FATE AND OCCURRENCE OF ALKYLPHENOLIC COMPOUNDS IN SEWAGE SLUDGES DETERMINED BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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An analytical method has been developed and applied to determine the concentrations of the non-ionic alkylphenol polyethoxylate surfactants and their metabolites alkylphenoxy carboxylates and alkyphenols in sewage sludges. The compounds were extracted with methanol/acetone (1:1 v/v) from sludge, and concentrated extracts were cleaned up by silica solid phase extraction prior to determination by liquid chromatography tandem mass spectrometry. The recoveries, determined by spiking sewage sludge at two concentrations, ranged from 51% to 89% with method detection limits from 6 μg kg^{-1} to 60 μg kg^{-1}. The methodology was subsequently applied to sludge samples obtained from a carbonaceous activated sludge plant, a nitrifying/denitrifying activated sludge plant and a nitrifying/denitrifying activated sludge plant with phosphorus removal. Nonylphenolic compounds were 2-3 fold higher in concentration compared to their octyl analogues. Long chain nonylphenol polyethoxylates ($NP_{3-12}EO$) ranged from 163 μg kg^{-1} to 11754 μg kg^{-1}. The estrogenic metabolite nonylphenol was present at concentrations ranging from 336 μg kg^{-1} to 6696 μg kg^{-1}.

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INTRODUCTION

Amongst the numerous organic micropollutants entering sewage treatment works [1] those with endocrine disrupting ability are major sources of concern [2-5]. Whilst estrogens constitute the major cause of endocrine disruption in wastewater [6] alkylphenoxy polyethoxylates (APEOs) also make a significant contribution [3,7]. During secondary aerobic biological wastewater treatment they are biodegraded by both percolating biological filters [8] and the activated sludge process [9,10] resulting in reduced concentrations in the final effluent discharged. Biodegradation is incomplete resulting in the formation of a range of breakdown products including alkylphenols, nonylphenols and octylphenols. The breakdown of APEOs during secondary biological wastewater treatment has been the subject of extensive investigation [10-14], however, the precise mechanism(s) of breakdown and the factors controlling this are not fully understood [7]. Considerable importance is attached to the formation and subsequent breakdown of carboxylated species in determining residual effluent concentrations. It has been established that in laboratory generated activated sludge APEO degradation is an intracellular biological process [14]. However, as presently operated, the ability of biological wastewater treatment to fully remove APEOs and their breakdown products is limited [15]. When discharged to the aquatic environment via sewage effluent the compounds may undergo further degradation leading to the formation of more persistent compounds such as nonylphenol [5]. During wastewater treatment some of the APEOs and their metabolites are absorbed to solid phases, crude sewage solids in the case of primary sludge and bacterial cells in the case of secondary sludge. High sorption coefficients (Log K_{ow} 4.12 – 4.48) for lipophlic nonyl- and octyl-phenolic compounds (NP, $NP_{1-2}EO$, OP and $OP_{1-2}EO$) in particular, result in adsorption of these compounds onto primary and secondary sludge solids (return activated sludge - RAS/ surplus activated sludge - SAS). Therefore these compounds, which will then be removed with the sewage sludges and subjected to further treatment prior to re-use, should be analysed in sludge samples.

The determination of these compounds in wastewater matrices is well established [8, 16-20]. However, it is necessary to analyse these compounds in sewage sludge, including their breakdown products and particularly the carboxylated species, if a full assessment of their environmental significance is to be made. Whilst

substantial data and methods exist for the determination of estrogens in sewage sludges and river sediments [7, 21], there are significantly less equivalent comprehensive methods for APEOs and their breakdown products in these matrices. This is due to the complexity of the sludge matrix and the major problems this poses to the extraction and clean-up of samples and the analysis of all organic micropollutants [22, 23].

Different analytical protocols have been proposed for the extraction of APEOs and their degradation products from solid samples (sewage sludge, sediment, soil) and have been extensively reviewed [17]. Numerous co-extractives interfere in the analysis of the target analytes, and the requirement for trace quantification, necessitates extensive extraction, clean-up and the application of sophisticated instrumentation to quantify these compounds. Only a few methods permit the simultaneous extraction and determination of the parent compounds and degradation products from solid environmental samples. These include sonication [24] or pressurized liquid extraction (PLE) [25, 26]. The main drawback of PLE is the high initial investment in PLE equipment [27] whilst a previous study found lower APEO recoveries when employing the sonication extraction procedure compared to that using shaking extraction method [18].

A quantitative LC/MS/MS method to analyse these compounds in wastewaters has previously been reported [8] and this study aims to develop, evaluate and apply the approach to generate a robust methodology for the determination of these alkylphenolic compounds to include the polar carboxylic degradation products in a range of sewage sludge samples with the intention of minimising solvent use through the reduction of sample size. The impact of sample size and concentration factors on matrix effects has previously been observed to be of significance in relation to reducing matrix effects in the analysis of alklyphenols [8].

