INFLUENCE OF SEWAGE SLUDGE TREATMENT ON POP LEVELS. ECOTOXICOLOGICAL CHARACTERISTICS

Roig N[1,2], Sierra J[1,3], Nadal M[2], Navalón P[4], Valor I[4], Schuhmacher M[1,2], Domingo JL[2]

[1]Environmental Engineering Laboratory, Departament d'Enginyeria Quimica, Universitat Rovira i Virgili, Av. Països Catalans 26, 43007 Tarragona, Catalonia, Spain; [2]Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Catalonia, Spain; [3]Laboratori d'Edafologia, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Catalonia, Spain; [4]LABAQUA S.A., Dracma Parcela 16-18, 03114 Alicante, Spain

Introduction

After the implementation of the Council Directive on Urban Wastewater Treatment[1], sewage sludge production has significantly increased in the European Union (EU). In 2009, the total quantity of sludge production in the EU-27 was estimated in 10.13 million tons (dry solids). The use of this sludge on agriculture, around 40% of the total generated[2], is known to be one of the best recycling routes vs. landfilling or incineration. Before its application as agricultural fertilizer, current legislations only consider the content of heavy metals in sludge, as well as that in soil. However, despite of the existence of a working document on sludge published by the EU in 2000[3], there are no legislative measures yet to limit other potential hazards such as organic pollutants or ecotoxicity levels.

It is largely known that sludge amendments improve soil properties such as organic matter, nutrient contents, soil porosity, bulk density, aggregate stability and water holding capacity[4], while the pollutants load can have a notable affection on soil functioning and biodiversity[5]. The aim of the present study was to assess the influence of various sewage sludge treatments on 3 groups of parameters: a) persistent organic pollutants (POPs), b) physico-chemical properties, and c) ecotoxicological characteristics. For that purpose, the levels of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), nonylphenols, and decabromodiphenyl ether (BDE-209) were analyzed in sewage sludge from Spanish wastewater treatment plants. In addition, some parameters related to sludge organic matter stability (respirometric assays) and fertility (pH, % of dry matter, C/N ratio, % of organic carbon) were also assessed, while phytotoxicity and Microtox® tests were performed with sludge samples.

Materials and methods

Twenty eight samples of sewage sludge were obtained from 24 urban wastewater treatment plants in Spain. Samples were classified in 5 different types according to the sludge treatment received: non-digested sludge & mechanical drying (Non. Dig. & M.D.), aerobic digestion & mechanical drying (Aer. Dig. & M.D.), anaerobic digestion & mechanical drying (Anaer. Dig. & M.D.), anaerobic digestion & thermal drying (Anaer. Dig. & T.D.), and anaerobic digestion & composting. (Anaer. Dig. & Compost).

All samples were dried at 60ºC during 48h, grinded, and stored at room temperature. Analysis consisted on:
-Physico-chemical parameters: pH and Electrical Conductivity (EC_{25}) were analysed in 1:2.5 water extracts.
-Chemical parameters: Oxidizable carbon and Nitrogen were measured according to Walkley Black and Kjeldahl methods, respectively[6,7]. Respirometric assay to determine the sludge organic matter stability was performed incubating sludges at 30ºC and at 50% of moisture during 4 days. Oxygen consumption was hourly analyzed (Oxitop®, WTW).
- Organic pollutants: prioritary organic compounds were extracted with organic solvents (hexane:acetone) and analysed by GC-ECD (Gas Chromatography with Electronic Capture Detection), GS-MS (Gas Chromatography Coupled to Mass Spectrometry) and HPLC-MS (Liquid Chromatography Coupled to Mass Detector)[8]. The levels of 16 US EPA priority PAHs, 7 PCB congeners, 4 PCN congeners, PFOS, PFOA, 12 nonylphenol congeners, and BDE-209 were determined. Phenolic compounds of aqueous extracts (1:10) were analyzed according to the Folin Ciocalteau method[9].

