Published online July 7, 2016

Journal of Environmental Quality

TECHNICAL REPORTS

WASTE MANAGEMENT

Path Analyses of Grain P, Zn, Cu, Fe, and Ni in a Biosolids-Amended Dryland Wheat Agroecosystem

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Abstract

Land application of biosolids is an effective means of recycling plant nutrients and is the primary method of biosolids reuse endorsed by the USEPA. One issue concerning biosolids application is the extent of the contribution of biosolids-borne plant nutrients to the overall crop concentration and uptake or removal of these nutrients. We studied the effects of biosolids application on wheat (*Triticum aestivum* L.) grain P, Zn, Cu, Fe, and Ni concentrations and uptake (removal) at two dryland agroecosystem sites from 1993 to 2014. We hypothesized that biosolids would have the greatest impact on wheat grain and uptake compared with ammonium bicarbonate–diethylenetriaminepentaacetic acid (AB-DTPA)-extractable nutrient levels, soil pH, or soil organic C concentrations. We used path analyses in combination with multiple linear regression to differentiate the direct, indirect, and total effects of cumulative biosolids applications, soil AB-DTPA, soil pH, and organic C. Biosolids rates, applied biennially from 1993 to 2014 at the beginning of a wheat–fallow rotation, were 0, 2.24, 4.48, 6.72, 8.96, and 11.2 Mg ha^{-1}. None of the parameters had significant direct, indirect, or total effects on grain concentrations. Biosolids applications had the greatest positive direct impact compared with AB-DTPA levels, soil pH, or soil organic C on P, Zn, Fe, and Ni uptake (removal), whereas AB-DTPA had the greatest positive direct impact on Cu uptake. Soil AB-DTPA, pH, and organic C directly affected some grain concentrations and cumulative uptake, but no consistent trends were noted. This pathway approach allowed differentiation between causation and simple correlation for the effects of cumulative biosolids applications on wheat P, Zn, Cu, Fe, and Ni cumulative uptake but did not provide these same results for grain concentrations.

Core Ideas

- Path analysis identifies nutrient source in biosolids-amended winter wheat.
- Cumulative biosolids applications affect P, Zn, Cu, Fe, and Ni grain removal.
- Cumulative biosolids applications did not affect P, Zn, Cu, Fe, and Ni grain concentrations.

Biosolids can provide plant nutrients such as P, Zn, Cu, and Ni to deficient soils in dryland agroecosystems (Barbarick and Ippolito, 2007). This is a major reason that USEPA 40 CFR Part 503 (USEPA, 1993) regulations endorse land application of biosolids (sewage sludge) for beneficial use. One question to consider is: To what extent do biosolids-borne nutrients contribute to overall crop concentration and uptake? One approach to answering this question is the use of the statistical technique known as path analysis. This procedure allows assessment of direct and indirect effects (i.e., paths) by partitioning correlation coefficients (Wright, 1921, 1934). Several soil–plant studies have used path analysis to discriminate between correlation and causation. Some statisticians argue, however, that path analyses are not useful or do not provide information on causation. Meehl and Walker (2002) suggest the disagreements are more about philosophical differences rather than being mathematical in nature.

Path analyses studies involving several soil systems have been reported. The assumptions of path analysis include (i) linearity (i.e., linear relationships for all variables), (ii) casual closure (i.e., all direct influences between variables have to be included), and (iii) unitary variables (i.e., variables cannot behave in different ways with different system variables) (Wright, 1968). Basta et al. (1993) used path analyses to differentiate between the effects of pH, cation exchange capacity, and organic C on trace metal adsorption by two Iowa soils. Zhang et al. (2005) and Richards et al. (2012) used a path analysis to determine what soil chemical properties had the greatest impact on P adsorption and trace element content in 28 Oklahoma benchmark soils, respectively. Through path analyses, Tang et al. (2007) showed how effective animal manure was in reducing Al toxicity in an acid soil, and Yan et al. (2013) looked at manure effects on P sorption in a paddy soil. Path analysis allowed Kang et al. (2009) to determine that the indirect effects of oxalate-extractable Al and Fe acting via soil organic matter had the greatest impact on P sorption in four North Carolina coastal plain soils. Muñoz et al. (2014) used a hierarchical path analysis to investigate the effect of a red clover (*Trifolium pratense* L.) cover crop on corn yields (*Zea mays* L.) as influenced by topography at Kellogg Biological Station in Michigan. In all of these studies, path

