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Aerobic treatment of selective serotonin reuptake inhibitors in landfill leachate

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Abstract

Background: Pharmaceuticals used in human medical care are not completely eliminated in the human body and can enter the municipal sewage sludge system and leachate water from landfill both as the parent compound and as their biologically active metabolites. The selective serotonin reuptake inhibitors (SSRIs) have a large potential for unwanted effects on nontarget organisms in the environment. Leachates from active or old closed landfills are often treated with continuous stirring and simple aeration in a pond/lagoon before infiltration into the environment. The aim of this work was to simulate the reduction of five SSRIs (citalopram, fluoxetine, paroxetine, sertraline and fluvoxamine) and three of their metabolites (desmethylcitalopram, didesmethylcitalopram and norfluoxetine) during aerobic treatment of leachate from landfills. This landfill leachate-simulation experiment was performed to see what happens with the pharmaceuticals during aerated treatment and continuous stirring of landfill leachate for 120 h. It is important to establish whether different pollutants such as pharmaceuticals can be removed (oxidized or otherwise degraded) or not before infiltration into the environment.

Results: All the SSRIs had a significant concentration reduction during the aeration treatment process. Total SSRI concentrations were reduced significantly during aerobic treatment, and the individual SSRIs were reduced by 89% to 100% after 120 h. Among the high-concentration samples, fluoxetine (10 mg L^{-1}) was the least degraded with 93% concentration reduction. Among the low-concentration samples, paroxetine was the least degraded with 89% concentration reduction. Fluvoxamine and citalopram were most effectively eliminated and were completely removed from both the high- and low-concentration samples. The samples were also investigated for the metabolites desmethylcitalopram, didesmethylcitalopram and norfluoxetine but only norfluoxetine in the high-concentration fluoxetine sample was detected.

Conclusions: Our results suggest that aeration is an effective method for eliminating pharmaceuticals such as SSRIs from landfill leachate water. Comparing the results of all SSRIs with different treatment methods, paroxetine and fluvoxamine seem to be the easiest compounds to eliminate independent of method, while fluoxetine and sertraline seem to be the most stable.

Keywords: Aeration; Biological treatment; Environment; Landfill leachate; Pharmaceuticals; SSRI

Background

For several years, the occurrence of pharmaceuticals and personal care products (PPCPs) in the aquatic environment has been recognized as one of the emerging issues in environmental risk assessment [1-4]. Pharmaceuticals used in human medical care are not completely eliminated in the human body and can enter the municipal sewage sludge system both as the parent compound and

as their biologically active metabolites. These compounds have a large potential for unwanted effects on nontarget organisms in the environment. Several investigators have examined the removal of pharmaceutical compounds during the passage through municipal sewage treatment systems [5-8]. Effluent concentrations and elimination rates for different compounds vary significantly. Various pharmaceutical compounds have been detected in concentrations up to μg L^{-1} in sewage effluents, downstream of sewage treatment plants and in surface and groundwater [2,9,10]. In addition to sewage treatment plants, landfill sites where unused drugs and different

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personal care products have been disposed may release these compounds into the environment. There are several old landfill sites that were established when the disposal of such compounds through garbage was tolerated, and drugs have been detected in leachates from such municipal landfills [11]. Leachates from landfill sites are also known to penetrate down to the groundwater causing pollution [12].

Six selective serotonin reuptake inhibitors (SSRIs) are on the Norwegian market today, fluoxetine, fluvoxamine, paroxetine, sertraline, citalopram and escitalopram (the pure S-enantiomer of citalopram). SSRIs are typically used as antidepressants and are used to treat conditions such as depression, anxiety disorders and some personality disorders. The total consumption of SSRIs has slowly increased in Norway the last years, although when adjusted to population growth, the number of SSRI users has been relatively stable at just below 4% of the population. Citalopram/escitalopram is most widely distributed and constitutes approximately 591 kg of the total amount, while sertraline with its higher defined daily dosage is sold in the largest amounts contributing with approximately 621 kg [13]. SSRIs have been found in wastewater in Norway [8,14], and it is reasonable to believe that not all pharmaceuticals are used or delivered back to the pharmacies but might end up on landfill sites through private garbage disposal. Contaminants from landfill sites might reach the environment through leachate water run-off, and water soluble chemicals can reach the aquatic ecosystems or the groundwater. SSRIs have been shown to have a number of unwanted effects on aquatic organisms, such as the behavioural effect fluoxetine has on fish [15] and the induction of spawning and parturition in bivalves [16,17]. Fluoxetine is probably the most studied SSRI when it comes to effects on nontarget organisms, but effects from the other SSRIs have been found as well.

