Method development and application to determine potential plant uptake of antibiotics and other drugs in irrigated crop production systems

Tammy L Jones-Lepp[*1], Charles A Sanchez[2], Thomas Moy[3], Reza Kazemi[4]

[1]U.S. Environmental Protection Agency, Research Chemist, Office of Research and Development, National Exposure Research Laboratory-Environmental Sciences Division, Las Vegas, NV 89119, (702) 798-2144, jones-lepp.tammy@epa.gov; [2]University of Arizona, Department of Soil, Water, and Environmental Sciences, Yuma Agricultural Center, Yuma, AZ; [3]Senior Environmental Employee Program, U.S. Environmental Protection Agency, 944 E. Harmon Ave., Las Vegas, NV 89119; [4]Student Services Contract, U.S. Environmental Protection Agency, 944 E. Harmon Ave., Las Vegas, NV 89119

Abstract

Studies have shown the detection of emerging contaminants (ECs), of which pharmaceuticals are a subset, in surface waters across the United States. The objective of this study was to develop methods, and apply them, to evaluate the potential for food chain transfer when EC-containing waters are used for crop irrigation. Greenhouse experiments were performed where select food crops were irrigated with water spiked with three antibiotics. Field experiments, at two different sites, were conducted. Select crops were irrigated with wastewater effluent known to contain ECs, EC-free well water, and Colorado River water containing trace-level ECs. The results of the greenhouse studies show the potential for uptake of one or more of the antibiotics evaluated, albeit at very low levels. In those food crops watered with wastewater effluent only an industrial flavoring agent, n,n'-dimethylphenethylamine (DMPEA) was consistently found. None of the evaluated contaminants were found in crops irrigated with Colorado River water.

Keywords: emerging contaminants; crop uptake; pressurized liquid extraction; liquid chromatography-electrospray-ion trap mass spectrometry/mass spectrometry; LC-MS/MS

Introduction

In the southwestern part of the United States, increasing demands on scarce water resources has forced water authorities to look for alternative water resources. Some water authorities use treated wastewater effluent for injection into ground water aquifers for the purpose of pumping it out later and re-use, with further treatment, as drinking water (*1*). Other municipalities use treated wastewater effluent for non-potable water reuse, e.g., watering of golf courses and municipal green spaces, as well as a source of irrigation water for crops (*2*). Of concern are the reports of numerous pharmaceuticals and other emerging contaminants (ECs) found in these groundwaters. Rowe et al. (*3*) reported that at least one EC was present in 76% of shallow urban wells sampled in the Great and Little Miami River Basins in Ohio and found that the number of ECs detected increased with increasing urban land use.

Although pharmaceuticals designed for human or veterinary use have a specific biological mode of action, the impact on non-target species is rarely known. Since pharmaceuticals are released into the environment as complex mixtures, and not as individual compounds, there exists the possibility for synergistic, or antagonistic, interactions resulting in unexpected biological effects. The concentrations of pharmaceuticals in drinking water supplies are likely to be below any level of direct risk to humans. However, it is the persistence and presence of antibiotics in the environment that could pose a serious threat to human health. (*4-7*). The principal existing concern with antibiotics is the identification of growing resistance in microbial populations (*7-10*). Resistance has been found in bacteria isolated from the innards of animals treated

with antibiotics, in their corresponding manure (*11*), and in agricultural soils receiving manure (*12, 13*). There is concern that non-pathogenic bacteria can serve as a platform for gene transfer to pathogenic organisms as a result of promiscuous exchange of genetic material among microbes (*5, 14*). Antibiotic-resistant bacteria have been found in surface water (*6, 7*), sediments (*15, 16*), and ground water (*10, 17*).

Recent studies have shown that human-use antibiotics (azithromycin, clindamycin, and roxithromycin) are environmentally available in wastewaters, source waters, and biosolids (*18-21*). Several researchers have demonstrated that certain veterinary antibiotics (e.g., florfenicol, trimethoprim, sulfamethazine, enrofloxacin, etc.) can be taken up into food crops (e.g., wheat, corn, lettuce, barley, and potato) produced on manure-amended soils (*22-25*). Recently, Herklotz et al. (*26*) published a study of the uptake of human pharmaceuticals (e.g. carbamazepine, salbutamol, sulfamethoxazole, and trimethoprim) into cabbage (*Brassica rapa var. pekinensis*) and Wisconsin Fast plants (*Brassica rapa*) in a hydroponic garden setting.

At the Imperial Diversion Dam (IDD) near Yuma, AZ almost 5 billion m^3 of water are diverted from the Colorado River to irrigate the approximately 400,000 ha of agricultural crops that are shipped nationally and internationally. Previous research has shown that the Colorado River is contaminated with low levels of perchlorate and this contaminant can be detected in most agricultural commodities irrigated with this water (*27, 28*). Macrolide antibiotics, pseudoephedrine, and illicit drugs have been identified in several municipal wastewater streams that discharge into the Colorado River (*29*). There

is a probability that the drugs present in water could potentially reach food crops. The research presented in this paper will focus on the development and ground-truthing of analytical methods for determining the fate of ECs (e.g., antibiotics, illicit drugs, over-the-counter (OTC) drugs) into food crops via a three-part study. There was an emphasis on method development for detecting three antibiotics - azithromycin, roxithromycin and clindamycin. Azithromycin and clindamycin due to their wide-spread usage in the US (*18*), http://drugtopics.modernmedicine.com/drugtopics/data/articlestandard//drugtopics/252010/674976/article.pdf, and roxithromycin due to its surreptitious usage. Roxithromycin is not prescribed in the US, but has been detected in wastewaters and biosolids in the US (*18, 20*).

