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INTERACTION OF ALKYLPHENOLS AND ALKYLPHENOL ETHOXYLATES WITH SEWAGE SLUDGES AND SOILS

- European Master in Quality in Analytical Laboratories -

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Barcelona, February 2010

This Master thesis, European Master in Quality in Analytical Laboratories (EMQAL), organized as an International program, by the European Union was divided into two parts: theoretical and experimental. The theoretical part was done in the University of Algarve in Portugal and the experimental part was performed in the Department of Analytical Chemistry in the University of Barcelona, in Spain.

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Dr. Miquel Vidal and Dra. Anna Rigol, Professors in the Departament de Química Analítica, Universitat de Barcelona

ATTEST

The current work entitled:

"INTERACTION OF ALKYLPHENOLS AND ALKYLPHENOL
ETHOXYLATES WITH SEWAGE SLUDGES AND SOILS"

which has been conducted by Jelena Milinovic in the Departament de Química Analítica, Universitat de Barcelona under our supervision.

Barcelona, February 2010

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ABSTRACT

A common practice for the reuse of sewage sludge from waste water treatment plants (WWTPs) is its application in agricultural soils as fertilizer. As sludge from WWTPs may be contaminated with numerous organic and inorganic compounds, once added to the soil it may contribute to the pollution of soil and groundwater. The current European Union Directive 1986/278 EUR only includes threshold concentration levels for heavy metals. The limit values for a few organic contaminants, such as alkylphenols (APs) and alkylphenol ethoxylates (APEOs) are proposed in European Union Working Document, in order to improve the present situation for sludge management. The implementation of a new directive including organic pollutants will require an exhaustive characterization of sewage sludge previous its application in soils, not only in terms of total concentration, but also from the point of view of its interaction and potentially incorporation into the soil or groundwater.

In this work the leachability of APs and APEOs in water, was evaluated in six sewage sludge samples originated from different WWTPs from Catalonia. Liquid-liquid extraction (LLE) followed by GC-MS was used to evaluate the concentration of octylphenol (OP), nonylphenol (NP), nonylphenol monoethoxylate ($NPEO_1$) and nonylphenol diethoxylate ($NPEO_2$) in the leachates from the sewage sludge samples. All target compounds were found to leach from the selected sludges less than 3%. The highest leachability was obtained for the sludge samples with the lowest pH and highest dissolved organic carbon content (DOC).

Since the addition of sewage sludge into the soils may contribute to the incorporation of APs and APEOs into soils, studies were undertaken to assess sorption and desorption behavior of these compounds, concretely NP and $NPEO_1$, in three soils of contrasted characteristics (e.g. pH and organic carbon) and from different areas in Spain. Batch experiments, followed by LLE and GC-MS, were allowed to conclude that sorption of NP and $NPEO_1$ is highly dependent on soil properties. Namely, NP and $NPEO_1$ were very little sorbed in Ascó soil, while they were almost completely sorbed in Delta 2 and Oviedo 1, soils with higher DOC contents. Sorption in all three soils was irreversible and NP and $NPEO_1$ were slowly desorbed, thus expected their accumulation in the top soil layer.

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LIST OF ABBREVIATIONS

AP	Alkylphenol
APEO	Alkylphenol ethoxylate
CAS	Chemical Abstracts Service
DOC	Dissolved Organic Carbon
dw	Dry weight
EEC	European Economic Community
EDC	Endocrine disruptive compounds
EU	European Union
GC-MS	Gas chromatography-mass spectrometry
ICP	Induced coupled plasma
IS	Internal standard
ISO	International Organization for Standardization
LLE	Liquid-liquid extraction
MeOH	Methanol
4-NP	4-nonylphenol
$NPEO_1$	Nonylphenol monoethoxylate
$NPEO_2$	Nonylphenol diethoxylate
OC	Organic carbon
OECD	Organization for Economic Cooperation and Development
4-t-OP	4-tert octylphenol
PHS	Priority hazardous substance
RSD	Relative standard deviation
SD	Standard deviation
SIM	Selected Ion monitoring
WFD	Water Framework Directive
WWTP	Wastewater treatment plant

1. INTRODUCTION

1.1 Background

The use of sewage sludge to agricultural soils as a fertilizer is an economic and environmentally acceptable method for municipal sludge management. Since sewage sludge contains essential plant nutrients and organic matter, it can be recycled beneficially. However, sewage sludge is the byproduct of wastewater treatment plants (WWTPs) and may contain heavy metals and organic contaminants that are not fully degraded and can represent serious risks to the environment.

The amount of sewage sludge derived from the WWTPs has greatly increased in the recent years within the European Union (EU). One of the factors responsible for this is the implementation of the Directives 91/271/EEC (Council Directive, 1991) and 98/15/EEC (Commission Directive, 1998) concerning wastewater treatment. Concretely, the implementation of the European Directive 91/271/EEC (Council Directive, 91/271/EEC, 1991) on urban waste water treatment, whose main objective was to protect the environment from the adverse effects of waste water discharges, led to a significant increase in the number of WWTPs with an ensuing production of large amounts of sewage sludge. During the WWTP treatment process, heavy metals and organic compounds can be accumulated in sludge, due to their intrinsic physico-chemical properties that make them resistant to a complete degradation.

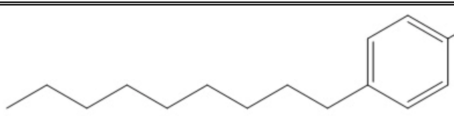
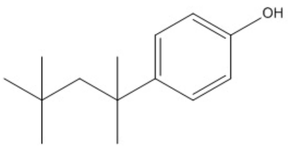
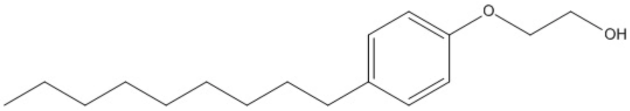
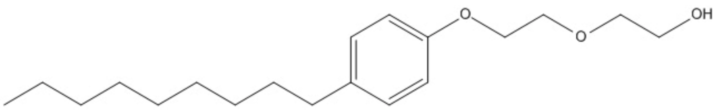
According to the Council Directive 86/278/EEC (Council Directive, 1986) on the protection of the environment including soil, when sewage sludge is used in agriculture, only limit values for concentrations of heavy metals in the sludge and soil, are regulated. Nowadays there is a progressive effort towards including the limit values for new pollutants, like APs and APEOs, acting as emergent organic pollutants of an increasing environmental interest.

1.2 Structure, origin and degradation of APs and APEOs

Alkylphenols (APs) are organic compounds synthesized through alkylation of phenols. Among APs, the most representative are nonylphenols (NPs) and octylphenols (OPs) which comprise both hydrophobic branched group nonyl, i.e. octyl and a hydrophilic moiety. NPs are released in the environment as a mixture of 18 isomers with branched nonyl groups, including the well-known linear isomer 4-n-nonylphenol (4-n-NP).

APEOs are widely used as intermediate chemicals in the cleaning and industrial processes. The spectrum of application ranges from dispersing agents in paper and pulp production to emulsifying agents in latex paints and pesticides formulations, flotation agents, industrial cleaners, cold cleaners for cars and household cleaners. The majority of APEOs are used in aqueous solutions and hence they are discharged into municipal and industrial WWTPs. NPEOs are the most widely used polyethoxylates of alkylphenols and they contribute to almost 80 % of the total APEOs.

Table 1.1 CAS registry numbers and chemical structures of 4-n-NP, 4-t-OP, NPEO$_1$ and NPEO$_2$

Compound	CAS number	Chemical structure
4-n-NP	104-40-5	
4-t-OP	140-66-9	
NPEO$_1$	9016-45-9	
NPEO$_2$	20427-84-3	

The CAS registry numbers and the chemical structures of the most important APs, 4-n-nonylphenol and 4-t-octylphenol, and APEOs, nonylphenol monoethoxylate ($NPEO_1$) and nonylphenol diethoxylate ($NPEO_2$) are given in table 1.1. The listed compounds deserve special attention not only because of their disrupting properties but rather for the widespread and high concentrations found in sewage sludge and soil (Jensen and Jepsen, 2005; Langford and Lester, 2002; Vikelsoe et al., 2002; Falkenberg et al., 2003; Auriol et al., 2006).

In WWTPs, APEOs are partly degraded to APs by shortening the hydrophilic ethoxylate chain (figure 1.1). These compounds are further degraded in anaerobically stabilized sewage sludge to the fully diethoxylated 4-nonylphenol, more lipophilic and toxic compound resistant to further microbial degradation.

