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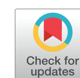
A preliminary study on the ecotoxic potency of wastewater treatment plant sludge combining passive sampling and bioassays



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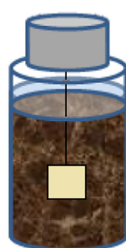
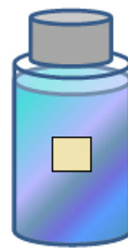
HIGHLIGHTS

- The passive samplers collected bioavailable and bioaccessible chemicals from the WWTP sludge samples.
- The sampler extracts were acutely and chronically toxic to water flea.
- The sampler extracts were cytotoxic and genotoxic.
- The sludge treatment such as composting and digesting diminished the toxicity.
- Effect-based methods should be part of the risk assessment of sludge recycling.

GRAPHICAL ABSTRACT

PDMS sheets were deployed in studying bioavailability of selected chemicals and to assess the toxicity of PDMS extract (*D. Magna*, umuC and NRR) and sludge filtrate (*A. fischeri*). Selected chemicals (PAHs, TCS, mTCS, active pharmaceutical ingredients) were analyzed from PDMS extract and sludge samples. PDMS extracts showed cytotoxicity and genotoxicity when deployed in digested sludge but only cytotoxicity in secondary sludge. All PDMS extracts presented toxicity for *D. Magna*. *A. fischeri* indicated potential toxicity in all sludge filtrates except for composted samples. Detected chemical concentration levels did not explain the toxicity of the samples judged by the reported toxicity thresholds. Bioavailability and bioaccessibility determination in conjunction with effect-based methods could improve the risk assessment of sludge as biosolids.





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ABSTRACT

Sewage sludge is an inevitable byproduct produced in wastewater treatment. Reusing nutrient-rich sludge will diminish the amount of waste ending in soil dumping areas and will promote circular economy. However, during sewage treatment process, several potentially harmful organic chemicals are retained in sludge, but proving the safety of processed sludge will promote its more extensive use in agriculture and landscaping.
Environmental risk assessment of sludge requires new methods of characterizing its suitability for various circular economy applications. Bioavailable and bioaccessible fractions are key variables indicating leaching, transport, and bioaccumulation capacity. Also, sludge treatments have a significant effect on chemical status and resulting environmental risks. In this study, the concentrations of polyaromatic hydrocarbons (PAHs), triclosan (TCS), triclocarban (TCC), methyl triclosan (mTCS), and selected active pharmaceutical ingredients (APIs) were determined in different sludge treatments and fractions. Passive samplers were used to characterize the bioavailable

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Sewage sludge
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Risk assessment
PAHs
APIs

and bioaccessible fractions, and the sampler extracts along the sludge and filtrate samples were utilized in the bioassays.
The TCS and PAH concentrations did not decrease as the sludge was digested, but the contents diminished after composting. Also, mTCS concentration decreased after composting. The API concentrations were lower in digested sludge than in secondary sludge.
Digested sludge was toxic for *Aliivibrio fischeri*, but after composting, toxicity was not observed. However, for *Daphnia magna*, passive sampler extracts of all sludge treatments were either acutely (immobility) or chronically (reproduction) toxic. Secondary and digested sludge sampler extracts were cytotoxic, and secondary sludge extract was also genotoxic. The measured chemical concentration levels did not explain the toxicity of the samples based on the reported toxicity thresholds.
Bioassays and sampler extracts detecting bioavailable and bioaccessible contaminants in sludge are complementing tools for chemical analyses. Harmonization of these methodswill help establish scientifically sound regulative thresholds for the use of sludge in circular economy applications.
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1. Introduction

Numerous chemicals are released to the environment from industry, agriculture, and households. A large proportion of such chemicals end up in wastewater, and many organic contaminants can be detected in wastewater treatment plant (WWTP) effluents and sludge. Even chemicals banned in the European Union (EU) are present in sewage as some imported products can still contain them (Fransson and Molander, 2013; Vestergren et al., 2015). When imported clothes, for instance, are used and washed in their destination countries, the coating chemicals and pesticides can end up in the WWTPs and even in the effluents or sewage sludge.

Sewage sludge is an inevitable byproduct produced in the wastewater treatment process. During the sewage treatment process, numerous nutrients and organic materials are retained in the sludge and recycling them as a fertilizer can promote sustainability. Unfortunately, sludge also collects many harmful substances, especially hydrophobic organic substances like PAHs, PCBs, and phthalates (Aparicio et al., 2009), and lists of the chemicals of most concern have been presented (e.g., Clarke and Smith, 2011). The use of sewage sludge for landscaping or as a fertilizer in agriculture has been under debate for decades. The benefits of sewage sludge reuse are not readily accepted (LeBlanc et al., 2009; Christodoulou and Stamatelatou, 2016), although most of its risks for human health are considered minor (Smith, 2009; Clarke and Smith, 2011; Eriksen et al., 2009). One concern is the accumulation of contaminants in crops, which is still insufficiently studied (Wu et al., 2015). Generally, publications imply low accumulation of personal care products and pharmaceuticals in plants (e.g., Hundal et al., 2008; Pannu et al., 2012; Bloem et al., 2017) and low risk for humans (Prosser et al., 2014), but wastewater-treatment-induced metabolites and their back-transformation in plants may complicate the risk assessment (RA) of biosolid-borne contaminants (Fu et al., 2018). The desorption of chemicals from sludge and their leaching into surface and ground waters may also be a threat (Mantis et al., 2005; Milinovic et al., 2014; Lapen et al., 2008; Topp et al., 2008; Wu et al., 2015), exposing a larger area and other matrices for contaminants.

In the EU, the treatment, collection, and discharge of urban wastewater are controlled by the European Commission (EC) Urban Waste Water Directive 91/271/EEC (EC, 1991), and Sewage Sludge Directive 86/278/EEC (EEC, 1986) encourages the reuse of sludge with minimum environmental risks. In 2000, the Joint Research Report of the EC suggested limit values for organic contaminants in sludge, such as a 6 mg kg^{-1} limit for the sum of nine PAHs (EC, 2000). In the EU, there are still regulative limits but only for heavy metals in reused sewage sludge, and the regulation does not involve the monitoring of organic substances in effluents or sludge (EEC, 1986). For example, according to the number of studies, APIs are not entirely removed or degraded at WWTPs and can end up in the environment via sludge reuse (Radjenovic et al., 2009; Jelic et al., 2011; Chen et al., 2013; Martín et al., 2015). Monitoring and regulating of only a few and single compounds is not enough to assess the risk posed by complex mixtures. Obtaining an analytical fingerprint of complex samples may be expensive, and the analytical fingerprint may be non-informative of the actual potential toxicity (e.g., due to additive and synergic effects). Therefore, the usage of bioassays provides a more direct and pragmatic response to the requirement of conducting environmental risk assessment.

While international guidelines and limit values are lacking, countries have very different policies concerning the reuse of sewage sludge. Some countries, such as Denmark and Germany, have set national limit values for organic contaminants in sludge for agricultural use (Project on Urban Reduction of Eutrophication (PURE), 2012). In Sweden, it has been proposed that only high-quality sludge is permitted to be used in agriculture, but the government's decision regarding this matter is still being awaited (SOU, 2020; Svenskt Vatten, 2020). In the Netherlands and Switzerland, the application of sewage sludge on agricultural soil is not allowed. In Finland, 40% processed sewage sludge was used as a fertilizer in agriculture in 2016, and 50% in landscaping (Vilpanen & Vilpanen and Toivikko, 2017). The use of sludge in agriculture is regulated according to the amount of nitrogen and phosphorus present in the sludge (FIWA, 2013). In practice, maximum application is limited to 30 t/ha every 5 years. Although sludge products are extensively used in Finland, the country's food industry is restricting their use in contract farming, and many farmers fear that sludge application would contaminate their properties (YLE News, 2018).

To assess the environmental and public health effects of chemicals in sludge, there is an urgent need to develop novel and readily applicable methods of assessing the risk posed by sewage sludge reuse. Sewage sludge contains an enormous amount of different chemicals and their transformation products, and if examining all of them were possible, it would be laborious and very expensive. Sludge is a complex matrix, and some harmful chemicals therein may remain below the method's limit of quantification (LOQ), which incorrectly implies that the substance is not present in the sample. However, harmful chemicals can produce negative effects even at concentrations below their analytical detection limits (the “something from nothing” phenomenon; Silva et al., 2002), and a mixture of chemicals can have an unexpected toxic impact because different compounds can contribute to one another's adverse effects.

The safe application of biosolids on agriculture and on landfills requires knowledge of ecotoxicological risks and of the desorption and subsequent leaching risks of sludge-associated substances (Kapanen and Itävaara, 2001; Alvarenga et al., 2007; Topp et al., 2008; Natal-da-Luz et al., 2009). The sludge that remains after the conduct of different treatments has been observed to induce toxic effects in various endpoints of ecotoxicity tests. For example, anaerobically digested municipal WWTP sludge was observed to be harmful for earthworms (mortality), water fleas (immobility), and bacteria (luminescence) (Alvarenga et al., 2007), and municipal WWTP sludge with different

shares of industrial influents showed the acute toxicity (*Vibrio fischeri*), genotoxicity (*Salmonella typhimurium*), and estrogenic, androgenic, and dioxin-like activity (yeast-cell-based assays) of different extracts (Kapanen et al., 2013). However, the pilot-scale composting of a sludge sample diminished the estrogenic and androgenic activities below the detection limits, and the dioxin-like activity and genotoxicity were also significantly decreased (Kapanen et al., 2013). Composting has been found to decrease toxic responses (Lopez et al., 2010), but aging plays a role in the responses and does not always follow the hypothesis "longer composting, less toxic" (Kapanen et al., 2013). The same has been observed with the soil-sludge contact time (Malara and Oleszczuk, 2013). Some sludge treatments conducted prior to composting often induce negative responses. Of these, Microtox or its equivalent (*Vibrio fisheri*) appears to be one of the most common test types (e.g., Mantis et al., 2005; Giannakis et al., 2020) among the aquatic standard tests (e.g., Malara and Oleszczuk, 2013; Chiochetta et al., 2014) and the terrestrial plant (e.g., Ramirez et al., 2008; Moreira et al., 2008) and invertebrate tests (e.g., Moreira et al., 2008).

As evidences of the potential risks of sludge application in agriculture have piled up, Huguier et al. (2015) have proposed a biotest battery for assessing the contaminant-associated risks of organic waste, including solid-phase testing with terrestrial plants and invertebrates and eluate-phase tests with bacteria and aquatic invertebrates. Plant growth and earthworm avoidance or reproduction tests are deemed the most sensitive and relevant for organic wastes, although other factors, such as ease of implementation and cost effectiveness, may also guide the test selection. One should not forget environmental realism either and consider using also other soil specific endpoints as performance of microbial communities (e.g. Sullivan et al., 2006; Hazard et al., 2014).

The multiple bioassay types and variable sample treatments (solid amendments, extracts, and eluates) call for harmonization of sludge assessment. The test circumstances (nutrients, color, and dissolved organic matter [DOM]/dissolved organic carbon [DOC]) most likely also play a significant role in both in-vivo and in-vitro tests (Kapanen et al., 2013; Li et al., 2013). Aside from these sample-related biological- and analytical-detector-specific reactions, the substance distribution within a matrix and sorption-related matters also matter (e.g., Nkinahamira et al., 2019). The conventional, exhaustive chemical analysis of sludge samples provides their total concentrations, including the readily bioavailable (freely dissolved), dissolved but bound (e.g.., to, DOM)), and particle-bound fractions of a compound. The exhaustive extraction-based concentration, however, does not relate very well to the possible toxic potencies of sludge. Bioavailable and bioaccessible fractions are more indicative of the hazards of harmful organic chemicals and the possibility of their transferring from the matrix to the biota (Reid et al., 2000; Cornelissen et al., 1997; Gouliarmou and Mayer, 2012). These fractions should thus also be considered in the RA of organic wastes.

Passive sampling can be used to study the bioavailable, freely dissolved fraction of chemicals. A number of equilibrium passive sampling devices have been developed to measure freely dissolved chemical concentrations (C_{free}) in soil and sediment (Fernandez et al., 2009; Smedes et al., 2009; Mayer et al., 2014; Mäenpää et al., 2015; Jahnke et al., 2012) and also in sludge (Sjoeholm et al., 2018). One of such devices includes a thin polymer layer of polydimethylsiloxane (PDMS) that acts as a receiving phase in a nondepletive fashion. A great innovation is jars coated with thin, multiple-thickness silicone (Reichenberg et al., 2008), which allows the easy detection of a sample's equilibrium status. It also reveals a thermodynamics-based chemical activity that can be used to analyze the direction of chemical diffusion (Reichenberg et al., 2008; Gobas et al., 2018).

