Science of the Total Environment (ISSN 0048-9697, eISSN 1879-1026)

Citation Details

Citation for the version of the work held in 'OpenAIR@RGU':

ZHANG, Z., LE VELLY, M., RHIND, S. M., KYLE, C. E., HOUGH, R. L., DUFF, E. I. and MCKENZIE, C., 2015. A study on temporal trends and estimates of fate of Bisphenol A in agricultural soils after sewage sludge amendment. Available from *OpenAIR@RGU*. [online]. Available from: http://openair.rgu.ac.uk

Citation for the publisher's version:

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A study on temporal trends and estimates of fate of Bisphenol A in agricultural soils after sewage sludge amendment

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Abstract

Temporal concentration trends of BPA in soils were investigated following sewage sludge application to pasture (Study 1: short term sludge application; Study 2: long term multiple applications over 13 years). The background level of BPA in control soils were similar, ranging between 0.67-10.57 ng g^{-1} (mean: 3.02 ng g^{-1}) and 0.51-6.58 ng g^{-1} (mean: 3.22 ng g^{-1}) for studies 1 and 2, respectively. Concentrations in both treated and control plots increased over the earlier sampling times of the study to a maximum and then decreased over later sampling times, suggesting other sources of BPA to both the treated and control soils over the study period. In study 1 there was a significant treatment effect of sludge application in the autumn (p=0.002) although no significant difference was observed between treatment and control soils in the spring. In study 2 treated soils contained considerably higher BPA concentrations than controls ranging between 12.89-167.9 ng g^{-1} (mean: 63.15 ng g^{-1}). This and earlier studies indicates the long-term accumulation of multiple contaminants by multiple sewage sludge applications over a prolonged period although the effects of the presence of such contaminant mixtures have not yet been elucidated. Fugacity modelling was undertaken to estimate partitioning of Bisphenol A (soil plus sewage: pore water: soil air partitioning) and potential uptake into a range of food crops. While Bisphenol A sorbs strongly to the sewage-amended soil a large proportion is likely to be degraded under the anaerobic conditions in the soil, with an immobilized fraction remaining in the soil, 4 % by mass was predicted to enter soil pore water resulting in significant uptake by crops particularly leafy vegetables (3.12 - 75.5 ng g^{-1}), but also for root crops (1.28 - 31.0 ng g^{-1}) with much lower uptake into cereal grains (0.62 - 15.0 ng g^{-1}). This work forms part of a larger program of research aimed at assessing the risks associated with the long-term application of sewage sludge to agricultural soils.

Keywords: Bisphenol A; sewage sludge; soil; temporal trend; modelling; risk assessment

1. Introduction

The purification of waste water leads to the formation of sewage sludge with a high organic matter content (e.g. 60-70% of dry matter during aerobic digestion, 40-50% of dry matter during anaerobic digestion) and which containins valuable nutrients, such as phosphorus and nitrogen [Tenenbaum 1997; European Commission 2001]. In Europe, the implementation of the Urban Waste Water Treatment Directive (91/271/EEC) has led to the production of increased quantities of sewage sludge which requires to be disposed of. At present, there are three main disposal routes for processed sludge from waste water treatment plants: agricultural application as fertilizer, land filling and incineration. The application of sewage sludge to agricultural land has many positive aspects and ensures the recycling of nutrients to improve soil fertility in a global situation where nutrient resources, particularly phosphorous, are being depleted [Van Vuuren et al., 2010]. Consequently, current trends in waste management policy favor agricultural application and composting as opposed to land filling or incineration [Petersen et al., 2003; European Commission, 2001; Laturnus et al., 2007]. the European Union produced more than 10 million tons (t) in 2006 with 8.7 million t DM in the EU-15 and an additional 1.2 million t DM for the 12 new Member States [European Commission, 2010]. As the level of wastewater treatment continues to improve throughout the European Union and specifically in the new member states, it is predicted that European sludge production will continue to increase. The application of treated sewage sludge to agricultural land is now the option of choice in many countries with more than half of the 27 EU member states recycling >50% of treated sewage sludge to agricultural land, either directly or after composting [European Commission, 2010; Kelessidis and Stasinakis, 2012]. There remains, however, great variation in the methods used to recycle or dispose of sewage sludge and methods often depend on local socio-political and legislative conditions. In some EU member states application of sewage sludge directly to land is not authorized due to growing public concern about the safety of the practice [European Commission, 2010]. In the

UK treated sewage sludge or biosolids are classified either as conventionally treated or advanced/enhanced treated biosolids. Conventionally treated biosolids in which at least 99% of pathogens have been destroyed cannot be surface spread but must be injected into the soil. The treated land can then only be grazed after a period of three weeks has passed. Advanced treated biosolids, in which virtually all pathogens are eliminated, can be surface spread but again three weeks must pass before animals are allowed to graze on the treated land [ADAS, 2001].

As well as being a valuable source of nutrients, sewage sludge contains a variety of potentially harmful substances, such as hydrophobic organic contaminants and metals not removed in the sludge treatment process [e.g. Tenenbaum 1997; European Commission, 2001; Kolpin et al., 2002; Darvodelsky and Fien 2005; Harrison et al., 2006; Rhind et al., 2013; Yager et al., 2014]. In laboratory studies, many of these compounds have been shown to cause a range of biological effects in organisms which are exposed to them. In field studies, however, direct causal links between biological effects and individual compounds are rare, if ever identified. The experiments described in this paper are part of a wider study originally devised to determine the potential physiological effects resulting from the exposure of sheep (pregnant ewes) to sewage sludge treated grass (via ingestion of both grass and soil) as well as to gather short- and long-term data on the accumulation of organic contaminants after sewage sludge treatment. The physiological studies demonstrated disruption of multiple organs in sheep grazing sludge-treated pastures and in their fetuses, however the underlying causes have not been identified [Rhind, 2009; Lind et al., 2009; Rhind et al., 2010; Lind et al., 2010; Hombach-Klonisch et al., 2013]. It is possible that exposure to a complex mixture of chemicals, ingested by the ewes during pregnancy, could be involved in the development of such biological effects but no causal links to such mixtures have so far been made. It is important therefore to clearly understand the behavior of such compounds, known to be present in the applied sewage sludge after application to the agricultural land on which they are grazing.

Bisphenol A (2,2-Bis-(4-hydroxphenyl)propane; BPA) is a high production volume plasticizer is one such compound, commonly detected in sewage sludge (0.1 - 3.2×10^{7} µg kg^{-1}) [Harrison et al., 2006; Song et al., 2014]. It is used industrially as a monomer for the production of polycarbonate and epoxy resins, unsaturated polyester-styrene resins and flame retardants and is used in a diverse range of products [Staples et al., 1998; Clarke and Smith, 2011]. The global consumption of BPA in 2011 was predicted to exceed 5.5 million metric tons. Once released into the environment, the fate of BPA is affected by its physico-chemical properties; it has a low vapor pressure of about 5×10^{-6} Pa (volatility), moderate water solubility (120-300 ppm), moderately high octanol water (Log K_{ow}: 3.4) and organic carbon (Log K_{oc}: 2.9) partition values. BPA interacts strongly with organic carbon rich particulate matter and as a result is found in sewage sludge produced during wastewater treatment [Mohapatra et al., 2011]. Reviews of reported environmental concentrations and its potential biological effects have recently been published [Greiner et al., 2007; Flint et al., 2012; Michalowicz 2014] and BPA is considered to have become ubiquitous in the environment within the past few decades [Flint et al., 2012; Huang et al., 2012].