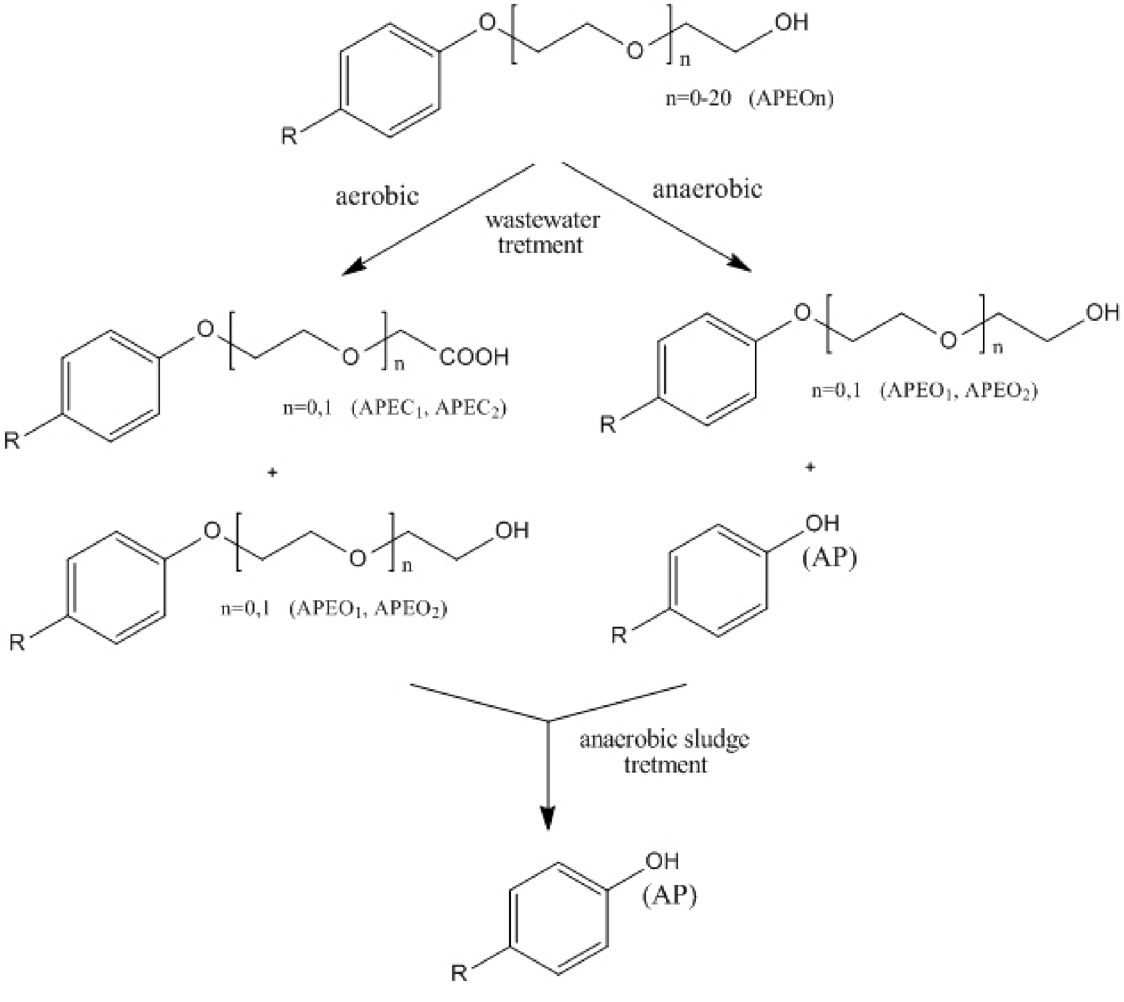


Figure 1.1 Degradation of alkylphenol polyethoxylate into alkylphenol

APs and APEOs occurrence in soil is related to anthropogenic activities such as sewage sludge application, land filling and accidental spillage. The activity that raises the major concern and has gained most attention is sewage sludge recycling to agricultural land. According to a Danish study, the fraction of sludge recycled by farmers in 2002 was 66% of the total production indicating the importance of investigating the occurrence and fate of contaminants such as nonylphenol (NP) that accumulate in sewage sludge (Jensen and Jepsen, 2005; Langford and Lester, 2002).

The concentrations of APs and APEOs in sewage sludges originated in some European countries may vary over a wide range depending on WWTPs, as summarized in table 1.2.

Table 1.2 APs and APEOs concentration in sewage treatment plant effluents

Country	Concentration range (µg/mL)				Reference
	OP	NP	$NPEO_1$	$NPEO_2$	
Canada	0.17-1.7	0.8-15	-	-	Lee and Peart, (1995)
Germany	0.002-0.67	0.03-0.08	-	-	Kuch and Ballschmiter, (2001)
Japan	0.02-0.48	0.08-1.2	0.21-3.0	-	Isobe et al., (2001)
Spain	-	6-289	-	-	Sole et al., (2000)
Switzerland	-	8	49	44	Ahel and Giger, (1985)
USA	0.002-0.67	0.001-37	-	-	Snyder et al., (1999)

APs and APEOs represent an environmental hazard because they may induce toxic effects. As they possess chemical structure similar to estrogens, they can compete with them by binding to characteristic hormone receptors in the cells, i.e. they can mimic biochemical activities of the estrogen hormones, several orders of magnitude below natural hormones (Warhurst, 1995). Hence, they show estrogenic activity and consequently are known as endocrine disrupting compounds (EDCs). As EDCs, APs and APEOs can develop different types of cancer and can reduce fertility (Routledge and Sumpter, 1996).

1.3 EU Directives concerning the application of sludges to agricultural soils

In the European Union, sewage sludge application in agriculture is regulated by EU Council Directive 86/278/ECC (Council Directive, 1986). This Directive requires that heavy metal contents (Cd, Cr, Cu, Hg, Ni, Pb, Zn) in both the sludge and soil must comply with the established limits, which are usually soil-pH dependent. However, this Directive does not establish limit values for trace organic contaminants in sewage sludge. Therefore, proposals to modify this directive aim at adding to the legislation limit concentration values for certain priority organic pollutants in both sewage sludge and sludge-amended soils. The European Union produced a Working Document on Sludge (Working Document on Sludge, 2000), described as "an EU-initiative to improve the present situation for sludge management". In this document limit values for the concentrations of a number of organic compounds are proposed. Besides dioxins, some of the organic compounds that will have to be monitored according to this document comprise alkylphenols (APs), such as nonylphenol (NP), and alkylphenol ethoxylates (APEOs) such as nonylphenol (NP), nonylphenol monoethoxylate ($NPEO_1$) and nonylphenol diethoxylate ($NPEO_2$), due to their environmental ubiquity and toxicity (EU, 2001; Leschber, 2004). The limit value established for the sum of the concentrations of these three compounds in sludge dry matter for land application is 50 mg/kg. If the total concentration of NP, $NPEO_1$ and $NPEO_2$ in sludge dry matter exceeds the concentration limit proposed, the reuse of sludge on agricultural soils is not allowed.

Only a few European countries like Denmark and Germany have introduced national legislations regarding this issue (Danish Ministerial Order, 1996; Sewage sludge ordinance, 1992). However, in European countries the content of NP, $NPEO_1$ and $NPEO_2$ in sewage sludges often exceeds the limits allowed by proposed regulations and thus they represent a major environmental problem.

In the Water Framework Directive 2000/60/EC (Directive, 2000) NP and its ethoxylates are designated as priority hazardous substances (PHS) and most of their uses are currently regulated by Directive 2003/53/EC (Directive, 2003). The widespread use of APEOs have led to the incorporation of NP and octylphenol (OP) in the list of 33 priority hazardous compounds of the European Union Water Framework European Directive (WFD).

1.4 Interaction of APs and APEOs with sludge and soil

For obtaining essential information of APs and APEOs mobility and their distribution in the sludge and soil, sorption and desorption studies are useful. The information derived from these studies can be used in the prediction of a number of the processes such as an estimation of the availability and transport (root uptake; leaching through the soil profile) of the compounds in terrestrial and water compartments, volatility from sludge or soil, and run-off from land surfaces into natural waters. Besides, the risk assessment of potential environmental hazards associated with their presence in the soil, as well as definition of possible remediation strategies, require an accurate evaluation and quantification of the soil response to these compounds.

Table 1.3 Physico-chemical properties of alkylphenolic compounds

Compound	Solubility in water (mg/L)	Partition coefficient, log K_{ow}	pKa
4-n-NP	4.9^a	4.48^c	10.28^d
4-t-OP	12.6^b	4.12^c	10.39^d
$NPEO_1$	3.02^b	4.17^c	-
$NPEO_2$	3.28^b	4.21^c	-

[a]Langford and Lester (2002); [b]Ahel and Giger (1993a); [c]Ahel and Giger (1993b); [d]Muller and Schlatter (1998)

Distribution of the APs and APEOs between solid and aqueous phase is a complex process depending on a number of different factors such as: physical and chemical properties of these compounds (table 1.3), sludge and soil properties and various climate factors such as temperature, humidity and rainfall properties.

The attention of environmental risk of APs has been mainly focused on water contamination whereas relatively little information is available about the impact and fate of APs and APEOs introduced in the soil. The soil sorption of APs is a key process governing their behavior and subsequent fate in the environment. As hydrophobic compounds, APs can be strongly retained to soil through nonspecific hydrophobic interactions which reduce their mobility in soils and their bioavailability to plants and microorganisms.

The response of soil to the estrogenic risk of APs is generally related to the distribution of these compounds in the various soil phases (figure 1.2). The APs and APEOs can be extensively and strongly bound to solid fractions in the terrestrial compartment and accumulate in top soil layer or, if they are weakly bound, they can easily transport to the water compartment and then transported to ground water.

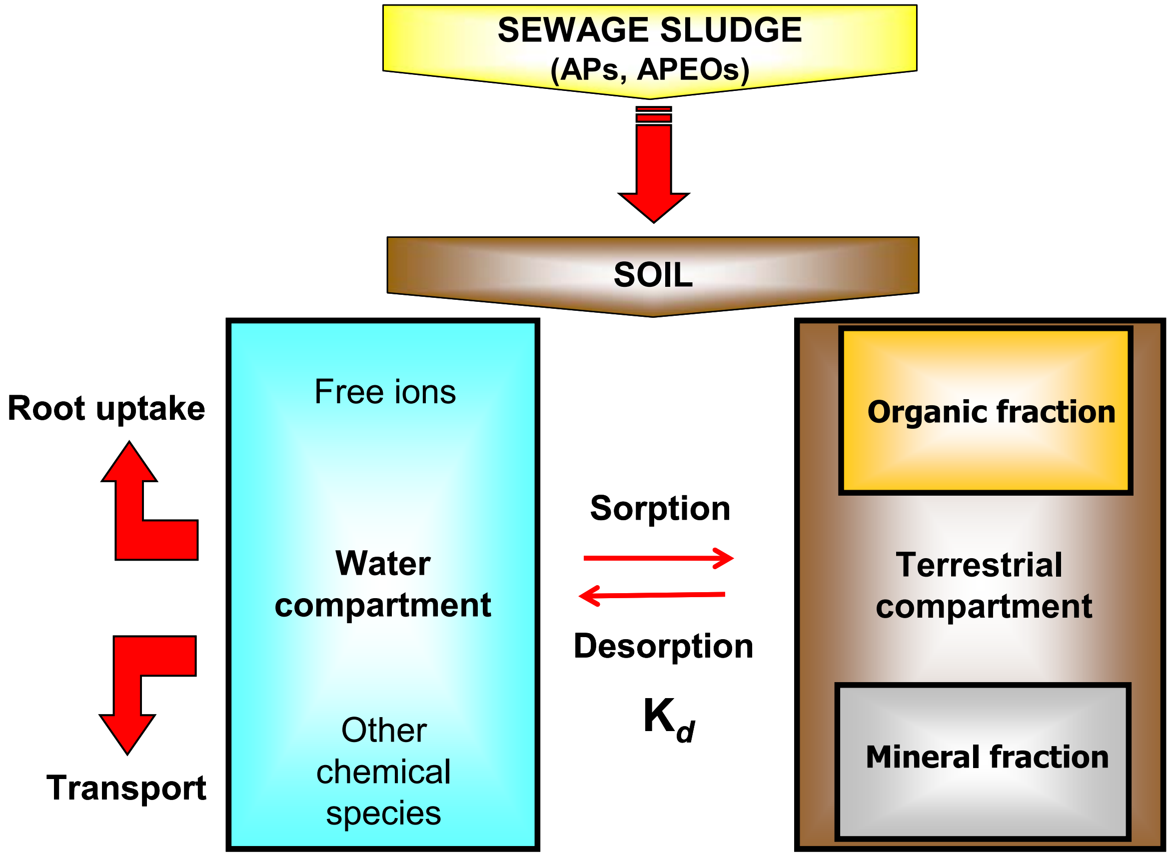


Figure 1.2 APs and APEOs interactions in soil

As it has been already mentioned, a significant portion of the NPEOs applied to soils, may be transformed into NP resulting in high NP concentrations that can reach aquifers (Montgomery-Brown et al., 2003). Hence, under normal soil conditions, NP is present in the soil as nondissociated compound, which can be sorbed at the organic fraction of soil due to a hydrophobic interaction between the ethoxy chain and the organic phase.

