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MINERALIZATION OF ORGANIC CARBON DOES NOT PRODUCE A "TIME BOMB EFFECT" IN BIOSOLIDS-AMENDED SOIL

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MINERALIZATION OF ORGANIC CARBON DOES NOT PRODUCE A "TIME BOMB EFFECT" IN BIOSOLIDS-AMENDED SOIL

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

APPENDIX I

LIST OF TABLES

LIST OF FIGURES

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DISCLAIMER

Mention of proprietary equipment and chemicals in this report does not constitute endorsement by the Metropolitan Water Reclamation District of Greater Chicago.

SUMMARY

The Metropolitan Water Reclamation District of Greater Chicago (District) has owned and operated a 6,300 ha land reclamation site in Fulton County, Illinois, for the past 28 years. Long-term monitoring data from eight fields amended from 1974 through 1984, with a total of 543 Mg ha^{-1} of biosolids, were used to test the hypothesis that mineralization of organic carbon (organic C) in the amended soil following cessation of biosolids applications would produce a "biosolids time bomb" effect leading to a dramatic increase in the phytoavailability of trace elements, which were otherwise unavailable for plant uptake when the biosolids were first applied.

During a 13-year period following cessation of biosolids applications, mean soil organic C decreased from 51.0 to 38.0 g Kg^{-1}. It was estimated that 46.2 percent of the total organic C added to the amended fields mineralized between 1974, when biosolids applications first began, and 1997, the final year of this study. This large net mineralization of soil organic C led to significant increases in the ratios of soil trace element concentrations to soil organic C concentration (relative trace element concentration). The mean relative

trace element concentration increased by 25.8, 21.6, 33.3, and 37.2 percent for Cd, Cu, Ni, and Zn, respectively, from the first three years following cessation of biosolids applications to the final three years of the study. These increases indicate that trace elements were being released from potential binding sites in biosolids organic C during the study period, a necessary condition for the biosolids time bomb effect, if it were to occur.

However, contrary to the hypothesis that the mineralization of soil organic C would lead to a release of trace elements into more phytoavailable forms, significant decreases in the concentration of Cd, Cu, Ni, and Zn in corn leaves, and Cd and Ni in corn grain were observed during the study period. The decreases in the concentration of these trace elements in corn leaves and grain indicate that significant mineralization of soil organic C does not lead to their increased phytoavailability. Modeling of organic C mineralization in biosolids-amended fields indicates that net mineralization had either ceased by the 12th year following cessation of biosolids applications, or it is proceeding so slowly that soil organic C levels in these amended fields won't reach their original levels in the soil that is not amended with biosolids (control) for another 103 years. Therefore, it does not appear that a time bomb effect will ever occur in these fields and that contrary to the hypothesis, the net mineralization of biosolids organic C in amended soils will not increase the phytoavailability of trace elements.

INTRODUCTION

Land application of biosolids is a widespread practice in the United States. The practice is attractive because the biosolids are rich in N and P and can be utilized in place of commercially produced fertilizers to supply crop requirements for these elements. In addition to benefits derived from the nutrients supplied in the biosolids, soil organic matter is also replenished. This is an important attribute of land application of biosolids because sustained conventional fertilization of continuous cropping rotations leads to depletion of soil organic C (Rasmussen et al., 1998; Odell et al., 1984; CAST, 1980), and the application of biosolids enriches soils with organic C instead of depleting it. The continuous input of organic matter from land application of biosolids can be a very important component of sustained productivity in the nation's agricultural soils.

The practice of land applying biosolids is regulated by the United States Environmental Protection Agency's (USEPA) 40 CFR Part 503 "Standards for the Use or Disposal of Sewage Sludge" (USEPA, 1993). Biosolids which meet the metals concentration limits defined in Section 503.13, the pathogen reduction criteria of Section 503.32, and the vector attraction reduction requirements of Section 503.33 may be perpetually land applied at agronomic rates to support sustained productivity of agricultural soils with minimal regulatory overview.

While continuous application of biosolids to agricultural soils might theoretically support sustained productivity, for a given parcel of land, it may not be practicable or feasible to carry such a practice into perpetuity due to changes in land ownership and land use. This fact has given rise to a concern, expressed by critics of land application of biosolids, about the accumulation and plant uptake of trace elements contained, in the biosolids that are applied on land after applications of biosolids cease. Biosolids organic matter is not inert in the soil environment and mineralizes after applications are made. For example, Beckett et al. (1979) were concerned about the mineralization of the organic content of biosolids, and the subsequent release of large quantities of applied trace elements after the biosolids applications on soil ceased. During biosolids applications the trace metals are tightly bound to the organic fraction of biosolids, and not available for plant uptake. Beckett et al. (1979) coined the phrase "time bomb" to describe the potential for increased bioavailability of trace elements that could occur as a result of their release from binding with organic matter due to its mineralization after biosolids applications cease. McBride (1995) recently reiterated the concern that the slow mineralization of organic matter in biosolids could release the bound trace elements into more soluble forms that are readily available for uptake by plants in a fashion similar to the time bomb effect.

Chang et al. (1997) recently tested the time bomb hypothesis utilizing data (Cd uptake by Swiss chard) from their long-term biosolids-amended plots. They concluded that there was no evidence that increases in metal uptake by Swiss chard occurred ten years after cessation of biosolids applications. McBride (1995) reviewed the global literature for evidence that trace element solubility and availability to plants increased following the cessation of biosolids applications. He found that there was no clear evidence for increased availability of trace elements with time following the cessation of biosolids applications. McBride (1995) further concluded that a fairly stable situation exists in biosolids-amended soils, in which the loss of a large fraction of added organic matter will not substantially release the soil metals into an available form for uptake by plants. These conclusions suggest that the biosolids time bomb hypothesis is not supported by evidence in the existing scientific literature.

This may be due to the fact that many researchers have reported that organic matter is in fact not the predominant binder of trace elements in biosolids or biosolids-amended soils. Brown et al. (1998) demonstrated that even after 84 percent of the organic matter had been mineralized from biosolids-amended soil, the concentration of Cd in lettuce had not significantly changed from initial concentrations, and it was much lower on biosolids-amended plots than on plots amended with equal amounts of Cd added as a soluble salt. This indicates that biosolids components other than organic matter are effective binders of trace elements. Dudka and Chlopecka (1990) reported that only 25 percent of the Cd, 21 percent of the Ni, and 17 percent of the Zn were contained in the organic fraction of a heavily Zn contaminated biosolids, while nearly all of the remainder of the trace elements were found as carbonate, Fe-Mn oxide, and residual (extractable only by concentrated mineral acids) fractions. Emmerich et al. (1982) reported that the biosolids organic fraction contained only 22.4, 60.4, 24.3, and 27.9 percent of the Cd, Cu, Ni, and Zn present in biosolids. Planquart et al. (1999) found that only 13 to 22 percent of the Zn and 14 to 32 percent of the Cu resided in the organic fraction of biosolids composts, with nearly all of the rest of the Cu and Zn residing in the inert oxide and residual fractions. McGrath and Cegarra (1992) observed that the percentage of Cd, Cu, Ni, and Zn in the organic fraction of long-term biosolids-amended fields was <10, <40, <20, and <20 percent, respectively, with most of the remaining trace elements residing in the residual and carbonate fractions. Kuo et al. (1985) reported that amorphous Fe oxides are the most important component of biosolids-amended soils controlling the plant concentrations of Cd and Zn, and that soil organic matter content had little relationship with the metal availability to plants.

Granato et al. (1999) reported that the concentrations of Cd, Cu, Ni, and Zn did not increase in corn grain or leaves during a 13-year period following the cessation of biosolids applications at a 6,300 ha land reclamation site in Fulton County, Illinois. This observation adds further credence and supports the results presented by other investigators that no time bomb effect occurs due to the release of trace elements from binding with biosolids organic matter, even after the cessation of the application of biosolids on land for many years, and with no fresh application of biosolids being made.

Although Granato et al. (1999) reported in their study that no significant increase in the plant uptake of trace metals occurred even 13 years after the cessation of biosolids applications, a detailed analysis of the data collected over the 13-year period was not made for that report. In this report, a critical analysis of the data was made to validate their earlier conclusions.

MATERIALS AND METHODS

Description of Experimental Fields

The experimental area is a functioning land reclamation site located approximately 300-km southwest of Chicago in Fulton County, Illinois. The site is 6,300 ha in size, approximately 1,751 ha of which have been developed into 75 fields for biosolids applications and crop production.

For the purposes of this study, we identified 8 fields, averaging 17.1 ha, that had received similar loadings of biosolids in the same years. The mean cumulative mass loading of biosolids and trace elements Cd, Cu, Ni, and Zn applied to these fields from 1974 through 1984 was 543 Mg ha^{-1}, and 135, 873, 213, 1,647 Kg ha^{-1}, respectively. The mean concentrations of Cd, Cu, Ni, and Zn in the biosolids applied between 1974 and 1984 were 248, 1,607, 392, and 3,033 mg Kg^{-1}, respectively. These fields have received no biosolids since 1984, and they are referred to as biosolids-amended fields. We also identified 18 fields, averaging 25.6 ha, that had never received biosolids and included these fields as experimental controls. These control fields are henceforth referred to as unamended fields.

The biosolids applied to fields in this study were produced from waste-activated sludge that was anaerobically digested, and then aged in lagoons. The biosolids had a mean volatile solids (organic matter) content of 41.9 percent. The mean soil pH of the biosolids-amended fields was observed to be between 6.59 and 6.91 for all years of this study, except 1989 when it was 7.14.

Analysis of Trace Elements and Organic Carbon

Trace element concentrations in soil and in plant tissue were analyzed for both biosolids-amended and unamended fields over the 13-year period subsequent to the cessation of biosolids amendment in 1984 (Granato et al., 1999). Organic C concentrations for this period were also analyzed for both amended and unamended fields (Nelson and Sommers, 1982).

Statistical Analysis of Data

Regression models for trace element concentrations in soil and plant tissues and organic C in soil in terms of time "t" in years since the amendment ceased were selected using cross-validation techniques (Appendix I). These models allow for a general nonlinear form. Models for organic C concentrations also allow for the expected concentration to be different for the two types of fields and allow for the expected concentration in the amended fields to reach an equilibrium level in a finite number of years, t_0. Trace element concentration curves are derived from actual trace element concentration data and are plotted in the figures of this paper, but the estimated parameter values are not reported since these curves are used to illustrate patterns over time in plotted concentration data, and are not meant to generalize beyond the reported data, or beyond the actual study period. (See Appendix I for a detailed treatment of these issues.) The solid lines in the figures are best-fit estimators of trace element concentration over time derived via the cross validation techniques presented in the Appendix I.

RESULTS AND DISCUSSION

We have identified four conditions necessary for successful testing of the biosolids time bomb hypothesis. We will first demonstrate that all four of these conditions for testing and detecting the biosolids time bomb effect existed in our study. We will then evaluate the data to determine whether or not a biosolids time bomb effect has occurred, and whether one is likely to occur in the future.

