ACCUMULATION OF PERFLUORINATED COMPOUNDS IN RADISH: A HIERARCHICAL MODEL APPROACH

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Introduction

Biogas technology is a competitive process for managing biodegradable wastes and by-products and a green method for energy production. Biogas plants digestates (BGD) can be used as fertilizers or soil improvers. This is of great importance, since phosphorus is a depleting nutrient and nitrogen fertilizers are manufactured in an energy-intensive process. However, BGDs may contain hazardous organic chemicals. When BGDs are used in agriculture, these compounds may accumulate in the soil and eventually in food of plant and animal origin.

Perfluoroalkyl compounds (PFC) comprise a large group of compounds that have been widely used in a myriad of applications such as electronic parts, firefighting foams, photo imaging, hydraulic fluids and stain-resistant coatings. PFCs are persistent and have harmful effects on humans and the environment. As a result of their water solubility, PFCs may end up in groundwater[1]. They can be taken up by plants via the roots[2] and they can accumulate in food of animal origin[3]. The most widely used compounds have been perfluorooctane sulphonic acid (PFOS) and perfluorooctanoic acid (PFOA). The production and use of PFOS, its salts and perfluorooctane sulphonyl fluoride (PFOS-F) have been severely restricted in the EU since 2006 ((EU) No 1907/2006), and were added to Annex B of the Stockholm Convention on Persistent Organic Pollutants in 2009. There are no restrictions on other PFCs.

The aim of this study was to assess the accumulation of PFCs in radish as a result of the use of biogas plant digestate as an agriculture fertilizer. We measured the concentrations of four PFCs (perfluorohexanoic acid PFHxA, perfluoroheptanoic acid PFHpA, PFOA, PFOS) in 19 digestates from ten biogas plant production lines in Finland. A Bayesian hierarchical model was used to predict the concentration of these compounds in agricultural soil after a single addition of digestate (15 t fresh matter (f.m.) ha^{-1}). Bioconcentration factors for the selected PFCs in radish (*Raphanus sativus* var. *sativus*) were determined in a pot experiment, in which a known amount of PFC was mixed in soil and the concentration of the compounds was determined in the soil and plants. Finally, we estimated the concentration of the compounds in radish grown on agricultural land using the predicted concentration of PFCs in the soil and the posterior distribution of the bioconcentration factors (BCFs) for radish.

Material and methods

Sampling

Nineteen digestate samples from ten biogas production lines in Finland were sampled between December 2010 and October 2012. Single samples from the reactor or the separator discharge valves were collected into sampling vessels. The dry matter concentrations of the digestates ranged from 2.4 to 10.1%. The studied biogas plants have been described by Suominen et al. (2012)[4].

Pot experiment

BCFs for the studied compounds were determined in a pot experiment in which a known amount (0, 1, 10 and 50 µg each PFC kg^{-1} soil) of the selected PFC was added to test soil (ISO 11269). Samples of the test soil were taken after mixing in the compounds. Radish (*Raphanus sativus* var. *sativus*) was used as the test plant species.

Plants were grown in 5-litre pots (6.5 kg of soil) under a glass roof during the summer of 2012. The pots were watered twice a week with equal amounts of water and no plant protection chemicals were used. After nine to ten weeks, the test plants were harvested and the roots were washed free from soil and delivered to the laboratory for PFC analysis. BCFs for the studied compounds were determined as the concentration of the compound in the tuber tissue of the plant divided by the concentration in soil (f.w. basis, observed data).

Chemical analysis
For PFC analysis, digestate and soil samples were freeze-dried, ground and extracted with methanol. The plant samples were extracted and cleaned with a modified QuEChERS procedure[5]. Mass-labelled internal standards were added prior to extraction. The obtained extracts were evaporated to dryness and redissolved in methanol. The extracts were analysed using ultra performance liquid chromatography (UPLC[®]) coupled with tandem mass spectrometry (MS/MS). LC effluents consisted of water and methanol buffered with ammonium acetate. An isolator column was placed before the injector to delay signals originating from the instrument. The analytes were quantified with a triple quadrupole mass spectrometer (Xevo TQ MS, Waters) using electrospray ionization (ESI) and multiple reaction monitoring. The final results were recovery corrected.

Description of the complete Bayesian hierarchical model
The model combined concentration data from the biogas plant digestates with experimental data from the pot experiments. Firstly, the posterior predictive distribution of concentrations in digestates was first simulated. It was then transformed into the distribution of concentrations in soil (ploughing depth 25 cm, assuming 15 t digestate (f.m.) ha^{-1} (360 to 1,500 kg dry matter (d.m.) ha^{-1})). Finally, the predictive distribution of concentrations in soil was used as an input distribution for the independent variable in a regression model describing the relationship between PFC concentrations in soil and in radish as determined from the pot experiment. The uncertainty in the regression coefficient (BCF) was therefore accounted for probabilistically together with the uncertainty in the input concentration of the studied compounds in digestates and consequently in ploughed soil.