2. EXPERIMENTAL

2.1 Sewage samples

Sewage sludge samples were obtained from three sewage treatment works (STWs) for this study. All three were activated sludge plants (ASP), one carbonaceous (CAS), one a nitrifying/denitrifying plant (N/DN) and the third a

nitrifying/denitrifying plant with phosphorus removal (N/DN-P) plant. At the CAS plant both primary sludge and the primary and surplus activated sludge (SAS) mixed sludge prior to anaerobic digestion was sampled on two separate occasions 12 months apart (Sample A April 2007 and B April 2008). For the N/DN plant primary sludge and return activated sludge (RAS) was sampled. At the N/DN-P plant, in addition to primary and RAS samples, liquors from the primary sludge drum thickeners were sampled (Table 1).

Please insert Table 1 here

2.2 Reagents and chemicals

The technical 4-nonylphenol mixture of various chain isomers and 4-*tert*-octylphenol were obtained from Sigma-Aldrich (Gillingham, Dorset, UK). The long-chain APEO, NPEO (Igepal CO210, CO520, CO720) and OPEO (Igepal CA210, CA520, CA720) were available in a commercial surfactant mixture containing different oligomers also purchased from Sigma-Aldrich. The commercial NPEO and OPEO formulations contained oligomers with an average of 5 ethoxylate groups containing oligomers from 2 to 12 units. Nonyl- and octyl-phenoxy acetic acid (NP_1EC, OP_1EC), 4-nonyl- and octylphenolmono- and diethoxylate ($NP_{1-2}EO$, $OP_{1-2}EO$) were obtained from QMX Laboratories (Thaxted, Essex, UK). Standards for NP_2EC, NP_3EC, OP_2EC, OP_3EC were not commercially available. NP_2EC and NP_3EC were quantified with the NP_1EC standard, assuming equal response factors. Similarly OP_2EC and OP_3EC were quantified with OP_1EC.

Acetone, ethylacetate, acetonitrile, methanol (MeOH) and dichloromethane were obtained from Rathburn (Walkerburn, Scotland, UK) and acetic acid from Sigma–Aldrich. Single standard stock solutions were prepared in acetonitrile. Reagent grade MilliQ-water (18.2 MΩ) (Millipore, Watford, UK) was used for spikes and preparation of solutions. The working standard solutions were prepared by further diluting the stock standard solutions with acetonitrile/MilliQ-water (50:50 v/v).

2.3 Analytical procedure

Frozen sewage sludge was freeze-dried and alkylphenolic compounds were solvent extracted on a multi-reax system (Heidolph Instruments, Schwubach,

Germany). The sludge samples were extracted using 10ml MeOH/acetone (1:1) and mechanically shaken for half an hour in a 25 ml Teflon tube. This procedure was repeated twice with centrifugation at 1500 g for 10 min each time. The combined supernatants were then subjected to clean up by passing through a pre-conditioned 500mg/3cc silica SPE cartridge (Waters Ltd, Hertfordshire, UK) which was eluted using 10% acetic acid in 10ml MeOH prior to quantification by LC/ESI/MS/MS consisting of an HPLC (Waters Alliance HPLC system 2695) coupled to a Waters Quattro Premier XE mass spectrometer with a Z-Spray ESI source (Micromass, Manchester, UK.) as described previously [8]. For method recovery work freeze-dried sludges were spiked with the alkylphenolic compounds to give final concentrations of 0.125 mg kg^{-1} (low recovery, LR) or 1.25 mg kg^{-1} (high recovery, HR) samples.

3. RESULTS AND DISCUSSION

3.1 Method development

The determination of alkylphenolic compounds in the sewage sludge matrix was established using solvent extraction, clean up with silica solid phase extraction cartridges and quantification using liquid chromatography tandem mass spectrometry in ESI mode (LC/ESI/MS/MS). The method had previously been developed for laboratory generated activated sludge mixed liquor samples without including the carboxylated metabolites employing SPE and LC/ESI/MS [13]. The determination of alkylphenolic compounds in sludge samples was similar to the methodology established for the soluble alkylphenolic compounds in sewages as previously reported in Koh et al., [8].

In order to prevent biotransformation of the long chain alkylphenolic compounds, sludge samples were frozen and freeze-dried following collection. The STW sludge (0.2 g) was initially extracted with various solvent combinations which included dichloromethane, MeOH and acetone. The combination of MeOH and acetone (10 ml) in 1:1 ratio gave a superior recovery, which ranged from 50% to 90%. This was similar to those reported where recoveries between 64% to 94% were obtained using the same solvent combination on river sediments [26].

