RESEARCH TO DEMONSTRATE THE SAFETY OF RECLAIMED WATER FOR EDIBLE CROPS

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Introduction

King County, WA has started an effort to identify clients for a reclaimed water program. Recognizing the potential for changes in fresh water availability as a result of decreased snow pack storage as well as increased demand as a result of population growth, the County has recognized the importance of beneficial use of reclaimed water. Although the area around Seattle, the Emerald City, is known for high rainfall and an overabundance of cloudy days, the climate here is Mediterranean with generally very low rainfall during the summer months. Effluent from the two King County wastewater treatment plants has traditionally been directly discharged into Puget Sound with very limited beneficial use of treated effluent. The potential customers that the water reuse program has identified include golf course and landscape irrigation and irrigation water for truck farms. The program recognizes that factors influencing the acceptance of reclaimed water as a resource include concerns over the safety as well as understanding the benefits of the practice. The program has identified university research as a key component of their efforts to demonstrate safety and utility of reclaimed water.

Research into the social acceptability of reclaimed water reveals that, support for water reuse decreases as the possibility of contact with the skin or risk of ingestion increases (Stevens et al., 2006). Common public concerns on beneficial use of reclaimed water center are generally centered on potential contaminants in the water. These contaminants can be broadly grouped into three categories: organics, metals and pathogens. For pathogens, there is a real potential to actually cause illness for people exposed to them at sufficient concentrations (Gerba and Smith, 2005). The most widely recognized pathogen is *E.coli,* an intestinal bacteria found in mammals that is an indicator of fecal contamination (Toranzos and McFeters, 1997). In 2006 a widely publicized outbreak of *E.coli* O157:H7 was associated with consumption of spinach. *E.coli* is generally harmless; however, a few strains are highly dangerous. The most well known of these is O157:H7; which causes acute bloody diarrhea and abdominal cramps and some cases results in hemolytic uremic syndrome (HUS). HUS has one of the highest mortality rates of waterborne diseases (Meschke, 2006). E.coli is also an abundant intestinal bacteria in humans and is a more specific indicator of the presence of fecal contamination than the broader fecal coliform group of bacteria (Toranzos and McFeters, 1997).

There are a wide range of metals that can be detected in wastewater treatment plants do to soil contamination, metal piping, and the wide use of tools and appliances that contain metals in domestic and industrial sites that feed into the wastewater system. Much of the initial focus on risks associated with the beneficial use of biosolids focused on metals for this reason (McBride, 1995). In general, all of the metals of concern in the 503 regulations will be only sparingly soluble and will bond either to organic matter or oxide minerals that are present in wastewater treatment systems (Basta et al., 2005). As a result metals will partition to the solids during wastewater treatment (Hettiarachchi et al., 2006). Subsequently the concentration of metals in the water portion is minimal. However, concern exists that repeated application of reclaimed water overtime will result in an accumulation of metals in a soil system. Of the range of metals, cadmium is of concern because of the potential for negative human and ecosystem impacts as a result of elevated exposure to this element (Brown et al., 1998; Chaney and Ryan, 1994). Cadmium is toxic to humans and can accumulate to concentrations in the edible portions of plants that are potentially harmful for humans and wildlife before causing plant toxicity (Mclaughlin and Singh, 1999).

Recent scientific literature has increased public awareness and concern about the fate, environmental effects and degradation of a range of organic chemicals in the environment [contaminants of concern or COCs](e.g. Kolpin et al., 2002; Kinney et al., 2006). These chemicals, while found in common household products and pharmaceuticals safe for human consumption, can have marked environmental effects at very low (ng kg^{-1}) concentrations. Recent studies have found a wide range of these compounds downstream from wastewater treatment plants. Effects on endocrine systems of aquatic organisms have been observed. There is also research to evaluate persistence of these compounds in aquatic systems with some decaying more rapidly in an aquatic environment than would be predicted by lab incubations and others persisting for longer than would be expected (Fono et al., 2006). Although there is no indication that land application of reclaimed water or biosolids can have any adverse affect on human health as a result of the presence of contaminants of concern, they are a significant source of public concern. Research on behavior of some of these compounds in biosolids amended soil has shown very short half lives for some and persistence of others (Xia and Jeong, 2004. Jacobsen et al., 2005). Degradation in an aerobic soil system may occur differently than when compounds are added to aquatic systems. In addition, when compounds are added with reclaimed irrigation water, persistence and degradation may differ than when compounds are added to soils in a biosolids matrix.