Bioaccessibility, on the other hand, can be detected with depletive passive samplers. Silicone-based sorptive sinks have been used to measure the accessibility of PAHs in soils and particulate matter (Mayer et al., 2000, 2011; Gouliarmou and Mayer, 2012; Allan et al., 2016). This approach is based on the principle of a larger mass of a sink versus the sorptive phase in a source. Accessibility estimations can reveal the fraction of contaminants available for biodegradation, mobility between phases (sludge as a fertilizer in agricultural soil), and biomagnification in soil or sediment systems.

Passive sampling has also been combined with bioassays either as sampler extracts or passive dosing (Muller et al., 2007; Vermeirssen et al., 2009; Booij et al., 2013; Li et al., 2013; Vrana et al., 2015; Vethaak et al., 2017; Sonavane et al., 2018). Bioassays integrate the effects of all substances and complement chemical analysis. The combination of passive samplers and bioassays among the effect-based tools is an efficient means of including mixture toxicity evaluation into RA schemes. The main aim of this study was to widen the application of bioassay-based tools in characterizing the potential toxicity of sludge-based biosolids by considering the bioavailability and bioaccessibility, and to apply the latter as an operational fraction in selected bioassays. To the best of the authors' knowledge, this study was the first to have utilized passive sampler extracts to identify the ecotoxicological risks of WWTP sludge.

In this study, the RA of WWTP sludge was investigated by (1) analyzing the common contaminants in the total extractions of secondary and digested sludge samples as well as in the PDMS passive sampler extracts, and (2) performing different bioassays with sludge, filtrate, and sampler extracts of secondary, digested, and composted sludge. The sampler extracts were subjected to a chronic daphnid reproduction test and to genotoxic and cytotoxic in-vitro tests to assess their specific toxicological effects. The secondary sludge and its filtrate were studied directly with a luminescence bacteria assay. The polyaromatic hydrocarbon (PAH), triclosan (TCS), triclocarban (TCC) and methyl triclosan (mTCS) concentrations were determined in the sludge and in the PDMS passive samplers. The contents of the active pharmaceutical ingredients (APIs) were determined in the aqueous and solid fractions of sludge to study their partitioning in sewage sludge.

2. Materials and methods

2.1. Sludge samples

The sludge samples that were used in this study were collected in 2015–2016 from two WWTPs (Table 1). WWTP1 treats the wastewater of 160,000 inhabitants, and about 7% of the sewage that it treats is industrial wastewater from the chemical, metal, food, and forest industries as well as waters from laundries, waste management, and power production. The average wastewater discharge in 2015–2016 was 40,000 $m^3\ d^{-1}$. The treatment process used is simultaneous biological and chemical precipitation. Biologically degradable organic matter is removed, ferric salt is added for precipitation, and the water is clarified with sedimentation. A schematic overview of the treatment process employed at WWTP1 has been provided in Lindholm-Lehto et al. (2018).

The first sample was taken from secondary sludge (A), and the second sample was taken after mesophilic anaerobic digestion at 38 °C (B) (Table 1). At the WWTP, digested sludge is further aerated to disable the bacteria. After the addition of polymer, the sludge is dried and prepared for composting. The third sample was taken at the beginning of composting (C), and about 50% of the sludge was digested and originated from WWTP1. The rest of the composted material included undigested sludge from smaller WWTPs and woodchips. The fourth sample was taken after three weeks of composting (D).

WWTP2 treats the sewage waters of 6000 inhabitants, and the treatment procedure that it employs is similar to the one employed by WWTP1. The average discharge of WWTP2 in 2015–2016 was 1133 $m^3\ d^{-1}$. The samples were taken from secondary sludge (E) and composted sludge (F). The composted sample consisted of sludge from septic tanks and cesspools, which was dried at the site before sampling (Table 1). The dry weights of the samples were determined from the weight losses of five replicate samples kept at 105 °C overnight.

Table 1
Sewage sludge samplesand the conducted chemical analysis and ecotoxicological tests.

ID	Sample	Dry weight (%)	Type	Chemical analysis	Ecotoxicological tests
A	WWTP1, secondary	0.50 ± 0.05	Solid sludge	PAHs, TCS, TCC, mTCS, APIs	n.d.
			Filtrate	APIs	*Luminescent bacteria*
			PDMS	PAHs, TCS, TCC, mTCS (bottles)	*Daphnia chronic, genotoxicity, cytotoxicity (sheets)*
B	WWTP1, digested	1.6 ± 0.6	Solid sludge	PAHs, TCS, TCC, mTCS, APIs	n.d.
			Filtrate	APIs	*Luminescent bacteria*
			PDMS	PAHs, TCS, TCC, mTCS	*Daphnia chronic, genotoxicity, cytotoxicity*
C	WWTP1, before composting	29 ± 1	Solid sludge	PAHs, TCS, TCC, mTCS	*Luminescent bacteria*
			Filtrate	APIs	n.d.
			PDMS	PAHs, TCS, TCC, mTCS	*Daphnia chronic*
D	WWTP1, after composting	51 ± 1	Solid sludge	n.d.	*Luminescent bacteria*
			Filtrate	APIs	n.d.
			PDMS	–	*Daphnia chronic*
E	WWTP2, secondary	0.08 ± 0.01	Solid sludge	PAHs, TCS, TCC, mTCS	n.d.
			Filtrate	APIs	*Luminescent bacteria*
			PDMS	PAHs, TCS, TCC, mTCS	*Daphnia chronic*
F	WWTP2, after composting	20 ± 1	Solid sludge	PAHs, TCS, TCC, mTCS	*Luminescent bacteria*
			Filtrate	n.d.	n.d.
			PDMS	PAHs, TCS, TCC, mTCS	*Daphnia chronic*

n.d. = not determined.

The timing of the sampling did not consider the retention time at the WWTPs and hence, the samples did not represent exactly the same material.

2.2. Chemicals and materials

The analytical-standard carbamazepine (CBZ), diclofenac (DCF), ibuprofen (IBU), ketoprofen (KET), and naproxen (NAP) (purity 98%) that were used in this study were purchased from Alfa Aesar (Karlsruhe, Germany). The solutions of TCS and TCC in methanol and mTCS in nonane, as well as their mass-labeled surrogates ($^{13}C_{12}$-TCS $^{13}C_{13}$-TCC and $^{13}C_{12}$-mTCS), were purchased from Wellington Laboratories (Guelph, ON, Canada). The commercial PAH mixture including 18 PAHs (PAH-Mix 9 in cyclohexane), benzo[*e*]pyrene, perylene, and deuterated PAHs (PAH-Mix 24 in hexane: naphthalene D8, acenaphthene D10, phenanthrene D10, chrysene D12, and perylene D12) were from Dr. Ehrenstorfer (Augsburg, Germany). The β-nicotinamide adenine dinucleotide (NADPH tetrasodium salt) was obtained from AppliChem (Panreach, Germany). Fluka Analytical liquid chromatography–mass spectrometry (LC-MS)-grade methanol, ammonium acetate, and acetonitrile were used for LC eluents and/or the extraction of TCS and TCC from the sludge and water samples, and pesticide residue analysis-grade hexane and cyclohexane were used for the extraction of PAHs and mTCS. The HPLC-grade methanol that was used for the extraction of passive samplers and the dichloromethane (for organic residue analysis) that was used for pharmaceutical analysis were produced by J. T. Baker. The acetone (SupraSolv) was produced by Merck. The water that was used in the analyses was ultra-high-quality (UHQ) water (Millipore, Bedford, MA, USA). The PDMS sheets were SSP-M823 (Specialty Silicone Products, Ballston Spa, NY, USA), and the PDMS paste that was used for the coated bottles was Silastic Medical adhesive silicone, Type A (Dow Corning, USA).

2.3. Passive samplers

2.3.1. PDMS sheets for the toxicity tests

PDMS passive samplers were cut from a larger SSP-M823 silicone sheet (Shielding Solutions Limited, UK) and were shaken for 2 h in n-hexane, 2 h in acetone, and 2 h in methanol. The samplers were then rinsed with UHQ water and dried in an oven at 105 °C for 2 h. They were then stored in UHQ water and were weighted before deployment. The sheets that were used for the toxicity test extracts had 6 × 6 and 4 × 4 cm sizes and 254 and 356 μm thicknesses, respectively. One sheet was deployed in one sample flask. The sludge sampler design was not intended to be nondepletive and to have access only to the freely dissolved fraction of contaminants. Instead, the mass of a PDMS sampler in a sludge bottle was designed to have access to the bioaccessible fraction (Reichenberg and Mayer, 2006). This is an important fraction of a contaminant as it determines the potentially desorbing fraction available for diffusion and long-range transport. The setup imitated a sludge amendment to soil where desorption and diffusion of contaminants from a more concentrated sludge fraction to a much larger receiving soil phase is expected, such as with the aid of rain or irrigation water. This sorptive bioaccessibility extraction (Bartolome et al., 2018) can be characterized with the sorption capacity ratio (SCR) (Hilber et al., 2017).

$$SCR = \frac{K_{PS} m_{PS}}{K_{OC} m_{OC}} \tag{1}$$

where K_{PS} is the partition coefficient between the passive sampler (PS) and water, m_{PS} is the mass of the sampler, K_{OC} is the organic carbon water partition coefficient of the chemical of concern, and m_{OC} is the mass of organic carbon in the sludge sample. Ratios above 1 would indicate depletive sampling. The ratios were calculated for ten PAHs. The K_{PS} was taken from Smedes et al. (2009), the K_{OC} was taken from Hawthorne et al. (2006), and the organic carbon contents in our sludge samples were assumed to be the same as that in a Swedish digested WWTP sludge (344 mg kg^{-1} dw; Svahn and Bjorklund, 2015).

The dry weight of the sludge varied between the treatments (Table 1), and additional UHQ water was needed in samples C, D, and F to obtain slurry. The total amount of sludge slurry was 900 g (fresh weight) per bottle containing one PDMS sheet fixed with an iron wire to keep its surface open and to maximize its contact with the sludge. Three replicate sample bottles were deployed for both PDMS thicknesses in this study. Extracts from sheets with 254 μm thicknesses were used in the genotoxicity (UmuC) and cytotoxicity (NRR) assays, and those with 356 μm thicknesses were used for the *D. magna* chronic tests. The bottles containing PDMS samplers were shaken in a dark, temperature-controlled room at 18 °C for 28 days. After the deployment, excess biofouling was gently removed, and the sampler was placed in a glass bottle and stored in a freezer (−20 °C) until extraction. The samplers were extracted with 12 mL methanol in an ultrasonic bath for 5 min. The extraction was continued through the shaking of the sampler in a bottle overnight. The methanol was then transferred to a new bottle, and the extraction process was repeated. The extracts were then combined and evaporated to a volume in which the ratio of the

PDMS mass to the methanol volume was 9:5. The ratio was kept equal in all the samples, and the methanol extract was applied in biotests.

2.3.2. Silicone-coated glass bottles

A known amount of PDMS paste (Silastic, Medical Adhesive Silicone Type A, Dow Corning) was weighted and diluted in pentane to produce PDMS paste, which was used to coat the inner walls of a 1 L glass bottle. An appropriate volume of this PDMS solution was transferred to a horizontally rotating 1 L glass bottle, and the bottle was rolled for 15 min to make the solvent evaporate. Only the vertical walls of the bottle were coated with PDMS (Reichenberg et al., 2008). The diameter of the bottle was 9 cm, and the walls were coated at a 14 cm height, producing a 396 cm^2 PDMS-coated area. A few drops of UHQ water were added to the bottom of the bottle to facilitate polymerization reaction, and the bottle was made to stand open without a cap for at least 5 days to dry the PDMS. The PDMS bottles were prepared with three different thicknesses (5, 9, and 18 μm), with three replicates of each. The PDMS bottles were cleaned by treating them sequentially with n-hexane, acetone, and UHQ water, each for 60 min.

The PDMS-coated bottles filled with 900 g (fresh weight) sludge were shaken in a dark, temperature-controlled room at 18 °C for 28 days. Previously, a 3 wk. equilibrium time had been enough for the PAHs in the sludge samples (Sjoeholm et al., 2018). This applies also to our study as the surface area-volume relationship was about the same as Sjoeholm et al. (2018) had. After the deployment, the sludge was removed from each bottle, and the bottle was quickly rinsed with UHQ water, dried with tissue paper, and stored in a freezer until analysis. Before the analysis, internal standards were added into the PDMS-coated bottles, and the samplers were extracted with 75 mL methanol by shaking the bottles for 24 h. The methanol extract was removed and evaporated to 10 mL with a rotary evaporator. Half of the methanol extract was taken for TCS and TCC analysis and was evaporated to dryness with an EZ-Envi centrifugal evaporator (Genevac, Ipswich, UK). The dried extract was then reconstituted to 0.5 mL methanol and transferred to 2 mL amber glass vials for instrumental analysis. The other half of the methanol extract was taken for PAH and mTCS analysis and was concentrated to 1 mL under nitrogen stream. The extract was cleaned with a solid phase extraction (SPE) cartridge (Biotage, ISOLUTE EPH, 5 g). The SPE cartridge was conditioned with 20 mL hexane. The methanol extract was added to the cartridge, and the aliphatic compounds were removed with 12 mL hexane. The PAHs and mTCS were eluted with 20 mL dichloromethane. 1 mL cyclohexane was added as a solvent keeper, and dichloromethane was evaporated gently under nitrogen flow.