Two commercially available SPE cartridges, silica and an anion-exchanger NH_2, were considered for use in the clean-up step. Commonly used organic solvents

such as ethyl acetate, hexane, MeOH and a combination of each of these solvents were evaluated and gave recoveries >70% from using silica SPE and elution with neat MeOH. However, the addition of 10% acetic acid to MeOH gave the maximum recovery (77-105%) of alkylphenolic compounds while retaining most interfering co-extracted chemicals. A silica SPE clean-up step prior to LC/MS/MS analysis was found to remove further matrix interferences (7-15%), which is consistent with other studies using silica based clean up [28, 29]. Following elution of these compounds from the silica SPE, the alkylphenolic compounds were quantified using the LC/MS/MS as shown schematically in Figure 1.

Please insert Figure 1 here.

The optimum LC/MS/MS conditions used for compound identification on sludge samples were reached when the highest intensities or superior signal-to-noise (S/N) resolution of >3 were achieved for each alkylphenolic compound. A chromatogram illustrating the separation can be seen in Figure 2. Calibration standards obtained through linear regression was r^2 = 0.998 for AP, APEO and APEC. The instrument variability of the LC/MS/MS was <5%. Elution times for each compound in solid samples were reproducible (within ±0.2 min) which demonstrated the robustness of the liquid chromatography and that addition of ammonium hydroxide did not affect the elution time/interaction of the alkylphenolic compounds (hydrophilic/hydrophobic properties) from the stationary phase of the Gemini C18 column as previously described [8] and confirmed by [30, 31].

Please insert Figure 2 here.

3.2 Matrix effects

Subsequent to the evaluation of dry weight of solid samples on alkylphenolic compounds recovery, matrix interference was evaluated for sludge based on a 0.2 g primary sludge sample (CAS) and a blank using (MQ water) which were unspiked or spiked with the alkylphenolic compounds (low and high spike of 0.125 mg kg^{-1} and 1.25 mg kg^{-1} respectively). The signal suppression was derived using the following equation:

$$\frac{A_s - (A_{sp} - A_{usp})}{A_s} \times 100 \quad (1)$$

where *As* is the peak area of the analyte in pure standard solution, *Asp* is the peak area in the spiked matrix extract and *Ausp* is the peak area in the unspiked matrix extract. For the water extract, signal suppression of 6-10% was observed for all determinands in both low and high spikes. The overall method recoveries decreased by up to 20% for the high spiked compared to the low spiked sample, thus showing some matrix interferences still persisted. Significantly higher matrix interference was observed for primary and mixed primary and RAS sludge samples of up to 27% for $AP_{1\text{-}12}EO$. Greater matrix interference was observed for primary sewage sludge for hydrophilic long chain $AP_{3\text{-}12}EO$ of up to 27% and up to 24% for hydrophobic short chain alkylphenolic metabolites $AP_{1\text{-}2}EO$, $AP_{1\text{-}3}EC$ and APs. The impact of sample size and concentration factors on matrix effects has previously been observed to be of significance in relation to reducing matrix effects in the analysis of alklyphenols [32].

3.3 Method recovery

The recoveries and relative standard deviations were determined in experiments where the analytes were in the concentration range of low spike and high spike using MQ water and primary sludge samples. Recoveries ranged from 51% to 89% for AP, APEO and APEC for low and high spikes (Figure 3). Generally, the relative standard deviations (RSD) were in the range of 1 to 14. Recoveries for NPEO and OPEO averaged >70% with an RSD of 5 and 3 respectively for high spiked MQ water.

Please insert Figure 3 here.

Recoveries for NPEO and OPEO were 76% and 75% with an RSD of 6 and 5 respectively for low spiked sludge. Approximately <3% recovery values were observed for high spiked sludge compared to the low spiked sludge with low RSD (2 – 10) obtained for the long-chain $AP_{3\text{-}12}EO$ ($NP_{3\text{-}12}EO$ and $OP_{3\text{-}12}EO$). This methodology exhibits consistent recoveries and low RSD for primary sludge samples.

The method recovery for sludge samples was on a par with or slightly lower for $NP_{1-12}EO$ and $OP_{1-12}EO$ which averaged 76% and 73% compared to a study performed on $NP_{3-15}EO$ (78%) and $OP_{3-15}EO$ (80%) [24]. Nonylphenol recovery observed in this study of 70% and 73% compared well with that reported previously [24]. In contrast, lower recovery was observed in this study for OP which was 63% (Figure 3) in comparison to 75% [24]. Higher recovery values were obtained in this study for the carboxylated species at 72% and 73% compared to 61% and 65% for NP_1EC and OP_1EC respectively in Petrovic et al. [33].

