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Mass Balance Calculations of Illicit Drugs in Stoke Bardolph Sewage Treatment Works, United Kingdom

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Abstract

Work encompassed in this study directly measures illicit drug removal rates in laboratory studies. Researchers employed removal rate data in calculating mass balances in sewage works which is an improvement over prior studies where assumptions on removal rates at Sewage Treatment Works were made. The batch tests data enabled determination of degradation of the compounds at different temperatures and times, using various sludge types after characterization. Mass balances for the Stoke Bardolph Sewage Treatment Works were constructed using the removal rate data from the batch studies. Final effluent concentrations of 10.0 ng L^{-1} *(morphine), and 80.0 ng* L^{-1} *(6-monoacetylmorphine), were recorded after a total of 8 hour hydraulic times (8 HRT) from an initial influent concentration of 50 mg* L^{-1}*. A projected influent concentration of morphine (1.4 g L-1) at Stoke Bardolph was derived from back-calculating measured final effluent concentrations using the same mass balance approach.*

Keywords: mass-balance, illicit drugs, removal rates, sewage treatment works.

1.0 Introduction

There appears to be limited published work on the mass balances for illicit drugs. However, a review of many reported approaches of heavy metals in full-scale sewage treatment has been undertaken to demonstrate mass balance calculation and highlight assumptions inherent in these [1- 6]. Herein are some approaches to highlight the merits and demerits often associated with the mass balances of this nature and then offer alternative methods based on the present batch studies.

To estimate the levels of cocaine in wastewaters, Zuccato *et al* [7] first related the concentration of benzoylecgonine (BZE), a main metabolite of cocaine to estimate the loads of parent cocaine in effluent wastewater and this was later applied by other researchers [8-10]. In Zuccato *et al* [7] approach, certain assumptions were made: (i) a total of 45% of ingested cocaine dose is excreted as BZE, (ii) no loss or leakage of wastewater along the sewage system, (iii) no accidental discharge or 'dumping' of large quantities of BZE into the sewage system and (iv) the main source of cocaine and metabolites comes from the human urination and the metabolite used in back calculation is the major product. The authors also proposed some parameters relevant to the calculations including (i) the concentration of the main metabolite be ng L^{-1}, (ii) the ratio of the molecular masses of the parent drug and metabolites (e.g. BZE/COC), (iii) the influent flow rate (m^3 sec^{-1}), (iv) the population size that are served by the Sewage Treatment Works (STW), (v) the percentages of drug dose excreted as major metabolites, (iv) correction factor and (vi) proven stability of the main metabolite (BZE) with respect to pH and temperature.

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In calculating the load (g day^{-1}); the concentration of BZE (ng L^{-1}), flow rate and molar fraction of cocaine and BZE as well as their molar mass ratio were estimated. Zuccato *et al* [7] used the approach to estimate the community consumption of cannabinoids, opiates and cocaine per day/1000 people but the percentage of drug dose excreted as drug target residue (DTR) and correction factor must be known. Bones *et al* [9] used 10% as a percentage of parent cocaine excreted to estimate the level of cocaine consumed with the assumption that cocaine was more stable in aqueous media and found in greater quantity than its main metabolite, benzoylecgonine. It appears literature differs on the actual percentage of excreted cocaine and BZE from cocaine dose and more information is therefore required to address discrepancies in the literature.

In 2007, mass balances of pharmaceutical products were undertaken at the Soseigawa Municipal Sewage Treatment Works, Japan. Grab samples were taken 11 times from the effluents from grit chamber, the effluent from secondary sedimentation basin and the effluents from the two pilots scale-submerged MBRs of 175 L effective volume installed at the STW, operated at the same membrane flux and HRT of 6.7 h as this study and the result are presented in Table 1.

Table 1: Average concentrations and mass balances of pharmaceuticals in the WWTP and MBRs as found in Soseigawa Municipal STW, Japan [11]

