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DISSIPATION OF CONTAMINANTS OF EMERGING CONCERN IN BIOSOLIDS APPLIED TO NONIRRIGATED FARMLAND IN EASTERN COLORADO[1]

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ABSTRACT: In 2007, a 1.5-year field-scale study was initiated by the U.S. Geological Survey to evaluate the dissipation of contaminants of emerging concern (CECs) following a first agronomic biosolids application to nonirrigated farmland. CECs with the greatest decrease in concentration in the surface biosolids at 180 days post-application included indole, d-limonene, p-cresol, phenol, and skatol. CECs that were present in the largest concentration in 180-day-weathered biosolids included stanols, nonylphenols, bisphenol A, bis(2-ethylhexyl) phthalate, hexahydrohexamethyl cyclopenta-benzopyran (HHCB), and triclosan. CECs that were detected in pre-application soil were 3-beta coprostanol, skatol, acetophenone, beta-sitosterol, beta-stigmastanol, cholesterol, indole, p-cresol, and phenol, most of which are biogenic sterols or fragrances that have natural plant sources in addition to anthropogenic sources, yet their concentrations increased (in some cases, substantially) following biosolids application. Preliminary data indicate the nonylphenols (including NPEO1, NPEO2), OPEO1, benzo[a] pyrene, diethyl phthalate, d-limonene, HHCB, triclosan, and possibly 3-beta coprostanol, skatol, beta-sitosterol, cholesterol, indole, and p-cresol, migrated downward through the soil by 468 days post-application, but indicated little uptake by mature wheat plants. This study indicates that some CECs are sufficiently persistent and mobile to be vertically transported into the soil column following biosolids applications to the land surface, even in semiarid regions.

(KEY TERMS: organic chemicals; soils; transport and fate; biosolids; crops; field dissipation.)

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INTRODUCTION

Biosolids are the treated solid-waste component of wastewater treatment plant (WWTP) effluent, and about 50% of the biosolids produced in the United States (U.S.) are land applied (http://water.epa.gov/polwaste/wastewater/treatment/biosolids/genqa.cfm, accessed 2/18/2013). The high levels of nutrients and organic matter in biosolids can be a valuable resource

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to agricultural fields (Giudice and Young, 2011). Biosolids that are generated from municipal sewage treatment are applied to farmland in Colorado as a soil amendment and fertilizer through a process permitted by the state of Colorado (CDPHE, 2003) in accordance with the requirements of the U.S. Environmental Protection Agency (USEPA, 1993). The biosolids are transported by truck from nearby WWTPs or by rail from distant WWTPs to private and WWTP-owned property and are applied to irrigated and nonirrigated fields as an alternative to landfilling or incineration. Biosolids are applied (by the WWTP, if of local origin) according to agronomic loading rates calculated for each field on the basis of soil nitrogen and expected crop uptake of nutrients (CDPHE, 2003). In Colorado, land-applied biosolids must meet Colorado regulatory limits, which specify Ceiling Concentration Limits and Pollution Concentration Limits for metals and pathogen-destruction criteria (CDPHE, 2003). Therefore, biosolids routinely are monitored at the WWTPs for these regulated constituents in addition to nitrogen, phosphorus, pH, and potassium.

Previous studies have documented that biosolids and biosolids products such as composted biosolids destined for land application can contain many additional, nonregulated, natural, and synthetic chemical compounds (O'Connor, 1996; Kinney *et al.*, 2006; USEPA, 2009; Langdon *et al.*, 2011). These compounds include hormones, detergent metabolites, fragrances, prescription and nonprescription drugs, fire retardants, disinfectants, and plasticizers, which collectively are referred to as contaminants of emerging concern (CECs) in this article. Concerns related to these compounds are still emerging as evidenced by recent papers addressing effects of these compounds (Kinney *et al.*, 2012; Liu *et al.*, 2012; Galus *et al.*, 2013; Li *et al.*, 2013; Rosi-Marshall *et al.*, 2013). Not all the CECs are toxic but some CECs have been linked to effects such as endocrine disruption, and other deleterious effects continue to be identified (Brodin *et al.*, 2013). In addition, select compounds can provide valuable indications of anthropogenic inputs (Oppenheimer *et al.*, 2011). CECs can be either synthetic or natural chemicals that have a number of sources and pathways to the environment. Most conventional WWTP processes were not designed to remove these chemicals, allowing some quantity of these compounds to persist in wastewater effluent (Langdon *et al.*, 2011). Treated liquid effluent and associated CECs in parts per trillion (ppt) or parts per billion (ppb) concentrations (Barber *et al.*, 2011) often are discharged to streams (Kolpin *et al.*, 2002; Glassmeyer *et al.*, 2005; Lee *et al.*, 2011). However, some CECs are sequestered in biosolids (Xia *et al.*, 2005; Kinney *et al.*, 2006; Citulski and Farahbakhsh, 2010; Nieto *et al.*, 2010; Gottschall *et al.*, 2012). Many CECs are concentrated in the biosolids, some as high as a milligram per kilogram (mg/kg, equivalent to parts per million or ppm) concentrations (Kinney *et al.*, 2006; USEPA, 2009; McClellan and Halden, 2010; Walters *et al.*, 2010).

The frequent occurrence of these CECs in biosolids destined for land application indicates that biosolids application can be a pathway into the terrestrial environment for these chemicals. The presence of CECs in the environment is a concern because various studies have shown that some CECs can have potentially harmful ecologic effects, including endocrine disruption in impacted aquatic systems (Painter *et al.*, 2009; Bringolf *et al.*, 2010; Guler and Ford, 2010; Sumpter and Jobling, 2013), but antagonistic and synergistic effects of multiple CECs on biota are not well understood (Schultz *et al.*, 2011). In addition, results from other studies indicate that CECs in municipal biosolids can be mobile in the environment because CECs can be leached from fresh and weathered biosolids (Yager *et al.*, 2013), can be present in runoff from biosolids-applied fields (Yang *et al.*, 2012), can be found in tile drainage following biosolids application (Lapen *et al.*, 2008; Edwards *et al.*, 2009; Gottschall *et al.*, 2012), and can transfer to the consumed portions of plants (Wu *et al.*, 2010; Calderón-Preciado *et al.*, 2011; Lahti *et al.*, 2011; Holling *et al.*, 2012). CECs also have been shown to biomagnify (Kinney *et al.*, 2008) and produce harmful effects (Lin *et al.*, 2012) in the terrestrial food web; however, the fate and transport of biosolids-borne CECs in the terrestrial pathway have not been well understood because of the number of variables involved, including climate and food-web participants, which can vary by geographic area. Thus, many questions remain yet to be answered. Do CECs dissipate quickly after application or do they persist in the environment? Do CECs degrade *in situ*, volatilize, migrate into soil, or assimilate into crops? To begin addressing some of these questions pertaining to the fate and transport of CECs from field-applied biosolids, the U.S. Geological Survey (USGS) initiated a 1.5-year field-scale study in eastern Colorado in 2007 to evaluate the dissipation and movement of CECs following biosolids application to farmland.