Condition 1: Trace Element Concentrations in Soil Must Be Significantly Elevated By Biosolids Amendments

The first condition necessary for producing and detecting the biosolids time bomb effect, if any, is that biosolids must be applied to land at rates that significantly elevate the trace element concentrations of the soil. This is necessary since the time bomb hypothesis is based on the premise that the biosolids will lose their ability to bind endogenous trace elements due to mineralization of organic C. The time bomb hypothesis postulates that as the biosolids release the trace elements bound originally with the organic C fraction during its mineralization, the soil's ability to bind these trace elements will be overwhelmed, and the bioavailability of the released trace elements will rapidly increase.

Biosolids were applied to the eight fields utilized in this study for eleven consecutive years at a mean annual application rate of 49.4 Mg ha^{-1}. During this time (1974 through 1984), the concentrations of Cd, Cu, Ni, and Zn were increased in the surface soil of these biosolids-amended fields by 4008, 1643, 215, and 1163 percent, respectively (Table 1). The total mass inputs of these trace elements was 135 Kg ha^{-1} for Cd, 873 Kg ha^{-1} for Cu, 213 Kg ha^{-1} for Ni, and 1,647 Kg ha^{-1} for Zn.

If the time bomb hypothesis is to be accepted, i.e., the biosolids organic C is the only important sink capable of effectively binding these trace elements, then these trace elements should be taken up by plants similarly to a soluble salt form as they are released during mineralization of organic C in biosolids-amended soils. Trace elements applied to soils as soluble salts, at rates much lower than described above, have been shown to produce extremely large increases in their concentration in crop tissues (Mahler et al., 1987; Hinesly, 1985; Mortvedt and Giordano, 1975; Bingham et al., 1975; Cunningham et al., 1975).

In this long-term study, far greater quantities of each trace element have been applied to soils than those for soluble trace element salts, which were reported to produce large

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TABLE 1

INCREASES IN CONCENTRATIONS OF CD, CU, NI, AND ZN IN SURFACE SOILS DUE TO BIOSOLIDS APPLICATIONS TO AMENDED FIELDS

	Mean Soil Concentration		
Element	Unamended	After Biosolids Applications Ceased	Increases in Soil Concentration
	------------ mg Kg^{-1}	-----------	------- % -------
Cd	1.3	53.4	4008
Cu	20.4	356.5	1648
Ni	27.7	87.3	215
Zn	62.7	792.0	1163

increases in plant tissue trace element concentrations. The first condition necessary for producing and detecting the biosolids time bomb has thus been met in this study.

Condition 2: The Trace Elements Applied to Biosolids-Amended Fields Must Remain in the Root Zone During the Test Period

The second condition necessary for the hypothesized time bomb effect to occur is that trace elements applied via the biosolids to the surface soil of the test fields must remain in the root zone where they are available in a form to be taken up by the test crop through the roots during the test period. Any increase in the concentrations of trace elements in tissues of the test crop can be attributed to the increased bioavailability of the trace elements released from biosolids due to organic C mineralization rather than to the amount of trace elements that are present in the soil.

During the 13-year test period that was used in this study, Granato et al. (1999) have demonstrated that there was no significant ($p<0.05$) change in the concentrations of Ni and Zn in the top 15 cm of biosolids-amended soil. While the apparent decrease in the concentrations of Cd and Cu were found to be statistically significant ($p<0.05$) in the surface 15 cm of soil during the same test period, the decreases were small (Cd decreased by 6.4 percent and Cu decreased by 8.4 percent).

Figure 1 displays scatter plots of the Cd, Cu, Ni, and Zn concentrations in the surface 15 cm of soil throughout the study period. While there is significant scatter in the data presented in Figure 1, the plots clearly illustrate that there is no trend of decreasing concentrations of trace elements in the surface soil of the biosolids-amended fields in this study. The curves running through the scatter plots show the mean soil trace element concentration estimated by the best fitting model and are included merely to highlight trends in the data. The fact that the best fit curves for Cd, Ni, and Zn oscillate between increasing and decreasing concentration with time is also indicative that no consistent loss of trace element from the surface soil is occurring for these elements, and the oscillation in the data may be attributed to random sampling and analytical errors.

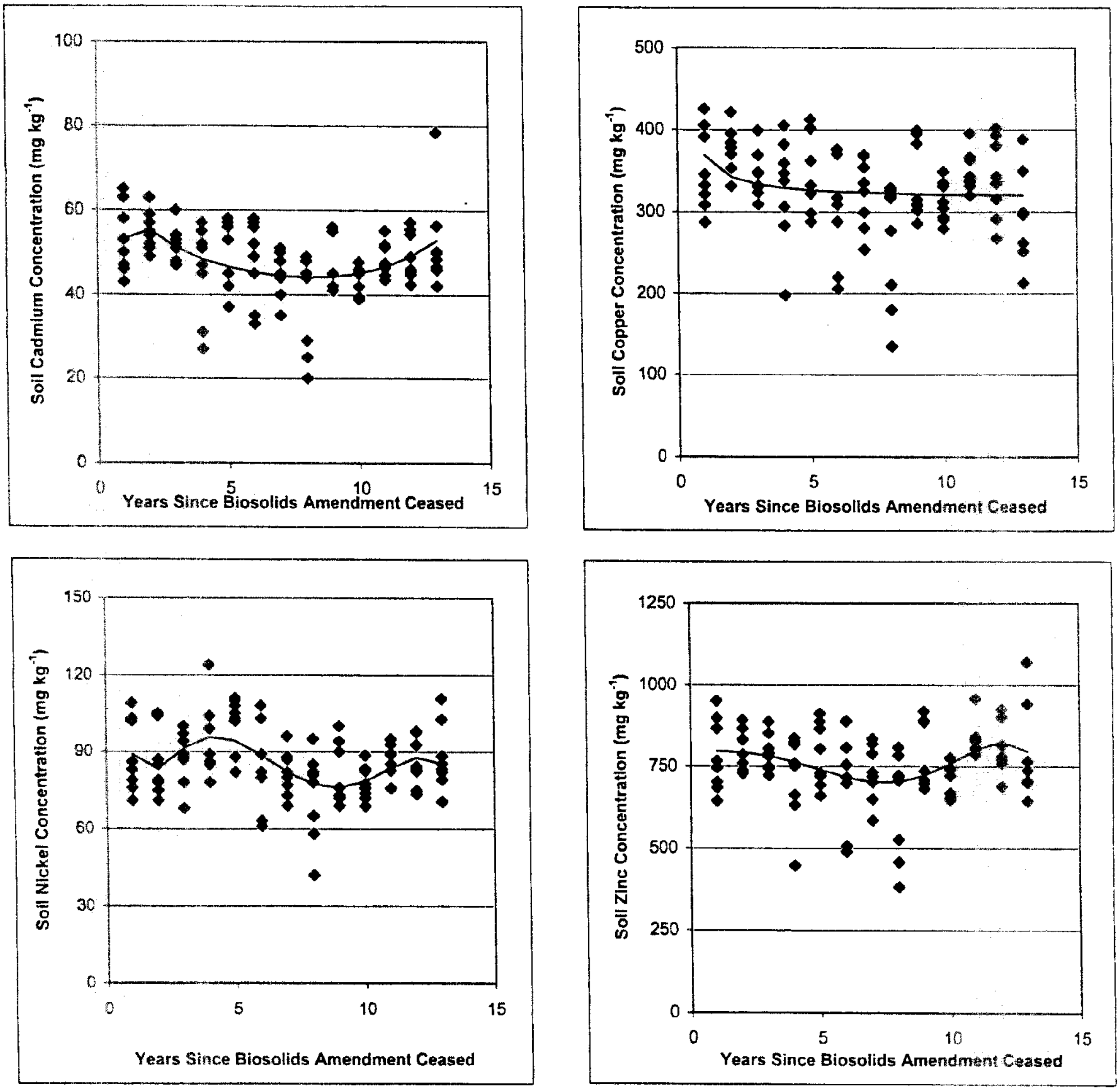
The second condition necessary for producing and detecting the biosolids time bomb effect has thus been met in this study.

Condition 3: Sufficient Organic C Must Mineralize in the Soil of Amended Fields to Provide A Significant Release of Trace Elements From Biosolids Binding Sites During the Test Period

The third condition necessary for the biosolids time bomb effect to occur is that significant mineralization of

FIGURE 1

TRACE ELEMENT CONCENTRATIONS IN SURFACE SOIL FROM BIOSOLIDS-AMENDED FIELDS DURING THE 13-YEAR TEST PERIOD FOLLOWING THE CESSATION OF BIOSOLIDS APPLICATIONS



biosolids organic C must occur during the test period. According to the time bomb hypothesis, this is essential to produce a substantial reduction in the capacity of the biosolids to bind trace elements. As the biosolids organic C is mineralized and this postulated binding capacity is lost, trace elements should become more available for plant uptake.

Granato et al. (1999) have demonstrated that during the 13-year test period, the concentration of soil organic C decreased significantly ($p<0.05$) from 51 to 38 g Kg^{-1}. When one considers that the organic C concentration of the unamended soil is 14 g Kg^{-1}, the organic C attributable to biosolids was decreased from 37 to 24 g Kg^{-1}, a decrease of 35.1 percent during the test period following cessation of biosolids applications. This is only the quantity of biosolids organic C that mineralized in the soil after the final biosolids application was made in 1984. However, organic C from biosolids applications that were made prior to 1984 was also mineralizing during the period of continuous applications.

Organic C was never measured directly on the biosolids applied to the fields in this study during the years that applications were made repeatedly, but it can be estimated by using a mean volatile solids concentration of 419 g Kg^{-1}. If the volatile solids concentration is assumed to be a good estimator of total organic matter and the common conversion factor of 0.58 is used to convert total organic matter to total organic C (Nelson and Sommers, 1982), the 543 Mg of biosolids applied to each ha of land contained 243 g Kg^{-1} of organic C.

Granato et al. (1999) reported that the biosolids-soil mixing zone for these fields is 23 cm deep, with the soil contributing 15 cm of depth and the biosolids contributing 8 cm of depth. If the 15-cm layer of soil is estimated to have a bulk density of 1.5 g cm^{-3}, and the organic C concentration is 14 g Kg^{-1} and it was uniformly mixed with 543 Mg of biosolids having an organic C concentration of 243 g Kg^{-1}, the surface soil in the biosolids-amended fields should have had an organic C concentration of 58.6 g Kg^{-1} at the end of the biosolids application period.

On the assumption that the surface soil from biosolids-amended fields had an organic C concentration of 58.6 g Kg^{-1} (had no mineralization occurred) and the background level of organic C was 14 g Kg^{-1}, the surface soil should have received 44.6 g Kg^{-1} of biosolids organic C. Therefore, 46.2 percent of the total biosolids organic C added to the fields in this study had mineralized by the end of the test period, because the soils from the biosolids-amended fields contained only 24 g Kg^{-1} of organic C at the end of the test period.