The biologically available, freely dissolved concentration (C_{free}) was calculated using the equation that was used by Reichenberg et al. (2008), as shown below (Eq. (2)), and the $K_{silicone,water}$ partition coefficients for Silastic silicone were obtained from Smedes et al. (2009) and Pintado-Herrera et al. (2016). The missing $K_{PDMS,water}$ values of 1 and 2 methylnapthalene were determined by plotting the partition coefficients of Smedes et al. (2009) with the ones of Yates et al. (2007), who had determined 1 and 2 methylnapthalene with different PDMSs (Altesil).

$$C_{free} = \frac{C_{PDMS}}{K_{silicone,water}} \quad (2)$$

The equilibrium was validated by plotting the mass of the PDMS (x) against the mass of the measured chemical (y) in the PDMS (Table S1). The determination coefficients (R^2) should approach 1 if the PDMS samplers were in equilibrium and did not have problems arising from abrasion or depletion. The R^2 values ≥0.6 were deemed acceptable (Schmidt et al., 2017; Sjoeholm et al., 2018), and only such R^2 values were reported. The C_{PDMS} determined as the amount of chemical in the sampler divided by the mass of the sampler (mg kg^{-1}) is a direct indication of a chemical activity in an equilibrated system and can be used to compare the activity changes during the sludge treatments (Sjoeholm et al., 2018). A positive activity ratio between the treatment processes will suggest an increase of the freely dissolved concentration and thus a potential for increased availability of a substance in a numerator treatment.

2.4. Chemical analysis of sludge and water samples

For TCS and TCC analysis, 5–50 mL liquid sludge (dry matter content <10%) (samples A, B, E, and F), 100 mL test water from the *D. magna* test (A and B), or 0.3 g solid sludge (C, D, and F) was spiked with internal standards. The liquid sludge samples were extracted with Bakerbond Speedisks® (C18, J. T. Baker). The disks were conditioned with 20 mL methanol followed by 15 mL UHQ water. The water samples were extracted with Oasis HLB SPE cartridges (6 cc, 200 mg, Waters), which were conditioned with 5 mL methanol and UHQ water. When the samples had passed through them, the sample bottles were washed twice with 10 mL UHQ water. The disks and cartridges were dried under vacuum, and the analytes were eluted from the disks with 10 mL methanol and from the cartridges with 6 mL methanol. The solid sludge samples were extracted twice with 5 mL acetonitrile in a shaker for 20 min, and the extracts were combined. All the extracts were evaporated to dryness with a centrifugal evaporator, after which 0.5 or 1.0 mL methanol was added.

For PAH and mTCS analysis, 100–300 mL liquid sludge (samples A, B, E, and F), 500 mL test water from the *D. magna* test (A and B), or 2–5 g solid sludge (C, D, and F) was spiked with internal standards. The liquid sludge and water samples were extracted with 25 mL hexane on a magnetic stirrer for 1 h. The solid sludge was extracted with 30 mL acetone/hexane (v/v, 1/2) by shaking the samples for 1 h. Cleaning of the extracts before PAH and mTCS analysis was conducted as described for PDMS-coated bottle extracts.

The APIs were determined from the aqueous and solid parts of two replicate sludge samples. Sludge samples A and B (900 g sludge fresh weight) in the 1 L glass bottle were kept in a temperature-controlled room at 18 °C for 14 days. The aqueous and solid phases of the sludge were separated through centrifugation (15 min at 3000 rad min^{-1}). The sampling procedure, sample treatment, and analysis methods are described in Lindholm-Lehto et al. (2015, 2018). The APIs were determined in the solid sludge and aquatic phases after the centrifugation of the sludge.

2.5. Instrumental analysis

TCS and TCC were analyzed with Acquity ultra-high-performance liquid chromatograph (UPLC) coupled with Xevo TQ MS with electrospray ionization (Waters, Milford, MA, USA). 7.5 μL of the sample was injected into an Acquity UPLC® BEH C18 column (1.7 μm, 1.2 × 50 mm; Waters). The LC eluents were 0.2 mM ammonium acetate in water (A) and methanol (B). The analytes were separated with the following 4-min gradient: initial 50% B, held for 0.5 min > 70% B by 1.0 min > 99.9% B by 3.0 min, held for 0.6 min > 70% B by 4.0 min. The eluent flow rate was 0.4 ml min^{-1}, and the column temperature was 40 °C. The mass spectrometer was operated in multiple reaction monitoring mode with the following mass transitions: TCS 287 > 35, 289 > 35, and $^{13}C_{12}$-TCS 299 > 35, TCC 313 > 160 and 313 > 126, $^{13}C_{13}$-TCC 326 > 166.

The mTCS was analyzed with a Thermo Scientific gas chromatograph (GC; Trace 1310 GC Ultra) connected to a TSQ Quantum XLS Ultra MS. The GC was equipped with a TriPlus RSH autosampler (CompiPal, CTC Analytics AG) fitted with a Zebron ZB-SemiVolatiles column (length 30 m, i.d. 0.25 mm, and film 0.25 μm). Helium was used as a carrier gas. 2 μL of the sample was injected with a programmed temperature vaporizing injector at 250 °C, and the oven program was as follows: 90 °C for 2 min, ramp from 90 to 250 °C at 10 °C min^{-1}, ramp from 250 to 320 °C at 25 °C min^{-1}, and hold time 3 min at 320 °C. Single

reaction monitoring (SRM) mode was used in the mass spectrometer to identify the mTCS.

The PAHs were analyzed with a GC (Varian CP-3800) equipped with a mass spectrometer (Varian 1200). An autosampler (CompiPal, CTC Analytics AG) fitted with a ZB-5MS (length 30 m, i.d. 0.25 mm, film 0.25 μm, Zebron) column and with helium as carrier gas was used. 1 μL was injected (split/splitless injector, 1177, Varian) at 250 °C. The oven program was as follows: 60 °C for 2 min, ramp from 60 to 190 °C at 15 °C min^{-1}, ramp from 190 to 250 °C at 4 °C min^{-1}, ramp from 250 to 270 °C at 10 °C min^{-1}, ramp from 270 to 300 °C at 4 °C min^{-1}, ramp from 300 to 320 °C at 20 °C min^{-1}, and hold time 5 min at 320 °C. Selected ion monitoring (SIM) mode was used in the mass spectrometer to identify the PAHs. Blank samples were prepared in each sample batch. A silicone-coated bottle was also checked for background, and no background was detected. Mass-labeled internal standards were used to correct the losses in the sample pretreatment and due to the matrix effects.

The APIs were analyzed and quantified through LC-MS/MS as described by Lindholm-Lehto et al. (2015).

2.6. Ecotoxicological tests

2.6.1. Toxicity of sludge filtrate to Aliivibrio fischeri

The inhibitory effect of the sludge samples as such (Table 1) was studied with a standard or kinetic luminescent bacteria test using BioTox Kit™ following the ISO standards (ISO 11348-3, ISO 21338). In colored and murky samples, using a kinetic test is advisable, and in this study, both the standard or kinetic luminescent bacteria test procedures were used depending on the sample. The samples were prepared according to the sample type, and all the sludge samples were analyzed (Table 1: A-F). The secondary (A and E) and digested (B) sludge samples were tested with a standard luminescent bacteria test. The samples were centrifuged at 4000 rpm for 5 min to remove the suspended solid particles. The required pH range was 6–8.5, and for the samples with pH values outside this range, the pH values were adjusted to pH 7 ± 0.2 with 2 M NaOH or HCl. The dissolved oxygen saturation of the samples was above 3 mg L^{-1}, and the samples with less oxygen were aerated for at least 3 min. Finally, solid NaCl was added to create a 2% NaCl environment.

More solid sludge samples (C, D, and F) were tested through a kinetic luminescent bacteria test where the sample acted as a reference to itself. The maximum signal was measured right after bacteria dispensing, and it was compared with the signal after the incubation time (Lappalainen et al., 2001). The samples were extracted by weighing 2 g of sludge (fresh weight) and adding 7 mL of the extraction solution (2% NaCl and 5 μg L^{-1} $NaHCO_3$). The pH and dissolved oxygen were checked and adjusted as described above. UHQ water was then added up to a 10 mL total volume, and the solution was then mixed with a vortex mixer for 5 min. After mixing, the solution was allowed to settle for 2 min, and the supernatant was collected as the sample. For the standard and kinetic tests, dilution series were prepared using a 2% NaCl solution, which was also used as the control sample. The 2% NaCl solution was prepared by dissolving 9 g NaCl (included in the BioTox Kit™) in 450 mL UHQ water.

The freeze-dried bacteria (*A. fischeri*) were added to a reconstitution solution (included in the BioTox Kit™) and were stabilized at +4 °C for 30 min. The bacteria suspension was then equilibrated in a cool bath (15 °C) for 30 min. An *A. fischeri* test was executed according to the aforementioned ISO standard method. In brief, 250 μL bacterial suspension for each concentration was transferred to test tubes and was stabilized at 15 °C for 15 min. After the baseline luminescence was measured from the bacterial suspension using Sirius L Single Tube Luminometer, 250 μL of the sample was added to the tubes. After mixing, the samples were incubated in a cool bath (15 °C), and the luminescence was measured after exactly 15- and 30-min contact in the duplicate samples.

In the kinetic method for the colored samples, the luminescence was continuously measured for 5 s, and the bacterial suspension was added exactly after 15 and 30 min. Two replicates were measured from the undiluted sample and dilutions of 1/2, 1/4, 1/8, and 1/16 in both methods. The results were shown as EC_{50} values, and they were calculated according to the ISO (2007, 2010) standards.

2.6.2. Chronic toxicity of the PDMS extract to D. magna

For the *D. magna* 21-day reproduction test, the test water was spiked with the methanol extract of the PDMS sampler. The test was performed as a modification of ISO 10706:2000 (ISO (International Organization for Standardization), 2000b) without media renewal because the normal water replacement schedule was not possible due to the small volume of the extract. The extract was evaporated to a low volume to increase the concentrations of the substances and to minimize the amount of methanol in the test. *D. magna* was cultured in M7 water (OECD, 2008) with neutral pH 7.8 ± 0.2 at 20 ± 2 °C with a 16/8 light/dark photoperiod. The tests were conducted in M7 water without Fe-EDTA. For the test, neonates (less than 24 h old) were randomly selected from 3- to 4-week-old adults (3rd to 8th brood). The neonates were individually placed on 600 mL glass flasks containing 200 mL of the test solution (M7 + 200 μL PDMS extract) with ten replicates, and the reproduction was recorded for 21 days. The experiments were performed in four separate sets, each with a control (0 control; M7 only) and a solvent control (200 μL methanol in 200 mL M7). The first set included extracts of the secondary and digested sludge of WWTP1 (A and B in Table 1). In the second set, the same extracts were used, but they were first diluted to half, 40 μL of which was mixed with 200 mL M7 (1:10 dilution). The third set had extracts of the WWTP1 sludge before and after composting (C and D), and the fourth set had extracts of the secondary and composted WWTP2 sludge (E and F), with both sets having a 200 μL extract in 200 ml M7.

The PAH, TCS, TCC and mTCS concentrations in the test solutions were determined in treatments A and B at the beginning and end of the chronic test. In addition to the four test setups, a control test was performed to evaluate the potential toxicological effects of the sole PDMS sheet when extracted with methanol. For this test, a purified but unused PDMS sheet was extracted with methanol, and the test water was spiked with 200 μL of this extract, as in the treatments.

The *D. magna* was fed daily with monoclonal algae (*Raphidocellis subcapitata*). The algae concentration was determined with microscope counting (Olympus BX-41, Japan) using a cellometer (Nexcelom CP2–001, USA). The volume of algae needed to reach the necessary carbon content was determined to be $1 \cdot 10^8$ algae cells L^{-1}, corresponding to 1 mg L^{-1} carbon (ASTM, 1997). In accordance with the amount of carbon per animal, the feeding increased during the test: 0.1 mg at days 0–7, 0.15 mg at days 8–14, and 0.2 mg at days 15–21 (ASTM, 1997). The offspring were counted and removed daily, and the pH and O_2 were measured three times a week. The evaporation from the flasks was periodically corrected by adding UHQ water. The control (M7) characterized the fitness of the population, and the methanol solvent control (MeOH) was used as a test control in the statistical analysis. The reproduction was evaluated with ANOVA and Dunnett post hoc test using the software Statistical Package for the Social Sciences (SPSS 23, 2015).