J. Environ. Qual. 45:1400–1404 (2016)
doi:10.2134/jeq2015.06.0303
Received 23 June 2015.
Accepted 1 Feb. 2016.
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Abbreviations: AB-DTPA, ammonium bicarbonate–diethylenetriaminepentaacetic acid analyses allowed delineation of direct and indirect effects on several soil or plant attributes.

We feel this approach may help determine the impact of biosolids-borne nutrients on wheat grain (*Triticum aestivum* L.) concentrations and uptake (removal) where uptake is expressed as:

$$\text{uptake}\left(\text{kg ha}^{-1}\right)=\text{yield}\left(\text{Mg ha}^{-1}\right) \times \text{grain concentration}\left(\text{mg kg}^{-1}\right) \times\left(\frac{10^3\,\text{kg}}{\text{Mg}}\times\frac{\text{kg}}{10^6\,\text{mg}}\right) \quad [1]$$

Our hypothesis is that biosolids application will have a greater total effect on wheat grain nutrient concentrations and cumulative uptake as compared with ammonium bicarbonate–diethylenetriaminepentaacetic acid (AB-DTPA)-extractable (plant-available) soil nutrients, pH, or organic C levels. Analyses of variance and path analyses will be used to determine the impact of biosolids applications, AB-DTPA concentrations, pH, and organic C and serve as basis for testing the hypothesis. Lindsay (1979) detailed the pH effects on P and micronutrient availability, whereas Sparks (2015) used molecular scale tools to better delineate mineral effects on plant availability that may supersede pH effects. Richards et al. (2011) found that changes in soil organic matter content associated with biosolids applications also affected P and micronutrient availability. Barbarick and Ippolito (2009) and Barbarick et al. (2010) completed ANOVA, showing the relationships between cumulative biosolids applications and AB-DTPA nutrient concentrations on grain yield and grain nutrient concentrations used in this study. We decided to determine how pH and organic C levels also affect long-term plant-available nutrient concentrations and uptake. To test our hypothesis, we used multiple linear regression and path analysis (SAS PROC REGR and PROC CALIS [SAS, 2015]) to determine direct and indirect effects of biosolids applications, soil AB-DTPA levels, pH, and soil organic C concentrations on grain P, Zn, Cu, Fe, and Ni concentrations and uptake (Fig. 1). We did not complete statistical analyses on other trace elements because many of the grain concentrations were below detection limits.

Materials and Methods

Study Site and Biosolids Applications

Our two North Bennett study sites were established in August 1993 and August 1994 about 20 km north of Bennett, CO (39.9563° N, 104.462° W) on Weld loam soils (fine, smectitic, mesic Aridic Argiustoll) (NRCS, 2015). The initial soil pH of the top 20 cm was 6.9 at both sites and ranged from 6.9 to 7.8 in 2013 and 2014. Further study site details are provided by Barbarick and Ippolito (2007, 2008).

After 60 d of sand drying in late July or early August immediately before September planting, we manually applied anaerobically digested biosolids from the Littleton/Englewood CO Wastewater Treatment Plant biennially at rates 0, 2.24, 4.48, 6.72, 8.96, and 11.2 dry Mg ha^{-1} to 1.8 by 17.1 m plots from 1993 through 2014. We have recommended that 4.48 dry Mg ha^{-1} serve as the agronomic rate (Barbarick and Ippolito, 2000, 2007). The biosolids treatments were half of a study that included six rates of N fertilizer (urea [46–0–0] at rates of 0, 22.4, 44.8, 67.2, 89.6, and 112 kg N ha^{-1}). Each site was arranged with four replications of every treatment (24 biosolids and 24 N-fertilizer plots) randomized as a completed block design.