Depletion of SSRIs in sewage sludge has previously been found during an aerobic composting process [18]. Vasskog [18] showed that the depletion rate was highest for fluoxetine (1.23 mg (kg ash/day)$^{-1}$) and paroxetine (1.31 mg (kg ash/day)$^{-1}$) and lowest for citalopram (0.88 mg (kg ash/day)$^{-1}$). In addition, three out of four known SSRI metabolites were detected in all compost samples, and two of them showed a significant increase in concentrations during the composting period.

PPCPs have also been investigated under anaerobic digestion of sewage sludge [19-22], but SSRIs were not part of these investigations. Carballa et al. [19] observed high removal efficiencies for antibiotics, natural estrogens, musk and naproxen, while for example carbamazepine showed no elimination under anaerobic treatment of sewage sludge. Vieno et al. [20] also observed no elimination of carbamazepine in their anaerobic treatment of raw sewage, while fluoroquinolones were eliminated by >80%.

Beta-blockers were found to be eliminated less than 65%. Degradation was found for acetylsalicylic acid, while a mixture of degradation and abiotic removal mechanisms on other pharmaceuticals was observed during an anaerobic degradability study by Musson et al. [21]. The fate of hormones and pharmaceuticals during combined anaerobic treatment and nitrogen removal in black water has been investigated by de Graaff et al. [23]. They found that only a few compounds were partly removed, and anaerobic treatment was effective only to remove the majority of paracetamol. Falås et al. [24] have demonstrated that there are large variations in removal of different pharmaceuticals within the same pharmaceutical class as well in activated sludge. In this experiment, the nonsteroidal anti-inflammatory drugs (NSAIDs) ibuprofen and naproxen showed the highest removal rates, while diclofenac, mefenamic acid and clofibric acid showed little or no removal. Bergersen et al. [25] have shown that anaerobic treatment of sewage sludge gives a reduction in SSRI concentrations from 32% (fluoxetine) to 98% (citalopram) in a 24-day experiment, again demonstrating large variations within the same pharmaceutical class.

Eggen et al. [26] have described that PPCPs from old landfill leachate with treatment based on aeration and sedimentation may represent a significant source of concern for new and emerging pollutants in groundwater.

To our best knowledge, no studies have been conducted on SSRIs in treatment of aerated landfill leachate. When pharmaceuticals enter the environment through landfill leachate systems, they may either be found in the water phase or bound to particles. Landfill leachate aeration ponds could treat the water via aeration and particulate matter sedimentation.

Based on earlier results showing that the removal of SSRIs from sewage sludge is different during aerobic and anaerobic treatment, it was interesting to compare this with what would happen in the aeration process that landfill leachate goes through.

The aim of this work was to simulate the possible elimination of five SSRIs (citalopram, fluoxetine, paroxetine, sertraline and fluvoxamine) and three of their metabolites (desmethylcitalopram, didesmethylcitalopram and norfluoxetine) during aerobic treatment of leachate from landfills. Today, landfill leachates are treated with aeration in a pond/lagoon before infiltration into the environment. This landfill leachate-simulation experiment was performed to see what happens with the pharmaceuticals during aerated treatment and continuous stirring of landfill leachate for 120 h. This experiment mimics the common process used for treatment of landfill leachate ponds and aims to predict the efficiency of pharmaceutical removal in aerated leachate ponds. It is important to establish whether different pollutants such as pharmaceuticals can be removed or not before infiltration into the environment.

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Results and discussion

Aerobic treatment and oxygen consumption

Due to the use of the pharmaceutical compounds as a carbon source combined with the carbon source in the leachate, we followed the changing concentrations of each SSRI in flasks with continuous stirring. Simultaneously, we followed the changing consumption of oxygen within the flasks headspace through 120 h (Figure 1). In landfill leachate, prescription and nonprescription pharmaceutical concentrations generally range from 100 to 10,000 ng L^{-1} [27]. The concentrations used in this experiment are

somewhat higher than what is found before to enhance the effects seen on the experimental outcomes but are still regarded as environmentally relevant. Each type of SSRI with different concentrations could also give different oxygen consumption when present in landfill leachate, which again will be valuable information about the aerobic treatment of the landfill leachate.