-Ecotoxicity tests: Aqueous extracts were done in aqueous 1:10 DIN 38414 S4 leachates[10]. Organic extractions were performed according to US EPA method 3546[11]. After extraction, organic solvent was evaporated at 45ºC and low pressure, and the resulting fraction was mixed with 4 mL of dimethyl sulfoxide (DMSO). These extracts were used for Microtox® Acute Basic Test[12] and Seed germination/Root Elongation Toxicity Test. Phytotoxicity test was performed with seeds of *Lolium perenne, Allium cepa,* and *Raphanus sativa* according to US EPA Ecological Effects Test Guidelines[13]. Germination Index (GI) was calculated by comparing the number of germinated seeds and root lengths of the samples with respect to control seeds[14]. Statistical treatment was performed by means of the SPPS 17.0 software package. Pearson correlation and analysis-of-variance (ANOVA) were executed to study correlations and significant differences, respectively. Probabilities lower than 0.05 ($P<0.05$) were considered as significant.

Results and discussion:

The results of physico-chemical and chemical parameters are shown in Table 1. It can be seen that the percentage of dry matter significantly increased with the intensity of the sludge treatment, whereas the pH remained near the neutrality, except for the non-digested sludge, which may be due to acid flocculants ($FeCl_3$). Electrical conductivity values were similar and quite high, but not enough to salinize the soil at usual application rates. The oxidizable carbon values were similar for the 5 sludge types. However, the mineralization rate, used as organic matter maturity, was considerably different. According to Barrena et al.[15], sludges uptaking <30 mg O_2/g can be considered as stable organic matter, while those >80 mg O_2/g are considered as raw organic matter. High mineralization rates can involve significant risk of soil and water pollution due to the release of elements contained in sludge (N, pollutants, etc). This may be particularly serious in the case of sludge with low C/N values (<8).

Table 1: Results of physico-chemical parameters and stability degree of various sewage sludge treatments.

SLUDGE TREATMENT	% dry matter	pH	EC (dS/m)	% OC	C/N	mg O_2/g sludge	mg O_2 / mg OC
Non. Dig. & M.D.	20.1±10.2^{a}	6.4±1.1^{a}	9.1±1.7^{bc}	20.3±3.5^{a}	2.5±0.6	202.7±25.5^{c}	1.01±0.17^{d}
Aer. Dig. & M.D.	18.9±7.4^{a}	7.3±0.6^{a}	6.3±2.0^{ab}	19.7±0.3^{a}	4.2±0.1	143.4±24.9^{bc}	0.73±0.13^{cd}
Anaer. Dig. & M.D.	24.6±5.5^{a}	7.3±0.4^{a}	5.0±1.5^{ab}	19.5±3.0^{a}	9.1±3.8	114.3±57.0^{b}	0.58±0.26^{bc}
Anaer. Dig. & T.D.	72.0±28.6^{b}	7.2±0.6^{a}	6.3±2.2^{bc}	18.2±4.7^{a}	6.3±0.3	38.7±27.0^{a}	0.25±0.19^{ab}
Anaer. Dig. & Compost	82.2±2.6^{c}	7.3±0.2^{a}	9.3±3.3^{c}	18.4±4.8^{a}	7.2±1.1	30.1±17.0^{a}	0.16±0.05^{a}

OC: oxidizable carbon. In a same column, different superscripts (a,b,c) indicate significant differences at P < 0.05. Results are expressed as dry matter.

The concentrations of free phenolic compounds values (Table 2) were notably higher than those concerning other pollutants (PAHs, PCBs, PCNs and nonylphenols). Although most phenols contained in sludges are of natural origin, phenolic compounds can also have antimicrobial and phytotoxic properties. In general terms, their levels were positively correlated with fresh organic matter, as values were lower in digested and stabilized samples. The mean concentrations of PAHs and PCBs fulfilled the threshold levels established in the 3rd EU working draft (6 and 0.8 mg/kg, respectively), even though some specific samples slightly exceeded those limits. The low concentrations of some PCB congeners (No. 126, 167, 69 and 77), PFOS, PFOA, and BDE-209 must be highlighted, being undetected in most samples. Generally, and as expected, the lowest values of all organic pollutants were found in compost samples. It could be associated to the microbial degradation and the immobilization with humidified organic matter through covalent bindings. PAHs tend to reduce their concentration in samples treated by thermal drying, as high temperatures (100-150ºC) enhance the volatilization of hydrocarbons, in general, and low-molecular weight PAHs in particular.