2.6.3. Cytotoxicity of PDMS extract assessed with neutral red retention assay

The neutral red retention (NRR) assay is an in-vitro biotest for the determination of cell viability via the uptake of the vital dye neutral red. The assay was performed as in the studies of Babich and Babich and Borenfreund (1992) and Klee et al. (2004), with slight modifications. The permanent cell line RTL-W1 extracted from the liver of rainbow trout (*Oncorhynchus mykiss*; Lee et al., 1993) was used in the present study. The cells were cultured at 20 °C in 75 cm^2 plastic culture flasks in an L15 medium with L-glutamine supplemented with 9% fetal

calf serum. The cells were exposed to serial dilutions of the methanol extract from the PDMS sheets deployed in the sludge from WWTP1 (secondary and digested sludge; Table 1: A, B) in 96-well microtiter plates with a sample concentration ranging from 0.04 to 2.5% (exposure concentration). In each test, there was a control sample with 2.5% methanol. 3,4-dichlorophenol (80 mg L^{-1}, which was diluted to 40 mg L^{-1} on the microtiter plate) was used as a positive control, and the medium was used for the negative control. The cells were exposed for 48 h at 20 °C, after which the exposure medium was discarded, and the cells were rinsed with phosphate-buffered saline (PBS) to remove any remaining medium. The cells in each well were stained with 100 μL of a 0.004% neutral red solution (2-methyl-3-amino-7-dimethylamino-phenanzine) in a medium, and were incubated in darkness for 3 h at 20 °C. After incubation, the neutral red solution was discarded, and the wells were washed twice with 100 μL PBS, and 100 μL of the extraction solution (2.5 mL acetic acid [glacial] in 125.0 mL ethanol and 122.5 mL distilled water) was added to each well. The plates were then shaken for 30 min. Subsequently, the absorption by neutral red was measured at 540 nm with a 690 nm reference wavelength, using a spectrophotometer (Victor[3], Perkin Elmer, Singapore).

The viability of the exposed cells was calculated as a percentage of the negative controls (unexposed cells), and the data were plotted as concentration-response curves to determine the NR_{50} values for the samples.

2.6.4. Genotoxicity of PDMS extract assessed with umuC assay

The umuC assay was performed according to ISO standard 13,829 (ISO (International Organization for Standardization), 2000b), with minor modifications. In short, the genetically modified bacterium *Salmonella enterica* subsp. *enterica* (*S. typhimurium*) strain (TA1535/pSK1002, DSMZ-German Collection of Microorganisms and Cell Cultures GmbH) was exposed to the methanol PDMS sheet sampler extracts from WWTP1 (secondary and digested sludge; Table 1: A, B) at four concentrations (0.44, 0.87, 1.8, and 3.5%, with three replicates in each). In each test, there was a control sample with 3.5% methanol, which showed no response. Six replicate samples were measured for each treatment. The samples were tested with and without metabolic activation by a rat liver S9 fraction using 96-well microplates. Positive control samples were prepared according to the guideline, except in the test with metabolic activation, where aminoantracene (2-AA) was diluted 200-fold instead of 500-fold. Furthermore, NADPH 16.88 mg in a 5 ml 10× tryptone, glucose, and ampicillin medium (TGA) was used as a cofactor solution. Additional negative controls (6 parallel) and blanks (3 parallel) for methanol were tested and applied in the calculations because the samples were in methanol instead of normal DMSO. The plates were incubated at 37 °C for 4 h, after which the bacterial growth was measured at 620 nm with the microplate reader iEMS Reader MF 1401 (Labsystems, Finland). Then the plates were incubated at 28 °C for 30 min, and the induction of the umuC gene was determined at 405 nm. A sample was evaluated as genotoxic if the induction factor was >1.5 while the growth factor was >0.5 (Cupi and Baun, 2016). If the growth factor was <0.5, the sample was considered cytotoxic, and by definition no evaluation of the genotoxicity was possible.

3. Results and discussion

3.1. Chemical analysis and chemical activity

PAHs, APIs, TCS, and mTCS are compounds that are commonly present in sewage sludge (Radjenovic et al., 2009; Ozaki et al., 2017). APIs are continuously released and are constantly present at WWTPs; especially, DCF and CBZ are observed as ubiquitous in WWTP sludge (Jelic et al., 2011). The presence of API residues was also evident in the authors' studies, and all the selected APIs were detected.

The ratio of API concentrations in the aqueous and solid fraction $C_{solid}/C_{aqueous}$ determined in this study was higher in the secondary sludge than in the digested sludge (Table 2). K_d determined by Martín et al. (2012) was clearly higher for CBZ and IBU, lower for KET and on equal range for NAP. The total amount was also higher in the secondary sludge than in the digested sludge for all the APIs, except for CBZ. This is possibly due to the faster transformation of APIs in digested materials (Martín et al., 2012). The sorption of APIs also depends on the characteristics and chemical properties of APIs as well as the different characteristics of sludge (Berthod et al., 2017; Svahn and Bjorklund, 2015). As previously reported, KET has the highest tendency to attach onto solid particles among the selected compounds (Ziylan & Ince, 2011; Lindholm-Lehto et al., 2015; Verlicchi & Zambello, 2015). This is also supported by the results of this study as the $C_{solid}/C_{aqueous}$ ratio was the highest in the case of KET for the secondary and digested sludge.

The total TCS concentration was the highest in the digested sludge and decreased in the composted sample to the same level as in the secondary sample (Table 3). mTCS that is more persistent, lipophilic, and toxic than its parent compound TCS (Bester, 2003, 2005; DeLorenzo et al., 2008; Coogan et al., 2007) was not detected in every solid sludge sample, but with the PDMS samplers, it was found in all the samples. Composting diminished the mTCS concentration, implying the presence of degrading bacteria.

PAHs are recognized as toxic, carcinogenic, and mutagenic compounds (Stołyhwo & Sikorsk, 2005; Kim et al., 2013) that are hydrophobic and mostly bound to sewage sludge in the wastewater treatment process. The limit concentration (6 mg kg^{-1}) for the sum of nine PAHs (acenapthene, phenanthrene, fluorene, fluoranthene, pyrene, benzo (b + j + k)fluoranthene, benzo(*a*)pyrene, benzo(*ghi*)perylene, and indeno(1,2,3-*cd*)pyrene) proposed by the EC (2000) was not exceeded in any sample (Table 3). The PAH concentrations considerably increased from the digested sludge sample to the sample taken just before composting. This probably relates to the local composting process, where the digested sludge from WWTP1 is mixed with sludge from other facilities without anaerobic digestion. The concentrations of these nine PAHs were generally lower in the digested sludge samples

Table 2
Concentrations of APIs in the aqueous and solid sludge fraction.

API	A			B			Sludge water partition factor in secondary sludge K_d[a]
	WWTP1, secondary			WWTP1, digested			
	C_{solid}	$C_{aqueous}$	$C_{solid}/C_{aqueous}$	C_{solid}	$C_{aqueous}$	$C_{solid}/C_{aqueous}$	
	(μg/kg)	(μg L^{-1})	(L kg^{-1})	(μg/kg)	(μg L^{-1})	(L kg^{-1})	
Carbamazepine	0.5	0.0056	89	0.7	0.018	39	1740–4570
Diclofenac	26	0.2	130	1.9	0.13	15	n.d
Ibuprofen	29	0.21	140	15	2	7.5	66–1020
Ketoprofen	250	0.035	7100	21	0.069	300	26–76
Naproxen	13	0.055	240	1.9	0.076	25	16–39

n.d = not determined.
[a] Martín et al., 2012.

Table 3
The C_{tot} values (μg/ kg per dry weight) were determined from the whole sludge samples, and C_{PDMS} (μg/ kg) and C_{free} (ng L^{-1}) were determined by PDMS coated bottles (5, 9 and 18 μm PDMS thicknesses). C_{free} was determined only for the compounds that were in equilibrium.

Compound	A			B			C			E			F		
	WWTP 1, secondary			WWTP 1, digested			WWTP 1, before composting			WWTP 2, secondary			WWTP 2, after composting		
	C_{tot} (μg/kg)	C_{PDMS} (μg/kg)	C_{free} (ng L^{-1})	C_{tot} (μg/kg)	C_{PDMS} (μg/kg)	C_{free} (ng L^{-1})	C_{tot} (μg/kg)	C_{PDMS} (μg/kg)	C_{free} (ng L^{-1})	C_{tot} (μg/kg)	C_{PDMS} (μg/kg)	C_{free} (ng L^{-1})	C_{tot} (μg/kg)	C_{PDMS} (μg/kg)	C_{free} (ng L^{-1})
1-Methylnaphthalene	61	65 ± 10	11 ± 2	20	39 ± 7		130	84 ± 8	14 ± 2	7.5	320 ± 180		nd	20 ± 4	3.4 ± 0.5
2-Methylnaphthalene	90	nd		30	77 ± 13	12 ± 2	200	140 ± 16	21 ± 3	15	560 ± 310		6.2	290 ± 100	
Acenaphthene	22	nd		19	nd		25	32 ± 6	8.9 ± 1.5	30	19 ± 8		nd	78 ± 25	
Acenaphthylene	68	20 ± 2	1.7 ± 0.2	52	36 ± 8		54	37 ± 6		11	23 ± 12		nd	nd	
Anthracene	7.1	nd		4.3	nd		5.5	nd		25	11 ± 5		nd	150 ± 10	
Benzo[*a*]anthracene	45	27 ± 6		27	20 ± 6		69	22 ± 5		18	30 ± 12		11	nd	
Benzo[*a*]pyrene	28	26 ± 17		9.9	nd		180	32 ± 17	0.089 ± 0.045	34	nd		nd	nd	
Benzo[*b*]fluoranthene	51	11 ± 2		30	nd		160	13 ± 3		nd	26 ± 14		54	nd	
Benzo[*e*]pyrene	20	29 ± 9		9.9	nd		210	17 ± 4		nd	nd		28	nd	
Benzo[*g,h,i*]perylene	14	nd		220	39 ± 1		57	43 ± 15		260	nd		nd	nd	
Benzo[*k*]fluoranthene	17	7.5 ± 2.2		15	25 ± 10		44	nd		nd	28 ± 12		nd	nd	
Chrysene	36	nd		15	nd		58	2.7 ± 0.1		nd	17 ± 7		nd	nd	
Dibenzo[*a,h*]anthracene	nd	66 ± 10		57	nd		nd	35 ± 12	0.030 ± 0.010	65			nd	nd	
Fluoranthene	100	63 ± 2	2.0 ± 0.1	4.2	75 ± 7	2.4 ± 0.3	280	74 ± 6	2.4 ± 0.2	29	36 ± 11	1.2 ± 0.4	66	40 ± 12	
Fluorene	46	34 ± 4		43	43 ± 6		79	75 ± 12		6.3	63 ± 26		18	79 ± 28	
Indeno[1,2,3-*cd*]pyrene	12	nd		5.3	nd		55	21 ± 8		26	v		nd	50 ± 1	
Naphthalene	42	30 ± 7		8.1	18 ± 6		0.13	99 ± 17		21	280 ± 160		nd	98 ± 33	
Perylene	8.6	21 ± 5		nd	34 ± 11		50	190 ± 150		nd	34 ± 3		85	36 ± 21	
Phenanthrene	140	59 ± 29		180	68 ± 37		220	180 ± 40		36	160 ± 60		61	93 ± 32	
Pyrene	100	84 ± 3		87	86 ± 9		250	120 ± 20		19	61 ± 20		68	33 ± 10	
Triphenylene	11	5.3 ± 0.3		8.1	8.5 ± 2.2		50	7.6 ± 0.1		nd			nd	nd	
Triclocarban	500	4.7 ± 0.6		430	13 ± 4		102	8.9 ± 2.9		13	1.4 ± 0.3		15	nd	
Triclosan	360	28 ± 1	26 ± 1	1600	95 ± 19		320	96 ± 17		430	15 ± 2	14 ± 2	120	18 ± 3	18 ± 3
Methyltriclosan	nd	28 ± 2	7.0 ± 0.5	nd	31 ± 3	7.8 ± 0.6	0.6	12 ± 4		2.1	19 ± 7		3.4	15 ± 2	3.7 ± 0.5

nd = not detected.

in this study (WWTP1, 0.5 mg kg^{-1} dw) than in the Danish WWTPs (0.7–4 mg kg^{-1} dw; Sjoeholm et al., 2018). This difference may have partly contributed to the low concentrations in the silicone-coated jars, and as such, only some of the substances had a validated equilibrium concentration in silicone. The digested sludge C_{free} of fluoranthene (WWTP1, 2.4 ng L^{-1}) in this study was exactly within the same range as that in the Danish WWTPs (1.1–3.1 ng L^{-1}) (Sjoeholm et al., 2018), suggesting the same thermodynamic potential of PAHs in these digested sludge samples. The digestion process in WWTP1 and the digestion and composting in WWTP2 appeared not to have decreased the PAH and TCS concentrations. The chemical activity was more or less the same between the secondary and digested sludge for fluoranthene and mTCS in WWTP1. According to Sjoeholm et al. (2018), as part of the organic matter decreases in the sludge digestion, it releases the attached compounds to their dissolved form. This increases the freely dissolved concentration and chemical activity. Unfortunately, the limited data do not allow further discussion of the role of organic matter degradation and the consequent solvent depletion (Macdonald et al., 2002) on the chemical activity and potential exposure concentrations in the field locations of composted or digested sludge.