METHODS

Study Area

The study area was located in the eastern plains of Colorado, northeast of Denver (Figure 1), on land

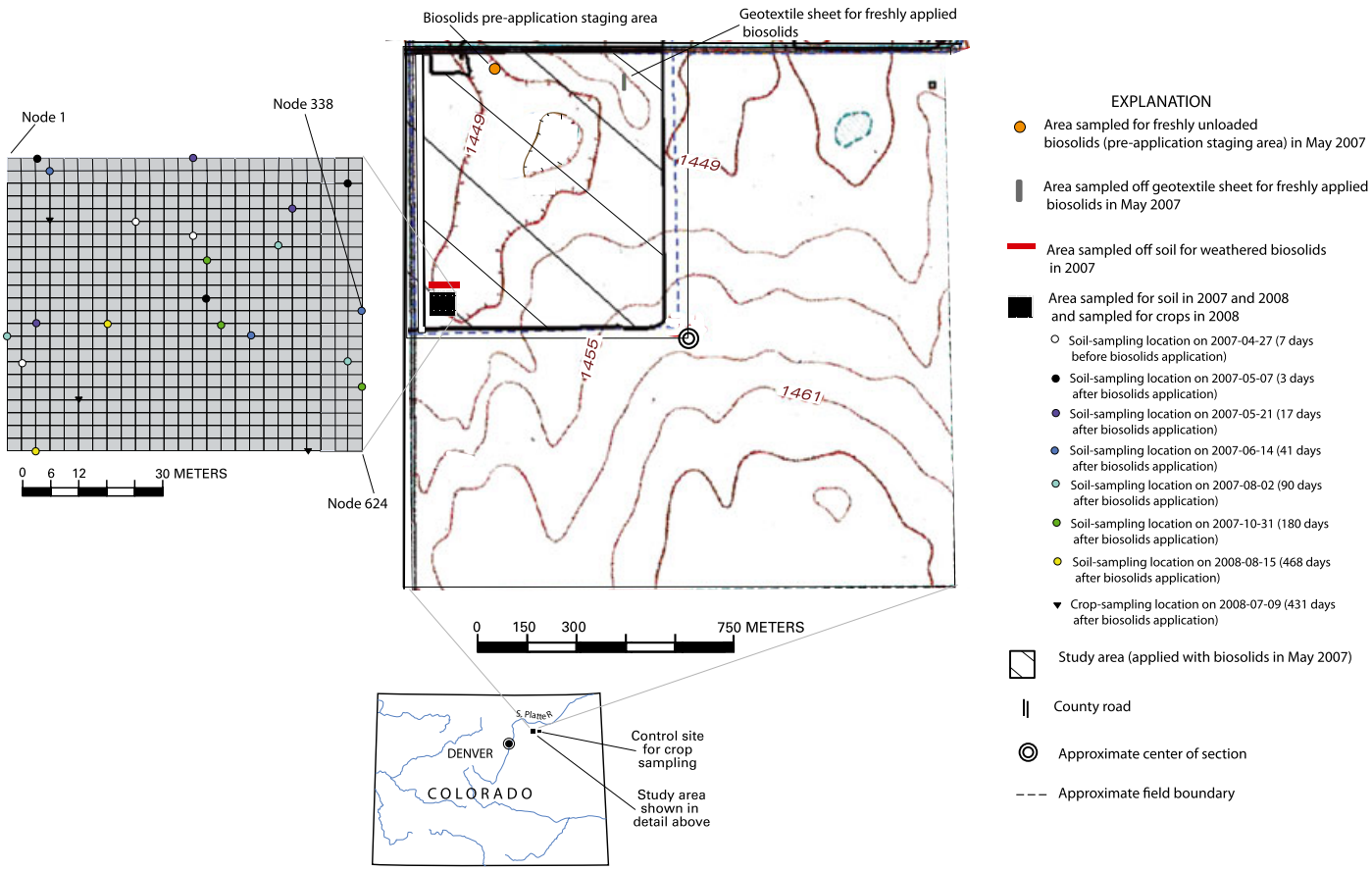

FIGURE 1. Location of Sampling Areas for Biosolids, Soil, and Crops. Topography is indicated by contour lines (elevation shown in meters; contour interval is 3 m).

that had a history of crop production but had not previously received biosolids or manure applications. The study area contained different sampling areas for freshly unloaded biosolids (pre-application staging area), freshly applied biosolids, weathered biosolids, and soil and crops (Figure 1). The area sampled for soil and crops was an organic-carbon-poor sandy loam; carbon and texture data are included as Supporting Information (Table S1). Weather stations in the vicinity of the study area indicated that about 300 mm of precipitation was received during the study; the precipitation information that was estimated for the study area is included as Supporting Information (Figure S1). Most of the precipitation was received as rainfall. During 2007 and 2008, land in the study area was used as cropland for growing a variety of hard, red, winter wheat. Low-till or no-till farming procedures were used without irrigation. Herbicides were applied to the study area during 2007-2008 to minimize weeds. Small county roads (dirt) bounded the field that contained the study area on two sides.

Dewatered municipal biosolids (type "Table 3 Class B" according to CDPHE, 2003) resulting from secondary treatment were applied as a fertilizer and soil amendment to the study area during May 2-7, 2007. Biosolids were applied only once during the study and were applied at an agronomic loading rate (calculated to be 4.60 dry metric tons of biosolids per hectare [dMT/ha] for this particular field based on soil analyses) to the study area after the first (pre-biosolids) soil sampling. The actual application rate in May 2007 was 4.17 dMT/ha (4.17 Mg-dw/ha), which yielded about 68 kilograms of nitrogen per hectare (kg-N/ha). Within a few days after application, the biosolids were partially incorporated in the soil to a depth of 15 cm using a rotating, tractor-pulled aerator (Aerway™ tilling system, Norwich, Ontario, Canada). After this step, considerable biosolids aggregates were still visible on the land surface, but some biosolids had been mechanically inserted into the subsurface. About four months after biosolids were applied, a crop of winter wheat was planted; about 14 months after biosolids were applied, the crop was harvested.

Because the entire study area was applied with biosolids, a control field for crop sampling was selected near but external to the study area. The

selection of the control field was determined by matching as many variables as possible to the field that would receive biosolids: previous land use, starting biosolids condition (none), slope, aspect, soil type, weather, farming methods and schedule, crop (winter wheat), field size, and proximity to roads, waterways, structures, and animal-feeding operations. The selected control field was farmed by the same grower as the biosolids-applied field. Additional details about the study area are included in the Supporting Information section.

