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Uptake of pharmaceuticals, hormones and parabens into vegetables grown in soil fertilized with municipal biosolids

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ABSTRACT

Several recent greenhouse studies have established the potential for uptake of human pharmaceuticals from soil fertilized with municipal biosolids into a variety of crops. In the present study, a field experiment was undertaken to evaluate the uptake of organic micropollutants from soil fertilized with municipal biosolids at a regulated application rate into tomatoes, carrots, potatoes and sweet corn produced under normal farming conditions. The vegetables were grown according to farming practices mandated by the province of Ontario Canada, the key feature being a one-year offset between biosolid application and the harvest of crops for human consumption. Biosolids at application, and crop samples following harvest were analyzed for 118 pharmaceuticals and transformation products, 17 hormones or hormone transformation products, and 6 parabens. Analyte concentrations in the biosolids were consistent with those detected in other surveys. Eight of the 141 analytes were detected in one or two crop replicates at concentrations ranging from 0.33 to 6.25 ng/g dry weight, but no analytes were consistently detected above the detection limit in all triplicate treated plots. Overall, this study suggests that the potential for micropollutant uptake into crops under normal farming conditions is low.

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1. Introduction

In many jurisdictions municipal biosolids are commonly applied to agricultural land as a valued source of nutrients for crop production and organic matter for improvement of soil quality. In the province of Ontario, Canada for example, about 120 thousand dry tonnes of sewage biosolid material is applied annually to agricultural fields as a fertilizer, recycling urban nutrients into crop production. The sustainable agronomic use of biosolids must be undertaken with consideration of the potential risk to environmental and human health posed by microbial, organic and inorganic contaminants that may be found in the material (NRC, 2002). Mandated management practices that mitigate risk associated with the agricultural use of biosolids can include pretreatment methods that reduce pathogen content; criteria for judicious choice of application location, rates and timing; and offset times between biosolid application and permitting livestock to graze on treated pastures, or the harvesting of crops destined for human consumption (O. Reg. 267/03). Recently, pharmaceuticals and personal care products (PPCPs) and other classes of micropollutants have received significant regulatory and scientific scrutiny as an important class of emerging organic contaminants detected in biosolids (USEPA, 2009; Clarke and Smith, 2011). We have characterized the soil persistence and transport potential via tile flow or surface runoff of numerous micropollutants from biosolids-treated land in the context of evaluating the risk of exposure to adjacent water (Lapen et al., 2008; Topp et al., 2008; Edwards et al., 2009; Sabourin et al., 2009; Topp et al., 2010). Consumption of crops by humans or forages by livestock grown in biosolids-fertilized land is a route of human exposure to micropollutants that has not been fully evaluated. This in light of a number of recent studies that have established the potential of crop uptake of micropollutants detected in biosolids or detected in wastewater which may ultimately end up in biosolids (Redshaw et al., 2008; Herklotz et al., 2010; Jones-Lepp et al., 2010; Wu et al., 2010; Eggen et al., 2011; Shenker et al., 2011). All of these studies are limited however in that they were undertaken under greenhouse or hydroponic conditions that are not representative of farming practices and soil conditions typical of commercial agriculture. In the present study, we evaluated the uptake of numerous PPCPs, hormones and parabens into vegetable crops grown in biosolids-treated field soil employing farming practices mandated by the Province of Ontario, the key element of which is a one-year offset

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between biosolid treatment and the harvest of crops destined for human consumption. This is to our knowledge the first time that such a comprehensive analysis has been made of micropollutant crop uptake from soil fertilized with biosolids.