MATERIALS AND METHODS

Chemicals. Clarithromycin was obtained from U.S. Pharmacopeia (Rockville, MD). Azithromycin, roxithromycin, clindamycin, and n,n'-dimethylphenethylamine (DMPEA), were obtained from Sigma-Aldrich (St. Louis, MO). Methamphetamine, MDMA, d_5-MDMA, and pseudoephedrine were obtained from Cerilliant Corporation (formerly Radian Corp., Round Rock, TX). HPLC-grade methanol was obtained from varying sources [e.g., Burdick and Jackson (Muskegon, MI); EK Industries (Joliet, IL); JT Baker (Phillipsburg, NJ)]. Acetic acid, glacial ACS reagent grade (VWR, West Chester, PA); acetonitrile (Burdick and Jackson, Muskegon, MI); formic acid ACS reagent grade (Anachemia, Rouses Point, NY); methyl tertbutyl ether (MTBE) (VWR, West Chester, PA); and deionized water (NANOpure™, Barnstead, Dubuque, IA).

Stock standard solutions were individually prepared in HPLC-grade methanol and stored in the dark at 4^{o}C. A high-level standard mix (containing the macrolide antibiotics and the other drugs/chemicals), at concentrations of 10 or 20 ng μL^{-1}, was prepared monthly in methanol, and a calibration standard mix was prepared weekly at environmentally relevant concentrations (0.5 to 1 ng μL^{-1}) in 99% methanol:1% acetic acid.

Samples

Multiple samples, e.g., soils, waters, plants, were collected and processed during the three phases of the study. A brief summary of the samples collected and their sources are listed in Table 1.

Phase I - Greenhouse study, plant materials and growth conditions. The first phase of the study was a controlled greenhouse experiment. Three crops, lettuce (*Lactuca sativa*), spinach (*Spinacia oleracea*), and carrots (*Daucus carota sativus*), were initially germinated in potting soil and irrigated with unspiked Colorado River water. At approximately the four-leaf stage, the plants were transplanted into 1.5 L pots filled with 1.5 kg of washed silica sand. From transplanting through harvest, the plants were irrigated with Colorado River water spiked with varying concentrations of three antibiotics: azithromycin, clindamycin, and roxithromycin. The antibiotics were dissolved in a small amount of methanol, and then diluted to 1000 ng L^{-1} with Colorado River water. All concentrations were achieved by serial dilutions with Colorado River

water. The dosing concentrations were selected relative to concentrations found in wastewater effluent streams (*29*) and were dosed at 0 (control), 0.1, 1, 10, 100, and 1000 ng L^{-1}. It was observed that the concentrations of the macrolide antibiotics in the prepared irrigation water declined with time, perhaps due to photodegradation, microbial degradation, or adhesion to the walls of the plastic container. Thus, solutions were prepared weekly to maintain the target concentrations. Four replicates, at each concentration, were performed. Plant selection per treatment was done following a complete randomized design. After harvest, the crop plants were partitioned into leaves and roots and then frozen. The frozen samples were freeze-dried, and weights before and after freeze-drying were recorded. The freeze-dried samples were ground and stored in vials for later extraction.

Phase II - Field studies UA-CAC. The second phase of the study was to ground-truth the methods developed during the first phase. This phase of the study was conducted at the University of Arizona Campus Agricultural Center (UA-CAC), Tucson, AZ. This was accomplished by applying the developed methods to field-grown crops irrigated with treated City of Tucson wastewater effluent that contained known amounts of ECs and, as a control, irrigated with well water known to be EC-free (Table 2). The growing field consisted of loam-textured soils and was split into two separate sections. The first half was irrigated by furrows filled with treated wastewater effluent and the other section, the control, was irrigated by furrows filled with well water (Table 3). On March 10, 2008, peppers (*Capsicum annuum*), tomatoes *(Lycopersicon esculentum*), melons (*Cucumis melo*), lettuce, and watermelon (*Citrullus lanatus*) transplants were

