Appendix 3. Quality-control sample results and discussion

Biosolids and Soils

Field-replicate samples ("field") of biosolids and split-replicate samples ("split") of soils were submitted blindly. Variability between replicates was determined as the relative percent difference (RPD = absolute difference divided by average, multiplied by 100) of elemental total recoverable concentrations. All precision data are shown in **Error! Reference source not found.**

Variability between biosolids field replicates generally was low, less than about 25 percent for most elements. This finding was quite good given the relatively small size of the subsamples, between 0.5 and 3 milliliters (mL) removed from about 1 liter of a largely aqueous sample, wherein much of the chemical mass is likely associated with the solids (present at less than 3 percent dry weight). Elements with high variability included antimony (>25 percent) and mercury (52 percent), both in biosolids pair #2. For antimony, one replicate was below the reporting level of 1.37 mg/kg (milligrams per kilogram) and the other value was slightly above that at 1.77 mg/kg. Therefore, the absolute values were fairly close despite the RPDs being quite different. In the case of mercury, the two values were three orders of magnitude above the reporting limit. This high variability was likely related to the relatively small (0.5 mL) subsample size that was used for mercury analysis although the other replicate set had a RPD for mercury of only 8 percent. In any case, all mercury values for biosolids were discussed in light of the potentially high variability.

Variability between soil split replicates was quite low for most elements and generally was less than about 25 percent. Three exceptions were chromium (60 and 61 percent), selenium (>24 percent), and mercury (25 percent). These results were especially acceptable because of the inherent difficulty of homogenizing and subsampling a solid phase sample. For chromium and mercury, the values for each replicate were well above the respective reporting limits. Although some variability might be due to the inability to collect true replicates, that does not explain the high variability seen between these replicate values, especially given the large number of the other elements that were less variable in these same replicates. For selenium, one replicate value was below the reporting limit of 1.1 mg/kg and the other was slightly above (1.4 mg/kg). Thus the absolute values were fairly close despite the RPDs being quite different.

Variability in *E. coli* and fecal coliform counts in solid phase samples was determined using four sets of field replicates—two for soils and two for biosolids. All bacterial counts in both biosolids replicate sets as well as in one set of soil replicate sets were below the detection level (data not shown). For the second soil replicate set, the RPD of the fecal coliform analyses was 0 percent. The RPD of the *E. coli* analysis was 146 percent, although the absolute difference was only 105.4 most probable number per gram total solids (MPN/g TS).

Nine of the 29 human-health pharmaceuticals were detected in at least one of two pairs of field replicate samples of biosolids (table 3-2). The RPDs ranged from 5 percent for cotinine to >183 percent for caffeine, both in the second replicate pair. Compounds with relatively large

absolute differences determined in replicates included caffeine (>57.14 micrograms per kilogram, µg/kg), citalopram (23.70 µg/kg and 10.52 µg/kg), fluoxetine (>13.17 µg/kg).

A pair of field replicates of biosolids was used to estimate the variability of the various hydrocarbon analyses (table 3-3). The RPDs ranged from 0 to 43 percent. Oil and grease, operationally determined as n-hexane extractable material (HEM), and non-polar material, operationally defined as silica gel treated n-hexane extractable material (SGT-HEM), were the most variable constituents. The RPDs were 43 percent for HEM and 42 percent for SGT-HEM. The absolute differences for these constituents were 33 milligrams per liter (mg/L) and 23 mg/L, respectively, for HEM and SGT-HEM.

To determine bias in elemental concentrations of solid phases, total recoverable (that is, leachable) elements were determined in two standard reference materials (SRMs) obtained from the National Institute of Standards and Technology (NIST, Department of Commerce). NIST 2711a is Montana II soil and NIST 2781 is domestic sludge (biosolids). These particular SRMs were chosen as either the only available choice in the case of domestic biosolids or as a best match to the elemental concentrations in early project samples in the case of soil. Note that no total recoverable values are certified; only the total concentrations (that is, "total-total") are certified. The reporting levels associated with all methods were sufficiently low to address the data quality objective dealing with determining any exceedances of the ceiling concentration limits for all biosolids applied to land as set in EPA Part 503 Biosolids Rule (table 3-4). Note that the units for mercury in table 3-4 are milligrams per kilogram (mg/kg) whereas all results reported for mercury as part of this study are in micrograms per kilogram (µg/kg).

