Accelerated Solvent Based Extraction And Enrichment Of Selected Plasticisers And 4-Nonylphenol, And Extraction Of Tin From Organotin Sources In Sediments, Sludges And Leachate Soils.

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Abstract

Enrichment techniques have become an important feature in the trace analysis of oestrogen mimicking chemicals in the environment. Recent developments such as accelerated solvent extraction (ASE) have improved extraction recoveries in a wide variety of solid matrices including sediments, sludges and leachate soils. Such samples taken from the Irish Midlands Shannon Catchment region during the winter of 2004/5 and suspected to contain certain xenooestrogens or hormonally active agents, were extracted using this technique, which was then coupled with high performance liquid chromatography (HPLC) for quantification purposes. ASE was thus employed to both isolate and pre-concentrate targeted analytes using the minimum amount of solvent hence making extractions more conservational. Two simple, yet extremely sensitive liquid chromatographic methods were developed based on UV detection; one for phthalates and one for alkylphenols, with recoveries reaching up to 92.0%. Acid digestion was used for the extraction of the tin and organotin compounds with analysis by polarography. In river sediment, levels of up to 24.4 mg kg^{-1} phthalate, 1.14 mg kg^{-1} 4-nonylphenol and 118 mg kg^{-1} tin were found. In leachate sediments, values up to 49.8 mg kg^{-1} phthalate, 1.57 mg kg^{-1} 4-nonylphenol, and 36.0 mg kg^{-1} tin were determined. In sludge, values up to 174 mg kg^{-1} phthalate and 22.8 mg kg^{-1} 4-nonylphenol were quantified. The highest value of tin (118 mg kg^{-1}) was found present in an area of high leisure craft activity. Typical sediment levels of tin at other river locations ranged between 1.20 and 37.5 mg kg^{-1}.

Keywords: Accelerated solvent extraction; phthalates; 4-nonylphenol; tin; sediments; xenooestrogens

1.0 Introduction

The oestrogenicity of a compound is measured by its ability to induce physiological effects similar to that of endogenous oestrogens giving rise to concerns about the endocrine disrupting potential of an array of chemical substances. Examples of such hormone modulators include organotins, alkylphenols, phthalates and certain metals. ASE and digestion techniques were used in the trace analysis of these chemicals in the Irish midlands Shannon Catchment in order to quantify levels in an Irish context and to provide benchmark data for future studies in this area. Dibutyl-, diethylhexyl-, diisononyl- and diisodecyl- phthalates (DBP, DEHP, DINP and DIDP respectively) have been shown to be oestrogenic in *in vitro* studies. [1, 2] These phthalates are among those which have been frequently used in Irish

manufacturing industries. A number of polymer-based industries are currently situated in the midlands region pertinent to this study. Phthalates have also been identified in leachate soils[3,4,5] and sewage sludges.[6, 7] The European Union (EU) have issued new regulations which requires that products marketed for use in children's toys may not contain more than 0.1% DEHP, DBP or BBP (butyl-benzyl phthalate) and essentially a total ban has been enforced in toys likely to be chewed by babies. For clarification on this emotive issue, it must be understood that only one of these phthalates DINP, is generally used in toys. Also both it and DIDP have been granted a somewhat favoured status as of April 13, 2006 when in the *Official Journal of the European Union*, it was concluded that in relation to both human health and the environment that there was no need for alteration to the currently 'sufficient' risk reduction measures for these two compounds. Contemporaneously, the EU imposed controls on occupational exposure to DBP, dermally or by inhalation, and are establishing mandatory occupational safety limits as DBP exposure is considered more noxious than that to BBP, DEHP, DINP or DIDP.[8] It is noteworthy that an estimated 90% of plasticisers produced are used in PVC products. DEHP is considered as a priority pollutant in several countries not least as annual production amounts to three to four million tonnes.[9] Phthalates tend to be lipophilic in nature so one would expect to see higher levels present in solid matrices, as they will partition onto organic matter. Oestrogenicity of phthalates has been reported in a number of both *in vivo* and *in vitro* studies.[1, 2, 6, 10]

Alkylphenols are a class of compounds frequently used in industrial and institutional cleaning products. Metabolised alkylphenol polyethoxylates are more noxious than the parent compound and are more persistent and lipophilic. Alkylphenols are degraded into a compound known as 4-nonylphenol (4-NP), which is also manufactured as an intermediate by the chemical industry for use as a preserving agent. Nonylphenol phosphates are used as co-stabilisers and antioxidants in various polymers like rubber and plastic. According to Cheng and Ding[11], household detergents contain between 21 and 57% nonylphenol ethoxylates (NPEOs) and hence implicated in their subsequent entry into waterways via effluents.[12] 4-NP has also been found to emanate from landfilled waste.[13,14] 4-NP induces cell proliferation in the human oestrogen-sensitive MCF-7 breast tumour cells[15,16] and levels as low as the ng L^{-1} range have been shown to induce the production of vitellogenin, a protein produced in fish liver in response to oestrogen stimulation.[17]

[insert figure 1 about here]

