Trace Element Concentrations in Soil, Corn Leaves, and Grain after Cessation of Biosolids Applications

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ABSTRACT

From 1974 to 1984, 543 Mg ha^{-1} of biosolids were applied to portions of a land-reclamation site in Fulton County, IL. Soil organic C increased to 5.1% then decreased significantly ($p < 0.01$) to 3.8% following cessation of biosolids applications (1985–1997). Metal concentrations in amended soils (1995–1997) were not significantly different ($p > 0.05$) (Ni and Zn) or were significantly lower ($p < 0.05$) (6.4% for Cd and 8.4% for Cu) than concentrations from 1985–1987. For the same biosolids-amended fields, metal concentrations in corn (*Zea mays* L.) either remained the same ($p > 0.05$, grain Cu and Zn) or decreased ($p < 0.05$, grain Cd and Ni, leaf Cd, Cu, Ni, Zn) for plants grown in 1995–1997 compared with plants grown immediately following termination of biosolids applications (1985–1987). Biosolids application increased ($p < 0.05$) Cd and Zn concentrations in grain compared with unamended fields (0.01 to 0.10 mg kg^{-1} for Cd and 23 to 28 mg kg^{-1} for Zn) but had no effect ($p > 0.05$) on grain Ni concentrations. Biosolids reduced ($p < 0.05$) Cu concentration in grain compared with grain from unamended fields (1.9 to 1.5 mg kg^{-1}). Biosolids increased ($p < 0.05$) Cd, Ni, and Zn concentrations in leaves compared with unamended fields (0.3 to 5.6 mg kg^{-1} for Cd, 0.2 to 0.5 mg kg^{-1} for Ni, and 32 to 87 mg kg^{-1} for Zn), but had no significant effect ($p > 0.05$) on leaf Cu concentrations. Based on results from this field study, USEPA's Part 503 risk model overpredicted transfer of these metals from biosolids-amended soil to corn.

The land application of sewage sludge (biosolids) for the purpose of conditioning the soil and fertilizing crops is a common practice in the United States. Bastian (1997) reported that as much as 4.13 × 10^6 Mg of biosolids are currently land-applied annually in the United States. The land application of biosolids is regulated by the 40 CFR Part 503 regulation (USEPA, 1993).

Trace element concentrations in crop tissues can be elevated by biosolids application to land (Chaney, 1973; Council for Agricultural Science and Technology, 1980; Logan and Chaney, 1983; Corey et al., 1987; Sloan et al., 1997; Barbarick and Ippolito, 2003). Because of this, the Part 503 regulation set limits on the total mass of nine trace elements that can be applied to a unit area of land. The regulation also established concentration limits for the nine trace elements that were determined by risk analyses that evaluated 14 terrestrial pathways (USEPA, 1992). The trace element concentration limits make land application of biosolids comparable with commercial fertilizer application in that biosolids that meet them can be applied without cumulative mass loading restrictions.

The Part 503 risk assessment for land application included five pathways where the transfer of trace elements from biosolids-amended soil to plant tissue was modeled using data from existing scientific literature to establish plant uptake coefficients (UCs) (USEPA, 1992). The Part 503 pathways in which the transfer of trace elements was modeled from soil to plant tissue and the trace elements included in each pathway are listed below:

1. Biosolids → soil → plant → human (home gardener) (As, Cd, Hg, Ni, Se, Zn)
2. Biosolids → soil → plant → human (consumer) (As, Cd, Hg, Ni, Se, Zn)
3. Biosolids → soil → plant → animal (As, Cd, Cu, Pb, Mo, Ni, Se, Zn)
4. Biosolids → soil → plant → animal → human (Cd, Hg, Se, Zn)
5. Biosolids → soil → plant (Cr, Cu, Ni, Zn)

The USEPA computed the UC as the slope of the linear regression of the trace element loading rate (kg trace element ha^{-1} of land) with the resulting concentration of trace element in the tissue of plants growing in the biosolids-amended soil (mg trace element kg^{-1} dry plant matter) from individual studies in the published literature. The UC values for specific trace element–plant type pairs (e.g., Cd uptake by cereals and grains, or Zn uptake by leafy vegetables) that were used in the Part 503 regulation were derived by calculating the geometric mean of the UC values from individual studies (USEPA, 1992).

The long-term land applications of biosolids permitted under the Part 503 regulation will cause a gradual buildup of trace element concentrations in amended soils over time. Most of the data used by the USEPA to calculate the UC values in the Part 503 risk assessment were from short-term studies where the trace element uptake by plants was measured during years when biosolids were applied to the land. The Part 503 risk assessment methodology implicitly assumed that bioavailability of the trace elements would not change after biosolids applications ceased.

Organic matter is known to comprise a significant fraction of biosolids, typically 20 to 60%, depending on processing. Consequently, concerns have been raised that the bioavailability and mobility of added trace ele-

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Abbreviations: UC, uptake coefficient.

ments in the amended soils may increase over time after cessation of biosolids applications, as the biosolids organic matter is mineralized. This has been attributed both to the loss of trace element binding capacity related to mineralization of organic matter associated with soil solids (Chaney, 1973; Beckett et al., 1979; McBride, 1995), and to mobilization of trace elements by dissolved organic carbon that is produced during mineralization of biosolids organic matter (Lamy et al., 1993; McBride et al., 1997; Antoniadis and Alloway, 2003).

If increases in trace element bioavailability were to occur due to mineralization of biosolids organic matter in the years following cessation of biosolids application, the UC values used in the Part 503 regulation would underpredict the transfer of trace elements from biosolids-amended soil to the tissues of plants growing in those soils after biosolids applications ceased. This means that the Part 503 risk assessment would underestimate the exposure of humans and animals to trace elements from the ingestion of plant tissue grown on biosolids-amended land and, ultimately, the Part 503 regulatory limits would not provide adequate protection for humans and animals from this route of exposure. Hence, it is important to compare the uptake of trace elements predicted by the Part 503 UC to occur in soil many years after biosolids applications have ceased with trace element uptake that has occurred at field application sites.

In long-term field plots, Chang et al. (1997) and Hyun et al. (1998) observed a reduction of organic C in the biosolids-amended plots of 40%, but did not observe increased bioavailability of Cd 10 yr following cessation of biosolids applications. Brown et al. (1998) did not observe any significant increase in uptake of Cd in biosolids-amended soils that had lost 84% of the biosolids-added organic C 13 to 15 yr following cessation of applications. White et al. (1997) reported that diethylenetriamine-pentaacetic acid (DTPA)-extractable metals (Fe, Zn, Cu, Cd, and Pb) were highest 4 yr following cessation of biosolids applications, but were near concentrations in control soils 8 yr following cessation of applications. Walter et al. (2002) observed that 35 to 44% of the biosolids organic matter was mineralized 9 yr following cessation of biosolids applications but found that DTPA-extractable metals (Zn, Cd, Cu, Ni, Pb, Cr) had decreased over the same period. Barbarick and Ippolito (2003) observed large decreases in DTPA-extractable Cu and Zn following cessation of biosolids applications, but did not observe concomitant decreases in uptake of these elements into wheat (*Triticum aestivum* L.) grain. In contrast, McBride et al. (1997) observed large losses of trace elements 15 yr following cessation of biosolids applications to an orchard, implying that they had become very mobile following application in biosolids. We wanted to test the hypothesis that trace element concentrations would not increase in crop tissues after cessation of repeated annual applications of biosolids at a large-scale land application site.