There is currently a lack of data on the behavior of these compounds in soils irrigated with reclaimed water. Although EPA has issued guidelines for water reuse, there are no federal regulations dictating water quality standards for different reuse categories (Harwood et al., 2005). Most state standards are based on the risk of public exposure to potential contaminants in reclaimed water. Pathogen and metal concentrations are generally regulated in states with reclaimed water programs. For COCs, although these compounds are not regulated, public perception and future regulatory questions require the development of a data base on persistence and behavior of these compounds to assure the safety of the use of reclaimed water. The Reclaimed Water division has attempted to proactively address these concerns by engaging in research with the University of Washington on the behavior of microconstituents, metals and pathogens in reclaimed water amended soils. This paper will describe the research conducted to demonstrate the safety of reclaimed water use for growing edible plants and turf.

Materials and Methods

The behavior of estrogens (estriol [E1], 17β-estradiol [E2], and Ethinylestradiol [EE2]) in reclaimed water amended soils was tested in a greenhouse study using turf grass collected from a golf course in King County. Squares of turf including approximately 5 cm of soil and roots were collected for the study. Turf grass collected from the golf course was potted on top of sand and then assigned one of four treatments. Treatments included a control (tap water + fertilizers, sand filtered water with different levels of fertilizer addition (0, 50% and 100% fertilizer addition) and municipal biosolids. Biosolids were added at a rate equivalent to7.3 Mt ha^{-1}. The experiment was set up in the greenhouse at the University of Washington Botanic Gardens using a randomized complete block design with 4 replicates. The greenhouse is equipped with supplemental lighting and an air cooling system so that daytime temperatures during the course of the study did not exceed 29° C.

The grass was maintained in the greenhouse from July until mid-December for a total of 6 months. The plants were watered with aliquots of 250 or 500 mL of reclaimed or tap water depending on need. On a per pot basis, each pot received a total of 2.75 l of reclaimed water during the course of the trial. Through the course of the study reclaimed water source samples and leachate samples from the base of the pots after watering were collected. Five hundred mL of water was added to pots and water that passed through the pots was collected for analysis. Each batch of reclaimed water used in the study was tested for E1, E2 and EE2. Water samples were collected in amber jars to prevent photodegradation and were immediately transferred to King County Environmental Laboratory and refrigerated at 4° C. A mass balance was done at the end of the trial to calculate total addition of each form of estrogen in the reclaimed water treatments. Plants were harvested at the end of the study and the leaf tissue was analyzed for total estrogen content. The plants were collected by cutting the stalk of the grass approximately 1 mm above the base. Soil was also analyzed for total estrogen content. The soil in each pot was cut so that the original soil layer and the underlying sand layer were analyzed separately. The soil was homogenized and stored in glass jars in the freezer at -20°C until analysis at the King County Environmental Laboratory.

Sample analysis was done at the King County Environmental Laboratory. A deuterated internal standard containing all three estrogens was added to each sample immediately prior to analysis to determine recoveries. Samples were analyzed with a gas chromatograph mass spectrometer. Each sample run included a method blank, spike blank and laboratory duplicate. The method blank and spike blank contained a known amount of deuterated E1, E2 and EE2 carbon labeled surrogate and 13C-Triclosan.

The presence of pathogens and plant metal uptake for edible crops grown using reclaimed water was tested in a greenhouse study. Soil from a local truck farm was used for this experiment. The study was set up as a randomized complete block design with 5 replicates. Water treatments included reclaimed water and tap water. Each soil sample location was used for a separate replicate in the greenhouse trial. Site soils are classified as Newberg silt loam (Ng in the above figure) or Nooksack (Nk in the figure) silt loam. The Newberg is an alluvial flood plain soil. The Newberg series a coarse-loamy, mixed, superactive, mesic Fluventic Haploxerolls (USDA NRCS, 2009). The Nooksack series is a coarse-silty, mixed, superactive, mesic Fluventic Haploxerolls (USDA NRCS, 2009).