The WWTP1 facility arranged a monitoring campaign to analyze the organic chemicals from the secondary sludge as well as before and after composting for 4 or 6 months (Table S3). The campaign was arranged two years after the samples in this study were taken. The sludge treatment process, including composting, diminished many chemicals (PAHs and linear alkylbenzene sulfonates) but was not effective for *per*- and polyfluoroalkyl substances (PFASs), polychlorinated dibenzodioxins (PCDDs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers. Also, Aparicio et al. (2009) noticed that although composting reduced the phthalate, LAS, octyl- and nonylphenol, and PAH contents, these compounds were still found in the composted materials. Generally, it can be concluded that the treatments did not remove all the toxic substances, and the most persistent (phthalates, nonylphenols, and some PAHs and PCBs) were still present after composting. Therefore, the sludge samples most likely contained various known and unknown substances that had a chance to accumulate in the PDMS sheet samplers and influence the toxicity of the sample extract.

3.2. Ecotoxicological tests

3.2.1. Toxicity of sludge filtrate to A. fischeri

All the sludge samples were subjected to a luminescence bacteria test. The test results indicate the toxic potential of all the secondary and digested sludge filtrate samples (Table 4). However, composting made the samples nontoxic.

The acute-toxicity half maximal inhibitory concentration (IC50) of TCS to *Vibrio fischeri* in aqueous samples is 53 μg L^{-1} (39–71 μg L^{-1}, 95% confidence limit), and that in sediment is 616 μg/ kg (470–808 μg L^{-1}, 95% confidence limit), as determined by DeLorenzo et al. (2008). The latter concentration was exceeded in the digested sludge samples (1600 μg/ kg) in this study. However, Villa et al. (2014) measured a 0.73 mg L^{-1} (0.36–1.14 mg L^{-1}) IC50 to *V. fischeri* for TCS, 0.91 mg L^{-1} (0.55–1.3 mg L^{-1}) for mTCS, and 1.76 mg L^{-1} (1.3–2.26 mg L^{-1}) for TCC, but the C_{free} concentrations of TCS in this study were far below the aforementioned IC50 concentrations (14–26 ng L^{-1}).

Di Nica et al. (2017) observed that CBZ was moderately toxic for *A. fischeri*, with a 94 mg L^{-1} IC50. For IBU and diclofenac sodium, the IC50 values were 18.3 and 15.9 mg L^{-1}, respectively. PAHs induce 50% inhibition at 0.2–0.7 mg L^{-1} levels for *V. fischeri* (Lee et al., 2013). These levels are far above those that were measured in the filtered samples in this study or that were calculated in the C_{free} fraction. Vethaak et al. (2017) studied the toxicity of the PDMS passive sampler extracts with *V. fisheri*. The samplers were deployed ex situ in the marine sediments collected from estuarine, coastal, and offshore sites. The researchers observed that only part of the toxicity could be explained by the analyzed chemicals, suggesting the presence of unknown biologically active components. This could also explain the results in this study. Furthermore, Ozaki et al. (2017) observed that composting decreased the toxicity of secondary sludge to *A. fischeri*, as in the present study.

The above examples indicate that the analyzed contaminants unlikely are solely responsible for toxicity. The sludge contains several hydrophobic organic substances (Table 3 and S2) that can penetrate phospholipid-constructed cell membranes with passive diffusion. Although the measured concentrations were low, additive, baseline narcotic effects are a possible explanation (Escher et al., 2002). The *V. fischeri* luminescence test is commonly used on sludge testing exhibiting negative responses on leachates or eluates (e.g., Domene et al., 2008; Chiochetta et al., 2014; Mantis et al., 2005; Alvarenga et al., 2007; Huguier et al., 2015). The use of solvents in preparing eluates can increase the toxicity (Kapanen et al., 2013), which likely relates to the increased desorption of hydrophobic contaminants.

3.2.2. Chronic toxicity of PDMS extract to D. magna

The SCR calculations (Table S4) were based on the mean values for ten PAHs and were also indicative of other unknown compounds in the mixture exposure in this study with the same K_{PS} and K_{OC} qualities. Both PDMS sheet thicknesses were depletive and hence sensed a bioaccessible fraction in treatments A, B, E, and F, but the sorptive capacity of the sheets did not match the high mass of organic carbon in the drier sludge samples taken before and after the composting treatments (C and D). These latter treatments probably did not sense the whole bioaccessible fraction, but as the intention was to characterize the potential toxicity of the sludge samples, this was deemed not to be a significant fault in the design. Despite the limited mass, these extracts also contained numerous unknown substances that could elicit a response in the tests in this study.

Both the WWTP1 and WWTP2 sludge extracts showed immobility–lethality or reduced offspring production in almost all the samples compared to the solvent control (Table 5). The immobility was reduced from 100% to 10% after the digested sludge of WWTP1 was composted, but the response in reproduction was still present. Surprisingly, the composting of the WWTP2 sludge did not alleviate the immobility–lethality response. The secondary sludge of WWTP1 as an additional 1:10 dilution was the only nontoxic sample. The dry weights of the daphnids did not differ when recorded in setup testing sludge

Table 4
EC50-values as % of the sludge extract of the *A. fisheri* test with different sludge samples and treatments.

ID	A	B	C	D	E	F
Sample	WWTP1, secondary	WWTP1, digested	WWTP1, before composting	WWTP1, after composting	WWTP2, secondary	WWTP2, after composting
A. fischeri EC50*	11	4.8	13	non toxic	2.4	non toxic
Method	Luminescence	Luminescence	Kinetic luminescence	Kinetic luminescence	Luminescence	Kinetic luminescence
Sample amount	50 mL	50 mL	2 g	2 g	50 mL	2 g

Table 5
Toxicological effect of the PDMS extracts on *D. magna*. Mean - average number of offspring per animal along the test; SD- standard deviation; C·V - coefficient of variation; Mean Brood - average number of broods per animal along the test. All the statistical results are from a comparison between methanol solvent control (MeOH) and the respective treatment.

Treatment	WWTP1				WWTP1			
	200 μL/200 mL M7				20 μL/200 mL M7			
	0-Control	MeOH	WWTP1, secondary (A)	WWTP1, digested (B)	0-Control	MeOH	WWTP1, secondary (A)	WWTP1, digested (B)[a]
Mean	90.0	73.9	n.d	n.d	70.5	48.2	44.1	37.6
SD	5.9	6.7	n.d	n.d	4.9	6.4	9.6	4.7
C·V %	6.5	9.1	n.d	n.d	6.9	13.4	21.8	12.5
Mean Brood	4.1	4.4	n.d	n.d	4.3	4.2	3.8	4.1
Mortality %	0	0	100	100	0	0	0	0
Mean dry weight of daphnids (mg)	0.41	0.41	0.34	0.40				

Treatment	WWTP1				WWTP2			
	200 μL/200 mL M7				200 μL/200 mL M7			
	0-Control	MeOH	WWTP1, before composting (C)	WWTP1, after composting (D)[b]	0-Control	MeOH	WWTP2, secondary (E)[c]	WWTP2, after composting (F)
Mean	71.1	59.1	n.d	48.4	68.9	57.6	17.0	n.d
SD	7.9	10.5	n.d	7.2	12.3	20.4	14.7	n.d
C·V %	11.1	17.7	n.d	14.9	17.9	35.4	86.7	n.d
Mean Brood	4.1	3.8	n.d	3.3	3.8	3.7	1.3	n.d
Mortality %	0	0	100	10	0	0	70	100

n.d = not determined.
[a] One-way Anova p < 0.01 F < 5.64; Post Hoc Dunnet p < 0.01)
[b] T-test $p < 0.05$.
[c] T-test $p < 0.01$.

treatments A and B (Table 5). Even though methanol reduced the population fitness compared to the 0 control ($p < 0.01$), no difference was observed when pure methanol was compared with the methanol extracted from the clean PDMS sheets. The mean numbers of offspring per parent were 33 ± 8 and 31 ± 11 for the pure methanol and the PDMS-extracted methanol, respectively (t-test $p > 0.05$), indicating that PDMS materials have no intrinsic toxic potential. All the *D. magna* control tests were in agreement with the ISO 10706:2000 validity criteria (ISO, 2000a, 2000b). The PAHs were analyzed, and 13 were detected in the *D. magna* test solution. The total concentration of the 13 detected PAHs was 1.8 μg L^{-1} at the beginning of the test in both solutions (secondary and digested sludge, A and B; Table S2). The initial concentrations of TCS were 42 and 32 ng L^{-1} in the solutions representing the secondary and digested sludge, respectively, and those of mTCS were 30 and 16 ng L^{-1}. Roughly 10% of the PAHs were present in the water phase at the end of the trial, and the TCS and mTCS contents were below the LOQ. The reported acute EC50 and chronic EC10/LOEC/NOEC values for the measured PAHs, TCS, mTCS, and TCC were at least at a low μg L level (Table S2), and although additivity is assumed in toxicity, it cannot by itself explain the observed responses in the daphnid test.

Leachates and eluates typically created with standards (e.g., CEN-EN 12457–2) are used in daphnid assays to test sludge samples. Several studies with variable sludge treatments indicate acute toxicity of the leachates (Fjällborg et al., 2005; Alvarenga et al., 2007; Domene et al., 2008; Chiochetta et al., 2014; Huguier et al., 2015; Giannakis et al., 2020), although nontoxic samples have been detected as well (Alvarenga et al., 2007; Huguier et al., 2015). Similarly, variable results have been found for leachates of soil-sludge mixtures (nontoxic, Giannakis et al., 2020; and toxic, Malara and Oleszczuk, 2013). When chemical analyses were used to explain the bioassay results, metals were suggested as being responsible for such results (Fjällborg et al., 2005), or no correlation was suggested to be evident (Domene et al., 2008); instead, the physicochemical parameters related to organic matter stability were connected to acute toxicity where decomposted, more stable organic matter reduced bioavailability and hence toxicity (Domene et al., 2008).

3.2.3. Cytotoxicity and genotoxicity of PDMS extract

Assays were performed with extracts from the PDMS sheets that were deployed in the secondary (A) and digested (B) sludge from WWTP1. Both types of sludge samples induced cytotoxic effects in the 0.3–2.5% sample concentrations in the RTL-W1 cells (Fig. 1). The calculated NR_{50} values were 0.63% for the digested sludge sample and 0.48% for the secondary sludge sample. The control samples showed that methanol did not have an effect on the toxicity of the sample. The cell-line-based cytotoxicity tests in the literature are far rarer than the Microtox (or equivalent)-based nonspecific cytotoxicity tests. The aqueous eluates of municipal sludge showed apoptosis and necrosis in the human-derived lymphocytes (Gajski et al., 2011). The extent of cell death differed between the measures and was associated with the sludge treatment. Klee et al. (2004) used the same fish cell line as in the present study and found cytotoxic responses. Explosives and their metabolites were discussed as a possible reason for this.

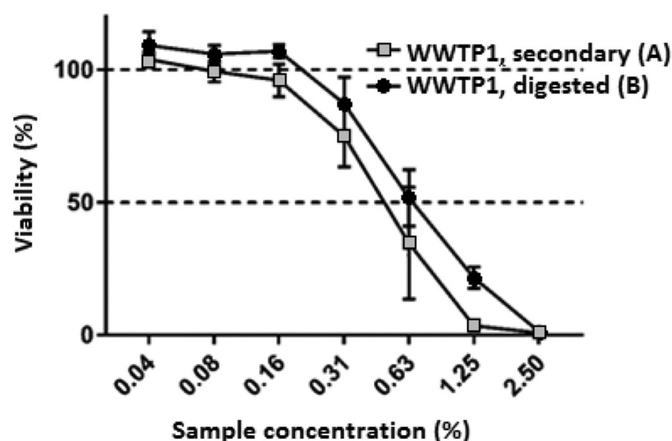

Fig. 1. The cytotoxicity in the PDMS extracts deployed in secondary and digested sludge was analyzed with the NRR assay using the RTL-W1 fish liver cell line (3 tests, mean ± SD).