The Metropolitan Water Reclamation District of Greater Chicago has owned and operated a 6300-ha land reclamation site in Fulton County, Illinois, since 1971. Biosolids are being used to reclaim strip-mined soils and fertilize non-mined soils to produce crops. We were able to identify a group of similarly treated fields that had received annual biosolids applications from 1974 through 1984. These fields have not received biosolids applications since then, and they are monitored annually for soil and plant tissue trace element concentrations. The monitoring data were used to determine whether the HNO_3–extractable concentrations of Cd, Cu, Ni, and Zn in soil, corn leaves, and corn grain from biosolids-amended fields change with time after biosolids applications cease. These elements were observed by Sloan et al. (1997) to have the greatest relative bioavailability among the trace elements they studied. Throughout this study it is assumed that changes in plant concentrations of these elements will result from changes in trace element bioavailability in the soil. We used these data to test the hypothesis that cessation of biosolids applications and subsequent mineralization of residual soil organic carbon would not result in increases in the bioavailability of Cd, Cu, Ni, and Zn and hence would not result in increased concentrations of these elements in corn at this site.

These monitoring data were also used to examine how closely select Part 503 plant uptake coefficients predicted actual transfer of Cd, Cu, Ni, and Zn from biosolids-amended soil to corn at this site. The Part 503 risk assessment Pathways 1, 2, and 3 assess risk to humans and animals from direct ingestion of food and feed crops, respectively. Risk to humans directly ingesting crops grown on biosolids-amended soils is assessed for consumers who purchase the fruits and vegetables they eat in Pathway 1 and for home gardeners in Pathway 2 (USEPA, 1992). Pathway 3 assesses risk to animals that consume forage grown on biosolids-amended soil (USEPA, 1992). Since the fields used in this project are virtually identical to midwestern farm fields that produce crops for consumers and feed for animals, we focused on Pathways 1 and 3. In Pathway 1, the USEPA determined UCs for potatoes, leafy vegetables, legumes, root vegetables, garden fruits, peanuts, and grains and cereals. We used the data from this study to test the hypothesis that the UC for grains and cereals overpredicts uptake of Cd, Ni, and Zn into corn grain and that the UC for animal forage overpredicts the uptake of Cd, Cu, Ni, and Zn into corn leaves at this site.

MATERIALS AND METHODS

Site Description

The experimental area is a functioning land reclamation site located approximately 300 km southwest of Chicago in Fulton County, Illinois. The site has mean annual precipitation of 948 mm. During the study years of 1985 through 1987, the site received 986, 969, and 661 mm of rainfall, respectively. During 1995 through 1997 it received 1087, 703, and 897 mm, respectively. The entire site consists of 6300 ha of non-mined land, calcareous strip-mine soil, acidic coal refuse material, mine lakes, and wooded areas. Approximately 1751 ha of non-mined land and calcareous strip-mine soil have been developed into more than 75 fields for biosolids applications and crop production. For the purposes of this study, we identified eight fields, each averaging 17.1 ha, that had received similar loadings of biosolids in the same years. The mean cumulative mass loading of biosolids and the trace elements Cd, Cu, Ni, and Zn applied to these fields from 1974 through 1984 was 543 Mg ha^{-1} and 135, 873, 213, 1647 kg ha^{-1}, respectively. These fields have not received any biosolids since 1984, and they are referred to as biosolids-amended fields. We also identified 18 fields, each averaging 25.6 ha, that had never received biosolids. These fields are henceforth referred to as unamended fields. The non-mined soils in the fields used in this study consisted of Aquic Argiudolls, Typic Endoaquolls, Typic Argiudolls, Udollic Endoaqualfs, Typic Hapludalfs, and Mollic Hapludalfs. The fields included in this study, which are spread across the 6300-ha site, contain various combinations of these soils.

Description of Biosolids

The biosolids applied to the biosolids-amended fields were anaerobically digested sludge from the Stickney and Calumet Water Reclamation Plants. From 1974 through 1979 liquid biosolids were applied to land by subsurface injection, and after 1979 they were land-applied as a cake using a manure spreader.

The biosolids applied to the biosolids-amended fields had a mean volatile solids (approximates organic matter) content of 41.9%. The volatile solids content of the biosolids was determined by heating in a muffle furnace at 550°C for 1 h. Organic C was not directly determined on the biosolids but was estimated using the common conversion factor of 0.58 (mass organic C/mass organic matter). Using this factor and the mean volatile solids content, the biosolids in this study were estimated to have an organic C content of 24.3%. The biosolids had mean Cd, Cu, Ni, and Zn concentrations of 248, 1607, 392, and 3033 mg kg^{-1}, respectively. To put these trace element concentrations into perspective, the mean concentrations of Cd, Cu, Ni, and Zn in the USEPA's 1988 National Sewage Sludge Survey were 6.9, 741, 42.7, and 1202 mg kg^{-1}, respectively (USEPA, 1990). The current Part 503 concentration limits for Cd, Cu, Ni, and Zn in exceptional quality biosolids (as defined in Table 3 of Section 503.13) are 39, 1500, 420, and 2800 mg kg^{-1}, respectively. The soil pH of all the fields, as required by Illinois law, must be maintained at 6.5 or higher. Dried biosolids were estimated to have bulk density of 0.69 g cm^{-3}. This was determined by filling metal cylinders of known volume with biosolids under water tension to allow the biosolids to settle under simulated natural conditions, then to dry before taking measurements (Simmons, 2003). This value was found to be in good agreement with values computed from scaled semi-trailers used to haul air-dried biosolids.

Soil and Plant Sampling and Analysis

Details of the soil and plant sampling and analysis that are conducted annually, as part of the environmental control system for the site, are reported by Granato et al. (1991, 1995). Plant tissue and soils were extracted with concentrated $HNO_3 + HClO_4$ and $HNO_3 + H_2O_2$, respectively before trace element analysis. Soil organic C was determined by the Walkley–Black method (Nelson and Sommers, 1982). Soils were sampled by dividing each field in half and collecting 20 to 25 cores (0–15 cm depth) from each half of the field. The cores were composited for each half of the field and were air-dried and ground before analysis. Corn leaves were sampled by dividing each field in half and collecting, from each half of the field, 15 leaves opposite and below the primary ear leaf. The leaf samples were composited for each half of the field and were dried at 65°C and ground before analysis. Corn grain samples are a single grab from the combine hopper after approximately half of the field is harvested (i.e., harvested grain does not include border rows but originates from plants from the interior of each field). Grain samples were dried at 65°C and ground before analysis. Sample analyses were conducted in duplicate with each set of 17 samples being accompanied by duplicate reagent blanks, and a set of duplicate check samples. Each set of check samples consisted of a certified soil, leaf, or grain sample from a biosolids-amended and an unamended source.