Organotin compounds are formed when tin combines with carbon, examples including dibutyltin, tributyltin, and triphenyltin. Both organic and inorganic tin compounds along with tin metal itself, may be found in environmental matrices where they are naturally present in the rocks or where they are mined, manufactured or processed. Tributyltin (TBT) i.e., when tin bonds covalently with three butyl groups and an associated anion (X; Figure 1) and related compounds, which have been used as biocides, notably in anti-fouling paints on the hulls of ships, have been implicated as a cause for imposex (hermaphrodite) marine organisms such as the dogwhelk, *Nucella lapillus* and other molluscs, resulting in a ban on such usage. Organotin compounds are added to PVC to prevent degradation with

an estimated 5 – 20 g/kg of organotins present in PVC. According to Bancon-Montigny *et al*. [18], "the most important non-pesticidal route of entry of organotins into the environment is leaching from organotin stabilised PVC." Hence, matrices were also tested for TBT (see Figure 1) along with tin. The half-life values for organotins vary with the intensity of light, temperature, organisms present, matrix type and pH, so degradation can vary from six days to several months in seawater, the latter clearly illustrating their recalcitrant nature. TBT concentrations as low as 1 ng/L have caused development of male sex organs in female snails and other gastropods and molluscs.[19]

The overall objective of this paper was to exploit ASE based extraction methods to quantify levels of oestrogen mimics in solid environmental samples from the Irish Shannon Catchment (See Table 1 for details of the origins of pollutants). Houtman et al., 2006 [20], used ASE combined with bioassays to measure the biological responses of oestrogenic mixtures, and recoveries from spiked sediment were estimated by determination of their mixture potencies in *in vitro* assays. However, in this study, direct recovery methods supported by HPLC or spectrophotometric or voltammetric analysis, as appropriate, were employed for the analysis of sediments and sludges.

2.0 Experimental

2.1 Materials

HPLC grade methanol, acetonitrile, dichloromethane and acetone were purchased from Labscan Analytical Ltd. (Ireland). Analytical grade reagents were used to prepare standard solutions of 100 μg ml^{-1} in HPLC grade methanol from which subsequent standard dilutions were made. The following reagents were purchased from Sigma – Aldrich; 1, 2-benzenedicarboxylic acid dimethyl ester CASRN 131-11-3 (dimethyl phthalate; DMP); 1, 2-benzenedicarboxylic acid dibutyl ester CASRN 84-74-2 (dibutyl phthalate; DBP); 1, 2-benzenedicarboxylic acid bis (2-ethylhexyl) ester CASRN 117-81-7 (diethylhexyl phthalate; DEHP); 1, 2-benzenedicarboxylic acid bis (3, 5, 5-trimethylhexyl) ester CASRN 28553-12-0 (diisononyl phthalate; DINP); and 1, 2-benzenedicarboxylic acid diisodecyl ester CASRN 26761-40-0 (diisodecyl phthalate; DIDP), all >99%; 4-nonylphenol (4-NP) techn.; and concentrated nitric acid 69% AnalaR® BDH. Standard analytical grade 1 M hydrochloric acid was used for polarography, purchased from Reagecon (Ireland). A standard 100 μg ml^{-1} solution of tributyltin oxide (TBT) was purchased from the Laboratory of the Government Chemist (LGC), UK. Dilutions of analyte mixtures were carried out as necessary in the appropriate solvent as required. Primary stock solutions were prepared individually from the pure compound at a concentration of 100 μg ml^{-1} and solutions were stored in amber glass bottles at 4^0C, remaining stable for at least eight months. Chem Tube-Hydromatrix for use as a bulking agent with ASE was purchased from Varian through JVA Analytical (Ireland) and 30 mm cellulose filters were purchased from Dionex (UK) and glass sludge storage vessels (250 mL) for the purpose of storing sludge and soil samples, were purchased from AGB Scientific Ltd. (Ireland).

2.2 Sampling

The sampling process itself was carried out using an inert stainless steel telescopic sampling rod and cup. Samples were then collected into 500 mL glass jars and were immediately transported to the laboratory where they were dried to constant weight and stored at 4 ^{0}C. Each sample was tested for pH, organic residue content (ashing) and carbonate content (back titration). Recovery experiments were performed for each of the target analytes after an optimal method of extraction was devised.

2.3 Methods for accelerated solvent extraction

As there were two separate HPLC methods, one for phthalates and another for nonylphenol, it was also necessary to develop two separate ASE protocols and to perform recovery experiments on both. For samples to be extracted using ASE, a Hydromatrix or diatomaceous earth dispersing agent was added to the sample in order to prevent aggregation of sample particles. Reducing the particle size improves the extraction by providing better contact of the solvent with the sample.[21] Portions of 2.0 g of sediment or 1.0 g dry weight of sludge were accurately weighed, and the quantity taken homogenised using a mortar and pestle with Chem Tube-Hydromatrix. The sample was then packed into a 66 mL cell containing *triple* filters at the end of the extraction cell to maximise separation of matrix components. The final extract was then dried down at 37 ^{0}C under nitrogen to prevent oxidation of the sample. The Chem Tube-Hydromatrix was purified using a bake-out process at 850 ^{0}C for 24 hours prior to mixing with the sample.

A Dionex 100 Accelerated Solvent Extractor with 66 mL extraction cells was used to perform pressurised liquid extractions of the solid samples. For the extraction of phthalates, an optimal solvent combination of 1:1% v/v dichloromethane: acetone at 110^{0}C was used and for 4-nonylphenol, a 1:1% v/v acetone: methanol at 100^{0}C was found to be the most suitable. The pressures employed were 1500 psi for each protocol. The extraction stages were: preheat, 5 min.; static solvent extraction time, 1 min.; purge 2 min.; static cycle (n = 3) 10 min. High purity nitrogen was employed as the purge gas.