Three species of edible crops including Paris Island Romaine lettuce (*Lactuca sativa*), Danvers Half Long carrot (*Daucus carota* subsp. sativus), and Everberry strawberry (*Fragaria ananassa*) were used in the study. All species except the strawberries were started from seed. Each pot received one strawberry plant. For lettuce and carrots there were three plants per pot, and five pots or replicates per water treatment. Plants were grown for 2-3 months prior to harvest. Each pot received > 5 l of reclaimed water during the course of the study. The edible portions of both lettuce and carrots were tested for metal concentration. All plants were tested for pathogen concentrations.

During the study, reclaimed water source samples were collected weekly and crop and soil samples were collected at the end of the study when the edible part of the plants were ready for consumption. The samples were tested for a range of parameters to assess the safety of using reclaimed water to irrigate food crops. Analysis used to evaluate the safety of reclaimed water included microbial analysis of soils, water samples and washed and unwashed edible portion of plant tissue. Total arsenic (As), cadmium (Cd), lead (Pb) and nickel (Ni) in reclaimed water, soils and edible portions of plant tissue were also determined to assess the potential for plant uptake of any metals introduced by the reclaimed water.

The most probable number or MPN method was used to determine the presence of total coliforms, fecal coliforms and Escherichia coli (E. coli) in the soil, water and edible crop samples. The MPN procedure requires observing evidence of bacterial growth in serial dilutions of media and using probability formulas to determine the density of organisms in a sample. The analytical procedure follows King County SOP guidelines including: # 05-02-005-000 (Total coliforms), #05-03-005-000 (Fecal Coliforms), and # 05-06-004-000 (E. coli). The King County MPN method for soils and edible crops are based on Neufeld (1984), while the water methods are taken from the American Public Health Association.

The lettuce, and carrot samples were analyzed for metals using King County SOP #06-03-003-002 or EPA SW-846 (1996). The lettuce and carrot samples were freeze-dried and homogenized by hand using a ceramic mortar and pestle. Dried samples were digested using concentrated HNO_3^- on a Hotblock™ digestion vessel. A spike solution containing known concentrations of As, Cd, Pb, and Ni was added to each sample. After digestion with acid, H_2O_2 was added to the sample to oxidize any remaining organic matter. Samples were allowed to cool, filled to volume with deionized water and analyzed using an ICP-MS.

Results

Estrogens

Total concentrations of each compound and total rate of addition per pot is shown in Table 1.

Table 1. Total estriol, 17β-estradiol and ethinylestradiol concentrations in reclaimed water and biosolids used in the greenhouse study. Total amount of each compound added to each experimental unit are also shown. Means +/- standard deviation are presented

Estriol (E1)	17β-estradiol (E2)	Ethinylestradiol

			(EE2)
Water Conc. (ug L^{-1})	0.068 +/- 0.049	0.019 +/- 0.0065	0.021 +/- 0.011
Total addition (ug)	0.187	0.052	0.058
Biosolids Conc. (ug kg^{-1})	152+/- 2	10.6+/-0.3	4.8+/-0.3
Total addition(ug)	2.58	0.18	0.08

The concentrations of the three estrogens varied significantly in the reclaimed water over time. For E1, concentrations ranged from 0.01 to 0.27 µg l^{-1}. For this study, the method detection limit (MDL) for water samples was 0.0012 µg l^{-1}. Variation for E2 and EE2 was less pronounced, with concentrations ranging from below detection limit to 0.035 µg l^{-1}. The MDL for E2 and EE2 in water was 0.002 µg L^{-1}. In the biosolids, the concentration of E1 was 152 ± 2 µg kg^{-1}, E2 was 10.6± 0.3 µg kg^{-1}, EE2 was 4.8 ± 0.3µg kg^{-1}. Estrogen (E1 and E2) was detected in the leachate samples from the reclaimed water treated pots in some, but not all, of the sampling intervals (Table 2). Concentrations were typically ten fold smaller than in the reclaimed water. For the majority of sampling intervals, EE2 was not detected in the leachate samples. In the biosolids amended soils, there was no detectable movement of any of the estrogens for all sampling intervals. Spike recoveries for the leachate for the biosolids amended soils ranged from approximately 75% to 125%.

