



Equilibrium sampling reveals increasing thermodynamic potential of polycyclic aromatic hydrocarbons during sewage sludge digestion

Sjøholm, Karina Knudsmark; Nørgaard Schmidt, Stine; Jahnke, Annika; Svensmark, Bo; Mayer, Philipp

Published in:
Chemosphere

Link to article, DOI:
[10.1016/j.chemosphere.2018.05.104](https://doi.org/10.1016/j.chemosphere.2018.05.104)

Publication date:
2018

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Sjøholm, K. K., Nørgaard Schmidt, S., Jahnke, A., Svensmark, B., & Mayer, P. (2018). Equilibrium sampling reveals increasing thermodynamic potential of polycyclic aromatic hydrocarbons during sewage sludge digestion. *Chemosphere*, 207, 421-429. <https://doi.org/10.1016/j.chemosphere.2018.05.104>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Equilibrium sampling reveals increasing thermodynamic potential of polycyclic aromatic hydrocarbons during sewage sludge digestion

Karina K. [Sjoeholm](#)^{a, b, *}

jessing@plen.ku.dk

Stine N. [Schmidt](#)^{a, b}

stnsch@env.dtu.dk

Annika [Jahnke](#)^c

annika.jahnke@ufz.de

Bo [Svensmark](#)^b

svensmark@plen.ku.dk

Philipp [Mayer](#)^a

philm@env.dtu.dk

^aDepartment of Environmental Engineering, Technical University of Denmark, Bygningstorvet B115, DK-2800, Kgs, Lyngby, Denmark

^bDepartment of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871, Frederiksberg, Denmark

^cDepartment of Cell Toxicology, Helmholtz Center for Environmental Research GmbH - UFZ, Permoserstraße 15, DE-04318, Leipzig, Germany

*Corresponding author. Department of Environmental Engineering, Technical University of Denmark, Bygningstorvet B115, DK-2800, Kgs, Lyngby, Denmark

Handling Editor: Keith Maruya

Abstract

The reuse of digested sludge from wastewater treatment plants (WWTPs) as soil fertilizer poses a risk for contamination of soil and water environments. The present study provides a new approach for investigating the exposure of hydrophobic organic chemicals in sewage sludge. The methodology of equilibrium sampling with multiple thicknesses of silicone was successfully validated and applied to complex sludge matrices. Polycyclic aromatic hydrocarbon (PAH) concentrations in silicone ($C_{silicone}$) were determined and compared across four WWTPs. Activity ratios (ARs), defined as $C_{silicone}$ at equilibrium with digested sludge (final product) over $C_{silicone}$ at equilibrium with secondary sludge (intermediate product), were in the range 0.85–20 with all except one $AR > 1$. These ARs thus revealed increased thermodynamic potential of both parent and alkylated PAHs in digested sludge compared with secondary sludge, and thereby higher exposure of PAHs in sludge after digestion than before digestion. This observation can be explained by the concept of “solvent depletion” as organic matter decreased by a factor of 1.3 during digestion, resulting in reduced sorptive capacity and increased freely dissolved concentrations (C_{free}). The PAHs with $\log K_{ow} > 6$ had ARs close to 1.3, whereas PAHs with $\log K_{ow} < 6$ showed higher ARs than the organic matter decrease factor of 1.3. C_{free} in digested sludge were higher than reported in rural soil and generally consistent with levels reported for Baltic Sea sediment.

Keywords: Equilibrium sampling; Solvent depletion; Waste reuse; Biosolids; Digestion; Thermodynamic potential

1 Introduction

It is common practice to reuse digested sludge from wastewater treatment plants (WWTPs) as soil amendment due to its high content of plant nutrients, such as phosphate and organic matter. In Denmark, 59% of the produced sludge is applied on agricultural land, and in the EU 39% is reused in this way ([Milieu Ltd., 2010](#)). The percentage of reused sludge is expected to increase with an increasing awareness of the shortage of natural resources and as the philosophy of circular economy gains ground. However, the reuse of sludge poses a risk for contamination of soil and water environments as many organic contaminants sorb to the organic material in the sludge and are then transferred to agricultural land during sludge application ([Clarke and Smith, 2011](#)).

Polycyclic aromatic hydrocarbons (PAHs) are examples of hydrophobic organic chemicals (HOCs) that sorb to organic material in sludge. The PAHs constitute a complex group of neutral, nonpolar compounds consisting of fused aromatic rings with or without alkyl groups attached at different positions on the ring structure (Stout et al., 2002). PAHs are associated with various health risks, and their carcinogenicity is well documented for several of the compounds (Boström et al., 2002). PAHs enter WWTPs via industrial effluent, domestic wastewater, and dry and wet deposition. For European areas, the total content of the 16 US EPA priority PAHs in sewage sludge is typically reported in the low mg/kg dry weight (DW) range (Busetti et al., 2006; Ozcan et al., 2013; Villar et al., 2009). Sludge amendment has been reported as an important PAH source to soil with the risk of causing substantially elevated soil levels (Oleszczuk and Baran, 2005). When applying sludge as soil fertilizer, the maximum allowed total PAH concentration is 6 mg/kg DW in the EU and 3 mg/kg DW in Denmark. However, “total PAH” in the context of this legislation includes only nine PAHs, of which none is alkylated (Milieu Ltd., 2010).

A typical wastewater treatment process in Danish WWTPs proceeds as follows: The influent passes through a primary settling tank and is then led through an aeration tank, where secondary sludge settles. The secondary sludge is then digested anaerobically at elevated temperatures. This digestion process typically proceeds over three weeks, and the final product is digested sludge. Anaerobic digestion is a low-cost, energy-efficient way to reduce excess organic material in the sewage sludge, leaving mineralized compounds like ammonium, phosphates and sulfides in solution (van Lier et al., 2008), and it involves several biochemical reactions performed by specialized bacterial consortia. Hydrolysis is generally the rate-limiting biochemical step in the overall digestion process (van Lier et al., 2008). However, the anaerobic digestion process does hardly degrade organic pollutants like PAHs (Larsen et al., 2009). After digestion, the sludge can be dewatered in different ways before further handling (incineration, composting, or use as soil amendment), and the water is either recycled in the WWTP or discarded as effluent. The discard of effluent is considered environmentally safe, as all problematic compounds (like the PAHs) are thought to be either degraded in the aerobic WWTP steps or strongly sorbed to the organic matter in the sludge and thereby essentially removed from the aqueous phase.