Statistical Analysis and Evaluation of Part 503 Uptake Coefficients

We examined the data collected from a total of 26 fields in this study, 8 biosolids-amended and 18 unamended. The fields were chosen because they consisted of non-mined land and they received similar biosolids loading rates in each of 11 consecutive years or, in the case of the control fields, did not receive biosolids applications at all. Specific soil surveys of these fields indicate that several soil series are present in various combinations in each of these fields as described earlier. For the purposes of this study we are treating the fields as replicate biosolids-amended and control plots although no attempt was specifically made to manage them as such during their history of use. The fields were not managed in a continuous corn rotation. In any given year, some of the fields were used to produce either wheat, oats, rye, sorghum, soybeans, or they were left fallow with a cover crop. The effect of these rotations on trace element uptake in this study is unknown; however, they produced an unequal number of replicate fields in which corn was grown each year. The soil data means reported throughout the paper were computed using the data from all 26 fields each year, regardless of whether a corn crop was produced on each field. Granato et al. (1999) have reported all of the soil, corn leaf, and grain data that were available for each of the 26 fields used in this study.

Metal concentrations in surface soil (0–15 cm), corn grain, and corn leaves were available for biosolids-amended and unamended fields from 1985 through 1997, the first 13 yr after the cessation of biosolids applications at the end of 1984. Organic C concentrations in surface soil were also available for these fields. The effect of time on these concentration levels can be complex. However, the issue of primary importance for biosolids-amended fields in this study was whether or not plant metal concentration was different at the end of the observation period from the start of the period. For this reason, we have compared the average concentration levels in corn grain and leaves at the start of the period (1985 through 1987) with those at the end of the period (1995 through 1997). Three-year periods were chosen to account for possible differences in growing seasons, while avoiding potentially substantial effects due to changes in metal bioavailability over longer time periods.

We used two-sample hypothesis testing methods to analyze the changes in the mean concentration levels between the two periods, 1985 through 1987 and 1995 through 1997. Under the models associated with these testing methods, measurements for any year and for any biosolids-amended field were treated as independent with population average constant over each 3-yr period.

The standard two-sample *t* test (SAS Institute, 1999) was applicable in this context, but the test requires approximate normality for the small sample sizes used in our analyses. For this reason, we used the Shapiro–Wilk test for normality (SAS Institute, 1999). This test was applied to the residuals to decide whether to use the *t* test (used if the test of normality is nonsignificant), or the nonparametric Wilcoxon rank sum test (SAS

Institute, 1999) (used if the test for normality is significant at the 5% level). When the *t* test was appropriate to use, we also used the F test for constant variance (SAS Institute, 1999) to decide whether or not to adjust the computations for nonconstant variance (SAS Institute, 1999).

Metal concentrations in surface soil, corn grain, and corn leaves, as well as organic C concentrations in surface soil, were available for fields that were not amended with biosolids over the period 1985 through 1997. We compared the mean levels for these concentrations during 1995 through 1997 for the unamended fields with those of amended fields. This 3-yr period was chosen for the same reasons as above, and to be consistent with earlier analyses. For these two types of fields during the same period, 1995 through 1997, we also compared the mean tissue concentration levels of trace elements observed for unamended fields with the residual levels in amended fields after adjusting the observed mean total tissue concentrations for the Part 503 regulation predictions of increased concentration levels that should result from biosolids applications. We also used two-sample hypothesis testing methods in these two contexts, and we used the same statistical methods as described above.

RESULTS AND DISCUSSION

Total Concentrations of Cadmium, Copper, Nickel, Zinc, and Organic Carbon in Surface Soil

The "plow layer" in biosolids-amended fields in this study was estimated to be 23 cm deep. Since the biosolids bulk density was estimated to be 0.69 g cm^{-3}, we made the assumption that 69 Mg ha^{-1} of dry biosolids makes a layer of 1 cm when applied to 1 ha of land. Since an average of 543 Mg of biosolids were applied to each ha of land, the cumulative biosolids applications comprised 7.9 cm of the plow layer. Therefore, we assumed the plow layer consists of approximately 15 cm of soil and 8 cm of biosolids. We also estimated that 543 Mg ha^{-1} of biosolids were mixed with 2240 Mg ha^{-1} of soil in the plow layer between 1974 and 1984 (2240 Mg ha^{-1} is the mass of 15 cm of soil over 1 ha of land for soil with bulk density of 1.5 g cm^{-3}). We then calculated the expected metal concentration based on the total metals in the control soil and the average metal concentration in the biosolids during the period that they were applied to the site. The mean concentrations of Cd, Cu, Ni, and Zn in unamended soils from 1985 through 1997 were 1.3, 20, 28, and 63 mg kg^{-1}, respectively. The biosolids applied to the fields from 1974 through 1984 had mean concentrations of Cd, Cu, Ni, and Zn of 248, 1607, 392, and 3033 mg kg^{-1}, respectively. The calculated concentrations of Cd, Cu, Ni, and Zn that were estimated to be present in the surface soil of biosolids-amended fields were 50, 330, 99, and 642 mg kg^{-1}, respectively.

The mean concentrations of Cd, Cu, Ni, and Zn measured in the surface 15 cm of soil in the biosolids-amended fields from 1985 through 1987 were 53, 357, 87, and 792 mg kg^{-1}, respectively (Table 1). The mean concentrations of Cd, Cu, Ni, and Zn measured in the surface 15 cm of soil in the biosolids-amended fields from 1995 through 1997 were 50, 327, 87, and 815 mg kg^{-1}, respectively (Table 1).