	WWTP	**MBR-A**	**MBR-B**
Water flow (m^3/day)	125 000	0.624	0.624
Excess sludge (kg/day)	7865	0.035	0.033
Influent concentration[b] (ngL^{-1}, n=11)			
Clofibric acid	28 ± 8		
Diclofenac	251 ± 100		
Ketoprofen	979 ± 237		
Ibuprofen	1966 ± 662		
Mafanamic acid	221 ± 62		
Naproxen	276 ± 115		
Effluent concentration[b] (ngL^{-1}, n=11)			
Clofibric acid	14 ± 4	14 ± 5	5 ± 4
Diclofenac	145 ± 32	124 ± 29	46 ± 17
Ketoprofen	445 ± 121	171 ± 60	<20[c]
Ibuprofen	40 ± 32	106 ± 68	35 ± 32
Mafanamic acid	62 ± 23	51 ± 1	15 ± 6
Naproxen	99 ± 18	11 ± 12	<10[c]
Amount of pharmaceuticals adsorbed on sludge[b] (ng/g of TSS[d], n=4)			
Clofibric acid	<4[c]	<4[c]	<4[c]
Diclofenac	35 ± 7	135 ± 200	31 ± 7
Ketoprofen	<40[c]	<40	<40[c]
Ibuprofen	51 ± 8	26 ± 8	18 ± 6
Mafanamic acid	130 ± 71	111 ± 27	92 ± 29
Naproxen	<20[c]	<20[c]	<20[c]
Total elimination during wastewater treatment [g/day (WWTP) or ug/day (MBR)]			
Clofibric acid	1.75	8.74	14.4
Diclofenac	13.3	79.2	128
Ketoprofen	66.8	504	598
Ibuprofen	241	1160	1200
Mafanamic acid	19.9	106	129
Naproxen	22.1	165	>166
Elimination due to sorption [g/day (WWTP) or ug/day (MBR)]			
Clofibric acid	<0.031	<0.14	<0.13
Diclofenac	0.28	4.7	1.0
Ketoprofen	<0.31	<1.4	<1.3
Ibuprofen	0.40	0.91	0.59
Mafanamic acid	1.0	3.9	3.0
Naproxen	<0.16	<0.70	<0.66
Sorption vs Elimination (%)			
Clofibric acid	<2	<2	<0.9
Diclofenac	2	6	0.8
Ketoprofen	<0.5	<0.3	<0.3
Ibuprofen	0.2	0.1	0.05
Mafanamic acid	5	4	2
Naproxen	<0.7	<0.4	<0.4

[a]Amount of sludge extracted from each process to maintain a target concentration of biomass, [b] Data are shown with standard deviations. [c]Concentrations were always <LOQ. [d] Dry weight is represented by grams of TSS

Simple mass balances were estimated by inflow concentration minus outflow concentration. However, the problems of representative samplings and effect of rainfall were not taken into account as sampling was in summer at low rainfall. This meant the dilution of the effluent was minimal and for this reason, errors often encountered as a result of rain dilution have been eliminated. Also, the presence of conjugates were overlooked but it has been reported that pharmaceuticals that enter the STWs are significantly underestimated in studies as dilution of influent wastewater in STW would have occurred and result is an overestimate of performance.

A detailed study of the occurrence and removal of selected pharmaceutical compounds in a STW utilising activated sludge treatment was undertaken by Jones *et al* [12] in England, UK. The mass balance was completed using municipal sewage samples collected over four days sampling periods for the study. An example of simple mass balance of the flow through the works was consistent with the large amount of data as presented in Table 2. To calculate a simple mass balance of the flow of pharmaceuticals along the sewage works processes, Jones *et al* used the formula:

$M_{rem} = m_{in} - m_{out}$ [equation. 1]

Where, M_{rem}= mass removed by the activated sludge, m_{in} = mass of compound from settled sewage and m_{out}= total mass leaving the works in the final effluent.

Table 2: Mass balance (g/d) of pharmaceuticals over 4 day sampling period in England, UK [12]

Compounds	**Day**				**Removal rate (%)**
		M_{in}	M_{out}	$(M_{in}-M_{out})$	
Ibuprofen	1	4.82	8.38	46.44	84.71
	2	40.89	8.06	32.84	80.29
	3	47.58	5.67	41.91	88.08
	4	45.54	4.27	41.27	90.62
	Mean	47.21	6.60	40.61	86.03
Paracetamol	1	28.37	3.77	24.60	86.71
	2	27.72	2.10	25.62	92.42
	3	24.92	1.23	23.70	95.08
	4	24.17	1.57	22.60	93.51
	Mean	26.30	2.17	24.13	91.93
Salbutamol	1	35.93	2.94	32.99	9183
	2	32.23	3.09	29.13	90.40
	3	44.13	2.11	42.02	95.21
	4	53.81	0.82	52.98	98.47
	Mean	41.52	2.24	39.28	94.60
Melfenamic acid	1	51.72	4.83	46.89	90.66
	2	60.36	5.40	54.96	91.05
	3	42.87	5.62	37.25	86.89
	4	47.79	1.31	46.48	97.25
	Mean	50.69	4.26	46.40	91.54

The main error were from the returned activated sludge (RAS) which was mixed with the settled sewage before entering the activated sludge unit and this was assumed to be 'locked in the system'. Removal of selected compounds during primary sedimentation ought to have been carried out in primary tanks prior to the experiment to completely eliminate bias, but this may be insignificant as the study was limited to activated sludge. As a useful tool in allowing the fate of drugs to be accounted for, as well as assessing analytical quality, mass balance calculation is important wherever the data is available. It appears the problems due to sampling logistics and desludging process often introduce high errors in the mass balances of heavy metals through sewage works and these make methods and methodology of reporting mass balances vary considerably [13]. Therefore more work is required to address the discrepancies in literature, which the current study hopes to provide.