2. Materials and methods

2.1. Field experiment

The experiment took place at the Environmental Sciences Western Experimental Field Station of the University of Western Ontario (latitude 43°4′47″N, longitude 81°20′24″W) from April 2009 to September 2010. The soil at the site is a silt loam (gray brown Luvisol) belonging to the Bryanston soil association with the following key properties: pH of 7.5, sand/silt/clay composition (%) of 18/67/15, and organic matter content of 3.4%. The experimental area had not previously received any biosolids. On April 22, 2009, dewatered municipal biosolids were manually applied to a 9 m × 12 m plot using plastic pails to treatment plots at a standard commercial rate of 8000 dry kg per ha. Anaerobically-digested sludge from nine mechanical activated sludge wastewater treatment plants and organic residuals from two water treatment plants in the Niagara Region are stored in a lagoon at the facility. The material is pumped into glass lined storage tanks, mixed and concentrated with a high speed centrifuge. The biosolids were dewatered to 28.8% total solids by weight, had a pH value of 8.0, and did not receive any further treatment prior to application. Biosolids' aggregate diameter ranged from about 1 to 5 cm and soil coverage was close to uniform. The biosolids were incorporated into the treated plot a few hours after surface application at a depth of 15 cm using a Kongskilde vibro-shank cultivator. A nearby control plot with the same dimensions as the treated did not receive biosolids but otherwise received the same cropping and agronomic management. On June 22, 2009, Roundup (glyphosate) was applied to control weeds. Weed control was subsequently done manually. Both plots were entirely cropped to barley (*Hordeum vulgare*; OAC Kawartha) as a cover crop. Barley was harvested in August 2009. In May 2010, both control and treated plots were subdivided into twelve 1 m × 3 m plots. These were cropped to a variety of vegetables that grow above and below ground. Three replicates of tomatoes (variety H9909; 5 cm spacing), potatoes (*Solanum tuberosum* variety; 5 cm spacing), carrots (*Daucus carota*, variety coreless Scarlet Nantes; thinned to 5 cm) and sweet corn (*Zea mays* var Honey Select; 5 cm spacing), for a total of twelve plots, were planted on the area previously cropped with barley. Samples of the edible portions of each crop were harvested in the fall of 2010 when they would visibly be market ready. These were washed with distilled water to remove any adhering soil, and then archived at −70 °C until analysis by a commercial laboratory.

2.2. Sample preparation and analysis

A biosolid sample was collected for analysis by pooling 15 subsamples obtained randomly from the biosolids pile with a clean (EtOH rinsed) stainless steel implement. The subsamples were thoroughly mixed before placing in sterile plastic containers. All vegetable samples were sent frozen to the commercial laboratory Axys Analytical Services (Sidney, BC, Canada) for extraction and analysis. Thawed samples were homogenized using either a Quaker City grinding mill (potato, carrot, unpeeled) or a Vertis mixer (sweet corn kernels, tomato) and sub samples (5 g wet weight) taken for PPCP analysis using USEPA Method 1694 (USEPA, 2007a). Two 0.5 g portions of each homogenized sample were taken for hormones and paraben analyses. Samples were adjusted to a pH of 2.0 and spiked with surrogates. This was followed with extraction by sonication with aqueous buffered acetonitrile and with pure acetonitrile. Extracts were concentrated by rotary evaporation, diluted with ultra pure water to 200 mL and cleaned up by solid phase extraction (SPE). After adding recovery standards, the extracts were analyzed by LC/ESI-MS/MS in positive and negative ionization modes. Data are reported on a dry weight basis.