These observations and computations indicate that a large and significant quantity of organic C was mineralized in biosolids-amended soils in this study. Since the major premise of the time bomb hypothesis is that the biosolids-amended soil loses its ability to bind trace elements as the organic C mineralizes, we computed relative trace element concentrations and the ratio of trace element concentration to organic C concentration in the soil of each field for each year of the study. As this relative trace element concentration increases, it is indicative of the loss of potential binding sites in the soil due to the mineralization of biosolids organic C. Significant increases in this relative trace element concentration should be indicative of significant changes in the ability of the biosolids to bind trace elements via their organic matter. If biosolids organic matter is as important in binding trace elements, as is postulated in the time bomb hypothesis, then trace element bioavailability should increase as this relative trace element concentration increases.

Regression models for these relative trace element concentrations in terms of time, (i.e., in years since amendment ceased), were selected using cross-validation techniques. These models allowed for the possibility of estimated relative trace element concentrations having complex time behavior (i.e., oscillating randomly up and down). Mean relative Cd, Cu, and Zn levels estimated by the best fitting models, represented by solid lines in <u>Figure 2</u>, increased monotonically over time with substantial increases occurring from the beginning to the end of the test period. Predicted mean nickel relative concentrations also increased substantially from the beginning to the end of the test period, but they did not follow a regular monotonic trend. <u>Figure 2</u> indicates quite clearly that substantially larger amounts of Cd, Cu, Ni, and Zn relative to organic C are available for uptake at the end of the test period than at the start as is required to produce a time bomb effect on plant uptake during this test period.

Further statistical analysis of our data indicates that the mean relative concentrations of Cd, Cu, Ni, and Zn calculated for the last three years of the test period, 1995 through 1997, were significantly larger ($p=0.0001$) than those calculated for the first three years of the test period, 1985 through 1987. When the relative concentrations for these two periods are compared, an increase of 25.8, 21.6, 33.3, and 37.2 percent is observed for Cd, Cu, Ni, and Zn, respectively, (<u>Table 2</u>).

Interestingly, Senesi et al. (1989) used soils that had received biosolids applications of up to 630 Mg ha^{-1} over a

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FIGURE 2

RATIO OF TRACE ELEMENTS TO ORGANIC C IN THE SURFACE SOIL FROM BIOSOLIDS-AMENDED FIELDS DURING THE 13-YEAR TEST PERIOD FOLLOWING CESSATION OF BIOSOLIDS APPLICATIONS

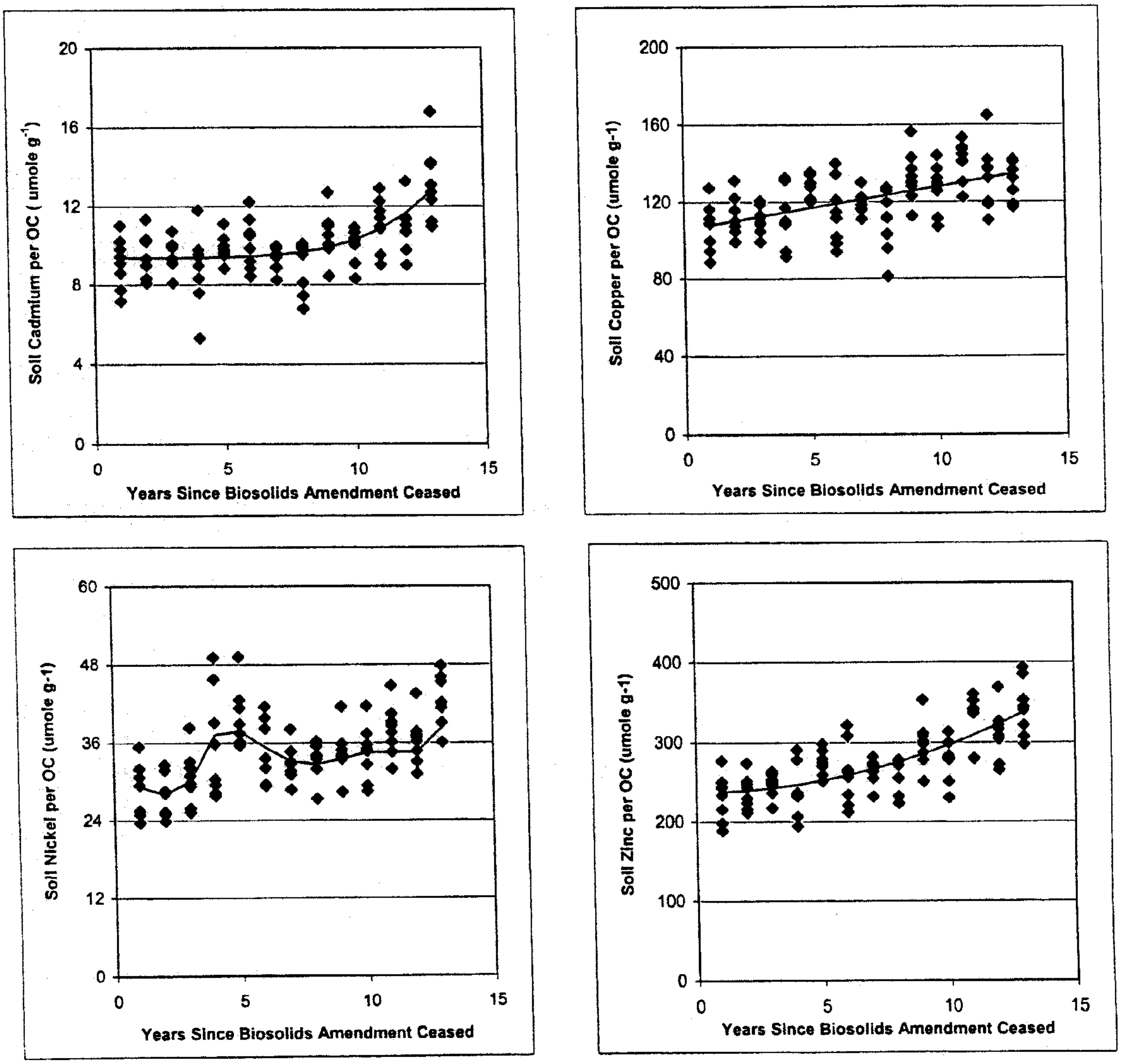

TABLE 2

CHANGE IN RATIO OF TRACE ELEMENT TO ORGANIC C IN SURFACE SOIL OF BIOSOLIDS-AMENDED FIELDS FOLLOWING CESSATION OF APPLICATIONS

	Trace Element/Organic C in Surface Soil			
Element	Mean 1985-1987	Mean 1995-1997	Equal Mean Test p-value	Type of Test[1]
	--------- μmole g^{-1} organic C ----------			
Cd	9.3	11.7	0.0001	adj.t
Cu	110.3	134.1	0.0001	t
Ni	29.1	38.8	0.0001	t
Zn	238.3	326.9	0.0001	adj.t

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[1] In all cases, the Shapiro-Wilk test for normality was nonsignificant. When the F test for constant variance was significant, we used the t-test adjusted for nonconstant variance (adj.t). Otherwise we used the standard t test, (t).

seven year period to demonstrate that as soil concentrations of Cd, Cu, Ni, and Zn reached as high as 18, 298, 132, and 1043 mg Kg^{-1}, their concentration in the humic acid fraction of these biosolids-amended soils reached a plateau at approximately 0.06, 122, 3.8, and 213 μmoles g^{-1}. In our study, the relative concentration of Cd, Cu, Ni, and Zn at the end of the test period was 11.7, 134.1, 38.8, and 326.9 μmoles g^{-1}. Hence, if one assumes that all of the trace element content of the biosolids-amended soils resides in the residual organic matter, as the time bomb theory postulates, then it appears that organic C in the biosolids-amended fields of this study may be at saturation with respect to binding Cu and is well past saturation with respect to binding Cd, Ni, and Zn.

The third condition necessary for producing and detecting the biosolids time bomb effect has therefore been met in this study.

Condition 4: An Appropriate Means of Detecting Changes in Bioavailability of the Trace Elements to Plants Must Be Available During the Test Period

The fourth condition necessary for the hypothesized biosolids time bomb effect to occur is that biosolids applications must cease, and a test crop must be grown at the beginning and end of the test period. This is the period of time following cessation of biosolids applications to allow for evaluation of changes in the bioavailability of the trace elements that may occur. In this study, concentrations of Cd, Cu, Ni, and Zn were measured in leaves and grain of corn from all of the amended fields where it was grown in each of the 13 years of the test period.

The fourth condition necessary for producing and detecting the biosolids time bomb effect has thus been met in this study.

Evaluation of the Occurrence of the Time Bomb Effect

From the foregoing, all of the four conditions that are necessary for the hypothesized biosolids time bomb effect to occur exist in this study.

If the time bomb effect had occurred during the test period of this study, an increase in the concentration of the trace elements in the leaves and grain of the corn crops that were grown in the biosolids-amended fields should have been observed. However, Granato et al. (1999) showed that none of the trace element concentrations in the corn leaves and grain were higher at the end of the test period, mean of the final three years, than they were at the beginning of the test period, mean of the initial three years (Table 3).

Figures 3 and 4 display the observed Cd, Cu, Ni, and Zn concentrations in corn leaves and grain harvested from crops grown on the biosolids-amended fields during the 13-year test period. Regression models for these tissue trace element concentrations in terms of time after cessation of biosolids applications were selected using cross-validation techniques. These models allowed for the estimation of expected trace element concentrations over time reflecting the behavior of trace element uptakes within the time period of the study only and not beyond.

Mean tissue trace element concentrations estimated by the best fitting models are represented by the solid curves in Figures 3 and 4, and they are meant only to illustrate the model that best fits the trends in the data. Due to the large scatter in the data, these predicted mean tissue concentration lines were inserted into plots to help discern trends or patterns that may be occurring with time. We have not reported the actual best fit functions here because they are not intended to be utilized to extrapolate beyond the time period reported or the data utilized in this study. While trends in predicted mean tissue trace element concentrations can be quite

TABLE 3

COMPARISON OF MEAN ORGANIC C AND TRACE ELEMENT CONTENT OF SURFACE SOIL AND TRACE ELEMENT CONTENT OF CORN GRAIN AND LEAVES DURING THE BEGINNING (1985-1987) AND FINAL (1995-1997) 3 YEARS OF THE 13-YEAR PERIOD FOLLOWING CESSATION OF BIOSOLIDS APPLICATIONS IN 1984[1]

Element[2]	Mean Concentration (Count)[3] 1985-1987	Mean Concentration (Count)[3] 1995-1997	Equal Mean Test P-Value	Type of Test[4]
	-----------mg Kg^{-1}	------------		
		Surface Soil		
Cd	53.4(24)	50.0(24)	0.0137	r.s.t.
Cu	356.5(24)	326.7(24)	0.0246	t
Ni	87.3(24)	86.7(24)	0.8703	t
Zn	792.0(24)	814.7(24)	0.3740	t
OC	51,000 (24)	38,000 (24)	0.0001	t
		Corn Grain		
Cd	0.2(23)	0.1(22)	0.0099	t
Cu	1.7(23)	1.5(22)	0.3761	adj.t
Ni	1.6(23)	0.9(22)	0.0004	r.s.t.
Zn	27.9(23)	28.4(22)	0.7156	t
		Corn Leaves		
Cd	10.2(13)	5.6(22)	0.0044	r.s.t.
Cu	12.7(18)	10.1(22)	0.0035	t
Ni	3.0(18)	0.5(22)	0.0001	r.s.t.
Zn	152.0(18)	87.0(22)	0.0001	t

---

[1] Biosolids were applied from 1974 through 1984.