Field Methods

Biosolids were sampled separately from soils before application, immediately after application, and at various dates post-application. Field methods for all samples collected are summarized here and are described in more detail in the Supporting Information. Biosolids were sampled from the pre-application staging area (Figure 1) as soon as the biosolids were unloaded from the transport truck and before land application. Biosolids were sampled off a geotextile sheet at the time of land application to keep the applied biosolids separate from the native soils. The only detections of CECs in samples of the clean geotextile sheet were small, estimated values (less than the minimum reporting level that was determined by the laboratory [MRL]) for acetylhexamethyl tetrahydronaphthalene (AHTN) (18.8 micrograms per kilogram [μg/kg]), N, N-diethylmetatoluamide (DEET) (29.2 μg/kg), hexahydrohexamethyl cyclopenta-benzopyran (HHCB) (64.1 μg/kg), and tributyl phosphate (126 μg/kg). Replicate biosolids samples were prepared for the pre-application and at-application samples (data are included in Tables S2, S3, and S9 of the Supporting Information). To prevent bias in the soil data that would be caused by removal of biosolids and to minimize disturbance of the soil-sampling area, the freshly applied biosolids and weathered biosolids deliberately were sampled from different parts of the study area than the area sampled for soil and crops. Weathered biosolids were collected from the land surface (separately from soils) at 17, 41, 90, and 180 days post-application. Biosolids aggregates were identified by visual inspection and composited from across the designated sampling area (Figure 1). After 180 days, biosolids aggregates on the land surface could no longer be identified with certainty by visual inspection and thus were no longer sampled separately from soil.

Soil was sampled seven days prior to biosolids application to represent the pre-biosolids condition and then sampled at an approximately exponential time frequency after application. Soil (along with any surface or incorporated biosolids) in the 0-126-cm deep soil interval were sampled 3, 17, 41, 90, 180, and 468 days post-application. The soil-sampling area was located at an interior part of the study area (Figure 1) and was subdivided into a grid where intersections of grid lines represented possible sampling locations (nodes) (Figure 1). For each sampling trip, sampling nodes were randomly selected in advance by using the Excel™ program (Microsoft Office Professional, Microsoft Corporation, Redmond, Washington). Soil samples usually were collected from three nodes every sampling trip. The target interval for soil sampling at each selected sampling node was 0-126 cm below land surface, which was sampled as seven separate (approximately equal) vertical depth increments. The sample for each depth interval was a composite prepared for that specific interval at that particular node from soil cored by using a bucket auger.

Various quality-control samples associated with the soil samples also were prepared at the time of sample collection. Soil-sample replicates were collected from the top depth interval because this interval was likely to contain the most biosolids, CECs, and pre-biosolids organic carbon, so the matrix was more complicated and CEC concentrations were more likely to be higher than in samples from lower depth intervals. A replicate soil sample was composited from depth-interval 2 at all three nodes that were sampled on May 7, 2007, for use as a matrix spike; this sample was spiked at the laboratory to minimize contamination from the field environment. A soil-sampling ambient blank was prepared in the field by slowly pouring clean, burned, silica sand into a clean sampling jar at a sampling node, then leaving the lid off the sampling jar during the collection of a soil subsample from a single depth interval, then closing the sample jar and storing it with the other samples; no CECs were detected in the ambient blank. A soil-sampling equipment blank was prepared in the field at one of the sampling nodes by slowly pouring clean, burned, silica sand over and through the cleaned bucket auger onto the cleaned tray, stirring the sand on the tray with the clean bucket auger for 5 min, homogenizing the sand on the tray by stirring with cleaned spatulas and a spoon for 5 min, using the spoon to transfer the stirred sand into a clean sampling jar, then closing the sample jar and storing it with the other samples; no CECs were detected in the equipment blank. All quality-control data are included in the Supporting Information (Tables S7, S8, S9, and S10). Variability observed in replicate analyses also is discussed in the Supporting Information.

Crop samples were collected from the study area (biosolids applied) and the control site when the farmer said the crop was ready to harvest, which was

431 days post-application. Crop-sampling locations within each field were selected randomly by using the same process that was used for selecting soil-sampling locations. At each randomly selected sampling node, three to four whole-plant samples were collected by using a cleaned metal shovel to remove the plants from the ground, including as many of the roots as possible. Crop samples were washed at the laboratory to remove any biosolids or soil particles and partially air dried. Whole-plant crop samples subsequently were composited across nodes by plant part (root wads, stems/leaves, and grain heads) and each composited plant part for each field was analyzed in triplicate. Replicate crop samples also were collected, and a matrix spike on a replicate of crop roots was prepared. No blank samples associated with crop sampling were prepared. All quality-control data for crop samples are included in the Supporting Information (Tables S7, S8, and S9). Variability observed in replicate analyses also is discussed in the Supporting Information.

For all samples collected for this study (including biosolids, soil, and crop samples), field personnel followed the methods of Lewis and Zaugg (2003) to minimize contamination of the samples. All the samples were placed in clean, burned, brown glass jars and were placed immediately on ice in a field cooler and delivered to the laboratory on the same day collected. Additional details about sample collection and processing are available as Supporting Information.

Analytical Methods

All biosolids, soil, and crop samples were analyzed by the USGS National Water Quality Laboratory for 57 CECs that included detergent metabolites, fragrances, pesticides, fire retardants, polycyclic aromatic hydrocarbons, steroids, oils and fuels, and disinfectants (referred to as wastewater indicators or WWIs in this article). Selected pre-application soil samples also were analyzed for organic carbon (estimated by loss on ignition using the universal conversion coefficient 0.58) (Schulte and Hopkins, 1996) and soil texture (hydrometer). Selected biosolids and soil samples also were analyzed by the USGS for 19 pharmaceutical compounds; results for the pharmaceutical analyses are included as Supporting Information but are not the focus of this article because few of these types of CECs routinely were detected in the soil samples.

The biosolids and soil samples were analyzed for WWIs by using pressurized solvent extraction, solid-phase extraction, and capillary-column gas chromatography/mass spectrometry (Burkhardt *et al.*, 2006). Crop samples were analyzed for WWIs by using an exploratory method that was a modification of the method described by Burkhardt *et al.* (2006). Pharmaceutical CECs were analyzed on selected fresh, wet samples that were frozen at −25°C then thawed before analysis; the research method involved pressurized solvent extraction followed by high-performance liquid chromatography coupled with electrospray ionization/quadrupole mass spectrometry similar to that described by Kinney *et al.* (2006).