PAH measurements in, e.g., sludge, sediment, and soil are most often reported as the total compound concentration (mg/kg or µg/kg) obtained based on exhaustive extraction. However, the total concentration does not give any information on the availability of the compound for bio-uptake nor the fraction of chemical that can be mobilized or take part in transformation processes (Reichenberg and Mayer, 2006). In addition, total concentrations are difficult to compare between similar matrices with very heterogeneous compositions (e.g., sludge or soil) and between different matrix types (e.g., air, water, soil, and human tissue). The freely dissolved concentration (C_{free}) and chemical activity (a) are thus more meaningful parameters for characterizing and quantifying exposure (Gobas et al., 2018). C_{free} is proportional to chemical activity, which quantifies the energetic state of a HOC relative to its reference state ($a = 1$) and thus expresses its thermodynamic potential (Schwarzenbach et al., 2002). The chemical activity of a substance is equal in all phases of an equilibrated system, and differences in chemical activity (i.e., differences in thermodynamic potential) drive spontaneous processes such as diffusion and partitioning (Reichenberg and Mayer, 2006). These rules are true regardless of the heterogeneity, the organic matter quality, and the sorptive capacity of the matrix. Assessing ratios in chemical activities between environmental media can thus be utilized to investigate environmental processes and to discover new phenomena: Jahnke et al. (2014) measured the thermodynamic potential of polychlorinated biphenyls (PCBs) in sediment and eels from the same lake and found PCBs in eel to be under-equilibrated relative to the sediment even though the biota to sediment accumulation factors exceeded one. Gilbert et al. (2014) found motile microorganisms to co-transport PAHs within a chemical activity gradient of 1 mm and by that enhance mass transfer of HOCs through diffusive boundary layers. Smith et al. (2013) found that PAHs exerting no or limited toxicity at saturation when tested individually can form toxic mixtures due to solubility and activity addition. † To the best of our knowledge, studies on chemical activity ratios of HOCs in WWTP sludge have not yet been reported.

Chemical activity can be determined with equilibrium sampling devices that utilize the partitioning of freely dissolved molecules between a matrix and a polymer reference phase (Mayer et al., 2003). The partitioning of molecules between matrix and polymer is driven by differences in the initially low chemical activity of the compound in the polymer compared to the higher chemical activity in the sample (Legind et al., 2007), until reaching a thermodynamic equilibrium (i.e., the same chemical activity in polymer and sample).

Artola-Garicano et al. (2003) applied Direct Immersion Solid Phase Microextraction (DI-SPME) to measure the C_{free} of polycyclic musks in aqueous suspensions of WWTP sludge. They investigated various fractions from influent to secondary sludge, which was the final product in the studied WWTPs. They found (1) that C_{free} varied much less than C_{total} during the treatment process and (2) that C_{free} were largely constant and in some cases even increased between influent and effluent despite a high removal rate of these compounds due to sorption to sludge constituents. This observation leads to the crucial question of what actually happens to the sludge-bound HOCs in the digestion process following the aerobic WWTP steps. Investigating this question, however, requires new analytical methods, since a major limitation of DI-SPME is the risk of damaging the fiber during agitation in solid matrices with high viscosity (Souza-Silva et al., 2015). So far other passive sampling methods have not been used for the analysis of trace contaminants in WWTP sludge (Lindholm-Lehto et al., 2017).

The present study applied negligible depletion equilibrium sampling with silicone-coated jars of multiple coating thicknesses (Reichenberg et al., 2008; Reichenberg and Mayer, 2006) to secondary and digested sludge. This equilibrium sampling methodology has a built-in quality control for (1) confirming equilibrium sampling without time series measurements and (2) detecting the absence of artefacts, such as silicone surface abrasion, adsorption on the silicone surface, and sample depletion. Due to proportionality between PAH concentrations in silicone ($C_{silicone}$) and chemical activity, $C_{silicone}$ values can without further conversion give valuable information as changes in $C_{silicone}$ follow changes in the thermodynamic potential (Reichenberg et al., 2008). Further, C_{free} can be determined from $C_{silicone}$ using silicone- and analyte-specific silicone to water partition coefficients ($K_{silicone, water}$):

$$C_{free} = \frac{C_{silicone}}{K_{silicone,water}}$$

Equilibrium sampling using jars coated with multiple thicknesses of silicone has successfully been applied for studying HOCs in soil (Reichenberg et al., 2008; Mäenpää et al., 2011), marine (Jahnke et al., 2012; Schmidt et al., 2017), lake (Jahnke et al., 2014), and river sediments (Schäfer et al., 2015).

Parent and alkylated PAHs were used as model HOCs. The objectives of the present study were: *i)* validation of the equilibrium sampling methodology when applied on sewage sludge, *ii)* determination of activity ratios to quantify differences in the thermodynamic potential of target PAHs within and between four different WWTPs, and *iii)* comparison of PAH exposure levels in sludge from Danish WWTPs to environmental background levels. The technical hypothesis was that coated jars with multiple silicone thicknesses enable equilibrium sampling of PAHs in very complex sludge matrices. If so, equilibrium sampling can reveal differences between the thermodynamic potential of PAHs in secondary and digested sludge. The scientific hypothesis was that chemical activities of PAHs remain rather constant during the digestion process due to limited PAH degradation and buffering of PAH exposure by the matrix. Contrarily, decreases in chemical activities would indicate PAH degradation or increased sorption, whereas increasing chemical activities could indicate a decrease in sorptive capacity.

2 Materials and methods

2.1 Chemicals and solvents

PAH quantification standard consisted of 19 parent PAHs including the 16 US EPA PAHs and benzo(e)pyrene, perylene, and dibenzothiophene. 2-methylphenanthrene, 3,6-dimethylphenanthrene, 1,2,6-trimethylphenanthrene, and 1,2,6,9-tetramethylphenanthrene were used to quantify alkylated phenanthrenes. For a full list of PAHs used for quantification and the corresponding deuterated internal standards see Table A.1 (supplementary data). All PAHs and deuterated PAHs were acquired from CIL (Cambridge, UK, purity >97%). From the mixture of unlabeled PAHs, seven calibration solutions were prepared in isooctane (pro analysis grade, Merck) in the concentration range 3–300 ng/mL. The mixture of deuterated internal standards was prepared in isooctane and had a concentration of each compound of 300 ng/mL. The PAH quantification standard and deuterated internal standard mixture were further diluted 20 times in ethyl acetate (>99.7%, Sigma-Aldrich) before addition of 200 µL to each of the 4 mL extracts. The final internal standard concentration was 0.75 ng/mL.

2.2 Sludge samples

Secondary and digested sludge samples were collected from three WWTPs in Denmark in October 2014. From a fourth Danish WWTP, dewatered digested sludge was sampled in July 2014 and used in a small validation test. All studied WWTPs are of the type mechanical, biological nitrification, denitrification, and chemical treatment and perform anaerobic digestion of the sludge. Digested sludge from WWTPs I–III is used directly as soil amendment, whereas sludge from WWTP IV is composted for ten weeks to reduce levels of regulated contaminants before land application. See Table 1 for a detailed description of the four WWTPs. All samplings were performed once as grab sampling from large containers with continuous circulation, and the samples are thus considered representative. For secondary sludge, the sampling was performed by grabbing sludge from the top of large basins with a plastic bucket. For digested sludge, the sampling was performed by tapping from the bottom of the digestion tank. In both cases, approximately 10 L sludge was collected. At WWTP IV, the dewatered digested sludge was collected from the rotor snail just before the sludge entered trucks for transportation away from the WWTP. Approximately 5 kg dewatered digested sludge was sampled. In all cases, 20-L plastic buckets were used as sampling and storage containers. The sludge samples were stored at 4 °C in a temperature-controlled room overnight before equilibrium sampling was started (within 24 h). Dry weight was determined gravimetrically by weighing sludge before and after drying at 105 °C for 24 h. The content of organic matter was determined gravimetrically by weighing sludge before and after loss on ignition of the dried sludge at 550 °C over night (Nelson and Sommers, 1996).

Table 1 Characteristics of the studied WWTPs, total PAH concentrations, and physical parameters.