[2] OC is organic carbon.

[3] Count is the number of observations used in statistical computations.

[4] If the Shapiro-Wilk test for normality was significant, we used the Wilcoxon rank sums test (r.s.t.). If the F test for constant variance was significant, we used the t test adjusted for nonconstant variance (adj.t). Otherwise we used the standard t test (t).

From: Granato et al.(1999)

FIGURE 3

TRACE ELEMENT CONCENTRATIONS IN CORN LEAVES DURING THE 13-YEAR TEST PERIOD FOLLOWING CESSATION OF BIOSOLIDS APPLICATIONS

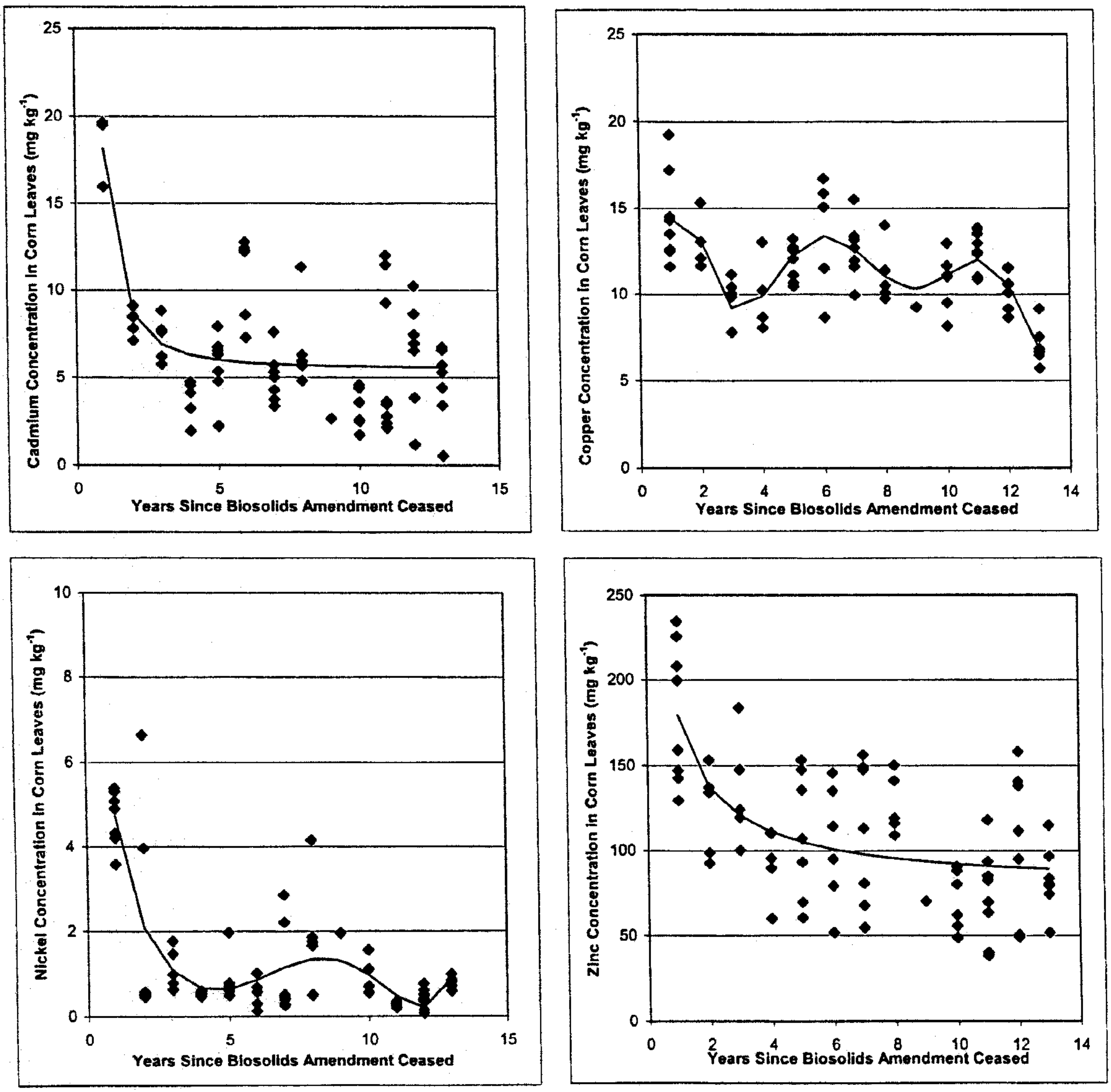
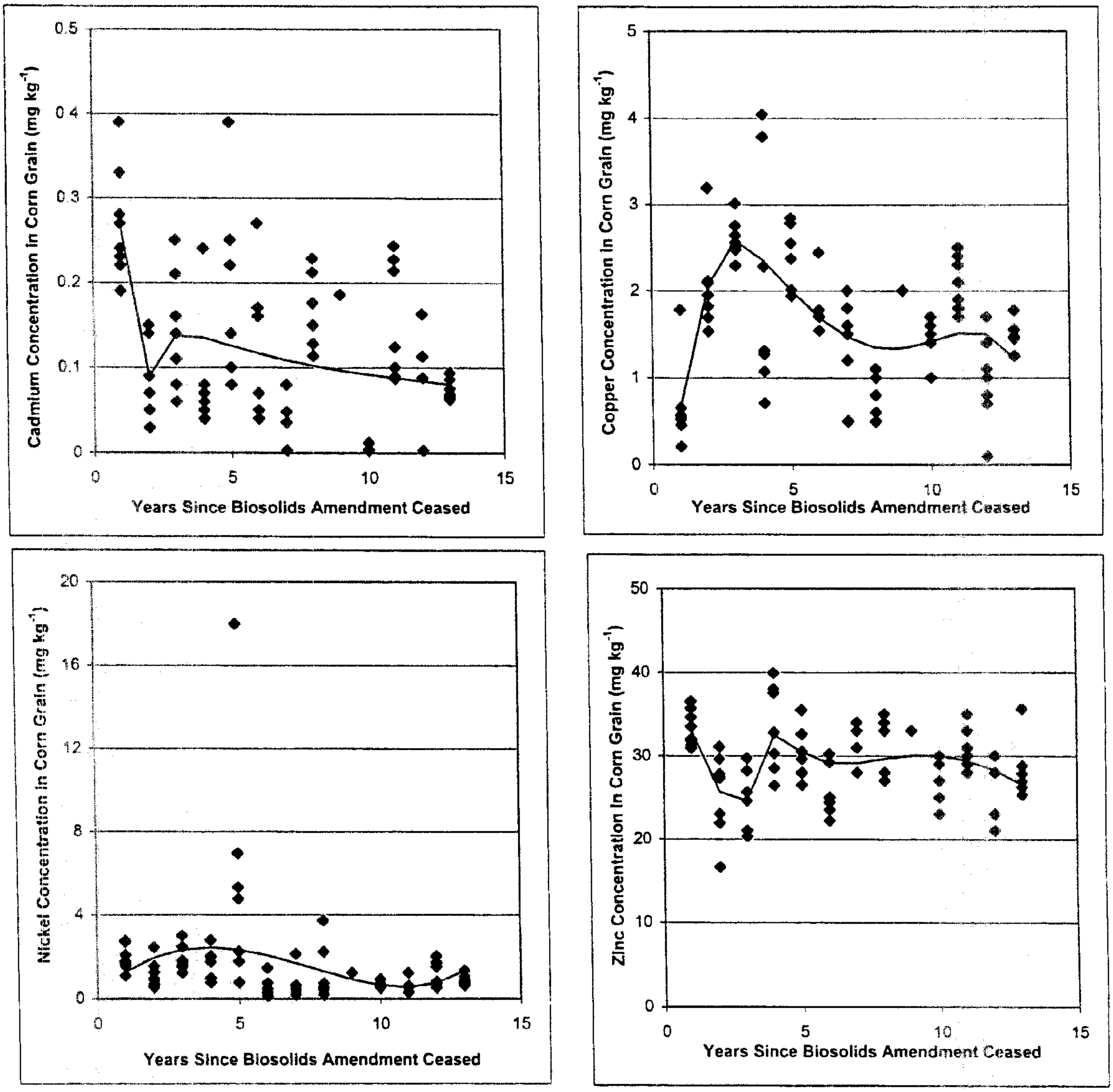


FIGURE 4

TRACE ELEMENT CONCENTRATIONS IN CORN GRAIN DURING THE 13-YEAR TEST PERIOD FOLLOWING CESSATION OF BIOSOLIDS APPLICATIONS



complex over time, for all cases except Cu in corn grain, they decrease from the beginning to the end of the period (Figures 3 and 4).

Granato et al. (1999) reported that the decreases in the mean corn leaf concentration of Cd, Cu, Ni, and Zn observed from the first three years of the test period to the final three years of the test period were all significant ($p<0.05$). This clearly contradicts the time bomb hypothesis, and indicates that not only has no biosolids time bomb effect occurred yet, but the opposite of a time bomb effect is gradually occurring with trace element concentrations in corn leaves decreasing with time after cessation of biosolids applications.

Granato et al. (1999) reported that the decreases in the mean corn grain concentration of Cd and Ni observed from the first three years of the test period to the final three years of the test period were also significant ($p<0.05$), and no significant ($p<0.05$) change in the concentration of Cu and Zn was observed in corn grain for the same periods. This also contradicts the time bomb hypothesis. It indicates that not only has no biosolids time bomb effect occurred yet, but the Cu and Zn concentrations in corn grain are not changing, and the Cd and Ni are gradually decreasing with time after cessation of biosolids applications.

In the case of Cu in corn grain, the tissue concentrations increased to a maximum in the first few years following cessation of biosolids applications and then decreased after that time. While the data for the first three years following cessation of biosolids applications show a definite increase, the Cu concentrations in corn grain show a steady trend downward after that point. This is occurring despite the fact that the soil organic C continues to mineralize and the relative concentration of Cu in the soil, defined as the ratio of soil Cu concentration to organic C concentration, continues to increase. This contradictory behavior of Cu does not support the time bomb hypothesis. Neither does the fact that Cu concentrations in corn leaves fail to show the same increasing trend for the first three years following cessation of biosolids applications as was just described for corn grain.

We have not observed a single instance where the concentration of Cd, Cu, Ni, and Zn have consistently increased in corn grain or leaves during the test period despite the fact that all the conditions necessary for a biosolids time bomb effect to occur were met. Most of the data indicate that the opposite of a time bomb effect is occurring, and that trace element concentrations in corn grain and leaves are tending to decrease with time after cessation of biosolids applications.