Mass spectrometry is an "information-rich" method, so the qualitative identification of a compound at concentrations that are less than the MRL is augmented by other information (Childress *et al.*, 1999; Burkhardt *et al.*, 2006). Analytes in the samples that were positively identified below the MRL and met other quality-control criteria were reported by the laboratory as estimated concentrations (e-coded values) because of increased uncertainty in quantitation (Childress *et al.*, 1999; Burkhardt *et al.*, 2006; Rounds *et al.*, 2009). These estimated concentrations are valid chemical detections and were used for this study. Quality-assurance protocols for processing and analysis of samples for CECs included contamination-minimization procedures for all personnel (Lewis and Zaugg, 2003), analysis of replicate samples, preparation and analysis of a matrix spike on a sample replicate, analysis of surrogate compounds with every sample, and analysis of reagent (burned-sand) laboratory blank and laboratory spike samples with every set of prepared samples. Multiple ions were monitored for the analysis of each analyte, and internal standards and continuing calibration verification samples were used for the analyses.

For a few CECs, particularly bisphenol A and tetrabromodiphenyl ether (BDE-47), analytical interferences present in biosolids and soil samples precluded unambiguous identification or quantitation. These interferences result from two sources: (1) the complex organic chemical mixture present in soils and especially biosolids, which was coextracted with the compounds of interest, and (2) compound-specific analytical artifacts, such as variable and lower procedural recovery (bisphenol A) and low response in the mass spectrometer (BDE-47).

RESULTS AND DISCUSSION

Number of Detections

This study indicated that the number of CECs detected differed among biosolids, soil, and crop samples (Figure 2). The largest number of CEC detections were for biosolids samples; a similar number of

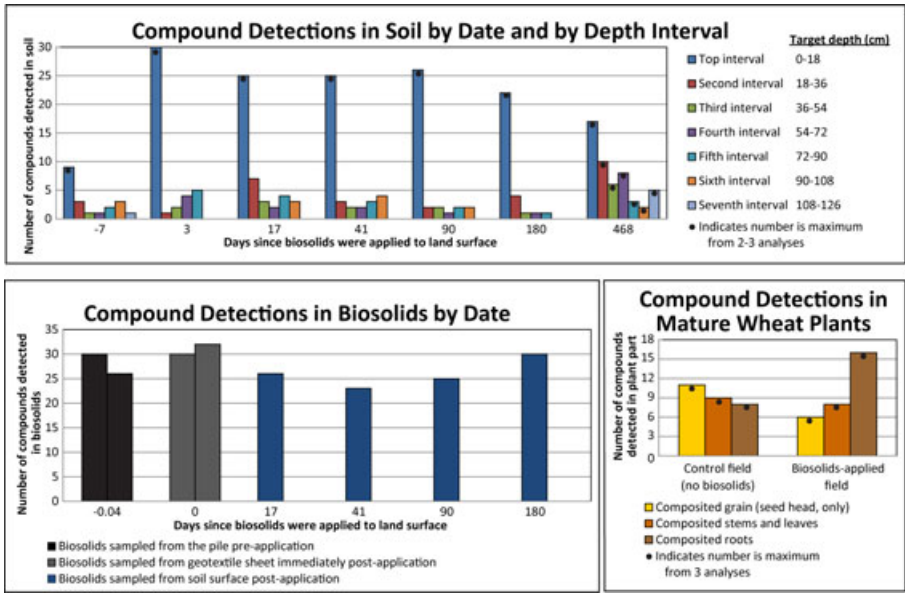

FIGURE 2. Number of Compounds Detected in Soil, Biosolids, and Crops. Detections included values estimated by the laboratory (both above and below the minimum reporting level). Each bar represents a single composited sample that was analyzed. A black dot indicates that the bar shows the maximum number of compounds detected in an individual sample where multiple samples were analyzed. All bars are shown in the same order in the graphs and in the explanations of the graphs.

CEC detections were for soil samples from the biosolids-incorporation zone. Fewer CECs were detected in the wheat-plant parts. All results, including detected compounds, are included in Supporting Information (Tables S2 through S8); the detections are discussed in more detail below.

Biosolids were expected to contain CECs based on the literature (Kinney *et al.*, 2006). This study indicated that a similar number of CECs (26-32 of 57 wastewater-indicator analytes) were detected in fresh biosolids from the pile (pre-application; day −0.04) and biosolids collected off the geotextile sheet at application (day 0), suggesting consistency before and at application. Any differences in number of CECs detected between −0.04 and 0 days post-application likely reflect chemical heterogeneity of the biosolids and analytical uncertainty. This study indicated that the number of CECs that were detected in biosolids throughout the 180 days of sampling (23-32 of 57 wastewater-indicator analytes) did not differ substantially from the number of CECs detected in the starting material (26-32 of 57 wastewater-indicator analytes) (Figure 2), suggesting relative stability of the CECs incorporated into biosolids.

The maximum number of CECs detected in soil samples (30 of 57 wastewater-indicator analytes) was similar to the number detected in the fresh and weathered biosolids samples (26-32 of 57 wastewater-indicator analytes). Not surprisingly, the largest number of CEC detections in soil were in the top soil-depth interval (Figure 2); this top soil interval contained the surficial and incorporated (subsurface) biosolids. The pre-application soil samples also indicate that the top 18 cm of soil contained as many as nine wastewater-indicator CECs (3-beta coprostanol, 3-methyl-1H-indole, acetophenone, beta-sitosterol, beta-stigmastanol, cholesterol, indole, p-cresol, phenol) before biosolids were applied to the study area; most of these listed CECs have natural sources, but some of the CECs could be from nearby roads, vehicles, and farming activities. However, the number of CECs detected in soil intervals 2, 3, 4, 5, and 7 was larger in the samples from day 468 post-application than for pre-application soil samples (Figure 2), indicating that CECs may have migrated vertically down into the soil profile. Note that the samples collected from depth-interval 7 were analyzed only for days −7 and 468 post-application because vertical migration of CECs was expected to be slow in this semiarid environment.

The number of CECs detected in mature-plant crop samples (day 431 post-application) were less than the number of CECs detected in biosolids or soil and were similar in samples from the control site and the biosolids-applied site, but varied by plant part (Figure 2). Slightly more CECs were detected in the grain and stems/leaves of the control-field plants than in the biosolids-applied-field plants (Figure 2); most of these detections were biogenic sterols or other naturally occurring CECs. The roots of the wheat plants from the biosolids-applied field had a larger number of CECs detected (maximum was 16 of 57 wastewater-indicator analytes) compared to the roots of the wheat plants from the control field (maximum was 8

of 57 wastewater-indicator analytes) (Figure 2). Detections in the wheat-plant roots from the biosolids-applied field included AHTN, diethyl phthalate, fluoranthene, HHCB, isopropyl benzene, pyrene, and triclosan (which were not detected in any of the analyses of roots from control-field samples), in addition to biogenic sterols and other naturally occurring compounds such as 3-beta coprostanol, beta-sitosterol, beta-stigmastanol, cholesterol, indole, p-cresol, and phenol. The wheat plants were washed before analysis, so the larger number of CECs detected in the roots from wheat plants collected from the biosolids-applied field indicates uptake by the wheat plants of various CECs into the roots, as opposed to CECs in residual soil on root surfaces. Thus, this study indicates that although the number of CECs detected in biosolids did not substantially diminish over 180 days, some mass of CECs did move from the biosolids downward into the soil profile and into wheat-plant roots by day 431-468 post-application.