The sorption of NP and OP from liquid to solid phase in the soil was found to be very fast in the first hours of contact (During et al., 2002; Loffredo and Senesi, 2006). The following slow increase in sorbed concentration was ascribed to the movement of the NP from external to internal sites of insoluble organic matter by intraorganic matter diffusion that is assumed to be the rate limiting process. Also, it was shown that pH and dissolved organic carbon (DOC) may influence affinity of NP to soil.

Hollrigl-Rosta et al. (2003) concluded that NP binds strongly to humic acid in the dissolved organic matter through nonspecific lipophilic interactions that are regulated by diffusion and are not completely reversible (During et al., 2002). Stronger sorption is often observed at pH values near the pK_a since a high degree of protonation results in increased interactions with the soil matrix.

Considering the soil properties, it was observed that K_d values of 4-n-NP and 4-t-OP increased with the organic carbon contents in soil (Ying and Kookana, 2005). The other soil properties had less effect on 4-n-NP and 4-t-OP sorption.

It has been also shown that degradability of APs is affected by their alkyl chain structure. Namely, in comparison with 4-t-OP, 4-n-NP is more amenable to biodegradation under aerobic conditions due to its straight side chain. Because degradation products of commercial surfactants APEOs are mainly branched, their behavior should be similar to 4-t-OP rather than 4-n-NP. However rapid aerobic degradation of both APs (4-t-OP and 4-n-NP) in the soil was proved (Ying and Kookana, 2005) and it was clearly demonstrated that APs are adsorbed onto the organic fraction of soil surface where degrade rapidly under aerobic conditions.

On the contrary, Trocme et al. (1988) found that the volatilization rate of NP from soils is not significant, only 0.22% over a period of 40 days. According to this, in another work it was shown that the mobility of NP is low in the soil, since it is strongly bound to the soil particles.

The same authors estimated that, after two years, almost 99% of the NP was still within the soil surface of 30 cm depth (Vogel et al., 2000).

1.5 Objectives and working plan

The first objective of the work was to examine and describe the leaching of NP, OP, $NPEO_1$ and $NPEO_2$ from sewage sludge samples of WWTPs with different urban origin. In that sense, leaching tests were applied to assess the release of the target compounds after contact of the sludge samples with water.

In order to accomplish this main objective, the working plan was designed as follows:

- Selection of sludge samples from different origin.

- Characterization of selected sludge samples.

- Application of a leaching test to sludge samples in order to characterize NP, OP, $NPEO_1$ and $NPEO_2$ leaching rates.

- Correlation of the leachability of the target compounds with sludge characterization.

The second major objective of the work was to investigate the sorption-desorption behavior of NP and $NPEO_1$ in soil samples from Spain. After the application of sorption-desorption tests, the relationship between the results obtained and physical and chemical properties of NP and $NPEO_1$ and soil extracts was examined.

The working plan for this objective comprised the following steps:

- Selection of soil samples exhibiting different physical and chemical characteristics.
- Characterization of soil samples.

- Analysis of NP and $NPEO_1$ interactions with soils after the application of sorption and desorption experiments.

- Examination of the relationship between the obtained results and properties of target compounds and soil samples.

2. EXPERIMENTAL PART

2.1 Preparation of standard solutions

Standards of 4-nonylphenol (4-NP, purity 100 %), 4-tert-octylphenol (4-t-OP, purity 98.5 %), 4-nonylphenol monoethoxylate (4-NPEO$_1$, purity 99 %) and 4-nonylphenol diethoxylate (4-NPEO$_2$, purity 99 %) were purchased from Dr. Ehrenstorfer (Cromlab, Spain). Stock standard solutions were prepared at approximately 1 g/L in methanol (Merck, Darmstadt, Germany) and stored at -18 °C.

Deuterated 4-n-nonylphenol D$_8$ (4-n-NP-D$_8$, concentration of 100 μg/mL) and 4-nonylphenol monoethoxylate D$_2$ (4-NPEO$_1$-D$_2$, concentration of 10 μg/mL) were used as internal standards and they were purchased from the same manufacturer.

2.2 Sample preparation and characterization

2.2.1 Sludge samples

Six sludge samples (SL 1 – SL 6) were selected for leaching studies. They were collected from WWTPs in Catalonia, located in different industrial zones. Sewage sludge samples SL 1 and SL 2 originate from zones with a low industrial activity and they were not digested in the WWTP. Sludge samples SL 3, SL 4 and SL 5 were collected from highly industrial zones and were treated under anaerobic conditions. Finally, sewage sludge SL 6 originates from the WWTP in a highly contaminated area and was selected due to its high levels of APs and APEOs (Lecina, 2006; Fernández-Sanjuan et al., 2009).

All sludge samples were homogenized in the MAT CONTROL Laboratory of the Universitat de Barcelona, which is specialized facility for the preparation of quality control materials. Samples were dried at 40°C and subsequently milled in an agate mortar. They were homogenized and kept at room temperature in amber glass containers until analyzed.

Characterization of the sludges was performed according to the Official Spanish Analysis Methods and ISO Norms (Ministerio de Agricultura, Pesca y Alimentación, 1994; ISO Norm 10694, 1995; ISO Norm 11466, 1995). Main characterization parameters, obtained in a previous study, are summarized in tables 2.1 and 2.2 (Lecina, 2006; Fernández-Sanjuan et al., 2009).

Table 2.1 pH, organic carbon (OC) and concentrations of major metals extractable in aqua regia

Sludge	pH	OC (%)	Ca (mg/kg)	Fe (mg/kg)	K (mg/kg)	Mg (mg/kg)
SL 1	8.3	22.8	43218 (1530)	15313 (146)	2978 (30)	7246 (96)
SL 2	8.6	37.1	37392 (923)	5444 (123)	3421 (95)	4844 (147)
SL 3	8.4	27.9	46308 (2074)	9959 (486)	3006 (91)	6791 (237)
SL 4	7.1	32.2	55566 (2202)	27982 (1176)	2298 (93)	5680 (140)
SL 5	6.1	28.2	22040 (763)	7381 (701)	5022 (75)	5994 (212)
SL 6	6.6	46.1	na	na	na	na

The results are summarized as the mean values (standard deviation); n = 3
na – not analyzed

Table 2.2 Concentrations of heavy metals extractable in aqua regia (mg/kg)

Sludge	Cd	Cr	Cu	Ni	Pb	Zn
SL 1	nd	135 (2)	425 (8)	73 (3)	86 (2)	140 (4)
SL 2	nd	137 (1)	666 (27)	25 (1)	59 (2)	455 (4)
SL 3	nd	150 (2)	635 (34)	94 (4)	41 (3)	1446 (84)
SL 4	nd	119 (2)	631 (21)	29 (1)	73 (2)	214 (19)
SL 5	nd	52 (5)	528 (36)	16 (1)	65 (2)	1210 (48)
SL 6	1.98 (0.01)	165 (2)	703 (3)	55 (1)	78 (4)	1351 (22)

The results are summarized as the mean values (standard deviation); n = 3
nd – not detected

2.2.2 Soil samples

Three soil samples, Ascó, Delta 2 and Oviedo 1, collected at different localities in Spain, were selected for the NP and $NPEO_1$ sorption and desorption experiments, as soils showing different physical and chemical characteristics. Samples were taken from the surface layer, dried at room temperature, sieved at 2 mm and homogenized before analyses. In table 2.3 a few chemical properties of the selected soils are shown (Gil-Garcia et al., 2008).

Table 2.3 pH, OC and concentrations of major metals in soil solution

Soil	pH	OC (%)	Ca (mmol/L)	K (mmol/L)	Mg (mmol/L)
Ascó	8.0	0.2	2.5	1.0	15.8
Delta 2	7.9	7.7	20.4	2.0	43.5
Oviedo 1	4.6	9.4	3.9	0.55	24. 6

2.3 Sludge leaching experiments

Approximately 8 g of the sludge was weighed into a glass tube and 80 mL of Milli-Q double deionized water (USF PureLaB Plus, 18.2 MΩ/cm) were added to sludge sample. The tube was closed with a plastic cap and put into the end-over-end shaker. The sludge suspensions were shaken for 24 hours at a speed of 30 rpm and in dark, to avoid degradation. After shaking, the suspension was centrifuged (Universal 30F) at 2000 *g*, for 30 minutes, and the obtained supernatant was filtrated through filters with pore size of 0.45 µm (Chromafil, Macherey-Nagel, Germany), using sterile plastic syringes BD Discardit (Becton Dickinson S.A., Spain), and kept for the next step (see figure 2.1).

Various aliquots of the supernatant were taken for subsequent analysis: 30 mL for the determination of AP and APEOs leachability and the rest for the leachate characterization - 20 mL for pH and DOC measurements; - 10 mL for anion determination by ionic chromatography and - 5 mL for major and trace metal determinations (see section 2.5).

