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Analysis of nonylphenol and nonylphenol ethoxylates in sewage sludge by high performance liquid chromatography following microwave-assisted extraction

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Abstract

Nonylphenol polyethoxylates (NPnEOs) constitute a significant portion of the non-ionic surfactant market. The presence of nonylphenol (NP) in the aquatic environment is often a product of the microbial breakdown of NPEOs through discharge of industrial effluents and sewage treatment plants. The aim of this work is to develop the microwave-assisted extraction for the determination of the NP and NPEO in sewage sludge and compare this method with more traditional methods such as Soxhlet extraction and sonication. The method efficiency was evaluated as to the linearity, repeatability, accuracy, and sensitivity. Recoveries were 61.4% for NPEO and 91.4% for NP with repeatability less than 5%. The detection limit was 1.82 μg/g for NPEO and 2.86 μg/g for NP. The developed method was applied on sewage sludge samples from the sewage treatment plants of three Greek cities: Athens, Patras and Heraklion and were ranged 12.8–233.5 mg/kg for NPEO and 3.6–93 mg/kg for NP.
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1. Introduction

Nonylphenol ethoxylates (NPEO) are common non-ionic surfactants (Fig. 1) and are still used in many applications. In particular, because of their favorable physicochemical characteristics. they have been used commercially as emulsifiers and solubilizers in pharmaceutical [1] and agrochemical [2] formulations, in cosmetics [3], as well as in various biotechnological processes [4]. Furthermore, polyethoxylated nonylphenols are used in the industrial production of cleaning products, textiles, petroleum, pulp and paper and pesticides formulation [5]. During biological wastewater treatment they are partially converted to more persistent and toxic metabolites (nonylphenol mono or di ethoxylates,

NPEO1,2 or nonylphenol NP) [6]. Additionally, NP is a raw material for the production of NPEO as well as other chemicals such as phosphate antioxidants, modified phenolic resins, additives to machine oils and metallurgical oils.

The toxic effect of NPEO metabolites has been attributed to the ability of these compounds to mimic natural hormones (estrogens) inducing endocrine disruption of aquatic organisms [7]. Since NPEOs and their metabolites are discharged to the environment directly or indirectly, through the wastewater treatment plant effluents, they have been detected in sediments and soil [8,9] and in aquatic organisms [10–12], raising a significant concern about the long-term impact of these compounds on human health.

In recent years, sewage sludge has been used as an organic fertilizer in agriculture. Current trends in waste management policies favor land applications (Council Directive 1999/31/EC). It has become clear that organic contaminants
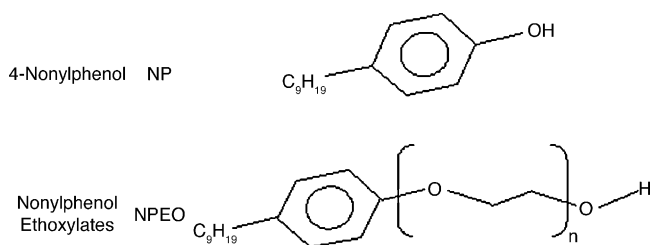
Fig. 1. Chemical structures and acronyms of the target compounds.

like NPEO and NP may accumulate in sludge, especially during anaerobic processing [13,14]. Therefore, it is important to develop methods, which allow routine measurements of sludge matrices. Usually, the nature and the amounts of organic contaminants in solid or semi-solid environmental samples, such as sludges, are determined by an exhaustive extraction of the target compounds into an appropriate solvent and subsequent analysis of the so-obtained extracts [15,16]. This approach generally involves the application of liquid (solvent, or gas) extraction techniques, such as Soxhlet extraction [17,18], sonication [19,20] microwave assisted extraction (MAE) [21,22], pressurized liquid extraction (PLE) [17,23–25] and supercritical fluid extraction (SFE) [15,26–28].

In spite of important drawbacks, Soxhlet extraction is still in routine use in many laboratories. The need for low-cost and less solvent- and time-consuming extraction techniques, amenable to automation, has generated significant research effort for the development of new techniques. As a result, Soxhlet extraction and steam-distillation, used almost exclusively in the 1980s and 1990s, have been partially replaced by more versatile sonicated extraction systems, and SFE, MAE and PLE.

Microwave-assisted extraction (MAE) is based on the non-ionising radiation that causes molecular motion by migration of ions and rotation of dipoles, without changing the molecular structure. This technique has several advantages [29]. One important advantage is that small volumes of solvents are needed for the extraction. Minimization of the required extraction time is another advantage of MAE; as microwave energy heats the samples and the solvents in a closed pressurized extraction vessel, the temperature can be rapidly increased, allowing the extraction of samples in minutes, in contrast with traditional methods, for which hours are needed.