Dissipation and transport of CECs also can be evaluated by considering concentration changes of individual CECs throughout the study period. All concentration data and associated quality-control data from this study for individual CECs in biosolids, soil, and crop samples are available in the Supporting Information (Tables S2 through S10).

Concentrations in Biosolids Samples

The concentration data for this study confirm that biosolids can contain many CECs and that many CECs occur in large concentrations (ppm range) relative to concentrations in liquid WWTP effluent (ppt to ppb range) (Barber *et al.*, 2011). A comparison of concentration data for day −0.04 post-application (the biosolids pile pre-application) with the concentration data for day 0 post-application (samples off the geotextile sheet) indicates that concentrations of some CECs in biosolids (such as beta-stigmastanol, HHCB, indole, 4-nonylphonol, phenol, and triclosan) might dissipate during land application through processes such as volatilization, although the heterogeneity of the biosolids and the large analytical uncertainty for this matrix makes the magnitude of this dissipation difficult to quantify. This study indicates that concentrations of many CECs in biosolids can dissipate during weathering at the soil surface after application. The mechanical weathering of the biosolids aggregates in the field at this study area was observed to be much more severe and rapid than the mechanical weathering of similar biosolids samples in the laboratory under forced air, fluorescent or infrared light, or mechanical abrasion observed during the study documented by Yager *et al.* (2013). This suggests that UV light, freeze-thaw cycles, and exposure to water could be important factors in mechanical weathering of land-applied biosolids in eastern Colorado. CECs that had the greatest decrease in concentration during the initial 180 days in the field-weathered biosolids remaining on the land surface included indole, d-limonene, p-cresol, phenol, and 3-methyl-1H-indole (skatol). The detected CEC that demonstrated the most rapid concentration dissipation (undetectable at 180 days post-application) was beta-sitosterol. Most of the CECs that were detected in biosolids were persistent through 180 days of weathering but demonstrated decreasing concentrations. Only one CEC (3-beta coprostanol) demonstrated little concentration dissipation through 180 days of weathering. CECs that were present in the largest concentration in six-month-weathered biosolids (day 180 post-application) included stanols, nonylphenols, bisphenol A, bis(2-ethylhexyl) phthalate (DEHP), HHCB, and triclosan. Previous research determined that the nonylphenols, bisphenol A, DEHP, HHCB, and triclosan were mobile when leached with laboratory-grade deionized water (Yager *et al.*, 2013), indicating that biosolids are a possible long-term source of CECs for multiple terrestrial exposure pathways. This is of potential concern because 4-nonylphenol, bisphenol A, phthalate metabolites, and triclosan have been linked to endocrine disruption and other detrimental environmental effects (Kusk *et al.*, 2011; Langdon *et al.*, 2012). Tris (2-butoxyethyl) phosphate (TBEP) also was detected in biosolids through 180 days post-application, was detected in leachates from fresh and weathered biosolids (Yager *et al.*, 2013), and has been linked to endocrine disruption (Liu *et al.*, 2012).

Concentrations in Soil Samples

The differences in CEC concentrations in soil over the duration of the study (468 days post-application) provide information about the dissipation of detected CEC analytes in a semiarid agricultural environment. The concentration data for the soil samples indicate that many of the CEC analytes had concentrations that were less than the MRL. Compositing of soil samples within each depth interval was done to ensure that CECs moving downward through the soil profile in significant concentration would not be missed. Shortcomings of this compositing approach were that a small mass of CECs in a single soil-depth interval could be diluted to undetectable concentrations and that the homogenization step needed to prepare the composite soil samples could have resulted in a loss of mass for some compounds due to volatilization.

The pre-application soil data indicate that biosolids applications are not the only source of CECs in soil of this area. CECs that were detected in the pre-application soil were 3-beta coprostanol, skatol, acetophenone, beta-sitosterol, beta-stigmastanol, cholesterol, indole, p-cresol, and phenol. Most of these CECs are biogenic sterols (e.g., 3-beta coprostanol, beta-sitosterol, beta-stigmastanol, cholesterol) or fragrances that have natural plant sources in addition to anthropogenic sources, yet their concentrations increased (in some cases, substantially) following application. Concentrations of some CECs showed increased detection frequency but little concentration change in soil post-application (e.g., anthracene, benzo[a]pyrene, TBEP). Concentrations of some CECs in soil dissipated rapidly, decreasing to pre-application levels within 90 days post-application (e.g., 4-nonylphenol monoethoxylate [sum of all isomers, NPEO1], 4-tert-octylphenol, AHTN, fluoranthene) or within 180 days post-application (e.g., skatol, 4-nonylphenol, DEHP, HHCB, pyrene). Some CECs detected in soil were more persistent; concentrations initially spiked upward but did not dissipate to pre-application levels within 468 days post-application (e.g., 3-beta coprostanol, beta-stigmastanol, cholesterol, indole, triclosan), although most detected CEC concentrations in soil dissipated during the 468 days post-application. The data from this study indicate that the concentration of some CECs in the soil such as triclosan might increase over time, at least temporarily. This concentration increase could result from initial chemical transformation into other compounds, such as glucuronides, that are present in biosolids that are subsequently retransformed into the original compound, but the concentration increase is more likely the result of biosolids weathering releasing more of the compound over time. In this study, many CECs in the top (approximately 18 cm) soil-depth interval dissipated markedly during 468 days post-application without a corresponding (quantifiable) concentration increase in the underlying soil intervals. Therefore, the CEC mass dissipation observed in this study likely is not simply from vertical advective transport to deeper soils. The triclosan and nonylphenol data from this study were used in mass-balance computations to evaluate mass-transfer processes and heterogeneity in this terrestrial system. The mass-balance calculations for these two CECs indicate that either the mass of these CECs in the biosolids samples was too heterogeneous or there was too much uncertainty in the various analytical results to be able to explore mass-transfer processes for this field-scale study. Moreover, the disparity in soil concentration for the different sampling nodes at the same depth interval and sample date reflects the heterogeneity in biosolids-application coverage at the core scale (the top depth interval did not contain surficial biosolids at every node that was cored), the heterogeneity in initial biosolids concentration, and the laboratory variability inherent in the analysis of the solids matrix. The complexity of the biosolids matrix can cause analytical interference and increase the analytical uncertainty of the resulting CEC data.