155 planted in raised beds on 1-m centers. Spinach and carrots were seeded in these same
156 beds. Identical crops were established in each of the two sections. The crops were
157 fertilized and pests were controlled using standard practices. The crops were irrigated as
158 needed and harvested as each crop species matured. The final harvest was June 15, 2008.
159 After harvest, the crop plants were partitioned into leaf and root segments, and where
160 appropriate fruit, and frozen. The frozen samples were subsequently freeze-dried, and
161 weights before and after freeze-drying were recorded. The freeze-dried samples were
162 ground and stored in vials for later extraction.
163
164 **Phase III - Field studies UA-YAC.** During the third phase of the study, the
165 same crops as used in the Tucson studies were grown and collected at the University of
166 Arizona-Yuma Agricultural Center (UA-YAC), Yuma, AZ. All crops were grown on
167 loam-textured soils and irrigated with Colorado River water diverted at the IDD, north of
168 Yuma (Table 3). An opportunity arose to sample Bermuda hay grass from a field, close
169 to UA-YAC, that had a long-term history of application (several years' worth) of EC-
170 containing biosolids. The biosolids used on the field were obtained from Hyperion
171 wastewater treatment plant (WWTP), Orange County, CA, whose biosolids had
172 previously been characterized for ECs (*20*). The Bermuda grass samples were sampled
173 for the purpose of studying the possible migration of ECs from the biosolids into
174 Bermuda grass grown as feedstock for livestock.
175
176 **Water samples. Phase I.** Colorado River water, used in the greenhouse studies,
177 was sampled during each collection period. **Phase II.** Well water and treated wastewater

effluent used in the UA-CAC field study were sampled approximately every other irrigation period. These water samples were kept on ice, or refrigerated, until processing. **Phase III.** Water, which was diverted at the IDD for agricultural use in the Yuma region of the lower Colorado River, was sampled monthly at the main Yuma conveyance siphon during the crop-growing period of the field crops being sampled.

Water extractions. Water samples were prepared for analysis using solid phase extraction (SPE) Oasis MCX cartridges (Waters Corp., Milford, MA) with an automated extractor (AutoTrace, Caliper Life Sciences, Hopkinton, MA). Oasis MCX cartridges were prepared for use by loading at a rate of 1mL min^{-1}, 5 mL each of methanol, deionized water, and 95:5 water:methanol. All water samples were pH adjusted to < pH 3, with 12 N HCl, and 500 mL were passed through the prepared Oasis MCX cartridges at a rate of 7 mL min^{-1}. The cartridges were then dried for 15 minutes (using N_2), then extracted with 5 mL of 90:10 MTBE/methanol, followed by 10 mL methanol/4%ammonium hydroxide. The resultant extracts were reduced to 0.5 mL using 4 to 10 psi of nitrogen, via an automated evaporator (TurboVap, Caliper Life Sciences, Hopkinton, MA). Sample extracts were analyzed by liquid chromatography-electrospray-ion trap mass spectrometry/mass spectrometry (LC-ESI-ITMS/MS).

Plant and soil extractions. Crop samples were freeze-dried for 48 hours, or longer, until moisture was no longer present. The freeze-dried samples were ground to a semi-fine state, such that they passed through a sieve size of 300 μm, and stored in vials until extraction.

Test plot and field soil samples were poured into clean 2-liter beakers and air-dried. The dried soils were ground to ~ 300 μm using a high impact ball mill (Mixer Mill 301, Retsch Inc, Newtown, PA).

Pressurized liquid extraction (PLE) of plant and soil samples. One gram each of prepared plant and soil samples was extracted using an Accelerated Solvent Extraction (ASE) system (Model ASE 200 Accelerated Solvent Extractor, Dionex Corporation, Sunnyvale, CA) in 22-mL stainless steel extraction cells according to the following procedures.

Extraction cell preparation. A glass microfiber filter, 2 cm (Ahlstrom, Helsinki, Finland) was placed at the bottom of the extraction cell. Dependent upon whether soils or plants were to be extracted the extraction cell(s) were prepared as follows:

Soil sample extraction cell preparation. Three grams of fluorosil were added to the cell, followed by a layer of 3 g of alumina.

Plant sample extraction cell preparation. Three grams of alumina were added to the cell, followed by a layer of 3 g fluorosil.

The final sample cell preparation, whether soil and plant samples, was the same. A mixture of 1 g of sample (soil or plant) and 1-g of Hydromatrix^{TM} was added to the

224 extraction cell, followed by 3 g alumina. Hydromatrix™ was filled to top and the
225 extraction cell was capped with another glass microfiber filter and sealed.
226
227 **PLE extraction procedure.** A two solvent extraction regime was necessary in
228 order to fully extract the analytes from the solid matrices. The prepared cells were placed
229 into the ASE and initially extracted with a mixture of MTBE:methanol (90:10) and
230 flushed at 80% of cell volume. Temperature and pressure were kept steady at 50°C and
231 1500 psi, respectively. After a static period of 15 minutes, the eluant was purged into a
232 clean collection vial. The cells were left *in situ*, and further extracted with a mixture of
233 methanol/1%acetic acid and flushed at 80% of cell volume. The temperature and
234 pressure were maintained at 80°C and 2800 psi, respectively. After a static period of 15
235 minutes, the eluant was purged into a clean collection vial.
236
237 **PLE extract concentration and cleanup**. The MTBE/methanol extract was
238 placed into a Turbovap™ tube and reduced to 5 mL, using 4 to 10 psi of nitrogen, via an
239 automated evaporator (TurboVap™, Caliper Life Sciences, Hopkinton, MA). The
240 methanol/acetic acid extract was then combined with the reduced MTBE/methanol
241 extract and evaporated until a combined extract sample volume of 5 mL was reached.
242 The 5-mL extracts were removed from the TurboVap™ and washed with 1 to 2 mL of
243 hexane. The number of hexane washes varied from one sample to another, but typically
244 washes were done as many times as necessary to clean the sample of any undesirable
245 compounds, such as chlorophyllic compounds, fatty and waxy materials. The cleaned