Changes in Ni and Zn concentrations between these two periods, 1985 through 1987 and 1995 through 1997, were not significant (Table 1). The mean soil concentration for these two elements for the final 3-yr period of the study was approximately 12% lower for Ni and 27% higher for Zn than the calculated concentrations based on the biosolids trace element content and loading rates. No consistent trends throughout the study period of increasing or decreasing concentrations were observed for Ni and Zn (Fig. 1). Walter et al. (2002) reported

Table 1. The mean organic carbon (OC) and trace element contents of surface soil and trace element contents of corn grain and leaves during the beginning (1985–1987) and final (1995–1997) years of the 13-yr period following the cessation of biosolids applications in 1984.†

	Concentration (count)‡							
	1985–1987			1995–1997				
Element	Mean	SD	MRS	Mean	SD	MRS	Equal mean test *p* value	Type of test§
	mg kg^{-1}							
				Surface soil				
Cd	53 (24)	5.7	29.5	50 (24)	7.5	19.5	0.01	RST
Cu	357 (24)	37		327 (24)	51		0.02	*t*
Ni	87 (24)	12		87 (24)	9.5		0.87	*t*
Zn	792 (24)	76		815 (24)	97		0.37	*t*
OC	51 000 (24)	5 000		38 000 (24)	5 000		<0.01	*t*
				Corn grain				
Cd	0.2 (23)	0.1		0.1 (22)	0.1		<0.01	*t*
Cu	1.7 (23)	0.9		1.5 (22)	0.6		0.38	adjusted *t*
Ni	1.6 (23)	0.7	29.8	0.9 (22)	0.5	15.9	<0.01	RST
Zn	28 (23)	5.4		28 (22)	3.9		0.72	*t*
				Corn leaves				
Cd	10 (13)	4.9	24.5	5.6 (22)	3.2	14.2	<0.01	RST
Cu	12 (18)	2.7		10 (22)	2.6		<0.01	*t*
Ni	3.0 (18)	2.1	29.2	0.5 (22)	0.3	13.4	<0.01	RST
Zn	152 (18)	43		87 (22)	33.0		<0.01	*t*

† Biosolids were applied from 1974 through 1984.
‡ Count is the number of observations used in statistical computations, SD is the standard deviation, and MRS is the mean rank score.
§ If the Shapiro–Wilk test for normality was significant, we used the Wilcoxon rank sums test (RST). If the *F* test for constant variance was significant, we used the *t* test adjusted for nonconstant variance (adjusted *t*). Otherwise, we used the standard *t* test (*t*).

similar inconsistent trends of increasing and decreasing total soil metal concentrations.

The mean soil Cd and Cu concentrations showed a small but significant decrease following cessation of biosolids applications (Table 1). No consistent trends throughout the study period of decreasing concentrations were observed for Cd and Cu (Fig. 1), and the mean soil concentrations for 1995 through 1997 were still very close to the soil concentrations estimated from the trace element loadings. For Cd and Cu the observed soil concentrations were 50 and 327 mg kg^{-1}, while the calculated concentrations based on loadings were 49 and 330 mg kg^{-1}, respectively. Therefore, it appears that there was no substantial leaching or redistribution of Cd, Cu, Ni, or Zn from the surface 15 cm of the biosolids-amended fields during the duration of our observation period (1985 through 1997).

The mean concentration of organic C measured in the surface 15 cm of biosolids-amended fields was 5.1% (Table 1 expressed in mg kg^{-1}) from 1985 through 1987. The mean concentration of organic C in the surface 15 cm of biosolids-amended fields in 1995 through 1997 was 3.8%. This was significantly lower than the measured concentration from 1985 through 1987 (Table 1). Therefore, during the 13 yr following the final application of biosolids to land in 1984, organic C concentration in biosolids-amended soil decreased by 25.5%. At the end of the study period, the organic C in the biosolids-

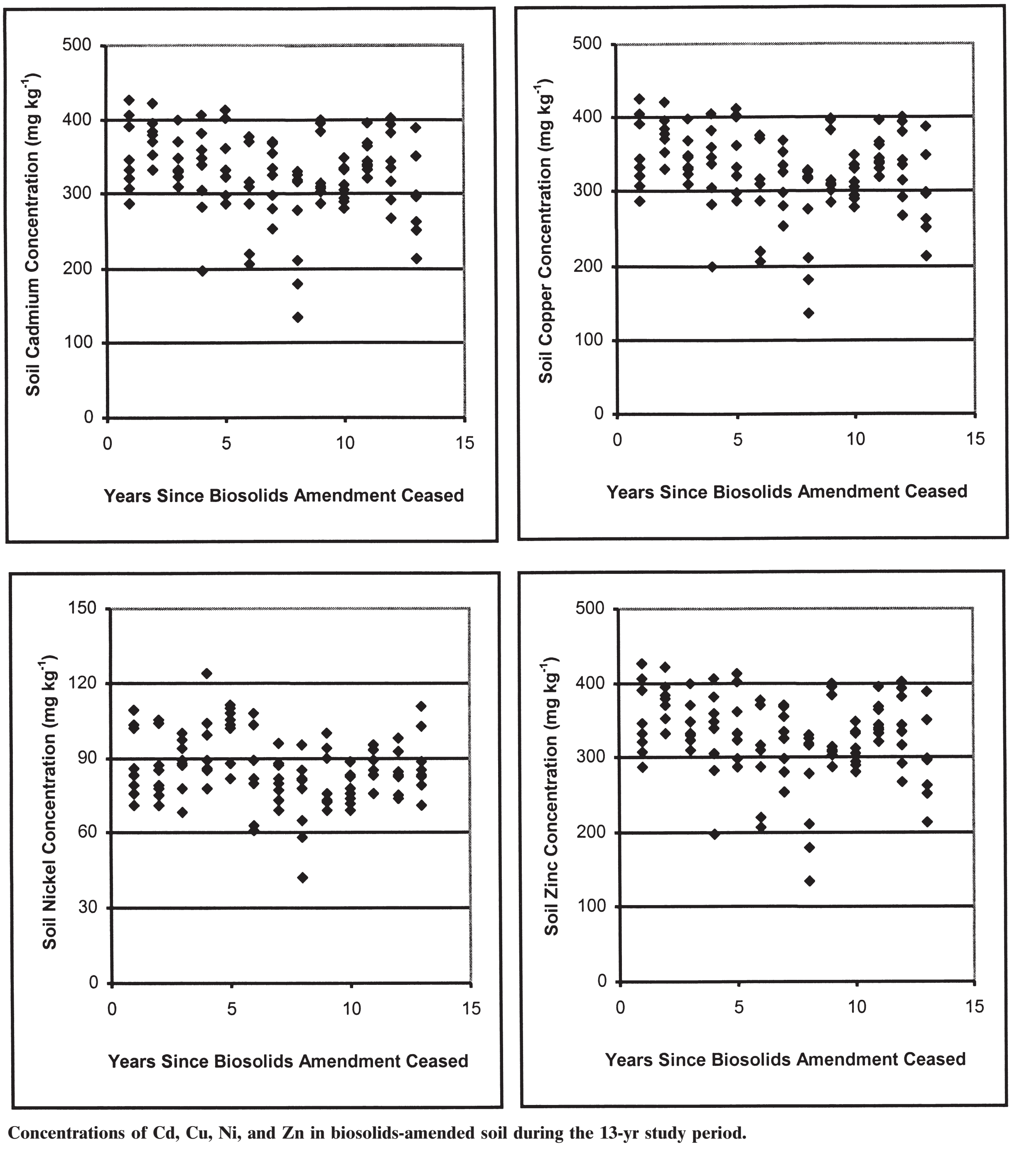

Fig. 1. Concentrations of Cd, Cu, Ni, and Zn in biosolids-amended soil during the 13-yr study period.

amended soil (3.8%) was still significantly greater ($p < 0.05$) than in unamended soil (1.4%).