The differences in CEC concentrations with soil depth over the duration of the study (468 days post-application) provide some information about the movement of CECs in this semiarid agricultural environment. The CECs that were detected in the lower depth intervals of soil (approximately 18-126 cm) post-application that were not present in any soil interval pre-application are summarized in Table 1. Thus, the preliminary data from this study indicate that d-limonene, 4-nonylphenol, NPEO1, 4-nonylphenol diethoxylate (NPEO2), 4-tert-Octylphenol monoethoxy (OPEO1), benzo[a]pyrene, diethyl phthalate, fluoxetine, HHCB, triclosan, warfarin, and possibly 3-beta coprostanol, skatol, beta-sitosterol, cholesterol, indole, and p-cresol could migrate downward through the soil by 468 days post-application. Although the biogenic sterols were present in the soil profile pre-application, post-application concentrations in the first, second, and third depth intervals were substantially higher than pre-application for CECs such as beta-sitosterol, and concentration distributions over time with depth indicated some vertical movement (e.g., 3-beta coprostanol, beta-stigmastanol). Recall that biosolids were incorporated to a depth of only 15 cm, so this finding is unlikely to result from just mechanical transport of biosolids aggregates. The sporadic detections of these CECs in the post-application soil profile indicate that lower laboratory reporting levels would be needed to further assess the vertical movement of CECs in soil over time in this area.

Concentrations in Crop Samples

In general, the preliminary concentration data for crop samples collected in this study indicated little uptake of target CECs by wheat plants. The roots of biosolids-grown wheat plants had the largest concentrations (as well as number) of CECs, particularly for the biogenic sterols (e.g., 3-beta coprostanol, beta-sitosterol, beta-stigmastanol, cholesterol). No substantial concentrations of CECs were present in the stems/leaves or grain of the biosolids-grown wheat plants. Concentrations of 3-beta coprostanol, beta-sitosterol, cholesterol, and triclosan were substantially higher in the roots from the biosolids-applied field compared to the roots from the control field.

TABLE 1. Summary of Contaminants of Emerging Concern That Were Detected in Lower Soil Depths That Were Not Present in Any Soil Interval Pre-Application.

Sampled Depth Interval	Target Depth below Surface, cm	CECs Detected Three Days Post-Application	CECs Detected 17 Days Post-Application	CECs Detected 41 Days Post-Application	CECs Detected 90 Days Post-Application	CECs Detected 180 Days Post-Application	CECs Detected 468 Days Post-Application
2	18-36	—	4-Nonylphenol, NPEO1, NPEO2	Diethyl phthalate	—	d-Limonene	HHCB, triclosan
3	36-54	—	Fluoxetine	—	—	d-Limonene	—
4	54-72	—	—	—	—	d-Limonene	OPEO1, triclosan
5	72-90	—	—	—	—	—	—
6	90-108	—	—	OPEO1	—	—	—
7	108-126	—	Warfarin	—	—	—	Benzo[a]pyrene

Note: CECs, contaminants of emerging concern; cm, centimeters; —, no CECs met the criterion; HHCB, hexahydrohexamethyl cyclopenta-benzopyran; NPEO1, 4-nonylphenol monoethoxylate; NPEO2, 4-nonylphenol diethoxylate; OPEO1, 4-tert-octylphenol monoethoxylate.

Concentration of indole was substantially higher in the roots from the control field compared to the roots from the biosolids-applied field. Concentrations of beta-sitosterol were substantially higher in the stems/leaves from the control field compared to the stems/leaves from the biosolids-applied field. Concentrations of skatol, NPEO2, beta-sitosterol, and p-cresol were substantially higher in grain from the control field compared to the grain from the biosolids-applied field. It is not known whether the larger concentrations of beta-sitosterol in stems/leaves and grain from the control site indicate a suppression of this natural CEC from biosolids application, a difference in the fields that is unrelated to biosolids, or natural heterogeneity in this compound. In general, uptake and bioaccumulation of CECs in plants is expected to be little, if any (O'Connor, 1996). Eggen *et al.* (2011) reported uptake of pharmaceuticals by plants (carrots, wheat and barley cereals, meadow fescue, turnip rape seed) and negative effects on growth and development of carrots; however, the wheat samples from the Colorado study were not analyzed for pharmaceutical CECs. Holling *et al.* (2012) reported pharmaceutical and triclosan uptake by cabbage roots and aerials.

Dissipation of Selected CECs

The dissipation and transport over time of selected CECs can be further explored through schematic cross-sectional diagrams that show concentration changes over time and compartment (Figure 3). For this analysis, concentrations were simplified into categories, such as less than the MRL, estimated by the laboratory but less than the MRL, or some factor of 10 times the MRL. Rounded data were used to avoid the appearance of large concentration changes when concentration differences were small. Compartments included biosolids, soil-depth intervals, and wheat-plant parts (Figure 3). Cross-sectional diagrams are included in Figure 3 for 3-beta coprostanol, HHCB, and triclosan. Additional cross-sectional diagrams are included as Supporting Information (Figure S2) for 4-nonylphenol (sum of all isomers), indole, and TBEP. These compounds were selected for the cross-sectional diagrams to show a range of dissipation and mobility for different types of CECs: 3-beta coprostanol is a biogenic sterol, HHCB and indole are fragrances, triclosan is an antimicrobial, 4-nonylphenol is a detergent degradate, and TBEP is a plasticizer and fire retardant. Where no dissipation in biosolids concentration is indicated in Figure 3 and Figure S2 (such as for 4-nonylphenol), the large magnitude of the initial concentration in biosolids and the few concentration classes depicted can mask smaller concentration