BPA is described by many researchers as an endocrine disrupting chemical (EDC), however there is much debate and conflicting evidence about its environmental and toxicological impact [Chapin et al., 2008; European Union, 2008; Flint et al., 2012; Molander et al., 2012; Rochester et al., 2013; Bergmann et al., 2013; Teeguarden and Hanson-Drury, 2013; EFSA 2014; Rezg et al., 2014; Tyl et al., 2014; vom Saal and Welshons, 2014]. Many studies have described endocrine disrupting effects across a range of species [Delclos et al., 2014; Hunt et al., 2012; Markey et al., 2003; Newbold et al., 2007]. In biological systems, once the BPA is taken up from the local environment, it is rapidly metabolized to its conjugated (glucuronidated) form, BPA-gluc, with the main site of metabolism being the liver. BPA but not the BPA-gluc is reported to be able to pass over the placenta. Fetuses and neonates, however, have a much lower (if any) ability to metabolise BPA to BPA-gluc and so may be exposed if unconjugated-BPA crosses the placenta [Domoradzki et al., 2004; Taylor et al., 2008, 2011; Corbel et al., 2014]. A number of recent studies have reported measurable biological effects in sheep exposed to BPA. These studies used exposure via daily sub-cutaneous injections over extended time periods during either the gestation (60 days 5000ug g^{-1} per day) or neonatal (14 days 50ng g^{-1} per day) period [Savabieasfahani et al., 2006; Rivera et al., 2011; Viega-Lopez et al., 2014]. In these latter studies, the exposure routes of BPA are not representative of those which will occur to grazing animals and do not take into account issues of the bioavailability of any BPA contained in the soil/herbage matrix. The main source of exposure to sewage sludge containing a complex mixture of chemicals at relatively low individual doses for sheep grazing on sewage sludge amended pasture is most likely to be ingestion of soil during feeding and subsequent compound and metabolite specific trans-placental transfer to the developing fetus.

This is potentially a substantial exposure route in sheep with soil reported to make up to 80% of the dry matter ingested by grazing animals in winter although this is considerably less in the summer months [Smith et al., 2009]. Previous studies have suggested that grazing sheep ingested up to 400g of soil per Kg body weight between May and November [MaGrath et al., 1982].

In laboratory based degradation experiments, spiked BPA in soils was shown to degrade relatively rapidly under aerobic conditions with little or no degradation occurring under anaerobic conditions [Ying and Kookana, 2005; Xu et al., 2009; Kim and Cunningham, 2014]. Langdon et al. [2011] introduced the BPA to soil in laboratory based experiments using biosolids containing 'inidenous' BPA and monitored BPA concentrations over 32 weeks after the addition of the biosolids to soils. BPA degradation followed a biphasic pattern, indicating a weakly bound fraction that was readily degraded and a recalcitrant fraction not available for aerobic degradation, In a later study the effect of application method in the laboratory studies was further investigated with the addition of indigenous BPA via biosolids method being compared to the spiking method. Differences were observed in both the rate and pattern of degradation. The BPA in the spiked samples was completely degraded within the study period whilst the indigenous BPA from the biosolids degraded at a rate five times slower than the spiked samples and a recalcitrant fraction remained in the soil with little or no apparent degradation. When BPA is added to soils as a spiking solution it had a half-life in the soil of 7 days. When the BPA was introduced to the soils as a component of a biosolid matrix a biphasic degradation was observed, with a readily bio-available fraction being relatively rapidly degraded in an exponential manner with a half life between 18-102 days and a recalcitrant fraction that was resistant to biodegradation. This biphasic degradation pattern supported the earlier laboratory observations of the group [Langdon et al., 2011] and was in agreement with observations in field trials carried out in Southern Australia [Langdon et al., 2013]. When the same biosolids were applied in the field trials, the same biphasic degradation pattern was observed but degradation rates in the field trials were considerably slower than in the laboratory studies using the same biosolid/soil combination. This clearly showed the influence of environmental factors on the rate of degradation of the bioavailable fraction. In the field trial study [Langdon et al. 2012] the climate was Mediterranean in nature with winter temperatures ranging from 11-20^{o}c and rainfall an average of 22mm with a maximum of 67mm. In the summer period temperatures ranged from 21-38^{o}c with almost no rainfall. These local conditions are likely to have had a large influence on the rate of biodegradation of the bioavailable BPA fraction. The proportion of the BPA retained in the soil is likely to be site specific and related to the nature of the specific biosolid applied (i.e the relative proportions of bioavailable/recalcitrant BPA). In the study reported here, climatic conditions are markedly different and the source of biosolids are potentially significantly different from the field studies reported by Langdon et al. [2013]. There is currently little or no information on the effects of soil amendment with sewage sludge with respect to BPA soil concentrations, under weather conditions relevant to Scotland and the UK. In East and Central Scotland where the studies reported here were carried out, environmental conditions are markedly different from those reported in the study of Langdon et al. [2012]. In Scotland rainfall is generally well distributed throughout the year with wettest months in autumn and early winter. Mean annual air temperatures lie between 6-9^{o}c depending on location. Lower soil temperatures may lead to a slower rate of aerobic BPA degradation but the increased soil moisture content may or may not mitigate this effect.

A simple equilibrium partitioning model to estimate the distribution of any BPA in the soil air, soil pore water and soil and can be used to direct further monitoring in these phases in future studies. It is not the purpose of this paper to consider what happens in the organisms after uptake or to study soil biodegradation rates of BPA.

The aims of the present study are: (i) to determine the effect of sewage sludge amendment on the concentration of BPA in agricultural soils relative to control soils under environmental conditions common in Scotland and the UK; (ii) to determine if there is a recalcitrant BPA fraction which has the potential to cause temporal changes in the surface concentrations of BPA in the short term and after multiple long term applications of sewage sludge to agricultural land; This is the first long term (13 year) study on the effect of repeated and multiple sewage sludge application on soil BPA concentrations to be reported; (iii) to estimate partitioning of BPA within the sewage sludge-amended soil matrix and subsequent potential uptake into sheep via herbage and soil ingestion and into crops; (iv) to assess the appropriateness of current UK legislation which states that grazing animals should be excluded from sewage sludge treated pasture for three weeks after application of sewage sludge.

2. Material and methods

2.1 Sewage Sludge

The sewage sludge pellets used in study 1 (short-term monitoring) were obtained from Oran Environmental Services, Alloa, UK with the sewage sludge being derived from a variety of waste water treatment plants operated by a range of large water service companies. The sewage sludge from these mixed sources were combined during the pellet manufacturing process. It is therefore not possible to state the original sources of any BPA in the sewage sludge, although would have been produced as a result of at least secondary wastewater treatment processes. The sewage sludge pellets used for study 2 (long-term monitoring) were originally sourced from Thames Water, originating from the Seafield waste water treatment plant in Edinburgh, UK, prior to being sourced from Oran Environmental Services. All sewage sludge pellets used are classified as advanced treated biosolids and therefore could be surface applied to the test plots prior to the commencement of sheep grazing of the land. The sewage sludge pellets used in study 1 were taken from the same batch as that used in study 2. The concentrations of BPA in sludge were determined using the methods described in sections 2.5-2.7 of this report.

2.2 Study 1

In order to characterise short-term temporal changes in soil concentrations of Bisphenol A, following sludge application to pasture, eight experimental plots (2m × 2m; 2 treatments × 4 replicates per treatment) were marked within a single field. The experimental plots were situated at the James Hutton Institute research station at Glensaugh, Aberdeenshire, UK. This is a hill farming site in the North East of Scotland, within an altitudinal range of 120 to 450 m above sea level and has an average annual rainfall of 1040 mm. The two periods of study were associated with contrasting environmental temperature profiles. During the autumn study period, ambient temperatures declined from approximately 12^0C to 6^0C while in the spring they increased from 4^0C to 10^0C. Treatments were allocated randomly within pairs of plots. Sewage sludge pellets were applied to the surface of 4 plots (Treated; T), at each of two contrasting times of year (September 2010 (autumn) and March 2011 (spring)), at a rate equivalent to 2.25 t ha^{-1}. Inorganic fertilizer containing an equivalent amount of N (150 kg ha^{-1}) was applied to the 4 remaining plots as a control. The sludge and inorganic fertilizer applied on each occasion was from the same batch.