2.0 Materials and Methods

2.1 Chemicals and Materials

Standard compounds of cocaine, benzoylecgonine, heroin, 6-acetylmorphine, morphine, diazepam, ephedrine, lidocaine, codeine, ibuprofen, procaine, amphetamine, ecgonine methylester, cocaethylene, nordazepam, caffeine, nicotine and bromacil (herbicide) were purchased under license from both Sigma Aldrich (Gillingham Dorset, UK) and LGC standards (Teddington Middlesex, UK). Analar grade hydrochloric acid (HCl), ammonium hydroxide (NH_4OH) and methanol (MeOH) used for pH adjustment and sample preparations were obtained from Aldrich. A derivatizing agent, *N*, *O*, bis (trimethylsilyl) trifluoroacetamide (BSTFA with 1% trimethylchlorosilane, TMCS) was purchased from Cerrilliant (Round Rock, TX, USA). The choice of BSTFA as a silylating agent for derivatizaion is due to its faster reaction and volatility of its by-products. Pyridine was also purchased from Aldich and was used to provide appropriate derivatization reaction medium. Reagent water was from a Millipore milliQ water purification system (ELGA Labwater, UK). Stock solutions of each chemical at 100 µg L^{-1} were prepared in methanol and were stored at -20 ^oC in the dark at pH = 2 with 37% HCl until analysis [16], while working solutions were prepared from appropriate dilutions. Oasis HLB® sorbent in a 47mm SPE disc format and disc holder were purchased from Waters (Elstree Herts, UK). A Phenomenex SPE Vacuum Manifold (Macclesfield Cheshire, UK) with 12 ports and a self-cleaning and drying vacuum were used for loading and elution of samples with appropriate solvent mixtures.

2.2 Experimental Preparation

A simple experiment to simulate conditions in actual STWs was designed to use raw sewage in batch studies to provide natural bacterial species and population that can allow continuous degradation of metabolites slowly and naturally in contrast to utilising synthetic activated sludges. Three hours duration for the degradation studies was chosen as this permitted an intensive subsampling and processing allowing data-intensive assessments for an anticipated fast (min hr^{-1}) degradation rate. Thirteen (13) different compounds, including cocaine, benzoylecgonine, codeine, diazepam, morphine, ephedrine, lidocaine, diacetylmorphine, ibuprofen, procaine, amphetamine, ecgonine methylester and bromacil (herbicide) were detected in random wastewater samplings from Nottingham STW effluents. However, only 3 compounds were used in spiking (batch) studies namely heroin and its metabolites; 6-monoacetylmorphine and morphine.

2.3 Description of the STWs Studied with Sampling Location

Royal Air Force (RAF) Base Molesworth is located in Molesworth, Suffolk, approximately 20 miles from Cambridge. The Base which is non-residential operates 24 hours per day with approximately 1,200 personnel (over 2 shifts), with an overnight staffing around 400 personnel. The STW is located within the RAF base and utilises activated sludge for secondary biological treatment. The plant is consented to discharge a maximum of 360 m^3 day^{-1} (0.1Mgal day^{-1}). The average volume treated by the works is approximately 78.4 m^3 day^{-1} (0.02 Mgal day^{-1}). RAF Molesworth has separate wastewater and surface water drainage networks and wastewater is pumped from across the base to a biological treatment works where it is treated prior to discharge to a tributary of 'Cock Brook'. The STW has four different stages: pre-treatment, primary treatment, secondary treatment and reed beds. The sewage plant process starts from terminal pumping station where sewage is pumped to a raised inlet works and screened. A storm overflow diverts excess flow to the storm tanks and settled material accumulates in the storm tanks which are always manually cleared. Wastewater gravitates to a primary tank which removes coarse materials and a submerged aerated filter (SAF) process removes biochemical oxygen, ammonia and finely dispersed solids. This new process replaced the previous plastic media filter system. Humus tanks are used to remove any secondary settleable material. The recirculation pumping system has not been used as it was installed to ensure the required wetting rate of the old plastic media filter was maintained. The reed beds polish the final effluent to required quality standards before being finally discharged to the neighbouring rivers.

The supernatant constitutes the effluents that are passed forward. The excess secondary sludge, the solids from primary sedimentation and sedimentation of solid wastes as well as liquid stream in humus tanks are recycled back into the inlet of the plant. Co-settled sludge is pumped from the primary sludge well to a sludge storage tank. Decant liquors can be removed from the tank by an adjustable decant arm. The sampling points for the analysis are: (i) inlet to the grit removal unit (influent wastewater), (ii) inlet to primary sedimentation tank (primary sludge), (iii) inlet to submerged aerated filter reactor (SAF-1), (iv) mixed SAF, (v) secondary sedimentation unit (humus sludge) and (vi) outlet of sedimentation unit (effluent) [14].

2.4 Mass Balance Calculation from Batch Studies Data with Molesworth Sewage Samples

The site flow data for 12 months in RAF Molesworth shown in Fig. 1 gives an indication of the average flows and the range. A meter measures and records the flow to the primary tank and the data is logged by site operatives. For performance assessment, the maximum flow was taken as the consented 360 m^3/d (0.10 Mgal/d), while the average volume treated by the works was approximately 78.4 m^3/d (0.02 Mgal/d). The hydraulic retention time (HRT) and average time the flow spends in each treatment unit is a function of the average flow as calculated in equation 3. The process calculation of each unit including the volume has been shown [14]:

Hydraulic Loading Rate (m/h) = flow to tanks (m^3/h) / total surface area (m^2) [eqn 2]

Hydraulic Retention Time (h) = flow to tanks (m^3/h) / total volume of tanks (m^3) [eqn 3]

Therefore, HRT = 360 m^3/d (15 m^3/h) / 78.4 x 3 m^3/d (9.81 m^3/h)
= ~ 1.5 hr

For mass balances, duplicate samples were collected every 15 minutes over 3h to provide composite batch samples for each process stage. Samples were taken through the process at the following four locations: (1) primary effluent (2) submerged aerated filter-1, (3) mixed submerged aerated filter and (4) humus tanks effluent.