246 extracts were placed back into the TurboVap^{TM}, further concentrated to 0.5 mL and
247 solvent exchanged with methanol/1% acetic acid before analysis by LC-ESI-ITMS/MS.
248
249 **Validation of plant extraction method.** The PLE method was validated by using
250 a modified extraction technique that had previously been published for extracting ECs
251 from biosolids (*20*). The spiked plant materials were extracted and analyzed by LC-ESI-
252 ITMS/MS. The resultant accuracy and precision data are shown in Table 5.
253
254 **LC-ESI-ITMS/MS analysis.**
255 **Liquid chromatography**. Chromatographic separations were performed using an
256 Ascentis Express C18 (Supelco-Aldrich, Bellefonte, PA) 2.7 μm particle size, 3 cm x 2.1
257 mm column, coupled with a Varian guard column (MetaGuard 2.0 mm Pursuit XRs 3μm
258 C18). Compositions of the mobile phases were as follows: (A) deionized water/0.5%
259 formic acid and (B): 82% methanol/18% acetonitrile/0.5% formic acid. The flow rate
260 through the column was 200 μL min^{-1}, with the following gradient elution conditions:
261 mobile phase A 100%, hold for 2 min; 3 min gradient to 30% A:70% B, hold for 5 min; 3
262 min gradient to 100% A, hold for 2 min; end run, 5 min equilibration time between
263 analyses.
264
265 **Mass spectrometry.** Mass spectrometric data were acquired with an iontrap
266 mass spectrometer, Varian 500MS (Walnut Creek, CA USA), configured with a liquid
267 chromatograph and an electrospray ion source. The 500MS was run in the positive
268 ionization mode under the following conditions:

- ES needle was 5 kV
- Drying gas was set at 20 psi and 350°C
- Housing chamber at 50°C
- Nebulizer gas at 40 psi
- Spray shield at 600V
- Capillary voltages were set dependent upon the optimized response of the product ions of interest.

The molecular weight of the ECs of interest, the precursor and product ions formed under LC-ESI-ITMS conditions, and the mass spectrometric limits-of-detection of the ECs are listed in Table 4. Due to the large amounts of interfering materials co-extracted with the ECs, the analyses were performed using the collision induced dissociation (CID) mode for both identification and quantitation of the analytes of interest (*18*).

RESULTS AND DISCUSSION

The steps in environmental method development involved: (1) the ability to extract the analytes of interest with some degree of precision and accuracy from an environmental matrix; and (2) the ability to accurately identify and measure at low (environmentally relevant) concentrations the analytes of interest. The focus of the results and discussion section is on the plant extraction procedures and the results of the finalized plant extraction method as applied to the various plant samples.

Analytical challenges. During the development and execution of this methodology for plants, various analytical difficulties were encountered, both in the extraction phase and the detection phase. For example, chlorophyll, waxy and fatty materials were co-extracted from plant materials, but they were not fully removed during the hexane cleanup phase, even after multiple (4x) washes. Injection of plant and root extracts into the mass spectrometer built up deposits on the inner spray shield, causing loss of sensitivity and necessitating cleanup of spray shield after every second injection of sample extracts into the mass spectrometer.

Injection of some plant and root extracts temporarily bound non-dissolvable materials to the column, even with a guard column in place, resulting in poor chromatography. This problem necessitated reversing the flow into the chromatographic column. The column was flushed first with methanol/0.5% formic acid and then with deionized water/0.5% formic acid before the column was usable again.

Results of water analysis. Phase I. All contaminants evaluated were below detection in the Colorado River water collected for spiking in the greenhouse studies. **Phase II.** The treated Tucson wastewater effluent, used at UA-CAC field studies, contained the macrolide antibiotic azithromycin, the OTC drug pseudoephedrine, the illicit drug methamphetamine, and an industrial compound, n,n'-DMPEA (an isomeric compound to methamphetamine), Table 1. All contaminants evaluated were below detection in the control well water used during the Phase II experiments at UA-CAC. **Phase III.** Previous studies have found a number of ECs in wastewater discharged at

various points along the Colorado River *(29)*. However, almost all ECs were below levels of detection for Colorado River water that was collected at the IDD (main Yuma irrigation siphon). The one exception was Ecstasy (MDMA), which was detectable but not quantifiable during the warmer months (June through September).

Validation of PLE method. It is difficult to compare the recoveries of ECs from crops in this study to the few other studies on plant uptake that have been published (*22, 24, 26*) because those studies did not indicate findings of percent recovery of spiked ECs. Boxall (*22*) does briefly mention, "*Although recoveries for most determinands were good, low but reproducible recoveries were obtained for selected substances in soil and/or plant material, so all measured values were recovery corrected. These low recoveries were observed for the highly sorptive study substances."* The actual spiked recovery data, however, was not published. Most recently, Herklotz et al. (*26*) reported percent recoveries of spiked ECs from carrots and cabbages. Their method, similar to the one reported in this paper, used PLE, and they reported > 70% recoveries of 6 different ECs. However, their methodology used either a mass labeled internal standard calibration or a combination of standard addition and mass labeled internal standard calibration, to calibrate and calculate the percent recoveries.