Microwave-assisted extraction has been used for the determination of triazine and chloroacetanilide herbicides in soils [30,31]. There are a few publications reporting the application of MAE for the determination of pesticides in soils [32–35] and plant matrices [36–38], whereas there are only a few reporting the use of MAE for the determination of surfactants in solid samples, mainly soil and sediment [39–42]. Microwave assisted-extraction is a novel method recently proposed for the extraction of NP and NPEO. To our knowledge, MAE has not been used for the determination of nonylphenolic compounds in sewage sludge [39].

Sewage sludge is considered as one of the most difficult environmental matrices to extract specific compounds from, because it contains a large variety of contaminants. The main goal of this work was to develop and optimize the MAE method for the extraction of NP and NPEO from sewage sludge samples specifically. The method is quick and suitable for the routine determination of NP and NPEO in sludge samples. It is based on microwave-assisted extraction of NP and NPEO from sewage sludge, followed by HPLC coupled with fluorescence detector. The effect of important parameters, such as the type of solvent, temperature and pressure was investigated, in order to achieve the best performance of the method. The developed method was compared with other more traditional methods such as Soxhlet extraction and ultrasonication. Finally, it was applied for the determination of NP and NPEO concentrations in sewage sludge samples, originating from different sewage treatment plants in Greece.

2. Experimental

2.1. Reagents

Analytical standards of 4-nonylphenol (NP) were obtained from Fluka (74430) and of nonylphenol ethoxylates (NPEO$_x$, $x \sim 2$) from Aldrich (23,863-5). The standard of NPEO$_x$ was actually a mixture of homologues with different ethoxy units, which will be simply referred to further in the present work as NPEO. All organic solvents were of analytical or HPLC grade from Merck. Water was prepared on Milli-Q purification system (Millipore).

2.2. Sample collection and preparation

The sludge samples used for the development of the methods were from the sewage treatment plant of the city of Patras, Greece. The concentrated secondary sludge that was used for the development of the method was completely characterised, some of the most important characteristics are: TSS: 33.49 g/l, VSS: 25.6 g/l, d-COD: 5208 mg/l and T-COD: 36245 mg/l. The methods were also applied for four types of sludge: (I) primary, (II) secondary, (III) concentrated primary and (IV) concentrated secondary. Environmental samples were collected from the sewage treatment plants of three main Greek cities: Athens, Patras and Heraklion. The STP of Athens (4,000,000 inhabitants) is a new biological treatment plant, which treats daily 750,000 m^3 of sewage from the greater area of Athens and has a nominal daily capacity of 1,000,000 m^3. The STP in Patras, Western Greece district, serves a population of approx. 180,000 and receives domestic sewage from Patras and Ovria (a small municipality near Patras). The annual treated volume is 5.1 Mm^3 with an average influent BOD$_5$ of about 300 mgO$_2$ l^{-1}. Finally, the STP of Heraklion, Crete, Southern Greece district, is a secondary treatment plant (including nitrogen removal) with

chlorine disinfection of the effluent and anaerobic digestion of the sludge for the production of biogas. The STP of Heraklion has a daily capacity of 28,000 m^3 and treats mainly domestic discharges from the city of Heraklion (150,000 inhabitants).

The samples were conserved by the immediate addition of 1% formaldehyde and, when not immediately analysed, were stored in the dark at 4 °C. Prior to the analysis of the NP and NPEO, the samples were filtered (10 ml of aliquot were filtered via 0.7 μm) and dried in the oven at 35 °C, and the resulting solids were grinded. As the compounds of interest are highly hydrophobic, they are expected to be completely adsorbed on the solid matrix. Tests had proved that NPEO and NP are mainly present in the solid fraction and very little amounts are present in the liquid phase, where after SPE with HLB cartridges, the concentrations were below the detection limit.

2.3. Sample extraction

2.3.1. Microwave-assisted solvent extraction

A dried sample (0.03–0.3 g, depending on the type of sludge sample) was transferred to the PFA Teflon lined extraction vessel of 100 and 20 ml of solvent were added. Two different mixtures of solvents were tested: (i) hexane/acetone: 1/1 (v/v) and (ii) dichloromethane/methanol: 3/7 (v/v). The extractions were performed also at various conditions of temperature (100 and 120 °C) and power (600 and 1200 W). The extraction time was 17 min (ramp to 100 or 120 °C in 2 min and then hold at 100 or 120 °C for 15 min). After cooling, the extracts were concentrated to an approximate volume of 1 ml, using a rotary vacuum evaporator. In case a further clean-up was needed, the extract was redissolved in 100 ml of HPLC water and the clean-up procedure was followed, as described later. For our samples, no further clean-up was needed and thus the extract was redissolved in 10 ml acetonitrile and after filtration, 20 μl were used for the analysis in the HPLC-FD.