Regarding sludge ecotoxicity, the different performed bioassays showed similar results (Figure 1). The least toxic treatment for plants and bacteria was composting, followed by thermal drying. Within the group of higher plants, *Raphanus sativus* was found to be the most sensitive species. It must be emphasized that phytotoxicity was notable for all samples, except compost (GI<70%). This indicates that the utilization of not well stabilized sludges for agricultural could reduce crop production of sensitive species. Concerning Microtox®, organic extracts showed more toxicity (lower EC_{50} VALUES) than aqueous extracts, indicating that organic and non-polar compounds present in sludge exhibit more toxicity than the soluble ones.

Table 2: Concentration of organic compounds in sewage sludge samples according to the treatment.

		Phenolic comp. (mg/kg)	PAHs (mg/kg)	PCBs (mg/kg)	PCNs (mg/kg)	Nonylp. (µg/kg)	PFOS (µg/kg)	PFOA (µg/kg)	PBDE209 (mg/kg)
Non. Dig. & M.D.	Mean±SD	2431±789	1.46±1.02	0.33±0.14	0.07±0.05	0.02±0.01	<DL	<DL	<DL
	Min-max	1701-3548	0.71-2.96	0.21-0.50	<DL-0.11	<DL-0.03			
Aer. Dig. & M.D.	Mean±SD	1951±873	2.96±2.58	0.33±0.16	0.03±0.03	0.03±0.02	<DL	<DL	<DL
	Min-max	843-2977	0.69-6.18	0.11-0.51	<DL-0.07	<DL-0.06			
Anaer. Dig. & M.D.	Mean±SD	1726±524	2.72±1.63	0.52±0.37	0.10±0.14	0.02±0.01	<DL	<DL	0.01±0.00
	Min-max	1037-2830	0.64-6.11	0.20-1.59	<DL-0.56	<DL-0.05			0.01-0.01
Anaer. Dig. & T.D.	Mean±SD	1614±666	0.87±0.63	0.59±0.68	0.05±0.05	0.01±0.01	<DL	<DL	<DL
	Min-max	927-2507	<DL-1.45	<DL-1.57	<DL-0.11	<DL-0.02			
Anaer. Dig. & Compost	Mean±SD	464±361	0.99±0.02	0.18±0.09	0.02±0.01	0.02±0.01	<DL	<DL	<DL
	Min-max	209-719	0.97-1.00	0.11-0.24	<DL-0.02	<DL-0.02			

Phenolic comp.: phenolic compounds; Nonylp: nonylphenols. DL: Detection limit. SD: Standard deviation.

