Effects of Composted Biosolids and Manure Applications for Prairie and Wetland Restoration on Remediated Mine Lands

Oronogo-Duenweg Mining Belt Site, Webb City, Mo

Final Report

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Executive Summary

The use of organic soil amendments to restore fertility to highly degraded post-remedial soils near former lead and zinc mines is a promising restoration technique. In order to evaluate this technique with regard to nutrient and metals leaching in runoff, metal uptake in plants and earthworms, the potential impacts of pharmaceuticals and personal care products on biotic receptors, and the efficacy of these amendments in supporting native vegetation, several different composts composed of various feedstock materials were applied to test plots in terrestrial and wetland settings. Project test plots were installed in non-remediated (chat) and remediated (residual sub-soil) areas to simulate the range of conditions present at potential restoration areas throughout the Tri-State mining district. Wetland "tubs" containing residual soils and effluent were created to simulate impacts to aquatic systems.

Run-off results show there is an initial flush in metals and nutrients following the application of the amendments, but that effect tapers off with time. Rates of application (40 tons/ac vs 80 tons/ac) had no impact on the run-off of metals. The average dissolved lead, zinc and cadmium run-off from unremediated plots were substantially greater than the average run-off from the remediated plots. Test organisms showed 97.5%-100% survival in all of the treatments' runoff as well as the control when the run-off was diluted to simulate treatment of 1/8 of the watershed. Test organism survival was only impacted (77.5% survival) by the high rate of Poultry Litter and the bare ground control when simulating treatment of half of the watershed.

Plant tissue metal concentrations vary directly with the soil metal concentrations. Soil metals measured after amendments were mixed into the remediated soil plots were 27% of the original levels. Within the unremediated plots the various treatments showed no reduction

(beyond soil dilution) on the plant uptake of lead or zinc, and mixed results on cadmium. Reduction of zinc uptake beyond simple dilution was observed within the remediated plots, but no treatment of lead or cadmium. Similar to the upland scenarios, simply adding the soil amendments to the simulated wetlands diluted the metals content of the sediment. Both the remediated and non-remediated study site's soils were inordinately high in metals compared to most surrounding remediated areas. When the metal concentration reducing effects observed in this study are calculated using soil concentrations more representative of typical field conditions, the only adverse impact from metals to any animal species used for analysis would be from cadmium in the biosolids high and poultry high treatments.

The initial simulated wetlands showed that the composted cow manure was able to grow the greatest quantity and diversity of native species, with minimal weedy species invading. Of the unremediated soil amendment treatments, only the cattle manure grew a significantly greater richness and diversity of seeded species than the control. Within the less contaminated remediated plots, both the biosolids low and the manure-biosolids high treatments grew a significantly greater richness and diversity of native plants. The richness of the seeded species in the less-contaminated remediated plots was much less than in the unremediated plots, likely due to interspecific competition from non-native species.

Worms raised in the Control soil had the highest average Pb and Zn concentration, demonstrating a treatment effect for all amendments. Only the Cow Manure amendments demonstrated any treatment effect on the uptake of cadmium. The highest PPCP concentrations in worm tissues were the antimicrobials triclosan and triclocarban, though all of the concentrations were less than an order of magnitude below any adverse effects concentrations reported in previous literature.

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1. Introduction

The Tri-State mining district, which includes portions of Missouri, Kansas, and Oklahoma, was once an important lead and zinc producing area from around the mid-19th to mid-20th centuries. In Jasper County, Missouri, remedial actions to address the legacy of mine waste (approximately 1800 ha) have involved excavating the chat, tailings, and contaminated soils, and disposing of the material in repositories or subsidence pits (EPA 2004). Residual soils in the remediated areas are of poor quality consisting of mostly clay and chert gravel sub-soil with elevated concentrations of metals (105-1200 mg Pb/kg, 800-7400 mg Zn/kg, 39-162 mg/Cd/kg, EPA unpublished data). Early in the remedial action topsoil was brought in to cap the remediated areas, however it has not been economically or logistically feasible to import enough topsoil to cap all of the area needed. In addition, given the length of time required for natural soils genesis, the relocation of large areas of topsoil from the surrounding environment could have significant long term ecological consequences (Brown et al. 2014). The result is large expanses of poor soil which are unable to support healthy vegetative communities, provides poor wildlife habitat, and are subject to soil erosion and leaching of residual metals, potentially impacting streams receiving runoff.