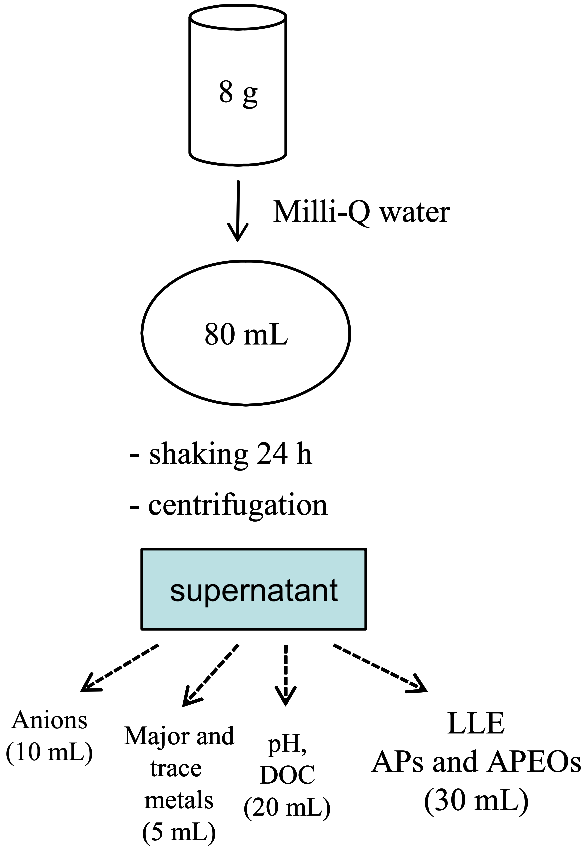


Figure 2.1 Scheme of sludge leaching test

All experiments were conducted in duplicates, with parallel blank runs and the extracts of sludge samples were kept in refrigerator before further analysis.

2.4 Sorption and desorption on soil samples

In sorption experiments, 3 g of soil samples were equilibrated with 30 mL of 0.01 mol/L $CaCl_2$ solution (p.a., Merck, Darmstadt, Germany) with 0.05 % NaN_3 (extra pure, Merck, Darmstadt, Germany) added as biodegradation inhibiting agent. After 24 h of equilibration, known amounts of NP and $NPEO_1$ were added to the soil suspension, as shown in table 2.4.

Table 2.4 Amount of NP and NPEO$_1$ added to the soil suspensions

No.	Amount of NP (µg)	Amount of NPEO$_1$ (µg)
1	0.51	0.99
2	1.02	1.99
3	2.05	4.97
4	5.12	9.93
5	10.25	19.86

The suspension samples were subsequently shaken for 24h at room temperature in dark and centrifuged at 2000 *g* for 30 minutes (see figure 2.2). Extracts were filtrated through filters of 0.45 µm (Chromafil, Macherey-Nagel, Germany), using sterile plastic syringes BD Discardit (Becton Dickinson S.A., Spain) and kept in refrigerator for the analysis.

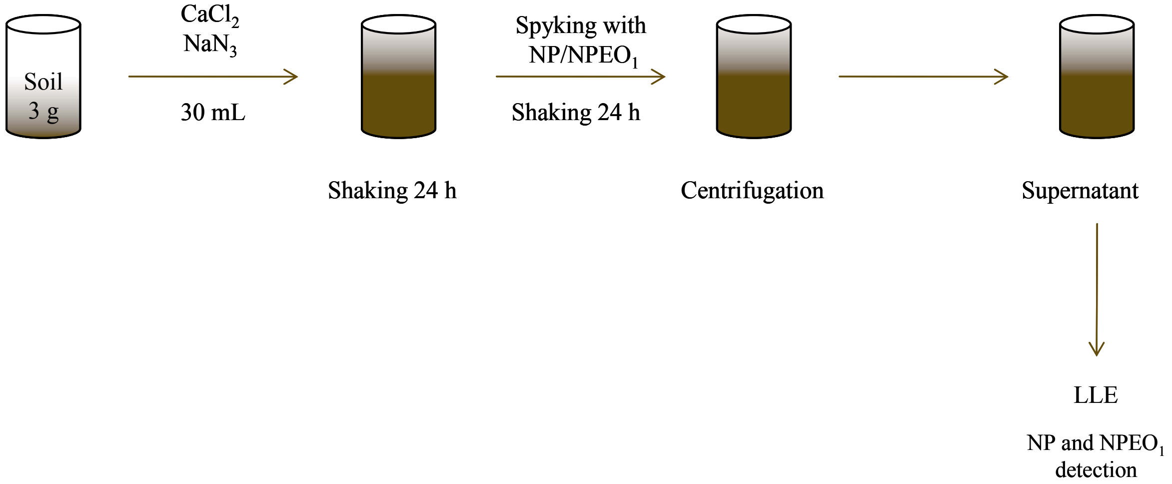


Figure 2.2 Scheme of soil sorption experiment

Blank and control samples were tested in parallel, in order to verify artifacts in the analytical method and matrix effects caused by soil sample, as well as to check stability of the target analytes in $CaCl_2$ solution and possible adsorption at the surfaces of the test vessels, respectively. All the experiments were performed in duplicate.

In order to evaluate the reversibility of the sorption process, the soil residue obtained after the sorption step was dried for 7 days at 40 °C to favor the incorporation of the target compounds in the soil. After this period dried soil samples were extracted with 30 mL of the same $CaCl_2$ solution as in sorption experiments. Supernatants were obtained after centrifugation and kept in glass tubes before analysis.

2.5. Characterization of sludge and soil solutions

Anion determinations were performed in diluted extracts with using Milli-Q water by using standard method of ion chromatography in anion-exchange column (Waters IC-PAK Anion) with UV detection (Kontron 332).

Organic carbon was determined using tin capsules and V_2O_5 as an additive, by introducing sample solutions into an elemental organic analyzer (Thermo Finnigan NA 2100, Milan, Italy). In order to eliminate the carbonates, the solutions were pretreated with 2 mol/L hydrochloric acid before the organic carbon analysis (ISO, 1995).

The concentration of major and trace elements in the solutions was analyzed by an ICP-OES (Perkin Elmer Optima, 3200RL). To determine the concentration samples were diluted in 1% HNO_3, following an ISO Norm 11466 (ISO 11466).

2.6 Liquid-liquid extraction

Liquid-liquid extraction (LLE) was the method applied for the extraction of OP, NP, $NPEO_1$ and $NPEO_2$ from aqueous solutions originated from either the leaching experiments with the sludges or the sorption-desorption tests with the soils. This conventional extraction is cheaper and is a reasonably fast method in comparison with some other advanced extraction procedures. Moreover, several works suggest this method of extraction as a method with good recoveries

(Navarro et al., 2009). Cyclohexane was selected as the organic solvent for extraction, due to its low toxicity.

5 µL of 4-n-NP-D_8 and 50 µL of 4-$NPEO_1$-D_2 were added as internal standards to 30 mL of the aqueous solution into a glass tube. Then, 10 mL of cyclohexane (Merck, Darmstadt, Germany) was added to the sample and the mixture was shaken vigorously for 2 minutes, in order to extract the target compounds into the organic phase. After separating two phases, cyclohexane layer was transferred into an amber glass vial (40 mL) and the extraction procedure with cyclohexane was repeated two times more. The collected 30 mL of cyclohexane solution was kept in freeze before evaporation.

Cyclohexane solutions were evaporated by a N_2 stream (Pierce, Reacti-Vap, TM III) to a volume less than 1 mL at temperature of 50 °C. The remaining volumes of organic extracts were transferred into smaller vials (Agilent, Germany), evaporated to dryness and reconstituted with ethyl acetate. The final solution was stored in freeze, before the GC-MS analysis.

2.7 Quantification of target compounds

2.7.1 Standard preparation for calibration

Calibration standards were prepared by appropriate volumetric dilutions of the stock standard solution in ethyl acetate (Merck, Darmstadt, Germany) in the following concentration ranges: 0.05 – 2.30 µg/mL for OP; 0.03 – 25.62 µg/mL for NP; 0.02 – 12.42 µg/mL for $NPEO_1$ and 0.26 – 12.75 µg/mL for $NPEO_2$.

In all standard solutions 5 µL of 4-n-NP-D_8 and 50 µL of 4-$NPEO_1$-D_2 were added as internal standards. 4-n-NP-D_8 was used to quantify OP and NP while 4-$NPEO_1$-D_2 was used for quantification of $NPEO_1$ and $NPEO_2$.

2.7.2 GC-MS conditions

Quantification of NP, OP, $NPEO_1$ and $NPEO_2$ in sludge leachates and NP and $NPEO_1$ in soil extracts, was carried out by gas chromatograph (GC) (Agilent, 6890 Series) coupled to a quadrupole mass spectrometer (MS) (Agilent Mass Selective Detector 5973) using electron impact as a source for mass fragmentation.

The main instrumental parameters used are shown in table 2.5.

Table 2.5 Instrumental parameters

Parameter	Value
Carrier gas flow He (mL/min)	1
Temperature of oven (ºC)	70 – 310
Potential of electronic ionization (eV)	70
Temperature of injector (ºC)	250
Temperature of interface (ºC)	270
Current of emission (µA)	100
Detector voltage (V)	400

A capillary column of fused-silica (15 meters of length, 25 mm of internal diameter and film thickness of 0.25 µm) with 5% of phenyl and 95% of methyl polysiloxane (HP-5MS) was used. The carrier gas was helium and it was used at a flow rate of 1 mL/min. A 2 µL volume of each sample was automatically injected three times (Agilent, 7683 Series) in splitless mode. Between the two consecutive samples, ethyl acetate was injected to clean the syringe and column.

3. RESULTS AND DISCUSSION

3.1 Determination of OP, NP, NPEO$_1$ and NPEO$_2$ by GC-MS

For the determination of OP, NP, NPEO$_1$ and NPEO$_2$ concentrations in standard solutions and samples derived from leaching and sorption-desorption experiments by GC-MS, data acquisition was performed in time-scheduled ion monitoring (SIM) using retention time windows. In table 3.1 quantification and confirmation ions used for each target compound and internal standards are presented.

Table 3.1 Characteristic ions for GC-MS analysis of OP, NP, NPEO$_1$ and NPEO$_2$

Compound	Retention time (min)	Quantification ion (m/z)	Confirmation ions (m/z)
OP	11.0 - 11.1	135	107, 207
NP	12.4 - 13.2	135	107, 149
NPEO$_1$	15.4 - 16.4	179	135, 193
NPEO$_2$	18.5 - 19.6	223	237, 135
4-n-NP-D$_8$	14.4 - 14.6	113	112, 228
NPEO$_1$-D$_2$	15.4 - 16.5	181	195

NP yields three major peak groups with m/z of 107, 135 and 149. The most intensive peak is that one with m/z ratio of 135 due to favored cleavage of benzyl group, which leads to a loss of the C_6H_{13}- group from the molecular ion. Elimination of the CO from the primarily benzyl ion produces an ion of m/z 107. Another abundant ion with m/z of 149 corresponds to the loss of pentyl group from the molecular ion.

Analogously, fragmentation of OP occurs by leaving the heptyl group, C_7H_{15}- and CO form OP molecular ion, which produces peaks at m/z of 135 and 107, respectively. Likely for NP, the most abundant peak of OP is one with m/z ratio of 135. Thus, by using different retention times, these compounds are quantified at the same m/z equal to 135. Figure 3.1 shows examples of GC-MS chromatograms obtained after injection of the calibration standards of the OP and NP.