RAF Molesworth currently operates no base housing, so the wastewater composition does not conform to typical ranges and conditions for wastewater. However, the actual Total Suspended Solids (TSS) and Chemical Oxygen Demand (COD) were easy to assess as the primary effluent did not include the recirculation flow which would have diluted the concentration by the recirculation flow. The plastic media filter has been replaced by the SAF (a COPA CB750, which has a process volume of 40 cubic meters) hence; this recirculation pumping system has not been used. It was installed to ensure the required wetting rate of the plastic media filter was maintained. In a SAF, the filter is entirely submerged and mechanical aeration is provided.

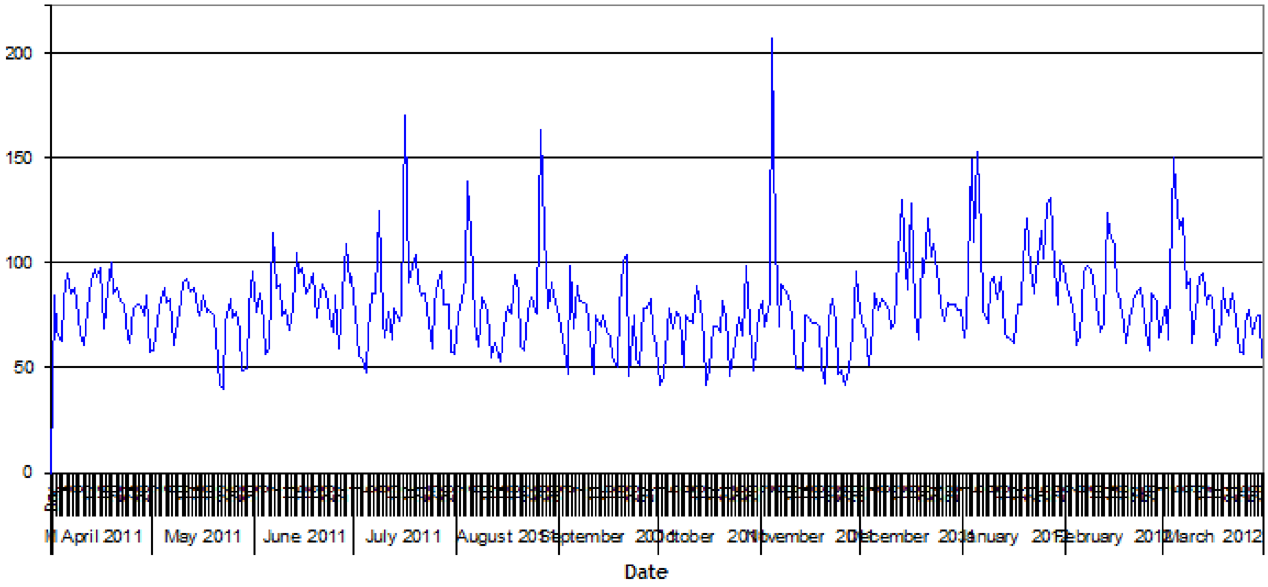

Fig. 1: RAF Molesworth - Waste Water Flow Data for Year 2011-2012

2.5 Procedures used in Mass Balance Calculation

1. In these batch studies, the removal of compounds was through adsorption to solids followed by degradation and data of different sludge experiments were obtained.

2. The per cent removal rate was calculated as follows:

$$\% \text{ removal} = \frac{100\ ([\text{initial drug}] - [\text{final drug in aqueous phase}])}{[\text{Initial drug}]}$$

The data obtained from different sludge of STW units are summarised in Tables 3 to 5 in the following order: Table 3 (morphine), Table 4 (6-monoacetylmorphine), and Table 5 (heroin).

3. The STW process at Molesworth goes through terminal pumping station, screens (no grit trap), primary tank, submerged aerated filter (SAF), humus tank, reed bed, and finally to the outfall chamber. Each dissolved compound in effluent of an STW unit is passed on to the next unit where the compound would again partition into aqueous-solid layers and the new partitioning concentration calculated as presented in Table 3 – 8. The successive partitioning of compound along through STW indicate the measure of mass balance of individual drug through different STW units using equation 2 as follows:

   Conc. of drug in effluent of each unit = (% removal rate) x [dissolved drug] [eqn. 2]

4. The final effluent (µg in 250 ml) was then multiplied by 4 and converted to final concentration expressed in µgL^{-1}

5. Back-calculation assessment is to cross-check and account for all the masses through the STW bringing the total mass-flow to original batch concentration (12.5 mg in 250 mL).