In order to avoid additional complexity associated with the recycling of pollutants by grazing animals, through natural excretion, none were maintained in the experimental field from the time of the initial treatment with either sludge or inorganic fertilizers (controls). The experimental area was mowed at intervals of 7 to 10 days, during the growing season, to maintain the pasture height at approximately 4 cm (a height comparable to that maintained for grazing sheep). Clippings were removed to simulate grazing but the nutrient and any pollutants that they contained were not returned to the plots as they would have been, if animals had grazed the plots, through faeces and urine.

Soil samples were collected at weeks 0 (before application of sludge), 1, 3, 6, 8 and 10 in each of the two seasons. Samples (0 - 3 cm) were collected using a 5 cm diameter corer from 5 marked points within each plot. All cores were collected from an area within 15cm of each marked point in order to minimize variance attributable to soil properties. Samples were pooled for each sampling time and within each plot, wrapped in aluminium foil (dichloromethane (DCM) washed) and stored at -20 ^{0}C. The samples were freeze-dried and sieved (≤ 2 mm) to remove stones and large particles, and stored in glass jars before analysis.

Results are given for 6 plots (3 Treated, 3 Control) due to reduced numbers of samples presented for chemical analysis (saving for analytical cost).

2.3 Study 2

In order to assess longer-term changes in soil profiles and the effect of repeated applications on concentrations of BPA in the soil, samples were collected from experimental plots, at the James Hutton Institute research station at Hartwood, Lanarkshire, UK. These plots have been subject to long term treatment with either sewage sludge or inorganic fertilizer, respectively over a 13 year period. The general area comprises mainly upland farms devoted to stock rearing. Hartwood covers 350 ha, most of which slopes gently southwards, rising from 150 to 300 metres above sea level. Annual rainfall is approximately 1100 mm with a similar temperature profile to that described in study 1.

Sewage sludge was applied to one set of plots from 1997 to 2010, twice annually (spring and early autumn) at the same rate as in study 1. A second set of plots was treated with inorganic fertilizer as described for study 1. All plots were grazed by sheep at conventional stocking densities through the period of the study, except during periods of 3 weeks, immediately after each of the sludge applications to the treated plots, as prescribed by UK legislation [Great Britain Parliament, 1989].

Approximately 40 soil samples (0-3cm deep; 5 cm diameter corer) were collected from each of the respective treatments, at intervals of approximately one month, from August to December 2009 and thereafter in February, March, May, July and August 2010. Samples were pooled within treatment and stored, until analysis, according to the same protocols as in study 1.

2.4. Reagents and materials

All the solvents used were of HPLC glade, including iso-hexane, acetone, DCM and methanol (Rathburns, Walkerburn, UK). All compounds (BPA, d_{16}-BPA, pyridine, and bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane (TMCS)) were purchased from Sigma-Aldrich (Dorset, UK). Separate stock solutions of individual compounds were prepared by dissolving an appropriate amount of each substance in methanol. From these standards, a mixture of working target standards or internal standards containing each compound at 0.2 μg g^{-1} were prepared by diluting the stock solution in methanol. These standard solutions were stored at 4 ^{o}C and diluted in methanol prior to use.

Silica gel (63-200 μm, Merck, Damstadt, Germany) and anhydrous sodium sulfate (Mallinchrodt Baker, Deventer, Holland) used in the study were washed in DCM, by Soxhlet, for six hours and baked in a muffle furnace at 550 ^{o}C, overnight, before use. All glassware used throughout the experiments was machine washed and then baked at 450 ^{o}C for 12 h to eliminate organic contamination.

2.5. Selective pressurized liquid extraction (SPLE)

In brief, SPLE was conducted using a fully automated Dionex Accelerated Solvent Extraction (ASE) 200 system; the ASE extraction cell (11 mL) was loaded by inserting two glass filters (10 μm, Dionex, Leeds, UK), followed by the addition of an adsorbent/retainer (silica: 3 g, for the clean-up of samples) and anhydrous sodium sulfate (to remove water), followed by the addition of a further filter. Soil (0.5 g dry weight) spiked with internal standard (d_{16}-BPA) was loaded on the top of the filter and the cell was then filled with sand (0.5 g) which had been heated to 550 °C to destroy all organic matter. The extraction cells were then subject to pressurized liquid extraction [Zhang et al., 2011].

All Pressurized Liquid Extraction (PLE) cells were washed with methanol, by ultrasonication (10 min), twice, prior to use. The PLE was automated and allowed unattended extraction of up to 24 samples. The ASE200 was programmed to run a series of extractions on each cell. After the extraction, the solvents were evaporated down to 0.1 mL under a gentle stream of nitrogen gas at between 30 and 35 ^{o}C before further derivatisation procedures and GC-MS analysis.

2.6. Derivatisation procedure

Owing to the high polarity of BPA chromatographic peaks were poorly defined and derivatisation was necessary to reduce the polarity of these compounds. The derivatisation procedure used has been described previously [Zhang et al., 2006; 2008]. Briefly, the standards or extracts were transferred into 2 ml reaction vials, and evaporated to dryness under a gentle stream of nitrogen. The dry residues were derivatised by the addition of 50 μl each of pyridine and BSTFA (1% TMCS), which were heated in a block at 60-70 °C for 30 min. The derivatives were cooled to room temperature and then evaporated to dryness under a gentle stream of nitrogen. The residues were re-dissolved in 100 μl isohexane and then transferred to vials for GC-MS analysis. To prevent against reaction inversion of derivatisation samples, the sample will be analysed immediately once being derivatised. And we don't analyse over 10 samples per day to ensure this.

2.7. GC-MS analysis

An Agilent 5975C MSD (mass selective detector) linked to 7890A GC with an autosampler (7683B) was used to analyse the soil extracts. Separations were effected on a Zebron ZB5-MS fused silica capillary column (30 m x 0.25 mm internal diameter x 0.25 μm film thickness, Phenomenex, Macclesfield, UK).

The oven temperature programme for BPA analysis started at 100°C for 1 minute; the temperature was then ramped at 10 °C min^{-1} to 200 °C and then ramped to 260 °C at 15 °C min^{-1}, ramped to 300 °C at 3 °C min^{-1} and held for 2 minutes. The carrier gas was helium and the flow rate was 1 mL min^{-1}. The samples (1 μl) were injected in splitless mode. The injector and mass spectrometer were held at 280 °C and 250 °C, respectively. The electron impact energy was set 70 eV for mass spectrometer.

Before sample analysis, relevant standards were analyzed to check instrumental performance, peak height and resolution. With each set of samples to be analysed, reference standard mixtures, quality control samples, and procedural blanks were run in sequence to check for contamination, instrumental performance, peak identification and quantification. Compounds were identified mainly by selected ion and by their retention times. The limit of detection (LOD) and percentage recovery range for the method were 0.37 ng g^{-1} and 90-128%, respectively. All results of soil sample analysis were reported on a dry-weight (dw) basis.

2.8. Statistical analysis

In study 1, the effects of treatment and time (weeks) from application were assessed, for each season separately, using a nested ANOVA, with the main effects being treatment and time, and blocking for plot to allow for samples being taken from the same plots over time. Within analyses, p-values < 0.05 were regarded as statistically significant. Owing to the fact that there were differences in concentrations of BPA in study soils before treatment applications, despite the fact that the experimental plots were in a single field that had not been previously treated with sludge or other organic fertiliser, pre-treatment values were included in the analyses as a covariate. The analysis of the spring data used the autumn pre-treatment values (August) as the covariate, and assesses the accumulated effects of the two sewage sludge applications on the spring BPA data. Response and covariate data were both log transformed prior to analyses to satisfy the assumption of constant variance. Results obtained in the respective seasons were not strictly comparable since the treated (T) soils had been exposed, previously, to sludge when it was applied in the autumn. However, in a separate ANOVA analyzing seasonal means, and again blocking for plot, effects of season and treatment were determined, in order to gain some insight into the potential importance of season and temperature effects. Statistical analyses were carried out using GenStat 16. In study 2, statistical analysis to assess evidence for a treatment effect was not possible, owing to the fact that the treatment plots were not replicated. However, since the soil samples were pooled within plots, and across sampling times, the data represented a robust measure of the soil BPA burden in these treatment plots.