3.4 Method detection limit

Method detection limit (MDL) was determined by subjecting the entire analytical extraction and detection procedure to evaluation using 0.2 g sludge samples. The MDL determined for the entire SPE and LC/MS/MS method ranged from 6 to 60 μg kg^{-1} for AP, APEO and APEC (Table 2). Generally, lower MDLs were achieved for nonylphenol compounds compared to the octylphenol analogues.

Please insert Table 2 here.

A comparison of this method (Table 2) with other methodologies based on MDL indicates that sensitivity was equivalent to other methods, with the exception of NP and NP_1EC [34]. The MDL for NP and NP_1EC in this study were 11 μg kg^{-1} (RSD=5%) and 12 μg kg^{-1} (RSD=2%) respectively whilst corresponding MDL of 0.5 μg kg^{-1} and 1.5 μg kg^{-1} were obtained by Petrovic et al [34]. However, these RSD values of 5% and 2% were lower in comparison to Petrovic et al., [34] at 9% and 8% respectively for NP and NP_1EC. At present, the high concentrations of APEOs commonly found in sludge imply that the current detection method is sensitive enough and achieves a higher degree of accuracy and reliability in terms of RSD in comparison to other reported methods (Table 2). This could be a direct result of using smaller sample size than those commonly employed of 2 g for sewage sludges. The significance of sample size on method performance has been previously demonstrated in sewage sludge and it was observed that minimising sample size reduces matrix interferences and gives low RSD [32].

3.5 Application to sludge samples

To test the validity of the method, alkylphenolic compounds in primary sludges, primary and surplus activated sludge mixed sludges, return activated sludge (RAS) and drum thickener sludge liquors from both CAS and N/DN and N/DN-P configurations were analyzed. There were varying concentrations of alkylphenolic compounds in the primary and mixed sludges from the CAS and the concentrations were much lower than in RAS and drum thickener sludge liquors. There appears to have been some biodedegradation in the sewerage system which could account for the high concentration of NP_2EC at 26515 μg kg^{-1} in CAS (Table 3)

Please insert Table 3 here.

Nonylphenolic compounds were 2-3 fold higher in concentration compared to their octyl analogues. The estrogenic non-ionic surfactant metabolite by-product NP and OP were found in the various sludge samples. Octylphenol was below the MDL detection limit in a number of samples up to a maximum of 237 μg kg^{-1} in the drum thickener sludge liquors (Table 3). Nonylphenol existed in the return activated sludge in both the N/DN-P and N/DN at 2087 μg kg^{-1} and 6696 μg kg^{-1} respectively but <1920 μg kg^{-1} for primary sludge, mixed sludge and drum thickener sludge liquors Since most of these compounds, due to their physiochemical properties, may preferentially bind to the sludge particulates, removal of these solids will improve the removal efficiency for these compounds [35, 36]. Furthermore, the implication of this finding is that sedimentation will result in the accumulation of the non-polar hydrophobic AP and short chain APEO in sludge and have further repercussions for sludge treatment and re-use. In the majority of the STWs, there were significant amounts of $NP_{1-2}EC$ compared to $OP_{1-2}EC$ in the RAS of the N/DN and N/DN-P works. The carboxylated products ($AP_{1-3}EC$) were present in relatively high concentrations in the RAS ($NP_{1-2}EC$=1978-4673 μg kg^{-1}, $OP_{1-2}EC$=<LOD-53 μg Kg^{-1}) and were not necessarily efficiently removed during activated sludge wastewater treatment [37].

Since the voluntary agreement to withdraw the use of these non-ionic surfactants and also the replacement to alcohol ethoxylates, worldwide concentration of APEOs and subsequently APECs have declined, as reflected by the 2 to 3 order of magnitude decrease of NPEC (highest concentration 2.4 μg l^{-1}) in the STW effluent

discharging into River Schelde, Belgium [31]. In a recent study in Spain, NP showed a 10-fold decrease in concentration over the last 5 years [38]. This phenomenon was similarly observed in a recent assessment of alkylphenolic compounds in various UK sewage treatment works [39].