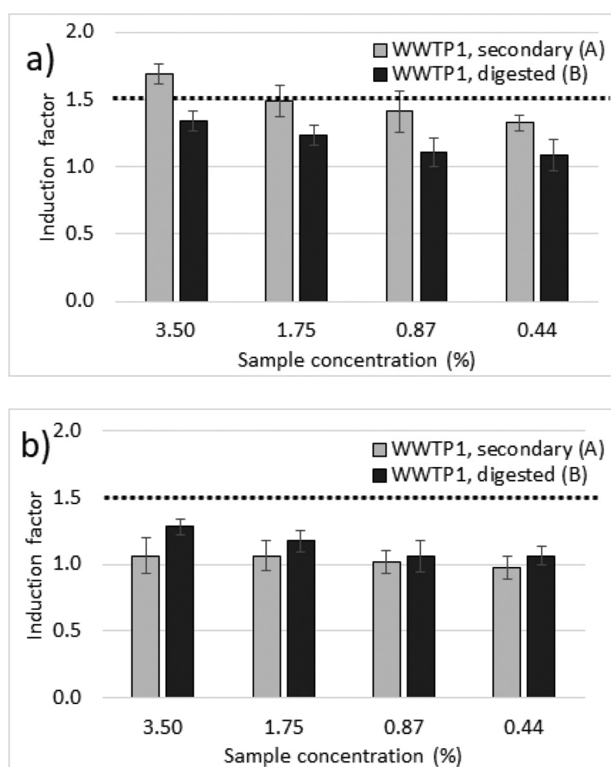

Fig. 2. The genotoxicity of secondary and digested sludge samples was analyzed with umuC-assay a) without metabolic activation and b) with metabolic activation by S9 (6replicates, mean ± SD).

Genotoxic effects (induction factor 1.71) in the UmuC tests were observed only in the highest sample concentration (3.5%) of the secondary sludge sample in the test without an S9 mix (Fig. 2a). However, in the tests with metabolic activation, the toxicity was reduced, which indicates that the compounds that caused genotoxicity in the sample were metabolized efficiently (Fig. 2b). The blanks showed no absorption and negative controls indicated no genotoxicity being on average 1.00–1.04 with standard deviations of 0.06–0.21. The mutagenic compounds can also be sorbed to the proteins in the S9 mix and hence give non-expression (Muller et al., 2007). The digested sludge samples were not genotoxic in any of the tested sample concentrations. Genotoxicity, per se, is a common response with aqueous eluates and has been recorded with the human-derived hepatoma (HepG2) cells (Mazzeo et al., 2016), the luminescent *Salmonella typhurium* strain (Kapanen et al., 2013), the *Vicia faba* root tips (Chiochetta et al., 2014) and the RTL-W1 fish cell line (Klee et al., 2004).

3.2.4. Bioassays and passive sampling

The toxicity test results with the luminescent bacteria in this study are in line with the results of many other studies indicating negative responses of the sludge eluates. It is difficult, however, to compare the extract of passive sampler that was deployed in the tests in this study with the literature observations as the extracts represent the bioaccessible fraction and as the samplers concentrate mainly hydrophobic organic contaminants and exclude heavy metals. Although sludge samples, their preparations for bioassays, and their bioassay types differ in the referenced publications, the adverse effects are very common. Among the sludge treatments, aging and composting appeared to diminish the effects (Ramirez et al., 2008; Kapanen et al., 2013; Mazzeo et al., 2016). The same progress was also evident in the present data, although not exclusively in all the test combinations.

It is a general view in literature that it is difficult to combine the analyzed chemical concentrations for characterizing exposure with the toxicological responses in sludge studies (e.g., Kapanen and Itävaara, 2001; Domene et al., 2008; Huguier et al., 2015). Considering the vast number of unknown substances and additive, synergistic, or antagonistic interactions, this may be an overwhelming task. Therefore, effect-based methods should have a stand-alone role in the RA process. Combining bioassays with passive samplers may further improve the testing setup and exposure scenario. The use of passive samplers in extract preparations has some clear advantages over the solvent and aqueous extract approaches. A sampler material mimics the bioconcentration of freely dissolved chemicals over the cell membranes or mimics the bioaccumulation of desorbing chemicals in a bioaccessibility study. Thus, sampler extract has high relevance in bioassays in reflecting exposure scenarios in field amendments. The sampler material can also be modified with absorbing phases to better represent bioavailable chemical mixtures (Smith et al., 2010; Jeong et al., 2018). Samplers can also be used in the passive dosing approach when the actual exposure scenario can be established in bioassays (e.g., Smith et al., 2010).

3.3. Bioavailability and bioaccessibility in the sludge risk assessment

Bioavailability as a concept and an operational term has been known and applied for decades in soil and sediment science (e.g., Ehlers & Ehlers and Luthy, 2003). The benefits of using bioavailability in the RA of contaminated sites are based on the understanding of the system, complementing the total concentration approach and essentially highlighting the ecological significance of the contamination. Bioavailability (Ortega-Calvo et al., 2015) and bioaccessibility (Bartolome et al., 2018) have been suggested to be part of the soil RA process, especially in retrospective assessment. The general acceptance has led to an ISO standard for soil quality assessment applying the bioavailability approach (ISO 17402 ISO (International Organization for Standardization), 2008). Bioavailability-related freely dissolved concentrations and chemical activities in WWTP sludge were recently introduced by Sjoeholm et al. (2018), confirming that the concepts are also suitable for the sludge matrix and can be used in evaluating sludge treatment efficiencies. In the present study, the usefulness of bioassays in detecting harmful chemical mixtures in bioaccessible fractions in the sludge treatment train was demonstrated.

The chemical RA of sludge-based biosolids in the EU is so far based only on the total heavy metal concentrations. Not only should more regulated substances be selected; their selection should also include mixture toxicity assessment using effect-based methods. Bioavailability and bioaccessibility can be used to characterize mobile and potentially harmful fractions. The methods would be appropriate for prospective RA methods, such as those indicating the suitability of the amendments made after different treatment steps (digestion and composting). The retrospective RA would also be important at amended field sites to follow the changes due to biodegradation and other aging-related effects. A safer dose range (t/ha) and application schedule could then be determined for each biosolid–field type combination (Table 6).

Table 6
Summary of the ecotoxicological test results.

ID	Sample	Toxicity for			
		A. fischeri, sludge extract	*D. magna*, PDMS extract	UmuC, PDMS extract	NRR, PDMS extract
A	WWTP1, secondary	+	+	+	+
B	WWTP1, digested	+	+	−	+
C	WWTP1, before composting	+	+	n.d	n.d
D	WWTP1, after composting	−	+	n.d	n.d
E	WWTP2, secondary	+	+	n.d	n.d
F	WWTP2, after composting	−	+	n.d	n.d

n.d. = not determined.

4. Conclusions

The disposal of municipal wastewater sludge is an important issue in the environmental management of recycling processes. Sewage sludge is a complex matrix that includes a number of polymeric, organic, and inorganic compounds. Due to the complexity of the matrix, the analysis and RA of the chemicals in municipal sewage sludge is not straightforward. For example, the size of the WWTP can have an effect on the efficiency of the sludge treatment process. In this study, the highest API concentrations were observed in the samples of a smaller wastewater treatment facility. This may also be related to the population demography and to the wider use of APIs among the aged WWTP2 customers.

Sludge treatments diminish the concentrations of chemicals and promote the recycling of the nutritious WWTP byproduct. However, sludge management techniques are required and have to be developed further before sewage sludge can be used in agricultural applications. In this study, standard procedures like digestion reduced the toxicity of sludge toward the light production of *A. fischeri*, and composting removed the toxic effects. However, the passive sampler extracts of a composted sludge still elicited adverse effects in the chronic exposures of *D. magna*. Also, the secondary and digested passive sampler extracts were cytotoxic, and the secondary sludge was genotoxic in the in-vitro tests. The amount of determined chemicals did not explain the toxicity of sludge. For example, the PAH concentrations in this study were clearly below the suggested regulatory limit value of 6 μg g^{-1} for soil amendments (EC, 2000). Also, additional analyses indicated concentrations below the German ordinance limit values for the other contaminants (Table S2).

The passive sampling method with silicone PDMS-coated jars can be used to estimate the freely dissolved concentrations of hydrophobic compounds in WWTP sludge, and to study chemical activity through the equilibrium concentrations in silicone. The PDMS sheet passive sampling approach, on the other hand, detects the bioaccessible fraction and provides a more useful view of the contaminants available for leaching and transfer in a recipient system. Thus, it senses a larger chemical fraction in a studied matrix. When these sampler extracts are applied in bioassays, they do not represent actual exposure conditions through pore water but may give an idea of a possibly desorbing chemical cocktail, such as that present in a digestive gut reactor of soil-dwelling biota (Penry and Jumars, 1987; Leppänen & Leppänen and Kukkonen, 1998). Assessing the hazards of sludge-based biosolids on different bioassays and considering the bioavailable or bioaccessible chemical fraction can provide a more representative picture of the harmfulness of sludge and sludge products to the environment.

The use of multiple ecotoxicological tests and environmental realism in the test selection should be promoted as the sensitivity of the organism and the endpoint can affect the accuracy and reliability of the results. Each ecotoxicity result should be accounted for as an evidence for a comprehensive environmental risk assessment because different organisms have different toxic responses. The harmonization of the passive sampler approach in sludge sampling and the effect-based methods in sludge testing will help establish scientifically sound regulative thresholds for the use of sludge in circular economy applications.

CRediT authorship contribution statement

Heidi Ahkola: Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Petra Lindholm-Lehto:** Investigation, Writing - original draft, Writing - review & editing. **Noora Perkola:** Investigation, Writing - review & editing. **Pia Välitalo:** Investigation. **Päivi Meriläinen:** Investigation. **Kimmo Mäenpää:** Methodology. **Julio Alberto Alegre Stelzer:** Investigation. **Ilse Heiskanen:** Investigation. **Johanna Järvistö:** Investigation. **Jari Nuutinen:** Investigation. **Matti T. Leppänen:** Conceptualization, Methodology, Validation, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.143700.

References

Allan, I.J., O'Connell, S.G., Meland, S., Baek, K., Grung, M., Anderson, K.A., Ranneklev, S.B., 2016. PAH accessibility in particulate matter from road-impacted environments. Environ. Sci. Technol. 50, 7964–7972.

Alvarenga, P., Palma, P., Goncalves, A.P., Fernandes, R.M., Cunha-Queda, A.C., Duarte, E., Vallini, G., 2007. Evaluation of chemical and ecotoxicological characteristics of biodegradable organic residues for application to agricultural land. Environ. Int. 33 (4), 505–513.

Aparicio, I., Santos, J.L., Alonso, E., 2009. Limitation of the concentration of organic pollutants in sewage sludge for agricultural purposes: a case study in South Spain. Waste Manag. 29, 1747–1753.

ASTM, 1997. Standard Guide for Conducting *Daphnia magna* Lifecycle Toxicity Tests. Report E 1193–97. Reapproved 2004. American Society for Testing and Materials, Philadelphia.

Babich, H., Borenfreund, E., 1992. Neutral Red Assay for Toxicology In Vitro. In Vitro Methods of Toxicology. CRC Press, Boca Raton, pp. 238–251.

Bartolome, N., Hilber, I., Sosa, D., Schulin, R., Mayer, P., Bucheli, T.D., 2018. Applying no-depletion equilibrium sampling and full-depletion bioaccessibility extraction to 35 historically polycyclic aromatic hydrocarbon contaminated soils. Chemosphere 199, 409–416.

Berthod, L., Whitley, D.C., Roberts, G., Sharpe, A., Greenwood, R., Mills, G.A., 2017. Quantitative structure-property relationships for predicting sorption of pharmaceuticals to sewage sludge during waste water treatment processes. Sci. Total Environ. 579, 1512–1520.

Bester, K., 2003. Triclosan in a sewage treatment process - balances and monitoring data. Water Res. 37, 3891–3896.

Bester, K., 2005. Fate of triclosan and triclosan-methyl in sewage treatment plants and surface waters. Arch. Environ. Contam. Toxicol. 49, 9–17.

Bloem, E., Albihn, A., Elving, J., Hermann, L., Lehmann, L., Sarvi, M., Schaaf, T., Schick, J., Turtol, E., Ylivainio, K., 2017. Contamination of organic nutrient sources with potentially toxic elements, antibiotics and pathogen microorganisms in relation to P fertilizer potential and treatment options for the production of sustainable fertilizers: a review. Sci. Total Environ. 607, 225–242.

Booij, P., Sjollema, S.B., Leonards, P.E.G., de Voogt, P., Stroomberg, G.J., Vethaak, A.D., Lamoree, M.H., 2013. Extraction tools for identification of chemical contaminants in estuarine and coastal waters to determine toxic pressure on primary producers. Chemosphere 93, 107–114.