In comparison to Herklotz et al.'s method(*26*) the method presented in this research used external standard calibration with no corresponding mass labeled compounds, for calibration and quantitation. The best recoveries of ECs (i.e., azithromycin, roxithromycin, and clindamycin) from the plant materials were generated

by packing the extraction cell with a layer of alumina, followed by a layer of fluorosil. With the PLE method reported in this article, the percent recoveries of the spiked ECs were low, on average 25%-30% recovery, but reproducible, as measured by percent relative standard deviation (RSD), most were < 17% RSD, Table 5. The EC amounts detected in the non-spiked plant materials were spike-corrected using an equivalent spiked matrix.

While the use of labeled compounds will give a sense of higher recoveries, in truth, the labeled compound is correcting for the low recovery of the native compound. One downside to the use of mass labeled compounds in these types of studies is the usually higher costs (compared to non-labeled standards) associated with their purchase and the lack of many of the ECs with an accordingly matched mass-labeled compound.

Results of plant uptake studies. Phase I Greenhouse - Above ground dry matter production averaged 1.5, 3.3, and 1.9 g for the spinach, lettuce, and carrots, respectively, and 2.0 g for carrot roots. There were no statistically significant differences in dry matter production among the macrolide treatment rates indicating no phytotoxocity to these macrolide antibiotics up to 1000 ng L^{-1} in irrigation water. The greenhouse study indicated that there were traces of uptake of clindamycin into the spinach roots, lettuce roots and carrot roots, Table 6. Trace amounts of roxithromycin were also detected in lettuce roots and carrot roots. Carrots showed the greatest amount of uptake of roxithromycin, an average of 110 ng g^{-1}, from the 1000 ng L^{-1} treatment. Neither clindamycin nor roxithromycin were detected at the lower than 1000 ng L^{-1} treatments.

The greenhouse study demonstrates potential for EC uptake from contaminated irrigation water.

Phase II – Field studies UA-CAC. The field study at UA-CAC was a side-by-side comparison and it did not include true replication so statistical evaluations of production were not possible. However, the observed production was generally lower in the plot receiving effluent compared to that receiving well water. Most of the crops evaluated are sensitive to salinity; therefore, the high salinity (1.2 dS m^{-1}) in the effluent, as compared to the well water (0.2 dS m^{-1}), may have caused the limited production.

Although several of the ECs studied were constantly present in the Tucson treated wastewater effluent, Table 2, only n,n'-DMPEA was consistently found in the UA-CAC food crops irrigated with wastewater effluent. No uptake of azithromycin was seen in any of the plant/root samples from Tucson effluent field crops. No detectable levels of the study pharmaceuticals were found in the soils collected from the root zones of the crops sampled in the Tucson effluent field crops.

The results of the greenhouse study, and the field study with treated effluent wastewaters, indicate a potential for uptake of pharmaceuticals from contaminated water, albeit at very low levels. At present, it seems that the pharmaceuticals tested are sufficiently diluted, or degraded, within the main channel of the Colorado River and that risks of uptake by crops irrigated downstream of municipal waste discharges are minimal.

Phase III – UA-YAC and biosolids amended field. None of the ECs evaluated were found in spinach crops grown in the UA-YAC fields irrigated with Colorado River water. This was not surprising considering no detectable levels of these contaminants were present in the Colorado River water diverted for irrigation at the IDD. However, in previous studies, perchlorate accumulation has been found in plants where the contaminant was not detectable in irrigation water (*27*), perhaps due to soil accumulation or plant bioconcentration. No detectable levels of the study pharmaceuticals were found in the soils collected from the root zones of the crops sampled in the Yuma area.

From a field nearby to UA-YAC, soils and Bermuda grass were collected. This field had been treated for several years with biosolids from the Hyperion WWTP (Orange County, California), and the Bermuda grass was being used for animal fodder. While none of the ECs evaluated were detected in either the soils or Bermuda grass grown in those soils, azithromycin, clarithromycin, and n,n'-DMPEA were detected in the roots of the bermuda grass, Table 7. Both azithromycin and clarithromycin had been previously detected in Hyperion biosolids (*20*).

The final analysis of data from Phase I, II, and III has shown the possibility, although small, of transfer of specific ECs into select crops. The amount of ECs that were transferred was minimal, part-per-trillion levels, but the likelihood does exist.

Although this study was designed to look at the possibility of transfer of human-use pharmaceuticals and other ECs into crops, the possibility exists for other avenues of

crop contamination via animal husbandry practices. Animal manures and composts are widely used on both feed and food crops in irrigated desert production systems to increase organic matter and improve overall soil fertility and tilth. Due to concerns of microbial food risks, state programs such as the Arizona and California Leafy Greens Marketing Agreements prohibit the application of raw manures for a one-year period preceding the production of leafy vegetables. However, the programs do allow for composted manure applications immediately before production, provided that testing shows the food systems are free of *coliform* indicators. Composts are widely used by organic producers as the principal forms of *N* and *P* fertilizers, and are also widely used by conventional growers due to soil quality improvements and production benefits. Therefore, further work with other pharmaceutical contaminants potentially present in irrigation waters and animal husbandry waste composts (i.e., combined animal feed lots), is warranted.