[insert figure 2 about here]

The extraction solvent for tin was 40 mL 3 M HNO_3 per 10.0 g of dry weight sediment whilst for TBT an extraction solvent consisting of 10 mL glacial acetic acid, 30 mL methanol and 20 mL 0.2 M sodium acetate buffer (pH 5.3) was added per 10.0 g of sediment. For the organotin, digestion methods combined with 60 minutes of sonication followed by 30 minutes of centrifugation at 2000 rpm on sample volumes of 50 mL, were used. After extraction, solutions were diluted to known volume with their appropriate diluents.

2.4 Chromatographic conditions

This consisted of a modular liquid chromatographic system comprising a Waters Autosampler 717, Waters Pump 510, and Shimadzu LC-6AD Detector, modified to enhance the sensitivity of the chromatographic output. The column used was a Pinnacle™ II Phenyl (150 x 2.1 mm, 5 μm) with an equivalent guard column, both purchased from Restek Ireland. Two individual isocratic methods were

developed, both using an optimum wavelength of 226 nm; one for the separation of phthalates (flow rate 0.2 mL min^{-1} and using an acetonitrile-water mix, 70:30 (v/v)) and another for 4-nonylphenol (flow rate 0.1 mL min^{-1} and using a methanol-25mM Na_2HPO_4 pH 4.8 buffer mix, 75:25 (v/v)). An injection volume of 5 μL was used for both methods.

2.5 Voltammetric conditions

The polarographic apparatus used was a Radiometer Pol 150 Polarographic Analyser and a Radiometer MDE 150 Polarographic Stand. The half wave potentials ($E_{½}$) for the analysis of tin and TBT, were –405mV and –780mV respectively. As organotin was not detected it was decided to modify the polarographic procedure subsequent to tin extraction using the United States EPA Method 3050B[22] with a multiple standard additions procedure for calibration. The electrolyte for tin used 0.5 mL 1.0 M HCl whilst for TBT an electolyte of 0.32 M ammonium chloride solution in 40 % aqueous ethanol adjusted to pH 2.5 using glacial acetic acid, was used. 3 M nitric acid was used to clean the electrolytic cell in between runs followed by washings with ultra pure water so as to avoid carry-over contamination. The differential pulse mode was used to acquire the standard addition diffusion currents from 10 μL of sample or added standard. The buffer consisted of 9.5 mL ultra pure water and 0.5 mL HCl mix. The growth time for the hanging mercury drop electrode was 0.2 s with every fourth drop being selected as the new pre-concentration surface. The nitrogen purge time was initially 300 s and 45 s for subsequent runs. The stirring rate was 400 rpm and electrolysis was carried out for 45 s with a waiting time of 10 s. The current range was 100 nA to 1 mA. The applied voltages were -1,100 mV (initial) to 120 mV (final) combined with a step duration of 0.1 s and a step amplitude of 1 mV. The pulse duration was 20 ms and the pulse amplitude was 50 mV. The standard diffusion currents (nA) were plotted against the amount of added standard for extrapolation to the concentration axis when the diffusion current read zero.

3.0 Results and discussion

Standard extraction methods for organic compounds in solid samples include sonication, Soxhlet extraction and shaking. These methods utilise substantial quantities of solvent by comparison with ASE and also extraction times are longer with possible poor recoveries. Microwave assisted solvent extraction (MASE) is another method which has become popular in recent years, however, extensive cleaning of sample cells is required in between runs, greater solvent volumes are required, carry-over and contamination may occur from sample cells and multiple filtrations are required at the end-stage to separate the extraction solvent from the solid sample. Indeed, in the early stages of the technique's adoption it was recognised that moisture and organic matter content were critical in determining the extent of recovery.[23] For this reason, ASE was the preferred choice in this study. Villar *et al.*, 2007 [24] used an alternative microwave digestion method which performed in a similar manner to the ASE method described here, however, we found ASE more efficient in terms of method development as there were only two parameters to optimise; extraction solvent and temperature and also there was no risk of localised charring with sample vessels. With ASE, pressure applied to the cell, kept the solvent liquidised during the extraction and the solvent was then flushed through filters placed in the bottom of

the sample cell, to an extract collection bottle. Using high pressure allows the extraction solvent to remain liquefied above the boiling point and also, allows the solvent to penetrate the sample matrix more efficiently.[25, 26] Temperature is the critical parameter in ASE as it decreases the viscosity of the solvent allowing it to penetrate the matrix and solubilise the analytes. Analytes may be retained in pores or other structures and a higher temperature will often disrupt strong analyte-matrix interactions. The solvent then undergoes a viscosity reduction allowing for improved matrix diffusion, hence increased efficiency of extraction.

Most applications operate at 75-125 ^{0}C with 100 ^{0}C typically used as a starting point in new method development. Reproducibility is expected to improve with higher temperatures. The improved contact of the analytes with the solvent enhances the extraction by reducing both time and solvent consumption compared with previous methods.[27] Prior validation of analyte solubilisation without producing matrix interference is required. The compatibility of the solvent with the analytical technique and the possible need for pre-concentration must also be considered. Water and buffered aqueous mixtures can also be used in the ASE, however, it is not recommended to use acidic solvents with this system and so the use of digestion techniques were utilised for the non-organic analytes.