Table 2. Concentrations of the 3 estrogens in leachate collected from control, reclaimed water and biosolids amended turf grass in a greenhouse study. Means +/- standard error are shown. There were 5 sampling events with 2-4 replicates for reclaimed water and a single replicate for biosolids. Values for reclaimed water are averaged across all fertilizer treatments. The minimum sample detection level (MDL) for E1 is 0.0012 µg L^{-1} and 0.002 µg L^{-1} for E2 and EE2.

	Estriol (E1)	17β-estradiol (E2)	Ethinylestradiol (EE2)
		µg L^{-1}	
Control	<MDL	<MDL	<MDL
Reclaimed water	0.011±0.014	0.005	<MDL
Biosolids	0.002*	<MDL	0.004

* Biosolids value for E1 has 3 of 5 <MDL
Biosolids value for EE2 has 4 of 5 < MDL
Reclaimed water has for E2 5 of 17 < MDL

At the end of the trial, the total concentration of each compound was measured in the soils. In the reclaimed water treatments, all estrogens were below detection limits in soils. The amount of E1, E2 and EE2 added the pots by the reclaimed water totaled approximately 0.187, 0.052, 0.058 µg respectively. The detection limit for soil is 0.03µg kg^{-1} for E1 and 0.05 µg kg^{-1} for E2 and EE2. The total loading rate was below the method detection limit. The results confirm that the concentration of the estrogens added to soil was below detection. As the total rate of addition was so low, it is currently not possible to test for degradation of this compound in the soil matrix. Estrogen was also below the detection limit for soils in the biosolids treatment (0.03 $\mu g\ kg^{-1}$). No estrogens were detected in leaf tissue for any treatment. In the biosolids treatments, dried and ground biosolids were added to the surface of the turf grass. Turf grass was cut several times during the study, but leaf tissue was not analyzed for any compounds until the final harvest and total plant removal of chemicals was not quantified. Thus, our results for soil concentrations of estrogens cannot be interpreted as proof of degradation of the added compounds. However, the collective data suggest minimal negative impacts of estrogen addition via land applied reclaimed water or biosolids on soil or water quality.

Pathogens

During collection, both subsamples of lettuce and carrots were washed with DI water to mimic the food preparation actions of the end user. Lettuce was also tested for pathogens without prior washing to mimic a worst-case scenario for pathogen transfer. Strawberries were only tested for pathogen concentration without washing. The washed and unwashed crop samples were compared for microbial presence. The results are listed in table 3. The probability formulas used to determine MPN result in a wide range of values that are not amenable to averaging. Therefore, if any of the five replicates of a treatment were positive for coliform bacteria than the specific bacterial counts of that replicate are shown as MPN/100g.

Table 3. Total coliform of strawberries, lettuce and carrots grown with tap water or reclaimed water. The strawberries were tested without washing, the lettuce was tested as both washed and unwashed samples, and the carrots were only tested after washing. Bacterial counts are shown as MPN/100g for individual samples

Crop			**Total Coliform (MPN/100g)**		
Unwashed Samples	1	2	3	4	5
Strawberry					
Reclaimed Water	<MDL	<MDL	<MDL	<MDL	<MDL
Control	<MDL	<MDL	<MDL	<MDL	<MDL
Lettuce					
Reclaimed water	<MDL	<MDL	<MDL	<MDL	<MDL
Control	<MDL	<MDL	940	2800	790
Washed Samples	1	2	3	4	5
Lettuce					
Reclaimed Water	<MDL	<MDL	<MDL	<MDL	<MDL
Control	<MDL	<MDL	1400	230	230
Carrot					
Reclaimed water	<MDL	<MDL	<MDL	230	20
Control	<MDL	230	78	220	260