All SRMs were submitted blind, and recoveries of elements in biosolids were necessarily determined in spiked samples rather than submitted neat (dry) in order to capture the likely largest source of bias—that associated with homogenization of a mostly aqueous sample. The biosolids spike consisted of 10.00 grams dry SRM material added to one of a pair of biosolids split replicates consisting of about 1 liter of sample that contained a few percent dry weight of solids. The goal was to approximately double the concentrations of as many elements as possible and the weight percent solids. Therefore, the overall bias in the biosolids elemental recovery reflected somewhat more than just the laboratory's analytical performance. The bias also reflected the ability to collect true split replicate biosolids samples and to spike each sample accurately.

Of the elements with EPA ceiling concentrations for biosolids, the total recoverable concentrations of arsenic, mercury, molybdenum, and selenium in NIST 2781 (domestic sludge) were not available. For these elements, total recoverable values were estimated using various methods (table 3-5). It was assumed that "total recoverable"/"total-total" ratios for arsenic, mercury, and selenium in NIST 2781 would be similar to ratios demonstrated in the NIST 2711a soil reference material. For molybdenum, it was assumed that recovery in the biosolids reference material would be similar to that achieved for chromium, because both are Group 6 transition metals.

Recovery efficiency (in percent) of metals and nutrients in soil and biosolids spikes was determined once for soil and twice for biosolids (table 3-6). Highlighted elements in table 3-6 are

those with criteria for ceiling concentration in biosolids (table 3-4). Two assumptions were used to construct table 3-6:

1. Recoveries for arsenic, molybdenum, selenium, and mercury in biosolids (shown in *italics*) were estimated from other information as noted in table 3-5.

2. Recoveries for phosphorus and total Kjeldahl nitrogen were calculated using the total values because no total recoverable values were reported. These calculations were reasonable because nitrogen likely does not exist in refractory mineral matrix form, and phosphorus likely exists largely in iron oxyhydroxides that would be included in the total recoverable analysis.

Elemental recoveries in biosolids were remarkably good given the multiple sources of variability. The two exceptions were 33 percent for aluminum in the second spike and –17 percent for mercury in the first spike. The low recovery of aluminum and mercury in biosolids was possibly related to the mass in the spike being only about a quarter of that in the environmental sample. Thus the RPD reflected the small difference between two large numbers. Also, the poor mercury recovery likely also reflected the potentially high variability of the analysis. The molybdenum recovery in the first spike was somewhat high (126 percent) though this might be related to the assumption that using the ratio for total to total recoverable concentrations for chromium was incorrect (see above). Otherwise, recoveries for the eight elements except mercury and molybdenum with EPA ceiling concentrations was good and ranged from 70 percent for chromium (second spike) to 101 percent for selenium in the second spike.

Elemental recoveries in soil were generally good except for antimony, which was quite low at 6 percent. The extremely large maximum value for beryllium was a mathematical artifact resulting from the SRM total recoverable concentration being 0.93 mg/kg and the laboratory result being <2.46 mg/kg. Recoveries for elements with EPA ceiling concentrations ranged from 70 percent for selenium to 111 percent for mercury. In addition to mercury, iron and manganese had recoveries in excess of 100 percent. None of these results compromised meeting project objectives of comparing chemical concentrations in soils before and after biosolids application.