The use of various soil amendments including phosphorus (P), lime, compost, and municipal biosolids (residual organic material recovered from the wastewater treatment process), have been found to reduce the phytoavailability of metals and increase soil fertility in mine waste areas (Yang et al. 2001,Yang and Mosby 2006, Brown et al. 2003, 2004, 2007, 2014, Miretzky and Fernandez-Cirelli 2008). Phosphate followed by lime addition has been shown to reduce bioavailability of Pb, Cd, and Zn. Phosphate treatment forms insoluble and less bioavailable

metal compounds by precipitating Pb and to a lesser degree Cd and Zn in the form of pyromorphite (Zwonitzer et al. 2003, Bolan et al. 2003a, Miretzky and Fernandez-Cirelli 2008, Wu et al. 2013). Subsequent lime addition will further immobilize Zn and Cd though an increase in soil pH. The addition of lime has also been effective at reducing bioavailability of metals when used alone (Bolan et al. 2003b, Geebelen et al. 2006, Gray et al 2006, Abd El-Azeem et al. 2013) and in combination with other amendments (Svendson et al 2007), primarily by increasing pH and adsorption. Compost and biosolids, both high P containing materials, increase the binding and precipitation of metals (Bolan et al. 2003c, Brown et al 2004, 2007), and when mixed with lime or P, have been shown to be more effective at reducing bioavailable metals than lime or P alone (Brown et al 2007).

Biosolids and composts have additional properties that make them especially promising for restoring mine waste and degraded soils; they are high in nutrients and organic matter, improve the structure and water holding capacity of the soils, and promote carbon sequestration (Tian et al. 2009, Lu et al. 2012). The use of composts and biosolids has been used successfully in the revegetation of mine waste and degraded soils (Brown et al. 2003, 2005, 2014, Halofsky and McCormick 2005) and in the restoration and establishment of native prairie and warm-season grass communities (Busby et al. 2006). Native prairie establishment is of particular interest in southwest Missouri, where tallgrass prairie communities were historically an important feature of the landscape (Nelson 1985). Nationally, native tallgrass prairie covers less than 1% of its pre-settlement expanse (Samson and Knopf 1994). Prairie restoration has the potential not only to increase wildlife habitat, but also to increase the capture and sequestration of atmospheric carbon, a greenhouse gas (Hernandez et al. 2012).

Despite the observed utility and benefits of soil amendments for remediation and restoration projects, concerns have been raised about the potential for soils treated with these materials to leach excess nutrients (Stehouwer et al. 2006), metals (Yang et al 2008), and hormonally active agents such as pharmaceuticals and personal care products (PPCPs, Wu et al. 2010). Studies have shown that PPCPs leaching from biosolids applied to land can persist for years following application (Walters et al. 2010) and can contaminate surface waters and groundwater (Gottschall et al. 2012), and be taken up by plants (Eggen et al. 2011) and earthworms (Kinney et al. 2008), with the potential to persist into higher trophic levels. However, the majority of research has focused on land application in an agricultural setting with typical agronomic application rates in the range of 8 Mg/ha (3.5 tons/ac) (Sabourin et al. 2009) to 22 Mg/ha (10 tons/ac) (Wu et al. 2010, Gottschall et al. 2012). The leaching potential of PPCPs from biosolids at application rates (134 Mg/ha (60 tons/ac) to 224 Mg/ha (100 tons/ac)) used in mine land remediation is unknown. The materials selected and the application rates used in restoration projects must balance the expected positive effects of metal immobilization and soil fertility increase with the potential unintended consequences of chemical leaching or contaminant exposure to wildlife or people.