CONCLUSIONS

A solvent extraction followed by liquid chromatography tandem mass spectrometry was developed to determine non-ionic surfactant, alkylphenol polyethoxylates and their metabolites alkylphenoxy carboxylates and alkyphenols with high efficiency in sludge. These compounds were concentrated and extracted by methanol/acetone (1:1 v/v) in sludge, and extracts were cleaned up by silica solid phase extraction proceeding determination by liquid chromatography tandem mass spectrometry using analytical standards for quantification. The recovery for these target compounds, determined by spiking sewage sludge at two concentrations ranged from 50% to 89% with method detection limit from 6 μg kg^{-1} to 60 μg kg^{-1}. The methodology was subsequently applied to sludge samples obtained from a carbonaceous activated sludge plant, a nitrifying/denitrifying activated sludge plant and a nitrifying/denitrifying activated sludge plant with phosphorus removal. The nonylphenolic compounds were 2-3 fold higher in concentration compared to the octyl analogues. The estrogenic metabolite nonylphenol was present at concentrations ranging from 336 μg kg^{-1} to 6696 μg kg^{-1}. The assessment of the various sludges from the sewage treatment works established that carboxylated products ($AP_{1-3}EC$) were present in relatively high concentrations in the RAS ($NP_{1-2}EC$=1978-4673 μg kg^{-1}, $OP_{1-2}EC$=<LOD-53 μg kg^{-1}) probably due to recirculation in the system.

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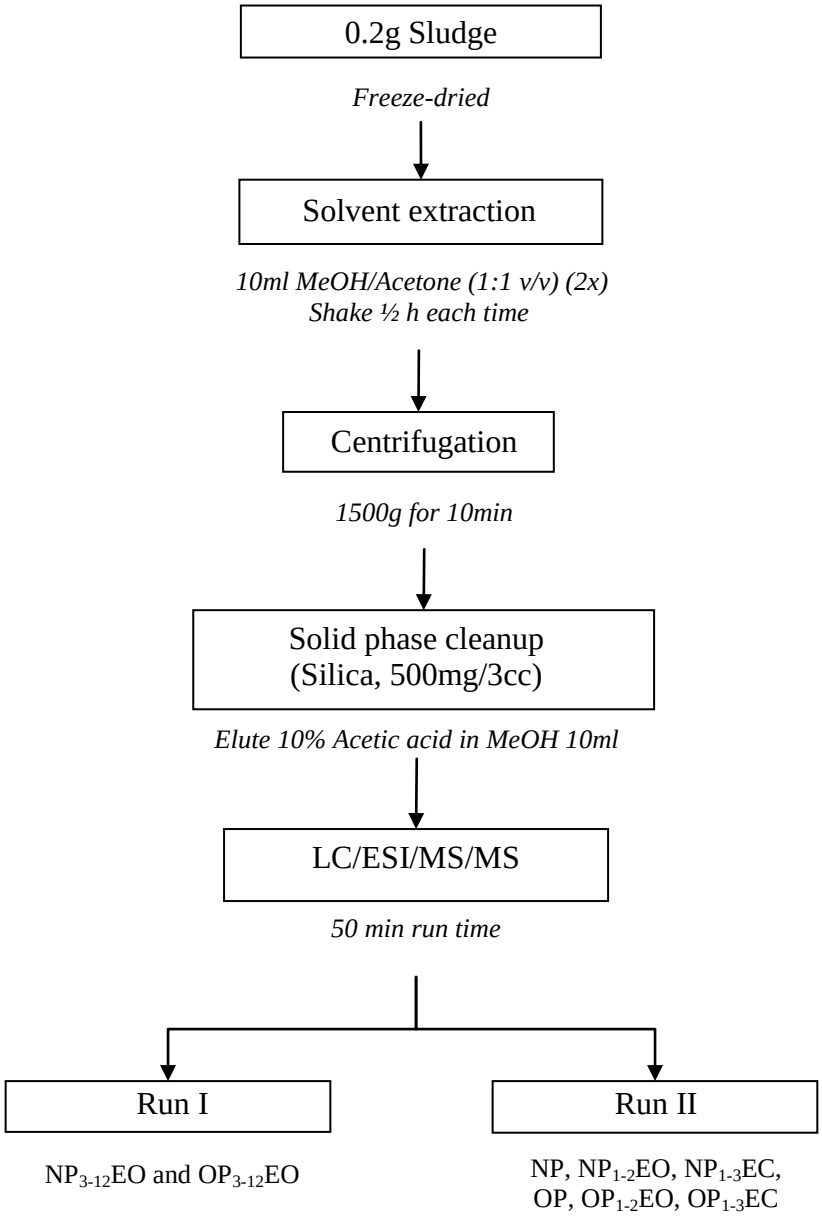

Figure 1. Scheme of the analytical method for extraction and determination of alkylphenolic compounds in sewage sludge.