Table 1 presents the ThOD for each SSRI, the total OC after 48 and 120 h and the measured OC minus the control sample (oxygen consumption of leachate) without SSRI. All the calculated ThODs show lower values
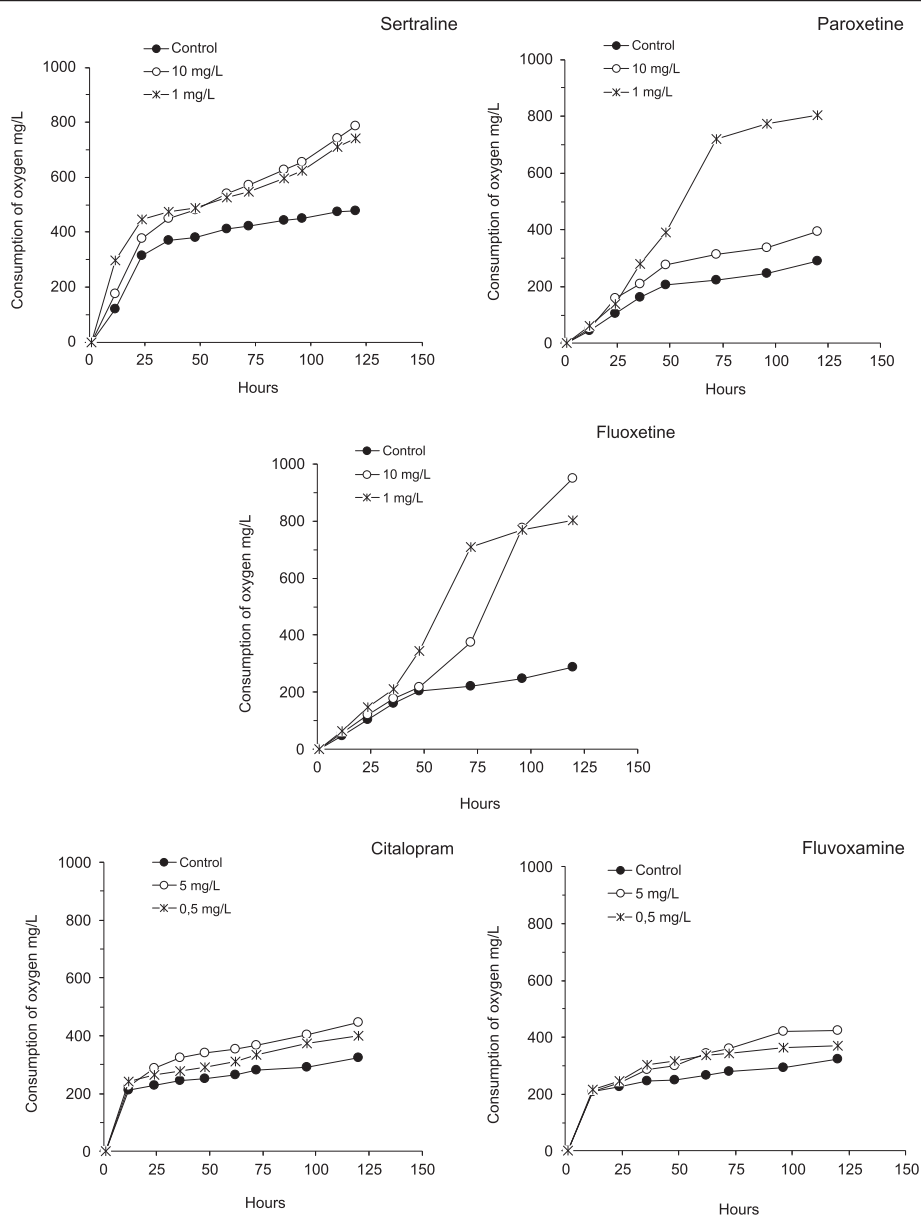

Figure 1 Total OC after 120 h in Voith Sapromat exposed to different concentrations of SSRIs in landfill leachate. Sertraline, paroxetine and fluoxetine 10 and 1 mg L^{-1}; fluvoxamine and citalopram 5 and 0.5 mg L^{-1}. Measured background respiration as control from leachate without SSRIs is included.

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Table 1 Theoretical and measured concentration of each SSRI spiked in leachate water before an aerobic treatment

Source	Theoretical SSRI	Measured SSRI	(ThOD) SSRI	Measured 48 h Tot. OC	Measured 120 h Tot. OC	Measured 48 h OC*	Measured 120 h OC*
	mg L^{-1}	mg L^{-1}	mgO2 L^{-1}	mgO2 L^{-1}	mgO2 L^{-1}	mgO2 L^{-1}	mgO2 L^{-1}
Fluvoxamine	5	6	11.0	300	424	49	99
	0.5	0.61	1.1	317	370	66	55
Citalopram	5	3.2	13.1	340	445	89	120
	0.5	0.39	1.3	290	401	39	76
Sertraline	10	10	30.2	482	787	101	311
	1	1.33	3.0	488	740	107	264
Fluoxetine	10	11	22.8	340	950	135	661
	1	0.84	2.3	290	802	85	513
Paroxetine	10	14	22.4	278	395	73	106
	1	1.31	2.2	393	803	188	514