1.2 Objectives

It is the goal of the United States Fish and Wildlife Service (USFWS) and the Missouri Department of Natural Resources (MDNR) to restore portions of the remediated mine waste areas in Jasper County to native prairie communities, and restore wetlands and riparian vegetation along streams and floodplains. If restoration of these areas is to be successful, they should not act as an attractive nuisance; attracting wildlife which would then be exposed to toxic

concentrations of metals in plants or soils (EPA 2007). In addition, many potential restoration project areas are located near streams (for example, Center Creek and Ben's Branch, Figure 1), making the effects of runoff from any soil amendments especially important. The objectives of this study are to evaluate the suitability of soil amendment mixtures for prairie and wetland restoration of remediated mine waste areas in Jasper County. The mixtures will be evaluated in terms of their effects on 1) metal bioavailability of residual contaminants, 2) nutrient, metal, and PPCP leaching in storm water runoff from amended soils, 3) establishment and success of native prairie and wetland species on amended soils, and 4) the potential for these soil amendment-native plant combinations to facilitate carbon sequestration.

Three different soil types and landscapes have been identified for the soil amendment investigation: remediated floodplain/wetland soils, remediated upland soils, and non-remediated soils. Remediated floodplain soils are generally organic-rich silty-clay loam. Remediated upland soils are generally B or C-horizon red cherty clay. Non-remediated contaminated soil at the study site is composed of compacted silty clay loam fill with intermixed chat. The soil amendment details are listed in Table 1. The project components and the objectives of each component are listed here:

Remediated upland field plots

1. Identify the capacity of amended soils to leach nutrients, metals and PPCPs.
2. Evaluate the toxicity of surface runoff to aquatic organisms.
3. Quantify differences in the potential for plants to uptake metals from amended soils.
4. Evaluate the suitability of amended soils to germinate and support native vegetation.
5. Quantify the bioaccessablility of metals in amended soils using earthworms

6. Assess potential risk to terrestrial wildlife from metals and PPCPs through the food chain.

Remediated wetland soil tubs

1. Identify the capacity of amended soils to leach nutrients, metals and PPCPs.
2. Evaluate the toxicity of surface runoff to aquatic organisms.
3. Quantify differences in the potential for plants to uptake metals from amended soils.

Non-remediated soil field plots (Discussed in Appendix A.)

1. Identify the capacity of amended soils to leach nutrients, metals and PPCPs.
2. Quantify differences in the potential for plants to uptake metals from amended soils.
3. Evaluate the suitability of amended soils to germinate and support native vegetation.