WWTP	Size, PE ^a :	Catchment area:	PAH concentrations, exhaustive extraction in digested sludge (mg/kg DW) ^{b,c}										Sludge sample type:	Physical parameters	
			Ace	Flo	Phe	Flu	Pyr	BbF	BaP	Ipyr	BghiP	Total PAH		DW (%) ^d	Organic matter (%) ^e
I	200.000	Mixed domestic and industry	0.06	0.12	0.56	0.78	0.71	0.76 ^f	0.43	0.26	0.32	4.0 ^g	Secondary	1.9 ± 0.35	66 ± 0.12
													Digested	3.1 ± 0.08	52 ± 0.12
II	420.000	Mixed domestic and industry										0.7	Secondary	1.7 ± 0.01	73 ± 0.63
													Digested	4.5 ± 0.01	62 ± 0.13
III	30.000	Domestic	<0.05	<0.05	0.257	0.504	0.469	0.345	0.221	0.137	0.279	2.6 ^h	Secondary	2.2 ± 0.15	68 ± 0.20

													Digested	2.9 ± 0.04	47 ± 0.46
IV	120.000	Mixed domestic and industry	<0.05	<0.05	0.545	0.416	0.324	0.215	0.151	0.108	0.128	2.0	Digested and dewatered	29.1 ⁱ	47.7 ⁱ

^a PE: Population Equivalents.

^b Ace: Acenaphthene, Flo: Fluorene, Phe: Phenanthrene, Flu: Fluoranthene, Pyr: Pyrene, BbF: Benzo(*b*)fluoranthene, BaP: Benz(*a*)pyrene, Ipyr: Indeno(1,2,3-*cd*)pyrene, BghiP: Benzo(ghi)perylene.

^c Total PAH concentrations are determined regularly in sludge from Danish WWTPs, and the data were kindly provided by the four WWTPs. The data are based on a composite sample obtained by grab sampling once a day for ten days distributed over a month. For WWTP I, the analysis was performed by Eurofins Miljø A/S (Ladelundvej 85, DK-6600 Vejen, Denmark) according to their M 0068 GC/MS method. For WWTPs III and IV, the analysis was performed by the ISO-accredited AGROLAB (Dr.-Pauling-Str. 3, DE-84079 Bruckberg, Germany) according to the VDLUFA VII, 3.3.3 method.

^d Dry weight was determined gravimetrically by weighing sludge before and after drying at 105 °C for 24 h (±absolute deviation, $n = 2$).

^e Content of organic matter (percentage of dry weight) was determined by loss on ignition at 550 °C for 24 h (±absolute deviation, $n = 2$).

^f Benzo(*b + j + k*)fluoranthene.

^g The specific batch of sludge was not used as fertilizer as the total PAH content exceeded Danish threshold values (max 3.0 mg/kg DW).

^h This value includes benzo(*j*)fluoranthene and benzo(*k*)fluoranthene.

ⁱ Data provided by the WWTP.

2.3 Equilibrium sampling

120-mL amber jars (Identipack B.V., the Netherlands) were coated on their inner vertical walls with different silicone coating thicknesses as described in detail by [Schmidt et al. \(2017\)](#). The jars were closed with airtight lids with PTFE lining (Identipack B.V., the Netherlands). The exact mass of silicone in each jar after curing and cleaning with 1 × 20 mL ethanol and 2 × 20 mL ethyl acetate (each for 30 min) was determined gravimetrically using a four digit balance. The corresponding average silicone thicknesses were 2.5 ± 0.2 μm ($n = 9$), 4.1 ± 0.4 μm ($n = 12$), 7.2 ± 0.5 μm ($n = 9$), and 13.5 ± 1.1 μm ($n = 11$), respectively.

The total of six secondary and digested sludge samples (each 10 L) from WWTPs I-III were homogenized before transfer of 100 g sludge aliquots of each of the samples to coated jars with 2.5, 4.1, 7.2, and 13.5 μm silicone, respectively. One mL aqueous 2% sodium azide (≥99%, Riedel-de Haën, Germany) solution was added to each jar to inhibit biodegradation and gas development during the time of equilibration. The jars were then sealed tight and rolled horizontally on a Ratek roller mixer (Ratek Instruments Pty Ltd, Australia) at 20 °C at the maximum speed of 60 rpm for three weeks. Coated jars with 99 mL deionized water and 1 mL 2% sodium azide solution were used as controls and treated like the jars with sludge.

For the validation test with dewatered digested sludge from WWTP IV, 20 g sludge was transferred to each jar with a metal spatula and 80 mL 0.0125% sodium azide solution was added before rolling. One set of jars was equilibrated for one week and another set of jars for three weeks in order to enable the comparison of $C_{silicone}$ obtained after different equilibration times. Coated jars with 20 mL deionized water and 80 mL 0.0125% sodium azide solution served as controls for this test.

After equilibration, the sludge was poured off and the jars were vigorously shaken with 2 × 2 mL deionized water before they were gently wiped with lint-free tissue to remove any remaining sludge and water. After cleaning, the sealed jars were stored at 4 °C until extraction. The jars were extracted within a week after equilibration using the following procedure: 2 mL ethyl acetate (GCMS grade, Merck, SupraSolv, Germany) was added to each jar, the jars were sealed and then rolled horizontally at 60 rpm for 30 min. The extracts were transferred with glass Pasteur pipettes to 10-mL glass vials with air tight lids. The extraction was repeated with a fresh portion of 2 mL ethyl acetate and the extracts were combined and stored at −80 °C until chemical analysis.

2.4 Chemical analysis

Without further treatment than addition of internal standards, the extracts were analyzed using a method based on gas chromatography coupled to mass spectrometry with electron ionization (GC-MS EI) as described in detail by [Gallotta and Christensen \(2012\)](#). The oven program was modified and a Programmed Temperature Vaporizing (PTV) solvent inlet was used instead of a split/splitless inlet to allow large volume injection. The injection volume was 20 μL. Detailed information on the GC-MS method is provided in [Appendix A](#) (Should this be "supplementary data" instead of Appendix A?). Both procedural blanks consisting of pure ethyl acetate and the extracts from the control jars were included in the chemical analysis.

Enhanced ChemStation, MSD ChemStation D.03.00.611 (Agilent Technologies), was used to integrate peaks, which were all manually checked. Calibration curves were linear and forced through the origin as the intercepts

were not significantly different from zero. Decision limits (*DLs*), defined as the minimum single result which, with a stated probability, can be distinguished from a suitable blank value, were used as detection limits (IUPAC, 2010). *DLs* were calculated using the formula:

$$DL = \frac{t_{\alpha,d.f.,1} \times s}{a} \quad (2)$$

where $t_{\alpha,d.f.,1}$ is the critical value of the *t* distribution at α significance level, i.e., the risk of obtaining a result $> DL$ for a blank sample with $n-1$ degrees of freedom (*d.f.*), *s* is the standard deviation on the lowest calibration concentration from four runs, and *a* the average slope of the calibration curves from these four runs. All data above *DL* with a significance level of 10%, i.e., confidence interval at 90%, was used. However, for perylene, indeno(1,2,3-*cd*)pyrene, dibenzo(a,h)anthracene, and benzo(ghi)perylene all data were used even though a few points were below the *DL* (but $> DL$ when the significance level was set to 20%), as the resulting single points of PAH masses were used in the function PAH mass plotted *versus* silicone mass subjected to quality control, as described in section 3.1. below. *DLs* ($\alpha = 10\%$) ranged from 0.03 to 1.99 ng/jar (see all *DLs* in Table A.2, supplementary data).

