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Abstract
We studied the fate of sludge spiked with ^{14}C-labelled diuron, glyphosate and nonylphenol applied to the soil by the way of contaminated sewage sludge in the soil- plant-water system. Here we show that the mineralization of the chemicals in mixture is reduced by 40-80% by comparison with a direct soil contamination. The persistence of the chemicals in soils is increased in the presence of sludge. We showed also that the chemicals present in the sludge are mobile and partly transferred to soil leachates and plant seedlings. These results allow postulating that these compounds may induce an ecotoxicological impact on the soil ecosystem.

Keywords
Sewage sludge; Herbicides; Transformation; Transfer

Introduction

In European Union member states, the byproducing of sewage sludge is increasing in wastewaters treatments plants (WWTPs). The sustainability of sludge spreading onto agricultural soils is now a socio-economic, environmental and health challenge. Sludge is recognized to be rich in organic matter, thus allowing soil stability, and to provide nitrogen and phosphorous. Unfortunately, it

contains numerous pollutants such as heavy metals and organics, of- ten able to produce eco-toxic effects. Only some persistent organic pollutants, such as polycyclic aromatic hydrocarbons and polychlorinated biphenyls, are taken into account by the actual regulation.

A draft for future European directive (Abad et al. 2005) is intended to improve sewage sludge management with respect to human, animal and plant health, quality of ground and surface waters, and long-term quality of the soil. It will prescribe limit values in sludge for selected families of organic pollutants, including dibenzo-p-dioxins and dibenzofurans, alkylbenzene sulphonates, di-2-(ethyl-hexyl)-phthalate, nonylphenol and related ethoxylates. Despite that evolution, no or few data are available concerning other types of bioactive organic contaminants in sludge.

Pesticides are mainly used as plant protection products in agriculture, but they enter also in domestic and industrial activities. By contrast with organochlorine compounds, little information is available concerning the occurrence and fate in of wastewater plants currently used pesticides, as well as their concentration in sludge. However, Gerecke et al. (2002), Neumann et al. (2003) and Nitschke and Schussler (1998) have measured significant amounts of pesticides in wastewaters entering wastewater plants. Recently, Kupper et al. (2005) reported the presence of the herbicide diuron in sludge from Switzerland.

In our laboratory, we demonstrated the presence of herbicides, namely glyphosate, diuron and their main metabolites in sludge originating from several urban wastewater plants located in the vicinity of Versailles, France (Ghanem et al. in preparation). Their concentrations ranged from ppb to ppm (dry sludge) in dried, composted and limed sludge samples. High levels of nonylphenols have also been noticed (Dubroca et al. 2005; Kollmann et al. 2003).

Nevertheless, previous studies concerning the relation- ships between sludge application and pesticides have always focused on the impact of this exogenous organic matter on the fate of pesticides, already present in the soil. Here, we studied the fate of ^{14}C-compounds (herbicides and nonylphenol in mixture) when applied to the soil by the way of spiked sludge samples. We used terrestrial model ecosystems (TMEs) to assess the biotransformation and availability of the labelled compounds, as well as their transfer to soil leachates and higher plants. We studied particularly a scenario of ‘worst case’ where contaminated sludge is applied onto the soil on one time at its

maximal amount allowed by the French regulation over a 10-year- period. The present study is a first part of a wider project intended to assess the ecotoxicological impact of sludge spreading onto agricultural soils.

Experimental

Chemicals

High purity glyphosate an diuron were purchased from Cluzeau Info Labo, whereas nonylphenol was from Lancaster. Other reagents have been obtained from Sigma-Aldrich. Phosphonomethyl-^{14}C glyphosate (2084 MBq mmol^{-1}) and ring-^{14}C-U-nonylphenol (1998 MBq mmol$_{-1}$) have been obtained from Dislab'S System, whereas ring-^{14}C-U-diuron (898 MBq mmol^{-1}) was from International Isotope. High purity grade solvents were from Carlo Erba.

Soil characteristics

The silt loam has been collected in the 10–20 cm layer of a field in Versailles, in winter (experiments with dried sludge) and spring (experiments with composted and limed sludge). It comprises 28.4% sand, 53.4% silt, and 18.2% clay. Its contents in organic carbon, total nitrogen and CaCO3 were 1.19, 0.12, and 1.26%, respectively. Soil pH_{wat} was 8.1 and its cationic exchange capacity was 14.4 cmol kg^{-1}. The soil was roughly homogenized and immediately used for TMEs filling.