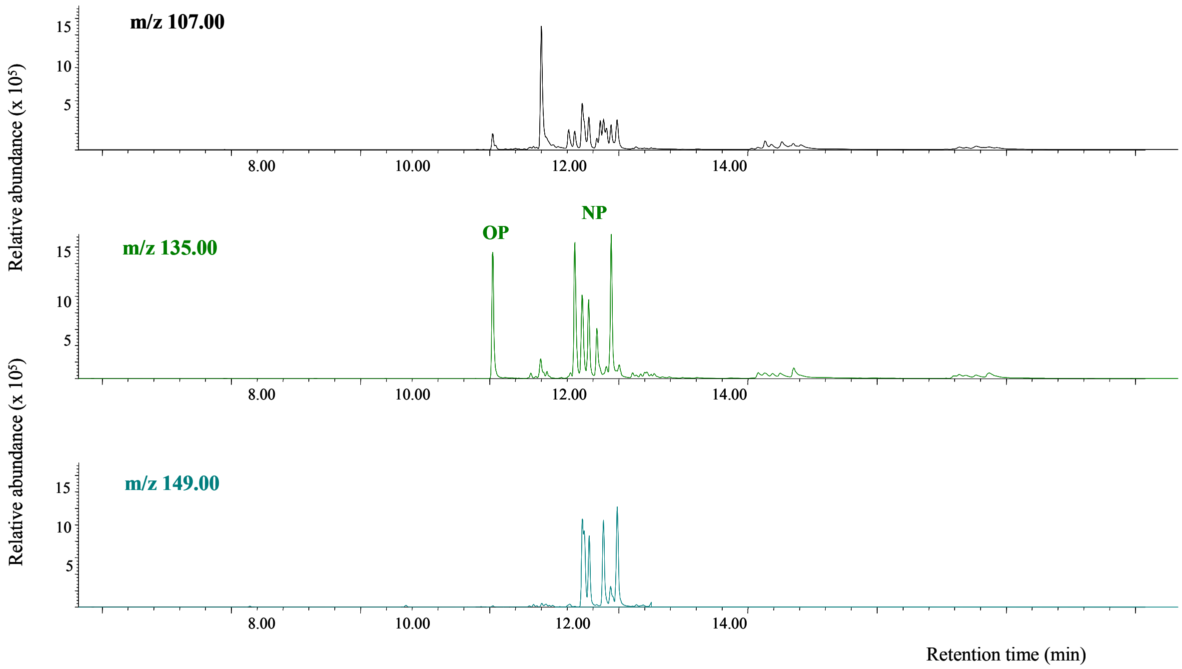


Figure 3.1 GC-MS chromatogram of standard solution of OP (2.30 µg/mL) and NP (25.62 µg/mL)

In the GC-MS chromatograms of $NPEO_1$ and $NPEO_2$ the main peaks with m/z equal to 179 and 223, are attributed to the addition of one or two ethoxylate groups to the most intensive peak of NP with m/z of 135 (figures 3.2 and 3.3).

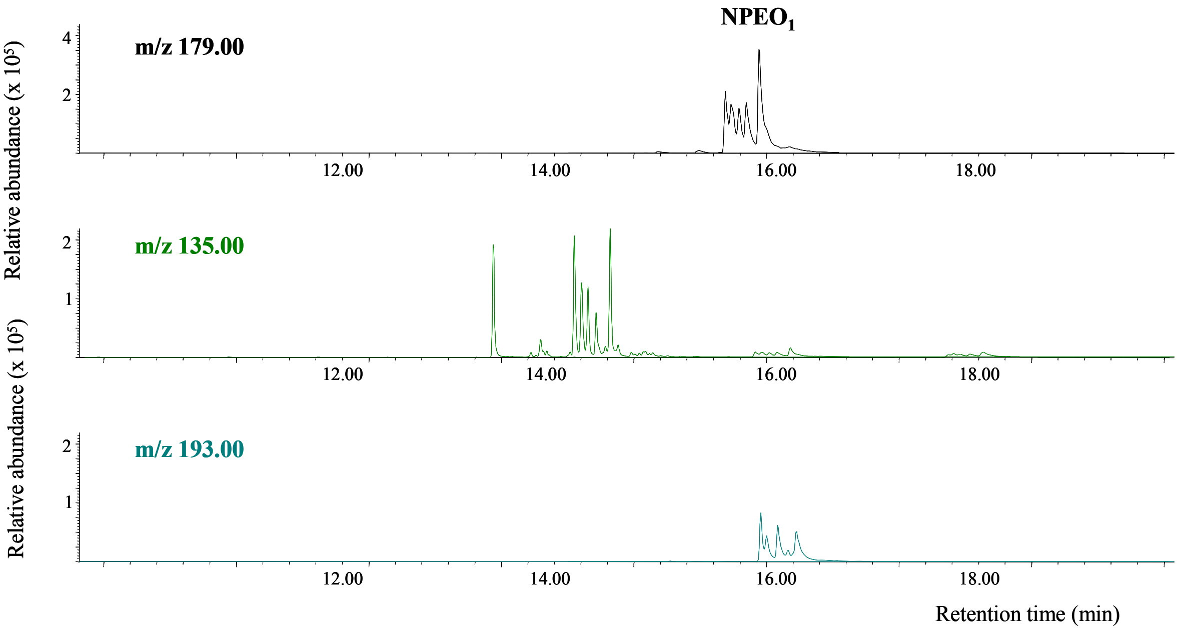


Figure 3.2 GC-MS chromatogram of standard solution of $NPEO_1$ (12.42 µg/mL)

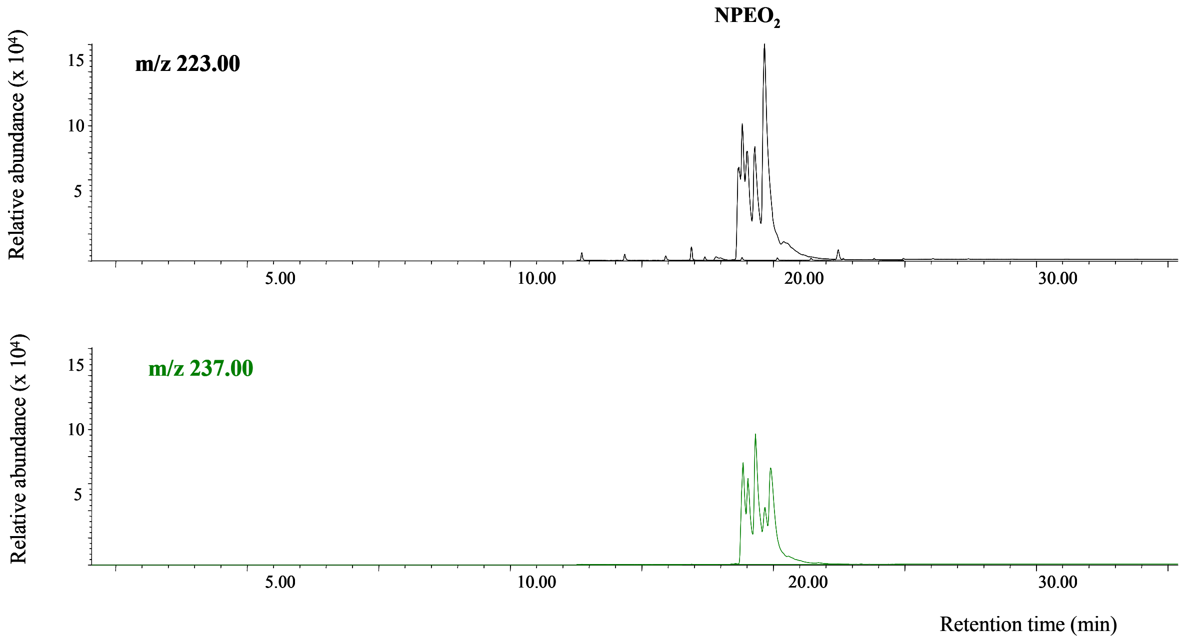


Figure 3.3 GC-MS chromatogram of standard solution of $NPEO_2$ (12.75 µg/mL)

Based on the chromatograms obtained, the calibration curve of target compound/internal standard peak area ratios *vs.* the target compound/internal standard concentration ratio of each alkylphenolic compound was plotted and the concentrations of the analyzed compounds in the samples were calculated based on the calibration curve equation.

The calibration curves had the correlation coefficients, higher than 0.996 for all the standard solutions, as it is shown in table 3.2. As can be observed, the slopes of the calibration curves for OP and NP had higher values in comparison with ethoxylated derivatives, due to their higher sensitivity by GC-MS determination.

Table 3.2 Parameters of OP, NP, $NPEO_1$ and $NPEO_2$ calibration curves

Parameter	**OP**	**NP**	**$NPEO_1$**	**$NPEO_2$**
Number of standards, n	6	8	8	5
Correlation coefficient, r	0.999	0.999	0.999	0.996
Slope	8.2	3.0	1.8	1.0

In figure 3.5 GC-MS chromatogram obtained after injection of sludge sample SL 6 is shown.