Measured oxygen consumption (Tot. OC) and OC* (Equation 1) after 48 and 120 h with each separate SSRI is compared with theoretical oxygen demand (ThOD) of SSRI tablets. The results of measured total OC are given as mean values ($n = 2$). *Equation 1.

than the measured OC after Equation 1 for each SSRI. All flasks spiked with the different SSRI tablets show dissimilar oxygen consumption, although considerably higher than the ThOD for each SSRI. This increased oxygen consumption in the flasks might be due to (i) co-metabolism between each SSRIs plus tablets excipients and low decomposable carbon sources in the leachate, which was not accessible alone in the control flask; (ii) microbial co-metabolism between SSRIs and the carbon source from the excipients in the tablets alone. Each tablet mixture used contained approximately 20% SSRIs and 80% excipients. Each SSRI tablet contains a large number of additives, e.g. carbon sources such as cellulose, starch and glycerol.

The high induced oxygen consumption observed in the flasks with paroxetine or fluoxetine in leachate substrate is most likely due to the excipients (Figure 1 and Table 1). Both these SSRI tablets have a higher amount of excipients than the other three SSRIs. The increased amount of excipient gave considerably higher oxygen consumption compared to the control samples and the other flasks spiked with fluvoxamine, citalopram or sertraline. Figure 1 and Table 1 also show that each SSRI gave an increased oxygen consumption compared to background consumption in the control flasks after a minimum of 50 h independent of SSRI concentration. After 120 h fluvoxamine shows less oxygen consumption in the flask with 0.5 mg L^{-1}, while the other SSRIs show increased oxygen consumption (Table 1). The diluted leachate solution without SSRIs had an initial pH of 7.1, and when spiking the samples with SSRIs, the pH increased in all samples due to the basic nature of the SSRIs (Table 2). The pH at start in

Table 2 SSRI concentrations and pH at the beginning and after 120 h of the experiment

SSRI	Measured at start				Measured at end				
	SSRI	STDEV	Rel. STDEV	pH	SSRI	STDEV	Rel. STDEV	pH	Reduction
	mg L^{-1}	mg L^{-1}	%		mg L^{-1}	mg L^{-1}	%		%
Fluvoxamine	6.0	1.104	18	8.7	0.006	0.004	68	8.4	100
	0.6	0.123	20	8.6	0.002	0.002	87	8.3	100
Citalopram	3.2	0.102	3	9.1	0.005	0.001	16	8.5	100
	0.4	0.026	7	9.0	0.002	0.001	11	8.6	100
Sertraline	10.0	1.227	12	8.6	0.355	0.046	13	7.9	96
	1.3	0.171	13	8.5	0.038	0.017	45	7.7	97
Fluoxetine	11.0	0.873	8	9.2	0.805	0.063	8	8.1	93
	0.8	0.156	18	9.0	0.003	0.001	14	8.0	100
Norfluoxetine	n.d.				0.006	0.001	11		
	n.d.			n.d	n.d.				
Paroxetine	14.0	0.163	1	9.8	0.105	0.011	11	8.0	99
	1.3	0.143	11	9.6	0.141	0.003	2	7.9	89

n.d., not detected.

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the samples varied between 8.5 (sertraline, low concentration) and 9.8 (paroxetine, high concentration). During the 120-h experiment, the pH was found to decrease in all samples, and the exact values can be seen in Table 2. This decrease in pH together with high oxygen consumption could support an explanation that the SSRIs spiked in the leachate have gone through an oxidation or are otherwise reduced in concentration.

Earlier SSRIs have been investigated under aerobic and anaerobic treatment processes of sewage waste to see if the SSRIs deplete or accumulate. Vasskog et al. [18] showed that all the SSRIs had a significant decrease in concentration during aerobic treatment in a composting process. Bergersen et al. [25] showed that paroxetine and citalopram were almost completely reduced during anaerobic treatment of sewage sludge while the lower reduction of fluoxetine, fluvoxamine and sertraline indicate that these three compounds have a higher potential for accumulation than citalopram and paroxetine under anaerobic conditions. A review describing removal or sorption of pharmaceuticals and other personal care products in water treatment such as natural wetland systems concludes that these systems have a high potential for removal of such chemicals [28]. One SSRI (fluoxetine) has been found to be removed from wetland systems by sorption into duckweed [29]. To our best knowledge, no studies have been conducted on SSRI depletion in landfill leachate ponds treated with aeration.