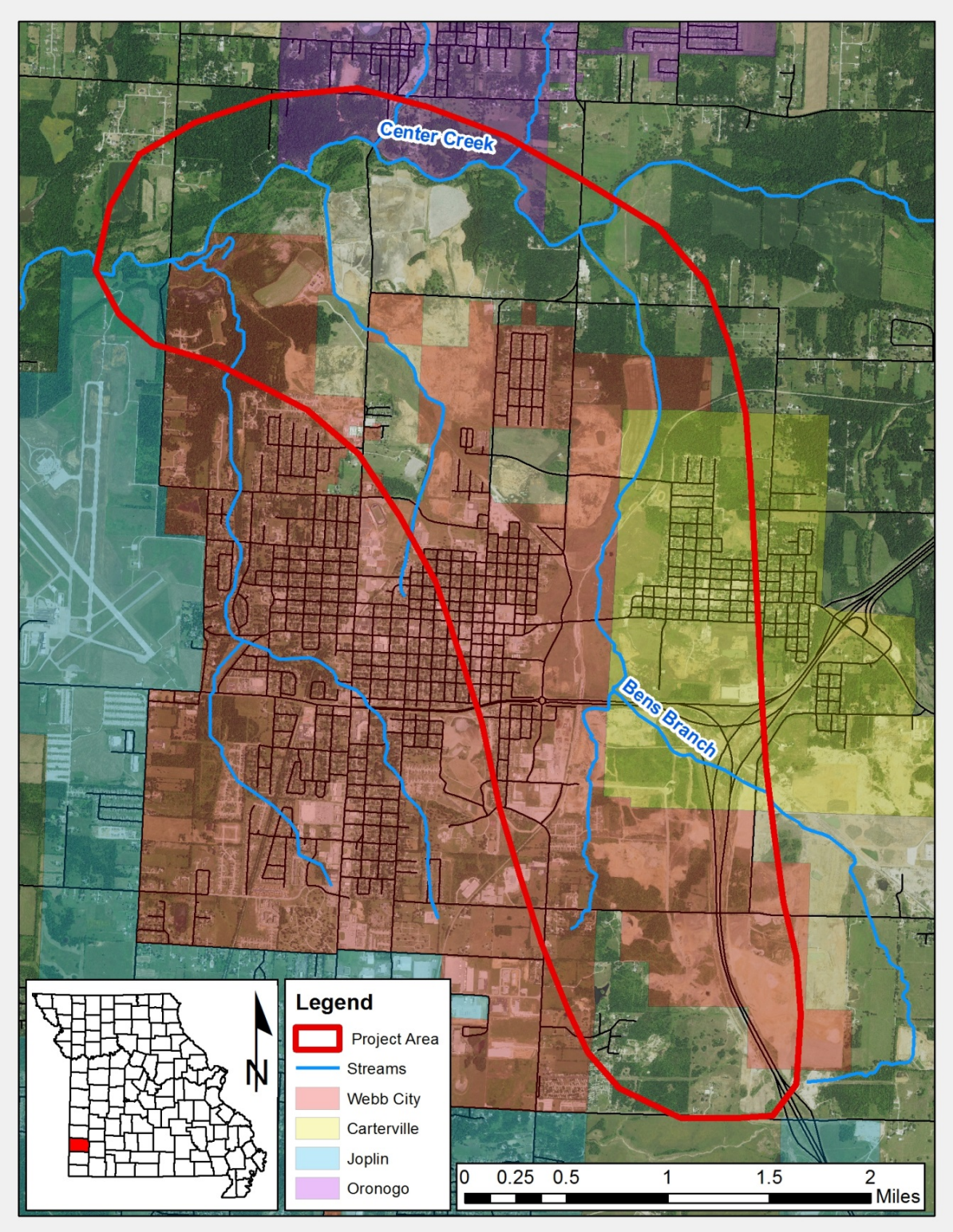

Figure 1. Map showing potential restoration project areas.

2. Non-Remediated Soil Field Plots

This portion of the study was intended to serve as a “worst-case scenario” due to the extremely high levels of metals in the soils. The soils in this area are a mixture of contaminated topsoil and residual chat. The majority of contaminated acres in the region are expected to be remediated with the residual soils resembling those described in the Remediated Upland Field Plot section below. Because it represents a special case, the Non-Remediated field plot portion of the study is included as an appendix to this report (Appendix A).

3. Remediated Upland Clay Soils

3.1 Experimental Soil Amendments

Three soil amendment mixtures were initially selected for evaluation in this study: a municipal biosolids based mixture (BS), a cattle manure based mixture (CM) and yard waste applied along with conventional agricultural fertilizer (AG). After receiving data from the first season of sampling the non-remediated soil field plots (reported in Appendix A), the project managers chose to use a combination of biosolids and cow manure (CM+), a combination of biosolids and poultry litter (PL+), and a limed yard waste treatment with less fertilizer (YF), as the treatments for the upland remediated soil type. All amendment mixtures were allowed to compost for approximately two months, being turned intermittently, prior to land application. A list of the amendments evaluated, along with the components of this study in which they were used is shown in Table 1.

Table 1. Names, descriptions, and abbreviations used for soil amendments used in this study, along with the study component in which they were used.

Soil Amendment Name	**Soil Amendment Description**	**Abbr.**	**Study Components Using Amendment**
Biosolids	Biosolids from Springfield, MO mixed 10% (by weight) with biosolids from Webb City, mixed with wood chips/leaf waste.	BS	Non-Remediated Plots Remediated Plots Wetland Tubs (Years 1&2) Earthworm Exposure
Cattle Manure	Cattle manure from local stockyard mixed with wood chips/leaf waste.	CM	Non-Remediated Plots Wetland Tubs (Year 1)
Yard Waste and Fertilizer	Mulched wood chips/leaf waste applied to plots with direct application of granular 10-20-10 NPK fertilizer.	AG	Non-Remediated Plots Wetland Tubs (Year 1)