We have reported that between 35 and 46 percent of the biosolids organic C has mineralized in the amended fields of this study. If a proportionate amount of each trace element were released from the organic C fraction due to its mineralization, and the biosolids-amended soil had no other effective binding sink for them, then approximately 40 percent of the added Cd, Cu, Ni, and Zn should be behaving similarly to their soluble salt form in these fields. If this were the case, as the time bomb hypothesis predicts, we should have observed dramatically higher concentrations of these trace elements in the leaves and grain of the corn harvested from these fields.

Hinesly (1985) reported the results of an experiment in which additions of $ZnSO_4$, $CuSO_4$, and $NiSO_4$ were made to a Plainfield sand into which corn was planted in a short-term greenhouse experiment. The corn stover Zn, Cu, and Ni concentrations resulting from application rates that were 42 percent, 42 percent, and 25 percent of the cumulative loading rates achieved through biosolids amendments in this study were 4,969 mg Zn Kg^{-1}, 28 mg Cu Kg^{-1}, and 64 mg Ni Kg^{-1}. When $ZnSO_4$ was added to an Elliot silt loam, the same series as utilized in this study, at the same rate, the corn stover Zn concentration that resulted was 1,000 mg Kg^{-1}. These concentrations are far above the mean corn leaf Zn, Cu, and Ni concentrations of

87.0, 10.1, and 0.5 mg Kg^{-1} reported for the final three years of this study. However, the amount of these trace elements added to the soil as soluble salts by Hinesly is approximately equivalent to the amount that should have been liberated via mineralization of biosolids organic C in this study.

In a similar study, Mortvedt and Giordano (1975) spiked a soil limed to pH 7.0 with $ZnSO_4$, achieving a Zn loading that was 29 percent of the cumulative loading rate achieved through biosolids amendments utilized in this study. The loading of Zn in the soluble salt form, which is approximately equivalent to the amount of Zn that should have been liberated by mineralization of biosolids organic C in this study, produced a corn leaf Zn concentration of 462 mg Kg^{-1}. This concentration was more than five times higher than that observed in biosolids-amended fields in our study.

Bingham et al. (1975) spiked a silt loam soil at pH 7.5 with $CdSO_4$, achieving Cd loadings that were 20 and 40 percent of those achieved in this study through biosolids amendments. The concentration of Cd observed in the leaves of corn grown to maturity in pots in a greenhouse was 27.0 and 40.0 mg Kg^{-1}. In the grain the Cd concentration was 1.4 and 2.4 mg Kg^{-1} for the soils spiked at 20 and 40 percent of our cumulative loadings. Although the quantities of Cd added as soluble salts by

Bingham et al. (1975) were roughly equal to the amount that should have been liberated by mineralization of biosolids organic C in amended fields, they observed tissue concentrations well above the mean Cd concentrations we detected in corn leaves and grain in the final three years of this study. These Cd concentrations were 5.6 mg Cd Kg^{-1} for leaves and 0.1 mg Cd Kg^{-1} for grain.

We analyzed the kinetics of organic C mineralization that is occurring in the soil of the biosolids-amended fields in this study. Figure 5 shows the soil organic C content of each of the 8 biosolids-amended fields and each of the 18 unamended fields that were used in this study. Regression models for soil organic C in terms of time t following cessation of biosolids applications were selected using cross-validation techniques. These models allowed for the possibility of the predicted mean soil organic C curves for the two types of fields to be nonlinear. They also allowed the predicted mean organic C in soil from biosolids-amended fields to reach an equilibrium level in a finite number of years, t_0.

Figure 5 also displays the mean soil organic C levels predicted over time by the models to provide the best fit to the data. The predicted mean soil organic C level in unamended fields is estimated to be constant over time. This

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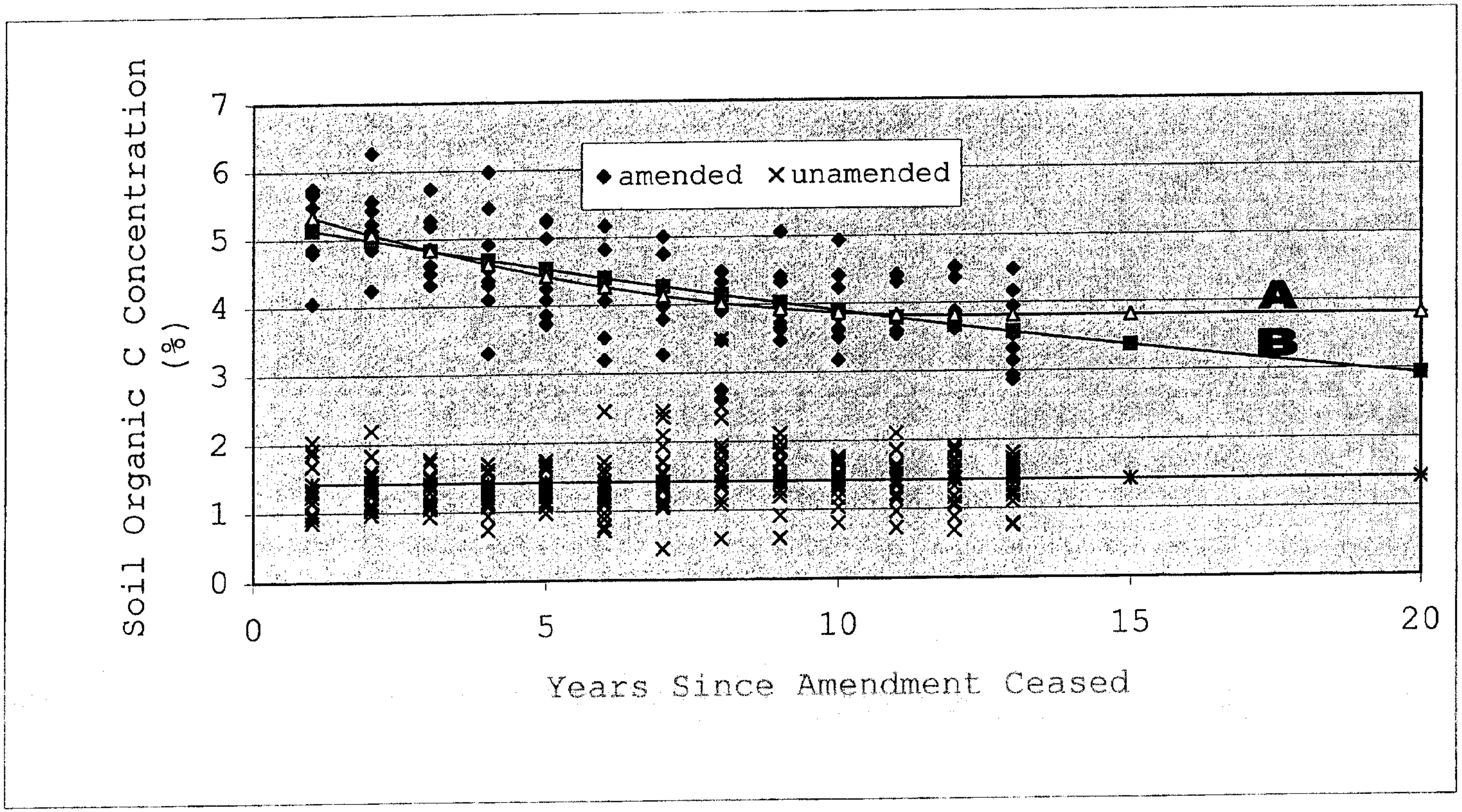
FIGURE 5

ORGANIC C CONCENTRATIONS IN THE SOIL OF BIOSOLIDS-AMENDED AND UNAMENDED FIELDS DURING THE 13-YEAR TEST PERIOD FOLLOWING CESSATION OF BIOSOLIDS APPLICATIONS



constant represents the background soil organic C level for the amended fields, the soil organic C level in the fields prior to biosolids amendments (14 g Kg^{-1}).

As mentioned above, the model for soil organic C in biosolids-amended fields was developed on the premise that soil organic C levels would come to an equilibrium, or a steady state value in a finite number of years following cessation of biosolids applications. We modeled two scenarios. The first scenario is that the soil organic C level descends to an equilibrium level that is possibly larger than the background soil organic C concentration (soil organic C level of unamended fields), and the second scenario is that the soil organic C reaches an equilibrium level the same as the background soil organic C concentration.

In the first scenario, the soil organic C concentration is found to reach an equilibrium level of 38.1 g Kg^{-1}, which is distinctly larger than the background soil organic C concentration of 14 g Kg^{-1}, at t_0=12 years (Curve A in Figure 5 with formula $3.81+0.126-Max(12-t,0)^2$). This is just before the end of the 13-year test period of this study.

Obviously, if this first scenario is correct and the soil organic C has reached equilibrium in the biosolids-amended fields (i.e., no further net mineralization is occurring), then a biosolids time bomb effect will never occur in these fields because the organic C binding pool has stabilized. Terry et al. (1979) indicated that between 55 and 80 percent of the organic C added to soils in biosolids was resistant to breakdown. The decomposition of biosolids applied organic C proceeded so slowly as to be imperceptible during experimental periods that were much shorter than the hundreds of years required for full mineralization to occur. This was borne out by the observations of Sloan et al. (1998). They found no change in soil organic C from the 1st to the 16th year following cessation of biosolids applications, and concluded that biosolids mineralization is so slow as to be imperceptible over that time period.

In the second scenario we modeled, where the biosolids-amended soil organic C descends and equilibrates to background levels, the soil organic C concentration reaches an equilibrium level at the background soil organic C concentration after t_0=116 years (Curve B in Figure 5 with formula $1.85+10^{-10}-\mathrm{Max}(116-t,o)^{5}$).

If the second scenario turns out to be correct, and the soil organic C continues to show net mineralization until it equilibrates at background concentrations, then the potential exists for further release of trace elements from binding with soil organic C over that time period. The best-fit model predicts that the soil organic C will not decay to background levels until 116 years after biosolids applications cease, or 103 years from the end of this test period. At this extremely slow rate of net soil organic C mineralization, one would not expect to ever experience a time bomb effect. This is because the trace elements bound to the biosolids organic C are being released so slowly that other binding and removal mechanisms can easily keep pace with their rate of release.

Evaluation of the Likelihood of a Future Occurrence of a Biosolids Time Bomb

It is quite clear that a biosolids time bomb effect has not occurred during the 13-year test period of this study. The time bomb effect hinges on the presumption that organic C is the principal binder of trace elements in biosolids, and that as it disappears, the trace elements become more bioavailable.

As we have previously mentioned, approximately 46 percent of all of the organic C added to the soils in this study with biosolids has already mineralized. In fact, the net mineralization of organic C may have already ceased in the soils of biosolids-amended fields, and there has been no evidence of a time bomb effect.

In many cases, as pointed out earlier, the trace element bioavailability appears to be decreasing with time after cessation of biosolids applications. This decrease in bioavailability that is accompanying the mineralization of biosolids organic C is consistent with evidence cited above. The inorganic fractions of the biosolids and soil are much more effective and tenacious binders of trace elements than is the organic C. The reason why no biosolids time bomb effect has materialized in this or other similar studies is that the basic premise of the time bomb hypothesis is flawed. Biosolids organic C is not the only effective binder of trace elements in biosolids-amended soils.