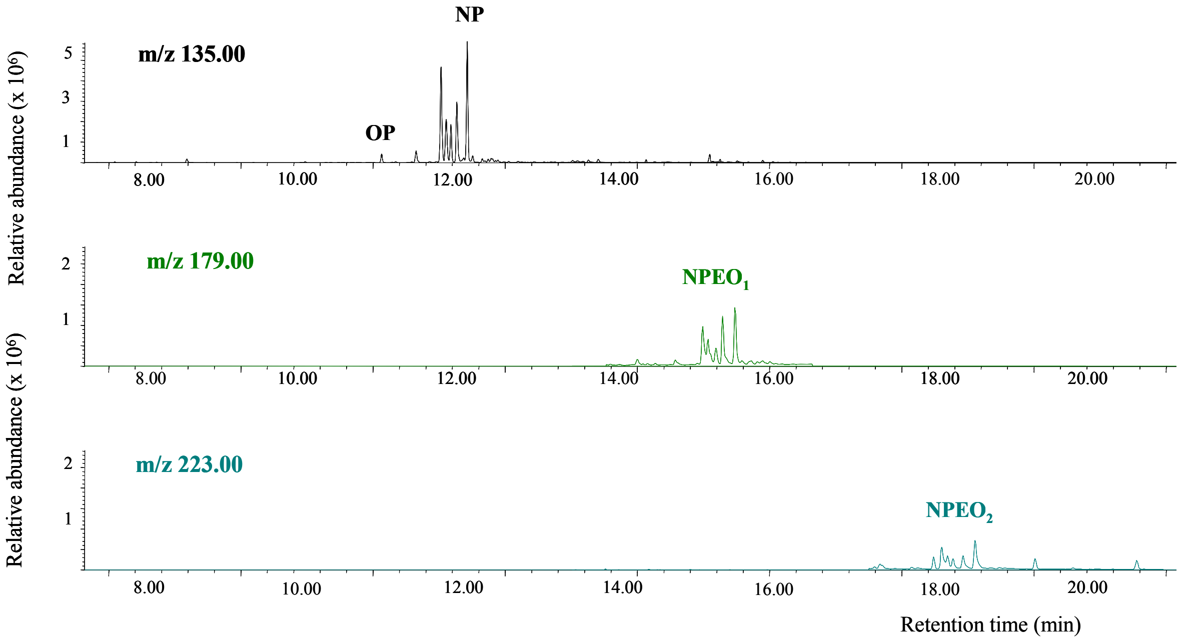


Figure 3.5 GC-MS chromatogram of sludge sample SL 6

3.2 Leaching behavior of APs and APEOs in sewage sludge samples

3.2.1 Characterization of sludge leachates

In order to have information about the factors that can affect leachability of APs and APEOs from sludge, several parameters like pH, DOC, major elements, heavy metal and anion contents were measured in the sludge leachates.

The results of pH and DOC determination in the sludge leaching solutions are presented in table 3.3. pH values for the six sludge samples examined, ranged approximately from 5.9 to 7.4. These results were unabled us to confirm that the acidic properties of the sludges and their leachates varied with their origin and WWTP treatment. The leaching solution of the sludge sample SL 2 showed the most acidic pH whereas the samples SL 1, SL 3 and SL 5 showed slightly weak acid characteristics. Sludge leachates SL 4 and SL 6 were neutral and slightly basic, respectively.

The values of DOC, ranging from 2 up to 7 % were the highest in the more acidic samples SL 1 (5.3 %) and SL 2 (7 %).

Table 3.3 Values of pH and DOC in sludge leachates

Sample	pH	DOC (%)
SL 1	6.24 (0.03)	5.3 (0.1)
SL 2	5.95 (0.01)	7.0 (0.2)
SL 3	6.71 (0.07)	3.59 (0.01)
SL 4	7.06 (0.04)	2.13 (0.01)
SL 5	6.78 (0.08)	2.14 (0.04)
SL 6	7.42 (0.06)	2.68 (0.04)

The results are summarized as the mean values (standard deviations); n = 2

The relationship between the pH and DOC content in the leachates was confirmed by the satisfactory correlation between the two parameters in the samples analyzed (correlation coefficient of 0.88).

Among the anions analyzed, chloride and sulfate were found in all sludge samples with similar high concentration values, in the range from 117 to 225 mg/L for chloride and from 123 to 262 mg/L for sulfate (see table 3.4). On the contrary, nitrate ion was not detected in the majority of the sludge leachates, with the exception of the SL 2 sample, in which its concentration was 16 mg/L.

Table 3.4 Concentrations of anions in sludge leachates (mg/L)

Sample	Cl^-	NO_3^-	SO_4^{2-}
SL 1	203.3 (0.4)	nd	262 (24)
SL 2	225 (16)	16 (1)	175 (21)
SL 3	150 (15)	nd	192 (20)
SL 4	117 (4)	nd	123 (1)
SL 5	134 (3)	nd	224 (2)
SL 6	146 (22)	nd	141 (25)

The results are summarized as the mean values (standard deviation); n = 2
nd – not detected

The concentrations of major elements in the analyzed sludge leachates were also determined, as summarized in table 3.5.

Table 3.5 Concentrations of major metals in sludge leachates (mg/kg)

Sample	Ca	Fe	Mg	K	Na
SL 1	2004 (168)	100 (13)	484 (37)	365 (43)	2478 (57)
SL 2	2166 (51)	42 (11)	2102 (23)	3353 (32)	3295 (92)
SL 3	1235 (120)	53 (12)	765 (77)	952 (49)	2212 (187)
SL 4	2004 (168)	100 (13)	484 (37)	365 (43)	1576 (149)
SL 5	966 (125)	27 (12)	512 (14)	876 (25)	1451 (38)
SL 6	1631 (102)	29 (11)	549 (14)	447 (15)	2094 (20)

The results are summarized as the mean values (standard deviation); n = 2

Based on the results obtained and initial concentrations of the major metals in dried sludge samples (table 2.1) the percentages of their lixiviation in the obtained sludge leachates can be calculated (table 3.6).

Table 3.6 Leaching rates of major metals in sludge leachates (%)

Sample	Ca	Fe	K	Mg
SL 1	3.61 (0.09)	0.09 (0.01)	86.9 (43)	16.1 (0.2)
SL 2	9.8 (0.2)	0.7 (0.1)	66.8 (32)	35.1 (0.4)
SL 3	2.9 (0.3)	0.34 (0.08)	32 (2)	11 (1)
SL 4	4.98 (0.09)	0.6 (0.3)	12 (2)	8 (2)
SL 5	2.1 (0.3)	0.3 (0.1)	29.1 (10.8)	7.5 (0.2)

The results are summarized as the mean values (standard deviation); n = 2

For Fe leaching rate was less than 1 %, but for Ca and Mg leaching rates were higher, in the range from 2 to 35 %. Leaching rates for K were the highest, i.e. up to 87 % for SL 1. Reasonably, those sludge samples with the lowest pH, SL 1 and SL 2 showed the highest mobility of the major elements analyzed.

In table 3.7 heavy metal concentration levels determined in sludge leachates are presented. As can be seen, Cd was the heavy metal with the lowest concentration in the sludge leachates. Actually, its concentration was over detection limit only in SL 6 which came from a highly contaminated locality.

Table 3.7 Concentrations of heavy metals in sludge leachates (mg/kg)

Sample	Cd	Cr	Cu	Ni	Pb	Zn
SL 1	nd	0.51 (0.09)	73 (14)	4.7 (0.2)	0.1 (-)	5.7 (0.6)
SL 2	nd	1.02 (0.04)	4.2 (1.8)	6.9 (0.1)	0.8 (0.3)	18.9 (0.5)
SL 3	nd	0.6 (0.1)	10 (2)	20 (1)	0.6 (-)	3.5 (0.1)
SL 4	nd	0.54 (0.06)	4.5 (0.6)	5.7 (0.3)	0.5 (-)	8 (2)
SL 5	nd	0.02 (0.01)	10 (7)	16.3 (0.5)	0.16 (0.01)	8 (5)
SL 6	0.02 (0.01)	0.41 (0.08)	19 (-)	7.6 (0.1)	0.3 (-)	7.2 (0.4)

The results are summarized as the mean values (standard deviation); n = 2
nd – not detected

Other heavy metals, such as Cr and Pb were also found to have low concentrations, up to approximately 1 mg/kg range. Finally, Ni, Zn and especially Cu, were present in the sludge leaching solutions in higher contents, ranging from 4.7 - 20, 3.5 - 18.9 and 4.2 - 73 mg/kg respectively.

Likewise for major elements, attention should be paid on the percentage of leachability of heavy metals (table 3.8) referred to the initial concentration in all sludge samples (table 2.2). For Cr, Pb and Zn the leaching rates were less than 3 %. Leaching rates were higher for Cu, i.e. up to 18 %. The highest leaching rates were obtained for Ni, in the range from 13.7 to 43.4 % which can suggest its presence in a form of a soluble chloride or carbonate in analyzed leachates.

Table 3.8 Leaching rates of heavy metals in sludge leachates (%)

Sample	Cd	Cr	Cu	Ni	Pb	Zn
SL 1	-	0.4 (0.1)	12 (2)	16 (1)	0.2 (-)	2.7 (0.3)
SL 2	-	1.97 (0.07)	0.8 (0.3)	43.4 (0.5)	1.2 (0.5)	1.56 (0.04)
SL 3	-	0.5 (0.1)	2.3 (0.5)	28.1 (-)	0.7 (-)	2.5 (0.1)
SL 4	-	0.39 (0.05)	0.9 (0.4)	21.8 (-)	0.6 (-)	4 (3)
SL 5	-	0.5 (0.3)	2 (1)	17.3 (0.5)	0.39 (0.03)	0.6 (0.4)
SL 6	1.2 (0.4)	0.25 (0.05)	18 (8)	13.7 (0.2)	0.2 (0.3)	0.53 (0.03)

The results are summarized as the mean values (standard deviation); n = 2

Although it was concluded that the sludge samples can be used in agricultural purposes (Fernández-Sanjuan et al., 2009) according to the limits of heavy metals proposed by EU Council Directive 86/278/ECC (EU, 1986), considerable Ni leaching rate can not be neglected concerning the contribution in environmental pollution of this heavy metal.

3.2.2 Leaching of APs and APEOs

The concentrations of OP, NP, $NPEO_1$ and $NPEO_2$ were determined in the sludge leachates and the results are shown in the table 3.9.

Table 3.9 Concentrations of OP, NP, $NPEO_1$ and $NPEO_2$ in sludge leachates (mg/kg)

Sludge	OP	NP	$NPEO_1$	$NPEO_2$
SL 1	0.01 (-)	0.05 (0.02)	nd	nd
SL 2	0.011 (0.004)	0.19 (0.04)	0.37 (0.02)	nd
SL 3	0.25 (0.02)	0.20 (0.01)	0.11 (0.02)	nd
SL 4	0.015 (0.001)	0.54 (0.03)	0.18 (0.02)	nd
SL 5	0.010 (0.001)	1.4 (0.1)	0.59 (0.03)	nd
SL 6	0.06 (0.01)	7 (2)	1.8 (0.4)	1.5 (0.3)

The results are summarized as the mean values (standard deviation); n = 2
nd – not detected

From the total concentrations of OP, NP, $NPEO_1$ and $NPEO_2$ in the sludge samples (Fernández-Sanjuan et al., 2009, Lecina, 2006), the percentages of the lixiviation of those compounds were calculated and summarized in table 3.10.