Soil Amendment Name	Soil Amendment Description	Abbr.	Study Components Using Amendment
Cattle Manure plus Biosolids	Cattle manure from stockyard mixed 10% (by weight) with biosolids from Webb City, mixed with wood chips/leaf waste.	CM+	Remediated Plots Wetland Tubs (Year 2) Earthworm Exposure
Poultry Litter plus Biosolids	Dry poultry litter from local source mixed 10% (by weight) with biosolids from Webb City, and wood chips/ leaf waste.	PL+	Remediated Plots Wetland Tubs (Year 2) Earthworm Exposure
Composted Yard Waste and Fertilizer	Wood chips/leaf waste mixed with granular 10-20-10 NPK fertilizer and allowed to compost	YF	Remediated Plots Wetland Tubs (Year 2) Earthworm Exposure

Biosolids (BS)

The biosolids produced from the Webb City WWTP routinely contain elevated levels of zinc (> 7500 mg/kg) due to sewer line inflow and infiltration from contaminated runoff and groundwater from the surrounding mining impacted lands. In order to dilute the Zn concentration, biosolids from the nearby city of Springfield, MO were mixed with the Webb City biosolids at a rate of roughly 1 part Webb City material to 10 parts Springfield material. The biosolids were limed to a circum-neutral pH, and mixed at an approximate 2:1 ratio, by weight, with wood chips and leaf yard waste to add bulk, increase the C:N ratio to an ideal 20-40:1 ratio, and to further dilute the overall concentration of zinc to near a target level of 1000 mg/kg. Analysis results of biosolids from each municipality are listed in Table 2. The biosolids were applied at a low rate of 40 dry tons/acre (90 Mg/ha) (BS Low) and a high rate of 80 dry tons/acre (180 Mg/ha) (BS High), not including the additional carbon material.

Table 2. Estimated metal and nutrient concentrations in biosolids

All values mg/kg	Webb City Biosolids	Springfield Biosolids	Anticipated 1:10 Mixture
	Metals		
Arsenic	ND	ND	ND
Cadmium	124	ND	14
Chromium	74.6	37.6	41
Copper	368	375	374
Lead	420	44.4	79
Mercury	1.1	0.9	1
Molybdenum	ND	57	53
Nickel	31.7	34	34
Selenium	ND	ND	ND
Zinc	11700	1288	2235
	Nutrients		
Total Kjeldahl Nitrogen	38500	74858	71553
Ammonia (NH3)	5970	13326	12657
Organic Nitrogen	32500	61533	58894
Total Phosporus	21000	34300	33091
Potassium	1770	1898	1886

Manure + Biosolids (CM+)

Cattle manure was sourced locally, from the Joplin Regional Stockyard. Generally, cattle manure has nutrient concentrations as follows: 9 to 21 lb/ton Nitrogen, 4 to 18 lb/ton phosphate, and 8 to 26 lb/ton potassium. The cattle manure and woodchips mixture was mixed 10:1 with a mixture of biosolids from Webb City and woodchips sufficient to balance the carbon:nitrogen ratio. The biosolids + manure mixture was applied at a low rate of 40 dry tons/acre(CM+ Low) and a high rate of 80 dry tons/acre(CM+ High), not including the additional carbon material.

Poultry Litter + Biosolids (PL+)

Uncomposted poultry litter was acquired from Opal Foods and was mixed with woodchips and leaves to increase the C:N ratio. Analysis of the poultry litter provided by the producer reported nutrient concentrations as follows: 56 lb/ton (Total Kjeldahl) Nitrogen, 40 lb/ton phosphate, and 31 lb/ton potassium. The biosolids + poultry litter mixture were applied at a low rate of 40 dry tons/acre (PL+ Low) and a high rate of 80 dry tons/acre (PL+ High), not including the additional carbon material.

Composted Yard Waste and Fertilizer (YF)

Yard waste consisting of leaves and wood chips was mixed with high phosphate fertilizer (10-20-10 NPK) and allowed to compost. The fertilizer was a mixed at a rate sufficient to achieve a carbon to nitrogen ratio of approximately 30:1. The application rate of this compost was similar in volume to the volume of material used in the biosolids application treatment, although, because this mixture was less dense than the other composts, the application rates may have been slightly less than 40 and 80 tons/acre.