Chen, Y.S., Yu, G., Cao, Q.M., Zhang, H.B., Lin, Q.Y., Hong, Y.W., 2013. Occurrence and environmental implications of pharmaceuticals in Chinese municipal sewage sludge. Chemosphere 93, 1765–1772.

Chiochetta, C.G., Goetten, L.C., Almeida, S.M., Quarana, G., Cotelle, S., Radetski, C.M., 2014. Leachates from solid wastes: chemical and eco(geno)toxicological differences between leachates obtained from fresh and stabilized industrial organic sludge. Environ. Sci. Pollut. Res. 21 (2), 1090–1098.

Christodoulou, A., Stamatelatou, K., 2016. Overview of legislation on sewage sludge management in developed countries worldwide. Water Sci. Technol. 73, 453–462.

Clarke, B.O., Smith, S.R., 2011. Review of 'emerging' organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. Environ. Int. 37 (1), 226–247.

Coogan, M.A., Edziyie, R.E., La Point, T.W., Venables, B.J., 2007. Algal bioaccumulation of triclocarban, triclosan, and methyl-triclosan in a North Texas wastewater, treatment plant receiving stream. Chemosphere 67, 1911–1918.

Cornelissen, G., van Noort, P.C.M., Govers, H.A.J., 1997. Desorption kinetics of chlorobenzenes, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls: sediment extraction with Tenax(R) and effects of contact time and solute hydrophobicity. Environ. Toxicol. Chem. 16 (7), 1351–1357.

Cupi, D., Baun, A., 2016. Methodological considerations for using umu assay to assess photo-genotoxicity of engineered nanoparticles. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 796, 34–39.

DeLorenzo, M.E., Keller, J.M., Arthur, C.D., Finnegan, M.C., Harper, H.E., Winder, V.L., Zdankiewicz, D.L., 2008. Toxicity of the antimicrobial compound triclosan and formation of the metabolite methyl-triclosan in estuarine systems. Environ. Toxicol. 23, 224–232.

Di Nica, V., Villa, S., Finizio, A., 2017. Toxicity of individual pharmaceuticals and their mixtures to Aliivibrio Fischeri: experimental results for single compounds and considerations of their mechanisms of action and potential acute effects on aquatic organisms. Environ. Toxicol. Chem. 36, 807–814.

Domene, X., Alcaniz, J.M., Andres, P., 2008. Comparison of solid-phase and eluate assays to gauge the ecotoxicological risk of organic wastes on soil organisms. Environ. Pollut. 151 (3), 549–558.

EC, 1991. Directive 91/271/EEC. COUNCIL DIRECTIVE of 21 May 1991 concerning urban waste water treatment. Off. J. Eur. Communities 135, 40–52.

EC, 2000. Working Document on Sludge. Third Draft. Brussels 27 April 2000.

EEC, 1986. Council Directive 86/278/EEC on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. Off. J. Eur. Communities 181/6.

Ehlers, L.J., Luthy, R.G., 2003. Contaminant bioavailability in soil and sediment. Environ. Sci. Technol. 37 (15), 295a–302a.

Eriksen, G.S., Amundsen, C.E., Bernhoft, A., Eggen, T., Grave, K., Halling-Sørensen, B., Källqvist, T., Sogn, T., Sverdrup, L., 2009. Risk Assessment of Contaminants in Sewage Sludge Applied on Norwegian Soils. Panel on Contaminants in the Norwegian Scientific Committee for Food Safety, Norway.

Escher, B.I., Eggen, R.I.L., Schreiber, U., Schreiber, Z., Vye, E., Wisner, B., Schwarzenbach, R., 2002. Baseline toxicity (narcosis) of organic chemicals determined by in vitro membrane potential measurements in energy-transducing membranes. Environ. Sci. Technol. 36 (9), 1971–1979.

Fernandez, L.A., Macfarlane, J.K., Tcaciuc, A.P., Gschwend, P.M., 2009. Measurement of freely dissolved PAH concentrations in sediment beds using passive sampling with low-density polyethylene strips. Environ. Sci. Technol. 43, 1430–1436.

FIWA, 2013. The use of processed sludge in agriculture Finnish Water Utilities Association (FIWA). ISBN 978-952-6697-91-8. In Finnish. https://www.proagria.fi/sites/default/files/attachment/puhdistamolieteopas_201320032014s.pdf.

Fjällborg, B., Ahlberg, G., Nilsson, E., Dave, G., 2005. Identification of metal toxicity in sewage sludge leachate. Environ. Int. 31 (1), 25–31.

Fransson, K., Molander, S., 2013. Handling chemical risk information in international textile supply chains. J. Environ. Plan. Manag. 56, 345–361.

Fu, Q.G., Liao, C.Y., Du, X.Y., Schlenk, D., Gan, J., 2018. Back conversion from product to parent: methyl triclosan to triclosan in plants. Environ. Sci. Technol. Lett. 5 (3), 181–185.

Gajski, G., Orescanin, V., Garaj-Vrhovac, V., 2011. Cytogenotoxicity of sewage sludge leachate before and after calcium oxide-based solidification in human lymphocytes. Ecotoxicol. Environ. Saf. 74 (5), 1408–1415.

Giannakis, I., Emmanouil, C., Mitrakas, M., Manakou, V., Kungolos, A., 2020. Chemical and ecotoxicological assessment of sludge-based biosolids used for corn field fertilization. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-020-09165-6 Published online: May 18 2020.

Gobas, F.A.P.C., Mayer, P., Parkerton, T.F., Burgess, R.M., van der Meer, D., Gouin, T., 2018. A chemical activity approach to exposure and risk assessment of chemicals. Environ. Toxicol. Chem. 37 (5), 1235–1251.

Gouliarmou, V., Mayer, P., 2012. Sorptive bioaccessibility extraction (SBE) of soils: combining a mobilization medium with an absorption sink. Environ. Sci. Technol. 46 (19), 10682–10689.

Hawthorne, S.B., Grabanski, C.B., Miller, D.J., 2006. Measured partitioning coefficients for parent and alkyl polycyclic aromatic hydrocarbons in 114 historically contaminated sediments: Part 1. K-OC values. Environ. Toxicol. Chem 25 (11), 2901–2911.

Hazard, C., Boots, B., Keith, A.M., Mitchell, D.T., Schmidt, O., Doohan, F.M., Bending, G.D., 2014. Temporal variation outweighs effects of biosolids applications in shaping arbuscular mycorrhizal fungi communities on plants grown in pasture and arable soils. Appl. Soil Ecol. 82, 52–60.

Hilber, I., Bastos, A.C., Loureiro, S., Soja, G., Marsz, A., Cornelissen, G., Bucheli, T.D., 2017. The different faces of biochar: contamination risk versus remediation tool. J. Environ. Eng. Landsc. Manag. 25, 86–104.

Huguier, P., Manier, N., Chabot, L., Bauda, P., Pandard, P., 2015. Ecotoxicological assessment of organic wastes spread on land: towards a proposal of a suitable test battery. Ecotoxicol. Environ. Saf. 113, 103–111.

Hundal, L.S., Cox, A., Granato, T.C., Abedin, Z., 2008. Levels of dioxins in soil and corn tissues after 30 years of biosolids application. J. Environ. Qual. 37 (4), 1497–1500.

ISO (International Organization for Standardization), 2000a. ISO 10706. Water Quality -Determination of Long Term Toxicity of Substances to *Daphnia magna* Straus (Cladocera. Crustacea). Geneva. Switzerland.

ISO (International Organization for Standardization), 2000b. ISO 13829. Water Quality – Determination of the Genotoxicity of Water and Waste Water Using the Umu-Test. Geneva. Switzerland.

ISO (International Organization for Standardization), 2007. ISO 11348-3. Water Quality. Determination of the Inhibitory Effect of Water Samples on the Light Emission of Vibrio Fischeri (Luminescent Bacteria Test). Part 3: Method Using Freeze-Dried Bacteria. Geneva. Switzerland.

ISO (International Organization for Standardization), 2008. ISO 17402. Soil Quality-Requirements and Guidance for the Selection and Application of Methods for the Assessment of Bioavailability of Contaminants in Soil and Soil Materials, Geneva, Switzerland.

ISO (International Organization for Standardization), 2010. ISO 21338. Water Quality- Kinetic Determination of the Inhibitory Effects of Sediment. Other Solids and Coloured Samples on the Light Emission of Vibrio Fischeri (Kinetic Luminescent Bacteria Test). Geneva. Switzerland.

Jahnke, A., Mayer, P., McLachlan, M.S., 2012. Sensitive equilibrium sampling to study polychlorinated biphenyl disposition in Baltic Sea sediment. Environ. Sci. Technol. 46, 10114–10122.

Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sanchez, R., Ventura, F., Petrovic, M., Barcelo, D., 2011. Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. Water Res. 45, 1165–1176.

Jeong, Y., Schäffer, A., Smith, K., 2018. Comparison of the sampling rates and partitioning behaviour of polar and non-polar contaminants in the polar organic chemical integrative sampler and a monophasic mixed polymer sampler for application as an equilibrium passive sampler. Sci. Total Environ. 627, 905–915.

Kapanen, A., Itävaara, M., 2001. Ecotoxicity tests of compost applications. Ecotoxicol. Environ. Saf. 49 (1), 1–16.

Kapanen, A., Vikman, M., Rajasärkkä, J., Virta, M., Itävaara, M., 2013. Biotests for environmental quality assessment of composted sewage sludge. Waste Manag. 33 (6), 1451–1460.

Kim, K.H., Jahan, S.A., Kabir, E., Brown, R.J.C., 2013. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ. Int. 60, 71–80.

Klee, N., Gustavsson, L., Kosmehl, T., Engwall, M., Erdinger, L., Braunbeck, T., Hollert, H., 2004. Changes in toxicity and genotoxicity of industrial sewage sludge samples containing nitro- and amino-aromatic compounds following treatment in bioreactors with different oxygen regimes. Environ. Sci. Pollut. Res. 11, 313–320.

Lapen, D.R., Topp, E., Metcalfe, C.D., Li, H., Edwards, M., Gottschall, N., Bolton, P., Curnoe, W., Payne, M., Beck, A., 2008. Pharmaceutical and personal care products in tile drainage following land application of municipal biosolids. Sci. Total Environ. 399 (1–3), 50–65.

Lappalainen, J., Juvonen, R., Nurmi, J., Karp, M., 2001. Automated color correction method for Vibrio fischeri toxicity test. Comparison of standard and kinetic assays. Chemosphere 45 (4-5), 635–641.

LeBlanc, R.J., Matthews, P., Richard, R.P., 2009. Global Atlas of Excreta, Wastewater Sludge. And Biosolids Management: Moving Forward the Sustainable and Welcome Uses of a Global Resource. United Nations Human Settlements Programme UN-Habitat, Nairobi, Kenya.

Lee, L.E.J., Clemons, J.H., Bechtel, D.G., Caldwell, S.J., Han, K.B., Pasitschniakarts, M., Mosser, D.D., Bols, N.C., 1993. Development and characterization of a rainbow-trout liver-cell line expressing cytochrome P450-dependent monooxygenase activity. Cell Biol. Toxicol. 9, 279–294.

Lee, S.-Y., Kang, H.-J., Kwon, J.-H., 2013. Toxicity cutoff of aromatic hydrocarbons for luminescence inhibition of Vibrio fischeri. Ecotoxicol. Environ. Saf. 94, 116–122.

Leppänen, M.T., Kukkonen, J.V.K., 1998. Relative importance of ingested sediment and pore water as bioaccumulation routes for pyrene to oligochaete (Lumbriculus variegatus, Muller). Environ. Sci. Technol. 32, 1503–1508.

Li, J.Y., Tang, J.Y.M., Jin, L., Escher, B.I., 2013. Understanding bioavailability and toxicity of sediment-associated contaminants by combining passive sampling with in vitro bioassays in an urban river catchment. Environ. Toxicol. Chem. 32, 2888–2896.

Lindholm-Lehto, P.C., Ahkola, H.S.J., Knuutinen, J.S., Herve, S.H., 2015. Occurrence of pharmaceuticals in municipal wastewater, in the recipient water, and sedimented particles of northern Lake Paijanne. Environ. Sci. Pollut. Res. 22, 17209–17223.

Lindholm-Lehto, P.C., Ahkola, H.S.J., Knuutinen, J.S., 2018. Pharmaceuticals in processing of municipal sewage sludge studied by grab and passive sampling. Water Qual. Res. J. Can. 53, 14–23.

Lopez, M.J., Vargas-Garcia, M.C., Suarez-Estrella, F., Guisado, G., Moreno, J., 2010. Flash microbial toxicity test as monitoring parameter at composting: Comparison of ecotoxicity level for different substrates. Proceedings in 14th Ramiran International Conference, Lisboa, Portugal.