From preliminary sample tests it was found (Table 1) that the overall pH was in the range 7.33 – 8.38 for the river sediments tested, confirming that the area is a carboniferous calcium region. The River Al at Ballydonagh was the most alkaline, whilst the River Hind appeared to be the least, these rivers being tributaries of the River Shannon. This may be due to the fact that the Hind passes through peat bog-land and forest areas, which would enhance the acidity. The Hind also receives discharge from a local sewage treatment plant in addition to a landfill and is downstream of a poultry farm. The alkalinity of the sediment was further confirmed by performing carbonate quantification by using a back titration to calculate the percent carbonate in the samples, the overall values from this ranging between 15.87% and 24.75%. The presence of carbonate would be expected to lower the bioavailable levels of metallic compounds by elevating the pH, hence immobilising them. The organic residue will adsorb pollutants from the water. Overall values ranging between 1.79 – 13.31% of organic residue were found for the sediments over the six-month period. The River Hind has elevated levels and this can be explained in terms of the reasons described above for this location and also, resulting from some rehabilitation works being carried out at the time of sampling on the bed of this river. The rehabilitation works involved dredging and consequent part removal of the bed, causing redistribution of organic matter in the sediment.

[insert table 1 about here]

Recovery extractions using ASE for the organic species were carried out in triplicate using 1.0 mL spikings of the analytes in acetonitrile, added to Chem Tube-Hydromatrix, at the concentrations indicated in Table 2.

[insert table 2 about here]

For the analysis of river sediment samples for the presence of phthalates and nonylphenol, 2.00 g of sample were extracted using the appropriate method, and the extract from this was dried under nitrogen and reconstituted in 2 mL of mobile phase. This 2 mL was then centrifuged prior to injection by HPLC. The recovery factors determined in the assay validation and subsequent processing steps were taken into account in the calculations.

[insert table 3 about here]

In river locations, there was an increasing trend in DEHP levels (Table 3) in March to April at the locations tested. This could be attributable to the start up of the boating season. Banagher is downstream, yet close to, Shannon Harbour. The levels found for Banagher were lower than for Shannon Harbour, indicating that both the local geography and the nature of boats congregating at the latter may have an influence on the exposure of the environment to anthropogenic chemicals at this location. By this we mean that Shannon Harbour, being a canal type water body, experiences a lower flow of water and also accommodates larger hulls from canal navigating boats. These boats are also wintered *in situ.* Levels found in both Burgess Park and Ballydonagh were high as a consequence of their respective proximities to landfilled wastes. The differences observed here on a monthly basis may be due to the amount of rainfall and the water level of the river. In high rainfall, analytes will be carried away and will also be diluted. At Ballydonagh the river has a much smaller volume and very narrow width compared with Burgess Park. Consequently, there will be less dilution of leachate sediment at Ballydonagh and one would expect to see higher levels and varieties of phthalates here. This was indeed observed in sediment from the same site. Much lower levels were observed in the Hind and at Athlone Lock than in either Ballydonagh or Burgess Park. This may be attributable to the higher gravel and silica content at both these locations, which would lower adsorption of the analytes. Using the method for 4-NP extraction, 2.0 g portions of sample were extracted, yielding the following results.

[insert table 4 about here]

NP (Table 4) was not found to be present at Ballydonagh but was however, quantified at all the other locations. Highest levels were observed in the River Hind, attributable to effluent discharging from the local sewage treatment plant. Both Shannon Harbour and Banagher had consistently high levels, with levels in Banagher (this being the reverse of the situation observed with DEHP described above) being the higher of the two. Possible sources include local domiciliary effluents and also discharge from boats. At Burgess Park and Athlone Lock, the levels at the former were initially higher but then reversed overall. These locations are in close proximity (199m apart) but differ in that on the east side of the bank (Burgess Park) lies an old unlined landfill facility, whilst the west side (Athlone Lock) is downstream of a sewer pipe and has lock gates allowing cruise boats and other vessels to pass the weir.

Sewage sludge from three treatment plants in the Midlands Shannon Catchment region was analysed. The first plant had secondary treatment only (X), the second plant had tertiary aerobic (Y) and the third plant had tertiary anaerobic treatment (Z). For the analysis of these samples, 1.00 g of dry weight sludge was ground up and homogenised with bulking agent and due to the complexity of the matrix, triplicate filters were inserted into the extraction cells to allow for sufficient sample clean-up. This eliminated particular matter from the sludge to some extent; however, extracts had to be centrifuged following ASE and underwent a final filtration down to 0.2 μm before injecting onto the analytical column. The results from sludge were as follows.

[insert table 5 about here]

[insert table 6 about here]

[insert table 7 about here]

A comparison of the three sewage treatment plant types and the quantities of each phthalate found are given in Tables 5, 6 and 7. DBP, DEHP and DINP were predominantly found and there would appear to be little difference between the plants. However, closer scrutiny of the levels in the various plants shows that for some 68 % of the time, levels in tertiary treatment were higher than in secondary treatment. This is as expected, since residence times in tertiary treatment are lengthened. The biggest difference to be observed was the data obtained for 4-NP. It is clear that levels following tertiary treatment were higher than post-secondary treatment, and of these, the highest levels appeared in the anaerobically treated sludge. The higher temperature of this sludge resulting from the biological digestion processes and the digestion process itself, increases the metabolism of alkylphenol polyethoxylates to 4-NP resulting in the levels thus observed in this study. Sole *et al.* [28], found 100 – 500 mg kg^{-1} NP in sewage sludge.