Matrix spike recoveries in sludge for constituents in the three categories of emerging contaminants—wastewater indicator compounds, hormones, and pharmaceuticals—are given in table 3-7. The recoveries ranged from –1,199 percent (indole in the wastewater indicator compounds group) to 2,270 percent (3-beta-coprostanol in the hormones group). Only a handful of compounds among the three schedules had reasonable recoveries defined as between about 70 and 130 percent. Factors affecting recovery include:

1. extremely complex aqueous matrix with a few percent solids by weight,

2. difficulty of collecting true replicates of a complex matrix,

3. presence of interferents (contributing to artificially high recoveries), and

4. relatively small differences between concentrations in the environmental and spiked replicates.

For compounds with very high or very low recoveries, results were interpreted with suitable caution.

Several matrix spikes were analyzed for the various hydrocarbon analyses of soil and biosolids (table 3-8). The median recoveries in soil samples were quite good and ranged from 69.5 percent for SGT-HEM to 103.5 percent for HEM. Two matrix spikes for three constituents were analyzed for biosolids samples. The median recoveries were 121 percent for HEM, 76 percent for motor oil range (C24-C36) organics, and 101.5 percent for SGT-HEM.

Surface-water and groundwater quality

Detections of target constituents in blanks for surface water and groundwater analyzed at the U.S. Geological Survey (USGS) National Water Quality Laboratory (NWQL) are summarized in table 3-9. For surface water, there were 19 detections of inorganic constituents and bacteria, mostly among the nutrients and ions but also some bacterial. The overall detection rate of inorganic constituents and bacteria was 10 percent. There were 15 detections of trace organic constituents, yielding a detection rate of 4 percent. Groundwater blanks had a 13 percent detection rate which was fairly evenly split between nutrients and ions and bacteria. Trace organics were detected in groundwater blanks at a rate of 2 percent. Among these detections in surface water and groundwater, most concentrations were near the respective reporting levels defined as being within a factor of five. The one exception was nitrate plus nitrite as nitrogen, which was present at 0.311 mg/L (reporting limits of 0.008 and 0.04 mg/L) in one surface-water blank (table 3-10). The source of this constituent was unknown but its presence was not pervasive. Finally, two constituents, ammonia-nitrogen in surface water and calcium in groundwater, were detected in seven blanks for each type of sample. Though numerous, none of these detections exceeded five times the respective reporting levels; however, the ammonia detections in the surface-water blanks enveloped approximately 60 percent of the detections in the environmental samples, which indicates a potential contamination issue. Further examination of the data revealed that ammonia represents a small portion (mean of 3 percent with a standard deviation of 3 percent) of the total nitrogen in the surface-water samples associated with this study, and more than a third of the environmental sample results were censored. Therefore, any ammonia contamination does not appear to be a pervasive problem or appreciably affect the analysis and conclusions of the study related to nitrogen in surface water. Overall, these blanks indicated no systematic positive bias.

Ten surface-water blanks were analyzed at the North Carolina Department of Environment and Natural Resources laboratory to assess contamination associated with the sampling equipment over the course of the study. Of the 430 analyses, there was only one detection of total dissolved solids (TDS) in an automated sampler (ISCO) blank at a concentration of 18 mg/L (reporting level = 12 mg/L) (table 3-11). One groundwater blank was analyzed, and there were no detections.

Variability of constituents and bacteria in surface-water samples was assessed with various types of replicates (fig. 3-1; table 3-11). Replicate sets consisted of EWI (equal width

increment) method sample pairs, ISCO sample pairs, and comparisons between concurrent EWI and ISCO samples. The latter comparisons were used to assess the representativeness of the point sampling done by the autosamplers when compared to the more spatially representative EWI technique. No replicates were available for trace organic constituents in surface water. The median RPD for analytes in the EWI replicates was 3 percent and ranged from 0 to 13 percent. The median RPD for ISCO replicates was 8 percent and ranged from 0 to 69 percent. Total manganese (RPD = 51 percent) was associated with a relatively small absolute difference of 21 micrograms per liter (µg/L) compared to the median concentration in the environmental samples of 170 µg/L. In contrast, dissolved aluminum (RPD = 48 percent) and dissolved iron (RPD = 44 percent) were associated with absolute differences of 290 µg/L and 170 µg/L, respectively. These concentrations were significant with respect to the median concentration in the environmental samples of 330 µg/L for dissolved aluminum and 670 µg/L for dissolved iron. The highest variability was among the EWI-ISCO comparison sets and specifically for ammonia, filtered (RPD >50 percent) and *E. coli* (RPD ~75 percent). The rest of the constituents among the replicate sets had RPDs less than about 30 percent. Although the high ammonia RPD resulted from small absolute differences in concentrations near the reporting level, the *E. coli* counts were well above the reporting level (10,400 MPN/100 mL and 9,700 MPN/100 mL for the EWI-ISCO comparison and the ISCO replicate set, respectively). This variability was considered in the discussion.