2.5 Determination of K_D values

Sludge to water distribution coefficients, K_D , were determined based on experimental data and compared to estimated K_D values. Experimental K_D values were calculated as the ratio of total concentration over C_{free} for PAHs where data was available. Total concentration data was kindly provided by the WWTPs and is listed in Table 1, and C_{free} was calculated from Eq. (1) using the $C_{silicone}$ data obtained by equilibrium sampling and silicone to water partition coefficients by Humel et al. (2017).

K_D values were estimated using the equation:

$$K_D = f_{oc} \times K_{oc} \quad (3)$$

where f_{oc} is the fraction of organic carbon in the sludge and K_{oc} is the organic carbon to water partition coefficient, obtained from EPI Suite (US EPA, 2017). As only the fraction of organic matter (and not the fraction of organic carbon) was measured on the studied samples, f_{oc} was estimated based on a reported total organic carbon content in digested sewage sludge of 344 mg/g DW (Svahn and Björklund, 2015), and the determined organic matter content in digested sludge from WWTPs I-III (Table 1). The average estimated f_{oc} was 0.67 which is in good agreement with the fraction of organic carbon in natural organic matter, generally being in the range of 40–60% (Schwarzenbach et al., 2002).

3 Results and discussion

3.1 Method validation

The secondary and digested sludge samples from WWTPs I-III were equilibrated for three weeks in jars with identical geometry but different silicone masses. The measured mass of a given PAH (ng) was plotted against the exact silicone mass (g) for the particular coated jar applied, and a linear regression (unweighted, forced through the origin) was then fitted to these data (Fig. 1). The slope of this regression is $C_{silicone}$ expressed in ng/g. Proportionality confirmed equilibrium as well as the absence of sampling artefacts resulting from, e.g., analyte depletion or silicone abrasion (Reichenberg et al., 2008). Equilibrium concentrations were (1) deemed valid when $R^2 > 0.60$ and the relative standard error (RSE) $< 20\%$ and (2) categorized as highly precise when $R^2 > 0.70$ and $RSE < 10\%$ (Schmidt et al., 2017). Examples of highly precise, valid, and non-valid concentrations are provided in supplementary data (Fig. A.1). Of all determined concentrations, 83% were deemed valid and of these 71% were categorized as highly precise.

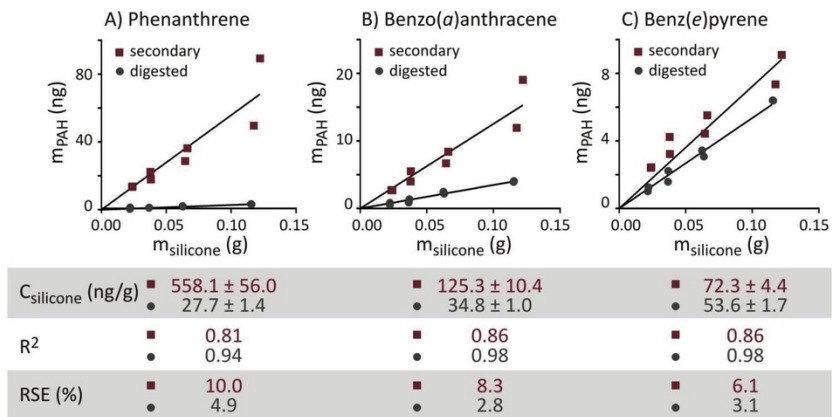


Fig. 1 Masses of three measured PAHs (ng) plotted against the silicone mass (g) in the jars equilibrated with sludge. A) Phenanthrene, B) Benzo(a)anthracene, and C) Benz(e)pyrene, representative for 3-ringed, 4-ringed, and 5-ringed PAHs. Proportionality confirms equilibrium partitioning of the analytes between silicone and sludge, and the slope is $C_{\text{silicone}} = \text{sludge}$. Circles: secondary sludge. Squares: digested sludge. Data from WWTP I. All data series were categorized as “highly precise”, except for phenanthrene in digested sludge which was categorized as “valid”. $C_{\text{silicone}} = \text{sludge} \pm \text{SEM}$ (ng/g), R^2 and RSE (%) for the shown data are provided below the graph.

alt-text: Fig. 1

For the validation test with sludge from WWTP IV, equilibrium sampling was performed for one and for three weeks. Fig. 2 shows C_{silicone} equilibrated with dewatered digested sludge for three weeks against the same measure for one week of equilibration. All C_{silicone} values, with benzo(k)fluoranthene as the only exception, were the same or slightly elevated (within a factor two) after three weeks of equilibration compared to results obtained after one week. Using a t -test for different slopes of two lines, 17 out of the 23 analyzed PAHs had no statistically significantly different C_{silicone} when equilibrated for one week compared to three weeks. Although all jars with sludge from WWTPs I-III were equilibrated for three weeks, the validation test confirms one week to be sufficient time for equilibrium sampling of PAHs in sludge. The quality of the data in Fig. 2 demonstrates the good quality of the methodology, both the equilibrium sampling and the chemical analysis.

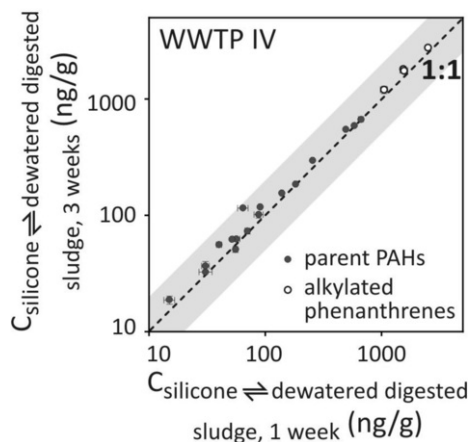


Fig. 2 For WWTP IV, equilibrium sampling was performed for one and three weeks on the same sludge sample. This graph shows C_{silicone} when equilibrated with dewatered digested sludge for three weeks against the same measure for one week of equilibration. The solid line is 1:1. Shaded area indicates a factor 2 deviation from the solid line. Closed symbols are parent PAHs, and open symbols are alkylated phenanthrenes. Error bars are SEM of the linear regression used to obtain C_{silicone} and are in many cases smaller than the symbols.

alt-text: Fig. 2

3.2 Thermodynamic levels and activity ratios of PAHs

The level and range in $C_{silicone}$ for the parent PAHs in digested sludge was similar in the four different WWTPs (WWTP I: 10-558 ng/g, WWTP II: 24-233 ng/g, WWTP III: 48-555 ng/g, and WWTP IV: 19-670 ng/g). For digested sludge, phenanthrene, fluoranthene, and pyrene had the highest $C_{silicone}$ in all four WWTPs (All $C_{silicone}$ values are shown in Table A.3, supplementary data). This observation correlates well with the reported high total concentrations for these three PAHs (Table 1). The level and range in $C_{silicone}$ for the alkylated phenanthrenes differed more in digested sludge, as WWTP I showed 589-1100 ng/g, WWTP II 548-2603 ng/g, WWTP III 1696-3526 ng/g, and WWTP IV 1202-2769 ng/g. To compare the thermodynamic potentials of PAHs in secondary and digested sludge, $C_{silicone}$ equilibrated with digested sludge ($C_{silicone=digested\ sludge}$) was plotted against $C_{silicone}$ equilibrated with secondary sludge ($C_{silicone=secondary\ sludge}$) in Fig. 3 (results from WWTP I-III). The activity ratio (AR) was defined as:

$$AR = \frac{C_{silicone \rightleftharpoons digested\ sludge}}{C_{silicone \rightleftharpoons secondary\ sludge}} \quad (4)$$