2.3.2. Ultrasonic extraction

Twenty millilitres of solvent mixture were added to dried sludge and were sonicated for 20 min. Two mixtures of solvents were used, a mixture of hexane/methanol: 1/1 and a mixture of dichloromethane/methanol: 3/7, according to Petrovic and Barcelo [20]. The extract was transferred to a flask, evaporated to an approximate volume of 1 ml, and redissolved in 10 ml of acetonitrile.

2.3.3. Soxhlet extraction

Two grams of dried sludge were transferred to a preextracted paper thimble. The thimbles were covered with preextracted cotton in order to prevent transfer of fine powder from the thimble into the extracting solvent. The samples were extracted in a Soxhlet apparatus with 100 mL of methanol for 18 h. The extracts were evaporated to 5 mL and then diluted to 50 mL with water and treated as described in the "Clean-up" section.

2.3.4. Clean-up

An SPE (solid phase extraction) method was used for clean-up and preconcentration of extracts, when this was needed. The sludge extracts were loaded to a Water Oasis HLB Vac RC 60 mg Extraction cartridge from Waters, which had previously been conditioned with methanol and HPLC water. The elution of the compounds was performed by adding 2 ml × 5 ml of acetonitrile.

2.4. Instrumentation

Microwave-assisted extraction was carried out using a microwave accelerated reaction system for extraction; model MARS 5 from CEM (North Carolina, USA). Although this model is able to extract 14 samples simultaneously in perfluoroalkoxy (PFA) copolymer resin Teflon-lined extraction vessels of 100 ml under the same conditions, the present work was performed with six samples being extracted at the same time. A standard Millipore filtration apparatus from Supelco was used for SPE extraction when needed. The sonication had been performed in a Vibracell 130 apparatus of Sonics & Materials, Inc. The HPLC system consisted of a Star 9010/9001 Solvent Delivery System from Varian connected with a variable wavelength UV–vis detector and a Prostar 363 Fluorescence detector, both from Varian. Chromatographic conditions were different for each group of compounds.

For the analysis of the NP and NPEO, the chromatographic separation was done using a reversed-phase XTerra® RP-18 analytical column of 250 mm × 4.6 mm and 5 μm particle diameter, preceded by a guard column (20 mm × 3.9 mm) of the same packing material, both from Waters. The mobile phase started with a 3 min isocratic step at 50% acetonitrile and 50% water, followed by a 17 min linear gradient to 66% acetonitrile and 34% water, and another 8 min isocratic step at the final mobile phase ratio. The detection was carried out using the fluorescence detector at λ_{ex}: 222 nm and at λ_{em}: 305 nm.

3. Results and discussion

3.1. Extraction methods

In order to determine the most appropriate method for the extraction and the analysis of nonylphenol and nonylphenol ethoxylates in sludge samples, several preliminary experiments were run.

For method selection, a concentrated secondary sludge sample, from the STP of Patras, was spiked with a mixture of the test compounds in known concentrations and was analyzed as described in Section 2. A blank (non-spiked) sample was subjected to the same extraction procedure with the spiked sample, in order to quantify the sludge background which was very low. The extracted amount of the test compounds was compared to the amount added in the sludge
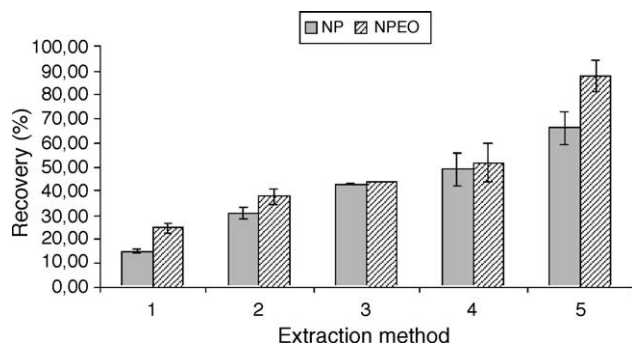
Fig. 2. Efficiency of different extraction methods: (1) SNC: hexane/acetone: 1/1, (2) SNC: DCM/MeOH: 3/7, (3) MAE: hexane/acetone: 1/1, (4) MAE: DCM/MeOH: 3/7, (5) Soxhlet: MeOH.