Grain Harvest and Analyses

We harvested grain and determined yields every year in July except for 2000 to 2001 (lost to hail damage) and 2008 to 2009 (sunflowers [*Helianthus anuus* L.] were grown to help control jointed goat grass [*Aegilops cylindrica* Host]). This resulted in 11 harvests at Site A and 9 harvests at Site B. After drying and grinding, grain samples were digested in concentrated HNO_3 and analyzed by inductively coupled plasma–atomic emission spectrophotometry (Soltanpour et al., 1996) for P, Zn, Cu, Fe, and Ni. Grain uptake (Eq. [1]) was calculated for each harvest, and cumulative grain uptake (measure of nutrient removal over time) was determined after each harvest. The ranges for yield and grain concentrations and uptake are presented in Table 1. The number of grain concentrations and uptake measured was either 90 (Zn, Cu, Ni) or 95 (P, Fe). We were not able to obtain accurate analyses for grain Zn, Cu, or Ni for the 2013–2014 harvest; hence, a smaller number of data points were observed.

Soil Analyses

We used a Giddings hydraulic probe to collect soil samples (composite of two or three cores) within 1 mo after harvest from a depth of 0 to 20 cm from the center of each plot to avoid the biosolids redistribution problem associated with tillage over time (Yingming and Corey, 1993). All samples were air-dried immediately, crushed, and sieved (<2 mm diam.).

The plant-available P, Zn, Cu, Fe, and Ni concentrations in AB-DTPA extracts (Barbarick et al., 1997) were determined with an inductively coupled plasma–atomic emission

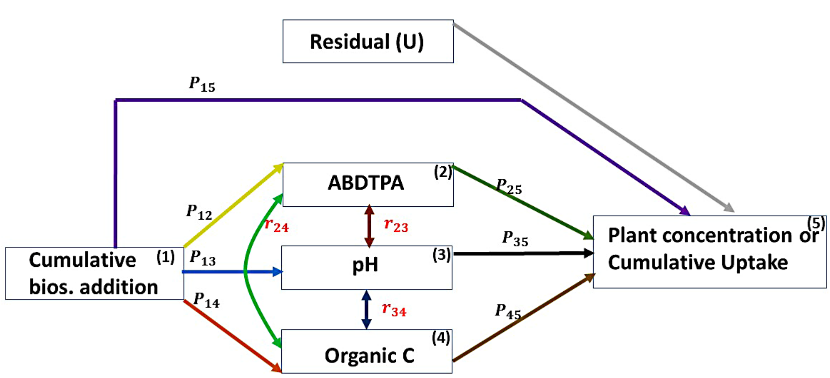

Fig. 1. Path analysis diagram for determining the effect of soil ammonium bicarbonate–diethylenetriaminepentaacetic acid (AB-DTPA), cumulative biosolids-borne nutrients, soil pH, and organic C on wheat grain concentrations or cumulative grain uptake (removal) for the two North Bennett sites, 1993–2014. Single-headed arrows indicate direct paths; two-headed arrows indicate indirect paths. r_{ij} represents the simple correlation coefficient between the parameters (1, cumulative biosolids applied; 2, AB-DTPA; 3, pH; 4, organic C; and 5, grain concentration or grain uptake). The P_{ij} values are path coefficients (direct effects), and (r_{ij})(P_{ij}) are the indirect effects on grain concentration or grain uptake.

spectrophotometer (Soltanpour et al., 1996). Soil pH (Thomas, 1996) was measured in saturated soil-paste extracts. The ranges for soil characteristics are presented in Table 1.

Statistical Analyses

SAS PROC CORR was used to determine simple correlation coefficients, and PROC REG was used to conduct multiple linear regression to calculate path coefficients and residuals (SAS, 2015). We used a backward-elimination regression analysis. The generalized model for grain concentration or cumulative uptake is shown in Eq. [2]:

$$\text{cumulative uptake} = v + w(\text{AB-DTPA}) + x(\text{cumulative nutrient addition}) + y(\text{pH}) + z(\text{organic C}) \quad [2]$$

where v, w, x, y, and z are regression coefficients.

We determined the residuals using Eq. [3]:

$$U = \sqrt{1 - R^2} \quad [3]$$

where U is the uncorrelated residual (influence of extraneous or unmodeled factors), and R^2 is the coefficient of determination from the multiple linear regression model.