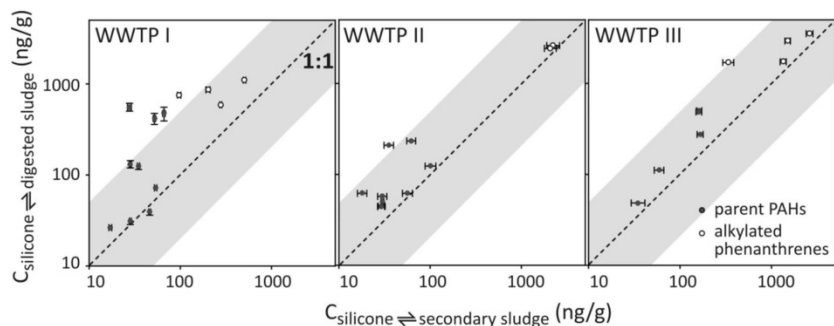


Fig. 3 Activity ratios (AR s) of parent (closed symbols) and alkylated (open symbols) PAHs for three Danish WWTPs, presented as $C_{silicone \rightleftharpoons digested\ sludge}$ against $C_{silicone \rightleftharpoons secondary\ sludge}$. The solid lines indicate equal AR s in digested and secondary sludge. Closed symbols are parent PAHs and open symbols are alkylated phenanthrenes. Error bars are the SEM of the linear regression used to obtain $C_{silicone}$ and are in many cases smaller than the symbols. Shaded areas are a factor 5 deviation from the 1:1 line.

alt-text: Fig. 3

The chemical activities in digested and secondary sludge are identical when AR is 1. With the exception of benzo(*b*)fluoranthene in WWTP I, all AR s were above one. Further, all AR s (except phenanthrene in WWTP I) were within a factor of 10. AR s above one thus revealed elevated thermodynamic potential of PAHs in digested compared to secondary sludge for all measured PAHs in all three WWTPs (total $n = 35$). The potential PAH exposure to organisms as well as the fraction of PAHs in the sludge available for uptake, leaching, and transformation processes was thus higher in digested compared with secondary sludge. Possible explanations for this observed increase in thermodynamic potential are: 1) Matrix heterogeneity and temporal variation in the PAH loading of the sludge, as the secondary and digested sludge from each WWTP were sampled at the same day and thus not from exactly the same sludge. 2) Formation of PAHs during the digestion process. 3) Decrease in sorptive capacity of the sludge matrix due to degradation of organic matter which leads to an increase in C_{free} (and thereby the thermodynamic potential). In the following, these possible explanations are discussed:

- 1 In previous studies, C_{free} showed less spatial heterogeneity and temporal variability than total concentrations (Artola-Garicano et al., 2003; Ter Laak et al., 2007), and were much less matrix-dependent. Measuring C_{free} thus reduces the potential problems arising from interpretation of highly variable total concentration data based on grab sampling. Major temporal variation in influent concentrations has previously been demonstrated to result from substantial rainfall leading to dilution (Fendinger et al., 1995). In our study, the six weeks prior sampling were relatively dry without extreme rainfall events (DMI, 2017), and exceptional variation in concentration levels in influent over time is thus not expected in the studied WWTPs. C_{free} of HOCs in suspensions compared to total concentrations are much less affected by dilution when the matrix buffers C_{free} (Ter Laak et al., 2007; Reichenberg and Mayer, 2006), based on observations by Artola-Garicano and coworkers (2003) on polycyclic musks in WWTP sludge suspensions. In addition to this, little variation was observed in PAH composition and temporal concentrations in WWTP influent in a recent Chinese study (Liu et al., 2017). Therefore, we expect the observed increase in chemical activity in digested compared with secondary sludge to be attributed to processes occurring during the digestion process rather than temporal variations in influent concentrations. Furthermore, the increased AR s are a consistent observation for all PAHs in all three WWTPs.
- 2 Besides having pyrogenic and petrogenic origin, selected PAHs can also form naturally by rapid diagenetic transformations of biological precursors. These biogenic PAHs include perylene, retene, and alkylated derivatives of chrysene and picene (Wakeham and Canuel, 2016). However, the increase in AR s in the present study was observed for all measured PAHs and not exclusively for perylene, the only biogenic PAH that was measured.
- 3 The degradation of organic carbon has earlier been used to explain an observed “disequilibrium” in chemical activities between environmental compartments (Jahnke et al., 2014). The content of organic matter in the studied sludge samples decreased by 11-21% (Table 1) during the sludge digestion process, and the observed consistent increase in chemical activity could thus be explained by a decrease in sorptive capacity. This observation is in principle analogous to the process of biomagnification, where the chemical activity of

HOCs increases in the gut of a predator compared to that in the prey because of the decrease of sorptive capacity, in that case digestion of lipids. In fact, the major part of the organic matter removed during the hydrolysis of the sludge digestion is lipids (van Lier et al., 2008), but as sludge digestion is a man-made process, the term “solvent depletion” seems more appropriate than “biomagnification”. Macdonald et al. (2002) introduced “solvent switching” and “solvent depletion” to explain bioconcentration and biomagnification in the environment. Solvent switching is basically describing partitioning, such as from water into organisms (bioconcentration) or to WWTP sludge. This phenomenon can lead to an increase in concentration but not in chemical activity. Contrarily, solvent depletion refers to the removal of part of the matrix that the chemical is dissolved in (as the digestion of lipids in biomagnification) and leads to increases in concentration and in chemical activity. Evaporative enrichment of solvent extracts in the analytical laboratory is a good example where solvent removal leads to an increase in analyte concentration and chemical activity. The concept of solvent depletion has recently been used to explain the increase in thermodynamic potential of persistent lipophilic organic pollutants in human serum/plasma during weight loss where lipid tissue is depleted (Li and Wania, 2017). For PAHs and other HOCs in the environment, it is the organic matter that serves as a “solvent” (i.e., the sorptive phase), and it is well established that part of this organic matter is degraded during the sludge digestion process (van Lier et al., 2008). The observed declines in organic matter (Table 1) and simultaneous increases in chemical activity (Fig. 3) during sludge digestion are in excellent agreement with the solvent depletion concept.

On average, the ratio of organic matter in secondary sludge to digested sludge was 1.3 ± 0.1 . It could then be expected that the observed *ARs* would be in the same range. However, in Fig. 4, the *ARs* are plotted against $\log K_{ow}$, showing the highest *ARs* for the less hydrophobic PAHs ($\log K_{ow} < 6$) and *ARs* close to 1.3 for the more hydrophobic PAHs ($\log K_{ow} > 6$).