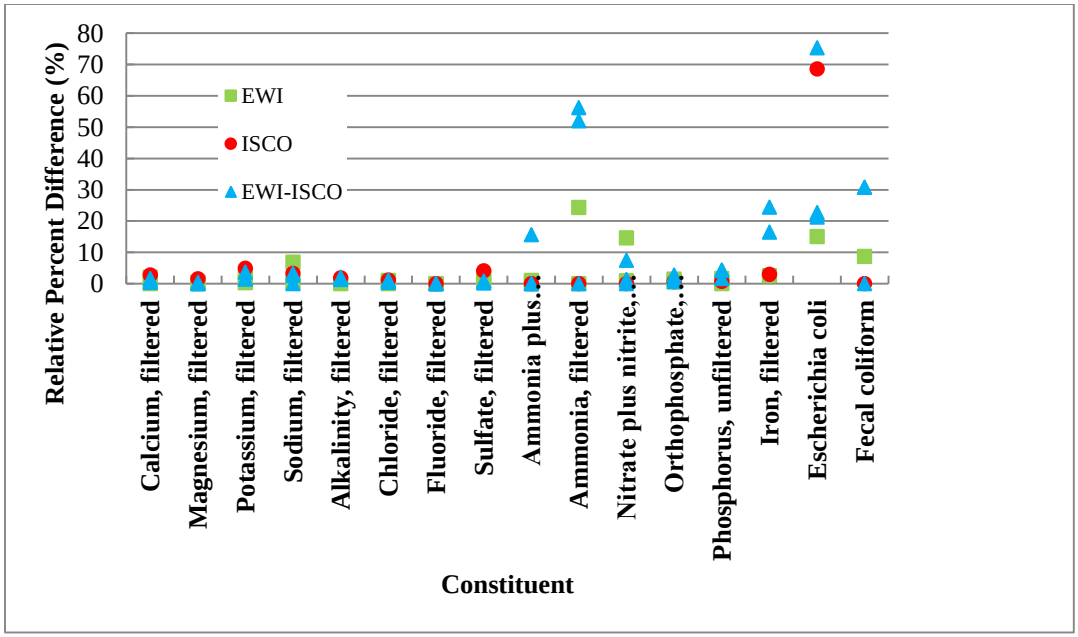

Figure 3-1. Precision of inorganic and bacterial constituents in replicate surface-water samples collected by the EWI (equal width increment) method, automated sampler (ISCO), and EWI-ISCO comparisons from surface-water monitoring sites on Collins Creek and Cane Creek in Orange County, North Carolina.

Variability of inorganic constituents and bacteria in groundwater samples were assessed with six replicate sets (fig. 3-2). Most constituents in most replicate sets had relatively low RPDs. The most variable constituents included fluoride, iron, and fecal coliform with RPDs ranging from 86 percent to almost 60 percent. That said, the concentrations and counts were near the respective reporting levels and so the absolute differences were small. One replicate set of groundwater samples was analyzed for trace organic constituents. Only two compounds were present above their respective reporting level in both samples, atrazine and bisphenol A. The RPD for these compounds was 13 percent and 44 percent, respectively, but the absolute differences were very small in both cases—0.01 µg/L for atrazine and 0.04 µg/L for bisphenol A.