Fig. 3. Efficiency of the MAE with hexane/acetone: 1/1, on the extraction of the NP/NPEO at different operation conditions.

sample and parameters, such as recovery, method detection limit and repeatability were estimated.

Fig. 2 shows the average recovery percentages obtained from triplicate analysis with different extraction methods. The recovery for each analyte was calculated as the ratio of the measured concentration during the analysis of the sludge sample over the concentration obtained by direct chromatographic injection of equivalent amounts of the standard mixtures in acetonitrile, multiplied by 100.

As it can be seen, the methods showed different extraction efficiency. Sonication with hexane/acetone: 1/1 had the lowest extraction efficiency: 15.1% for the NP and 24.6% for the NPEO. Changing the extraction solvent to dichloromethane/methanol: 3/7, according to Petrovic and Barcelo [20], the extraction efficiency was almost doubled (NP: 30.7% and NPEO: 37.7%).

Higher recoveries were obtained by MAE. Using hexane/acetone: 1/1 as the extraction solvent, the recoveries were 42.8 and 44.1% for the NP and NPEO, respectively. The change of the solvent to DCM/MeOH: 3/7 had no significant effect on the recovery of the NPEO (48.9%), but increased dramatically the recovery of the NP (167.9%). The power selected was 1200 W and the temperature was 120 °C.

During this study, Soxhlet extraction was also tested, as it is a standard method for analysis of solid samples, like sediments and sludge. The recoveries obtained for NP and NPEO by Soxhlet were 66.2 and 87.7%, respectively. These recoveries are comparable with those reported by other authors, e.g. Shang et al. 65–93% [43] and Croce et al. 79–82% [40].

3.2. Optimization of MAE

Several experiments were run, in order to determine the optimal conditions of MAE that result in high recovery of the analytes from sludge, with the least requirements in power and temperature.

Two different extraction mixture solvents (DCM/MeOH: 3/7 and hexane/acetone: 1/1) were tested under various operation conditions. Fig. 3 shows the recovery percentages obtained by MAE using hexane/acetone: 1/1 as the extraction solvent at different extraction powers and temperatures.
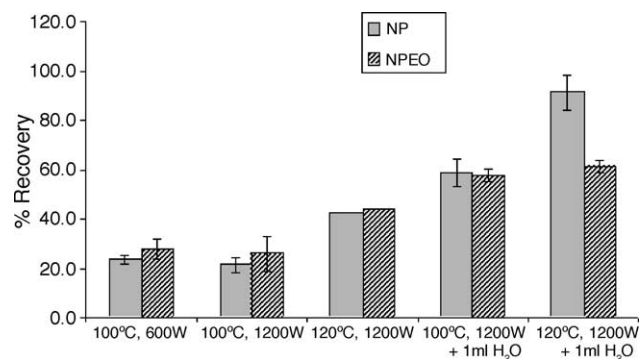
The increase in extraction power from 600 to 1200 W, at 100 °C, did not have a significant effect on the recoveries of the target compounds, which ranged between 20 and 28%. In contrast, increasing the extraction temperature, at 1200 W, increased the recoveries for both NP and NPEO. The recoveries at 100 °C were 21.5 and 26.2% for the NP and NPEO, respectively, and they almost doubled when the temperature was increased to 120 °C (NP: 42.8%, NPEO: 44.1%).

It was also noted during this study, that whenever the sludge was not dried before the extraction, the recoveries of the MAE at 1200 W and 100 °C were much higher (NP: 67%, NPEO: 62%). This is consistent with other studies that also verified the extraction power of water when present at traces in medium polar solvents [44–46]. Therefore, 1 ml of water was added to the dried sample and the extraction efficiency was tested at 100 and 120 °C, and at 1200 W extraction power. As seen in Fig. 3, higher extraction recoveries were obtained at both 100 and 120 °C compared with the ones without the water addition. The recoveries of both NP and NPEO were almost 60%, instead of 21.5 and 26.2%, respectively, when determined without water addition at an extraction temperature of 100 °C. The results for the extraction at 120 °C and 1200 W with the addition of 1 ml of water were similar. The recovery for NP was 91.4% instead of 42.8% without water and the recovery of NPEO increased to from 44.1 to 61.4%, respectively.