This study and others (Bidwell and Dowdy, 1987; Hinesly et al., 1979; Kelling et al., 1977) have observed a decrease in the bioavailability of trace elements after cessation of biosolids applications. Information and findings from reports such as these, which contradict the time-bomb hypothesis, have been available for as long as the hypothesis has existed and from even before. Unfortunately, they have been ignored by proponents of the time bomb over the past three decades.

Juste and Mench (1992) concluded from a survey of 23 long-term studies worldwide that the trend of a progressive decrease in metal uptake is commonly observed following cessation of biosolids applications. This may be due in part to trace elements being released from more soluble and available organically bound or complexed forms to less soluble and less available inorganically bound or occluded forms. As biosolids applications are occurring, biosolids organic C is mineralizing and the pool of soluble organic C in the amended soil is being sustained at high levels. This soluble organic C can be a very effective chelator of trace elements, which can increase their solubility and facilitate increased plant uptake.

In their recent literature review, Alloway and Jackson (1991) cite numerous references showing that soluble organic C is elevated in the solutions of soils undergoing degradation of biosolids organic matter, and these soluble organic compounds are effective chelators of trace elements which decrease their sorption to soil solids. Han and Thompson (1999) demonstrated that biosolids amendments to mollisols significantly increased soluble organic C in soil solution. Almas et al. (1999) demonstrated that total organic C concentrations were 50 percent higher in extracts recovered from soil receiving fresh organic pig manure amendments as compared to soils not receiving amendments, and that this resulted in a two to three order increase in soil solution Cd and Zn concentrations.

When biosolids applications cease, the pool of soluble organic C dwindles as the rate of net soil organic C mineralization slows and, in the absence of organic chelators, the trace elements released from binding with soil organic C can revert to less soluble inorganically bound forms. This would explain the decline in trace element bioavailability that we and others have observed following the cessation of biosolids applications. Emmerich et al. (1982) observed that the decreases in the amount of trace elements associated with the organic fraction of biosolids after application to land were accompanied by increases in the amount of trace elements associated with the insoluble residual fraction of the biosolids-amended soil. Many researchers have shown that trace elements such as Cd, Cu, Ni, and Zn are sorbed to or incorporated into hydrous Fe (Bell et al., 1991; Kuo, 1986; Kalbasi et al., 1978; Kalbasi and Racz, 1978; Hem, 1977), Al and Mn oxides (Kalbasi et al., 1978; Kalbasi and Racz, 1978; Gongmin et al., 1991), crystalline oxides such as goethite (Johnson, 1990; Forbes et al., 1976), and other clays such as montmorillonite (Bar-Tal et al., 1988) and pyrophyllite (Ford et al., 1999).

As discussed above, the possibility exists that net mineralization of soil organic C will persist in biosolids-amended fields until the soil organic C concentrations equilibrate to background levels. The only way that we can conceive of this producing a time bomb effect would be if the rate of release of the trace elements from the mineralizing organic matter was greater than the rate of binding by the other soil fractions described above. Because, in the case of equilibration to background, our best-fit model predicts that the remaining biosolids organic C, in excess of background concentrations, will gradually mineralize over the next 103-year period. Consequently, it seems unlikely that trace elements will be released at a rate great enough to overtake the kinetics of binding to inorganic fractions. Ford et al. (1999) demonstrated that within one month after addition to soil, Ni sorbed to surfaces of pyrophyllite was precipitated in Ni-Al double layered hydroxides that were gradually converting to a very stable Ni-Al phyllosilicate. Almas et al. (1999) observed that Cd and Zn added to soils were sorbed very rapidly, predominantly in the oxide fractions and with stable, non-organic, soil fractions binding up to 70 percent of the added trace elements. Even when the soils were spiked with 4 percent organic matter, added as pig manure, the same partitioning in the soil was observed.

Because the net mineralization rate is predicted to be so slow, it is not likely that soluble organic C pools will approach the elevated levels sustained during biosolids applications and in the few years immediately following cessation of those applications. Therefore, it seems very unlikely that a time bomb effect will ever occur in these fields.

CONCLUSIONS

In this study, we demonstrated that conditions necessary to produce a biosolids time bomb effect were present in the biosolids-amended fields at a large-scale land application site. Eleven annual biosolids amendments increased the concentration of Cd, Cu, Ni, and Zn in the surface soil of amended fields by 4,008, 1,643, 215, and 1,163 percent, respectively. In the 13 years following cessation of biosolids applications, 35.1 percent of the biosolids organic C mineralized, and up to 46.2 percent of the biosolids organic C mineralized since the start of applications. During this time period, the relative concentrations of trace elements (μmole trace element per g soil organic C) in the surface soil of amended fields increased significantly, and appear to be well beyond the saturation point for binding. If Cd, Cu, Ni, and Zn in biosolids-amended fields were only bound by organic matter, as the time bomb hypothesis purports, then the trace elements released from binding should have behaved similarly to soluble metal salts. This should have resulted in large increases in the concentrations of these elements in the tissues of corn that was grown on the fields.

Contrary to the assertion of the time bomb hypothesis, we observed that the concentrations of all four elements decreased in the leaves of corn, while in the grain Cd and Ni concentrations decreased and Cu and Zn concentrations remained constant. This leads us to conclude that no time bomb effect occurred in the biosolids-amended fields of this study. We have concluded that organic C is not the predominant binder of Cd, Cu, Ni, or Zn in biosolids-amended soils, as postulated by the time bomb hypothesis. Trace element availability to plants decreased as organic C concentration decreased in the soils of amended fields in this study. While the mechanisms which determine how the trace elements are partitioned and bound in the biosolids-amended soils of this study remain to be conclusively defined, it seems likely that they are bound to or occluded within Fe, Al, and Mn oxides, and are precipitated as complex sparingly soluble salts.

We also conclude that the time bomb effect will never be observed in these fields due to the mineralization of organic C in the biosolids-amended soils. We believe this for several reasons. First, because trace elements are bound by soil and biosolids constituents other than organic C, this precludes their extensive release from binding upon mineralization of organic matter. Second, while some of the trace elements are bound by soil organic matter, mathematical modeling indicates that if organic matter in these biosolids-amended soils is mineralized to background levels, the best estimate for this study is that it will take 103 years beyond the current observation period. This mineralization rate is far too slow to produce the sudden release of soluble and bioavailable elements required for a time bomb effect. Finally, mathematical modeling indicates that there may be no further net mineralization of organic matter in the biosolids-amended fields of this study. This would seem to preclude any further release of trace elements from the organic matter fraction.

Therefore, we conclude that no time bomb effect will ever occur in these fields due to the mineralization of organic C. The Cd, Cu, Ni, and Zn bioavailability in these soils will only be increased in the future by processes that affect the mineral chemistry of the soils. Since such processes normally occur over geologic time scales that span centuries and millennia, it is not likely that these trace elements will ever be rapidly released from binding.

REFERENCES

Alloway, B. J. and A. P. Jackson, "The Behavior of Heavy Metals in Sewage Sludge-Amended Soils," The Science of the Total Environment, 100: 151-176, 1991.

Almas, A, B. R. Singh, and B. Salbu, "Mobility of Cadmium-109 and Zinc-65 in Soil Influenced by Equilibration Time, Temperature, and Organic Matter," Journal of Environmental Quality, 28:1742-1750, 1999.

Bar-Tal, A., B. Bar-Yosef, and Y. Chen, "Effects of Fulvic Acid and pH on Zinc Sorption on Montmorillonite," Soil Science, 146:367-373, 1988.

Beckett, P. H. T., R. D. Davis, and P. Brindley, "The Disposal of Sewage Sludge onto Farmland: The Scope of the Problem of Toxic Elements," Water Pollution Control, 78:419-445, 1979.

Bell, P. F., B. R. James, and R. L. Chaney, "Heavy Metal Extractability in Long-Term Sewage Sludge and Metal Salt-Amended Soils," Journal of Environmental Quality, 20:481-486, 1991.

Bidwell, A. M. and R. H. Dowdy, "Cadmium and Zinc Availability to Corn Following Termination of Sewage Sludge Applications," Journal of Environmental Quality, 16:438-442, 1987.

Bingham, F. T., A. L. Page, R. J. Mahler, and T. J. Ganje, "Growth and Cadmium Accumulation of Plants Grown on a Soil Treated with a Cadmium-Enriched Sewage Sludge," Journal of Environmental Quality, 4: 207-211, 1975.

Brown, S. L., R. L. Chaney, J. S. Angle, and J. A. Ryan, "The Phytoavailability of Cadmium to Lettuce in Long-Term Biosolids-Amended Soils," Journal of Environmental Quality, 27:1-7, 1998.

CAST, "Organic and Conventional Farming Compared," Report No. 84, Council for Agricultural Science and Technology, Ames, Iowa, 1980.

Chang, A. C., H. Hyun, and A. L. Page, "Cadmium Uptake for Swiss Chard Grown on Composted Sludge Treated Field Plots: Plateau or Time Bomb?," Journal of Environmental Quality, 26:11-19, 1997.

Cunningham, J. D., D. R. Keeney, and J. A. Ryan, "Phytotoxicity and Uptake of Metals Added to Soils as Inorganic Salts or in Sewage Sludge," Journal of Environmental Quality, 4: 460-462, 1975.

Dudka, S. and A. Chlopecka, "Effect of Solid-Phase Speciation on Metal Mobility and Phytoavailability in Sludge-Amended Soil," Water, Air, and Soil Pollution, 51:153-160, 1990.

Efron, B. and R. J. Tibshirani, "An Introduction to the Bootstrap," Chapman and Hall, New York, 1993.

Emmerich, W. E., L. J. Lund, A. L. Page, and A. C. Chang, "Solid Phase Forms of Heavy Metals in Sewage Sludge-Treated Soils," Journal of Environmental Quality, 11:178-181, 1982.

Forbes, E. A., A. M. Posner, and J. P. Quirk, "The Specific Adsorption of Divalent Cd, Co, Cu, Pb, and Zn on Goethite," Journal of Soil Science, 27:154-166, 1976.

Ford, R. G., A. C. Scheinost, K. G. Scheckel, and D. L. Sparks, "The Link Between Clay Mineral Weathering and the Stabilization of Ni Surface Precipitates," Environmental Science and Technology, 33:3140-3144, 1999.

Gongmin, F., H. E. Allen, and C. E. Cowan, "Adsorption of Cadmium and Copper by Manganese Oxide," Soil Science, 152:72-81, 1991.

Granato, T. C., R. I. Pietz, G. Knafl, C. R. Carlson, Jr., P. Tata, and C. Lue-Hing, "Effect of Time After Cessation of Biosolids Applications on the Concentration of Cadmium, Copper, Nickel, and Zinc in Soil, Leaves, and Grain of Corn," Research and Development Department Report No. 99-23, Metropolitan Water Reclamation District of Greater Chicago, September 1999.