   Conc. of drug in effluent of each unit ÷ (% removal rate) = [dissolved drug] [eqn. 3]

3.0 Results and Discussion

3.1. Mass Balance Modelling

In Table 3**,** the concentration of morphine from the batch studies experiment using primary (PS), submerged aerated filter-1 (SAF-1), mixed submerged aerated filter (MSAF) and humus sludge are shown. The removal rates data for sorbed and degraded samples (underlined) and amount dissolved in aqueous phase for every 15 min timescale are presented in Table 3. In a STW with a HRT of 2 – 3 hours, for a mass balance the initial concentration goes through the STW, using the corresponding removal rates and the per cent dissolved in the aqueous phase to estimate the concentration of drugs that moves through the processing units of STW, and then measured the effluent.

The final effluent concentrations (µg L^{-1}) were calculated in the last column by multiplying the final measured drugs by 4 (concentration in µgL^{-1}) since the initial batch concentration was 12500 µg in 250 mL. We then used the data to estimate the mass balance calculation and this similarly applies to 6 – monoacetylmorphine and heroin in Table 3 - 5, respectively.

3.2 Mass Balance Modelling Calculation

The per cent removal rates data of compounds from all sewage types as presented in Tables 3 – 5 were used in the mass balance modelling work. For example, mass balance in the first row of Table 3 after 15 min degradation goes thus:

Removal rate of morphine by sorption & degradation from 12500 µg of morphine in 250 mL flask:

First PS row = 12500 µg **x** 84.8% (0.848) = 10600.0 µg (removed by sorption/degradation)
= 12500 µg **x** 15.2% (0.152) = 1900.0 µg (dissolved in aqueous phase) →

Second SAF row = 1900.0 µg (dissolved in aqueous) **x** 94.4% (0.944) = 1793.6 µg (removed/sorbed)
= 1900.0 µg **x** 5.6% (0.0056) = 106.4 µg (dissolved in aqueous phase) →

Third MSAF row = 106.4 µg (dissolved in aqueous) **x** 64.7 % (0.647) = 68.8 µg (removed)
= 106.4 µg **x** 35.3% (0.353) = 37.6 µg (dissolved in aqueous phase) →

Fourth HS row = 37.6 µg (dissolved in aqueous phase) **x** 99.0% (0.99) = 37.2µg
= 37.6 µg **x** 1.0% (0.001) = 0.38 µg (dissolved in aqueous phase) → **to effluent**

Total removed by degradation & sorption = 10600.0 + 1793.6 + 68.8 + 37.2 = **12499.6** µg
Final effluent (L) = 0.38 in 250 mL **x** 4 = **1.52** (µg L^{-1})

It should be noted that the calculation applies to all compounds passing the columns from 15 to 180 min.

Table 3: Concentration and mass balance of morphine from the batch studies experiment using primary sludge (PS), submerged aerated filter-1 (SAF-1), mixed submerged aerated filter (MSAF) and humus sludge

Time	**Removal rate (%) from each STW units**				**Morphine partition in aqueous and solid phases (µg in 250 mL)**					**Mass Balance**	**Final Effluent (µg L^{-1})**
(min)	PS	SAF	MSAF	HS	PS	SAF	MSAF	HS	Total degraded & sorbed		
15	15.2	5.6	35.3	1.0	1900.00	106.40	37.56	0.38		12500	1.50
	84.8	94.4	64.7	99.0	10600.00	1793.60	68.84	37.18	12499.62		
30	13.4	3.3	24.0	0.7	1675.00	55.28	13.27	0.09		12500	0.37
	86.6	96.7	76.0	99.3	10825.00	1619.73	42.01	13.17	12499.91		
45	11.5	2.9	22.0	0.6	1437.50	41.69	9.17	0.06		12500	0.22
	88.5	97.1	78.0	99.4	11062.50	1395.81	32.52	9.12	12499.95		
60	9.4	2.6	18.6	0.5	1175.00	30.55	5.68	0.03		12500	0.11
	90.6	97.4	81.4	99.5	11325.00	1144.45	24.87	5.65	12499.97		
75	8.2	2.0	14.8	0.5	1025.00	20.50	3.03	0.02		12500	0.06
	91.8	98.0	85.2	99.5	11475.00	1004.50	17.47	3.02	12499.98		
90	6.7	1.7	12.5	0.3	837.50	14.24	1.78	0.01		12500	0.02
	93.3	98.3	87.5	99.7	11662.50	823.26	12.46	1.77	12499.99		
105	5.1	1.4	10.9	0.3	637.50	8.93	0.97	0.00		12500	0.01
	94.9	98.6	89.1	99.7	11862.50	628.58	7.95	0.97	12500.00		
120	3.9	1.2	9.1	0.3	487.50	5.85	0.53	0.00		12500	0.01
	96.1	98.8	90.9	99.7	12012.50	481.65	5.32	0.53	12500.00		
135	3.2	0.7	7.6	0.3	400.00	2.80	0.21	0.00		12500	0.00
	96.8	99.3	92.4	99.7	12100.00	397.20	2.59	0.21	12500.00		
150	2.3	0.3	5.7	0.2	287.50	0.86	0.05	0.00		12500	0.00
	97.7	99.7	94.3	99.8	12212.50	286.64	0.81	0.05	12500.00		
165	1.3	0.2	4.0	0.2	162.50	0.33	0.01	0.00		12500	0.00
	98.7	99.8	96.0	99.8	12337.50	162.18	0.31	0.01	12500.00		
180	0.5	0.1	2.4	0.1	62.50	0.06	0.00	0.00		12500	0.00
	99.5	99.9	97.6	99.9	12437.50	62.44	0.06	0.00	12500.00		