Similar experiments were performed using a mixture of DCM/MeOH: 3/7 as extraction solvent. Fig. 4 shows the recovery percentages obtained for NP and NPEO in three different operating conditions. The poorest results were obtained when the temperature was set at 100 °C and the power was at 600 W, with recoveries for both NP and NPEO of about 50%. Increasing the extraction temperature to 120 °C and the extraction power to 1200 W did not lead to a significant change in the efficiency of the extraction of NPEO (48.9%), but increased dramatically the recovery percentage of NP (168%), a number exceeding 100% attributed probably to the co-extraction of other compounds, contained in the sludge. This dramatic increase in recovery can probably be attributed to the enhanced extraction efficiency of the selected solvent, exhibited at high temperature and extraction power.
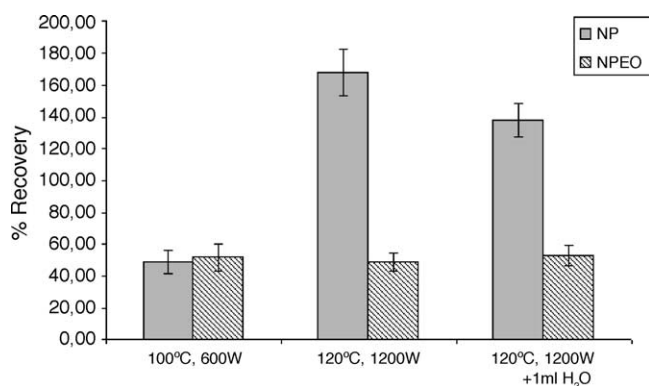
Fig. 4. Efficiency of the MAE with DCM/MeOH: 3/7, on the extraction of the NP/NPEO at different operation conditions.

The addition of 1 ml of water under the same conditions did not have an important effect on the efficiency of extraction (NP: 138%, NPEO: 53%) and thus this mixture of solvents for the MAE was rejected, as it was shown that the extraction was too strong and it was not possible to achieve a satisfactory separation of NP from the other compounds.

As it is apparent from the above discussion, the best results were achieved using hexane/acetone: 1/1 as the extraction solvent with the addition of 1 ml of water to the dried sample at 120 °C and 1200 W.

3.3. Method performance

The performance of the method was evaluated through the estimation of the linearity, repeatability and sensitivity, according to Standard Methods [47]. For quantification, five-point calibration curves were constructed, using a least-square linear regression analysis, from the HPLC-FD analysis of standard solutions of NP/NPEO in acetonitrile at concentrations ranging from 0.1 to 5 μg/ml. The calibration curves were linear with correlation coefficients (R^2) higher than 0.999 for both target compounds (see Table 1).

The overall method repeatability, calculated as the relative standard deviation (RSD) of the replicate ($n = 6$) analysis of secondary sludge spiked with a standard mixture of the analytes at 13.7 μg/g d.w., was satisfactory, with RSD values of 4.69 for the NP and 3.62 for the NPEO, respectively (Table 1).

Although for most samples, no further clean-up was needed, the recovery of SPE was determined for the cases

we had to use it. Two different volumes of extraction solvent were tested 5 and 10 ml. In both extraction volumes the recoveries were above 70% for both NP and NPEO. Slightly higher were the recoveries with 10 ml of extraction solvent, about 90%.

The limits of detection (LOD) and quantification (LOQ) of the method were experimentally estimated from the analysis of secondary sludge at the minimum concentration of each analyte, giving a signal to noise ratio of 3 and 8, respectively. As shown in Table 1, the LODs obtained for NP and NPEO were 2.86 and 1.82 μg/g d.w., respectively, using an injection volume of 20 μl. In case a bigger loop (during this study we could inject up to 100 μl) was used, these concentrations could be much lower. The LOQs, which were approximately 2.7 times higher than the corresponding LODs, were determined 7.72 and 4.90 μg/g d.w. for NP and NPEO, respectively. Although the minimum concentrations that can be detected are relatively high, compared with the ones reported using different methods, during this study the concentrations determined were rather high, and so we did not try to improve detection limits further. It should also be noted that the LODs determined in the water samples were 55 and 35 μg/l for NP and NPEO respectively.

3.4. Environmental samples

The developed analytical method was used to monitor the presence of NP and NPEO in sludge samples collected from different sewage treatment plants and to evaluate the fate of these compounds at different stages in a particular sewage treatment plant, that of the city of Patras, in order to evaluate the removal efficiency of the treatment applied.

Table 2 lists the concentrations of NP and NPEO obtained in this study at the different sewage treatment plants and the characteristics of each STP. The use of NPEO in the Greek household detergent formulation is evident, despite the fact that its use has been discontinued in many countries, because of the toxicity of their biodegradation products [48], The highest concentrations were determined in the primary sludge from the STP of Heraklion (233.5 mg/kg) and the lowest in the secondary sludge at the STP of Patras (12.8 mg/kg).

