintained at that level until the end of the runtime (10 min). Mass spectra were measured in positive ionization mode via multiple reaction monitoring. The compound-specific parameters are summarized in Table 1.

2.5. Method validation

Extraction recoveries of the selected compounds were determined for each extraction solution through samples spiked with the standard concentrations, which ranged from 20–1700 μg·kg^{-1} (see Table 1 for the concentration range). Recoveries were assessed by comparing the measured concentrations achieved versus the spiked concentrations. The drug concentrations were calculated by the ratio of analyte/internal standard through isotope dilution. Deuterium-labeled standards of each analyte served as internal standards to account for analyte loss during the extraction process. The limit of quantification (LOQ) and limit of detection (LOD) were respectively determined as ten times and three times the signal-to-noise ratio for each compound. Linearity was established by analyzing the standards at different concentrations and the LOQ was chosen as the lowest measurable concentration that fitted the standard curve within ±15%.

2.6. Statistical analysis

Statistical analyses were conducted using Prism® 7.03 (GraphPad Software Inc., USA). The Shapiro–Wilk normality test was performed, followed by a two-tailed *t*-test to compare the recoveries obtained with extraction solutions 1 and 2 (n = 15). Similarly, the differences between the concentrations of the drugs before and after sludge treatment for the various treatment processes were assessed by *t*-test.

3. Results and discussion

3.1. Extraction recoveries and data quality assurance

The performance of both extraction solutions followed by SPE or direct injection methods was determined for absolute recovery by comparing the concentration measured with the spiked concentrations in the samples, as shown in Table 1. The recovery of all the compounds with Solution 1 (methanol + 0.1 mol·L^{-1} acetic acid) and Solution 2 (formic acid + methanol) after SPE ranged from 93%–116% and 61%–186%, respectively, as summarized in Fig. 4. The variation in recovery could be related to matrix interference, which caused suppression (codeine/morphine) or amplification (MDMA/methamphetamine) of detection. Direct injection of the extracts gave a poor signal response, making SPE necessary. However, there was a significant difference in the recoveries obtained for all compounds with Solution 1 compared with Solution 2, both followed by SPE extraction (t_{28} = 2.11, p = 0.044). Based on our results, a pretreatment followed by SPE extraction is a recommended method for the extraction of targeted compounds in wastewater solids matrices. Similarly, Kaleta et al. [20] applied SPE extraction for the analysis of amphetamine in sludge samples due to the complex consistency of sludge. Thus, Solution 1 in combination with SPE extraction was used throughout the present study.

3.2. Analysis of selected compounds in sludge and biosolids samples

The extraction method outlined above was applied in order to determine the concentration of drugs in wastewater solids samples from different WWTP stages. The results obtained for each sample (mean ± SEM) are summarized in Fig. 5. The most ubiquitous

Table 1
Compound-specific mass spectrometric parameters used for the analysis of target compounds and the corresponding limit of detection (LOD) and limit of quantification (LOQ).

Targeted drugs/internal standards	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	Declustering potential (V)	Collision energy (V)	Standard concentration range (μg·kg^{-1})	LOD (μg·kg^{-1})	LOQ (μg·kg^{-1})
Morphine	3.88	286	165	50	65	400–850	3.2	9.6
Morphine-d3	3.89	289	165	50	65			
Codeine	6.62	300	215	50	35	800–1700	1.6	5.0
Codeine-d3	6.63	303	215	50	35			
MDMA	7.90	194	163	35	18	40–85	0.7	2.2
MDMA-d5	7.92	199	165	35	18			
METH	7.47	150	119	20	35	80–170	19.3	58.4
METH-d5	7.48	155	121	20	35			
BE	9.85	290	168	50	25	20–43	0.4	1.2
BE-d3	9.86	293	171	50	25			

Other MS/MS parameters: Nebulizer gas: 12 pounds/square inch gauge (psig); curtain gas: 10 psig; ion spray voltage: 5000 V.