Concentrations of Cadmium, Copper, Nickel, and Zinc in Corn Leaves and Grain from Biosolids-Amended Fields after Cessation of Applications

Corn leaves and grain grown on biosolids-amended fields were sampled and analyzed annually. The concentrations of Cd, Cu, Ni, and Zn for all years of the study period are shown in Fig. 2 for corn leaves and in Fig. 3 for corn grain. As discussed previously, we computed the mean concentrations for these elements in these tissues for the first three years following cessation of biosolids applications (1985 through 1987), and for the most recent 3-yr period for which data were available (1995 through 1997) to determine whether the concentrations of these elements were different at the end of the study period than they were at the beginning of the period.

The mean concentrations of Cd, Cu, and Ni in corn

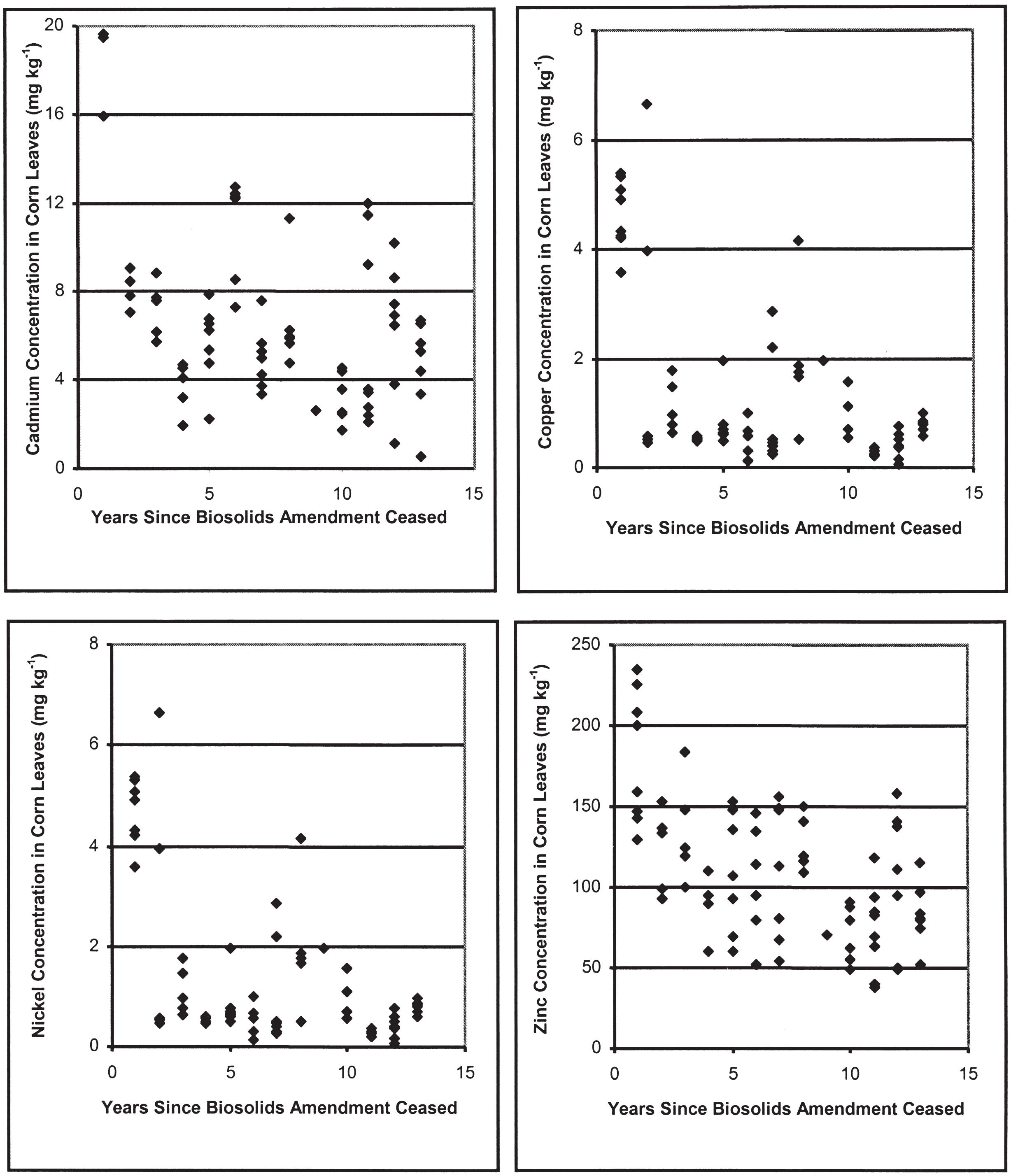

Fig. 2. Concentrations of Cd, Cu, Ni, and Zn in corn leaves during the 13-yr study period.

grain decreased from 0.2, 1.7, and 1.6 mg kg^{-1}, respectively, in 1985–1987 to 0.1, 1.5, and 0.9 mg kg^{-1}, respectively, from 1995–1997 (Table 1). The decreases observed for Cd and Ni were statistically significant ($p < 0.01$). The mean concentration of Zn in corn grain from 1985 through 1987 was 28 mg kg^{-1}, and it remained 28 mg kg^{-1} in 1995 through 1997 (Table 1).

The mean concentrations of Cd, Cu, Ni, and Zn in corn leaves significantly decreased from 10, 12, 3.0, and 152 mg kg^{-1}, respectively, in 1985–1987 to 5.6, 10, 0.5, and 87 mg kg^{-1}, respectively, in 1995–1997 (Table 1). This represents a 44, 17, 83, and 43% reduction in corn leaf Cd, Cu, Ni, and Zn, respectively.

These results are consistent with the findings of several other researchers who have also observed that trace element concentrations remained stable or decreased in plant tissue grown on biosolids-amended soil after biosolids applications ceased (Touchton et al., 1976; Hinesly et al., 1979; Bidwell and Dowdy, 1987; Chang et al., 1997; Canet et al., 1998; Brown et al., 1998).