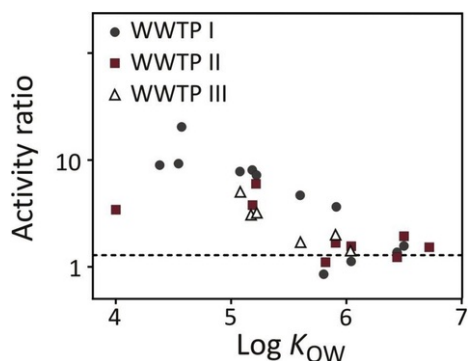


Fig. 4 Activity ratio (*AR*) as a function of the analyte $\log K_{ow}$ (Mackay et al., 2006) for WWTPs I-III (total $n = 27$). The broken line is 1.3 which corresponds to the secondary:digested organic matter ratio.

alt-text: Fig. 4

Furthermore, the K_D values determined from measured data are, with the exception of flourene in WWTPs III and IV, 0.4–2 log units higher than estimated K_D values derived from K_{oc} and the estimated fraction of organic carbon (shown in Fig. A.2., supplementary data). Although both the determined and the estimated K_D values are partly based on assumptions, the data indicate that simple phase partitioning into amorphous organic matter is not the only sorption mechanism for PAHs in WWTP sludge. Adsorption to high-affinity sites as, e.g., black carbon may be another important mechanism. The source of black carbon to WWTPs is road runoff and there might also be differences in the black carbon contribution to the wastewater. Further investigations and research are needed to elucidate the phenomenon of solvent depletion and its effect on thermodynamic potential of HOCs and its impact on complex matrices such as sewage sludge, which consist of several different sorptive phases (including lipids, amorphous carbon, black carbon, and dissolved organic carbon).

Time series measurements of the thermodynamic potential of PAHs and other HOCs, as well as measurements of changes in the different pools of sorbents during the sludge digestion process, are needed in future work to further elucidate the observed high *ARs* for PAH with $\log K_{ow} < 6$. The change in thermodynamic potential of other HOCs than PAHs, both during a digestion process and over the whole WWTP chain, is needed to better understand the treatment process with respect to HOCs and to better characterize exposure levels. From an environmental point of view, the less hydrophobic PAHs are less bioactive than the more hydrophobic PAHs, but the up to 20 fold increase in exposure concentrations in a matrix which is commonly used as soil amendment needs further investigation and consideration.

3.3 Comparison of freely dissolved PAH concentrations in digested sludge to environmental background levels

C_{free} was calculated in digested sludge from the four WWTPs by applying Eq. (1) and silicone-specific partition coefficients (Humel et al., 2017) (Table 2). As digested sludge is applied to agricultural land, sludge is a potential source of PAHs to the soil as well as to the aquatic environment, having the sea sediment as final recipient. If C_{free} (and thereby chemical activity) in the sludge was substantially higher than the reported levels in soil and sediment, then these environmental compartments could potentially act as a sink for the sludge associated PAHs. The optimal comparison would have been to C_{free} data from pore water in Danish soils as these are the recipients of the studied sludges. However, to the best of our knowledge, C_{free} values of PAHs have not been reported for Danish soils yet; hence, the comparison is made with data from Switzerland. The obtained C_{free} data (Table 2) were 4–9 times elevated compared to C_{free} in pore water of rural Swiss soils (Bartolomé et al., 2018), which was in the low ng/L range (Table 2). Lang et al. (2015) found that the sum of nine PAHs was 2.7–8.5 ng/L in the top 2 cm of Baltic Sea sediment, as determined by SPME.

C_{free} of anthracene, fluoranthene, and pyrene in digested sludge were at a similar level (1-3 ng/L) as reported in Baltic Sea sediment, whereas C_{free} for phenanthrene in the digested sludge was 2-3 times higher in three of the four studied WWTPs. For the fourth WWTP, the level was the same as in sediment (Table 2). Application of digested sludge to rural soils could potentially lead to increased PAH exposure as the C_{free} in sludge was higher than in the studied rural soils. As the determined C_{free} levels in digested sludge were at a similar level as Baltic Sea sediment levels for most compounds, the sludge investigated here could only be a potential source of phenanthrene. Comparing to industrially contaminated soils, PAH levels were on average 1000 (Bartolomé et al., 2018.) and 50-400 times (Humel et al., 2017) higher in Swiss soils and landfill-contaminated Austrian soil, respectively (Table 2) than the measured levels in the digested sludge.

Table 2 Freely dissolved concentrations of selected PAHs (ng/L) in digested sludge calculated from Eq. (1), applying partition coefficients from Humel et al., 2017 (Should be Humel et al., (2017)) For WWTP IV, C_{free} from both one and three weeks of equilibration are included in the table.

alt-text: Table 2

Location	C_{free} (ng/L)			
	Phenanthrene	Anthracene	Fluoranthene	Pyrene
WWTP I, 3 weeks	9.1	1.2	2.4	2.0
WWTP II, 3 weeks	2.2	0.8	1.1	1.1
WWTP III, 3 weeks	9.1	1.0	2.6	2.3
WWTP IV, 1 week	8.0	0.9	3.0	3.2
WWTP IV, 3 weeks	9.0	1.5	3.1	3.2
Baltic Sea sediment, close to Denmark ^a	3.9	0.8	2.2	1.7
Baltic Sea sediment, inner Gulf of Bothnia ^a	2.9	0.2	0.6	0.3
Swiss rural soil ^b	1.7	nd	0.28	0.35
Swiss skeet-contaminated soil ^b	2220	115	1500	969
Austrian landfill contaminated soil ^c	386	138	1012	755

^a Data from Lang et al. (2015).

^b Data from Bartolomé et al. (2018).

^c Data from Humel et al. (2017).

4 Conclusion

Equilibrium sampling with multiple coating thicknesses of silicone was extended to sewage sludge to investigate exposure levels of HOCs, represented by parent and alkylated PAHs. The methodology was successfully transferred to the very complex sludge matrix. Differences in thermodynamic potential were determined based on measured equilibrium concentrations of PAHs in silicone, which for parent PAHs were similar across four WWTPs when comparing the same type of sludge. The activity ratios between digested and secondary sludge were above one, indicating increased thermodynamic potential of both parent and alkylated PAHs in digested sludge. This means that the exposure concentration of PAHs was higher in digested sludge compared to secondary sludge, and this observation can be explained by solvent depletion: The organic matter content was reduced during the digestion process on average by factor 1.3, resulting in reduced sorptive capacity and increased C_{free} of PAHs. Plotting activity ratios against the corresponding $\log K_{ow}$ revealed the tendency that the activity ratios decreased with increasing hydrophobicity. PAH with $\log K_{ow} > 6$ had ARs close to the organic matter decrease factor of 1.3, whereas PAHs with $\log K_{ow} < 6$ showed higher ARs than 1.3. Comparing with existing data, application of digested sludge on rural soil could potentially be a source of PAH contamination as the C_{free} in sludge exceeded the reported levels in rural soil. Further research is needed in order to investigate the thermodynamic potentials of PAHs and other HOCs in WWTP sludge in terms of elucidating phenomena such as solvent depletion and to address the fate of HOCs in the soil environments receiving the sludge.

Acknowledgement

The authors are thankful to the participating WWTPs. We thank Prof. Jan H. Christensen and Lab Manager Peter Christensen for substantial support on the chemical PAH analysis and the Danish Research Council, Technology and Production for funding (Grant no.: DFF 1337-00033).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.chemosphere.2018.05.104>.