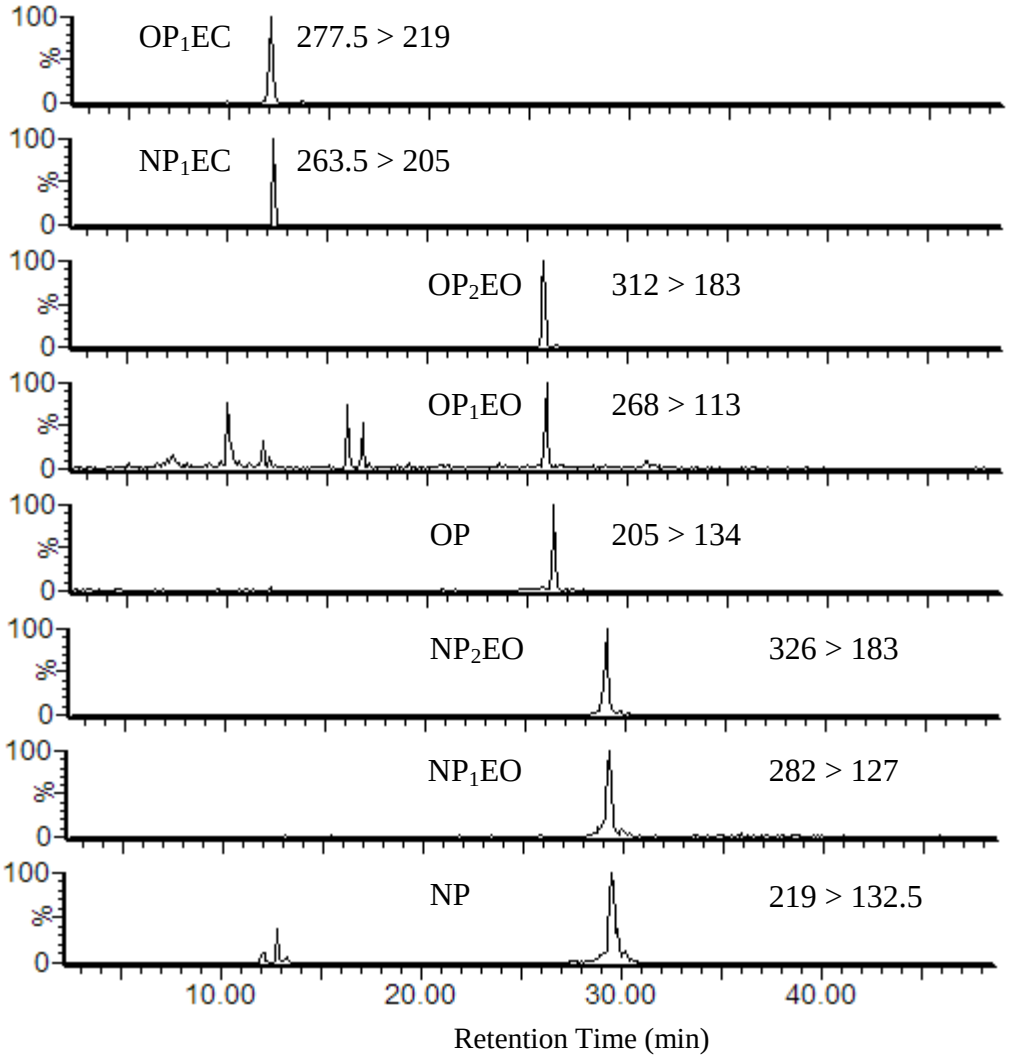

Figure 2. LC/MS/MS mass spectra of alkyphenolic compounds in 0.2 g dwt primary sludge sample spiked at 1.25 µg g^{-1} (only one product ion for each alkylphenolic oligomer is shown).