NOTICE: The United States Environmental Protection Agency through its Office of Research and Development funded and managed the research described here. It has been subjected to Agency's administrative review and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

References

1. Wu, L.; Chen, W.; French, C.; Chang, A. *Safe application of reclaimed water reuse in the southwestern United States.* ; **2009**.
2. Bryck, J.; Prasad, R.; Davis, T. L. S.; Carpenter, G. *National Database of Water Reuse Facilities Summary Report*; WaterReuse Foundation: Alexandria, VA, 2008.
3. Rowe, G. L.; Reutter, D. C.; Runkle, D. L.; Hambrook, J. A.; Janosy, S. D.; Hwang, L. H. *Water Quality in the Great and Little Miami River Basins, Ohio and Indiana, 1999-2001*; Circular 1229; Reston, VA, 2004.
4. Josephson, J., The Microbial "Resistome". *Environmental Science & Technology* **2006,** 40, (21), 6531-6534.
5. Kümmerer, K., Resistance in the environment. *Journal of Antimicrobial Chemotherapy* **2004,** 54, (2), 311-320.
6. Schwartz, T.; Kohnen, W.; Jansen, B.; Obst, U., Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. *FEMS Microbiology Ecology* **2003,** 43, (3), 325-335.
7. Schwartz, T.; Volkmann, H.; Kirchen, S.; Kohnen, W.; Schon-Holz, K.; Jansen, B.; Obst, U., Real-time PCR detection of Pseudomonas aeruginosa in clinical and municipal wastewater and genotyping of the ciprofloxacin-resistant isolates. *FEMS Microbiology Ecology* **2006,** 57, (1), 158-167.
8. Zhang, Y.; Marrs, C. F.; Simon, C.; Xi, C., Wastewater treatment contributes to selective increase of antibiotic resistance among Acinetobacter spp. *Science of the Total Environment* **2009,** 407, 3702-3706.
9. Gilchrist, M. J.; Greko, C.; Wallinga, D. B.; Beran, G. W.; Riley, D. G.; Thorne, P. S., The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. *Environmental Health Perspectives* **2007,** 115, (2), 313-316.
10. Batt, A. L.; Snow, D. D.; Aga, D. S., Occurrence of sulfonamide antimicrobials in private water wells in Washington County, Idaho, USA. *Chemosphere* **2006,** 64, (11), 1963-1971.
11. Berger, K.; Petersen, B.; Buening-Pfaue, H., Persistence of drugs occurring in liquid manure in the food chain. *Archives fuer Lebensmittelhygeine* **1986,** 37, (4), 99-102.
12. Esiobu, N.; Armenta, L.; Ike, J., Antibiotic resistance in soil and water environments. *International Journal of Environmental Health Research* **2002,** 12, (2), 133-144.
13. Vaclavik, E.; Halling-Sorensen, B.; Ingerslev, F., Evaluation of manometric respiration tests to assess the effects of veterinary antibiotics in soil. *Chemosphere* **2004,** 56, (7), 667-676.
14. Baquero, F.; Martínez, J.-L.; Cantón, R., Antibiotics and antibiotic resistance in water environments. *Current Opinion in Biotechnology* **2008,** 19, (3), 260-265.
15. Andersen, S. R.; Sandaa, R. A., Distribution of Tetracycline Resistance Determinants among Gram-Negative Bacteria Isolated from Polluted and Unpolluted Marine Sediments. *Applied and Environmental Microbiology* **1994,** 60, (3), 908-912.