2.9 Fugacity and plant uptake modelling

Soil characteristics (Table 1) were used to parameterize an equilibrium partitioning model (Level I fugacity model, MacKay, 2001) in order to predict the general partitioning behavior and preferential partitioning in an agricultural soil amended with sewage sludge. While it was not possible to validate the results of this model with actual measurements, it does provide an indication of how BPA might 'behave' in the soil environment. A three-compartment soil matrix [Hough et al., 2012; Pollard et al., 2008] consisting of: air ('A', pore space), water ('W', soil pore water) and sewage sludge amended soil ('SS'), for control sites it was assumed no sewage sludge was added to the soil ('S'). If these compartments are assumed to be in equilibrium, the total mass of BPA in the system (T, mol) is described by:

$$T_{BPA} = V_A C_A + V_W C_W + V_{SS} C_{SS} \qquad [1]$$

Where T_{BPA} is the total mass of BPA in the system, V represents the volume of each compartment (m^3), and C represents the concentration of BPA in each compartment (mol m^{-3}). The total mass of BPA, T_{BPA}, is known, thus Equation 1 is used to estimate the partitioning of BPA between the three phases (A, W, SS) within the soil matrix.

To quantify the equilibrium between the compartments), the relationships between C_A, C_W, and C_{SS} were estimated by deriving partition coefficients (Equations 2 and 3).

$$C_A = H\, C_W \qquad [2]$$

$$C_{SS} = k_d\, \rho_b\, C_W \qquad [3]$$

Where H is the Henry's Law constant, k_d is the distribution coefficient in soil, l kg^{-1}, and ρ_b is the bulk density, kg l^{-1}.

The partition coefficients can be used to characterize the distribution of BPA within the system (Equation 4).

$$T_{BPA} = V_A\, H\, C_W + V_W\, C_W + V_{SS}\ \rho_b\, k_d\, C_W \qquad [4]$$

Subsequently, the fractions of BPA in water (W_{BPA}), air (A_{BPA}), and sludge-amended soil (SS_{BPA}) can be derived from Equations 5-7:

$$W_{BPA} = \frac{V_W}{(V_W + H\, V_A + \rho_b\, k_d\, V_{SS})} \qquad [5]$$

$$A_{BPA} = \frac{H\, V_A}{(V_W + H\, V_A + \rho_b\, k_d\, V_{SS})} \qquad [6]$$

$$SS_{BPA} = \frac{\rho_b\, k_d\, V_{SS}}{(V_W + H\, V_A\, \rho_b\, k_d\ V_{SS})} \qquad [7]$$

To parameterise Equation 1, the volumetric composition of the sewage sludge-amended agricultural soil was derived by assuming a unit area of 1 m^2 and a plough depth of 0.25 m in order to calculate a total working volume of 0.25 m^3. All other parameter values required are detailed in Table 1.

Estimated concentrations of BPA in the three phases of the soil matrix (air, water, sewage sludge-amended soil) as derived using the Level I fugacity model (Equations 1 - 7) were used as input to a standard steady-state plant uptake model as described by Trapp and Legind [2011]. This model assumes that no removal of contaminants occurs due to degradation, infiltration and volatilization, and as such can be considered a precautionary approach. Finally, deposition of particles on the surfaces of leaves or grains is neglected and uptake from air is assumed solely by diffusive exchange in the gas phase. The steady-state expressions are given by:

$$C_{roots} = \frac{Q}{\frac{Q}{K_{rw}} + k_r M_r} C_W \qquad [8]$$

$$C_{plant} = \frac{\frac{Q_p}{K_{pw}} C_{roots} + A_p g_p C_A}{\frac{H}{K_{pw}} A_p g_p + k_p M_p} \qquad [9]$$

Where C_{roots} and C_{plant} are the concentrations in the roots and plant (here: leaves or grains), Q and Q_p are the transpiration stream (l d^{-1}) and the transpiration stream for leaves/grains (l d^{-1})

respectively, M_r and M_p are the root mass (kg ww) and leaf/grain mass (kg ww) respectively, A_p is the area of leaves or grains (m^2), g_p is the conductance of leaves/grains (m d^{-1}), and k_r and k_p are first-order growth rate coefficients for the roots and leaves/grains, respectively. K_{rw} and K_{pw} are the equilibrium partition coefficients (l kg^{-1}) between roots and water and between leaves/grains and water, respectively. These can be determined through the following empirical expressions:

$$K_{xW} = W_x + 1.22L_x(K_{OW})^b \qquad [10]$$

Where W_x and L_x are the water and lipid content of roots, leaves or grains and b is a correction factor for differences between solubility in octanol and sorption to plant lipids. Based on previous studies, b can be assumed to be 0.77 for roots and 0.95 for leaves/grains (Trapp and Legind, 2011).

3. Results and discussion

3.1 Concentrations of Bisphenol A in sewage sludge pellets

The concentrations of BPA determined in the sewage sludge pellets used in this study were 614-651 ng g^{-1} (n=3, mean: 636 ng g^{-1}, dw). In comparison, the level of BPA in these pellets is lower than that found in sludge from the Midwest USA (4600 ng g^{-1}; Kinney et al., 2008) , and Canada (33-36700 ng g^{-1}; Lee and Peart 2000) and similar to those reported in several areas, such as Greece (620 ng g^{-1}; Gatidou et al., 2007), Spain (5-680 ng g^{-1}; Dorival-Garcia et al., 2012), and Germany (4-1363 ng g^{-1}; Fromme et al., 2002). However, they are higher than those of Gran Canaria, Spain (1.4-54.9 ng g^{-1}; Vega-Morales et al., 2011), South California, USA (66-217 ng g^{-1}; Yu and Wu et al., 2012) and Ontaria, Canada (4-74 ng g^{-1}; Chu et al., 2005). These data indicate that the concentration of BPA in the sewage sludge used in this study is of a moderate level and within the range previously reported.

3.2. Background levels of BPA in Scottish soils and their source implications

Although there are numerous measurements of BPA in surface water and sediment, very little research has been carried out to determine concentrations in soils distant from potential point source pollution [Cousins et al., 2002; USEPA, 2010]. Background BPA concentrations at the study sites can be derived from the concentrations of BPA in the untreated soils used as experimental controls. In Study 1 (Glensaugh short-term trial, Figure 1), the concentrations of BPA were 0.67-10.57 (mean: 3.17), 0.68-8.18 (mean: 2.69) and 0.70-7.52 (mean: 3.34) ng g^{-1} in three different untreated soils, respectively. In Study 2 (Hartwood long-term trial, Figure 2), the concentration varied from 0.51 to 6.58 (mean: 3.22) ng g^{-1} in the background (control) soils. The data showed that the levels of this compound in different untreated soil (background level) from the two experiments (Glensaugh and Hartwood, Scotland) are very close (mean 3.02 and 3.22 ng g^{-1}, respectively). To our knowledge, the only BPA data reported in soil was similar to the background soil concentrations in Glensaugh and Hartwood, which were from 4 to 14 ng g^{-1} (mean: 6-7 ng g^{-1}) in United States [NTP-CERHR, 2008]. One of the main sources of organic pollutants in locations unaffected by direct point sources of pollution is atmospheric deposition. There have been a number of studies on BPA in air samples. Matsumoto et al. [2005] detected BPA in air deposition samples in Tokyo, Japan. Total deposition of BPA was detected in five out of eight samples in the range of 0.04 - 0.2 μg m^{-3} per day. Also Wilson et al. [2007] sampled indoor and outdoor air in North Carolina and Ohio, USA. BPA concentrations (with LOD of 0.9 ng m^{-3}) in indoor air were in the range from below the LOD to 0.19 μg m^{-3} while concentrations in outdoor air were in the range from below the LOD to 0.005 μg m^{-3} [Wilson et al., 2007]. Berkner et al. [2004] reported that BPA in aerosol from three different locations in north-east Bavaria, Germany. Two sampling sites were situated approximately 400 miles apart in the Waldstein mountain range (about 700m above sea level), one in a spruce forest and the other in a clearing. The third site was located near the city of Bayreuth. Despite the variation in sampling locations, BPA was detected at all three sites and at similar concentrations. The concentrations were in the range of 5 - 15 pg m^{-3} for the clear area in the forest, 10 - 15 pg m^{-3} in the forest and 10 pg m^{-3} in the urban area. An extensive study by Fu and Kawamura [2010] detected a wide range of global atmospheric concentrations 0.001-17.4 g m^{-3}. Such data suggests that wet or dry deposition could be an important source of the BPA detected in surface soil samples in addition to the primary source of land application of sewage sludge [Kang et al., 2006; Bailey and Hoekstra, 2010; Lemos et al., 2009; Flint et al., 2012]. Such findings indicate that background concentrations of BPA in soils not associated with direct inputs of BPA containing material (e.g the control soils in this study) may have a rather ubiquitous level of contamination and that inputs from the atmosphere are ongoing.