References

- Artola-Garicano E., Borkent I., Hermens J.L.M. and Vaes W.H.J., Removal of two polycyclic musks in sewage treatment plants: freely dissolved and total concentrations, *Environ. Sci. Technol.* **37**, 2003, 3111–3116, <https://doi.org/10.1021/es020226x>.
- Bartolomé N., Hilber I., Sosa D., Schulin R., Mayer P. and Bucheli T.D., Applying no-depletion equilibrium sampling and full-depletion bioaccessibility extraction to 35 historically polycyclic aromatic hydrocarbon contaminated soils, *Chemosphere* **199**, 2018, 409–416.
- Boström C.-E., Gerde P., Hanberg A., Jernström B., Johansson C., Kyrklund T., Rannug A., Törnqvist M., Victorin K. and Westerholm R., Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air, *Environ. Health Perspect.* **110**, 2002, 451–488 <https://doi.org/10.1289/ehp.02110s3451>.
- Busetti F., Heitz A., Cuomo M., Badoer S. and Traverso P., Determination of sixteen polycyclic aromatic hydrocarbons in aqueous and solid samples from an Italian wastewater treatment plant, *J. Chromatogr. A* **1102**, 2006, 104–115 <https://doi.org/10.1016/j.chroma.2005.10.013>.
- Clarke B.O. and Smith S.R., Review of “emerging” organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids, *Environ. Int.* **37**, 2011, 226–247 <https://doi.org/10.1016/j.envint.2010.06.004>.
- DMI, Danish Weather Archive, 2017, [WWW Document] <https://www.dmi.dk/vejr/arkiver/vejrarkiv>.
- Fendinger N.J., Begley W., McAvoy D. and Eckhoff W., Measurement of alkyl ethoxylate surfactants in natural waters, *Technology* **29**, 1995, 856–863, <https://doi.org/10.1021/es00004a004>.
- Gallota F.D.C. and Christensen J.H., Source identification of petroleum hydrocarbons in soil and sediments from Iguaçú River Watershed, Paraná, Brazil using the CHEMSIC method (CHEMometric analysis of Selected Ion Chromatograms), *J. Chromatogr. A* **1235**, 2012, 149–158 <https://doi.org/10.1016/j.chroma.2012.02.041>.
- Gilbert D., Jakobsen H.H., Winding A. and Mayer P., Co-transport of polycyclic aromatic hydrocarbons by motile microorganisms leads to enhanced mass transfer under diffusive conditions, *Environ. Sci. Technol.* **48**, 2014, 4368–4375 <https://doi.org/10.1021/es404793u>.
- Gobas F.A.P.C., Mayer P., Parkerton T.F., Burgess R.M., van der Meer D. and Gouin T., A chemical activity approach to exposure and risk assessment of chemicals, *Environ. Toxicol. Chem.* **37**, 2018, 1235–1251.
- Humel S., Schmidt S.N., Mayer P. and Loibner A., Enhanced accessibility of PAHs and heterocyclic PAHs in industrially contaminated soil after passive dosing of a competitive sorbate, *Environ. Sci. Technol.* **51**, 2017, 8017–8026 <https://doi.org/10.1021/acs.est.7b01198>.
- IUPAC, Chemical Compendium (“The Gold Book”), 2010.
- Jahnke A., Mayer P. and McLachlan M.S., Sensitive equilibrium sampling to study polychlorinated biphenyl disposition in Baltic Sea sediment, *Environ. Sci. Technol.* **46**, 2012, 10114–10122 <https://doi.org/10.1021/es302330v>.
- Jahnke A., Mayer P., McLachlan M.S., Wickström H., Gilbert D. and MacLeod M., Silicone passive equilibrium samplers as “chemometers” in eels and sediments of a Swedish lake, *Environ. Sci. Process. Impacts* **16**, 2014, 464–472 <https://doi.org/10.1039/c3em00589e>.
- Lang S.C., Hursthouse A., Mayer P., Kötke D., Hand I., Schulz-Bull D. and Witt G., Equilibrium passive sampling as a tool to study polycyclic aromatic hydrocarbons in Baltic Sea sediment pore-water systems, *Mar. Pollut. Bull.* **101**, 2015, 296–303 <https://doi.org/10.1016/j.marpolbul.2015.10.069>.

- Larsen S.B., Karakashev D., Angelidaki I. and Schmidt J.E., Ex-situ bioremediation of polycyclic aromatic hydrocarbons in sewage sludge, *J. Hazard Mater.* **164**, 2009, 1568-1572
<https://doi.org/10.1016/j.jhazmat.2008.08.067>.
- Legind C.N., Karlson U., Burken J.G., Reichenberg F. and Mayer P., Determining chemical activity of (semi)volatile compounds by headspace solid-phase microextraction, *Anal. Chem.* **79**, 2007, 2869-2876
<https://doi.org/10.1021/ac061880o>.
- Li L. and Wania F., Mechanistic pharmacokinetic modeling of the bioamplification of persistent lipophilic organic pollutants in humans during weight loss, *Environ. Sci. Technol.* **51**, 2017, 5563-5571
<https://doi.org/10.1021/acs.est.7b00055>.
- Lindholm-Lehto P.C., Ahkola H.S.J. and Knuutinen J.S., Procedures of determining organic trace compounds in municipal sewage sludge—a review, *Environ. Sci. Pollut. Res.* **24**, 2017, 4383-4412
<https://doi.org/10.1007/s11356-016-8202-z>.
- Liu Z., Li Q., Wu Q., Kuo D., Chen S., Hu X., Deng M., Zhang H. and Luo M., Removal efficiency and risk assessment of polycyclic aromatic hydrocarbons in a typical municipal wastewater treatment facility in Guangzhou, China, *Int. J. Environ. Res. Publ. Health* **14**, 2017, 861 <https://doi.org/10.3390/ijerph14080861>.
- Macdonald R.W., Mackay D. and Hickie B., A new approach suggests that phenomena, such as bioconcentration, biomagnification, and bioaccumulation, result from two fundamental processes, *Environ. Sci. Technol.* December 2002, 456-462, <https://doi.org/10.1021/es022470u>.
- Mackay D., Shiu W.Y., Ma K. and Lee S.C., Handbook of Physical-chemical Properties and Environmental Fate for Organic Chemicals, 2nd Ed, Chemphyschem a European Journal of Chemical Physics and Physical Chemistry, 2006, Taylor & Francis Group, LLC; FL, USA.
- Mayer P., Tolls J., Hermens J.L.M. and Mackay D., Equilibrium sampling devices, *Environ. Sci. Technol.* 2003, May, 184A-191A.
- Milieu Ltd, Environmental, Economic and Social Impacts of the Use of Sewage Sludge on Land Final Report Part II: Report on Options and Impacts, 2010.
- Määnenpää K., Leppänen M.T., Reichenberg F., Figueiredo K. and Mayer P., Equilibrium sampling of persistent and bioaccumulative compounds in soil and sediment: comparison of two approaches to determine equilibrium partitioning concentrations in lipids, *Environ. Sci. Technol.* **45**, 2011, 1041-1047 <https://doi.org/10.1021/es1029969>.
- Nelson D.W. and Sommers L.E., Total carbon, organic carbon and organic matter, In: Sparks D.L., Page A.L., Helmke P.A., Loeppert R.H., Soltanpour P.N., Tabatabai M.A., Johnston C.T. and Sumner M.E., (Eds.), *Methods of Soil Analysis, Part 3 Chemical Analysis*, 1996, Soil Science Society of America, Inc.; Madison.
- Oleszczuk P. and Baran S., Concentration of polycyclic aromatic hydrocarbons in sewage sludge - amended soil concentration of polycyclic aromatic hydrocarbons in sewage, *Commun. Soil Sci. Plant Anal.* **36**, 2005, 1083-1097 <https://doi.org/10.1081/CSS-200056865>.
- Ozcan S., Tor A. and Aydin M.E., Investigation on the levels of heavy metals, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls in sewage sludge samples and ecotoxicological testing, *Clean. - Soil, Air, Water* **41**, 2013, 411-418 <https://doi.org/10.1002/clen.201100187>.
- Reichenberg F. and Mayer P., Two complementary sides of bioavailability: accessibility and chemical activity of organic contaminants in sediments and soils, *Environ. Toxicol. Chem.* **25**, 2006, 1239-1245, <https://doi.org/10.1897/05-458R.1>.
- Reichenberg F., Smedes F., Jönsson J.-A. and Mayer P., Determining the chemical activity of hydrophobic organic compounds in soil using polymer coated vials, *Chem. Cent. J.* **2** (8), 2008 <https://doi.org/10.1186/1752-153X-2-8>.
- Schmidt S.N., Wang A.P., Gidley P.T., Wooley A.H., Lotufo G.R., Burgess R.M., Ghosh U., Fernandez L.A. and Mayer P., Cross validation of two partitioning-based sampling approaches in mesocosms containing PCB contaminated field sediment, biota, and activated carbon amendment, *Environ. Sci. Technol.* **51**, 2017, 9996-10004 <https://doi.org/10.1021/acs.est.7b01909>.
- Schwarzenbach R.P., Gschwend P.M. and Imboden D.M., Environmental Organic Chemistry, 2002, John Wiley & Sons, Inc; Hoboken, NJ, USA <https://doi.org/10.1002/0471649643>.