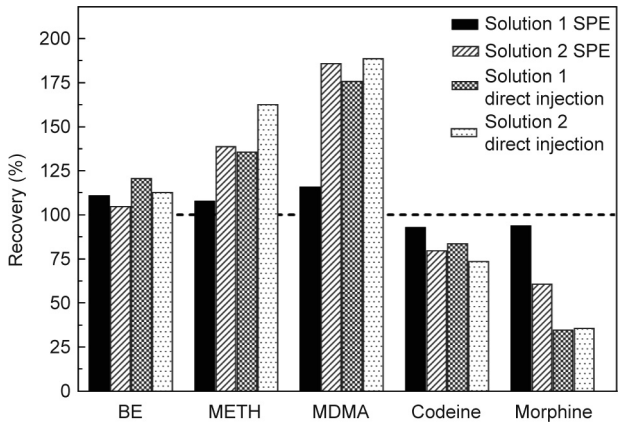

Fig. 4. Recoveries obtained for the extraction ($n = 3$) of target drugs with two solution mixtures (Solution 1 and Solution 2) followed by SPE or direct injection. BE: benzoylecgonine; METH: methamphetamine.

compounds were methamphetamine, codeine, and morphine, which were found in all sludge and biosolids samples. In contrast, benzoylecgonine and MDMA were present at the initial WWTP stages (primary sludge and digester samples) but were not detected in the treated biosolids, confirming either their complete removal by the sludge treatment processes or their biodegradation.

The concentrations of the targeted compounds ranged from 1 to 78 $\mu g \cdot kg^{-1}$ in primary sludge, with the highest concentration being reached for methamphetamine (Fig. 5). Morphine concentration was significantly higher in anaerobically digested sludge than in primary sludge ($t_6 = 2.598$, $p = 0.0408$). This increase (~four-fold) might be due to the glucuronide process—in which morphine-6-glucuronide, a major metabolite of morphine, is converted back to free morphine [40,52–54]—or to the conversion of codeine into morphine [55] during the treatment process. Another possibility is that morphine might be adsorbed and accumulated during the activated-sludge process, and then added to the digesters via the thickened WAS. Prior work at this site demonstrated that morphine was effectively removed from the incoming wastewater by this plant, following activated-sludge treatment (for a raw wastewater morphine concentration of about 900 $\mu g \cdot L^{-1}$, with > 90% removal) [40]. Assuming a primary-to-WAS ratio of 4:1, the observed four-fold increase in morphine levels between the primary treatment and WAS is feasible.

Benzoylecgonine and MDMA were not detected in the treated sludge (centrifuged or lagoon dewatered). These compounds may have undergone degradation based on their shorter half-life values or lesser affinity for solids partitioning. For example, the half-life of MDMA is much lower (15–59 d) than that of methamphetamine (131–502 d) [56,57]. Langford et al. [58] have described benzoylecgonine as a polar compound with a very low affinity for solids due to a small $\log K_{OW}$ of 2.15; this should result in a greater proportion of the compound being present in the aqueous phase and relatively less being present in the solids fraction. Another possibility regarding MDMA non-detection may be MDMA metabolism, which favors the degradation of MDMA to hydroxylated amphetamines. These compounds (i.e., hydroxylated amphetamines) may still be bioactive in the treated biosolids, with unknown toxicity. However, there was no significant difference in the methamphetamine mean concentrations in the centrifuged or lagoon-dewatered sludge (mean ± SEM; (61.6 ± 1.3) or (54.2 ± 1.1) $\mu g \cdot kg^{-1}$, respectively). The results for benzoylecgonine and MDMA in the present study are comparable to those of a recent Slovakian study [22], in which benzoylecgonine was below the LOQ and MDMA was present at very low levels, on average, in digested sludge (< 5 $\mu g \cdot kg^{-1}$). The methamphetamine concentrations recorded in this study are comparatively higher than those previously reported in sludge samples from an Australian WWTP (2 $\mu g \cdot kg^{-1}$) [43], but remain within the range reported by Mastroianni et al. [17] (i.e., 6.7–111 $\mu g \cdot kg^{-1}$) in sludge samples. It should be noted that in 2013, when the study by Mastroianni et al. was published, methamphetamine was being used—and therefore detected—at far lower levels than at present, which probably resulted in lower levels being present in solids as well at that time. For example, the methamphetamine concentration detected in influent wastewater in Spain was 50 $ng \cdot L^{-1}$ in 2011 [59], whereas it was about 3000 $ng \cdot L^{-1}$ in Australian wastewater in 2016 [40]. The results indicate that methamphetamine is a stable compound that undergoes minimal degradation under mesophilic anaerobic digestion conditions.