Sludge characteristics

The samples of sludge have been collected in urban WWTPs in the vicinity of Versailles. Their characteristics are reported Table 1.

Soil and sludge spiking

Three experimental conditions have been retained for incubations in model ecosystems: soil alone and soil amended by sludge at two ratios.

In the first model ecosystem used as a control, 0.7 kg of soil were spiked with labelled glyphosate, diuron and nonylphenol (370 kBq each) and unlabelled chemicals to ensure final amounts of 163, 480 and 203 µg chemical per model ecosystem.

In the second model ecosystem, the 'worst case' hypothesis of contamination, the sludge (28.5 dry sludge corresponding to 30 T dw ha^{-1}) was mixed with the soil. It has been previously spiked with the same amounts of chemicals as the soil alone These two model ecosystems can be directly compared together.

In the third model ecosystem, the soil was amended with 5.7 g dry sludge (equivalent to 6 T dw ha^{-1}) as an agronomic reality, with final amounts of chemicals being 33, 96 and 40 µg per model ecosystem.

For both sludge samples, concentrations of added glyphosate, diuron and nonylphenol were 6, 17 and 7 ppm (dry sludge). After addition of the chemicals, the solvents (MeOH and water) were allowed to evaporate and the spiked samples were 'aged' for 3 days at 4°C under nitrogen to avoid biotransformation.

Incubations in model ecosystems

Glass cylinders (10 cm i.d. and 50 cm depth with inlets and outlets) were filled with 1.8 kg soil at 80% of its moisture holding capacity, and incubated during 3 days. After that period, the upper layer comprising spiked soil alone or soil/spiked sludge mixtures was added and the model ecosystems were closed. A stream (0.5 l min^{-1}) of wet air was continuously flushed through the model ecosystems to allow $^{14}CO_2$ trapping in 1 N NaOH solutions. Model ecosystems were incubated for 91 days at 23◦C under 16 h light and 8 h darkness. Leachates were collected after 45, 60 and 90 days of incubation by watering the model ecosystems with 190 ml water (equivalent to 20 mm rainwater). Radish and wheat seedlings (obtained by the sowing of two seeds after 15 days of incubation) were harvested after a further 15- and 45-days-period of growth, and dried.

Analytical procedures

NaOH solutions were changed every 7 days. Soil cores (1.5 cm i.d. and 20 cm depth) were performed to determine extractable and bound 14C in the soil and soil/sludge mixtures after 15, 30, 60 and 90 days of incubation. Radioactivity was extracted by sequential shaking with acetone/EtOH 80/20 (v/v) (diuron and 4-n-nonylphenol) and 0.1 N NaOH (glyphosate), using a sol/liquid ratio of 1/5. Each extraction (1 h) was performed in duplicate and followed by filtration. The radioactivity was measured in all liquid fractions (extracts, leachates) by liquid scintillation counting. Non-extractable radioactivity (soil, soil/sludge samples, seedlings) was determined by combustion, followed by liquid scintillation counting.

HPLC analysis was performed by injecting 100 µl of the organic extracts onto an analytical column TSK ODS- 80TM (25 cm 4.6 mm i.d.) set at 30◦C. The mobile phase consisted of a mixture of acetonitrile/water (30/70; v/v) at a flow rate of 1 ml min^{-1}. After 1 min, it was increased to 100% acetonitrile in 20 min and maintained during the following 5 min. Radioactivity eluted from the column was monitored.

Results and discussion

Mineralization of the mixture of chemicals

We assessed the impact of the type and amount of sludge on the mineralization of labelled compounds in mixture, considering the soil alone as a control. Because our data have been obtained from two sets of experiments, results were expressed as normalized values with respect to soil alone to allow an easier comparison (Fig. 1).