Han, N and M. L. Thompson, "Soluble Organic Carbon in a Biosolids-Amended Mollisol," Journal of Environmental Quality, 28:652-658, 1999.

Hem, J., "Reactions of Metal Ions at Surfaces of Hydrous Iron Oxide," Geochimica et Cosmochimica Acta, 41:527-538, 1977.

Hinesly, T. D., "Comprehensive Finding During Fourteen Years of Assessing the Value of Municipal Sewage Sludge as a Fertilizer and Soil Amendment, Including Animal Health Effects," Department of Agronomy, University of Illinois, Urbana-Champaign, submitted to the Metropolitan Sanitary District of Greater Chicago, Chicago, Illinois, 1985.

Hinesly, T. D., E. L. Ziegler, and G. L. Barrett, "Residual Effects of Irrigating Corn with Digested Sewage Sludge," Journal of Environmental Quality, 8:35-38, 1979.

Johnson, B., "Effect of pH, Temperature, and Concentration on the Adsorption of Cadmium on Goethite," Environmental Science and Technology, 24:112-118, 1990.

Juste, C. and M. Mench, "Long-Term Application of Sewage Sludge and its Effects on Metal Uptake by Crops," In: Biogeochemistry of Trace Metals, D. C. Adriano ed., Lewis Publishers, Boca Raton, Florida, 1992.

Kalbasi, M. and G. J. Racz, "Association of Zinc with Oxides of Iron and Aluminum in Some Manitoba Soils," Canadian Journal of Soil Science, 58:61-68, 1978.

Kalbasi, M., G. J. Racz, and L. A. Lowewn-Rudgers, "Mechanism of Zinc Adsorption by Iron and Aluminum Oxides," Soil Science, 125:146-150, 1978.

Kelling, K. A., D. R. Keeny, L. M. Walsh, and J. A. Ryan, "A Field Study on the Agricultural Use of Sewage Sludge: III. Effect on Uptake and Extractability of Sludge-Borne Metals," Journal of Environmental Quality, 6:352-358, 1977.

Kohavi, R., "A Study of Cross-Validation and Bootstrap for Accuracy Estimation and Model Selection," In: Proceedings International Joint Conference on Artificial Intelligence, C. S. Mellish, ed., Morgan Kaufman, San Francisco, 1137-1143, 1997.

Kuo, S., "Concurrent Sorption of Phosphate and Zinc, Cadmium, or Calcium by a Hydrous Ferric Oxide," Soil Science Society of America Journal, 50:1412-1419, 1986.

Kuo, S. E., J. Jellum, and A. S. Baker, "Effects of Soil Type, Liming, and Sludge Application on Zinc and Cadmium Availability to Swiss Chard," Soil Science, 139:122-130, 1985.

Mahler, R. J., J. A. Ryan, and T. Reed, "Cadmium Sulfate Application to Sludge-Amended Soils I. Effect on Yield and Cadmium Availability to Plants," The Science of the Total Environment, 67: 117-131, 1987.

McBride, M. B., "Toxic Metal Accumulation from Agricultural Use of Sludge: Are USEPA Regulations Protective?," Journal of Environmental Quality, 24:5-18, 1995.

McGrath, S. P. and J. Cegarra, "Chemical Extractability of Heavy Metals During and After Long-Term Applications of Sewage Sludge to Soil," Journal of Soil Science, 43:313-321, 1992.

Mortvedt, J. J., and P. M. Giordano, "Response of Corn to Zinc and Chromium in Municipal Wastes Applied to Soil," Journal of Environmental Quality, 4:170-174, 1975.

Nelson, D. W. and L. E. Sommers, "Total Carbon, Organic Carbon, and Organic Matter," In: Methods of Soil Analysis Part 2 - Chemical and Microbiological Properties, A. L. Page, R. H. Miller, and D. R. Keeney, eds., American Society of Agronomy, Madison, Wisconsin, pp. 539-579,1982.

Odell, R. T., W. M. Walker, L. V. Boone, and M. G. Oldham, "The Morrow Plots a Century of Learning," Bulletin 775 University of Illinois Agricultural Experiment Station, 1984.

Planquart, P., G. Bonin, A. Prone, and C. Massiani, "Distribution, Movement and Plant Availability of Trace Metals in Soils Amended with Sewage Sludge Composts: Application to Low Metal Loadings," The Science of the Total Environment, 241:161-179, 1999.

Rasmussen, P. E., K. W. T. Goulding, J. R. Brown, P. R. Grace, H. H. Janzen, and M. Korschens, "Long-Term Agroecosystem Experiments: Assessing Agricultural Sustainability and Global Change," Science, 282:893-896, 1998.

Senesi, N., G. Sposito, K. M. Holtzclaw, and G. R. Bradford, "Chemical Properties of Metal-Humic Acid Fractions of a Sewage Sludge-Amended Aridisol," Journal of Environmental Quality, 18:186-194, 1989.

Sloan, J. J., R. H. Dowdy, and M. S. Dolan, "Recovery of Biosolids-Applied Heavy Metals Sixteen Years After Application," Journal of Environmental Quality, 27:1312-1317, 1998.

Stoer, J. and R. Bulrich, "Introduction to Numerical Analysis," New York: Springer-Verlag, 1980.

Terry, R. E., D. W. Nelson, and L. E. Sommers, "Carbon Cycling During Sewage Sludge Decomposition in Soils," Soil Science Society of America Journal, 43:494-499, 1979.

United States Environmental Protection Agency, "Standards for the Use or Disposal of Sewage Sludge," Federal Register, 58:9387-9415, 1993.

APPENDIX I

STATISTICAL ANALYSIS PERFORMED IN THIS PAPER

The figures included in this paper display expected concentration curves over time in a variety of cases. These curves are generated using selected regression models applied to observed concentrations as response variables y in terms of possible multiple transforms of time t in years since biosolids amendment ceased. This appendix provides a detailed treatment of these regression models and the cross-validation methods used to select them.

The possible response variables include trace element concentrations in the soil or plant tissue, organic C concentration in the soil, and relative trace element concentrations in the soil relative to the organic C concentration in the soil. Organic C concentrations are modeled over time t for amended and unamended fields, but all other types of concentrations are modeled only for amended fields.

Modeling Amended Field Data by Itself

Models applied to concentration y for amended fields only have a conditional expected value with the form

$$E(y|t)=\beta_1\cdot T_1(t)+\cdots+\beta_J\cdot T_J(t) \qquad (1)$$

where the $T_j(t)$ for $1\le j\le J$ are a set of distinct transforms of time t chosen from among the identity transform $T(t)=1$, power transforms $T(t)=t^p$ for $p\neq 0$, and the natural log transform $T(t)=\ln(t)$. The slope parameters β_j are estimated using ordinary least squares techniques. The slope parameter associated with the identity transform is an intercept parameter.

Any of these models may be estimated from available data, but it is important to know which model is most consistent and fits the data well. In general, it is necessary to decide which model within a given set of models is most consistent with available data. To answer such questions, a scoring function is needed that measures the predictive capability of the models. Cross-validation is an established method for achieving this (Efron and Tibshirani, 1993). It provides a measure of the predictive capability of a model for a given set of data, with better scores corresponding to models more consistent with the data. Cross-validation computations may be described as follows:

1. Delete the y values for a subset of the observations.
2. Estimate the model using the remaining observations.
3. Calculate predictions $\hat{y}$ for the deleted y values

of step 1 from the estimated model of step 2 at the x values for the deleted observations of step 1.

4. Calculate a score measuring how close the predictions $\hat{y}$ of step 3 are to the actual y values for the deleted observations of step 1.
5. Repeat steps 1-4 over subsets of observations forming a partition of the data into disjoint subsets, called folds, of nearly equal size.
6. Combine the scores of step 4 for all subsets of step 5 into a composite cross-validation score.

The scores we choose to use in step 4 are the likelihood terms for the y values and the associated predictions $\hat{y}$. We combine these terms in step 6 by multiplying all such terms together and then take the n^{th} root where n is the sample size. The generated cross-validation score CV is a geometric average deleted likelihood score. Models with larger cross-validation scores are better models with better predictive capability, and they are more consistent with the available data. However, models which are close to the best scores, for example, within 1 percent or 2 percent of the best score, provide competitive predictions for the available data. The preferable competitive model is the one which has a simpler structure and/or one which has a more natural interpretation and/or explanation of the underlying phenomenon and/or problem.

When the data are partitioned into k different folds in step 5, the procedure is called k-fold cross-validation. When k equals the number of observations, the procedure is called a full or leave-one-out cross-validation. The choice of k=10, a 10-fold cross-validation, is used often in practice, especially when full cross-validation would be computationally prohibitive. This choice is recommended by Kohavi (1997). We choose the leave-one-out cross-validation. In many situations, as long as the sample size is not too small, the choice of the number of folds has little effect on the results.

The set of models under consideration is often quite large, making an exhaustive search impractical. Heuristic search schemes are then needed to select models that balance the need for achieving optimal predictive capability with search time limitations. We employed the following rules in conducting our search for regression models of concentrations for amended fields in terms of time.

Model building proceeds in three consecutive stages:

1. Model expansion
2. Model transformation
3. Model contraction

Model expansion proceeds as follows:

1. Initialize the base model M and compute its cross-validation score CV(M).
2. Initialize the set S of possible expansion predictors.
3. Initialize the current best cross-validation score CV^* to be the score CV(M) for the base model.
4. Compute cross-validation scores CV(M+x) for models M+x determined by adding predictors x in S one at a time to the base model M.
5. Determine the best predictor x^* from S to add to the base model, that is, $CV(M+x^*)=\max\{CV(M+x):x\in S\}$.
6. If adding the best predictor to the base model does not degrade the model too much as compared to the best model so far, that is, if $CV(M+x^*)>(1-\alpha_1)\cdot CV^*$, then;
    a. Update the set of expansion predictors by deleting any predictors which degrade the base

model tangibly as compared to the best model so far and the current best predictor, that is, $S \leftarrow S \backslash \{x \in S : CV(M+x) \leq (1-\alpha_2) \cdot CV^*\} \backslash \{x^*\}$.

b. Expand the base model by x^*, that is $M \leftarrow M+x^*$.

c. Possibly update the score for the best model so far, that is, if $CV(M+x^*) > CV^*$, then $CV^* \leftarrow CV(M+x^*)$.

otherwise stop the expansion and use the current base model M as the final expanded model.

7. Iterate steps 4-6 until stopping in step 6.

Model transformation proceeds as follows:

1. Initialize the base model M to the model produced by the expansion stage with the cross-validation score CV(M).
2. Initialize the set S of transformation predictors to be the current set of predictors in M.
3. Compute cross-validation scores $CV(M(x \leftarrow T(x)))$ for models $M(x \leftarrow T(x))$ determined by replacing each of the predictors x in S

one at a time by the transform $T(x)$ of x with the improved CV score (see transformation selection rules below).