Analysis and reduction of selective serotonin reuptake inhibitors

The five selective serotonin reuptake inhibitors citalopram, sertraline, paroxetine, fluvoxamine and fluoxetine were analysed at the beginning and at the end of a 120-h experiment in a Voith Sapromat respirometer to investigate their concentration reduction during the process. The concentrations were calculated as mg L^{-1} and compared with the total theoretical amount of each SSRI if no reduction had occurred in the samples. Table 1 shows the theoretical concentration, measured concentration and recovery of each SSRI before treatment. After treatment for 120 h, all the SSRIs were measured at lower concentrations at the end of the experiment. The results shown in Table 2 give a detailed view of the concentration of each SSRI and the depletion throughout the experiment. All compounds showed a reduction in concentration of 89% to 100%, independent of high- or low-start concentration. The recovery was measured at the beginning of the experiment and was generally high (between 64% and 140%). The high recoveries of the SSRIs also imply that there is low or no binding to particles and humic acids in this experimental setup. There was no possibility to measure the recovery at the end of the experiment, but it is expected to be equal to that at the

beginning. The highest recoveries (above 100%) might be explained partly by the standard deviation but maybe more likely by the fact that the added SSRI is taken from crushed tablets. The weighed amount of the tablet might not have been homogenous and the sample withdrawn not representative. However, the main outcome of the experiment would be the same, showing the concentration reduction of the SSRIs during the experiment. There are small differences between the recovery of the high and low concentrations at the beginning of the experiment, which also indicate that the recovery should be more or less equal at even lower concentrations. Therefore, it is most likely that the low concentrations found at the end of the 120-h treatment were due to depletion of the compounds and not low recovery.

Likewise, their metabolites desmethylcitalopram, didesmethylcitalopram and norfluoxetine were analysed from the treated samples to determine whether they were formed during the experiment. Only one metabolite was found in the samples, and norfluoxetine was detected in a very low concentration (0.006 mg L^{-1}) in the sample with an initially high fluoxetine concentration (Table 2).

The experiment is set up to simulate the conditions in a landfill leachate pond, and it is reasonable to believe that the pharmaceuticals will have a similar degradation profile in a full-size treatment system treated with aeration for days or weeks.

The results in this experiment differ from earlier experiments, where the same SSRIs were measured during an aerobic composting and anaerobic sewage sludge treatment [8,25]. By comparing the results from the aerobic and anaerobic experiment with this experiment, both similarities and differences are found.

Table 3 illustrates a comparison of three different treatment processes that show reduction efficiency and reduction rate for all five SSRIs. First: All SSRIs show considerable higher reduction rate in an aqueous aerated media (Table 3). Second: In the aerobic composting process [18], fluvoxamine and paroxetine had the highest reduction rates, while in the anaerobic experiment

Table 3 Comparison of the reduction rates of SSRIs with different treatment methods

SSRI	Aerobic sludge composting [18]	Anaerobic sludge treatment [25]	Aeration treatment landfill leachate
	Reduction rate (% per 21 days)	Reduction rate (% per 24 days)	Reduction rate (% per 5 days)
Fluvoxamine	88	53	100
Citalopram	26[a]	85	100
Sertraline	46[a]	38	96
Fluoxetine	35[a]	32	93[a]
Paroxetine	46	98	90

[a]Metabolites detected.

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[25], paroxetine still had a high reduction rate while fluoxetine had a very low reduction rate. The metabolite norfluoxetine was detected in both aerobic experiments while it was absent after anaerobic treatment. Third: Citalopram was the most stable SSRI in the aerobic composting experiment, while in the anaerobic sewage sludge and in this aerated treatment experiment, it was quickly reduced in concentration. Based on this experiment, we can only assume that the differences in stability in these three experiments might be due to the different bacteria present in aerobic and anaerobic systems, but it could also originate from different chemical reactions in the three systems since we cannot determine whether the concentration reduction is due to chemical or biological degradation.

We are limited in the extent to which it is possible to compare three functionally different systems, especially when we consider the variability in pharmaceutical stability under different conditions. Table 3 clearly shows that the availability factor for degradation of each SSRI is higher in aqueous medium compared to solid-phase medium like the composting system. SSRI degradation in a solid-phase medium also gave detection of three different metabolites from five different SSRIs after the biological treatment. Independent of anaerobic or aeration treatment, SSRIs in aqueous solution gave considerably higher reduction rates.

Conclusions

Selective serotonin reuptake inhibitors can be effectively reduced in concentration by aerobic treatment of landfill leachates. The concentration reduction was close to complete for all compounds in both concentrations during the experimental time of 120 h. Only one of the known human metabolites (norfluoxetine) was found in the aeration samples, which suggests other degradation products than those formed by the human metabolism. Comparing the results of all SSRIs with different treatment methods, paroxetine and fluvoxamine seem to be the easiest compounds to eliminate independent of method, while fluoxetine and sertraline seem to be the most stable.