3.2 Methods

3.2.1 Study Area

The remediated plots are located approximately 3000 ft south of the WWTP. Remediated upland soils, the intended target for our restoration actions, are generally B or C-horizon red cherty clay, with elevated levels of metals, but levels that are below the EPA remedial action objectives (RAOs). Prior to installation of study plots, whole metal concentrations throughout the remediated upland and non-remediated study areas were measured with a handheld X-ray

fluorescence instrument (XRF, Olympus NDT Inc., Waltham, MA). Soils in the plots have average metal concentrations of 642 mg Pb/kg, 3322 mg Zn/kg, and 48 mg Cd/kg. Lead concentrations is individual plots ranged from 251 to 1525 mg/kg while Zn concentrations ranged from 858 to 8845 mg/kg and Cd concentrations ranged from 27 to 74 mg/kg.

3.2.2 Plot Design

The BS+, CM+, PL+, and YF amendment mixtures were applied at a high and a low application rate, creating a total of 8 experimental treatments. Each experimental treatment, in addition to a control treatment with no soil amendments, was replicated 3 times in a randomized block design, making a total of 24 plots. The plots measure 2 m wide by 4 m long (8 m^2) and are situated on a slight grade oriented parallel to the slope. The total area for the upland plots was 168 m^2 (21 plots x 8m^2 plot size) or 1815 ft^2, or 0.04 ac. The soil amendments and lime were applied to the experimental plots in the fall and incorporated 3-4" into the soil. All plots were tilled, including control plots. After a period of approximately 1 week, a mixture of native prairie plant species, including warm-season grasses, forbs, and legumes, were sown by hand to lower 7.7 m of each plot. The delay in seeding is to allow time for ammonia from the manure to volatilize, which could kill seeds (Center for Urban Horticulture 2002). Approximately 6 lbs/ac of grasses and 7 lbs/ac of forbs was used. The individual species used and the seeding rate are listed in Table 3. Spring oats (50lbs/ac), which germinate in cool temperatures but die after the soil freezes, were used as a cover crop.

Table 3. Native prairie plant species used in seed mixture.

Species	Common name	Plant Type	Seeding rate
Andropogon scoparius	Little bluestem	grass	1.5 lbs/ ac
Sorghastrum nutans	Yellow Indian grass	grass	1.5 lbs/ ac
Chasmanthium latifolium	Broad-leaf wood-oat	grass	1.5 lbs/ ac
Elymus virginicus	Virginia wildrye	grass	1.5 lbs/ ac
Strophostyles leiosperma	slick-seed wildbean	legume	0.75 lbs/ ac
Aesclepias tuberosa	butterfly milkweed	forb	0.75 lbs/ ac
Aesclepias syriaca	Common milkweed	forb	0.75 lbs/ ac
Symphyotrichum novae-angliae	New England aster	forb	0.75 lbs/ ac
Rudbeckia hirta	Black-eyed-Susan	forb	0.75 lbs/ ac
Dalea purpurea	Purple Prairie Clover	legume	0.75 lbs/ ac
Coreopsis lanceolata	Lanceleaf coreopsis	forb	0.75 lbs/ ac
Penstemon digitalis	Foxglove Penstemon	forb	0.75 lbs/ ac
Echinacea pallida	Pale Coneflower	forb	0.75 lbs/ ac
Liatris pycnostachya	Thick-spike gayfeather	forb	0.75 lbs/ ac

Figure 2. Remediated Upland Clay Soils plot with Teledyne Isco Propack removed

3.2.3 Water Quality Analyses

Samples for each plot were collected from Teledyne Isco Propacks (2 gallon sample collection bags) placed immediately downgradient of an 18” sodded buffer. Run-off water was directed into the bags via a wooden weir (see Figure 2). The collected runoff was distributed into bottles for transportation to the laboratory. Metals sample bottles were preserved with nitric acid and were analyzed for total and dissolved iron, cadmium, copper, lead, nickel and zinc, as well as dissolved calcium and magnesium, which were used to calculate hardness. Nutrient samples were preserved with sulfuric acid and analyzed for total nitrogen (TN), total phosporus (TP), and ammonia. Run-off water samples for the high application rates were analyzed for a chronic-exposure Whole Effluent Toxicity (WET) test, and the replicates were combined in order to achieve the greater sample volume needed. Dilution rates of 12.5% and 50% were used to portray the likely run-off contribution into a larger creek and a small tributary within our restoration area.