Observed fresh weight concentrations of the four PFCs in biogas plant digestates were modelled as gamma distributions, $x_i \sim$ gamma(a,b), based on the 19 measured concentrations of the compounds in digestates. Uninformative prior distributions were specified for the mean $\mu = a/b$ and precision $\tau = b^2/a$. The posterior predictive distribution for x* was then sampled with MCMC in OpenBUGS, representing the predicted concentration in a forthcoming sample digestate, to be used for the predicted fertilizing event. The regression model for the pot experiment was defined as $\log(z_i) \sim N(\log(BCF\ y_i), \sigma^2)$, for the measured concentrations z_i in radish and the measured concentrations y_i in the pot soil (after applying a given dose to the pot). Uninformative prior distributions were specified for log(BCF) and σ^{-2}. Since some of the measurements for x, z_i or y_i were below the limit of quantification, they were treated as statistically left-censored observations and were modelled accordingly to take into account the uncertainty concerning their exact value. The final predictive distribution for the concentration in radish was simulated in three stages. Firstly, a random concentration in digestate from the 10 biogas plants was predicted from the distribution $f(x^* \mid x_1, \ldots, x_{19})$, based on their 19 measurements. Secondly, these values were transformed to represent the concentration in ploughed soil x*'. Thirdly, the effect of accumulation was predicted via the regression model $z^* \sim N(\log(BCF\ x^{*'}), \sigma^2)$, where the parameters BCF and σ^2 were sampled from their posterior distribution based on the pot experiment data. Therefore, the uncertainties in all model parameters (a, b, BCF, σ^2) and in the predicted concentrations of PFCs in digestates were all included in the combined prediction for radish.

Results and discussion

The dry weight (d.w.) and fresh weigh (f.w.) mean, median, minimum and maximum concentrations of the studied compounds in digestates are presented in Table 1. Similar to many other studies on organic fertilizers (e.g. Bossi et al., 2008[6]), PFOS was the dominant compound in our samples. PFOS is also commonly the most abundant PFC in soils that have received organic fertilizers (e.g. Sepulvado et al., 2011[7]).

Table 2 presents the predicted increases in median concentrations and the 2.5[th] and 97.5[th] percentiles for the studied compounds in soil after a single addition of biogas plant digestate (15 t f.m. ha^{-1}). Table 2 also shows the estimated BCFs for the studied compounds in radish and the increase in the concentration of PFCs in radish predicted by our model. These results suggest that PFHxA, in particular, may have a rather high potential to accumulate in radish, even though its concentration in digestate can be relatively low. The predicted 97.5[th] percentile for the increase in the concentration of PFOS in soil and in radish is very high. This is due to the large variation in the PFOS concentration in our samples and the high degree of uncertainty in the BCF for PFOS.

Table 1. Measured dry weight (d.w.) and fresh weight (f.w.) concentrations of the studied compounds in biogas plant digestates.

Compound	Measured concentration in digestates µg kg^{-1} (d.w.)				Measured concentration in digestates µg kg^{-1} (f.w.)			
	Mean	Median	Min.	Max.	Mean	Median	Min.	Max.
PFOS	30.1	10.5	0.44	141	1.79	0.58	0.01	8.32
PFOA	3.26	1.35	0.23	14.6	0.17	0.09	0.01	0.71
PFHpA	0.45	0.22	<0.1	1.68	0.02	0.01	<0.003	0.09
PFHxA	1.61	0.65	<0.2	9.13	0.08	0.04	<0.006	0.24
Sum*	42.3	18.5	0.97	168	2.44	0.96	0.03	9.92

* Sum concentration of PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS and PFOS[8].

Table 2. Predicted median, 2.5[th] and 97.5[th] percentiles for the concentrations of the studied compounds in soil and in radish. Estimated bioconcentration factors (BCF) of the studied compound in radish.

Compound	Median	2.5 percentile	97.5 percentile
Predicted increase in concentration in soil ng kg^{-1} f.w.			
PFOS	4.3	0.01	54
PFOA	0.5	0.01	3.4
PFHpA	0.07	0.00	0.6
PFHxA	0.2	0.00	1.5
Estimated BCF in radish (tuber)			
PFOS	0.005	0.003	0.008
PFOA	0.019	0.011	0.030
PFHpA	0.048	0.033	0.069
PFHxA	0.10	0.081	0.13
Predicted increase in concentration in radish ng kg^{-1} f.w.			
PFOS	0.02	0.00	0.44
PFOA	0.01	0.00	0.11
PFHpA	0.00	0.00	0.04
PFHxA	0.02	0.00	0.18

The predicted median concentration of PFOS in radish after a single addition of digestate to agricultural land described in this paper was 1/200 of the concentration of PFOS in vegetables in Sweden (4.1 ng kg^{-1})[9], while the predicted concentration of PFOA was 1/2400 of the concentrations in vegetables in Sweden (22 ng kg^{-1})[9]. The 97.5[th] percentiles of the estimates reported in this paper for PFOS and PFOA, respectively, were 1/9 and 1/200 of the concentrations in vegetables in Sweden[9].

In a study by Blaine et al. (2013)[10], BCFs for the studied compounds were 64- to 380-fold higher in greenhouse lettuce (*Lacuta sativa*) and <37-fold higher in tomato (*Lycopersicon lycopersicum*) than the BCFs for radish presented in this paper. Blaine et al. (2013)[10] reported a significantly higher agronomic use of biological fertilizers (25 t d.m. ha^{-1}) than in this paper (0.36 to 1.5 t d.m. ha^{-1}). Concentrations of PFC in organic fertilizers can also be higher (e.g. Zhang et al., 2010; 692 µg kg^{-1} d.w.[11]) than reported in this study. This means that with other plants and under other circumstances, the increase in the concentration of PFCs in plants after the application of digestates as biological fertilizers may be higher than estimated in this study.

PFCs are persistent in the soil environment. Short-chain perfluorinated acids, in particular, can be leached down to deeper soil layers[1], resulting in a decrease in the concentrations of the PFCs in topsoil. Furthermore, the adsorption of organic compounds onto soil particles may reduce their bioavailability. PFOS, PFOA, PFHxA and PFHpA can also be formed from their precursors in soil[12]. These factors may have an impact on the concentrations of PFCs in soil, on their bioavailability and eventually on their concentrations in plants.

At their present level in European food, PFOS and PFOA are unlikely to exceed health-based guidance values[13]. However, because PFCs are persistent and because they have the potential to accumulate in plants and in food of animal origin, more information on their behaviour in the food chain is needed.

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