Our results suggest that aeration is an effective method for minimising the impact of pharmaceuticals such as SSRIs from landfill leachates water. However, further studies are required to determine what kind of degradation products are formed, and based on such results, preventive actions in the form of more sophisticated water treatment systems can be made.

Methods
Aerobic treatment experiment
Leachate from an active landfill (3 L) was diluted with water (10 L), and the composition of the leachate is given in Table 4. Table 4 shows the content of the leachate with carbon source as total organic carbon (TOC), organic acids and Total-N that will enhance microbial activity. The strategy of the experiment was to measure the microbial respiration in the leachate solution spiked with SSRI tablets and compare it to control flasks with diluted leachate water without addition of tablets.

Pharmaceutical pollution from landfill leachates will be as unused tablets or from tablet packages. Therefore, the stock solution was made up of crushed tablets of each SSRI that was dissolved in 30 mL methanol (MeOH, Merck, Darmstadt, Germany) and then diluted with 270 mL distilled water, stirred and heated for 2 h at 35°C and stored at 4°C until used. Diluted leachate in 500-mL flasks was spiked with a stock solution of the different SSRIs. The theoretical starting concentration was 5 and 0.5 mg L^{-1} for fluvoxamine and citalopram and 10 and 1 mg L^{-1} for sertraline, fluoxetine and paroxetine.

The flasks with leachate water were incubated in a water bath in a Voith Sapromat respirometer (Voith, Heldenheim/Brenz, Gemany) [30]. The Voith Sapromat continuously measure consumption of oxygen in solutions and the developed CO_2 gas in the flask is trapped with NaOH (Merck). The experiment was performed in duplicates, and the samples were incubated for 120 h at 20°C in darkness. The samples were stirred continuously in closed flasks with a magnet. Total oxygen consumption (Tot. OC) was calculated from the whole tablet mixture with all excipients, with the background respiration from the leachate after 48 and 120 h, while the

Table 4 Chemical analysis of leachate water after dilution used in the Voith Sapromat respirometer experiment

Parameter	Units	Raw material	Diluted leachate
TOC	mg L^{-1}	773	178
Tot-N	mg L^{-1}	322	74
Tot-S	mg L^{-1}	21	4.8
SO4	mg L^{-1}	0.3	0.1
Fe	mg L^{-1}	16	3.7
Formic acid	mg L^{-1}	31	7.2
Acetic acid	mg L^{-1}	5.6	1.3
Propionic acid	mg L^{-1}	1.1	0.25
Butanoic acid	mg L^{-1}	1.5	0.3
Pentanoic acid	mg L^{-1}	3.2	0.7
Hexanoic acid	mg L^{-1}	2.4	0.6
Heptanoic acid	mg L^{-1}	1.3	0.3
DL- lactat	mg L^{-1}	5.5	1.3
BOD 7	mg L^{-1}	430	
Redox potential	mV	−148	
pH		7.1	6.9

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oxygen consumption (OC)* (Equation 1) was calculated from the whole tablet mixture with all excipients, with the background respiration from the leachate (Equation 1) subtracted. Theoretical oxygen demand (ThOD) for each SSRI was calculated using the Buswell equation [31]. The SSRI tablets contain a large number of additives, e.g. carbon sources such as cellulose, starch and glycerol. The tablet mixture used in our experiment contains approximately 20% SSRIs and 80% excipients.

$$\text{Measured } OC_j = \text{Measured}(OC\ h_j \text{-} OC\ h, \text{control}) \quad (1)$$
$$\left(\text{mg oxygen L}^{-1}\right)$$
$$OC\ hours_j = \begin{array}{l}\text{Measured oxygen consumption of each}\\ SSRI\ \left(mg\ oxygen\text{L}^{-1}\right)\end{array}$$
$$OC\ hours, \text{control} = \begin{array}{l}\text{Measured oxygen consumption}\\ \text{leachate}\ \left(mg\ oxygen\text{L}^{-1}\right)\end{array}$$