The overall levels of DBP in anaerobically treated sludge (Z) were highest and levels appeared quite consistent over the six months, whilst in the other two plants, levels tended to fluctuate to a greater extent. For DEHP and DINP, levels were higher for the former, and equivalent for the latter, at plant type Y. Interestingly, this treatment plant is situated at a location where there are a number of polymer-based industries. Overall, plant type Z appeared to have the most consistent efficiency. Compared with sediment, the sludge contained a greater diversity and higher levels of phthalates, as one would expect (Figure 3). The graph illustrates the levels of phthalates found in sludge from plant type Y at Athlone, compared with the amount of DEHP from sediment collected at Athlone Lock, a riverine location, one of numerous tested but for which rainfall data was immediately available and is presented here.

[insert Figure 3 about here]

[insert Figure 4 about here]

Figure 4 demonstrates that rainfall inversely impacted upon the levels in the sludge. Associated with periods of high precipitation we have observed overflow from the Athlone plant and, as residence times for the analytes will consequently be reduced, less will adsorb onto the sludge. When rainfall is lower, the levels in the sludge will be higher, for example, months number two and four, which correspond to December and February. The sludge produced during sewage treatment accumulates many lipophilic substances and succeeding usage of the sludge as a fertilizer for agricultural land results in application of harmful micro-contaminants and metals to land and hence, the food chain and the water table. [29, 30] Even trace levels present in the ng/L range may provoke reproductive disturbances in riverine fish.[31] Solid environmental samples in particular can exhibit wide variations in their physico-chemical properties and particle dimensions, and these factors can affect the sorption and retention of certain analytes. In a study carried out by Kirk, McClure Morton (now RPS) [32], the quantity of NP in sediment was 6.4 mg/kg (March 2000) and 0.6 mg kg^{-1} (July 2000). For DEHP in sediment, values were < 0.1 mg kg^{-1} (March 2000) and 0.7 mg kg^{-1} (July 2000) and these were the only substances tested for by the monitoring group [28]. In this study, the more comprehensive method of extraction of ASE was used. A further advantage of our application of the technique of ASE is that there is no possibility of carryover between extractions, this being acutely important in minimising sample contamination in the laboratory.[24, 33]

In terms of the organotin testing, over 40 samples including both sediment and silt were extracted and analysed for both tin and TBT. Preliminary testing was only carried out for tin but since levels for tin was found to be significant it was decided to investigate if its source was from TBT degradation or from PVC leachate or other sources. A set of validation experiments for this method showed an RSD for repeatability of 0.7%, and for a series of three reproducibility determinations over each of a range of five concentrations, RSD values spanned 4.3 to 6.3%. Many boats are repaired, sanded and repainted in early spring and are set back out on the water. Particularly high values occured in Banagher and in Shannon Harbour, which are very busy cruise boating areas.

The results obtained were as follows (Table 8).

[insert table 8 about here]

Shannon Harbour clearly contained by far the greatest levels of tin. This canal location is also the busier boat overwintering area, which may account for these levels. Craft in this area are, unlike Banagher, frequently in use year-round. Furthermore these boats tend to be much older and will have the potential of accumulated layers of paint built up over a period, in some cases of over one hundred years. The next highest was Burgess Park, which can be explained in terms of leaching from an adjacent, now disused, landfill facility. For all the other locations, levels for the most part, were below

20 mg kg^{-1}. However, TBT was not detected in any of the sample extracts. Half-life values of organotins in sediment are very short and so this would account for us finding tin but not TBT along with the fact that the use of TBT has been restricted in recent years due to its detrimental effects. Hallas and Cooney [34], determined 3.0 – 7.9 mg kg^{-1} Sn in river sediments where there were a number or leisurecraft activities, with levels up to 239.6 mg kg^{-1} in one particular area. Lopez-Garcia *et al.*[35], found 82.8 – 207.1 mg kg^{-1} in industrial soils, 2.1 mg kg^{-1} in agricultural soils, 6.6 – 18.7 mg kg^{-1} in river sludge and 58.1 mg kg^{-1} in domestic sludge. The levels in this study by comparison ranged from <LOD to 118 mg kg^{-1}, with the higher values being observed in areas of high boating activity. Overall, levels were found to be comparable with our European counterparts and were present at concentrations capable of exerting oestrogen-modulating effects in the environment.

Conclusion

Samples taken for this study were taken according to anthropogenic activity, proximity to urbanised areas, targeting sewage treatment plants, landfills, boating areas, and the immediate surroundings of these locations.

In terms of extraction methodology, Fountoulakis *et al.*, [36] used MASE to extract NP from sludge and had recovery factor was 91.4 % with a limit of detection of 2.86 mg kg^{-1}. For this study, a method recovery factor of 95.4 % was obtained and a LOD of 0.015 mg kg^{-1} determined, which confirms the efficiency of the ASE methodology. The method in this study was also a more efficient extraction protocol that did not require the analyst to separate the sample from the solvent after extraction and thus eliminated the multiple filtration and centrifugation procedures frequently required for the clarification of analytes from difficult matrices.