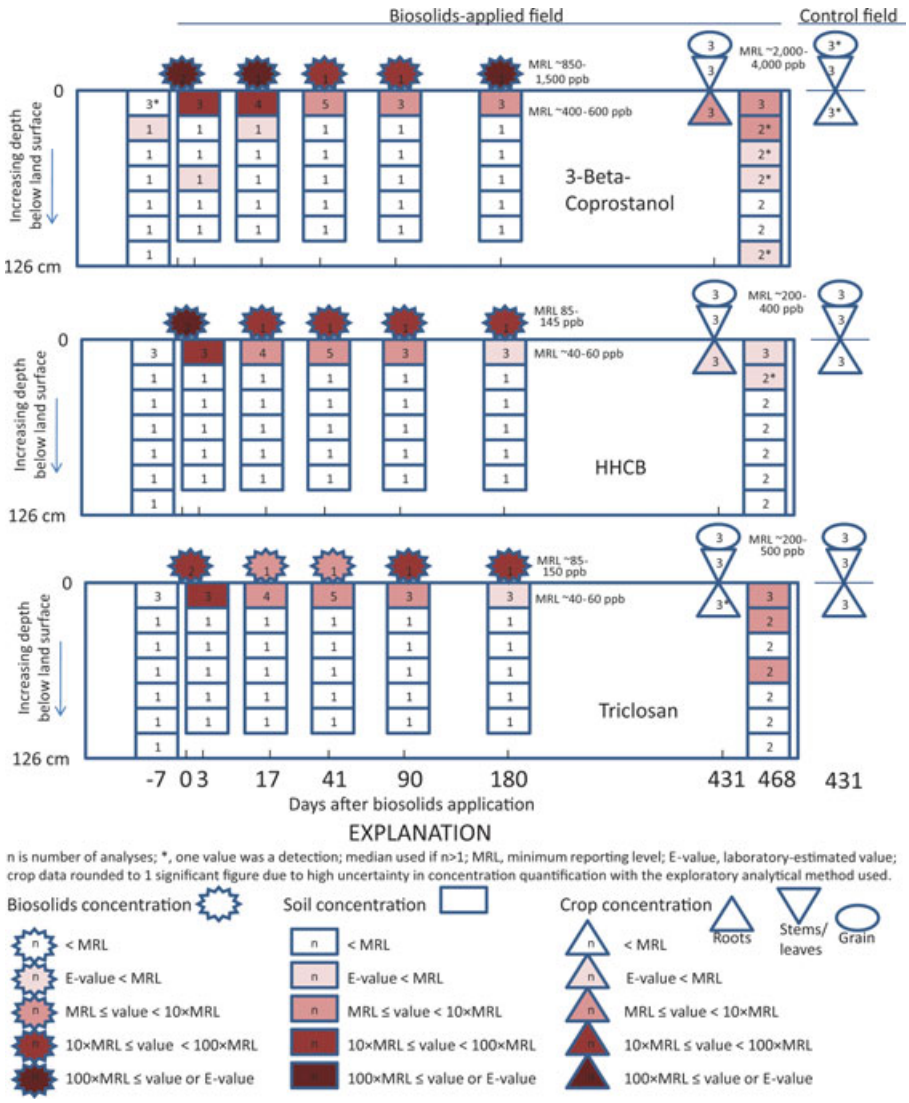

FIGURE 3. Cross-Sectional Diagrams Showing Occurrence and Concentration Dissipation in Biosolids, Soil, and Crops after a Single Application of Biosolids. Neither the biosolids-applied field nor the control field were irrigated.

decreases. The reader is referred to the tables in the Supporting Information for a complete listing of the analytes and concentration data.

3-Beta coprostanol was not only persistent in biosolids and soils but also was somewhat mobile (Figure 3). The mobility of 3-beta coprostanol indicated by the soil data for day 468 post-application is somewhat surprising considering the low water solubility and high octanol-water partition coefficient of this compound. The diagram indicates that mass of this CEC in biosolids likely migrated into soil to wheat-plant roots and from upper soil to deeper soil. The results also indicate that concentrations of 3-beta coprostanol dissipated to some extent in the surface soil over time (after 17 days), but concentrations dissipated little in biosolids through 180 days post-application. Of the samples collected, only the day-468 samples indicated much vertical transport of 3-beta coprostanol through the soil profile; little evidence of vertical transport of 3-beta coprostanol through the soil profile is indicated by the samples collected during the initial 180 days post-application when two-thirds of the precipitation was received (Figure S1).

A synthetic fragrance, HHCB, was persistent in biosolids and soils and had slight mobility (Figure 3). The results also suggest that a small mass of HHCB in biosolids likely migrated into soil and to wheat-plant roots. There appears to be little downward movement of HHCB from the upper soil-depth interval to deeper soil intervals, however. The results indicate more dissipation in soil HHCB concentrations over time (after 17 days) than for 3-beta coprostanol, but not much dissipation in biosolids through 180 days post-application. The increased dissipation

may reflect the greater volatility of HHCB relative to 3-beta coprostanol. Of the samples collected, only the day-468 samples indicated any vertical movement of HHCB through the soil profile; no evidence of vertical transport of HHCB through the soil profile was indicated by the samples collected during the initial 180 days post-application when two-thirds of the precipitation was received (Figure S1).

Triclosan, a synthetic antimicrobial compound, was persistent in biosolids through the 180 days of sampling (Figure 3). Large concentrations in the first (top) soil interval dissipated little over time. Of the samples collected, only the day-468 samples indicated any vertical movement of triclosan through the soil profile; no evidence of vertical transport of triclosan through the soil profile was indicated by the samples collected during the initial 180 days post-application when most of the precipitation was received. Little evidence of plant uptake was indicated by the data for roots, stems/leaves, or grain; only one of three analyses yielded a detection of triclosan, and that was in the roots of the biosolids-grown wheat plants. The dissipation and degradation potential of triclosan in biosolids-applied soil that are documented in the literature vary substantially (Ying *et al.*, 2007; Nakada *et al.*, 2008; Al-Rajab *et al.*, 2009; Xu *et al.*, 2009; Lozano *et al.*, 2010, 2012; Waria *et al.*, 2011; Butler *et al.*, 2012). A rapid dissipation of triclosan concentrations from biosolids (half-life of about 107.4 days) was estimated by Lozano *et al.* (2010) for Canadian agricultural soil that received about five times the biosolids and three times the precipitation of the Colorado fields. Triclosan was persistent in Canadian soil through about 180 days but not through about 365 days post-application where the greater precipitation at the Canadian site flushed triclosan through the soil into tile drainage (Gottschall *et al.*, 2012). Neither the Canadian study nor the Colorado study detected triclosan in wheat grain from the biosolids-applied fields.

Concentrations of 4-nonylphenol (sum of all isomers), a detergent or metabolite, were substantial in the Colorado biosolids (approximately 200 ppm) and persisted through at least 180 days post-application (Figure S2). Although they dissipated over time in the weathering biosolids, concentrations of 4-nonylphenol remained large (approximately 19 ppm, which is greater than 10 times the MRL) through 180 days post-application. Concentrations of 4-nonylphenol in soil were variable but persistent in the first (top) depth interval with no evidence of vertical movement through the soil profile through 468 days post-application (Figure S2). The results of this study indicated limited movement of 4-nonylphenol in soil and no uptake of 4-nonylphenol by any part of the wheat plants; however, the persistence of this CEC in biosolids and soil indicates the potential for long-term environmental effects.