Chemicals and solvents
Standards of the SSRIs citalopram (1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl)-1,3-dihydro- 5-isobenzofuran carbonitrile), sertraline ((1S-cis)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl-1-naphtylamine), paroxetine (trans-(−)-3-[(1,3-benzo-dioxol-5-yloxy) methyl]benzene propanamine), fluvoxamine (5-methoxy-1-[4-(trifluoro methyl)-phenyl]-1-pentanone-O-(2-aminoethyl)oxime) and fluoxetine ((±)-N-methyl-γ-[4(trifluoromethyl)phenoxy] benzenepropanamine), and the metabolites desmethyl citalopram (1-(4-fluorophenyl)-1,3-dihydro-1-[3-(methy lamino)propyl]-5-isobenzofurancarbonitrile), didesmet hylcitalopram (1-(3-aminopropyl)-1-(4-fluorophenyl)-1,3-dihydro-5-isobenzofurancarbonitrile) and norfluoxe-tine (γ-[4-(trifluoromethyl)phenoxy]-benzenepropanamine),

and the deuterated standards D_3-sertraline, D_4-paroxetine, D_5-fluoxetine and D_6-citalopram were purchased from Toronto Research Chemicals (Toronto, ON, Canada). The mass spectrometric identification criteria, recoveries, LOD and LOQ and pKa-values for the compounds are given in Table 5. pKa values are calculated by Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris, obtained from SciFinder Scholar 2010.

The isotope labelled compounds were used as internal standards (IS) for quantification. All standards were delivered with purity above 98%. Escitalopram, which is the pure S-enantiomer of citalopram, is not possible to distinguish from the R-enantiomer with our analytical techniques, and these enantiomers are measured as the same compound.

The standards were dissolved in HPLC-grade MeOH from Merck in concentrations of 100 or 200 μg mL^{-1}, stored at −18°C in the dark and used as stock solutions. From this stock solution, the daily work solutions were made by dilution with purified water.

Solvents for HPLC were HPLC-grade acetonitrile from Merck, purified water obtained from a MilliQ purification unit from Millipore (Bedford, MA, USA) and pro-analysis-grade formic acid from Merck. The same water and formic acid were used for liquid-phase microextraction (LPME), while dihexyl ether (purum ≥ 97%) from FLUKA (Buchs, Switzerland) was used as organic solvent to fill the pores of the fibre.

Chemical analysis
The samples were mixed with deionized water (ratio 1:2 by volume) and the pH was measured 30 min after mixing with a Ross electrode (Orion Instruments, Baton Rouge, USA). The redox potential in the leachate was

Table 5 Identification criteria, extraction recoveries, LOD, LOQ, pKa and mass spectrometric detection parameters for the different SSRIs

Compound	pKa	[M + H] + (measured)	Fragment (measured)	Cone voltage	Collision energy	Retention time (min)	LOD (pg L^{-1})	LOQ (pg L^{-1})	Extraction recovery (%)
Citalopram	9.59	325.5	108.6	35	25	3.3	20	60	64/78[a]
Desmethylcitalopram	9.41	311.1	108.5	28	25	3.1	230	760	-
Didesmethylcitalopram	10.14	297.1	108.5	29	20	2.9	200	780	-
Citalopram-D6 (IS)	-	331.2	108.6	41	27	3.3	-	-	-
Paroxetine	10.05	330.2	69.6	40	22	4.1	50	205	140/131[a]
Paroxetine-D4 (IS)	-	334.2	73.7	40	29	4.1	-	-	-
Fluvoxamine	9.39	319.1	70.6	24	17	4.8	130	380	120/122[a]
Fluoxetine	10.05	310.1	43.8	22	15	6.0	150	490	110/84[a]
Norfluoxetine	9.05	296.1	133.7	15	5	5.5	160	540	-
Fluoxetine-D5 (IS)	-	315.2	43.8	20	12	6.0	-	-	-
Sertraline	9.47	306.1	158.6	18	25	6.4	160	520	100/133[a]
Sertraline-D3 (IS)	-	309.1	274.8	17	12	6.4	-	-	-

[a]Extraction recoveries at high/low concentrations. LOD, limit of detection; LOQ, limit of quantitation.

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analysed by an ATI ORION (Orion Instruments) and conductivity by a Phillips PW 9527 digital meter (Philips, Eindhoven, Netherlands).

Inorganic parameters of the leachate illustrated in Table 4 were analysed by an accredited laboratory (Eurofins AS) using Norwegian standards regarding total nitrogen (Tot-N), TOC, biochemical oxygen demand (BOD7), total sulphur (Tot-S), SO_4 and iron [32]. The leachate water was also characterized with respect to content of short-chained organic acids. The organic acids in the leachate water used in the experiment were alkaline extracted by adding 0.1 M NaOH (Merck) to the samples. Concentrated HCl (pro analysis, Merck) was then added to the water phase and undissociated acids were then extracted with diethyl ether (>98%, Merck) and further derivatized by t-butyldimethylsilyl (>99% Sigma Aldrich, St. Louis, USA) and measured on a gas chromatograph-mass spectrometer (GC-MS) with electron impact ionization (EI) after a method described by Schooley et al. [33].