Nonylphenol, the main degradation product of NPEO, was detected in all samples at concentrations ranging from 3.6 mg/kg in the secondary sludge from Patras to 93 mg/kg in the primary sludge from Heraklion. As it is well known, the concentration level of these xenobiotics in the sludge depends on various factors, such as the discharge of industrial wastewaters in the STP influent, the population size of the area being served by the STP, and the type of sludge, i.e. whether it is primary, secondary, mixed, composted or anaerobically digested. It was no surprise that the highest concentrations were detected in the primary sludge, as this sludge contains particulate organics that serve well as a matrix for retention of adsorbed xenobiotics, especially the most hydrophobic ones.

Another observation was the higher concentrations of both NP and NPEO measured in the sludge from the STP of Patras

Table 1

Linearity of the calibration curves (R^2), repeatability as relative standard deviations (RSD), and limits of detection (LOD) and quantification (LOQ) characterizing the MAE-HPLC-FD analysis of the target compounds in sludge

	R^2	Repeatability (%RSD)	LOD (μg/g d.w.)	LOQ (μg/g d.w.)	LOD (μg/l)
NP	0.99	4.69	2.86	7.72	55
NPEO	0.99	3.62	1.82	4.90	35

Table 2
Characterization of sludges from different STPs and the levels of the NP/NPEO determined on these samples

Parameter (type of sludge)	Athens (07/2004) (concentrated primary)	Heraklion (07/2004) (primary)	Patras (02/2004) (concentrated secondary)	Patras (07/2004) (concentrated secondary)
TSS (g/l)	53.8	24.1	39.6	39.1
TS (g/l)	55.8	30.0	44.3	46.6
VSS (g/l)	33.3	15.7	26.9	24.7
VS (g/l)	34.4	19.5	30.1	31
d-COD (mg/l)	875.3	1290	980	1100
Total-COD (g/l)	30.9	34.1	34.8	35.5
Alkalinity (mgCaCO3/l)	1750	1875	4500	5062
pH	6.32	6.68	6.8	6.83
NP (mg/kg)	27.6	93	59	3.6
NPEO (mg/kg)	90.5	233.5	45	12.8

during winter, compared with the ones determined during the summer of the same year (last column of Table 2). This could indicate a seasonal variation on the profile of the concentrations of the nonylphenolic compounds, although further monitoring is needed in order to come to a confident conclusion.

Although there are numerous reviews and research papers that have reported concentrations of nonylphenol and nonylphenol ethoxylates, this information concerns the environmental fate of these compounds mostly in water, sediments or soil and little is known about their occurrence in sludge. Additionally, as the presence of the NP/NPEO depends on various factors, as mentioned above, and on many occasions these factors are not reported, the comparison of the results becomes a rather difficult if not impossible undertaking.

Generally, the measured concentrations were relatively high, but comparable with some that have been reported previously. Petrovic and Barcelo [20] determined NP and NPEO in dehydrated sludge from various STPs at the region of Catalunya, in Spain (NP: 172–601 mg/kg, NPEO: 21–135 mg/kg), at Porto, Portugal (NP: 234 mg/kg, NPEO: 2.1 mg/kg) and at Dresden, Germany (NP: 25.5 mg/kg, NPEO: 21 mg/kg). De Voogt et al. [49] determined NP levels of: 6.822 μg/g and NPEO: 40.16 μg/g in a sludge sample from an industrial wastewater treatment plant in the Netherlands, which are relatively low, compared with the ones reported by Petrovic and Barcelo and the concentrations determined during this work. Another example is from Bjergmarken STP, a plant for Roskilde (Denmark), where the concentrations of the NP and NPEO$_{1-2}$ were 0.675 and 23.723 mg/kg, respectively [50]. The occurrence of the nonylphenolic compounds in Southern Europe at higher concentrations compared with the ones reported in Northern Europe is in agreement with the fact that, in Northern Europe, the use of NP and NPEO was forbidden several years ago.