Pharmaceuticals and personal care products were analyzed using EPA method 1694, and hormones following methods MLA-068, based on EPA method 1698, and modified to include extra labeled internal standards and additional target analytes, and MLA-072, an in-house, performance-based method. All QA/QC protocols described in the original EPA method 1698 were followed, including the use of surrogates and recovery standards as well as blanks, and similar protocols were followed for MLA-072 (USEPA, 2007b). Results were reported down to the sample specific detection limit (SDL) for method 068, which is defined as the sample concentration equivalent to an analyte peak 2.5 times the height of the noise in the m/z channel of interest. For method 072, results were typically reported down to the lower method calibration limit (LMCL), which is the lowest calibration standard prorated for extract volume and sample size. However, where the SDL was greater than LMCL, the SDL was reported instead. Briefly, whole water samples were spiked with labeled surrogate standards and then cleaned up by solid phase extraction (SPE) on HLB cartridges. Extracts were then dried using sodium sulfate, split as necessary and derivatized with BSTFA prior to analysis by capillary gas chromatography and low resolution mass spectrometry (LRMS) or high resolution mass spectrometry (HRMS) for both hormones and sterols. For all solid samples, sterols were analyzed following the same method as for liquid samples, with the additional step of extraction by sonication with aqueous buffered methanol prior to cleanup by SPE. For hormone analyses, solid samples were first adjusted to the required pH (2.0) and then spiked with labeled surrogates. Samples were then extracted by sonication with aqueous buffered acetonitrile and pure acetonitrile, before cleanup with SPE. Extracts were then filtered and analyzed by LC/ESI-MS/MS. The initial DMB and the 60-day post-application incorporated DMB sample were originally analyzed using the GC-LR/HRMS method described above. However, better detection limits were achieved for most analytes in subsequent re-analysis using the LC/ESI-MS/MS method. Better detection limits were achieved with androsterone and estriol following the original analysis method and these results are reported in the text for these two analytes. Desogestrel and beta-estradiol 3-benzoate are not included in the LC/ESI-MS/MS method, so for the initial DMB concentrations, results from the GC-LR/HRMS were also used.

3. Results and discussion

3.1. Micropollutants in the biosolids

The biosolid material used in the experiment was analyzed for a total of 118 PPCPs or PPCP transformation products, 6 parabens, and 17 hormones or hormone transformation products. Most analytes were not detected in the biosolids (Supplementary Table 1) but a number were (Table 1). Five of the analytes were detected at concentrations about 1000 ng/g, 13 between 1000 and 100 ng/g and 35 below 100 ng/g (Table 1). Prominent in the list of detected PPCPs were agents that are toxic to microorganisms. These include antimicrobial biocides triclosan (TCS) and triclocarban (TCC), a variety of classes of antibacterials including fluoroquinolones (ciprofloxacin, norfloxacin, ofloxacin, enrofloxacin), tetracycline (tetracycline, doxycycline), and macrolides (azithromycin, clarithromycin), and the antifungal agent miconazole. A number of transformation products of antibacterial agents were detected including 4-epitetracycline, anhydrotetracycline, anhydrochlortetracycline, and erythromycin-H_2O. The antihelminthic thiabendazole was also detected. Otherwise, numerous classes of pharmaceuticals were present including a number of antidepressants (sertraline, amitriptyline, paroxetine, fluoxetine), non-steroidal anti-inflammatory drugs (ibuprofen, naproxen),

Table 1
Pharmaceuticals, personal care products, hormones and parabens detected in biosolids. Concentrations of analytes are in ng/g dry weight, and rank ordered from highest to lowest. Detection limits (ng/g) are indicated in brackets.

Triclocarban	6030 (6.1)	Norverapamil	94.7 (0.305)	Cotinine	14.8 (2.94)
Ciprofloxacin	5870 (12.2)	Carbamazepine	94.3 (3.05)	Codeine	14.6 (5.88)
Triclosan	4680 (122)	Fluoxetine	89.8 (3.05)	Naproxen	14 (6.34)
Norfloxacin	1750 (30.5)	Valsartan	76.5 (8.13)	Hydrocodone	11 (2.94)
Ofloxacin	1068 (30.5)	Verapamil	70.2 (0.31)	Diltiazem	10.1 (0.846)
Diphenhydramine	781 (1.22)	Clarithromycin	67.4 (3.05)	Enrofloxacin	10.1 (7.14)
Sertraline	497 (0.813)	Estrone	62 (6.67)	Gemfibrozil	7.89 (3.13)
Miconazole	477 (3.05)	Norfluoxetine	59.6 (30.5)	Methyl Paraben	7.14 (6.86)
Amitriptyline	448 (0.854)	Anhydrotetracycline	55.8 (38.4)	DEET	6.89 (0.305)
4-epitetracycline	386 (12.2)	Androstenedione	51 (3.34)	Erythromycin-H_2O	4.06 (0.61)
Tetracycline	341 (15.3)	Doxycycline	42.4 (12.2)	Ranitidine	3.26 (1.18)
Azithromycin	213 (3.05)	Cimetidine	42.1 (1.18)	Propoxyphene	2.9 (0.61)
Ibuprofen	167 (30.5)	Digoxigenin	38.1 (19.9)	Atenolol	2.88 (1.18)
Triamterene	153 (0.588)	Propranolol	35.4 (4.07)	Benztropine	2.46 (0.61)
Amphetamine	147 (2.94)	Anhydrochlortetracycline	32.9 (30.5)	Testosterone	16.1 (2.12)
Paroxetine	130 (8.13)	10-OH-amitriptyline	23.3 (0.31)	Desmethyldiltiazem	2.05 (0.305)
Progesterone	130 (2.79)	Thiabendazole	16.5 (3.05)	Diazepam	0.845 (0.61)
Amlodipine	120 (8.13)	Atorvastatin	15.1 (9.14)		