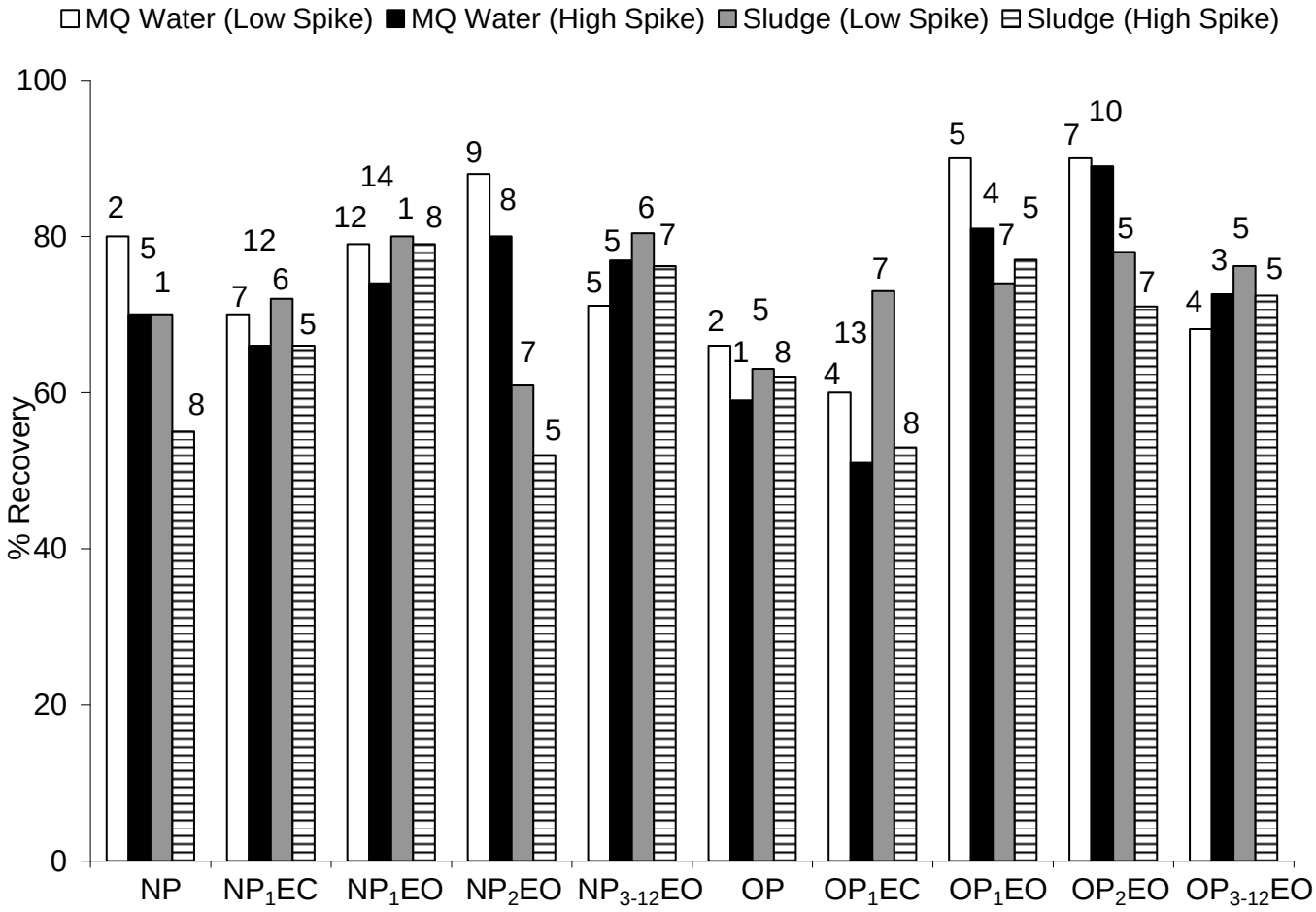

Figure 3. Method recoveries (%) and relative standard deviations (number at the top of each bar in %) in reagent water (MQ water) and primary sewage sludge (n=3) Data based on spiking at 0.125 mg kg^{-1} (low spike) and 1.25 mg kg^{-1} (high spike) of APs, AP_1EC, $AP_{1\text{-}2}EOs$ and $AP_{3\text{-}12}EOs$.

Table 1 Description of the sludge samples taken from each sewage treatment works

	Primary Sludge	Primary and Surplus Activated sludge (mixed)	Return Activated Sludge	Drum thickener primary sludge liquors
CAS Sample A (April 2007) and Sample B (April 2008)	✓	✓	×	×
N/DN	✓	×	✓	×
N/DN-P	✓	×	✓	✓

Note - CAS – Carbonaceous activated sludge plant; N/DN – nitrifying/denitrifying activated sludge plant; N/DN-P nitrifying/denitrifying with phosphorus removal activated sludge plant

Table 2. A summary of various analytical procedures for the analysis of alkylphenolic compounds in sewage sludge matrices.

Compound	Extraction	Detection method	MDL	Reference
NP, OP, $NP_{1-15}EO$, $OP_{1-15}EO$, $NP_{1-2}EC$, $OP_{1-2}EC$	C_{18} SPE extraction; clean up by sonication	LC/ESI/MS	5 – 25 μg kg^{-1}	Petrovic et al., 2001 [24]
$NP_{1-15}EO$, $OP_{1-15}EO$, NP, OP	Pressurized liquid extraction; clean up by C_{18} SPE	LC/ESI/MS	0.3 – 1 μg kg^{-1} (OPEOs & NPEOs) 30 μg kg^{-1} (OP & NP)	*Andreu et al., 2007 [40]
OP, NP, $NP_{1-15}EO$	Pressurized liquid extraction	LC/ESI/MS	5 to 23 mg kg^{-1}	Cespedes et al., 2008 [41]
NP, $NP_{1-2}EC$, $XAP_{1-2}EC$	Pressurized liquid extraction; clean up by C_{18} SPE	LC/ESI/MS/MS	0.5 μg kg^{-1} (NP) 1.5 μg kg^{-1} ($NP_{1-2}EC$)	Petrovic et al., 2003 [34]
OP, NP, $NP_{1-12}EO$, $OP_{1-12}EO$, $NP_{1-2}EC$, $OP_{1-2}EC$	Solvent extraction; clean up by silica SPE	LC/ESI/MS/MS	#11 μg kg^{-1} (NP) 12 μg kg^{-1} (NP_1EC) 6 μg kg^{-1} (NP_1EO) 12 μg kg^{-1} (NP_2EO) 7 μg kg^{-1} ($NP_{3-12}EO$) 34 μg kg^{-1} (OP) 60 μg kg^{-1} (OP_1EC) 48 μg kg^{-1} (OP_1EO) 25 μg kg^{-1} (OP_2EO) 8 μg kg^{-1} ($OP_{3-12}EO$)	This study, 2009