Our results clearly demonstrate that the mineralization of the mixture of chemicals is mostly reduced by 40-80% after 21 days when the chemicals enter the soil as previously aged into sludge. If the highest dose of composted sludge was without a clear effect, the strongest inhibitions are noticed with dried sludge (whatever the dose) and limed sludge (at the highest dose) with a 7-day lag phase in that last case. We observed recently similar effects concerning the mineralization of nonylphenol present in limed sludge (Dubroca et al. 2005). The depressive effect of the sludge on

chemical mineralization can be attributed to several factors: (1) toxicity of the sludge towards the soil microflora, because of the presence of numerous organic contaminants and high amounts of metal ions, thus requiring the selection of resistant micro-organisms, (2) a high biological oxy- gen demand, due to the high organic matter content of the sludge, (3) an increase of herbicides and nonylphenol adsorption in the mixtures by comparison with the soil alone. Nevertheless, a few studies have been published, which ad- dress the effects of sludge-bound chemicals on microbial processes in soils (Gejlsberg et al. 2001).

Mass-balance analysis of chemicals in soil and soil/sludge mixtures

Evolution of the pesticides was calculated from mass- balance analysis of soil alone and soil supplemented with the three types of spiked sludge, at the two doses. Relative amounts of radioactivity bound in the soil, extracted by organic solvents or by NaOH are shown in Fig. 2. The amounts of $^{14}CO_2$ released are also reported for comparison.

The main differences can be evidenced concerning organic extracts. At the highest dose, and whateve its form, sludge generally maintains high amounts of solvent extractable radioactivity during the incubation by comparison with the soil alone. HPLC analysis revealed that the products mainly present in the extracts were nonylphenol, diuron and in lesser amounts its metabolite 3-(3,4-chlorophenyl)-1-methylurea. Radioactivity in the alkaline extract was only reduced in the case of the limed sludge, whose pH was not modified by the extractant. Because the amounts of radioactivity in the alkaline extracts exceeded 33% (the initial contribution of glyphosate), it is likely that NaOH extracts contain also organic matter on which other labelled chemicals are bound. For that reason, we propose to combine this fraction non extractable by organic solvents with the really non extractable radioactivity. As a consequence, results show that the total non extractable fraction decreased (dried and composted sludge) or remained steady (limed sludge) by comparison with their corresponding values in soil references.

At high dose (the worst case hypothesis), the type of sludge impacted the transformation pathways of pesticides. Dried and limed sludge decreased the mineralization/stabilisation ratio by 75–80%, whereas composted sludge led to a slight increase (by 30%). Low dose (agronomic situation) gave less contrasted results. All these results suggest that the persistence of the chemicals is generally

increased in the presence of sludge.

Transfer of radioactive chemicals to soil leachates and higher plants

Mobility of radioactive materials to soil leachates or higher plant (radish and wheat) has been studied (Table 2). La- belled carbon is transferred to leachates in low amounts when expressed in percentage of the applied dose. However, when expressed as calculated concentrations of herbicides and nonylphenol in water, values are significant and often exceed the $\mu g\ l^{-1}$ range. They are well related to the amount of spiked sludge and generally decrease with respect to the soil periods of watering. The highest contamination of leachates was measured following the spreading with the limed sludge, and was two-fold higher than the corresponding value with the soil alone. HPLC analysis revealed that 80–100% of the radioactivity in the leachates was nonylphenol.

Labelled carbon was also absorbed by higher plants (Table 2). Seedlings were allowed to grow for 15 days (radish) and 45 days (wheat). If values were also very low expressed as percentage of the applied dose, calculated concentrations amounted to the $\mu g\ g^{-1}$ range. Radish seedlings were very small in the experiment with the dried sludge. In addition, none of the seed was able to germinate in the presence of the highest amount of limed sludge, because of a dramatic development of fungal hyphae. More detailed studies are necessary to characterise the nature of the labelled carbon absorbed by the plants. Because of the continuous stream of air through the model ecosystems, it was assumed that the radioactivity enters the plants through roots and corresponded to degradation products formed from the chemicals.

Conclusion

This paper reports for the first time the fate of herbicides contained in sludge, after spreading onto agricultural soil. The fate of pesticides entering the soil by the mean of contaminated sludge is depending on the type of sludge treatment. In most cases, mineralization of the chemicals is decreased by comparison with that occurring after direct treatment of the soil, and organic extracts contain high levels of non transformed chemicals. Our results also clearly demonstrate that the organic contaminants contained in sludge are mobile, and can be in part transferred to soil leachates and higher plants. Their levels are significant, and remain higher than these provided by regulations

concerning drinking water or edible foods, that may be of concern in assessing indirect exposure of living beings.