Indole, a fragrance with natural and anthropogenic sources, was detected in biosolids, soil, and crop samples (Figure S2). This CEC was detected in pre-application soil to a depth of approximately 108 cm, as well as in control-field wheat plants (all parts). Concentrations of indole in biosolids were persistent but dissipated through 180 days post-application (Figure S2). Concentrations of indole in soil were variable but persistent. Indole was mobile in soil, or else this fragrance was introduced into deeper soil depths during the coring procedure. Note that indole was not detected in the ambient blank or the equipment blank prepared in the field at 41 days post-application, and detected concentrations in soil post-application were greater than detected concentrations pre-application. Indole was detected in soil throughout the upper 108 cm through 90 days post-application (Figure S2), indicating substantial and rapid vertical movement. As was the case for triclosan concentrations, indole was detected in the first (top) soil-depth interval at higher concentration 468 days post-application than at some of the earlier days (e.g., day 180) post-application. The results from this study indicate indole movement through the soil profile could be related to precipitation because concentrations in all sampled soil intervals were detectable during the initial 90 days post-application when one-third of the precipitation was received, then subsequently dissipated, possibly by dilution. Concentration of indole in the wheat plants (all parts) were similar for the biosolids-applied field and the control field.

A plasticizer and flame retardant, TBEP, was detected in biosolids at relatively small concentrations compared to other CECs but was consistently detected in biosolids through 180 days post-application (Figure S2). Although TBEP was detected in the first (top) soil-depth interval through 180 days post-application, this CEC was not detected in the top soil-depth interval at day 468 post-application. The data from this study indicate little to no vertical movement in soil post-application, although perhaps a lower MRL for TBEP would indicate a different result because much of the data were censored at less than the MRL. An interesting result from this study is that day 17 post-application had the most detections of TBEP (two of four samples) in the top soil-depth interval, not day 3 when biosolids were first applied or day 180 when the TBEP concentration in biosolids was highest; however, all the detected concentrations of TBEP in the top soil-depth interval were less than the MRL. The maximum TBEP concentration detected in the top soil-depth interval was on day 3 post-application (estimated below the MRL as 42.6 μg/kg or ppb). In addition to limited persistence and mobility of TBEP, this study indicated no uptake of TBEP by wheat plants.

Previously published research also determined the dissipation of selected CECs in biosolids-amended soil in Australia (Langdon *et al.*, 2012) where some of the study plots were applied with centrifuged biosolids (about 40% moisture, as in the Colorado study) but at an application rate of 25 Mg/ha (about six times the application rate used at the Colorado field). Langdon *et al.* (2012) determined that triclosan concentration showed no statistically significant dissipation in the upper 0.1-m depth of biosolids-amended soil in Australia during the 336-day trial, whereas 4-nonylphenol, 4-tert-octylphenol, and bisphenol A dissipated to 50% of the initial concentration by day 289 post-application. In contrast to the results from Langdon *et al.* (2012), the data from our Colorado study indicate that concentrations of 4-nonylphenol (sum of all isomers) and 4-tert-octylphenol in the upper 0.2-m depth (target interval of 0-18 cm) of biosolids-amended soil took less than 180 days to dissipate to 50% of initial concentration; however, this study indicated that triclosan concentration can take more than one year to dissipate to 50% of initial concentration. As noted by Langdon *et al.* (2012), dissipation rates in the field can greatly exceed those determined through laboratory experiments and can be site specific because of differences in application/incorporation approaches, climate, and soil type.

The results of this study indicate that CECs in biosolids persisted in a real field-application environment. Between 180 and 468 days post-application, select CECs migrated deeper into the soil profile or were taken up by plant roots. The processes of transport could be direct infiltration, leaching, diffusion of volatilized compounds, or other processes that were not specifically identified in this study. The analytical methodology used in this study was not designed to comprehensively assay parent CECs and their degradation products; thus, the data from this study have limited applicability to the identification of CEC-transformation processes, and some decreases attributed to dissipation or volatilization may instead reflect transformation to unmeasured degradates. This study suggested little effect of transport on degradation rate, which indicates that the CECs in this study may be more affected by the mechanical and chemical weathering effects of precipitation on the biosolids than by simple advection at the time of precipitation.

CONCLUSIONS

This study demonstrates that agronomic applications of biosolids result in detectable concentrations of CECs in soil, and that CECs can persist in surficial biosolids and in soil-biosolid mixtures at detectable concentrations on time scales exceeding one year, particularly under the semiarid conditions present at this study site. This study also demonstrates that concentrations of many CECs in biosolids can dissipate during weathering at the soil surface after application and demonstrates the vertical mobility of some CECs down through soil horizons over the same one-year time scale under relatively arid, low-precipitation conditions. Although Yang *et al.* (2012) reported lateral CEC transport from simulated 100-year rain events at an adjacent biosolids-amended study plot, the study discussed in this article documented vertical transport and long-term storage of select CECs in surface soils with natural precipitation. Results from this and similar studies will be essential to understanding the effects of land application of biosolids on the storage or mobilization of CECs. As biosolids application continues as a means for amending organic-carbon- or nutrient-deficient soils and managing the solid products of wastewater treatment, the results from this study will help define the transport time frames and enable estimation of the potential effects of CEC transport from biosolids-amended soils. As population growth continues in the arid Western U.S., wastewater-management strategies such as land application of biosolids will need to be assessed to predict potential effects of biosolids-associated CECs, particularly those CECs that are persistent or mobilized.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article, including: further description of the study area, field methods, and quality-control sample results; all data tables; a graph of cumulative precipitation; and additional constituent diagrams.

Figure S1. Cumulative precipitation since biosolids application, in millimeters, estimated for the study area. Markers indicate biosolids- or soil-sampling dates (number in parentheses is number of days post-application). Data estimated from Kersey, Colorado KCOKERSE1 (unpublished data from Weather Underground accessed April 2007 through October 2008 at http://www.wunderground.com/weatherstation/WXDailyHistory.asp?ID=KCOKERSE1) and from Bennett, Colorado KCOBENNE3 (unpublished data from Weather Underground accessed April 2007

through October 2008 at http://www.wunderground.com/weatherstation/WXDailyHistory.asp?ID=KCOBENNE3).

Figure S2. Cross-sectional diagrams showing occurence and concentration dissipation in biosolids, soil, and crops after a single application of biosolids. Neither the biosolids-applied field nor the control field were irrigated.

Table S1. Texture, bulk density, and organic carbon data for pre-biosolids soil.

Table S2. Chemical data from wastewater-indicator analyses for biosolids composite samples.

Table S3. Chemical data from pharmaceutical analyses for biosolids composite samples.

Table S4. Chemical data from wastewater-indicator analyses for soil samples.

Table S5. Chemical data from pharmaceutical analyses for soil samples.

Table S6. Chemical data from wastewater-indicator analyses for crop samples.

Table S7. Chemical data from wastewater-indicator analyses for quality-assurance samples.

Table S8. Chemical data from pharmaceutical analyses for quality-assurance samples.

Table S9. Relative percent difference for chemical data from wastewater-indicator analyses for environmental sample-replicate pairs.

Table S10. Relative percent difference for chemical data from pharmaceutical analyses for environmental sample-replicate pairs.

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