Our path (causal) model (Fig. 1) illustrates the relationship between various parameters and grain concentration or uptake. Subscripts are labeled as (1) cumulative biosolids applications, (2) B-DTPA, (3) pH, (4) organic C, and (5) grain concentration or grain uptake. We completed path analyses using SAS PROC CALIS (SAS, 2015) through the following normal equations (Williams et al., 1990):

$$r_{15} = P_{15} \quad [4]$$

$$r_{25} = r_{23}P_{35} + r_{24}P_{45} + r_{23}r_{34}P_{45} + r_{24}r_{34}P_{35} + P_{25} \quad [5]$$

$$r_{35} = r_{23}P_{25} + r_{34}P_{45} + r_{23}r_{24}P_{45} + r_{34}r_{24}P_{25} + P_{35} \quad [6]$$

$$r_{45} = r_{34}P_{35} + r_{24}P_{25} + r_{34}r_{23}P_{25} + r_{24}r_{34}P_{35} + P_{45} \quad [7]$$

where r_{ij} represents the simple correlation coefficient between the above parameters 1 through 5. The P_{ij} values are path coefficients (direct effects), and (r_{ij}) (Pij) are the indirect effects on grain concentration or grain uptake (Fig. 1).

We provide simple correlation coefficients (r) between grain concentration or uptake and biosolids applications, AB-DTPA, pH, and organic C to compare with path analysis results. Although the simple r values indicate correlation, partitioning the correlation coefficient into direct and indirect path effects allows a view of what parameters are causing the correlation (i.e., causation).

Results and Discussion

The multiple linear regression analyses of grain concentrations of P, Zn, Cu, Fe, and Ni as affected by cumulative biosolids applications, AB-DTPA–extractable concentrations, pH, and organic C produced R^2 values <0.20 (data not shown). Consequently, we reject the hypothesis that biosolids would have a greater impact than AB-DTPA nutrient concentrations, pH, or organic C. Actually, Barbarick et al. (1995) showed that grain concentrations in biosolids-amended soils compared with cumulative biosolids application rates follow an exponential rise to a maximum nonlinear model, supporting rejection of the hypothesis.

Path analyses results for cumulative grain P, Zn, Cu, Fe, and Ni uptake showed that none of the indirect effects had a significant effect. Also, pH did not have a total effect on cumulative grain P, Zn, Cu, Fe, and Ni uptake. The uncorrelated residual (U) values ranged from 0.57 to 0.75.

Grain P Cumulative Uptake

The grain P uptake model accounted for 63% of the variability with cumulative biosolids applied, producing the largest partial R^2 (0.53) (Table 2). Biosolids applications, soil AB-DTPA, and organic C produced positive direct outcomes on cumulative grain P uptake (Table 3) and correlated significantly with P uptake. The biosolids applications had the largest impact on uptake.

We accept the null hypothesis that biosolids applications would significantly affect grain P uptake (removal) to a greater extent than AB-DTPA concentrations, pH, or organic C. Because both the cumulative uptake and biosolids applications increase concurrently each year, a direct relationship is plausible.

Grain Zn Cumulative Uptake

For the grain Zn uptake, the regression model accounted for 68% of the variability (Table 2). Cumulative biosolids applied and pH had significant effects on the model results, with biosolids having a partial R^2 of 0.65. Biosolids applied and AB-DTPA produced significant direct effects, and biosolids applied, AB-DTPA, and organic C had significant direct effects on cumulative grain Zn removal, with the largest effect associated with AB-DTPA concentrations (Table 3). We accept the null hypothesis that biosolids applications would produce the largest direct impact on grain Zn uptake compared with AB-DTPA concentrations, pH, or organic C.

Table 1. Range of grain yields, concentrations and uptake, final cumulative element biosolids applications, and soil characteristics at the two North Bennett study sites, 1993 to 2014.