During this work, the fate of NP and NPEO at different treatment stages within the same plant was also studied. The profile followed by the measured concentrations along the plant is illustrated in Table 3. A slight decrease was observed for both NP and NPEO from the influent of the STP to the

Table 3
The effect of the treatment on the presence of the NP/NPEO, in the STP of Patras

	NP (mg/l)	NPEO (mg/l)
Influent of STP	0.56	0.074
Influent to activated sludge	0.44	0.057
Concentrated activated sludge (mg/kg TS)	1.28	2.69
Dehydrated sludge	<LOD	<LOD
Effluent of STP	<LOD	<LOD

<LOD: below the method detection limit.

influent to the secondary treatment stage (activated sludge). A significant decrease in the concentrations was observed during the aerobic treatment; the concentration of NP from 476 mg/kg TS (corresponded to 0.44 mg/l) to 1.28 mg/kg TS and the concentration of NPEO from 61.5 mg/kg TS (corresponded to 0.06 mg/l) to 2.7 mg/kg TS, leading to an almost 100% removal of these compounds in the concentrated secondary sludge. This is in agreement with previous studies [51,52], where it was reported that both NP and NPEO are degraded under aerobic conditions. Finally, at the dehydrated sludge as well as in the STP effluent, the concentration of both NP and NPEO was below the detection limit of the method.

4. Conclusions

Microwave assisted extraction (MAE) extraction for determination of NP and NPEO in sewage sludge was developed and optimized. Various conditions were tested in order to obtain the maximum sensitivity and selectivity. This study allowed optimization of the extraction conditions: hexane/acetone: 1/1 as extraction solvent with the addition of 1 ml of water to the dried sample at 120 °C and 1200 W. The developed method provides good performance in terms of precision, linearity, LODs and LOQs.

The comparative study with Soxhlet and sonication methods demonstrated that MAE is a suitable alternative extraction method for NP and NPEO determination in sewage sludge.

The method was applied on real sewage sludge from different STPs in Greece and also was used to study the fate

of NP and NPEO in the various stages of the wastewater treatment in the STP of Patras. The analysis of sludge from the different STPs showed that the concentrations of NP and NPEO ranged between 12.8 and 233.5 mg/kg for NPEO and between 3.6 and 93 mg/kg for NP. The study for the fate of NP and NPEO in the STPs indicates aerobic biodegradation of these compounds.