16. Samuelsen, O.; Torsvik, V.; Ervik, A., Long-range changes in oxytetracycline concentration and bacterial resistance towards oxytetracycline in a fish farm sediment after medication. *Science of the Total Environment* **1992,** 114, 25-36.
17. McKeon, D. M.; Calabrese, J. P.; Bissonnette, G. K., Antibiotic resistant gram-negative bacteria in rural groundwater supplies. *Water Research* **1995,** 29, (8), 1902-1908.
18. Loganathan, B.; Phillips, M.; Mowery, H.; Jones-Lepp, T. L., Contamination profiles and mass loadings of select macrolide antibiotics and illicit drugs from a small urban wastewater treatment plant. *Chemosphere* **2009,** 75, (1), 70-77.
19. Jones-Lepp, T. L.; Alvarez, D. A.; Petty, J. D.; Huckins, J. N., Polar Organic Chemical Integrative Sampling and Liquid Chromatography–Electrospray/Ion-Trap Mass Spectrometry for Assessing Selected Prescription and Illicit Drugs in Treated Sewage Effluents. *Archives of Environmental Contamination and Toxicology* **2004,** 47, (4), 427-439.
20. Jones-Lepp, T. L.; Stevens, R., Pharmaceuticals and personal care products in biosolids/sewage sludge: the interface between analytical chemistry and regulation. *Analytical and Bioanalytical Chemistry* **2007,** 387, (4), 1173-83.
21. USEPA Targeted National Sewage Sludge Survey Report. http://earth1.epa.gov/waterscience/biosolids/tnsss-fs.html, http://www.epa.gov/waterscience/biosolids/tnsss-overview.html, http://www.epa.gov/waterscience/biosolids/
22. Boxall, A. B.; Johnson, P.; Smith, E. J.; Sinclair, C. J.; Stutt, E.; Levy, L. S., Uptake of veterinary medicines from soils into plants. *Journal of Agricultural and Food Chemistry* **2006,** 54, (6), 2288-97.
23. Migliore, L.; Cozzolino, S.; Fiori, M., Phytotoxicity to and uptake of enrofloxacin in crop plants. *Chemosphere* **2003,** 52, (7), 1233-1244.
24. Grote, M.; Schwake-Anduschus, C.; Michel, R.; Stevens, H.; Heyser, W.; Langenkamper, G.; Betsche, T.; Freitag, M., Incorporation of veterinary antibiotics into crops from manured soil. *Landbauforschung Volkenrode* **2007,** 57, (1), 25-32.
25. Dolliver, H.; Kumar, K.; Gupta, S., Sulfamethazine Uptake by Plants from Manure-Amended Soil. *Journal of Environmental Quality* **2007,** 36, (4), 1224-1230.
26. Herklotz, P. A.; Gurung, P.; Vanden Heuvel, B.; Kinney, C. A., Uptake of human pharmaceuticals by plants grown under hydroponic conditions. *Chemosphere* **2010,** In Press.
27. Sanchez, C. A.; Barraj, L. M.; Blount, B. C.; Scrafford, C. G.; Valentin-Blasini, L.; Smith, K. M.; Krieger, R. I., Perchlorate exposure from food crops produced in the lower Colorado River region. *Journal of Exposure Science and Environmental Epidemiology* **2008,** 19, 359-368.
28. Sanchez, C. A.; Blount, B. C.; Valentin-Blasini, L.; Lesch, S. M.; Krieger, R. I., Perchlorate in the Feed Dairy Continuum of the Southwestern United States. *Journal Agricultural and Food Chemistry* **2008,** 56, 5443-5450.

29. Sanchez, C. A.; Jones-Lepp, T.; Wilson, D.; Alvarez, D. In *Pharmaceuticals in Waste Streams and Surface Waters of the Colorado River Basin*, Lake Mead Science Symposium, Las Vegas, NV, 2009; Las Vegas, NV, 2009.

Tables

1. Chart of samples collected.
2. Tucson wastewater effluent ECs concentrations.
3. Chemical and physical properties of loam soil used in field experiment Phase II (UA-CAC) and Phase III (UA-YAC).
4. Emerging contaminants, MW, precursor and product ions, and LODs.
5. Accuracy and precision spiked recovery parameters (0.5 μg g^{-1} and 1 μg g^{-1}) from Bermuda grass, lettuce, spinach, carrots (roots and leaf).
6. Phase I – Results from greenhouse study.
7. Phase II and III – Results from UA-CAC field study and UA-YAC field study.

Table 1. Chart of samples collected.

Sample type	Phase I Greenhouse	Phase II UA-CAC	Phase III UA-YAC
Bell pepper, green		X	
Bermuda grass			X
Cantaloupe		X	
Carrots	X	X	
Lettuce	X		
Spinach	X	X	X
Soils	X	X	X
Water			
IDD	X		X
Tucson WWTP		X	
Tucson well water		X	
Watermelon		X	

UA-CAC = University of Arizona Campus Agricultural Center, Tucson, AZ
UA-YAC = University of Arizona Yuma Agricultural Center
IDD = Imperial Diversion Dam, Colorado River

Table 2. Tucson wastewater effluent ECs concentrations.

		ng L^{-1}					
		azithromycin	roxithromycin	clarithromycin	methamphetamine	n,n'-DMPEA	pseudoephedrine
	collection date						
Tucson effluent	02/28/08	255	ND	ND	144	ND	566
Tucson dup	02/28/08	255	ND	ND	222	ND	713
Tucson well	03/24/09	ND	ND	ND	ND	ND	ND
Tucson effluent	04/01/08	686	ND	ND	288	ND	680
Tucson effluent	04/10/08	162	880	ND	155	21	229
Tucson effluent	04/29/08	323	ND	ND	99	ND	86
Tucson effluent dup	04/29/08	285	ND	ND	135	ND	76
Tucson effluent	05/29/08	259	ND	ND	309	ND	158
Tucson effluent dup	05/29/08	267	ND	ND	289	ND	216
Tucson well	05/29/08	ND	ND	ND	ND	ND	ND
Tucson effluent	07/02/08	176	ND	ND	568	ND	608

ND = not detected. n,n'-DMPEA = n,n'-dimethylphenethylamine

Table 3. Chemical and physical properties of loam soil used in field experiment Phase II & Phase III.

Parameter (unit)	Value	
	Phase II	Phase III
pH	8.2	7.8
EC (dS m^{-1})	2.2	1.8
ESP (%)	3.3	4.1
Organic C (%)	0.7	1.2
Sand (%)	50	9
Silt (%)	38	53
Clay (%)	12	38
Nitrate-N (mg kg^{-1})	42	20
Bicarbonate Soluble Phosphate (mg kg^{-1})	24	27

EC=electrical conductivity and ESP=exchangeable sodium percentage.