Table 3.10 Leaching rates of OP, NP, $NPEO_1$ and $NPEO_2$ in sludge leachates (%)

Sludge	OP	NP	$NPEO_1$	$NPEO_2$
SL 1	0.84 (0.09)	1.3 (0.5)	-	-
SL 2	1.7 (0.6)	1.8 (0.5)	2.8 (0.2)	-
SL 3	1.7 (0.1)	0.87 (0.03)	0.9 (0.1)	-
SL 4	0.89 (0.03)	0.86 (0.04)	1.0 (0.1)	-
SL 5	0.55 (0.02)	0.73 (0.04)	1.1 (0.1)	-
SL 6	0.4 (0.1)	0.4 (0.1)	0.32 (0.07)	1.4 (0.2)

The results are summarized as the mean values (standard deviation); n = 2

Target compounds were leached from the sludge samples with rates lower than 3 % and with reasonably low standard deviations. For all compounds and sludge samples, the percentages ranged from the lowest values in the SL 6 to the highest leaching percentages in the SL 2 sample. Relatively low values obtained for lixiviation can be explained by the fact that the sludge samples were dried at 40 °C before analysis (section 2.2.1). It is possible that for the sewage sludge samples non treated or treated in different conditions (sludges kept at room temperature), the percentage of lixiviation could be higher. This would be the case of applying the sewage sludge directly to the soil without any drying treatment.

The results obtained for OP and NP lixiviation show that the highest percentage of extraction is found in sludge sample SL 2, 1.7 and 1.8 % respectively. The high leaching rate found for $NPEO_1$ in the same sample confirmed that in this sample the target compounds were more easily leachable. This can be explained by the fact that this sludge has the lowest pH value and the highest content of DOC (see table 3.3).

On the contrary, the sludge with the most basic pH value, SL 6 (pH 7.4) showed the lowest leaching percentages for OP, NP and $NPEO_1$ compounds. Moreover, the SL 6 was the single sample in which the leaching percentage of $NPEO_2$ could be determined, since $NPEO_2$ was only found in this sewage sludge (Fernández-Sanjuan et al., 2009).

By applying regression analysis, it was estimated that the correlation between NP and $NPEO_1$ leaching from the sludge samples and DOC content, was high with squared relation coefficients equal to 0.92 and 0.91, respectively. This suggests that DOC is an important factor influencing leaching processes. In addition, if pH is also considered, the relationship became slightly better ($R^2 = 0.93$) for both target compounds, which confirmed that two soil parameters, DOC content and pH, explained better variation of the leaching processes.

If the results of APs and APEOs leaching rates are compared with major (table 3.6) and trace element leachabilities (table 3.8) in the same sludge samples, it can be noticed that leachate of SL 2 sample was the most contaminated with respect to Ni, Cr and Pb concentration, whereas SL

6 had the minimum level of those metals among all other sludge samples. This may indicate potential correlations between inorganic and organic contaminants in the analyzed samples.

It should be stressed that there are no available literature data about leachability of these compounds from the sewage sludge. In that sense, these preliminary results obtained can give useful information about the contamination of sludge leachates and eventually their impact on soil contamination when used in agricultural practice. Hence, these preliminary results of APs and APEOs leaching rates found in sludge leachates, followed by the known information of the sludge doses applied, can be used for an estimation of the organic pollutants incorporated into the agricultural soils.

3.3 Sorption and desorption of NP and NPEO$_1$ in the soils

3.3.1 Characterization of soil equilibrium solutions

The contact solutions derived from sorption experiments (0.01 mol/L $CaCl_2$ – 0.05 % NaN_3 solutions, after equilibration with the soil samples) were characterized by three parameters: pH, DOC and heavy metal contents, which are considered to have a potential impact on the interaction between soils and target alkylphenolic compounds.

The results of pH show that Ascó and Delta 2 soils are weakly basic, whereas Oviedo 1 soil is acidic with pH 4.8 (table 3.11). The highest DOC content among the three soil samples was found in the Oviedo 1 soil (7.2 %). The Delta 2 soil had also a high DOC content (4.6 %), while Ascó soil showed a very low percentage of DOC (0.19 %).

Table 3.11 Values of pH and DOC in the soil equilibrium solutions

Sample	pH	DOC (%)
Ascó	7.3 (0.1)	0.19 (0.01)
Delta 2	7.7 (0.1)	4.6 (0.1)
Oviedo 1	4.8 (0.1)	7.2 (1.3)

The results are summarized as the mean values (standard deviation); n = 2

The heavy metal content in the solutions was also determined and presented in table 3.12.

Table 3.12 Concentrations of heavy metals in soil solutions (mg/kg)

Sample	Cd	Cr	Cu	Ni	Pb	Zn
Ascó	nd	0.08 (0.02)	0.07 (0.01)	0.06 (0.01)	nd	0.46 (0.04)
Delta 2	nd	0.08 (0.03)	0.13 (0.01)	0.06 (0.02)	nd	0.35 (0.05)
Oviedo 1	0.06 (0.01)	0.15 (0.01)	3.7 (0.2)	0.21 (0.03)	nd	14.1 (0.3)

The results are summarized as the mean values (standard deviation); n = 2
nd – not detected

As the data on total concentration of heavy metals in the soils were not available, only total concentration of heavy metals found in the soil equilibrium solutions was used for characterization. Pb was not found in the soil solutions and Cd was found only in Oviedo 1 soil sample. Actually, the equilibrium solution with the highest concentrations of heavy metals was that obtained with Oviedo 1 soil. The concentrations for Cr and Ni were in the range from 0.08 to 0.15 and 0.06 to 0.21 mg/kg, respectively. Similar results were obtained for Cu in Ascó and Delta 2 soil solutions, whereas its concentration in Oviedo 1 was found to be 3.7 mg/kg. In all soil solutions, Zn was found in the highest concentrations with respect to other trace elements analyzed.

3.3.2 Sorption of NP and NPEO$_1$ in the soils

3.3.2.1 Sorption isotherms

Sorption of NP and NPEO$_1$ was investigated in Ascó, Delta 2 and Oviedo 1 soil samples, under batch equilibrium conditions. Sorption isotherms of the two analytes were obtained for the three soils investigated by varying the initial concentration of the analytes.

The most common parameter for studying the sorption behavior of compounds is the solid-liquid distribution coefficient, K_d. In order to calculate $K_{d,}$ the concentrations of the target compound sorbed to soil and in the equilibrium solution must be known:

$$C_{sor} = (C_{in} - C_{eq})\ V_{sol} / w_s$$

C_{sor} is the concentration of target analyte (NP or NPEO$_1$) sorbed in the solid phase (mg/kg);

C_{in} is the initial concentration of NP or NPEO$_1$ in the equilibrium solution (mg/L);

C_{eq} is the concentration of NP or NPEO$_1$ in the equilibrium solution at the end of sorption experiment (mg/L);

V_{sol} is the volume of solution (mL);

w_s is the mass of the soil sample (g).

An equilibrium distribution coefficient, K_d can be calculated by using the ratio of NP or NPEO$_1$ found in the liquid and solid phase:

$$K_d = C_{sor} / C_{eq}$$

Tables 3.13 and 3.14 show the concentrations of NP and NPEO$_1$ in the liquid and solid phases of the three soils investigated. For NP in Ascó sample, the equilibrium concentrations in the liquid and the solid phases were positively correlated with the initial concentration of NP added to soil. For Delta 2 and Oviedo 1 soil samples, the linearity between initial and equilibrium NP concentrations was only observed for the solid phase. Similarly, the concentration of the sorbed

$NPEO_1$ increased with an increasing of initial concentration of $NPEO_1$ used for sorption experiments of the three soils studied.

Table 3.13 Concentrations of NP determined in the liquid and solid phase after the sorption experiments

C_{in} of NP (mg/L)	C_{eq} (µg/mL)			C_{sor} (µg/g)		
	Ascó	Delta 2	Oviedo 1	Ascó	Delta 2	Oviedo 1
0.02	na	0.0003	0.004	na	0.17	0.14
0.03	0.03	0.07	0.0003	0.02	na	0.33
0.07	na	0.002	0.0008	na	0.69	0.67
0.17	0.17	0.002	0.005	0.10	1.63	1.63
0.33	0.35	0.012	0.002	0.24	3.28	3.35

na – not analyzed

Table 3.14 Concentrations of $NPEO_1$ found in liquid and solid phase after the sorption experiments

C_i of $NPEO_1$ (mg/L)	C_{eq} (µg/mL)			C_{sor} (µg/g)		
	Ascó	Delta 2	Oviedo 1	Ascó	Delta 2	Oviedo 1
0.03	0.01	0.001	0.0008	0.25	0.30	0.32
0.07	0.90	0.002	0.024	na	0.64	0.46
0.17	0.10	0.01	0.006	0.66	1.54	1.60
0.33	1.62	0.01	0.008	na	3.23	3.23
0.65	0.23	0.01	0.006	4.25	6.41	6.52

na – not analyzed

From the C_{in}, C_{sor} and C_{eq} data it was possible to determine the percentages of analyte sorbed in the solid phase, as well as the K_d of the two analytes in the three soils investigated. Results are summarized in tables 3.15 and 3.16.

In Ascó sample, which was the soil with the lowest organic C content (table 2.3), after addition of NP at increasing concentrations, the sorption percentage had the lowest values among the soils tested. The values ranged from approximately 6 to 8 % (table 3.15).

Table 3.15 Sorption of NP into the soils

C_i of NP (mg/L)	Sorbed NP (%)			K_d (mg/L)		
	Ascó	**Delta 2**	**Oviedo 1**	**Ascó**	**Delta 2**	**Oviedo 1**
0.02	na	98	na	na	608	na
0.03	5.8	na	99	0.7	na	1128
0.07	na	98	99	na	455	971
0.17	6.2	99	98	0.6	753	378
0.33	7.7	97	99	0.8	304	2424

na – not analyzed

Unlike Ascó, Delta 2 and Oviedo 1 soil samples showed almost a quantitative sorption of the NP. Namely, it was found that Delta 2 sorbed more than 97 % of NP added, for all the initial concentrations tested, and in the case of Oviedo 1 soil, the percentages were also high (over 98 %). The high percentages of NP sorption can be explained by the fact that the last two soils had the highest organic matter content and DOC, among the soil tested and thus a higher capacity to interact with nonylphenol.

As a very sensitive parameter, K_d was used to evaluate the relationship between the percentages of sorbed target compounds and their relative distribution in solid and liquid phases. K_d values obtained for Ascó soil sample ranging from 0.6 to 0.8 mg/L showed independence on initial concentration of NP. On the other hand, K_d constants for Delta 2 and Oviedo 1 samples were much higher, in the range from 304 to 753 and 378 to 2424 mg/L, respectively.