The other two compounds detected in the dewatered centrifuge/lagoon-treated sludge were codeine and morphine, whose mean concentrations ranged in 10.6–15.4 and 39.6–139.4 $\mu g \cdot kg^{-1}$, respectively. Here, the mean dewatered

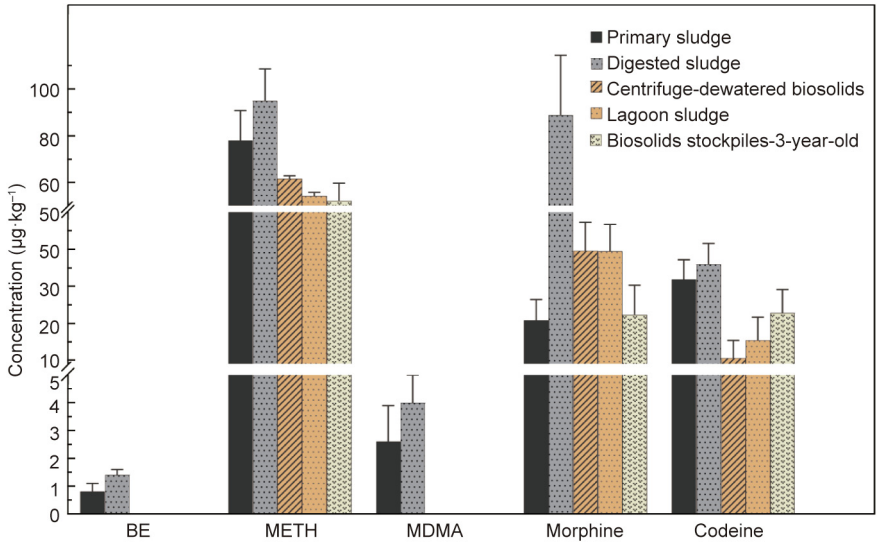

Fig. 5. Concentrations (mean ± SEM) of targeted compounds ($\mu g \cdot kg^{-1}$ of dry weight) found in primary sludge, anaerobically digested sludge, treated sludge dewatered by centrifuge/lagoon, and biosolids from stockpiles.

sludge values for codeine were almost double the levels (6.3 $\mu g \cdot kg^{-1}$) reported by Gago-Ferrero et al. [60] in sludge collected from WWTPs in Greece, where sludge is treated by aerobic digestion prior to dewatering. However, the digested and dewatered sludge codeine concentrations found in the present study were similar to those of Ivanová et al. [22], who reported median codeine levels of 16 $\mu g \cdot kg^{-1}$ in primarily anaerobically digested (some aerobic) and centrifuge-dewatered sludge from several Slovakian WWTPs. The difference in the levels between our data and those of Gago-Ferrero et al. [60] and, to a lesser extent, Ivanová et al. [22] might be due to the use of anaerobic digestion for sludge treatment in this study, as it may be less effective than aerobic digestion for codeine degradation or removal. For example, the literature reports that aerobic digestion is better at trace organics removal than anaerobic digestion [61,62]. Furthermore, given that the numbers are still within the same order of magnitude (< two-fold difference), these differences could also feasibly relate to sampling variability, or to differences in analytical measurement or recovery. The morphine mean concentration ($\sim$ 40 $\mu g \cdot kg^{-1}$) was the same in both treated sludges in this study (centrifuged or lagoon dewatered), and was comparatively higher than the range reported by Mastroianni et al. [17] (2.2–19.1 $\mu g \cdot kg^{-1}$) in treated sludge from 15 Spanish WWTPs with predominantly activated-sludge operations. The sludge treatment processes in their study were comparable to those used in the current investigation, and included anaerobic digestion followed by centrifuge dewatering. Overall, there was no significant difference in the concentrations of drugs (methamphetamine, codeine, and morphine) in the biosolids generated after dewatering by centrifugation or lagoon.