Note: ***** Methodology was based on soil sample amended with 10% sewage sludge; **#** Method detection limit of alkylphenolic compounds in freeze-dried 0.2g dwt primary digested sludge (*n*=3).

Table 3. Concentrations of alkylphenolic compounds in the sewage sludge samples from various STWs.

Alkylphenolic compounds (μg kg^{-1} dwt)	CAS				N/DN		N/DN-P		
	Primary Sludge (Sample A) (n = 5)	Primary and SAS Mixed (Sample A) (n = 5)	Primary Sludge (Sample B) (n = 5)	Primary SAS Mixed (Sample B) (n = 5)	Primary sludge (n = 3)	RAS (n = 4)	Primary Sludge (n = 4)	RAS (n = 4)	Drum thickener liquors (n = 4)
NP	304 (210 – 384)	157 (24 – 385)	232 (143 – 368)	133 (50 – 209)	378 (216 – 688)	6696 (4682 – 12093)	1307 (529 – 1652)	2087 (1572 – 2579)	1920 (551 – 3853)
NP_1EC	13 (12 – 14)	62 (26 – 107)	<LOD	28 (14 – 40)	<LOD	3318 (1801 – 6864)	20 (<LOD – 35)	2044 (674 – 2916)	38 (13 – 54)
NP_2EC	26515 (9644 – 89527)	241449 (201265 – 289544)	<LOD	37 (23 – 45)	585 (223 – 1128)	4673 (3564 – 5763)	121 (43 – 185)	1978 (674 – 3252)	506 (126 – 778)
NP_1EO	1950 (1138 – 2975)	1565 (987 – 2259)	14981 (14237 – 15889)	89533 (68558 – 102689)	309 (300 – 325)	4377 (1904 – 7844)	296 (137 – 427)	2812 (2022 – 3701)	1590 (452 – 2752)
NP_2EO	197 (112 – 269)	110 (73 – 175)	61 (29 – 117)	47 (30 - 67)	81 (47 – 133)	8995 (4489 – 20264)	87.4 (30 – 139)	2035 (1741 – 2583)	2536 (1445 – 4299)
$NP_{3\text{-}12}EO$	154	75	135	70	2702*	11754	698	1144	2311
OP	35 (35 – 36)	35 (34 – 38)	35 (34 – 36)	60 (48 – 69)	<LOD	<LOD	125 (<LOD – 208)	<LOD	237 (127 – 367)
OP_1EC	61 (60 – 63)	62 (60 – 65)	62 (60 – 64)	61 (60 – 62)	<LOD	117 (<LOD – 327)	<LOD	53 (<LOD – 112)	<LOD
OP_2EC	4374 (3982 – 5003)	16755 (15642 – 17692)	3659 (2460 – 4541)	13471 (11869 – 15079)	<LOD	<LOD	<LOD	<LOD	<LOD
OP_1EO	49 (48 – 51)	50 (49 – 52)	499 (375 – 672)	93 (52 – 163)	<LOD	1062 (<LOD – 4249)	<LOD	277 (<LOD – 561)	320.5 (222 – 446)
OP_2EO	25 (25 – 26)	26 (25 – 27)	125 (99 – 156)	112 (93 – 128)	<LOD	457 (136 – 1307)	<LOD	81 (<LOD – 112)	122 (64 – 184)
$OP_{3\text{-}12}EO$	196	194	83	37	129*	2017*	80*	375*	161*

Note: <LOD denotes below the MDL. To calculate the total concentration of the alkylphenolic compounds, half of the MDL was used for those that are <LOD. *When calculating the combination of alkylphenolic compounds, the concentration that is <LOD of a given analyte was set to half the MDL.

CAS – Carbonaceous activated sludge plant; N/DN – nitrifying/denitrifying activated sludge plant;N/DN-P nitrifying/denitrifying with phosphorus removal activated sludge plant; RAS – return activated sludge; SAS – surplus activated sludge.