Acknowledgments

References

[1] M.J. Lawrence, Eur. J. Drug Metab. Phamacokin. 19 (1994) 257.
[2] C. Reich, C.R. Robbins, J. Soc. Cosmet. Chem. 44 (1993) 263.
[3] J.J. Bolling, J.R. Seiler, S.M. Zedaker, J.W. Thompson, D. Lucero, Can. J. Forest Res. 25 (1995) 425.
[4] C.E. Forney, C.E. Glatz, Biotechnol. Prog. 11 (1995) 260.
[5] D.T. Bennie, C.A. Sullivan, H.B. Lee, J. Maguire, J. Water Qual. Res. J. Can. 33 (1998) 231.
[6] W. Giger, P. Brunner, C. Schaffner, Science 225 (1984) 623.
[7] E.J. Routledge, D. Sheahan, C. Desbrow, G.C. Brighty, M. Waldock, J.P. Sumpter, Environ. Sci. Technol. 32 (1998) 1559.
[8] G.G. Ying, B. Williams, R. Kookana, Environ. Int. 28 (2002) 215.
[9] A. Marcomini, G. Pojana, A. Sfriso, J.M.Q. Alonso, Environ. Toxicol. Chem. 19 (8) (2000) 2000.
[10] M.H.I. Comber, T.D. Williams, K.M. Stewart, Water Res. 27 (2) (1993) 273.
[11] A. Arukwe, A. Goksoyr, R. Thibaut, J.P. Cravedi, Marine Environ. Res. 50 (2000) 141.
[12] M. Ahel, J. McEvoy, W. Giger, Environ. Pollut. 79 (1993) 243.
[13] W. Giger, M. Ahel, M. Koch, H.U. Laubscher, C. Schaffner, J. Schneider, Water Sci. Technol. 19 (1987) 449.
[14] M. Ahel, W. Giger, M. Koch, Water Res. 28 (1994) 1131.
[15] H.-B. Lee, Wat. Qual. Res. J. Can. 34 (1999) 33.
[16] A. Marcomini, M. Zanette, J. Chromatogr. A 733 (1996) 193.
[17] A. Marcomini, W. Giger, Anal. Chem. 59 (1987) 1709.
[18] R.C. Hale, C.L. Smith, P.O. de Fur, E. Harvey, E.O. Bush, M.J. La Guardia, G.G. Vadas, Environ. Toxicol. Chem. 19 (2000) 946.
[19] M. Castillo, E. Martinez, A. Ginebreda, L. Tirapu, D. Barcelo, Analyst 125 (2000) 1733.
[20] M. Petrovic, D. Barceló, Anal. Chem. 72 (2000) 4560.
[21] K.K. Chee, M.K. Wong, H.K. Lee, Anal. Chim. Acta 330 (1996) 217.
[22] K.K. Chee, M.K. Wong, H.K. Lee, J. Liq. Chromatogr. Relat. Technol. 19 (1996) 259.
[23] S. Valsecchi, S. Polesello, S. Cavalli, J. Chromatogr. A 925 (2001) 297.
[24] W.-H. Ding, J.C.H. Fann, J. Chromatogr. A 866 (2000) 79.
[25] M. Petrovic, S. Lacorte, P. Viana, D. Barceló, J. Chromatogr. A 959 (2002) 15.
[26] R.J.W. Meesters, H.F. Schroder, Anal. Chem. 74 (2002) 3566.
[27] H.-B. Lee, T.E. Peart, D.T. Bennie, R.J. Maguire, J. Chromatogr. A 785 (1997) 385.
[28] J.-G. Lin, R. Arunkumar, C.-H. Liu, J. Chromatogr. A 840 (1999) 71.
[29] J.L. Luque-Garcia, M.D. Luque de Castro, Trends Anal. Chem. 22 (2003) 90.
[30] C. Molins, E.A. Hogendoorn, H.A.G. Heusinkveld, D.C. van Harten, P. van Zoonen, R.A. Baumann, Chromatographia 43 (1996) 527.
[31] Z. Vryzas, E.N. Papadakis, E. Papadopoulou-Mourkidou, J. Agric. Food. Chem. 50 (2002) 5026.
[32] C. Molins, E.A. Hogendoorn, H.A.G. Heusinkveld, D.C. van Harten, P. van Zoonen, R.A. Baumann, Chromatographia 43 (1996) 527.
[33] S.J. Stout, A.R. daCunha, D.G. Allardice, Anal. Chem. 68 (1996) 653.
[34] I. Silgoner, R. Krska, O. Gans, E. Rosenberg, M. Grassebauer, Fresenius J. Anal. Chem. 362 (1998) 120.
[35] O. Zuloaga, N. Etxebarria, L.A. Fernadez, J.M. Madariaga, Fresenius J. Anal. Chem. 367 (2000) 733.
[36] S.J. Stout, A.R. daCunha, G.L. Picard, M.M.J. Sfarpour, J. Agric. Food. Chem. 44 (1996) 3548.
[37] A. Bouaid, A. Martin-Esteban, P. Fernadez, C. Camara, Fresenius J. Anal. Chem. 367 (2000) 291.
[38] Z. Vryzas, E.N. Papadakis, E. Papadopoulou-Mourkidou, J. Agric. Food. Chem. 50 (2002) 2220.
[39] S. Heise, N. Litz, Extraktion von Tensiden aus Festmatrizes Tenside Surf. Det. 36 (3) (1999) 185.
[40] V. Croce, L. Patrolecco, S. Rolesello, S. Valscecchi, Chromatographia 58 (2003) 145.
[41] V. Croce, S. Paggio, A. Pagnoni, S. Rolesello, S. Valscecchi, CPS: envchem/0109001, 2001.
[42] R. Liu, J.L. Zhou, A. Wilding, J. Chromatogr. A 1038 (2004) 19.
[43] D.Y. Shang, M.G. Ikonomou, R.W. Mcdonald, J. Chromatogr. A 849 (1999) 467.
[44] A. Pastor, E. Vasquez, R. Ciscar, M. de la Guardia, Anal. Chim. Acta 334 (1997) 241.
[45] C.S. Eskilsson, E. Bjorklud, J. Chromatogr. A 902 (2000) 227.
[46] V. Camel, Trends Anal. Chem. 16 (1997) 584.
[47] Standard Methods for Water and Wastewater, 1995.
[48] D.T. Bennie, C.A. Sullivan, H.B. Lee, J. Maguire, J. Sci. Total Environ. 193 (1997) 263.
[49] P. de Voogt, K. de Beer, F. Van de Wielen, Trends Anal. Chem. 16 (1997) 584.
[50] J. Vikelsoe, M. Thomsen, L. Carlsen, E. Johansen, National Environmental Research Institute, Denmark, NERI Technical Report No. 402, http://technical-reports.dmu.dk, 2002, p. 100.
[51] N. Jonkers, T.P. Knepper, P.De Voogt, Envirn. Sci. Technol. 35 (2001) 335.
[52] A. Corti, S. Frassinetti, G. Vallini, S. D'Antone, C. Fichi, R. Solaro, Environ. Pollut. 90 (1995) 83.