3.3. Short-term sewage sludge application study

During the autumn study period, ambient temperatures declined from approximately 12 to 6 ^{o}C. As summarized in Figure 1, the concentration of BPA in the three treated soil plots from Glensaugh are 1.16 - 9.15 (mean: 4.12), 1.14 - 9.87 (mean: 3.59), 1.37 - 12.07 (mean: 5.52) ng g^{-1}, respectively. The results of ANOVA showed that there was a significant treatment effect of sewage sludge application in the autumn study (application date: 09/09/2010), with treated soils having a significantly higher concentration of BPA over the entire study period ($F_{1,4}$ = 8.86, p = 0.041), the evidence for a treatment effect being strongest when differences in background concentrations of BPA in the soils prior to treatment are taken into account ($F_{1,3}$ = 93.8, p = 0.002). Concentrations of BPA varied significantly over the 85 days of the autumn study period ($F_{4,16}$ = 3.64, p = 0.027), irrespective of treatment applied. This variation in concentrations did not occur in a linear fashion e.g. a high soil concentration after the application of sewage sludge pellets and decreasing over time, but rather there was a significant quadratic trend over time ($F_{1,16}$ = 12.1, p = 0.003) with concentrations in both treated and control plots increasing over the earlier sampling times of the study to a maximum and then decreasing over later sampling times.

When the sewage sludge was applied for a second time to the same plots in the spring (application date: 07/03/2011), although the mean concentrations of BPA in the treated plots were consistently higher than in the control plots at all but the last of the sampling points, ANOVA showed that the effect of treatment overall was not significant. This could be a result of the small sample size (n=6 plots). As observed in the autumn study the BPA concentrations varied significantly over the study period ($F_{4,16} = 11.3$, $p < 0.001$) with concentrations once again varying in a quadratic manner ($F_{1,16} = 35.1$, $p < 0.001$) independently of whether or not sewage sludge had been applied. The mechanistic reasons for this observation are unclear and require further study.

The results from the ANOVA comparing seasonal mean BPA concentrations from autumn and spring showed no evidence of significant differences between seasons, irrespective of treatment applied.

3.4. Long-term sewage sludge application study

In Hartwood soils, BPA concentrations in the two treated soil plots were 12.9 - 167 ng g^{-1}, with a mean value of 62.6 ng g^{-1}, following bi-annual applications of sewage sludge over a 13 year period (Figure 2). The BPA concentrations in the sewage sludge amended soils are considerably higher (over 10 times) than those of the control plot 1 (0.51 to 6.58, mean: 3.22 ng g^{-1}). In addition to this observation, it is interesting to note that the BPA levels in treated plots from Hartwood were much higher than those of both untreated soils (0.67-10.6 ng g^{-1}, mean: 3.06 ng g^{-1}) and treated plots (1.14-12.1 ng g^{-1}, mean: 4.41 ng g^{-1}) in Glensaugh. The sewage sludge used for both sites (Glensaugh and Hartwood) treatment was from the same source. The difference in BPA concentrations between these two sites suggested that long-term sewage sludge application at Hartwood causes the gradual accumulation of organic contaminants over time.

There have been no previous studies on the fate of BPA in amended soils after repeated long-term sewage sludge treatment; however there have been a number of published studies detailing the behavior of other organic pollutants (e.g. polychlorinated biphenyls (PCBs), Chlorobenzenes (CBs) etc.; Alcock et al., 1995; Wang et al., 1995). Our recent results provided preliminary evidence of greater increases in soil burdens of some selected organic contaminant groups (PCBs, PBDEs, PAHs and DEHP) from the repeated sludge applications (over 13 years) although single applications did not show the same trend for all of those target chemicals, as observed for BPA in this study [Rhind et al., 2013]. In other studies, it was shown that the trend in the total PCB burden of the sludge-amended plots closely followed that of the control which suggested that air-soil exchanges exerted a strong influence on the trends in the sludge amended or untreated plots, although other, as yet unknown, mechanisms may also be involved . However, the sludge-amended soils (over 20 years of treatment) contained over 5 times more total PCBs than the control plot after the last application, a finding similar to that found for Bisphenol A in this study and PBDEs in other studies [Alcock et al., 1995; Eljarrat et al., 2008]. Wang et al., [1995] also reported that the concentrations of individual PCBs in both control and sludge-amended soil were very low and almost indistinguishable in the short term at the start of the study but as the sewage sludge application continued over the long term (over 20 years of treatment) the concentrations of all the PCBs in the sludge-amended soil increased and remained higher than those in the control plot even after sludge applications ceased. The study indicated that multiple sewage sludge applications increased the concentrations of PCBs in soil to levels that remained detectable at a higher level for a long period. Unlike many of the persistent organic pollutants which are generally resistant to anaerobic degradation, theoretically BPA can be degraded in soils under anaerobic conditions. However, BPA in treated soils from the long-term Hartwood study were considerably higher than those detected in the control plot. This may suggest that there is a recalcitrant soil bound BPA fraction that is not bio-available and therefore does not biodegrade. This is in agreement with the findings of Langdon et al., 2013, despite the significant differences in the temperature and likely soil moisture content between the two studies. This suggests that the rate of biodegradation of bioavailable BPA fraction in the sewage sludge may change but the recalcitrant fraction is always present, the concentration and the proportion of the bioavailable and recalcitrant fractions may be related to sewage sludge source and treatment method.

3.5 Equilibrium partitioning modeling

Looking at mean results over time for each of the experimental plots sampled in this study, the results of the Level I fugacity modelling (Equations 1 - 4) indicate that the majority of BPA (~96 % by mass) remains strongly sorbed to the sewage sludge-amended soil, where the proposed bioavailable fraction will be available to aerobic degradation and potential partitioning with almost 4 % (by mass) solubilized in the soil pore water, and less than 1 % estimated to enter the gaseous phase (Figure 3). These results seem consistent with the environmental behavior of BPA; it has a low vapor pressure (volatility), moderate water solubility, moderately high octanol water (K_{ow}) and organic carbon (K_{oc}) partition values. These latter properties explain why BPA interacts strongly with organic carbon rich particulate matter and why is found in solid matrices, such as sewage sludge produced during wastewater treatment [Mohapatra et al., 2011] and the proposed recalcitrant BPA fraction, strongly bound to soil components is predicted to be immobilised. Estimated uptake by plants (Equations 5 - 7) suggest that while only 4 % of the bioavailable BPA could enter the soil pore water, which is readily taken up into various food crops (Figure 4a) with leafy vegetables being particularly susceptible to BPA accumulation, followed by root vegetables and cereal grains. Uptake of BPA has been demonstrated in lettuce and brassicas in the laboratory using hydroponic systems which likely over-estimate uptake compared to that likely to occur in soil cultivation where only a small proportion of the BPA is predicted to be present in the pore water and is available for plant uptake [Dodgen et al., 2013]. However, it must be noted that uptake into crops is different from subsequent human exposure as the latter is dependent on the amount of the crops consumed. Taking the human exposure parameters published by Legind and Trapp [2011] as indicative, the estimates of BPA uptake can be converted into exposure (Figure 4b). It can clearly be seen that while uptake into cereal grains is relatively low (Figure 4a), exposure via cereal grains may be significant due to cereals making up a large proportion of the western diet [Legind and Trapp, 2011] but anaerobic degradation of a bioavailable BPA fraction in the soil is likely to lower such exposure and uptake significantly and the recalcitrant fraction would be unlikely to be available for plant uptake .