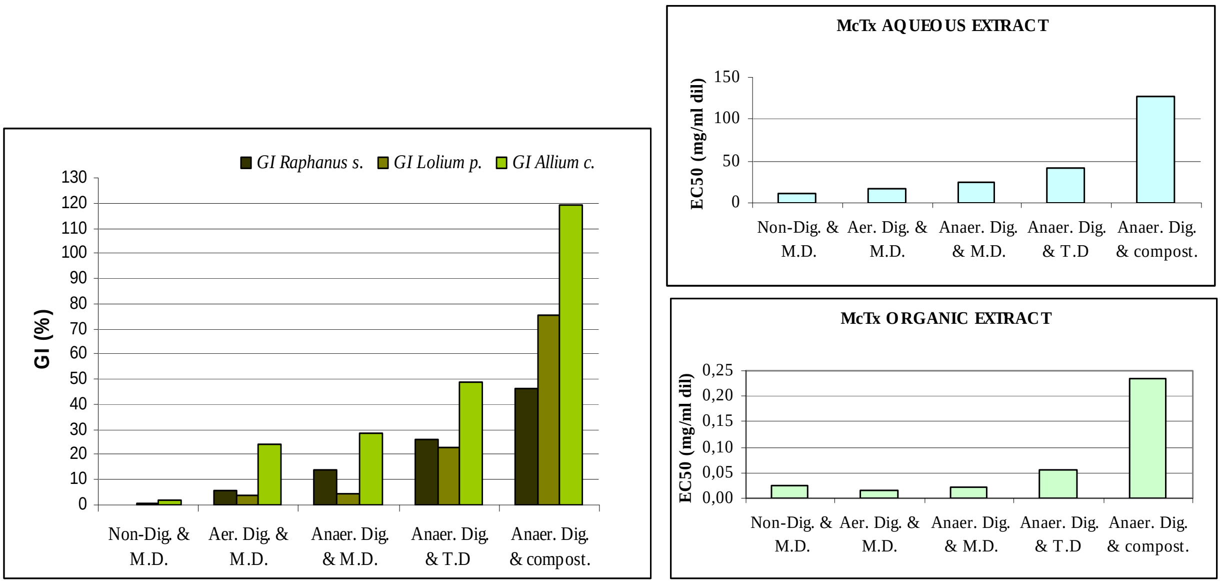

Figure 1: Ecotoxicity results. GI: Germination index in *Raphanus sativa, Lolium perenne,* and *Allium cepa.* McTx Aqueous extract: EC_{50} obtained with Microtox test in aqueous extract. McTx organic extract: EC_{50} obtained in Microtox test in organic extract

More than 50 parameters were correlated to discriminate the effect of sludge treatment on its properties and ecotoxicity. From these results, it can be seen that sludge treatment influenced positively and significantly on the dryness (p = 0.717, P<0.01) and pH (p = 0.405, P<0.05), and negatively with the sludge mineralization rate (p = -0.742, P<0.01) and, therefore, on the sludge organic matter stability. The sludge treatment also correlated positively with the Germination Indexes of all plants assayed (p = 0.437-0.596, P<0.01) and with the EC_{50} values of the Microtox® assay (p = 0.564-0.599, P<0.01). It indicates that the intensity of the sludge treatment significantly influences its toxicity, being less toxic the sludge treated at a greater extent. In turn, sludge treatment had low influence on the presence of specific organic pollutants, except for the phenolic compounds, which are related with organic matter maturity. Only benzo(a)pyrene (p =0.332, P<0.05) and PCB-81 (p = -0.337, P<0.05) correlated significantly. Regarding plant germination indexes, pH correlated positively (p = 0.389-0.416, P<0.05). Naphthalene and PCB-153 inhibited plant germination (p=-0.347, P<0.01 and p=-0.329, P<0.01, respectively), while the phenolic compounds had the strongest influence on phytotoxicity (p = -0.751, P<0.01). Finally, concerning bacteria toxicity, fluoranthene, naphthalene and PCB-153 showed the highest negative correlations (p = -0.324, P<0.05; p = -0.397, P<0.05; and p = -365, P<0.05, respectively) after phenolic compounds (p = -0.656, P<0.01).

In summary, the results of the current study demonstrate that the intensity of sludge treatment is very important in order to increase the stability of organic matter, to reduce the organic pollutant content and therefore, to reduce its ecotoxicity.

Acknowledgements:
This work was supported by the Ministry of Education and Science of Spain, through the project CTM2007-64490-TECNO, and the SOSTAQUA project, founded by CDTI in the framework of the *Ingenio 2010* Program under the CENIT call.

References:
1. CEC. Directive 91/271/EEC. *Official Journal*, L135, 30 May 1991
2. Milieu Ltd (2010). Final Report. Study contract DG ENV G4/ETU/2008/0076r
3. EU (2000) Working document on sludge, 3rd draft. Brussels, Belgium
4. Singh RP, Agrawal M. (2008); *Waste Manage.* 28: 347-58
5. Ros M, Pascual JA, García C, Hernéndez MT, Insam H. (2006); *Soil Biol Biochem.* 38: 3443-52
6. Walkley A, Black LA. (1934); *Soil Sci.* 37: 29-38
7. Kjeldahl JZ. (1883); *Anal Chem.* 22: 366-82
8. Nadal M, Schuhmacher M, Domingo JL. (2007); *Chemosphere.* 66(2): 267-76
9. Box JD. (1983); *Water Res.* 17: 511-25
10. DIN (1984). Deutsche Norm, Teil 4 Okt: 464-475
11. US EPA. (2000). Cincinnati, OH, USA 3546: 1-13
12. Azur (1999). Carlsbad, CA, USA
13. US EPA (1996). EPA 712-C-96-154
14. Zucconi F, Monaco A, Forte M, de Bertoldi M. (1985) In: Gasser, JDR. Elsevier Banking, p. 73-85
15. Barrena Gómez R, Vázquez Lima F, Sánchez Ferrer A. (2006); *Waste Manage Res.* 24(1): 37-47