Amount of drugs (sorbed & degraded) are underlined; others represent removal rate of drugs dissolved in aqueous phase

Table 4: Concentration and mass balances of 6-monoacetylmorphine from the batch studies experiment using primary (PS), submerged aerated filter-1 (SAF-1), mixed submerged aerated filter (MSAF) and humus sludge

Time	**Removal rate (%) from each STW units**				**6MAM partition in aqueous and solid phases (μg in 250 mL)**					**Mass Balance**	**Final Effluent (μg L^{-1})**
(min)	PS	SAF	MSAF	HS	PS	SAF	MSAF	HS	Total degraded & sorbed		
15	1.7	81.1	13.3	33.6	212.50	172.34	22.92	7.70		12500	30.81
	98.3	18.9	86.7	66.4	12287.50	40.16	149.42	15.22	12492.30		
30	1.3	56.7	11.6	29.1	162.50	92.14	10.69	3.11		12500	12.44
	98.7	43.3	88.4	70.9	12337.50	70.36	81.45	7.58	12496.89		
45	1.0	48.2	10.1	23.3	125.00	60.25	6.09	1.42		12500	5.67
	99.0	51.8	89.9	76.7	12375.00	64.75	54.16	4.67	12498.58		
60	0.8	49.6	9.0	14.2	100.00	49.60	4.46	0.63		12500	2.54
	99.2	50.4	91.0	85.8	12400.00	50.40	45.14	3.83	12499.37		
75	0.6	41.9	7.5	10.9	75.00	31.43	2.36	0.26		12500	1.03
	99.4	58.1	92.5	89.1	12425.00	43.58	29.07	2.10	12499.74		
90	0.5	29.8	6.1	9.9	62.50	18.63	1.14	0.11		12500	0.45
	99.5	70.2	93.9	90.1	12437.50	43.88	17.49	1.02	12499.89		
105	0.5	18.4	5.2	8.2	62.50	11.50	0.60	0.05		12500	0.20
	99.5	81.6	94.8	91.8	12437.50	51.00	10.90	0.55	12499.95		
120	0.4	14.0	4.4	6.7	50.00	7.00	0.31	0.02		12500	0.08
	99.6	86.0	95.6	93.3	12450.00	43.00	6.69	0.29	12499.98		
135	0.2	10.9	3.6	5.0	25.00	2.73	0.10	0.00		12500	0.02
	99.8	89.1	96.4	95.0	12475.00	22.28	2.63	0.09	12500.00		
150	0.2	8.3	2.9	3.4	25.00	2.08	0.06	0.00		12500	0.01
	99.8	91.7	97.1	96.6	12475.00	22.93	2.01	0.06	12500.00		
165	0.1	6.4	2.0	2.4	12.50	0.80	0.02	0.00		12500	0.00
	99.9	93.6	98.0	97.6	12487.50	11.70	0.78	0.02	12500.00		
180	0.0	3.2	1.1	1.4	0.00	0.00	0.00	0.00		12500	0.00
	100.0	96.8	98.9	98.6	12500.00	0.00	0.00	0.00	12500.00		

Amount of drugs (sorbed & degraded) are underlined; others represent removal rate of drugs dissolved in aqueous phase

Table 5: Concentration and mass balances of heroin from the batch studies experiment using primary (PS), submerged aerated filter-1 (SAF-1), mixed submerged aerated filter (MSAF) and humus