WET test samples were collected from rain-water runoff. All other samples were collected using simulated rainfall. To simulate rainfall, municipal drinking water was purified using a reverse osmosis process and acidified using sulfuric acid to an approximate pH of 6.5. The resulting hardness and alkalinity of the synthetic rainwater were below laboratory detection limits (<0.66 mg/L hardness and <5 mg/L total alkalinity). Lead and zinc concentrations were 1.28 μg/L and 7.06 μg/L respectively, and the Cadmium concentration was below the detection limit of 0.10 μg/L.

Metals and nutrient runoff data were compared to the Missouri Aquatic Life Criteria (10 CSR 20-7). The water quality criteria used are listed in Table 4. Sample concentrations were also compared to ambient conditions in the receiving streams, Center Creek and Ben’s Branch.

Ambient concentrations were estimated by finding the average of data reported in the Missouri Water Quality Assessment Database for a 10 year period from January 1, 2005 to December 31, 2014. Ambient concentrations are listed in Table 5.

Table 4. Selected water quality criteria for ammonia and dissolved metals.

Contaminant	Criteria Value	Units	Source
Ammonia			
- Acute	10.1 - 46.8	mg/L	MO WQS[1]
- Chronic	2.2 - 5.9	mg/L	MO WQS[1]
Dissolved Zinc			
- Acute	65 - 255	µg/L	MO WQS[2]
- Chronic	65 - 225	µg/L	MO WQS[2]
Dissolved Lead			
- Acute	30 - 172	µg/L	MO WQS[2]
- Chronic	4 - 7	µg/L	MO WQS[2]
Dissolved Cadmium			
- Acute	2.4 – 11.6	µg/L	MO WQS[2]
- Chronic	0.2 – 0.5	µg/L	MO WQS[2]

1: WQS for Ammonia are dependent on water temperature and pH.

2: WQS for metals are dependent on hardness.

Table 5. Ambient nutrient and metal concentrations in Center Creek and Bens Branch, from samples collected during the period January 1, 2005 to December 31, 2014.

Analyte	Units	Center Creek			Bens Branch		
		Average	Max	Min	Average	Max	Min
Total Nitrogen	mg/L	2.58	4.2	1.35	No Data		
Total Phosphorus	mg/L	0.15	1.26	0.03			
Ammonia	mg/L	0.04	0.38	0.01			
Dissolved Lead	µg/L	4.9	103.7	0.17	2.03	10	0.5
Dissolved Zinc	µg/L	234.47	2260	2.01	2776.54	9230	572
Dissolved Cadmium	µg/L	1.95	13.35	0.06	11.73	61.2	0.12
Hardness	mg/L	180.53	240	74.1	503.82	750	290
pH		7.79	8.6	6.4	7.51	7.9	6.77

3.2.4 PPCP Analysis

PPCPs were evaluated for their potential for adverse ecological effects in the aquatic environment by evaluating upland runoff and wetland water sample results and terrestrial effects by evaluating water concentrations compared to aquatic literature values representing a Lowest Observed Adverse Effect Concentration (LOAEC) and a No Adverse Effects Concentration (NOAEC) where applicable.

To evaluate potential PPCP concentrations of the amendments and their potential contribution to the environment, samples of raw and composted BS, CM+, and PL+ organic amendments were collected and analyzed. Water samples were also collected from the wastewater treatment effluent, wetland tub water, and remediated soil plot runoff to analyze for PPCPs. Water samples were collected from 15 test plots including 10 wetland tubs and 5 runoff samples from the remediated upland soils. The runoff samples were composited from two to three separate plots due to insufficient volume for individual samples.

Samples were analyzed for 71 separate PPCP constituents by AXYS Analytical Services Ltd using AXYS Method MLA-075. The specific suite of PPCP constituents were chosen for analysis based on relatively high levels of persistence in the environment and/or high potential for toxicity as identified by Walters et al. (2010). PPCP analytical parameters are segregated into three “lists” based on their properties and extraction technique. The lists analyzed for this study include Acid Extraction in Positive Ionization, Tetracyclines in Positive Ionization, and Acid Extraction in Negative Ionization. All the constituents that were analyzed and detected with appropriate LOAEC or NOAECs are given in Appendix B. After extraction, samples are analyzed using a liquid chromatograph/mass spectrometer.