3.6 Assessment of potential exposure risk

BPA is classed as an endocrine disrupting chemical (EDC), capable of disrupting endocrine functions as an estrogen agonist or an androgen antagonist hormones. The assessment of the risk of BPA to organisms inhabiting or utilizing the soils in the reported studies is extremely complex, especially when considering that BPA is one compound within a mixture of molecules present in the soil matrix which produce similar biological effects in isolation. In comparison to natural estrogens (e.g. 17β-estradiol, estrone and estriol), BPA has been reported in many studies to have a lower binding affinity to estrogen receptors, however there is much debate about how much less (up to 3-4 times less in nuclear estrogen receptors (ER) whereas at membrane bound ER it has been reported to have a similar affinity to that of 17β-estradiol) but is generally present at much higher concentrations (μg L^{-1}) in the environment than such natural steroid estrogens (ng L^{-1}) [Molina-Molina et al., 2012, FitzGerald and Wilks, 2014]. When an organism is exposed to BPA there will be species and life stage differences in its toxicokinetic behavior. If BPA is taken up by an organism, for example via the ingestion of contaminated soil by a grazing sheep, relatively rapid conjugation to non-estrogenic glucuronide and sulphate metabolites and rapid excretion occurs leaving only a small proportion of active BPA (2.8% reported in rats, 0.9% reported in monkeys) available for ER binding [EFSA 2014]. Of concern though is the exposure of organisms to BPA and other EDCs when the metabolic system is under-developed, for example at the fetal and neonatal development phase [EFSA 2014]. What seems clear from the current study and earlier research published by this group [Rhind et al., 2013] and others, however, is that long-term amendment of soils with sewage sludge increases the concentrations of BPA and a mixture of other EDCs. The risk of exposure of organisms living in or ingesting the soil, to such compounds is inherently increased, especially where agricultural soils have been amended with sewage sludge over a long period of time (in this study, 13 years). The toxicological significance is currently unknown and is related to the bioavailability of the complex mixture (potentially strongly bound to soil particulate material) to organisms via either the soil and herbage or via food crops and their ability to metabolize and excrete such compounds. The current findings suggest that banning grazing for three weeks after sewage sludge amendment is appropriate in sites which have not undergone long tem and repeated sewage sludge amendment. Repeated, long term amendment of the same site with sewage sludge will lead to a significant increase in the recalcitrant BPA fraction concentrations in soil over time and this should be avoided as the risk of an organism ingesting such soil is currently unknown.

4. Conclusion

The effect of sewage sludge amendment of agricultural soils on soil BPA concentrations has been investigated at two contrasting locations at Scotland, in both short-term and long-term experiments. Initial results showed that BPA was present at moderate concentrations in the sewage sludge pellets used in the study and that atmospheric wet/dry deposition is likely to be an important source of soil BPA particularly in the short term study, in addition to the primary source of land application of sewage sludge. The long-term multiple sewage sludge applications to pastures in study 2 (Hartwood) resulted in a considerably higher soil concentration of BPA in comparison to controls, an observation that was not made in the short term study carried out at Glensaugh. This appears to support the theory that there is a recalcitrant BPA fraction in the soil, resistant or unavailable to anaerobic degradation and that this recalcitrant fraction could build up in soils over the long term. Modelling the environmental partitioning of BPA indicates a low risk of uptake by plants if the bioavailable BPA fraction is relatively rapidly biodegraded and the recalcitrant fraction is strongly bound and effectively immobilized in the soil. The greatest theoretical risk of exposure would therefore be to those organisms who directly ingest the soil matrix in fields which have been amended regularly with sewage sludge over a long time period. This study shows that a proportion of the inputted BPA does accumulate in soil after long term and repeated amendment with sewage sludge. Current legislation banning the grazing of animals for three weeks after the addition of sewage sludge may be sufficient in sites which have not undergone repeated and long-term sewage sludge amendment. Further study is required to determine the rate of degradation of the proposed biodegradable fraction of BPA under weather and soil conditions found in Scotland and the UK.

Acknowledgements

This work was funded by the Scottish Government' Rural and Environment Science and Analytical Service Division. M. Le Velly acknowledges the support of the ERASMUS student exchange program which funded her project placement from IUT 'A' Universite Paul Sabatier, Castres, France. This paper is dedicated to the memory of Dr. Stewart Rhind who was a highly regarded colleague and friend of his co-authors and was killed in a car accident prior to the publication of this manuscript.

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Table 1 Input data set for the standard model for the calculation of the Phase I fugacity equilibrium (Equations 1 - 4) and subsequent plant uptake (Equations 5 - 7; normalised to 1 m^2 of soil). From Trapp and Legind (2011).

Symbol	**Input [unit]**	**Value**
Fugacity equilibrium		
R_A	Sewage sludge application rate [t ha^{-1}]	50
A	Unit area [m^2]	1
ρ_{ss}	Sewage sludge density [kg L^{-1}]	0.6
V_{ss}	Applied sewage sludge volume [m^3]	0.0083
pd	Plough depth [m]	0.25
V	Soil-air-water volume [m^3]	0.25
θ_a	Air content of soil	0.1
θ_w	Water content of soil	0.3
n	Total porosity	0.4
Vs	Soil volume [m^3]	0.15
$f_{oc,\ soil}$	Fraction of organic carbon in soil	0.05
$f_{oc,\ ss}$	Fraction of organic carbon in sludge	0.5
P_a	Partial/vapour pressure [Pa]	$9.7x10^{-5}$
T	Temperature [K]	298
R	Gas constant [J/(mol*K)]	8.313
S	Solubility in water [mg/l]	300[a]
ρ_b	Bulk density [kg/l]	1.6
K_{OW}	Octanal:Water partition coefficient [l/kg]	3.32[b]
K_{OC}	Organic carbon distribution coefficient [l/kg] =10^(1.04 logK_{OW} – 0.84)	410.02
f_{OC}	Fraction of organic carbon in mixed system =(Vs $f_{OC,\ soil}$ + V_{SS} $f_{OC,\ SS}$)/(V_S + V_{SS})	0.068
K_d	Distribution coefficient in soil [l/kg] =K_{OC} f_{OC}	27.88
H	Henry's constant [dimensionless] =(P_a MW/(RT)/S)	1.75
Roots		
W_r	Water content of roots [L/kg]	0.89
L_r	Lipid content of roots [L/kg ww]	0.025
Q	Transpiration stream [L/d]	1
M_r	Root mass [kg ww]	1
k_r	First-order growth rate coefficient [1/d]	0.1
Leaves/grains		
A_p	Area of leaves [m^2]	5
	Area of grains [m^2]	1
W_p	Water content of leaves [L/kg]	0.8
	Water content of grains [L/kg]	0.15
L_p	Lipid content of leaves/grains [L/kg ww]	0.02
M_p	Mass of leaves/grains [kg ww]	1
ρ_p	Density of leaves/grains [kg ww/L]	1
g_p	Conductance of leaves/grains [m/d]	86.4
k_p	First-order growth rate coefficient for leaves/grains [1/d]	0.035
Q_p	Transpiration stream for leaves [L/d]	1
	Transpiration stream for grains [L/d]	0.2