Time (min)	**Removal rate (%) from each STW units**				**Heroin partition in aqueous and solid phases (µg in 250 mL)**					**Mass Balance**	**Final Effluent (µg L^{-1})**
	PS	SAF	MSAF	HS	PS	SAF	MSAF	HS	Total degraded & sorbed		
15	3.8	0.4	0.4	12.3	475.00	1.90	0.0076	0.0009		12500	0.0037
	96.2	99.6	99.6	87.7	12025.00	473.10	1.8924	0.0067	12500		
30	3.3	0.4	0.4	8.9	412.50	1.65	0.0066	0.0006		12500	0.0023
	96.7	99.6	99.6	91.1	12087.50	410.85	1.6434	0.006	12500		
45	2.3	0.4	0.3	7.3	287.50	1.15	0.0035	0.0003		12500	0.0010
	97.7	99.6	99.7	92.7	12212.50	286.35	1.1465	0.0032	12500		
60	2.2	0.3	0.3	6.3	275.00	0.83	0.0025	0.0002		12500	0.0006
	97.8	99.7	99.7	93.7	12225.00	274.18	0.8225	0.0023	12500		
75	1.6	0.3	0.2	4.5	200.00	0.60	0.0012	0.0001		12500	0.0002
	98.4	99.7	99.8	95.5	12300.00	199.40	0.5988	0.0011	12500		
90	1.3	0.2	0.2	3.0	162.50	0.33	0.0007	0.0		12500	0.0001
	98.7	99.8	99.8	97.0	12337.50	162.18	0.3243	0.0007	12500		
105	1.1	0.2	0.2	2.5	137.50	0.28	0.0006	0.0		12500	0.0001
	98.9	99.8	99.8	97.5	12362.50	137.23	0.2744	0.0006	12500		
120	0.6	0.1	0.1	2.1	75.00	0.08	0.0001	0.0		12500	0.0000
	99.4	99.9	99.9	97.9	12425.00	74.93	0.0749	0.0001	12500		
135	0.5	0.1	0.1	1.1	62.50	0.06	0.0001	0.0		12500	0.0000
	99.5	99.9	99.9	98.9	12437.50	62.44	0.0624	0.0001	12500		
150	0.3	0.1	0.1	0.9	37.50	0.04	0.0	0.0		12500	0.0000
	99.7	99.9	99.9	99.1	12462.50	37.46	0.0375	0.0	12500		
165	0.2	0.1	0.1	0.9	25.00	0.03	0.0	0.0		12500	0.0000
	99.8	99.9	99.9	99.1	12475.00	24.98	0.0250	0.0	12500		
180	0.1	0.0	0.0	0.5	12.50	0.00	0.0	0.0		12500	0.0000
	99.9	100.0	100.0	99.5	12487.50	12.50	0.0	0.0	12500		

Amount of drugs (sorbed & degraded) are underlined; others represent removal rate of drugs dissolved in aqueous phase

3.3 Back-Calculation Assessment of Compounds

The result of back-calculation to estimate initial concentration of analytes in raw influent wastewaters using their respective removal rates in reversed order (from Table 3) is presented in Table 6. This was used to arrive back at initial influent concentration of 12500 µg L^{-1} and to confirm the correctness of the mass balance calculation operation.

Table 6: Mass Balance back-calculation in reversed order in humus sludge (HS), mixed submerged aerated filter (MSAF), submerged aerated filter-1 (SAF-1) and primary sludge (PS).

	Removal rate (%) from each STW units				**Cocaine partition in aqueous and solid phases (µg in 250 mL)**					
Time (min)	HS	MSAF	SAF	PS	HS	MSAF	SAF	PS	Total degraded & sorbed	**Batch Conc. (µg in 250ml)**
15	1.0	35.3	5.6	15.2	0.38	37.56	106.40	1900.00		12500
	99.0	64.7	94.4	84.8	37.18	68.84	1793.60	10600.00	12499.62	
30	0.7	24.0	3.3	13.4	0.09	13.27	55.28	1675.00		12500
	99.3	76.0	96.7	86.6	13.17	42.01	1619.73	10825.00	12499.91	
45	0.6	22.0	2.9	11.5	0.06	9.17	41.69	1437.50		12500
	99.4	78.0	97.1	88.5	9.12	32.52	1395.81	11062.50	12499.95	
60	0.5	18.6	2.6	9.4	0.03	5.68	30.55	1175.00		12500
	99.5	81.4	97.4	90.6	5.65	24.87	1144.45	11325.00	12499.97	
75	0.5	14.8	2.0	8.2	0.02	3.03	20.50	1025.00		12500
	99.5	85.2	98.0	91.8	3.02	17.47	1004.50	11475.00	12499.98	
90	0.3	12.5	1.7	6.7	0.01	1.78	14.24	837.50		12500
	99.7	87.5	98.3	93.3	1.77	12.46	823.26	11662.50	12499.99	
105	0.3	10.9	1.4	5.1	0.00	0.97	8.93	637.50		12500
	99.7	89.1	98.6	94.9	0.97	7.95	628.58	11862.50	12500.00	
120	0.3	9.1	1.2	3.9	0.00	0.53	5.85	487.50		12500
	99.7	90.9	98.8	96.1	0.53	5.32	481.65	12012.50	12500.00	
135	0.3	7.6	0.7	3.2	0.00	0.21	2.80	400.00		12500
	99.7	92.4	99.3	96.8	0.21	2.59	397.20	12100.00	12500.00	
150	0.2	5.7	0.3	2.3	0.00	0.05	0.86	287.50		12500
	99.8	94.3	99.7	97.7	0.05	0.81	286.64	12212.50	12500.00	
165	0.2	4.0	0.2	1.3	0.00	0.01	0.33	162.50		12500
	99.8	96.0	99.8	98.7	0.01	0.31	162.18	12337.50	12500.00	
180	0.1	2.4	0.1	0.5	0.00	0.00	0.06	62.50		12500
	99.9	97.6	99.9	99.5	0.00	0.06	62.44	12437.50	12500.00	

Amount of drugs (sorbed & degraded) are underlined; others represent removal rate of drugs dissolved in aqueous phase