All samples taken during the experiment were stored in a freezer at −18°C before chemical extraction and analysis. Three replicate liquid-phase microextraction analyses of the SSRIs and metabolites were performed.

An already developed extraction method known as hollow fibre-supported liquid-phase microextraction (HF-LPME or just LPME) was employed to extract the SSRIs and their metabolites from the samples. The method development is described elsewhere [34], and only a brief description will be given here. Since the SSRI concentration in the leachate water samples was relatively high, and the LPME method has proved to be very sensitive for the SSRIs [8], the samples were diluted to a concentration in the high end of the calibration curve prior to extraction (200 ng L^{-1}). The dilution was done with deionized water to a total volume of 1.1 L. The isotope labelled internal standards was added in a concentration of 50 ng L^{-1}, and the pH was adjusted to 12 with 5 M NaOH (Merck) before the samples were extracted by LPME. Three replicates were analysed from each of the selected days.

A 28-cm-long plasmaphan polypropylene hollow fibre (Membrana, Wuppertal, Germany) was used for the extraction, and the pores of the fibre were filled with dihexyl ether by dipping the hollow fibre into the solvent for 10 s. The excess ether on the fibre surface was removed by dipping it in an ultrasonic bath for 2 to 3 s. Then the fibre lumen was filled with 20-μL acceptor phase (purified water/formic acid at pH 2), and the ends of the fibre were sealed with a thin metal wire. The same wire was used to keep the fibre hanging in the middle of the sample bottle. The samples were stirred for 2 h at 800 RPM. After extraction, the extracts were transferred to Waters (Milford, MA, USA) total recovery HPLC-vials and analysed using HPLC-MS/MS. The separation

was conducted on a Waters Acquity UPLC BEH C_{18} column (1.0 × 150 mm with 1.7-μm particles), and the mobile phases consisted of A: H_2O and 0.1% formic acid and B: 90% acetonitrile, 9.9% H_2O and 0.1% formic acid. The gradient started with a 1-min isocratic elution with 70% A and 30% B; from 1 to 7 min, there was a linear change in composition to 55% A and 45% B and finally a linear change from 7 to 12 min to 50% A and 50% B. The flow rate was set to 50 μL min^{-1}. The mass spectrometric detection was done on a triple quadrupole instrument (Waters Quattro Premier XE) in multiple reactions monitoring mode with positive electrospray ionization for optimal selectivity and sensitivity. The protonated molecular ion $[M + H]^+$ was used as a precursor ion for all compounds, and the most intense product ion was selected for quantification (see Table 2).

Calibration curves

Calibration curves were made in the concentration range 1 to 250 ng L^{-1}. The standard samples were set up and extracted in the same way as the leachate water samples. Standard samples were extracted and analysed throughout the experiment period to ensure that the calibration curves were constant over time. Four internal standards were used for quantification. Sertraline was quantified with D_3-sertraline as IS; paroxetine with D_4-paroxetine; fluvoxamine, fluoxetine and norfluoxetine with D_3-fluoxetine; and citalopram, desmethylcitalopram and didesmethylcitalopram with D_6-citalopram as IS. The linearity (R^2) of the method ranged between 0.9997 (paroxetine) and 0.9894 (fluvoxamine) and was considered as good for all compounds in this concentration range. The linearity of fluvoxamine was a little lower than for the rest of the compounds, most likely because no isotope labelled IS was available for this compound.

The concentration measurements were done on the basis of the peak area of the analyte divided by the peak area of the IS, and all concentrations were analysed in triplicate for each concentration.

Quality control

The identification criteria of the SSRIs are given in Table 5 and consist of correct retention time and the correct ion transition. Ideally, two or more product ions should be analysed in the mass spectrometric analysis, but for four of the five SSRIs, the second product ion is of too low intensity to be seen at the relevant concentrations; hence, only the most intense ion was used.

The methods limit of detection and limit of quantification was found by extracting and analysing a dilution series of standards in leachate water, and the limits are given in Table 5. Three parallels of each concentration were analysed.

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All leachate water samples were extracted and analysed in triplicate. Between every third sample (nine injections), a blank sample of purified water was analysed to check for carry-over signals. No carry-over was detected.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
The authors OB and TV are responsible for the concept and design of the study. OB has also performed the aerobic treatment of the landfill leachate including the measurements of TOC, Tot-N, Tot-S, SO_4 and Fe, as well as writing of the manuscript. TV also developed the extraction and LC-MS methods used in the experiment, as well as result interpretation and writing of the manuscript. KØH performed the extractions of the samples, set up standard curves and calculated concentrations, as well as participated in discussions of methods and results. All authors read and approved the final manuscript.

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