For the significant urbanised area of Athlone, situated at the centre of the Catchment, levels of DEHP in sediment, with the exception of one value below the limit of detection, ranged between 0.45 – 42.2 mg kg^{-1} over the test period, and between 0.08 – 1.14 mg kg^{-1} for NP. As expected levels in leachate sediments and sewage sludge were, in the main, higher. By comparison, Tienpont *et al.*, [37] reported total phthalate levels of 19-409 mg kg^{-1} for sludge sourced from Belgian waste-water treatment plants and Sole *et al.*, [28] in Denmark, found 50 – 200 μg kg^{-1} NP in river sediment upstream of an STP, so the overall values for these analytes in samples at the interface with Irish waterways are, on the studies reported, tending to be lower than those elsewhere in continental Europe. Given the low relative population density in Ireland, this is not a surprise.

The values determined for tin will provide timely benchmark levels given the adoption of the call for an international ban on TBT by the Convention on the Control of Harmful Anti-fouling Systems on Ships by both the Marine Environment Protection Committee and the International Maritime Organization and its entering into force in September 2008.[38] Furthermore, given our focus on the freshwater environment in this work, it is noteworthy that TBT has been classified as one of the priority substances, for which monitoring is mandated on foot of the EU Water Framework Directive.[39] Some countries have regulated the levels of TBT in sediment. One such limit is that deposition of dredged material is forbidden in Swedish sea waters, if the organotin loading exceeds 300 μg TBT/kg sediment.[38] TBT was not detected in this study, so this level could not be exceeded in the

399 determinations reported here. Referencing local authority determinations, the Irish EPA has reported
400 widespread TBT contamination in the recent past, and with the advent of the above ban and earlier EU
401 prohibitions; it expects a 'gradual diminution' of TBT levels going forward.[40] The proposed maximum
402 allowable concentration for Irish fresh- and marine waters is 0.0015 μg TBT L^{-1}.[41]
403 These developments supplement the earlier initiative from Ireland in 1987 of banning the use of
404 organotins for painting boats of length less than 25 m in length intended for either fresh or marine
405 waters.[42]
406
407
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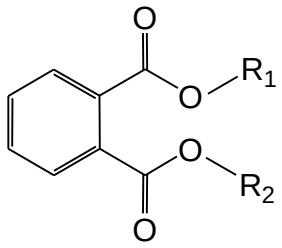
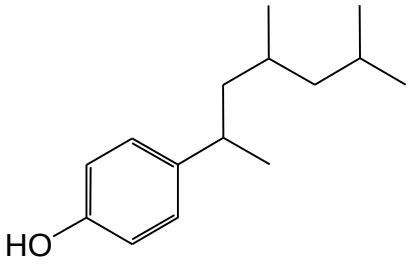
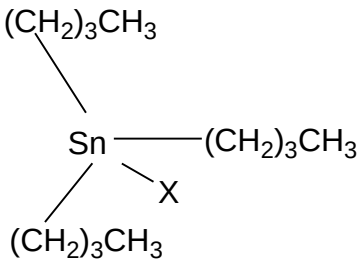


Phthalate Ester

R_1 and R_2 = alkyl group



4-Nonylphenol (one isomer)



Tributyltin

Fig. 1: General phthalate structure, 4-nonylphenol and tributyltin.

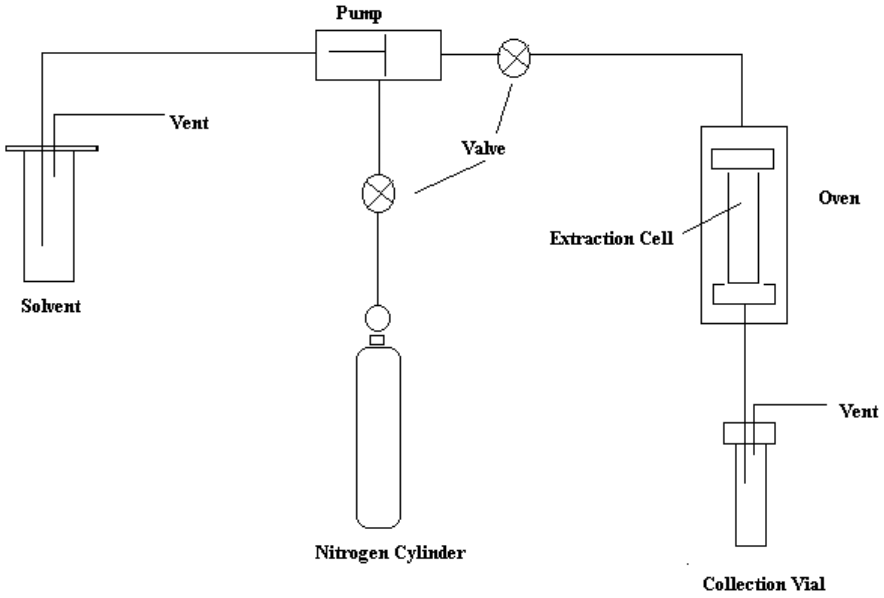


Fig. 2: Schematic drawing of the workings of the ASE system.