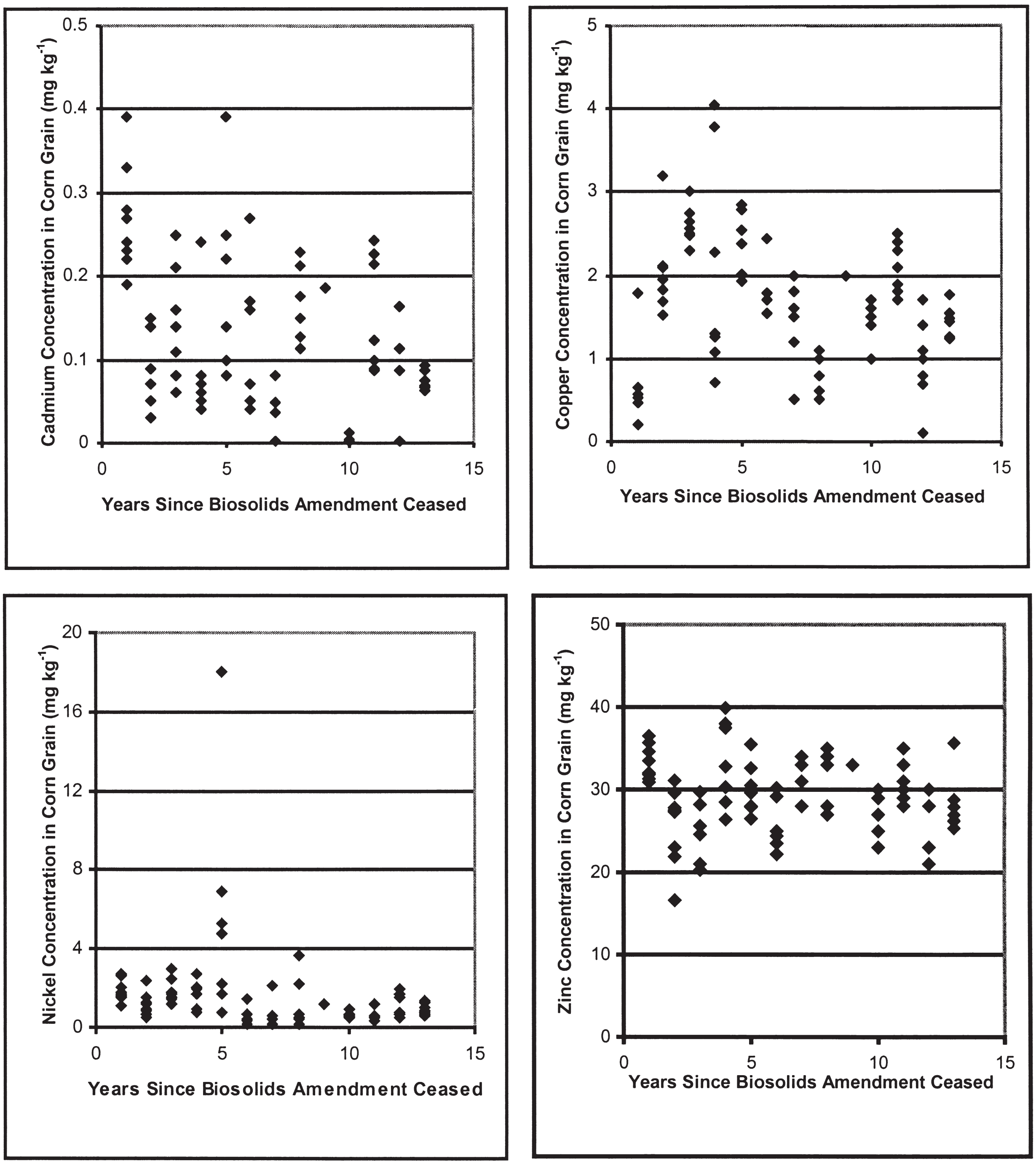

Fig. 3. Concentrations of Cd, Cu, Ni, and Zn in corn grain during the 13-yr study period.

Comparison of Cadmium, Copper, Nickel, and Zinc Concentrations in Corn Leaves and Grain from Biosolids-Amended and Unamended Fields

Data from 1995–1997 were used to compare mean concentrations of Cd, Cu, Ni, and Zn in corn leaves and grain from biosolids-amended and unamended fields.

As expected, the sizeable cumulative applications that were made to the amended fields in this study using high metal biosolids (248 mg kg^{-1} for Cd and 3033 mg kg^{-1} for Zn) from 1974 through 1984 increased the concentrations of Cd and Zn in corn grain compared with grain grown in the unamended soil. Both the Cd and Zn concentrations increased significantly from 0.01 to 0.10 and 23 to 28 mg kg^{-1}, respectively (Table 2). While the increases in corn grain are significant, they are small compared with the increases in total soil Cd and Zn. Biosolids applications increased the soil Cd concentrations from 1.6 to 50 mg kg^{-1} and the soil Zn concentration from 70 to 815 mg kg^{-1}. However, the concentration of Cd and Zn in corn grain increased by only 0.09 and 5 mg kg^{-1}, respectively (Table 2). Apparently, the bioavailability of the biosolids-borne Cd and Zn is rather small.

The biosolids amendments had no significant effect on the Ni concentration in corn grain, and decreased the concentration of Cu in corn grain significantly from 1.9 mg kg^{-1} in unamended fields to 1.5 mg kg^{-1} in biosolids-amended fields (Table 2).

The biosolids applications significantly increased the concentrations of Cd, Ni, and Zn in corn leaves, but they had no significant effect on the concentration of Cu in corn leaves (Table 2). The corn leaf Cd concentration was increased from 0.3 to 5.6 mg kg^{-1}, the Ni concentration was increased from 0.2 to 0.5 mg kg^{-1}, and the Zn concentration was increased from 32 to 87 mg kg^{-1} by the biosolids applications. The Cu concentration in corn leaves was 9.9 mg kg^{-1} in the unamended fields and 10 mg kg^{-1} in the biosolids-amended fields (Table 2).

Comparison of Field Data with USEPA Part 503 Biosolids Regulation Risk Assessment Model Outputs

Our data indicate that biosolids applications produced significant increases in the mean concentrations of Cd, Ni, and Zn in corn leaves, and Cd and Zn in corn grain that have persisted through 1995–1997 (Table 2). We used the data from the final three years of this study on the mean trace element concentrations in corn leaves and grain to evaluate how closely the Part 503 UC values predict the actual transfer of trace elements from biosolids-amended soils to corn in these fields.

The USEPA developed the land application section of their Part 503 regulation based on a risk assessment that determined the potential human and animal exposure to trace elements in biosolids through 14 terrestrial pathways. Five of the pathways included the transfer of trace elements from biosolids-amended soil to plants as a component of the pathway model (Fig. 4). Granato et al. (1995) provide a detailed discussion on the use of UC in the Part 503 risk assessment. The UC values for Cd, Ni, and Zn in grains and cereals and for Cd, Cu, Ni, and Zn in animal forage are displayed in Fig. 4. The USEPA did not compute a UC value for Cu in the pathways where humans consume grain directly because it concluded that Cu did not pose a significant risk (USEPA, 1992). Our results corroborate this conclusion since the mean Cu concentration in corn grain harvested from the biosolids-amended soils was significantly lower than the mean Cu concentration in corn grain from unamended soil (Table 2).

The Part 503 risk assessment algorithms for biosolids-amended soils are structured such that the tissue trace element concentrations are computed as the sum of a

Table 2. The mean concentrations of organic carbon (OC), Cd, Cu, Ni, and Zn in soil, corn grain, and corn leaves from biosolids-amended and unamended fields for 1995 through 1997.