[a] EINECS, 2010

[b] Hansch, Leo et al.,1995; Canada, 2008

Figure legends

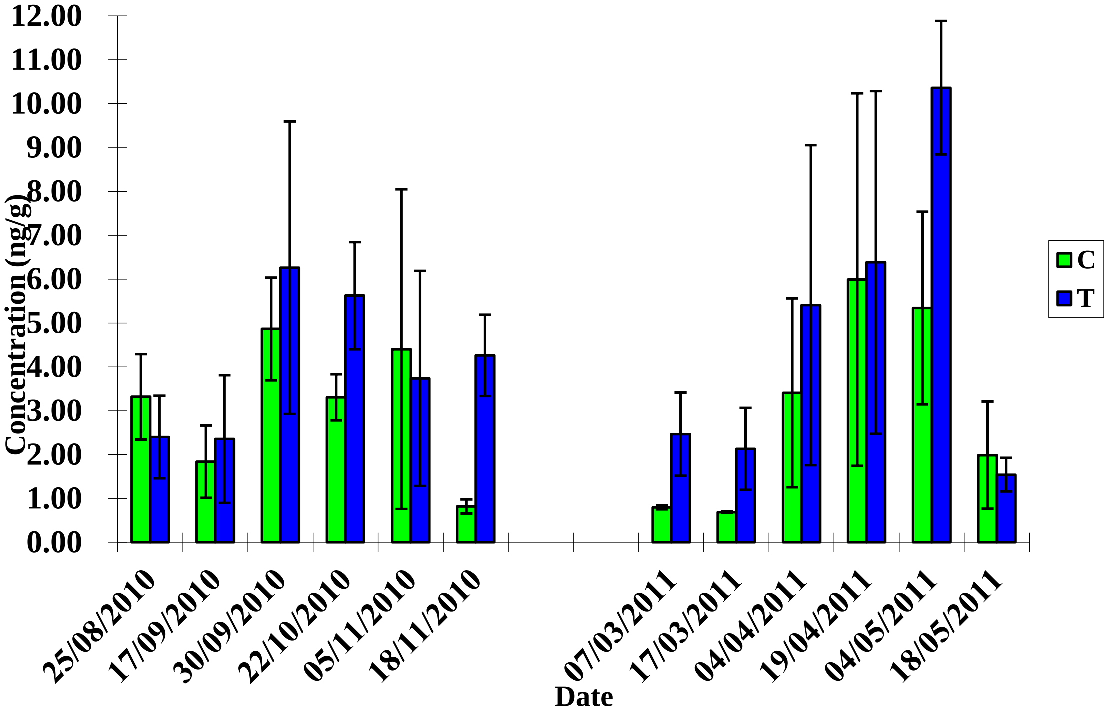
Figure 1. Mean BPA concentrations (ng g^{-1}, n=3, error bars: standard deviation) in control and treated soils of Study 1 (Glensaugh of Scotland, UK).

Figure 2. BPA concentrations (ng g^{-1}) in control and treated soils of study 2 (Hartwood of Scotland, UK).

Figure 3. Partitioning equilibrium of BPA within the three-phase fugacity model (Equations 1 - 4) showing partitioning to sewage sludge-amended soil, soil pore water and soil air. GS represents plots from the Glensaugh site (study 1), HW represents plots from the Hartwood site (study 2).

Figure 4. Results of the plant uptake and human exposure (Equations 5 - 7). (a) predicted uptake of BPA into root vegetables, leafy vegetables and cereal grains from agricultural soils of Glensaugh (GS, Study 1) and Hartwood (HW, Study 2; (b) predicted exposure of humans to BPA from water, air, soil, root vegetables, leafy vegetables, and cereal grains grown on agricultural soils of Glensaugh (GS, Study 1) and Hartwood (HW, Study 2).

Fig 1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0.00
Concentration (ng/g)
25/08/2010
17/09/2010
30/09/2010
22/10/2010
05/11/2010
18/11/2010
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17/03/2011
04/04/2011
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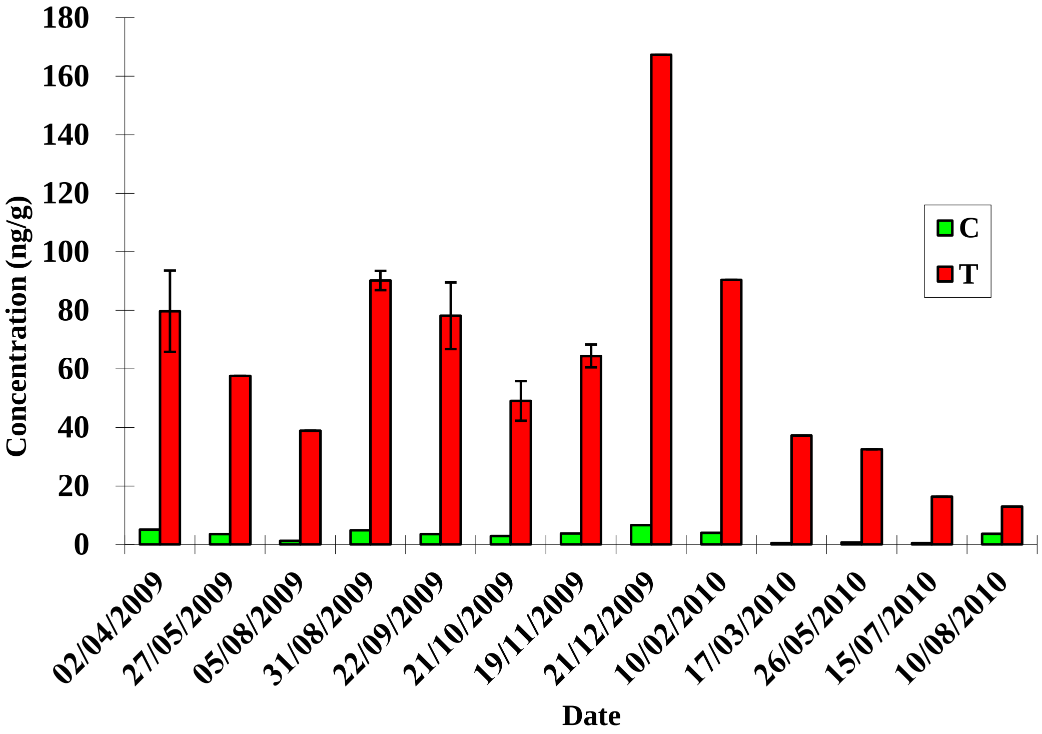
Fig 2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C
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22/09/2009
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15/07/2010
10/08/2010
Date