A similar pattern to that observed for NP was obtained for the monoethoxyl derivative of NP. Namely, after addition of $NPEO_1$ in the concentration ranges from 0.03 to 0.65 mg/L, more than

95 % of this compound was sorbed in Delta 2 and Oviedo 1 samples (table 3.16). However, for this analyte the sorption percentage in Ascó soil was much higher, since the results ranged from 43 - 73 %.

Table 3.16 Sorption of $NPEO_1$ into the soils

C_{in} of $NPEO_1$ (mg/L)	Sorbed $NPEO_1$ (%)			K_d (mg/L)		
	Ascó	Delta 2	Oviedo 1	Ascó	Delta 2	Oviedo 1
0.03	73	96	98	29	215	460
0.07	nd	97	na	na	368	na
0.17	43	95	97	7	173	323
0.33	nd	97	98	na	293	438
0.65	66	98	99	19	571	1229

na – not analyzed

K_d values for sorption of $NPEO_1$ in Ascó soil, were for 1 order of magnitude higher than those obtained for NP sorption. Similar to NP, the same conclusion for $NPEO_1$ sorption on Delta 2 and Oviedo 1 soils can be deduced that there is no relationship between the percentage of the sorbed target compounds and K_d values obtained.

At this stage it is difficult to explain sorption behavior of NP and $NPEO_1$ on the soils analyzed, but these preliminary results indicate that a role of the analyte in the sorption behaviour, besides the effects due to the soil matrix is important. At the pH in which the sorption experiments took place (from acidic to neutral), it is expected that both analytes would be present in undissociated (for example, pK_a of NP is about 10.3) and hence more hydrophobic form (Muller and Schlatter, 1998). Hence, the role of analytes will be further examined in the future experiments.

3.3.2.2 Fitting of the sorption isotherms

The data derived from the sorption isotherms may be attempted to be fitted to non linear models to permit a better comparison between the sorption pattern observed in the three soils. A simple model for this exercise is the Freundlich model, which relates the C_{sor} and C_{eq} through a non liner relationship, that could be linearized through the logarithmic equations derived from the model. Therefore, the Freundlich model can be defined by the following equation:

$$C_{sor} = K_F \; x \; C_{eq}{}^{n}$$

In this equation, K_F is Freundlich constant related to a weighted K_d for all the initial concentrations tested, whereas n refers to site heterogeneity.

By applying the logarithmic form of this equation:

$$logC_{sor} = logK_F + n \; logC_{eq}$$

it is easy to determine the K_F and n parameters (table 3.17).

Values of n Freundlich exponent, ranged from 0.8 to 1.1 for Ascó and Delta 2 soils. The squared correlation coefficients for NP and $NPEO_1$ in these two soils were higher than 0.95 and 0.81 respectively, and hence this approach fitted the experimental data relatively good. On the contrary, for Oviedo 1 soil, Freundlich fitting was not adequate good due to low parameters obtained, and the mean K_d value was used for evaluating the relations between soil properties and sorption behavior.

Table 3.17 Freundlich parameters for NP and $NPEO_1$ sorption on soil samples

Soil	R^2		n		K_F		mean K_d	
	NP	$NPEO_1$	NP	$NPEO_1$	NP	$NPEO_1$	NP	$NPEO_1$
Ascó	0.99	0.81	1.1	0.8	0.8	8	0.7	18
Delta 2	0.95	0.86	0.8	1.1	169	482	530	324
Oviedo 1	0.57	0.07	0.7	0.3	104	6	1225	612

Correlation analyses are here of a limited statistical justification, since only three soils were tested in the sorption experiments. However, it is of interest to examine the relationship between K_F and the mean K_d values and soil properties that potentially may affect NP and $NPEO_1$ soil sorption, basically the organic matter basically the organic matter of the soil expressed as organic carbon and the DOC content of the liquid phase in equilibrium with the soil during the sorption process.

By applying regression analysis, it was estimated that the correlation between the mean value of K_d constants for sorption of NP and $NPEO_1$ and DOC content, was very high ($R^2 > 0.97$). Furthermore, when using K_F, so called 'weighted' constant for $NPEO_1$ sorption to the soils, correlation was even higher, $R^2 = 0.99$. This suggests that DOC is an important factor in sorption mechanism, probably acting like a solvent for hydrophobic compounds, such as NP or $NPEO_1$.

Relatively good correlation between K_d and organic carbon was obtained for both analytes with R^2 greater than 0.86, which is good in comparison with those obtained by Düring et al., (2002).

3.3.2.3 Relationship between K_d and K_{OC} values

Sorption of NP or $NPEO_1$ can also be expressed by the organic carbon normalized sorption coefficient, K_{OC} which relates the distribution coefficient K_d with a content of organic carbon (OC) in the soil samples:

$$K_{OC} = K_d \ x \ 100 \ / \ \% \ OC$$

K_{OC} value characterizes distribution of mainly nonionic organic compounds, such as NP or $NPEO_1$ and due to the % OC normalization becomes independent on the soil type. In
table 3.18 K_{OC} values for NP and $NPEO_1$ for the three soils studied are presented.

Table 3.18 K_{OC} values for NP and $NPEO_1$ sorption on soil samples

Soil	**K_{OC}**		**log K_{OC}**	
	NP	**$NPEO_1$**	**NP**	**$NPEO_1$**
Ascó	350	9000	2.54	3.95
Delta 2	6883	4208	3.84	3.62
Oviedo 1	13032	6511	4.12	3.81

The K_{OC} values for NP and $NPEO_1$ ranged from 350 in Ascó soil to 13032 in Oviedo 1 and from 4208 in Delta 2 to 9000 mg/L in Ascó, respectively. This sorption test suggests that NP was less mobile than NP monoetoxylate in those soils with a higher content of organic carbon (the same pattern was obtained comparing K_d values). Similar conclusions concerning distribution of the two compounds of interest were obtained by the investigations of sorption on the aquatic suspended matter (Houet al, 2006).

The log K_{OC} values were in the narrow range from 2.5 to 4.2 for both compounds in all soils. Düring et al., (2002) and Navarro et al., (2009) obtained similar resulting K_{OC} values for sorption NP onto the solid matrices.

3.3.3 Desorption of NP and $NPEO_1$ from the soils

Likewise with the sorption study, desorption experiments were performed in parallel. An equilibrium distribution coefficient for desorption, K_{des} was calculated by using the ratio of NP or $NPEO_1$ found in the liquid and solid phase after performed desorption experiments:

$$K_{des} = C_{sor} / C_{eq}$$

The percentage of desorbed target compounds was calculated as the ratio between their concentrations found in equilibrium solution and in the solid phase, at the end of applied desorption experiment. The results for desorbed NP and $NPEO_1$ and appropriate K_{des} for the three soils investigated, are presented in tables 3.19 and 3.20.

Desorption of NP from Ascó soil was about 70% for two initial concentration tested (table 3.19). For Delta 2 and Oviedo 1, desorption of NP is much lower, i.e. less than 2% and 8% for Delta 2 and Oviedo 1, respectively.

Table 3.19 Desorption of NP in the soils

C_{in} of NP (mg/L)	Desorbed NP (%)			K_{des} (mg/L)		
	Ascó	Delta 2	Oviedo 1	Ascó	Delta 2	Oviedo 1
0.51	na	1.5	2	na	671	534
1.03	63	na	8	6	na	122
2.05	na	0.7	0.5	na	1471	1986
5.13	na	0.5	0.3	na	2099	3209
10.25	74	0.4	0.4	3	2589	2911

na – not analyzed

The mean values of K_{des} for NP desorption from Ascó, Delta 2 and Oviedo 1 are in increasing order, as follows: 4.5, 1707 and 1752 mg/L, respectively. If those values are correlated with the DOC content in the soils, a good correlation is obtained with R^2 equal to 0.88. Hence, as for sorption, the DOC content is an important parameter influencing desorption mechanism.

If desorption partition coefficients K_{des} for the three soils examined, are compared with the appropriate values of K_d (table 3.15), it can be noticed that K_{des} values are higher for the tested concentrations (with exception for the 1 mg/L of NP initial concentration, in Oviedo 1 soil), indicating slow desorption and hence moderate irreversibility of the compounds. This means that NP is expected to accumulate in the top soil layers.

Compared with the values of Düring et al. (2002) obtained for sorption of NP on a set of terrestrial soils, these K_{des} values obtained are much higher. These data indicate that not only an amount of organic matter but also the composition of soil is important factor affecting sorption.

Table 3.20 Desorption of $NPEO_1$ in the soils

C_{in} of $NPEO_1$ (mg/L)	Desorbed $NPEO_1$ (%)			K_{des} (mg/L)		
	Ascó	Delta 2	Oviedo 1	Ascó	Delta 2	Oviedo 1
0.99	25	2.3	2.8	32	426	361
1.99	na	1.8	2.1	na	555	452
4.97	49	1.1	5.3	10	836	194
9.93	na	1.1	1.3	na	965	802
19.86	23	0.9	1.3	33	1063	974

na – not analyzed

Desorption of $NPEO_1$ from Ascó soil is in the range from 23 to 49 % with no correlation with initial concentration of analyte (table 3.20). In Delta 2 and Oviedo 1 soil, desorption of $NPEO_1$ is less than 3 %, similar to NP desorption results obtained.

In this case, likewise for NP desorption, the correlation between DOC content and mean desorption partition coefficients can be established. The high correlation coefficient obtained (0.79) confirms that there is also an influence of DOC content in the soils on the $NPEO_1$ desorption process.

5. CONCLUSIONS

From the work carried out, the following conclusions can be drawn:

- Aqueous leachability of OP, NP, $NPEO_1$ and $NPEO_2$ from the sewage sludge samples was considerably low, less than 3%.

- Leachability was found to be dependent on pH and DOC. Thus, higher leachability was obtained in sewage sludges with low pH and high DOC.

- The low leachability of the target compound from the sewage sludge was attributed to the drying of the sample at 40ºC, which might contribute to an increase in the retention of the target compounds in the sludge. Therefore, it might be interesting to test the leachability in other sample conditions as wet sludge or sludge dried at room temperature.

- Sorption behavior of NP and $NPEO_1$ in soils is influenced not only by the properties of the target compounds but also by the soil characteristics. The good correlations obtained between K_d and DOC demonstrated that DOC is a key parameter affecting the sorption process.

- Sorption of NP and $NPEO_1$ was almost quantitative at the concentrations tested for those soils with a high content of organic matter.

- The preliminary results of APs and APEOs leachability obtained in this work together with the evaluation of their sorption and sorption reversibility in soils, may be useful in predicting contamination of soils amended with sewage sludge.

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