Property	All soils	P	Zn	Cu	Fe	Ni
Cumulative grain yield, Mg ha^{-1}	19–30					
Grain concentration, mg kg^{-1}		2200–6400	11–33	1.8–6.9	6.2–117	0.20–6.00
Cumulative uptake, kg ha^{-1}		79.9–91.5	0.45–0.69	0.122–0.162	0.517–0.684	0.033–0.040
AB-DTPA, mg kg^{-1} †		2.14–32.5	0.16–3.6	1.09–8.21	0.56–6.48	0.26–1.79
Cumulative element biosolids applications, kg ha^{-1} ‡		2340–2810	50.7–63.4	53.1–67.5	2470–2580	2.39–2.88
pH	6.7–7.8					
Organic C, g kg^{-1}	6.6–11					

† Ammonium bicarbonate–diethylenetriaminepentaacetic acid.
‡ Site B–Site A.

Grain Cu Cumulative Uptake

The model for cumulative grain Cu uptake explained 44% of the variability, with biosolids applied contributing the largest partial R^2 (0.26). This multiple-linear regression model produced the smallest accounting of variability compared with the models for cumulative grain uptake for P, Zn, Fe, and Ni (Table 2). For path analyses, biosolids applied, AB-DTPA, and pH had significant direct effects, and biosolids applied, AB-DTPA, and organic C had significant total effects on cumulative Cu grain removal, with the largest effect associated with AB-DTPA Cu concentrations (Table 3). We, therefore, reject the null hypothesis that cumulative biosolids Cu applications would account for the largest change in cumulative grain Cu uptake.

Grain Fe Concentrations and Cumulative Uptake

The regression model accounted for 51% of the variability for cumulative Fe uptake, with biosolids applications producing a partial R^2 of 0.45 (Table 2). Biosolids applied, AB-DTPA, and organic C had significant direct path and total effects on cumulative Fe uptake.

Table 2. Multiple regression model parameters for wheat cumulative uptake of P, Zn, Cu, Fe, and Ni for the two North Bennett study sites, 1993 to 2014.

Parameters	Regression coefficients†	Partial R^2	R^2	Data points
P				
Intercept	−92	–	0.63	95
Biosolids applied	0.64	0.53		
AB-DTPA‡ P	0.56	0.07		
pH	13	0.02		
Organic C	28	0.01		
Zn				
Intercept	−0.56	–	0.68	90
Biosolids applied	0.0044	0.65		
AB-DTPA Zn	−0.001	0.00		
pH	0.088	0.03		
Organic C	0.11	0.00		
Cu				
Intercept	−0.33	–	0.44	90
Biosolids applied	0.00046	0.26		
AB-DTPA Cu	0.016	0.12		
pH	0.037	0.04		
Organic C	0.088	0.02		
Fe				
Intercept	0.41	–	0.51	95
Biosolids applied	0.0045	0.45		
ABDTPA Fe	0.025	0.03		
pH	−0.079	0.01		
Organic C	0.29	0.02		
Ni				
Intercept	−0.04	–	0.61	90
Biosolids applied	0.00025	0.53		
AB-DTPA Ni	−0.003	0.03		
pH	0.0075	0.05		
Organic C	0.0072	0.00		

† All regression equations were significant at the 0.10 probability level.

‡ Ammonium bicarbonate–diethylenetriaminepentaacetic acid.

About 2500 kg Fe ha^{-1} has been added to both sites through 2014, the most of any element applied except P (Table 1). These applications are due primarily to $Fe_2(SO_4)_3$ application at the Littleton/Englewood Wastewater Treatment Plant inflow so as to reduce H_2S in the digester gas. The $Fe_2(SO_4)_3$ is added to reduce the acid formation in the biogas, or engine oil, used to generate electricity at the treatment facility. After digestion, the added Fe is probably amorphous and more available for plant uptake than the native soil Fe (Ippolito et al., 2007). We accept the hypothesis that biosolids Fe would significantly influence grain Fe removal.