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Table 1. Characteristics of wastewater plants and sludge samples used in the study

	Dried	Composted	Limed
Equivalent inhabitants	50,000	25,000	40,000
Organic carbon (C) (g kg^{-1})	396	336	231
Total nitrogen (N) (g kg^{-1})	70	41	37
C/N ratio	5.6	8.1	6.1
pH_{wat}	5.9	7.7	12.5
Dry matter (%)	94	75	30

Table 2. Transfer of radioactive chemicals to soil leachates and higher plants

Conditions	Leachates						Higher plants			
	45 days		60 days		90 days		Radish (15 days)		Wheat (45 days)	
	(%)	($\mu g\ l^{-1}$)	(%)	($\mu g\ l^{-1}$)	(%)	($\mu g\ l^{-1}$)	(%)	($\mu g\ g^{-1}$)	(%)	($\mu g\ g^{-1}$)
Soil	0.01	2.1	0.03	1.6	0.02	1.2	Traces	–	0.021	0.9
Soil + dried sludge (6 T ha^{-1})	0.01	0.5	0.03	0.3	0.02	0.2	Traces	–	0.011	0.07
Soil + dried sludge (30 T ha^{-1})	0.01	2.5	0.02	0.9	0.02	1.2	Traces	–	Traces	–
Soil	0.5	14.5	0.4	16.2	0.3	11.0	0.007	6.0	0.015	0.7
Soil + composted sludge (6 T ha^{-1})	0.8	4.8	0.7	5.2	0.6	4.7	0.006	1.1	0.051	1.2
Soil + composted sludge (30 T ha^{-1})	0.5	14.9	0.6	12.5	0.4	15.2	0.002	1.8	0.008	1.6
Soil	0.5	14.5	0.4	16.2	0.3	11.0	0.007	6.0	0.015	0.7
Soil + limed sludge (6 T ha^{-1})	0.6	3.4	0.4	3.8	0.4	3.7	0.005	4.3	0.017	0.1
Soil + limed sludge (30 T ha^{-1})	1	29.6	0.6	25.2	0.6	25.4	ng	–	0.011	1.1

ng: no germination of seeds

Fig. 1. Time-dependent mineralization of the mixture of chemicals with respect to the type and amount of sludge. (X) Soil alone; (O) dried sludge 6 T dw ha^{-1}; (●) dried sludge 30 T dw ha^{-1}; (□) composted sludge 6 T dw ha^{-1}; (■) composted sludge 30 T dw ha^{-1}; (△) limed sludge 6 T dw ha^{-1}; (▲) limed sludge 30 T dw ha^{-1}

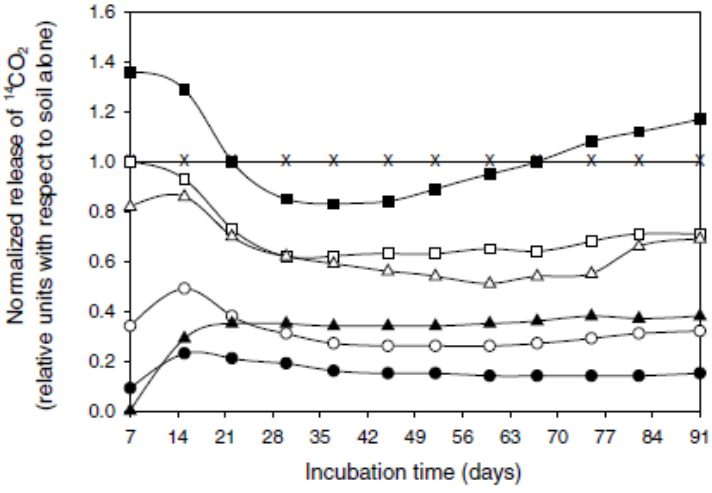

Fig. 2 Mass balance analysis of soils and soil/sludge mixtures (DS, dried sludge; CS, composted sludge; LS, limed sludge) after treatment with the mixture of ^{14}C-pesticides. Colours refer, from the bottom of the figure to the top, to non extractable ^{14}C, black; ^{14}C extracted by NaOH, white; ^{14}C extracted by organic solvents, black; and $^{14}CO_2$, white