	Concentration (count)†							
	Unamended			Biosolids-amended				
Element	**Mean**	**SD**	**MRS**	**Mean**	**SD**	**MRS**	**Equal mean test *p* value**	**Type of test‡**
	mg kg^{-1}							
				Surface soil				
Cd	1.6 (54)	0.8	27.5	50 (24)	7.5	66.5	<0.01	RST
Cu	21 (54)	4.9	27.5	327 (24)	51	66.5	<0.01	RST
Ni	27 (54)	6.5		87 (24)	9.5		<0.01	adjusted *t*
Zn	70 (54)	15	27.5	815 (24)	97	66.5	<0.01	RST
OC	14 000 (54)	3 000		38 000 (24)	5 000		<0.01	adjusted *t*
				Corn grain				
Cd	0.01 (21)	0.04	13.9	0.10 (22)	0.07	29.8	<0.01	RST
Cu	1.9 (22)	0.4		1.5 (22)	0.6		0.04	*t*
Ni	0.8 (22)	0.9	19.1	0.9 (22)	0.5	25.9	0.08	RST
Zn	23 (22)	3.1		28 (22)	3.9		<0.01	*t*
				Corn leaves				
Cd	0.3 (23)	0.3	12.2	5.6 (22)	3.2	34.3	<0.01	RST
Cu	9.9 (23)	1.9		10 (22)	2.6		0.78	*t*
Ni	0.2 (23)	0.1		0.5 (22)	0.3		<0.01	adjusted *t*
Zn	32 (23)	9.0	12.6	87 (22)	33	33.9	<0.01	RST

† Count is the number of observations used in statistical computations, SD is the standard deviation, and MRS is the mean rank score.
‡ If the Shapiro–Wilk test for normality was significant, we used the Wilcoxon rank sums test (RST). If the *F* test for constant variance was significant, we used the *t* test adjusted for nonconstant variance (adjusted *t*). Otherwise, we used the standard *t* test (*t*).

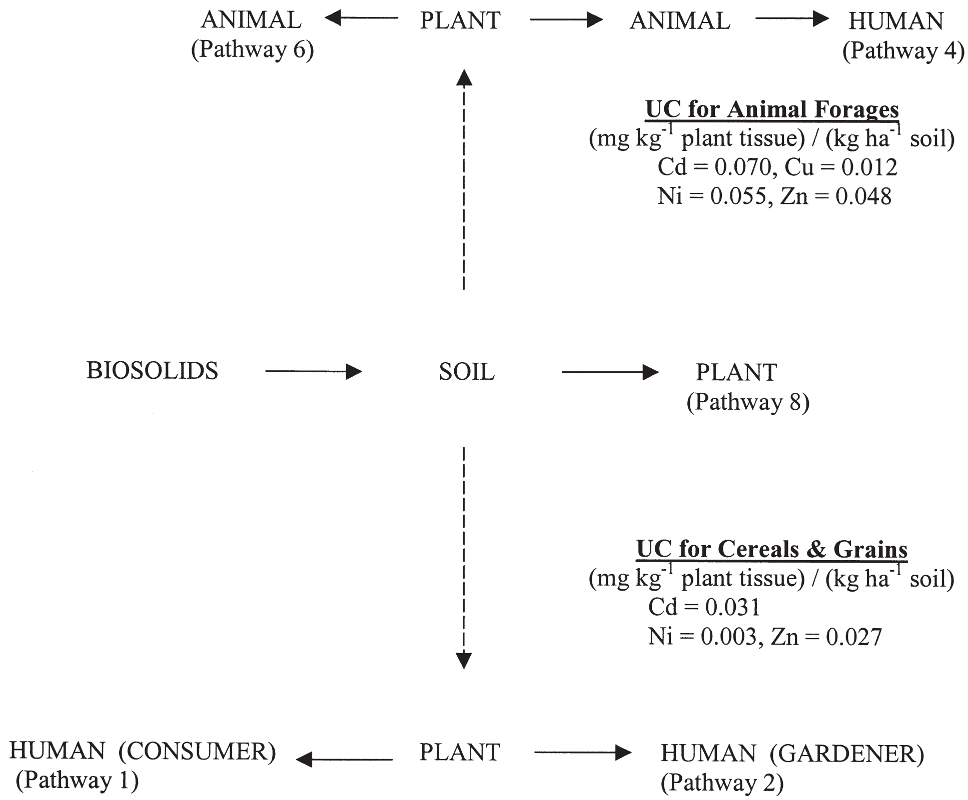

Fig. 4. Part 503 regulation risk assessment pathways that use plant uptake coefficients to model the transfer of trace elements from biosolids-amended soil to plant tissues.

background component, resulting from uptake from the background soil trace element pool, and a biosolids component, resulting from uptake of trace elements added to soil in biosolids. The biosolids components of the tissue trace element concentrations were calculated by multiplying the Part 503 UC for each trace element–tissue pair (e.g., Cd and corn grain) by the corresponding cumulative trace element loading rate for each field. The mean values for biosolids-amended fields are presented in Column C of Table 3. These concentrations were then added to the corresponding background component (mean concentrations in grain and leaves from unamended fields, Column E of Table 3) to obtain the tissue trace element concentrations that the Part 503 risk assessment predicts would be observed on amended fields in this study (Column B of Table 3). The actual total tissue trace element concentrations resulting from uptake from the background and biosolids components were measured in corn grown on the biosolids-amended fields in this study (Column A of Table 3). For this study, in all cases where comparisons were possible, the tissue concentrations predicted by the Part 503 risk assessment for grain and leaves of corn grown on biosolids-amended soil are greater than the actual observed concentrations. The Cd, Ni, and Zn concentrations in corn grain are overpredicted by 4100, 56, and 143%, respectively, and the Cd, Cu, Ni, and Zn concentrations in corn leaves are overpredicted by 75, 110, 2300, and 28%, respectively.

We also compared the mean computed background component of tissue trace element concentrations (Column D in Table 3) with the actual mean background component observed in corn grown on the unamended fields in this study (Column E in Table 3). The computed background component was arrived at by subtracting the biosolids component for each amended field (mean is reported in Column C in Table 3) from the corresponding total tissue trace element concentrations for each amended field for 1995–1997 (means are reported in Column A of Table 3).

If the Part 503 UC values exactly describe the transfer of trace elements from biosolids-amended soils to corn that occurred in the amended fields of this study, then there would be no significant difference between the computed and actual background tissue trace element concentrations in Table 3 (Columns D and E, respectively). This is because the biosolids component predicted by the Part 503 UC values would precisely account for the difference between the tissue trace element concentrations for biosolids-amended fields and unamended fields, which would result in the computed and actual background tissue trace element concentrations being equal. In fact, all of the computed background tissue trace element concentrations were significantly less than the corresponding actual background tissue trace element concentrations (Table 3). This further shows that the Part 503 uptake values were overly conservative for this site.

Of particular note are the results for Cd. Biosolids applications made to fields used in this study ceased nearly a decade before the promulgation of the Part 503 regulation. Cumulative biosolids applications were large

Table 3. Trace element concentrations in corn grain and leaves from biosolids-amended fields, from uptake from biosolids predicted by Part 503 uptake coefficients, and from computed and actual uptake from soil background.