antihistamine (diphenhydramine), calcium channel blockers (amlodipine, diltiazem) beta blockers (propranolol, atenolol), the diuretic triamterene, the anticonvulsant carbamazepine, regulators of lipid (gemfibrozil) and cholesterol (atorvastatin) metabolism, h2 histamine receptor antagonists (cimetidine, ranitidine) used to treat dyspepsia and opiate analgesic agents (codeine, hydrocodone). The steroidal hormones progesterone, estrone, testosterone and androstenedione were detected as was methyl paraben. The range of contaminants present and their concentrations are in general agreement with other recent surveys of chemical composition of biosolids (USEPA, 2009; Walters et al., 2010; McClellan and Halden, 2010).

3.2. Micropollutants in vegetables at harvest

Triplicate samples of vegetable crops grown in ground treated with a commercial rate of biosolids were evaluated for uptake of any of the full panel of analytes, and compared to a single sample of vegetables grown in ground that had not been treated with biosolids. The vast majority of the analytes were not detected in any vegetable crop (Supplementary Table 2). However, 24 analytes were detected in vegetables at least once (Table 2). In nine cases an analyte was detected in the three vegetable replicates from biosolids-treated soil:DEET (all values in ng/g dry weight; 0.06 +/− 0.02) and progesterone (4.7 +/− 1.95) in sweet corn; cocaine (0.14 +/− 0.18) and DEET (0.03 +/− 0.01) in carrots; minocycline (13.83 +/− 1.00), atenolol (3.95 +/− 1.62) and testosterone (4.2 +/− 1.32) in tomatoes; metformin (12.5 +/− 2.38) and testosterone (5.1 +/− 0.37) in potatoes. In every one of these nine cases the analyte was also detected in the control vegetable sample grown in the absence of biosolids. In two cases, ciprofloxacin in sweet corn (1.76, 2.14) and progesterone in potatoes (0.32, 0.34), an analyte was detected in two treated samples and not in the control sample. In six cases an analyte was detected in one treated sample and not in the control: glyburide (0.6) and naproxen (0.8) in sweet corn; triamfarene (0.064) in carrot; 4-epianhydrotetracycline (6.25), caffeine (4.24) and atenolol (0.5) in potato. The frequency of analyte detection in the unpeeled root vegetables was no higher than in the sweet corn or tomatoes. Based on the

Table 2
Analytes detected in vegetables at harvest. A single crop sample from untreated control, and three pooled crop samples from triplicate biosolids-treated plots were analyzed. Data are expressed as ng/g dry weight, and where triplicate samples were positive, data mean +/− std. dev is provided. U = undetected, NQ = not quantified.