Table 1: Range of sample parameters for the river sediments tested over a six month period

Location	County	pH (RSD: 0.3%)	% Carbonate (RSD: 0.6%)	% Organic residue (no precision data available)	Type or source of contamination
Hind	Roscommon	7.56 - 7.83	15.87 - 23.57	1.79 – 13.31	Effluent/leachate
Banagher	Offaly	7.93 - 8.15	21.96 - 24.28	2.44 – 7.00	Leisurecraft activities
Shannon Harbour	Offaly	7.97 - 8.10	20.12 - 24.52	1.97 – 2.89	Leisurecraft activities
Athlone Lock	Westmeath	7.33 - 8.10	22.19 – 24.75	1.94 – 4.02	Leisurecraft activities
Burgess Park	Westmeath	7.51 - 7.91	17.96 – 24.70	2.67 – 12.19	Leachate
Ballydonagh	Westmeath	8.08 - 8.38	21.98 – 24.67	2.34 – 7.33	Leachate

Table 2: Recovery values for the developed ASE methods

Analyte	% Recovery Value					
	Spike Quantities					
	0.5 mg kg^{-1} n = 3	2.0 mg kg^{-1} n = 3	5.0 mg kg^{-1} n = 3	10.0 mg kg^{-1} n = 3	Average % Recovery	Average % RSD
DBP	88.2	91.8	90.9	95.7	91.7	3.4

DEHP	83.1	88.0	91.4	84.8	86.8	4.2
DINP	74.8	85.0	90.7	83.6	83.5	7.9
DIDP	94.9	103	86.4	83.7	92.0	9.6
NP	75.6	78.6	83.7	84.3	80.6	5.2

Table 3: Levels of DEHP quantified in river sediments (mg kg^{-1}).

Location	Nov	Dec	Jan	Feb	Mar	Apr
Hind	0.52	1.10	13.7	7.57	6.64	4.48
Athlone Lock	7.50	5.43	0.49	0.45	20.5	5.10
Shannon Harbour	0.26	24.4	5.01	15.6	24.1	24.4
Banagher	<LOD*	9.15	0.71	Δ	Δ	Δ
Burgess Park†	12.7	<LOD*	22.2	42.2	40.9	40.9
Ballydonagh†	29.7	42.9	25.7	5.06	49.8	38.9

* Limit of detection = 0.014 mg DEHP kg^{-1}

Δ Data available from corresponding author on request but did not meet system suitability criteria.

Table 4: Levels of NP quantified in river sediments (mg kg^{-1}).

Location	Nov	Dec	Jan	Feb	Mar	Apr
Hind	0.13	1.57	1.56	0.07	0.81	0.12
Athlone Lock	0.13	0.19	0.10	1.02	0.79	0.78
Shannon Harbour	0.62	0.64	0.78	0.31	0.50	0.91
Banagher	1.14	0.71	0.81	1.01	0.72	0.41
Burgess Park†	0.93	1.14	0.21	0.08	0.16	0.14
Ballydonagh†	<LOD*	<LOD*	<LOD*	<LOD*	<LOD*	<LOD*

- Limit of detection = 0.015 mg NP kg^{-1}
- † The data so marked has been previously reported in the context of developing high performance liquid chromatographic methodology for EDCs. See Reid, A.M., Brougham, C.A., Fogarty, A.M., Roche, J.J. (2007). Isocratic LC methods for the trace analysis of phthalates and 4-nonylphenol in varying types of landfill and adjacent run-offs. *Toxicological & Environmental Chemistry* **89**, 3, 399-410.

Table 5: Phthalates and NP (mg kg^{-1}) in a sewage treatment plant with secondary treatment only (type X).

Phthalate	Nov	Dec	Jan	Feb	Mar	Apr
DBP	11.8	174	84.2	19.5	52.8	105
DEHP	3.77	36.9	18.8	27.2	18.7	27.0
DINP	2.60	29.4	9.86	31.3	28.6	29.3
NP	6.51	6.23	9.34	7.08	8.54	14.1

Table 6: Phthalates and NP (mg kg^{-1}) in a sewage treatment plant with tertiary aerobic treatment (type Y).

Phthalate	Nov	Dec	Jan	Feb	Mar	Apr
DBP	94.2	<0.01	79.2	140	146	60.7
DEHP	38.9	31.9	33.2	63.6	39.8	16.3
DINP	37.1	<0.03	21.6	31.0	33.5	34.0
NP	8.52	11.0	10.7	9.53	15.3	13.1

Table 7: Phthalates and NP (mg kg^{-1}) in a sewage treatment plant with tertiary anaerobic treatment (type Z).

Phthalate	Nov	Dec	Jan	Feb	Mar	Apr
DBP	61.8	45.6	67.5	121	135	116
DEHP	13.6	47.6	20.8	40.1	34.8	31.1
DINP	17.0	17.6	35.4	27.5	29.6	33.0
NP	16.0	19.3	17.9	20.5	22.8	20.5

Table 8: Concentrations of Tin in sediment (mg kg^{-1})

Location	Nov	Dec	Jan	Feb	Mar	Apr
Hind	9.50	4.00	1.40	28.8	1.20	3.00
Athlone Lock	7.50	37.5	4.00	2.00	11.8	14.4
Shannon Harbour	70.0	32.5	45.0	118	56.0	41.3
Banagher	2.50	4.50	5.20	17.0	1.20	13.5
Burgess Park	22.5	28.8	16.5	22.5	36.0	18.8
Ballydonagh	3.60	6.50	<LOD*	3.00	6.00	2.20