3.4 Application of Mass Balance to Calculate Influent Concentration of Analytes from Nottingham STW Effluent

In this section, back-calculation was applied for selected analytes from Stoke Bardolph STW Nottingham effluent to estimate influent concentrations (Table 7). Employing the removal rates (%) obtained from batch studies data using sewage samples collected from Molesworth STW and applying it to the Stoke Bardolph STW Nottingham to estimate influent (ng L^{-1}) in back calculation. The following assumptions that may influence the removal rate (%) and its application in the back-calculation were made:

1. Operational design and treatment policy for Stoke Bardolph Nottingham and Molesworth STWs are assumed to be similar. There was no recirculation section in Molesworth STW and no submerged aerated filter (SAF) processing unit at Stoke Bardolph STW Nottingham.
2. The nature of sewage and its characteristics were assumed to be representative of most municipal sewage types.
3. The STWs hydraulic retention times were assumed to be the same (though a total of 8 HRT in Molesworth and 16 HRT including recycling process in Stoke Bardolph STW Nottingham exist).
4. The batch experiments data obtained with the real sewage samples collected from each processing units of the Molesworth STW were assumed to be representative of real-time STW runs, the real time pilot run in the STW was not possible due to site restrictions.

Table 7: Estimation of analytes from effluents concentration at 2 HRT using the percent removal rates for morphine (Table 3) in back calculation to estimate influent concentration

Analyte	**Analytes (%) in aqueous phase of each STW units.**				**Concentration of analytes (ng L^{-1}) in STW units.**				**Estimated influent (g L^{-1})**
	HS	MSAF	SAF-1	PS	HS	MSAF	SAF-1	PS	
MOR	0.3 99.7	9.1 90.9	1.2 98.8	3.9 96.1	4.6 1528.7	1533.3 15282.5	16849.5 1387275.5	1404125.0	1.4

Amount of drugs (sorbed & degraded) are underlined; others represent amount of drugs dissolved in aqueous phase (Note the reverse order of sewage sludges: HS →MSAF→SAF-1→PS).

Using the concentration of drugs found from Stoke Bardolph STW Nottingham effluent and applying the per cent removal rates of drug at 2 h timescale for back- calculation as shown in Table 8.

The First HS row:
4.6 ng L^{-1} of Morphine (effluent)
Dissolved rate = 4.6 ÷ 0.3% (0.003) = 1533.3 ng (dissolved in aqueous phase that goes to MSAF) →
Removal rate = 99.7% = 1528.7ng (removed by sorption/degradation)

Second MSAF row:
Dissolved rate = 1533.3 ng ÷ 9.1% (0.091) = 16849.5 ng (dissolved in aqueous that goes to SAF-1) →
Removal rate = 90.9% = 15282.5 ng (removed by sorption/degradation)

Third SAF row:
Dissolved rate = 16849.5 ÷ 0.012 ng = 1404125.0 ng (dissolved in aqueous phase that goes to PS) →
Removal rate = 98.8% = 1387275.5ng (removed by sorption/degradation)

Fourth PS row:
Dissolved rate = 1404125.0 ng (influent)
Removal rate = 96.1% = 1349364.1 ng (removed by sorption/degradation)
Table 8 makes it easy to compare data of effluents concentrations of morphine obtained from Stoke Bardolph STW Nottingham with the back calculated influent concentrations for morphine (1.4 g L-^{1}).

Table 8: Comparing literature influent measurements of drugs

Influent measurements of morphine quoted from other places (16-21)			**Calculated influent based on back calculations from effluent using batch study removal rates**
Analytes	**Matrix**	**Measured Influent (ng L^{-1})**	**(g L^{-1})**
Morphine	5 STPs, NE Spain	25.9 – 96.7	1.4
	3 Rivers, Italy	-	
	5 STPs, Ireland	874 ± 86	
	2 STPs, Italy	83.3– 204.4	
	Barcelona, Spain	68.1 – 162.9 ± 20	
	12 STPs, Germany	123 ± 6	
	STP, Italy	7.1 – 96.7	

The seemingly high values obtained in this study – (1000s ng L^{-1}) compared with those quoted in other places (Table 8) may possibly be due to uncertainty in primary removal in our batch studies as no recovery values from the solid phase were used [25].

The capabilities of the current experimental batch data in generating removal rates as used in our current mass balance approach have improved on the complications associated with assumptions of Zuccato *et al* [15] by using 45% of total ingested cocaine dose to calculate the concentration excreted as BZE while Bones *et al* [10] used 10%. This brought about apparent differences in the actual percentage of excreted cocaine and BZE from cocaine dose. Also, the problems due to sampling logistics and desludging as experienced by prior studies have made methodologies of reporting mass balances reported to vary considerably and this is what the present approach has addressed.

The removal rates of morphine (10.0%) and 6MAM (80%) in total of 8 HRT compare to the removal efficiencies of some pharmaceuticals like ciprofloxacin (37-86%), ofloxacin (33-66%), norfloxacin (58-87%) and Iomefloxacin (21-72%) are presented, respectively [27].

4.0 Conclusion and recommendation

Clear and simple steps in mass balance calculation of compounds in STWs have been presented in the current work; and with some refinement, the conceptual approach may be useful.

5.0 Conflict of Interest

The authors report no conflict of interest in the publication of this article. The authors alone are responsible for the content and writing of the paper.

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