Macdonald, R.W., Mackay, D., Hickie, B., 2002. A new approach suggests that phenomena, such as bioconcentration, biomagnification, and bioaccumulation, result from two fundamental processes. Environ. Sci. Technol. 36, 457A–463A.

Mäenpää, K., Leppanen, M.T., Figueiredo, K., Mayer, P., Gilbert, D., Jahnke, A., Gil-Allue, C., Akkanen, J., Nybom, I., Herve, S., 2015. Fate of polychlorinated biphenyls in a contaminated lake ecosystem: combining equilibrium passive sampling of sediment and water with total concentration measurements of biota. Environ. Toxicol. Chem. 34, 2463–2474.

Malara, A., Oleszczuk, P., 2013. Application of a battery of biotests for the determination of leachate toxicity to bacteria and invertebrates from sewage sludge-amended soil. Environ. Sci. Pollut. Res. 20 (5), 3435–3446.

Mantis, I., Voutsa, D., Samara, C., 2005. Assessment of the environmental hazard from municipal and industrial wastewater treatment sludge by employing chemical and biological methods. Ecotoxicol. Environ. Saf. 62, 397–407.

Martín, J., Camacho-Munoz, D., Santos, J.L., Aparicio, I., Alonso, E., 2012. Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: removal and ecotoxicological impact of wastewater discharges and sludge disposal. J. Hazard. Mater. 239, 40–47.

Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2015. Pharmaceutically active compounds in sludge stabilization treatments: anaerobic and aerobic digestion, wastewater stabilization ponds and composting. Sci. Total Environ. 503, 97–104.

Mayer, P., Vaes, W.H.J., Hermens, J.L.M., 2000. Absorption of hydrophobic compounds into the poly(dimethylsiloxane) coating of solid-phase microextraction fibers: high partition coefficients and fluorescence microscopy images. Anal. Chem. 72, 459–464.

Mayer, P., Olsen, J.L., Gouliarmou, V., Hasinger, M., Kendler, R., Loibner, A.P., 2011. A contaminant trap as a tool for isolating and measuring the desorption resistant fraction of soil pollutants. Environ. Sci. Technol. 45, 2932–2937.
Mayer, P., Parkerton, T.F., Adams, R.G., Cargill, J.G., Gan, J., Gouin, T., Gschwend, P.M., Hawthorne, S.B., Helm, P., Witt, G., You, J., Escher, B.I., 2014. Passive sampling methods for contaminated sediments: scientific rationale supporting use of freely dissolved concentrations. Integr. Environ. Assess. Manag. 10, 197–209.
Mazzeo, D.E.C., Fernandes, T.C.C., Marin-Morales, M.A., 2016. Attesting the efficiency of monitored natural attenuation in the detoxification of sewage sludge by means of genotoxic and mutagenic bioassays. Chemosphere 163, 508–515.
Milinovic, J., Vidal, M., Lacorte, S., Rigol, A., 2014. Leaching of heavy metals and alkylphenolic compounds from fresh and dried sewage sludge. Environ. Sci. Pollut. Res. 21, 2009–2017.
Moreira, R., Sousa, J.P., Canhoto, C., 2008. Biological testing of a digested sewage sludge and derived composts. Bioresour. Technol. 99 (17), 8382–8389.
Muller, R., Tang, J.Y.M., Thierb, R., Mueller, J.F., 2007. Combining passive sampling and toxicity testing for evaluation of mixtures of polar organic chemicals in sewage treatment plant effluent. J. Environ. Monit. 9, 104–109.
Natal-da-Luz, T., Tidona, S., Jesus, B., Morais, P.V., Sousa, J.P., 2009. The use of sewage sludge as soil amendment. The need for an ecotoxicological evaluation. J. Soils Sediments 9 (3), 246–260.
Nkinahamira, F., Suanon, F., Chi, Q.Q., Li, Y.Y., Feng, M.L., Huang, X.Y., Yu, C.P., Sun, Q., 2019. Occurrence, geochemical fractionation, and environmental risk assessment of major and trace elements in sewage sludge. J. Environ. Manag. 249, 109427.
OECD, 2008. OECD Guidelines for the Testing of Chemicals. Test No. 211: *Daphnia magna* Reproduction Test. Organization for Economic Co-operation and Development, Paris.
Ortega-Calvo, J.J., Harmsen, J., Parsons, J.R., Semple, K.T., Aitken, M.D., Ajao, C., Eadsforth, C., Galay-Burgos, M., Naidu, R., Oliver, R., Peijnenburg, W.J.G.M., Rombke, J., Streck, G., Versonnen, B., 2015. From bioavailability science to regulation of organic chemicals. Environ. Sci. Technol. 49 (17), 10255–10264.
Ozaki, N., Nakazato, A., Nakashima, K., Kindaichi, T., Ohashi, A., 2017. Loading and removal of PAHs, fragrance compounds, triclosan and toxicity by composting process from sewage sludge. Sci. Total Environ. 605, 860–866.
Pannu, M.W., Toor, G.S., O'Connor, G.A., Wilson, P.C., 2012. Toxicity and bioaccumulation of biosolids-borne triclosan in food crops. Environ. Toxicol. Chem. 31 (9), 2130–2137.
Penry, D.L., Jumars, P.A., 1987. Modeling animal guts as chemical reactors. Am. Nat. 129, 69–96.
Pintado-Herrera, M.G., Lara-Martin, P.A., Gonzalez-Mazo, E., Allan, I.J., 2016. Determination of silicone rubber and low-density polyethylene diffusion and polymer/water partition coefficients for emerging contaminants. Environ. Toxicol. Chem. 35, 2162–2172.
Project on Urban Reduction of Eutrophication (PURE), 2012. Good practices in sludge management, appendix a. ISBN 978-952-5725-92-6. Available online:. http://www.purebalticsea.eu/download.php///dms/pure/PURE_Good_practices_in_sludge_management.pdf.
Prosser, R.S., Trapp, S., Sibley, P.K., 2014. Modeling uptake of selected pharmaceuticals and personal care products into food crops from biosolids-amended soil. Environ. Sci. Technol. 48 (19), 11397–11404.
Radjenovic, J., Petrovic, M., Barcelo, D., 2009. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res. 43, 831–841.
Ramirez, W.A., Domene, X., Ortiz, O., Alcaniz, J.M., 2008. Toxic effects of digested, composted and thermally-dried sewage sludge on three plants. Bioresour. Technol. 99 (15), 7168–7175.
Reichenberg, F., Mayer, P., 2006. Two complementary sides of bioavailability: accessibility and chemical activity of organic contaminants in sediments and soils. Environ. Toxicol. Chem. 25, 1239–1245.
Reichenberg, F., Smedes, F., Jonsson, J.A., Mayer, P., 2008. Determining the chemical activity of hydrophobic organic compounds in soil using polymer coated vials. Chem. Central J. 2.
Reid, B.J., Jones, K.C., Semple, K.T., 2000. Bioavailability of persistent organic pollutants in soils and sediments - perspective on mechanisms, consequences and assessment. Environ. Pollut. 108 (1), 103–112.
Schmidt, S.N., Wang, A.P., Gidley, P.T., Wooley, A.H., Lotufo, G.R., Burgess, R.M., Ghosh, U., Fernandez, L.A., Mayer, P., 2017. Cross validation of two partitioning-based sampling approaches in mesocosms containing PCB contaminated field sediment, biota, and activated carbon amendment. Environ. Sci. Technol. 51, 9996–10004.
Silva, E., Rajapakse, N., Kortenkamp, A., 2002. Something from "nothing" - eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. Environ. Sci. Technol. 36, 1751–1756.
Sjoeholm, K.K., Schmidt, S.N., 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.
Smedes, F., Geertsma, R.W., van der Zande, T., Booij, K., 2009. Polymer-water partition coefficients of hydrophobic compounds for passive sampling: application of cosolvent models for validation. Environ. Sci. Technol. 43, 7047–7054.
Smith, S.R., 2009. Organic contaminants in sewage sludge (biosolids) and their significance for agricultural recycling. Philos. Trans. R. Soc. A Math. Phys. Eng. Sci. 367 (1904), 4005–4041.
Smith, K.E.C., Oostingh, G.J., Mayer, P., 2010. Passive dosing for producing defined and constant exposure of hydrophobic organic compounds during in vitro toxicity tests. Chem. Res. Toxicol. 23 (1), 55–65.
Sonavane, M., Schollee, J.E., Hidasi, A.O., Creusot, N., Brion, F., Suter, M.J.F., Hollender, J., Ait-Aissa, S., 2018. An integrative approach combining passive sampling, bioassays, and effect-directed analysis to assess the impact of wastewater effluent. Environ. Toxicol. Chem. 37, 2079–2088.
SOU, 2020. Statens Offentliga Utredningar.2020:3. Hållbar slamhantering. Betänkande av Utredningen om en giftfri och cirkulär återföring av fosfor från avloppsslam. Elanders Sverige AB, Stockholm.
SPSS 23, 2015. IBM SPSS Statistics for Windows. Version 23.0. IBM Corp, Armonk. NY.
Sullivan, T.S., Stromberger, M.E., Paschke, M.W., 2006. Parallel shifts in plant and soil microbial communities in response to biosolids in a semi-arid grassland. Soil Biol. Biochem 38 (3), 449–459.
Stołyhwo, A., Sikorski, Z.E., 2005. Polycyclic aromatic hydrocarbons in smoked fish - a critical review. Food Chemistry 91 (2), 303–311.
Svahn, O., Bjorklund, E., 2015. 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, 73–82.
Topp, E., Monteiro, S.C., Beck, A., Coelho, B.B., Boxall, A.B.A., Duenk, P.W., Kleywegt, S., Lapen, D.R., Payne, M., Sabourin, L., Li, H.X., Metcalfe, C.D., 2008. Runoff of pharmaceuticals and personal care products following application of biosolids to an agricultural field. Sci. Total Environ. 396 (1), 52–59.
Vatten, Svenskt, 2020. https://www.svensktvatten.se/om-oss/nyheter-lista/kommentar-fran-svenskt-vatten-angaende-fosforutredningen/.
Verlicchi, P., Zambello, E., 2015. Pharmaceuticals and personal care products in untreated and treated sewage sludge: Occurrence and environmental risk in the case of application on soil - A critical review. Sci. Total Environ. 538, 750–767.
Vermeirssen, E.L.M., Bramaz, N., Hollender, J., Singer, H., Escher, B.I., 2009. Passive sampling combined with ecotoxicological and chemical analysis of pharmaceuticals and biocides - evaluation of three Chemcatcher (TM) configurations. Water Res. 43, 903–914.
Vestergren, R., Herzke, D., Wang, T., Cousins, I.T., 2015. Are imported consumer products an important diffuse source of PFASs to the Norwegian environment? Environ. Pollut. 198, 223–230.
Vethaak, A.D., Hamers, T., Martinez-Gomez, C., Kamstra, J.H., de Weert, J., Leonards, P.E.G., Smedes, F., 2017. Toxicity profiling of marine surface sediments: a case study using rapid screening bioassays of exhaustive total extracts, elutriates and passive sampler extracts. Mar. Environ. Res. 124, 81–91.
Villa, S., Vighi, M., Finizio, A., 2014. Experimental and predicted acute toxicity of antibacterial compounds and their mixtures using the luminescent bacterium Vibrio fischeri. Chemosphere 108, 239–244.
Vilpanen, M., Toivikko, S.R., 2017. https://www.vvy.fi/ohjeet-ja-julkaisut/jatevedet/yhdyskuntalietteen-kasittelyn-ja-hyodyntamisen-nykytilannekatsaus-vvy/.
Vrana B, Smedes F, Rusina T, Okonski K, Allan I, Grung M, Hilscherova K, Novák J, Tarábek P, Slobodník J. 2015. Passive sampling: Chemical analysis and toxicological profiling. In: Liška I, Wagner F, Sengl M, Deutsch K, Slobodnik J. (eds.) Joint Danube Survey 3. A Comprehensive Analysis of Danube Water Quality, ICPDR, pp. 304–315.
Wu, X.Q., Dodgen, L.K., Conkle, J.L., Gan, J., 2015. Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review. Sci. Total Environ. 536, 655–666.
Yates, K., Davies, I., Webster, L., Pollard, P., Lawton, L., Moffat, C., 2007. Passive sampling: partition coefficients for a silicone rubber reference phase. J. Environ. Monit 9 (10), 1116–1121.
YLE News, 7.4, 2018. https://yle.fi/uutiset/3-10148145.
Ziylan, A., Ince, N.H., 2011. The occurrence and fate of anti-inflammatory and analgesic pharmaceuticals in sewage and fresh water: Treatability by conventional and non-conventional processes. J. Hazard. Mater. 187 (1-3), 24–36.