- Schäfer S., Antoni C., Möhlenkamp C., Claus E., Reifferscheid G., Heininger P. and Mayer P., Equilibrium sampling of polychlorinated biphenyls in River Elbe sediments - linking bioaccumulation in fish to sediment contamination, *Chemosphere* **138**, 2015, 856–862 <https://doi.org/10.1016/j.chemosphere.2015.08.032>.
- Smith K.E.C., Schmidt S.N., Dom N., Blust R., Holmstrup M. and Mayer P., Baseline toxic mixtures of non-toxic chemicals: “solubility addition” increases exposure for solid hydrophobic chemicals, *Environ. Sci. Technol.* **47**, 2013, 2026–2033 <https://doi.org/10.1021/es3040472>.
- Souza-Silva É.A., Jiang R., Rodríguez-Lafuente A., Gionfriddo E. and Pawliszyn J., A critical review of the state of the art of solid-phase microextraction of complex matrices I. Environmental analysis, *TrAC Trends Anal. Chem. (Reference Ed.)* **71**, 2015, 224–235 <https://doi.org/10.1016/j.trac.2015.04.016>.
- Stout S.A., Uhler A.D., McCarthy K.J. and Emsbo-Mattingly S.D., Chemical fingerprinting of hydrocarbons, In: Murphy B. and Morrison R., (Eds.), *Introduction to Environmental Forensics*, 2002, Academic Press; New York, 135–260.
- Svahn O. and Björklund E., Describing sorption of pharmaceuticals to lake and river sediments, and sewage sludge from UNESCO Biosphere Reserve Kristianstads Vattenrike by chromatographic asymmetry factors and recovery measurements, *J. Chromatogr. A* **1415**, 2015, 73–82 <https://doi.org/10.1016/j.chroma.2015.08.061>.
- Ter Laak T.L., Mayer P., Klamer H.J.C. and Hermens J.L.M., Effects of dilution on the exposure in sediment toxicity tests- buffering of freely dissolved concentrations and changes in mixture composition, *Environ. Toxicol. Chem.* **26**, 2007, 2187–2191 <https://doi.org/10.1897/07-113R.1>.
- US EPA, Estimation Programs Interface Suite™ for Microsoft® Windows, 2017 v. 4.11.
- van Lier J.B., Mahoud N. and Zeeman G., Anaerobic wastewater treatment, In: Henze M., van Loosdrecht M.C.M., Ekama G.A. and Brdjanovic D., (Eds.), *Biological Wastewater Treatment: Principles, Modelling and Design*, 2008, IWA Publishing; London, U.K, 401–442.
- Villar P., Villar M., Callejon M., Perez J.L., Jimenez J.C., Alonso E. and Guiraum A., Evolution of polycyclic aromatic hydrocarbons (PAHs) and heavy metals in sludge samples from conventional activated sludge wastewater treatment plants, *Environmetrics* **20**, 2009, 561–574 <https://doi.org/10.1002/env>.
- Wakeham S.G. and Canuel E.A., Biogenic polycyclic aromatic hydrocarbons in sediments of the San Joaquin River in California (USA), and current paradigms on their formation, *Environ. Sci. Pollut. Res.* **23**, 2016, 10426–10442 <https://doi.org/10.1007/s11356-015-5402-x>.

Appendix A. Supplementary data

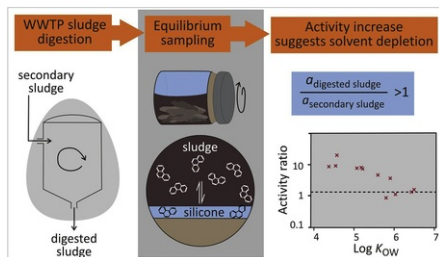
The following is the supplementary data related to this article:

[Multimedia Component 1](#)

Multimedia component 1

alt-text: Multimedia component 1

Graphical abstract



alt-text: Image 1

Highlights

- Equilibrium sampling with multiple thicknesses of silicone was applied on sludge.
- Exposure of PAHs was higher in digested compared to secondary sludge.
- Solvent depletion is suggested to cause increasing C_{free} during digestion.
- The activity ratios of PAHs decreased with increasing hydrophobicity.
- C_{free} rural soil < C_{free} sludge = C_{free} marine sediment < C_{free} contaminated soil.

Queries and Answers

Query: Please confirm that the provided email jessing@plen.ku.dk is the correct address for official communication, else provide an alternate e-mail address to replace the existing one, because private e-mail addresses should not be used in articles as the address for communication.

Answer: Please change my email address to kaknje@env.dtu.dk

Query: Please check the address for the corresponding author that has been added here, and correct if necessary.

Answer: Correct

Query: Please confirm that given names and surnames have been identified correctly and are presented in the desired order and please carefully verify the spelling of all authors' names.

Answer: All names are correct and presented in the right order.

Query: Your article is registered as a regular item and is being processed for inclusion in a regular issue of the journal. If this is NOT correct and your article belongs to a Special Issue/Collection please contact p.das@elsevier.com immediately prior to returning your corrections.

Answer: Regular issue is correct,