Of the five targeted drugs, methamphetamine, codeine, and morphine were detected in all biosolids samples (Fig. 5). In general, methamphetamine concentration (52.2 $\mu g \cdot kg^{-1}$) was approximately twice the mean concentration of codeine and morphine ($\sim$ 23 $\mu g \cdot kg^{-1}$). A likely reason for the higher concentration of methamphetamine in biosolids is its higher influent mass loads [40,49,63]. Methamphetamine has been shown to be relatively stable in the environment [57,64], which may also explain its prevalence and persistence in the stabilized biosolids examined here. The codeine levels were within the same range as that reported by Langdon [65], but were slightly higher than the concentrations found by Sabourin et al. [48] (up to 14.6 $\mu g \cdot kg^{-1}$) in Canadian biosolids.

To the best of our knowledge, this is the first study providing information on the presence of illicit and pharmaceutical drugs in Australian biosolids. In order to try to understand the potential environmental risk associated with these compounds in biosolids in the context of Australian land application practice, we conducted a hazard assessment using risk quotient (RQ). The RQ was calculated as a ratio of the measured concentrations in the biosolids from our study to the predicted no-effect concentration (PNEC) in water from the literature [66,67], since no equivalent PNEC soil values were available. Based on their RQ values, the compounds were categorized as being of high ($RQ \geq 1$), moderate ($RQ = 0.1–1$), or low ($RQ < 0.1$) risk to the environment [68,69]. This analysis resulted in RQ values of < 0.1 (low environmental risk) for all compounds except codeine (RQ = 0.4; moderate risk). We should stress that since these RQ values were calculated using measured concentrations in 100% biosolids, this result substantially overestimates the risk in the context of Australian agricultural biosolids reuse, in which biosolids are typically applied at low rates of 10 $t \cdot hm^{-2}$, or 1 $kg \cdot m^{-2}$ by dry mass [70]. Assuming that biosolids are incorporated into the top 10 cm of the soil, a further 100-fold dilution occurs of biosolids-associated compounds in the environment, which reduces the above RQ values for all substances to well below the low-risk threshold value. Other jurisdictions should reassess this risk determination in line with the local context (i.e., biosolids drug concentrations and biosolids application rates/practices) in order to properly assess local environmental risk. We should also reiterate that the PNEC values used to calculate the RQ relate to water environments, so the above RQs are only estimates for soil.

4. Conclusions

This study surveyed sludge and biosolids from a large Australian WWTP in order to understand the occurrence and fate of methamphetamine, MDMA, codeine, morphine, and the cocaine metabolite benzoylecgonine during wastewater solids treatment. Benzoylecgonine and MDMA were readily removed during sludge treatment and were not detected in biosolids following long-term stabilization treatment. Methamphetamine, codeine, and morphine were always detected at low levels ($\mu g \cdot kg^{-1}$) in treated sludge (centrifuged and lagoon-dewatered) and biosolids. In this study, the average concentration of methamphetamine in wastewater solids was higher than the levels reported internationally; this may be a reflection of the fact that methamphetamine is the main stimulant of choice for Australians, leading to relatively higher levels in the wastewater and consequently in the solids fraction. This study also found that the solids treatment processes used at the surveyed WWTP (i.e., anaerobic digestion, agitated air drying, lagoon stabilization, and stockpiling) do very little to remove methamphetamine, morphine, and codeine, even after exposure to long-term (multiple year) solids stabilization processes. A simple environmental risk assessment showed that for all five drugs, the risks associated with the land application of biosolids are likely to be very low at typical Australian biosolids land application rates. Little is known on their long-term persistence and accumulation in the environment, however, and the use of biosolids for agriculture may benefit from more research to establish a better risk profile for these compounds in the environment and to assess their potential to enter the food chain. For such research, a long-term monitoring study would be required that should include a sampling of biosolids-amended soils before and after application at various rates, along with monitoring of the crops grown in order to assess plant uptake potential.

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Compliance with ethics guidelines

Meena K. Yadav, Cobus Gerber, Christopher P. Saint, Ben Van den Akker, and Michael D. Short declare that they have no conflict of interest or financial conflicts to disclose.

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