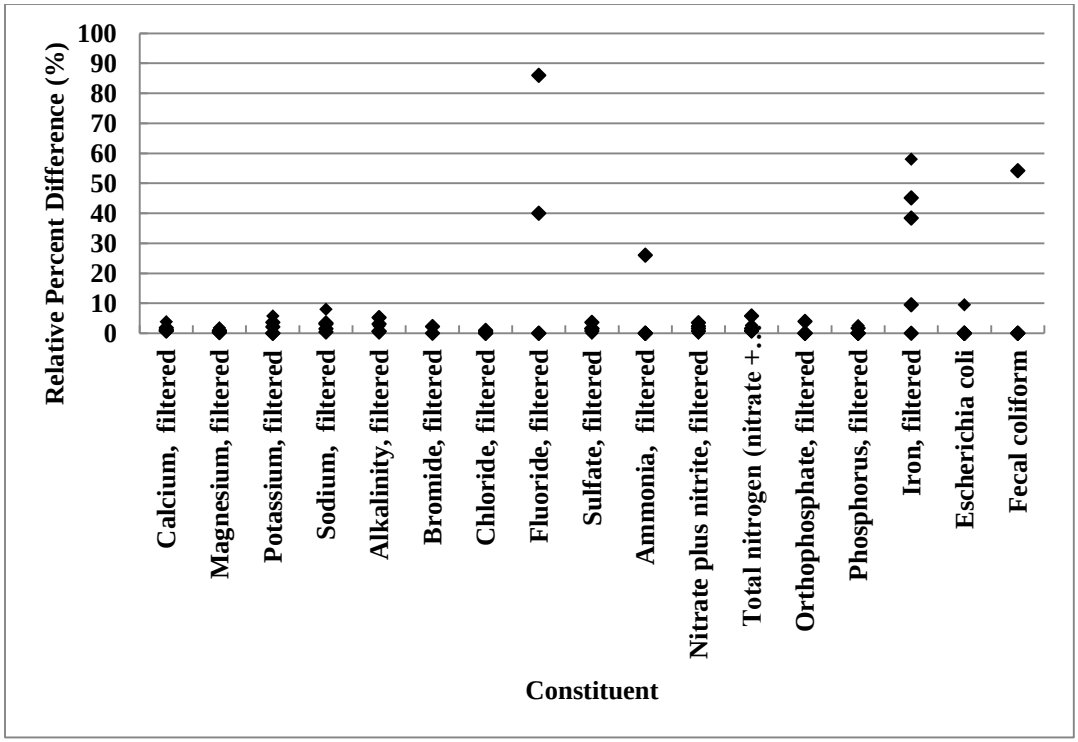

Figure 3-2. Precision of inorganic and bacterial constituents in replicate water-quality samples from the study groundwater wells in Orange County, North Carolina.

Matrix spike recoveries in surface water for constituents in the three categories of emerging contaminants—wastewater indicator compounds, hormones, and pharmaceuticals—are given in table 3-12. The recoveries ranged from a minimum of –239 percent for bis(2-ethylhexyl) phthalate in the wastewater indicator compounds group) to 144 percent for cholesterol in the hormones group. In contrast to the case of recovery in the sludge matrix, many compounds among the wastewater indicator compounds and hormones had reasonable recoveries defined as between about 70 and 130 percent. The same factors that affected recovery in sludge were operative for recovery from surface water. Results for compounds with very high or very low recoveries were appropriately qualified.

Two Standard Reference Samples (SRSs) were submitted blind to NCDENR, one for total metals (aluminum, barium, beryllium, copper, iron, manganese, molybdenum, nickel, lead, and zinc) and one for dissolved metals (silver, arsenic, cadmium, chromium, copper, iron, manganese, molybdenum, nickel, lead, antimony, selenium, thallium, and zinc) though both only contained dissolved metals (table 3-11). The median RPD was within 4 percent of the nominal concentrations of all metals for both total and dissolved metal analyses and ranged from 0 to 18 percent.