	Mean tissue trace element concentration				
Element	A: Biosolids + background components (amended fields)	B: Biosolids + background components (predicted by Part 503); C + E	C: Biosolids component predicted by Part 503 UC†	D: Background component (computed); A − C‡	E: Background component (unamended fields)§
	mg kg^{-1}				
			Corn grain		
Cd	0.1	4.2	4.2	−4.1¶	0.01
Ni	0.9	1.4	0.6	0.3¶	0.8
Zn	28	68	45	−16#	23
			Corn leaves		
Cd	5.6	9.8	9.5	−3.9¶	0.3
Cu	10	21	11	−0.4#	9.9
Ni	0.5	12	12	−11¶	0.2
Zn	87	111	79	8#	32

† Component of the total trace element concentration in tissue from biosolids-amended fields predicted by the Part 503 regulations for trace elements added to soil in biosolids. Computed for each amended field as uptake coefficient (UC) × soil trace element loading rate.

‡ Component of the total trace element concentration in tissue from the biosolids-amended fields attributed to the uptake of the background soil trace elements. Computed for each amended field for 1995–1997 as total tissue concentration minus concentration attributed to uptake from biosolids by Part 503 UC. Apparent discrepancies between differences of Column A − Column C and values in Column D are due to rounding of values to correct significant figures for presentation in table.

§ Mean tissue concentration for unamended fields resulting from the actual uptake of background soil trace elements in 1995–1997.

¶ Computed background tissue trace element concentration (Column D) is significantly smaller than the actual background component (Column E) under the rank sums test, indicating that the UC overpredicts the biosolids component.

Computed background tissue trace element concentration is significantly smaller than the actual background component under the *t* test adjusted for nonconstant variance, indicating that the UC overpredicts the biosolids component.

and the Cd loading to the soils was 3.5 times greater than the maximum presently allowed under the Part 503 regulation. The Part 503 regulation allows 39 kg Cd ha^{-1} to be applied, and in this study 135 kg Cd ha^{-1} were applied. The Part 503 risk assessment computes the total Cd concentrations for corn leaves and grain from biosolids-amended fields to be 9.8 and 4.2 mg kg^{-1}, respectively (Column B of Table 3). Yet, the actual observed mean concentrations for biosolids-amended fields were 5.6 mg kg^{-1} for leaves and 0.1 mg kg^{-1} for grain.

CONCLUSIONS

In this study we compared the mean concentrations of Cd, Cu, Ni, and Zn in corn grain and leaves grown from 1985–1987 (the first three years following cessation of biosolids applications) with the mean concentrations observed from 1995–1997. None of the mean trace element concentrations were significantly higher at the end of the observation period than at the beginning. The mean concentrations of Cd, Cu, Ni, and Zn in corn leaves and Cd and Ni in corn grain all decreased significantly following cessation of biosolids applications.

These observations have led us to conclude that the bioavailability of Cd, Cu, Ni, and Zn to corn did not increase following the cessation of biosolids applications at this site. This study encompasses the first 13 yr following the cessation of biosolids applications when 25.5% of the biosolids-amended soil OC mineralized and trace element concentrations all decreased in corn leaves. We conclude that in this study, mineralization of biosolids OC following cessation of biosolids applications did not increase the bioavailability of these elements.

The significant decrease in the organic C concentration observed in surface soils in this study (25.5%) was accompanied by a significant decrease in the trace element concentration in corn leaves (44, 17, 83, and 43% for Cd, Cu, Ni, and Zn, respectively). For corn grain the Cd and Ni concentrations decreased by 50 and 44%, respectively, while Zn and Cu concentrations were not significantly changed. For the soils used in this study, trace element availability to corn in biosolids-amended soil remained constant or decreased as the soil organic C decreased following the cessation of biosolids applications.

Trace elements may be more soluble or mobile in the rhizosphere of biosolids-amended soil when they are initially bound by organic matter and released from their bound or chelated state as organic matter mineralizes (Antoniadis and Alloway, 2003; Lamy et al., 1993). Stacey et al. (2001) have shown that the degree to which this occurs is variable and is dependent on biosolids properties. When biosolids applications cease, biosolids organic carbon continues to slowly mineralize, but the rate at which it mineralizes and the concentration of dissolved organic carbon it supports in soil solution are reduced from that which occurs while applications are being made. As a result, trace elements that are released from organic matter following cessation of biosolids applications may also react with the inorganic constituents of the biosolids and soil, such as Fe, Al, and Mn oxides, which renders them less soluble and reduces their availability to plants.

This is corroborated by many researchers who reported that the inorganic fraction of biosolids has a high affinity for trace elements, and that large proportions of the total biosolids trace element concentration can associate with these inorganic rather than organic constituents (Canet et al., 1998; Sloan et al., 1997; Stover et al., 1976; Council for Agricultural Science and Technology, 1980; Lund et al., 1980; Emmerich et al., 1982; Sposito et al., 1982; Logan and Chaney, 1983; Chang et al., 1984; Lake et al., 1984; Hettiarachchi et al., 2003).

Brown et al. (1998) found that after 84% of the biosolids organic C mineralized, the uptake of Cd was still significantly greater for soils spiked with Cd salts than from soils receiving equal loading of biosolids Cd. Zhenbin et al. (2001) and Hettiarachchi et al. (2003) have recently demonstrated that removal of organic matter does not account for differences in adsorption of Cd between biosolids-amended soils and control soils. It is quite possible in this study that the higher concentration of trace elements observed in corn at the beginning of the cessation of applications was due to greater presence of soluble organic carbon, which facilitated uptake. However, as biosolids organic matter continued to mineralize, concentrations of trace elements decreased in corn, probably because the remaining fraction was bound to inorganic biosolids constituents.

Cumulative biosolids additions of 543 Mg ha^{-1} produced significant increases, relative to unamended fields, in the concentration of Cd, Ni, and Zn in corn leaves, and Cd and Zn in corn grain in the final 3-yr period (1995–1997) of this study. However, biosolids additions did not significantly increase the mean Cu concentration in corn leaves or the mean Cu and Ni concentrations in corn grain. In fact, biosolids additions significantly reduced the mean Cu concentration observed in corn grain during the final 3-yr period of the study (1995 through 1997).

Although biosolids applications significantly increased Cd, Ni, and Zn concentrations in corn leaves and Cd and Zn concentrations in corn grain, the increases are less than those predicted by the Part 503 risk assessment.

In the case of Cd, the cumulative loadings in this study exceeded those that are currently allowed by the Part 503 regulation by 250%. Yet the observed Cd concentrations in corn leaves and grain were approximately one-half and one-fiftieth, respectively, of those predicted by the Part 503 risk assessment. Hence, we have concluded that the Part 503 risk assessment overpredicts the uptake of Cd, Cu, Ni, and Zn into corn in biosolids-amended fields at this site.

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