Fig 3

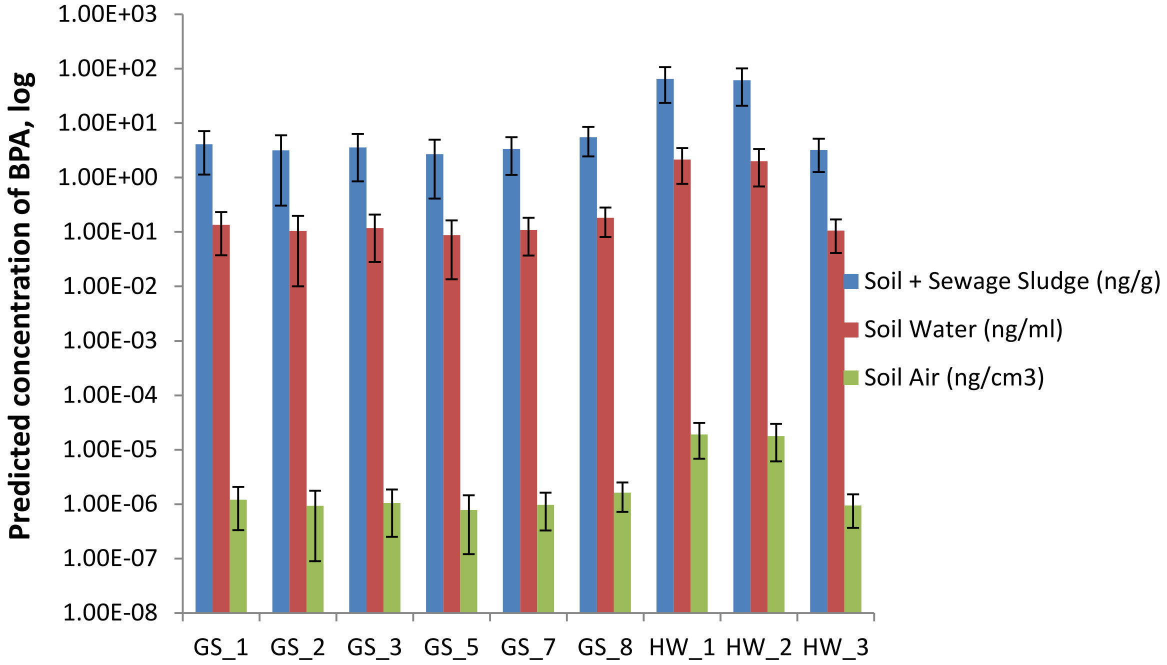
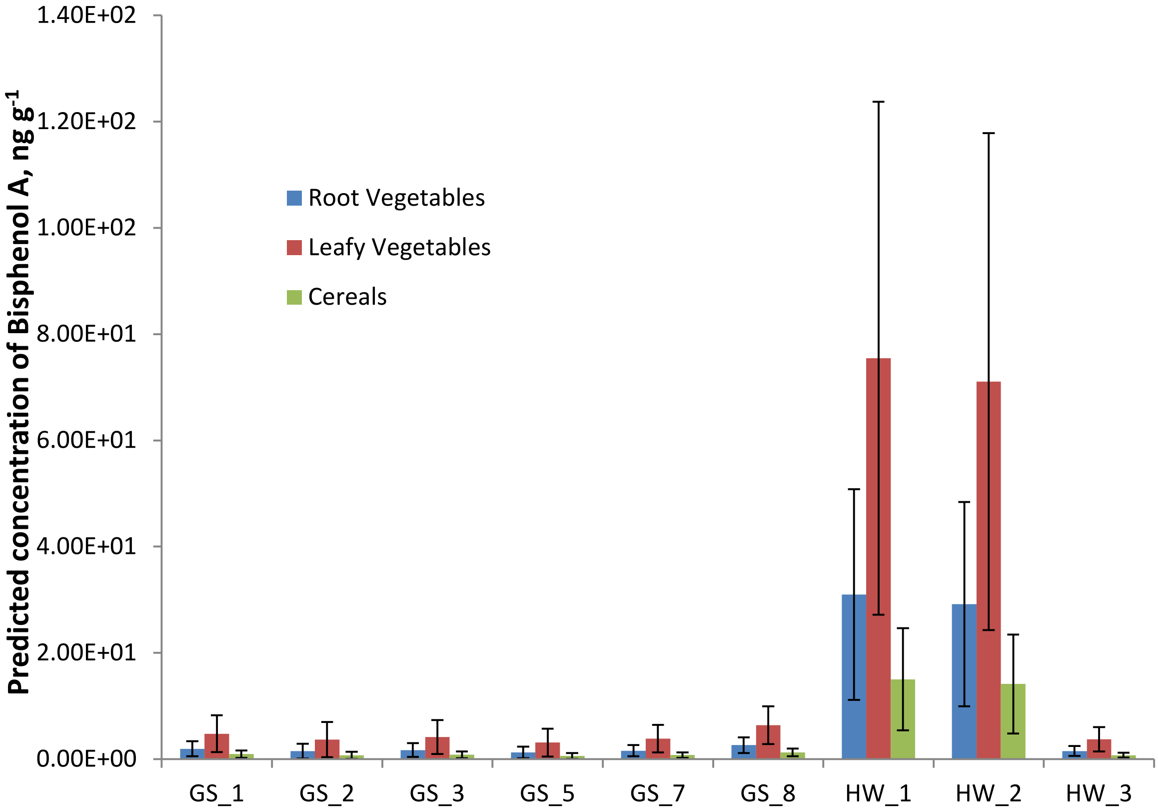
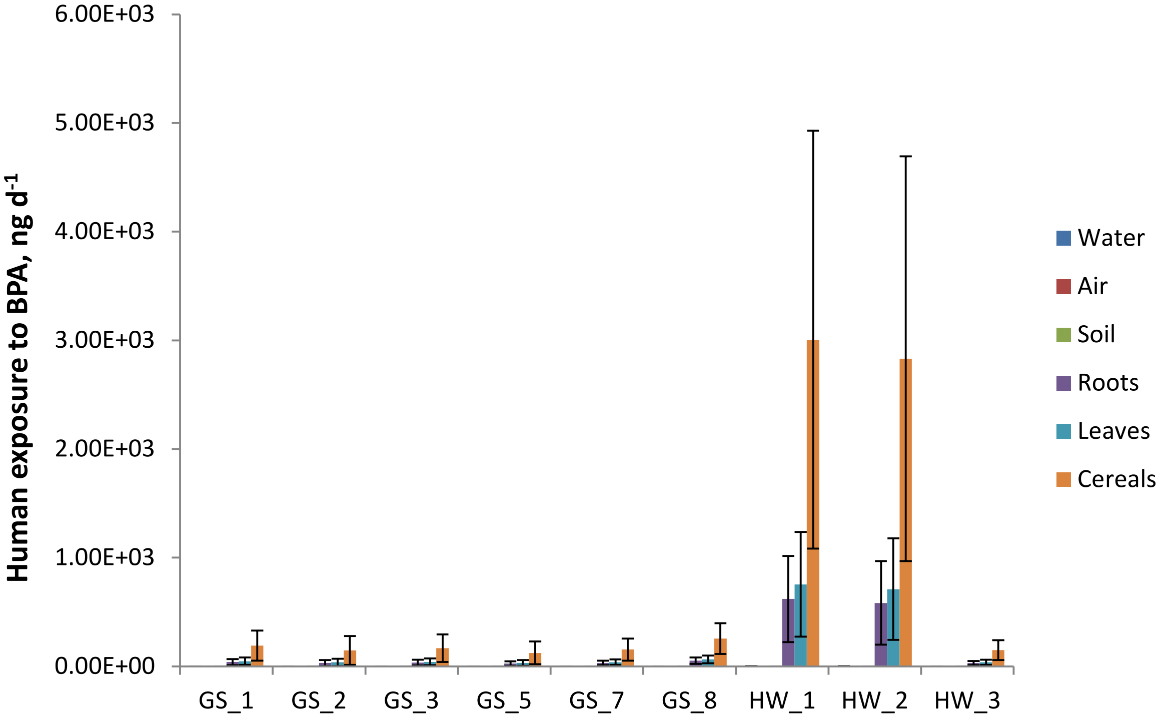

Predicted concentration of BPA, log
1.00E+03
1.00E+02
1.00E+01
1.00E+00
1.00E-01
1.00E-02
1.00E-03
1.00E-04
1.00E-05
1.00E-06
1.00E-07
1.00E-08
GS_1
GS_2
GS_3
GS_5
GS_7
GS_8
HW_1
HW_2
HW_3
Soil + Sewage Sludge (ng/g)
Soil Water (ng/ml)
Soil Air (ng/cm3)

Fig 4a



Predicted concentration of Bisphenol A, ng g^{-1}
1.40E+02
1.20E+02
1.00E+02
8.00E+01
6.00E+01
4.00E+01
2.00E+01
0.00E+00
Root Vegetables
Leafy Vegetables
Cereals
GS_1
GS_2
GS_3
GS_5
GS_7
GS_8
HW_1
HW_2
HW_3

Fig 4b


Human exposure to BPA, ng d-1
6.00E+03
5.00E+03
4.00E+03
3.00E+03
2.00E+03
1.00E+03
0.00E+00
GS_1
GS_2
GS_3
GS_5
GS_7
GS_8
HW_1
HW_2
HW_3
Water
Air
Soil
Roots
Leaves
Cereals