4. Determine the best predictor x^* from S to transform in the base model, that is, $CV(M(x^*\leftarrow T(x^*)))=\max\{CV(M(x\leftarrow T(x))):x\leftarrow S\}$.
5. If transforming the best predictor in the base model improves the model tangibly, that is, if $CV(M(x^*\leftarrow T(x^*)))>(1+\alpha_3)\cdot CV(M)$, then
   a. Update the set of transformation predictors by deleting any predictors whose transformation does not improve the base model tangibly, that is, $S\leftarrow S\setminus\{x\in S:CV(M(x\leftarrow T(x)))<(1+\alpha_4)\cdot CV(M)\}$.
   b. Adjust the base model by replacing x^* with its associated improved transform $T(x^*)$, that is $M\leftarrow M(x^*\leftarrow T(x^*))$.

   Otherwise stop the transformation and use the current base model M as the final transformed model.

6. Iterate steps 3-5 until stopping in step 5.

Model contraction proceeds as follows:

1. Initialize the base model M to the model produced by the transformation stage with the cross-validation score CV(M).
2. Initialize the set S of possible contraction predictors to be the current set of predictors in M.
3. Initialize the current best cross-validation score CV^* to be the score CV(M) for the base model.
4. Compute cross-validation scores $CV(M \backslash x)$ for models $M \backslash x$ determined by removing each of the predictors x in S one at a time from the base model M.
5. Determine the best predictor x^* from S to remove from the base model, that is, $CV(M \backslash x^*) = \max\{CV(M \backslash x) : x \in S\}$.
6. If removing the best predictor in the base model does not degrade the model tangibly as compared to the best model so far, that is, if $CV(M \backslash x^*) > (1-\alpha_5) \cdot CV^*$, then

a. Update the set of contraction predictors by deleting any predictors whose removal degrades the base model tangibly as compared to the best model so far and the current best predictor, that is, $S \leftarrow S \setminus \{x \in S : CV(M \setminus x) < (1+\alpha_6) \cdot CV^*\} \setminus \{x^*\}$.

b. Adjust the base model by removing x^* from the base model, that is $M \leftarrow M \setminus x^*$.

c. Possibly update the score for the best model so far, that is, if $CV(M \setminus x^*) > CV^*$, then $CV^* \leftarrow CV(M \setminus x^*)$.

Otherwise stop the contraction and use the current base model M as the final contracted model.

7. Iterate steps 4-6 until stopping in step 6.

Transformation selection for a fixed variable x in the base model M proceeds as follows:

1. Initialize the change $\Delta p = \Delta p^{(0)}$ for searching through powers p for trans-

forms $T(x;p)$ of x having the form

$$
\begin{aligned}
T(x;p) &= x^{p} && \text{if } x>0 \\
&= 0 && \text{if } x=0 \\
&= -|x|^{p} && \text{if } x<0;
\end{aligned}
$$

2. Initialize the current power $p=1$ for x as currently used in the base model M with cross-validation score $CV(M)$;
3. Initialize the search direction d as follows:

   $d=-1$ if $CV(M(x^{*} \leftarrow T(x^{*};p-\Delta p))) > CV(M)$

   $d=+1$, otherwise;
4. Compute cross-validation scores $CV(k)=CV(M(x \leftarrow T(x;p+d \cdot k \cdot \Delta p)))$ for models $M(x^{*} \leftarrow T(x;p+d \cdot k \cdot \Delta p))$ determined by replacing x in the base model by transforms $T(x;p+d \cdot k \cdot \Delta p)$ of x as long as the model is improved tangibly, that is, for integers $k=2,\cdots,k'$ where k' is the first integer satisfying $CV(k') \leq (1+\alpha_{7})CV(k'-1)$.
5. Return $p+d \cdot (k'-1) \cdot \Delta p$ as the selected power.
6. Repeat steps 2-5 using $\Delta p \leftarrow \Delta p/10$ until

the CV scores for powers within $\pm\Delta p$ of the selected power are not too much smaller than the score for the selected power, that is, until the following condition is met:

$\min(CV(k'-2), CV(k')) \geq (1-\alpha_8) \cdot CV(k'-1).$;

Special handling is required for computing $T(x;p)$ when $p=0$. For example, if $x \geq 0$, then $T(x;0)$ as defined above equals the indicator $1_{(x>0)}$. However, the limit as $p \rightarrow 0$ of the model $M(x \leftarrow T(x;p))$ depends on what the other variables are in the model. Specifically, if the model contains an intercept term for the case $(x>0)$, for example, if another variable x' in the model is a nonzero multiple of $1_{(x>0)}$, then the limit as $p \rightarrow 0$ of the model $M(x \leftarrow T(x;p))$ is the model $M(x \leftarrow 1_{(x>0)} \cdot \ln(x))$. Otherwise, it is $M(x \leftarrow 1_{(x>0)})$. In order to avoid the specific determination of which of these cases to use when $p=0$, we adaptively redefine $T(x;p)$ for small values of p, that is, for $|p| \leq p_*$. In particular, we replace $T(x;p)$ as defined earlier by

$$
\begin{aligned}
T'(x;p) &= T(x;p) && \text{if } |p| > p_* \\
&= T(x;p_*) && \text{if } 0 < p \leq p_* \\
&= T(x;-p_*) && \text{if } -p_* \leq p < 0 \\
&= T(x;d \bullet p_*) && \text{if } p = 0
\end{aligned}
$$

where $d=\pm 1$ is the search direction determined in step 3 of the transformation selection procedure. This definition requires that the initial power be nonzero. In practice, we set $p_*=10^{-5}$.

To conduct the search using amended field data, the initial set of possible predictors was set to

$$S=\{t^p:p=-5,-4.5,\cdots,4.5,5\}\cup\{\ln(x)\}.$$

Note that the case $t^0=1$ corresponding to the identity transform (since $t>0$) is included in S. Thus, an intercept term might possibly be added to the model in the expansion stage. If it is, then transforming this predictor has no effect on the model, and it might possibly be removed in the contraction stage. Since S contains a fairly broad set of transforms of t, expanded models require at most mild amounts of transformation. The tolerance parameters were set to $\alpha_1=0.01$, $\alpha_2=0.05$, $\alpha_3=0.01$, $\alpha_4=0.01$, $\alpha_5=0.02$, $\alpha_6=0.1$, $\alpha_7=0.001$, $\alpha_8=0.02$. These settings were chosen on the basis of experience by using these heuristics on several other data sets prior to using them in the analyses reported in this paper.

Modeling Organic C for Amended and Unamended Fields

In the case of modeling organic C concentrations y in the soil, the conditional expected value may have the arbitrary form of equation (1). However, different choices are possible for the transforms T_j for amended fields than for unamended fields. This latter fact means that a more extensive search is needed for this case than when modeling concentrations for amended fields only. In particular, the search must be conducted over transforms T_{Aj} for amended fields and transforms T_{Uj}

for unamended fields.

To conduct this search, we used the following initial set of transforms:

$$S=S_A \cup S_U \cup \{1\}$$

where

$$S_A=\{1_A \cdot t^p : p=-5,-4.5,\cdots,4.5,5\} \cup \{1_A \cdot \ln(x)\}$$

$$S_U=\{1_U \cdot t^p : p=-5,-4.5,\cdots,4.5,5\} \cup \{1_U \cdot \ln(x)\}.$$

We used the same set of transforms as before, but with possibly different powers and estimated slopes for amended than for unamended fields. The cases $p=0$ with transforms equal to the indicator variables 1_A and 1_U allows for the possibility of different intercept parameter values for the two types of fields. We also include the identity function 1 in the search to allow for the possibility of the same intercept parameter value for both types of fields.

We used the same settings of the search parameters as before, but first we adjusted the parameter $\alpha_1=0$. This guarantees that transforms will only be added to the model in the expansion stage if they improve the model. The resulting model had the score $CV_1=0.5325$, and the form

$$E(y|t)=\beta_1 \cdot 1_U+\beta_2 \cdot 1_U+\beta_3 \cdot 1_A \cdot t^{0.5},$$

indicating that organic C was constant β_1 over time for unamended fields but varied with time for amended fields. However, that dependence had a simple monotonic form since only one transform of time was included in the model. Furthermore, the estimate of the slope β_3 was negative, indicating that organic C in amended fields decreases over time to a different equilibrium level β_2 than the background level β_1 for unamended fields.

Then, we repeated the search, but now with α_1=0.01 to see if there was any impact on the choice of this parameter. The resulting model had the slightly better score of 0.5331. It included only one transform for the amended fields, the same one $t^{0.5}$ as before. However, seven different transforms of time were included for unamended fields as well as the identity transform. Thus, it may be that the equilibrium level for amended fields agrees with the background level for amended fields if one allows for possibly complex variability around that equilibrium level for unamended fields.

Finally, we conducted more searches using the simpler initial transform set

$$S=\{1_U, 1_A, 1_U \cdot t, 1_A \cdot t\},$$

with α_1=0.01 and all other settings the same as before. The transformation stage had a much larger impact on the final model for this search than for prior searches. The model produced by this search had the same score of 0.5331 when rounded as the model produced by the second search. However, it was an adjusted version of the model produced by the first search with the form

$$E(y|t)=\beta_1 \cdot 1_U+\beta_2 \cdot 1_U+\beta_3 \cdot 1_A \cdot t^{0.3},$$

thus supporting the conclusion that the dependence on time for both types of fields is not too complex.

The kinds of transforms considered may allow the expected organic C concentration in amended fields to converge to a constant equilibrium level over time, but not to actually achieve equilibrium in a finite amount of time. This made these types of transforms undesirable since we wanted to ascertain the time to equilibrium as well as the equilibrium organic C concentration. To model the attainment of equilibrium at a constant organic C concentration over an amount of time, t_0, we use a simple form of spline (see, for example, Stoer & Bulrich, 1980). Specifically, we start with the simple spline

$$S(t;t_0)=\max(t_0\ t,\ 0)$$

that is linear prior to time t_0 and constant with value zero afterwards. By including a transform of $S(t;t_0)$ in the model, we allow the expected organic C concentration to have general monotone behavior prior to t_0 at which point it becomes constant. By further including an intercept in the model, we allow the expected organic C concentration to remain at an arbitrary constant equilibrium level, at a value equal to the intercept, after t_0.

Since the results obtained prior to the consideration of splines indicates that the dependence on time is not too complex, we conducted our search for the appropriate transform of a spline in the same way as our third search based on time t directly. We used the initial transform set

$$S(t_0)=\{1_U,1_A,1_U\cdot S(t;t_0),1_A\cdot S(t;t_0)\}$$

with $\alpha_1=0.01$ and all other settings the same as before. We conducted a search using transforms of the splines $S(t;t_0)$ for each fixed choice of the equilibrium level t_0 separately, and then chose the equilibrium level to be the integer that produced the best overall cross-validation score. To form a 99 percent pseudo-prediction interval for the equilibrium level, we used the integers generating cross-validation scores within 1 percent of the best score. First, we searched models having different intercept parameters for the two types of fields, thereby allowing the equilibrium level for amended fields to be different from the background level determined by the unamended fields. Then, we searched models having equal intercepts for both types of fields, thereby forcing the equilibrium level for amended fields to be the same as the background level determined by unamended fields. The generated models are displayed in Figure 5.