Analyte	Sweet corn		Carrot		Tomato		Potato	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Atenolol	U	U	U	U	2.77	3.95 +/− 1.62	U	0.5
Benzoylecgonine	U	U	0.304	U	U	U	U	U
Caffeine	U	U	U	U	U	U	U	4.24
Ciprofloxacin	U	2.14, 1.76	2.1	2.15, 1.41	U	U	U	U
Cocaine	U	0	0.395	0.14 +/− 0.18	0.039	U	0.1	0.34, 0.44
Cotonine	U	U	0.364	U	U	U	U	U
DEET	0.33	0.06 +/− 0.02	0.22	0.03 +/− 0.01	0.272	0.019	U	U
Digoxigenin	U	U	U	U	U	U	4.2	U
4-Epianhydrotetracycline	U	U	U	U	U	U	U	6.25
Glyburide	U	0.6	U	U	U	U	U	U
Ibuprofen	U	U	U	U	2.99	U	U	U
Lincomycin	U	U	U	U	1.19	U	U	U
Metformin	U	U	U	U	U	U	14	12.5 +/− 2.38
Minocycline	U	U	U	U	16.2	13.83 +/− 1.00	NQ	NQ
Naproxen	U	0.8	U	U	U	U	U	U
Norfloxacin	U	U	26.6	4.5	U	U	U	U
Penicillin G	5.7	2.5	U	U	U	U	U	U
Progesterone	4.82	4.7 +/− 1.95	0.772	U	U	U	U	0.32, 0.34
Sulfadimethoxine	U	U	U	U	0.064	U	U	U
Sulfamerazine	U	U	U	U	0.526	0.343, 0.343	U	U
Testosterone	U	U	U	U	5.65	4.2 +/− 1.32	4.9	5.1
Triamfarene	U	U	U	0.064	U	U	U	U
Trimethoprim	U	U	U	U	0.387	0.432	U	U

criterion that plant uptake must be evidenced by detection in each of the three replicated treated samples and not the control sample, results are not significant (i.e. are below detection limit) for any of the 141 analytes evaluated in this study. The apparently aberrant detection of analytes in some control samples is presumably due to cross-contamination, perhaps with soil, or at some point in the laboratory manipulations. The fact that no single analyte was detected in all four control samples (one for each vegetable) indicates that there was no consistent background soil contamination or contamination from irrigation water. The absence of micropollutant residues in irrigation water was verified and confirmed by chemical residue analysis (data not shown).

A small number of analytes detected in a few vegetable samples were not detected in biosolids: glyburide (one sweet corn sample), 4-epianhydrotetracycline (one potato), monocycline (detected in all 4 tomato samples), caffeine (one potato), Penicillin G (one corn sample from treated plot and one from control plot), sulfadimethoxine (tomato untreated) and sulfamerazine (tomato untreated and two treated), trimethoprim (tomato untreated and one treated), benzoylecgonine (carrot control), cocaine (all carrot samples, untreated tomato, all potato samples), and metformin (all potato samples). Presumably this could be due to a higher detection limit in biosolids than in crops in some cases, and sample cross contamination in others. The detection of metformin at comparable concentrations in all treated and control potato samples suggests that some other chemical in this vegetable was misidentified as this drug, but this would have to be confirmed.

Results from the present study indicate that the potential for drug uptake into vegetables grown under normal farming conditions is very low, in contrast with greenhouse studies that concluded that the potential for uptake into crops is significant (Redshaw et al., 2008; Herklotz et al., 2010; Jones-Lepp et al., 2010; Wu et al., 2010; Eggen et al., 2011; Shenker et al., 2011). During the mandated year between biosolid application and crop harvest, residue degradation, formation of soil-bound residues and loss by leaching are all factors that would limit the availability of micropollutant residues for subsequent uptake from the soil into crops. These processes would not be adequately represented in short term greenhouse or laboratory studies, and therefore these experiments would not represent the risk of crop uptake under normal farming conditions. Crop micropollutant uptake from soil fertilized with biosolids treated by composting or other methods could similarly be evaluated.

In summary, the present study revealed no significant uptake of a range of micropollutants that vary widely in their chemical properties into any of a variety of crops. On this basis, we suggest that there is little risk of PPCP uptake into vegetable crops produced according to current mandated regulations that specify a one-year offset between biosolid application and crop harvest.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2012.05.017.

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