Grain Ni Cumulative Uptake

The multiple regression cumulative grain Ni uptake model accounted for 61% of the variability (Table 2). Biosolids applications had a significant positive direct impact on cumulative Ni uptake, whereas soil AB-DTPA had a significant negative impact (Table 3). Biosolids applications and organic C had a significant positive total effect on cumulative Ni uptake, whereas soil AB-DTPA had a significant negative impact. The negative impact of AB-DTPA on cumulative Ni uptake may be due to the nature of the Ni mineral phases. Sparks (2015) indicates that Ni forms an inner-sphere complex at4low surface-covering rates and a Ni-Al hydroxide precipitates at

Table 3. Path analyses direct effects and indirect effects of cumulative biosolids applications, soil ammonium bicarbonate–diethylenetriaminepentaacetic acid (AB-DTPA), soil pH, and organic C on wheat grain P, Zn, Cu, Fe, and Ni concentrations and cumulative P, Zn, Cu, Fe, and Ni grain uptake (removal) from the two North Bennett study sites, 1993 to 2014.

	Path coefficients		Total effects (r)	R^2	U†
	Direct effect	Indirect effect			
P cumulative uptake					
Biosolids applied	**0.73**‡	**–**	**0.73**	**0.63**	0.61
AB-DTPA	**0.33**	0.04	**0.37**		
pH	−0.01	0.04	0.05		
Organic C	**0.22**	0.06	**0.27**		
Zn cumulative uptake					
Biosolids added	**0.81**	–	**0.81**	**0.68**	0.57
AB-DTPA	**0.33**	0.04	**0.37**		
pH	0.07	0.02	0.09		
Organic C	0.16	0.09	**0.25**		
Cu cumulative uptake					
Biosolids added	0.51	–	**0.51**	**0.44**	0.75
AB-DTPA	**0.55**	0.03	**0.58**		
pH	**0.19**	−0.04	0.15		
Organic C	0.16	**0.17**	**0.33**		
Fe cumulative uptake					
Biosolids applied	**0.67**	–	**0.67**	**0.51**	0.70
AB-DTPA	**0.28**	0.03	**0.31**		
pH	−0.03	−0.04	−0.07		
Organic C	**0.23**	0.04	**0.27**		
Ni cumulative uptake					
Biosolids applied	**0.72**	–	**0.72**	**0.61**	0.62
AB-DTPA	**−0.19**	−0.05	**−0.24**		
pH	0.14	0.03	0.17		
Organic C	0.21	0.04	**0.25**		

† Uncorrelated residual.

‡ Effects significant at the 0.10 probability level are highlighted in bold.

higher coverage. Thus, possibly Ni was influenced to a greater degree by organo-mineral surface complexation and not by precipitation reactions governed by soil pH. All other nutrient removals showed a positive correlation with AB-DTPA levels. We accept the hypothesis that cumulative biosolids applications would have the largest effect on grain Ni uptake.

Conclusions

The implication of this study regarding environmental quality is that cumulative biosolids application will directly affect cumulative wheat grain uptake (removal) of P, Zn, Fe, and Ni to a greater extent than AB-DTPA–extractable concentrations, soil pH, or soil organic C content. In fact, for P, Zn, Fe, and Ni in our dryland wheat agroecosystem study, cumulative biosolids additions explained the greatest amount of the variability associated with nutrient uptake (removal). For Cu uptake, AB-DTPA concentrations had the largest direct impact. This study also indicates that path analyses may be useful in determining causation of environmental management effects on plants and soils.

Cumulative biosolids applications did not affect grain P, Zn, Cu, Fe, and Ni concentrations. Soil pH did not have a direct path effect on cumulative grain P, Zn, Cu, Fe, and Ni uptake, whereas cumulative biosolids application, AB-DTPA concentrations, and organic C did have direct path effects on grain uptake. We also did not find any significant indirect path effects on cumulative grain uptake. Consequently, we accepted our hypotheses that biosolids applications would directly affect P, Zn, Fe, and Ni cumulative grain uptake. Soil AB-DTPA had a direct positive effect on grain P, Zn, Cu, and Fe, supporting the utility of AB-DTPA as a soil-test extractant for these nutrients.

Path analyses provided useful information on the impact of biosolids applications on grain uptake (removal) of P, Zn, Cu, Fe, and Ni. This approach also allows potential differentiation between causation and simple correlation effects.

Acknowledgments

This research was funded by the Littleton/Englewood Wastewater Treatment Plant and the Colorado State Agricultural Experiment Station.

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