Table 4. Emerging contaminants, molecular weight, precursor and product ions, and LODs.

Analyte CAS #	Molecular weight (amu)	Precursor ions	Product ion (confirmation ions)	LOD ng, on-column
Azithromycin (83905-01-5)	748.5	749.5 $(M+H)^+$	591.4 $(M+H-C_8H_{16}O_2N)^+$	0.5
Roxithromycin (80214-83-1)	836.5	859.5 $(M+Na)^+$	755.4 $(M+Na-C_4H_9O_3)^+$	1
Clarithromycin (81103-11-9)	747.5	748.4 $(M+H)^+$	590.1 $(M+H-C_8H_{16}O_2N)^+$	1
Clindamycin (18323-44-9)	424.2	425.2 $(M+H)^+$	377.2 $(M+H-SH-CH_3)^+$	1
Methamphetamine (537-46-2)	149.3	150 $(M+H)^+$	119 $(M+H-CH_3NH_2)^+$	1.5
MDMA (69610-10-2)	193	194 $(M+H)^+$	163.0 $(M-CH_3NH_2+H)^+$	1
Pseudoephedrine (90-82-4)	165.2	166 $(M+H)^+$	148.2 $(M+H-H_2O)^+$	0.5
n,n-dimethylphenethylamine (1126-71-2)	149.2	150 $(M+H)^+$	105 $(M-N(CH_3)_2)^+$	0.5

MW = molecular weight; LOD = limit-of-detection

Table 5. Accuracy and precision spiked recovery parameters (0.5 μg g^{-1} and 1 μg g^{-1}) from Bermuda grass, lettuce, spinach, carrots.

	% Recovery (standard deviation; % relative standard deviation) [†]						
Sample type Compound	Bermuda Roots	Lettuce leaf	Lettuce root	Spinach leaf	Spinach root	Carrot root	Carrot tops
Azithromycin	20 (± 4; 20%)	22 (± 2; 10%)	2 (± 1)	45 (± 9; 20%)	5 (± 1; 20%)	19 (± 6; 32%)	19 (± 1; 5%)
Roxithromycin	40 (± 3; 8%)	32 (± 5; 16%)	26 (± 2)	29 (± 4; 14%)	48 (± 4; 8%)	76 (± 17; 23%)	35 (± 5; 13%)
Clarithromycin	22 (± 6; 25%)	20 (± 2; 11%)	10 (± 1)	22 (± 4; 20%)	16 (± 3; 17%)	32 (± 9; 28%)	21 (± 3; 12%)
Clindamycin	33 (± 7; 22%)	30 (± 8; 26%)	22 (± 1)	23 (± 6; 26%)	38 (± 9; 24%)	35 (± 5; 15%)	32 (± 4; 12%)
Methamphetamine	44 (± 6; 14%)	24 (± 4; 16%)	15 (± 0)	21 (± 2; 7%)	33 (± 9; 28%)	30 (± 4; 15%)	36 (± 5; 13%)
MDMA	45 (± 8; 17%)	23 (± 1; 6%)	11(± 0)	23 (± 4; 18%)	22 (± 15; 69%)	26 (± 6; 21%)	26 (± 1; 4%)
n,n'-dimethylphenethylamine	47 (± 10; 21%)	29 (± 1; 5%)	17 (± 2)	22 (± 3; 13%)	23 (± 6; 28%)	29 (± 5; 16%)	38 (± 1; 2%)
Pseudoephedrine	50 (± 3; 6%)	27 (± 0; 0%)	17 (± 1)	24 (± 2; 8%)	20 (± 15; 74%)	23 (± 6; 28%)	28 (± 1; 4%)

[†]n=3 for all sample types, except lettuce roots: n= 2; and carrot roots: n=6

Table 6. Phase I – Results from greenhouse study.

ng g^{-1} , n = 2						
Spiked Compound	Lettuce leaf	Lettuce Root	Spinach leaf	Spinach root	Carrot greens	Carrot root
Azithromycin	ND	ND	ND	ND	ND	ND
Roxithromycin	ND	< 10 ng g^{-1} LOQ	ND	ND	ND	115
Clindamycin	ND	< 10 ng g^{-1} LOQ	ND	< 10 ng g^{-1} LOQ*	ND	53

ND = not detected; *not enough sample for duplicate extraction.

Table 7. Phase II and III – Results from UA-CAC* field study and UA-YAC** field study.

	ng g^{-1}							
Sample type / Analyte Detected	Bermuda Grass**	Bermuda roots**	Cantaloupe*	Carrot Roots*	Green bell pepper*	Spinach*	Spinach**	Watermelon*
n,n'-DMPEA	ND	125	53	ND	58	48	ND	180
Azithromycin	ND	90	ND	ND	ND	ND	ND	ND
Clarithromycin	ND	135	ND	ND	ND	ND	ND	ND

ND = not detected; n,n'-DMPEA = n,n'-dimethylphenethylamine