MDL = 18 MPN/100g

The only positive results for bacteria in the edible crops tissue detected was limited to total coliforms. There were no fecal coliform or E. coli detected in any of the treatments for any of the crops (data not shown). Fecal coliforms are a direct indication of fecal contamination and can be from humans or other animals. E. Coli are also a direct indication of fecal contamination and exposure to E. Coli has been linked to severe illness and death in a number of cases. The source of the most virulent E Coli strain is cattle, and its' presence is an indication of contamination by animal manure. E Coli is not found in human excrement and so is a sign of animal fecal matter rather than human fecal matter. Total coliform is a broad category of organisms with a majority of these organisms not responsible for human illness. The majority of the coliforms detected were in the control treatments with only two detects in the reclaimed water treated plant samples. Total coliforms are not a specific group of bacteria and include species that are not indicators of fecal contamination. This suggests that the positive detection of coliforms is most likely of non-fecal origin. It also suggests that the plants grown in reclaimed water were safe for human consumption with or without prior washing. These results should not be taken to mean that it is not necessary to wash fruit and vegetables before eating.

Metal uptake
Both lettuce and carrots were harvested and analyzed for metals concentration. Lettuce was chosen for this study because it is a common garden crop that has a high potential for metal uptake. Lettuce has previously been used as an indicator crop to evaluate metal availability in contaminated soils (Brown et al., 1996). It has also been used because of its' high metal uptake characteristics to evaluate the potential of food chain transfer of Cd (Brown et al., 1998). Carrots were selected for this study because the edible portion of the plant is grown directly in the soil. It was thought that public concern would be greater for unacceptable concentrations of metals in plant tissue for a crop that had direct contact with the soil. The average metal concentrations are presented in table 4.

Table 4. Average dry weight metal concentration +/- standard error of lettuce and carrot grown in the greenhouse and watered with reclaimed water or tap water.

	Lettuce		Carrot	
	Reclaimed Water	Control	Reclaimed Water	Control
	- mg kg $^{-1}$ -			
Arsenic	0.102 ± 0.036	0.091 ± 0.023	0.036 ± 0.003	0.038 ± 0.036
Cadmium	1.52 ± 0.16	1.34 ± 0.15	0.47 ± 0.08	$0.59 + 0.1$
Lead	0.156 ± 0.04	0.08 ± 0.04	0.041 ± 0.02	0.095 ± 0.056

Nickel	0.719 ± 0.11	0.62 ± 0.08	0.33 ± 0.07	0.576 ± 0.09

The concentration of metals in the vegetables grown with reclaimed water and tap water were not statistically different ($p>0.5$), with the exception of lead in the lettuce and nickel in the carrots. Lettuce concentration of Pb was significantly higher ($p >0.05$) in plants grown in reclaimed water irrigated pots in comparison to tap water irrigated pots. Nickel concentration of carrots was significantly higher ($p>0.05$) in plants grown using tap water in comparison to those grown using reclaimed water. For all other elements for both carrots and lettuce there was no significant difference in plant metal concentration as a result of the type of water used to irrigate the crop. In other words, using reclaimed water to irrigate the plants did not change the metal concentration of As or Cd in the edible parts of the plants. For Pb, use of reclaimed water did increase plant lead concentration in lettuce. The mean concentration of lead in the lettuce grown with reclaimed water was 0.16 + 0.04 mg kg-1. Higher concentrations of metals in lettuce in comparison to carrots was expected, as lettuce tends to uptake metals at greater concentrations than other vegetables (Brown et al., 1996). However, greater lead concentration due to reclaimed water wasn't expected as the reclaimed water lead concentration was similar to the tap water. These results suggest that metal uptake of vegetables grown using reclaimed water is not of concern from a human health perspective.

Conclusions

Results from these two greenhouse studies suggest that using reclaimed water to irrigate crops is a safe practice. There was no increase in soil estrogens from reclaimed water. Pathogen content and metal concentration of edible crops was similar for tap water and reclaimed water irrigated fruits and vegetables. In addition to being safe, the use of reclaimed water for irrigation of crops is preferable to tap water due to the higher concentration of essential plant nutrients present in reclaimed water. It is possible that these trials, using local soils and reclaimed water from local treatment plants will increase the likelihood that public acceptance of reclaimed water in King County will be high.

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