*Limit of detection = 0.04 mg Sn kg^{-1}

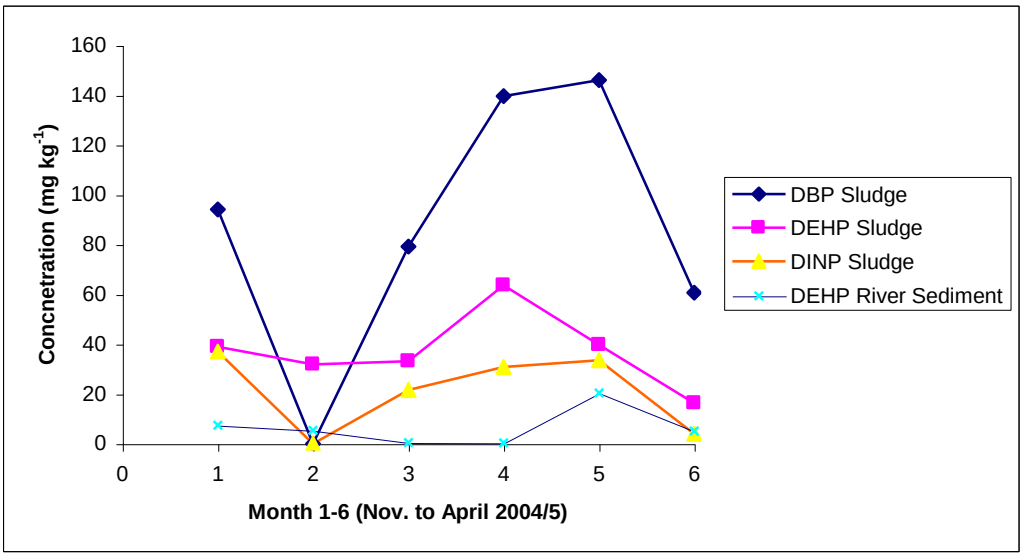


Fig. 3: Comparison of phthalate concentrations in river sediment (Athlone Lock) and sewage sludge from Athlone Sewage Treatment Plant (tertiary aerobic, type Y).

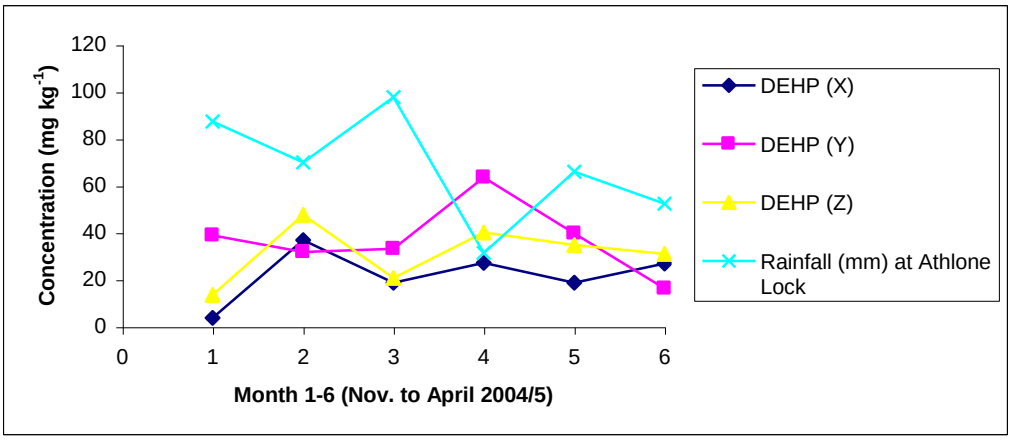
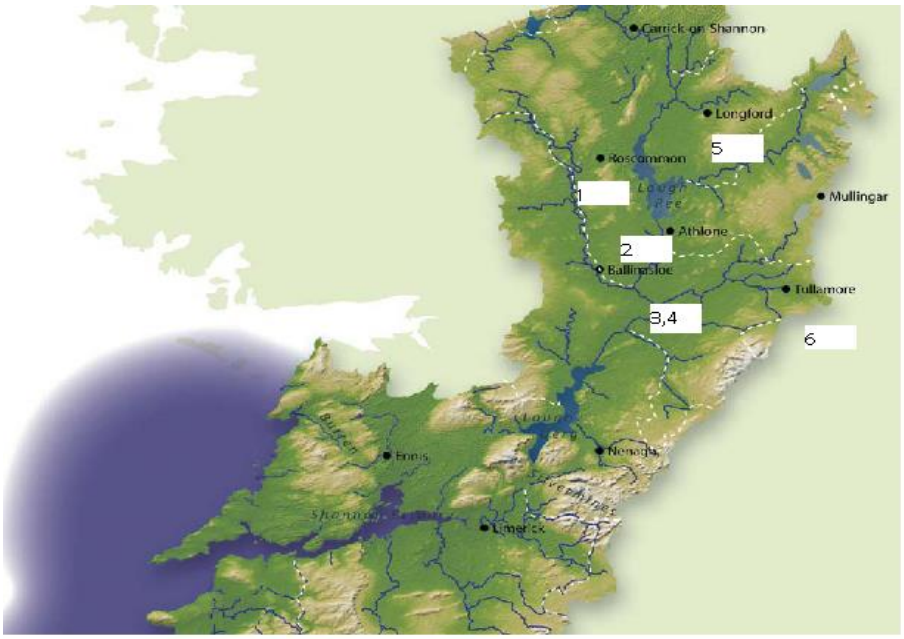


Fig. 4: Comparison of DEHP concentrations in sewage sludge between sewage treatment plant types and rainfall.



1. Hind
2. Athlone Lock, Burgess Park, Ballydonagh, Athlone STP (Type Y)
3. Shannon Harbour
4. Banagher
5. Longford STP (Type X)
6. Tullamore STP (Type Z)

534 Fig. 5: The sampling locations in the Irish Midlands Shannon Catchment.
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