3.3 Runoff Water Quality Results

3.3.1 November 2014 Toxicity Testing

In November of 2014, shortly after installation of the remediated upland pilot plots, sufficient rainfall fell to be able to collect run-off samples. Unfortunately not all plots captured run-off in sufficient volume to analyze. The run-off from the high application rate plots of CM+, PL+, and BS were collected and sent to a contract lab for Whole Effluent Toxicity (WET) testing. WET tests assess the aggregate toxic effect to aquatic organisms from all pollutants contained in the run-off, and thus impart important information not only about analytes suspected to be potential problems, but also unknown contaminants as well. In these chronic WET tests, water fleas (*Ceriodaphnia dubia*) and fathead minnows (*Pimephales promelas*) were placed in a dilution of the run-off for 7 days. Growth and survival of larval minnows was monitored, as was the survival and reproduction of the water fleas. A dilution of 12.5% was used to mimic a restoration scenario in which 1/8 of the watershed of a stream would be restored.

The *Ceriodaphnia* survived 100% in all of the treatments as well as the internal control. The water fleas in the internal control produced an average of 20.5 young, and the experimental treatments all produced greater numbers (CM+ was the highest). This data indicates that the run-off from the test plots was more beneficial to the Ceriodaphnia than the water used as the control at the lab.

On average, the minnows survived 97.5% of the time within the lab control water, 97.5% of the time in the PL+ treatment, and survived 100% of the time in the BS and CM+ treatments. The mean dry weight of the minnows in the control at the end of the 7 days was 0.536 mg, and

all of the treatments produced a greater amount of fish biomass than the control. Again, this data indicates that the run-off from the test plots, at the 12.5% dilution, was more beneficial to the minnows than the water used as the control at the lab.

Since the first round of WET tests indicated run-off from our treatment was not likely to adversely impact water quality at the 12.5% dilution, and we had an incomplete set of data, we decided to repeat the WET test with simulated rain. On December 11, 2014, five composited samples were collected from the test plots. The treatments represented were BS High application rate, YF, bare-ground control, CM+ High application rate, and PL+ High application rate. This time a 50% dilution was used, which would simulate a worst-case scenario in which amendments would be applied to half of a small stream's watershed, and then subjected to a rainfall event before vegetation could be established.

The run-off from the lab control, BS High, bare-ground control, CM high, and YF treatments had no significant effect on the survival of the minnow larvae at the 50% concentration, while PL+ High did (77.5% survival rate). Significant reduction in growth of the minnows was observed in the PL+ High and bare ground control treatments. The reason for the reduced survival in the PL+ and bare ground controls are unclear. The dissolved oxygen in the poultry treatment was much lower than the other treatments, and may be the cause for the lower survival. However, this was not the case in the bare ground control. The highest growth came from the CM+ treatment, which produced 32% more biomass than the bare-ground control.

None of the treatments had a significant impact on the survival of the Ceriodaphnia, even with the 50% dilution rate. However, significant reductions in reproduction were observed in the PL+ and bare ground control.

3.3.2 December 2014 Samples

Following the initial November run-off event, subsequent rainfall events were of insufficient magnitude to capture adequate samples for water quality analysis. A simulated rainfall event was conducted in December in order to collect samples to analyze for metals and nutrients. Samples were collected from 18 of the 24 plots, including at least two replicate plots from each of the treatments.

Average TN concentrations ranged from 2.26 mg/L in the Control plot samples to 45.5 mg/L from the PL+ Low treatment plots. There were no significant differences in TN concentrations between application rates of any amendment mixtures. Average ammonia concentrations ranged from 0.34 in both the Control and CM Low plot samples to 13.4 mg/L from the PL+ High treatment plots. There were no significant differences in ammonia concentrations between application rates of any amendment mixtures. Average TP concentrations ranged from 0.47 mg/L of the Control samples to 56.2 mg/L of the PL+ High samples.

Average dissolved Pb, Zn and Cd concentrations in the PL+ High treatment (375.5 μg/L, 2320 μg/L, 48.5 μg/L respectively) were higher than all other treatments, while the BS High samples had the lowest average concentrations (4.84 μg/L, 31.9 μg/L, 0.58 μg/L respectively). Average dissolved Pb, Zn, and Cd concentrations in the control samples were lower than all other treatments except the BS High treatment.

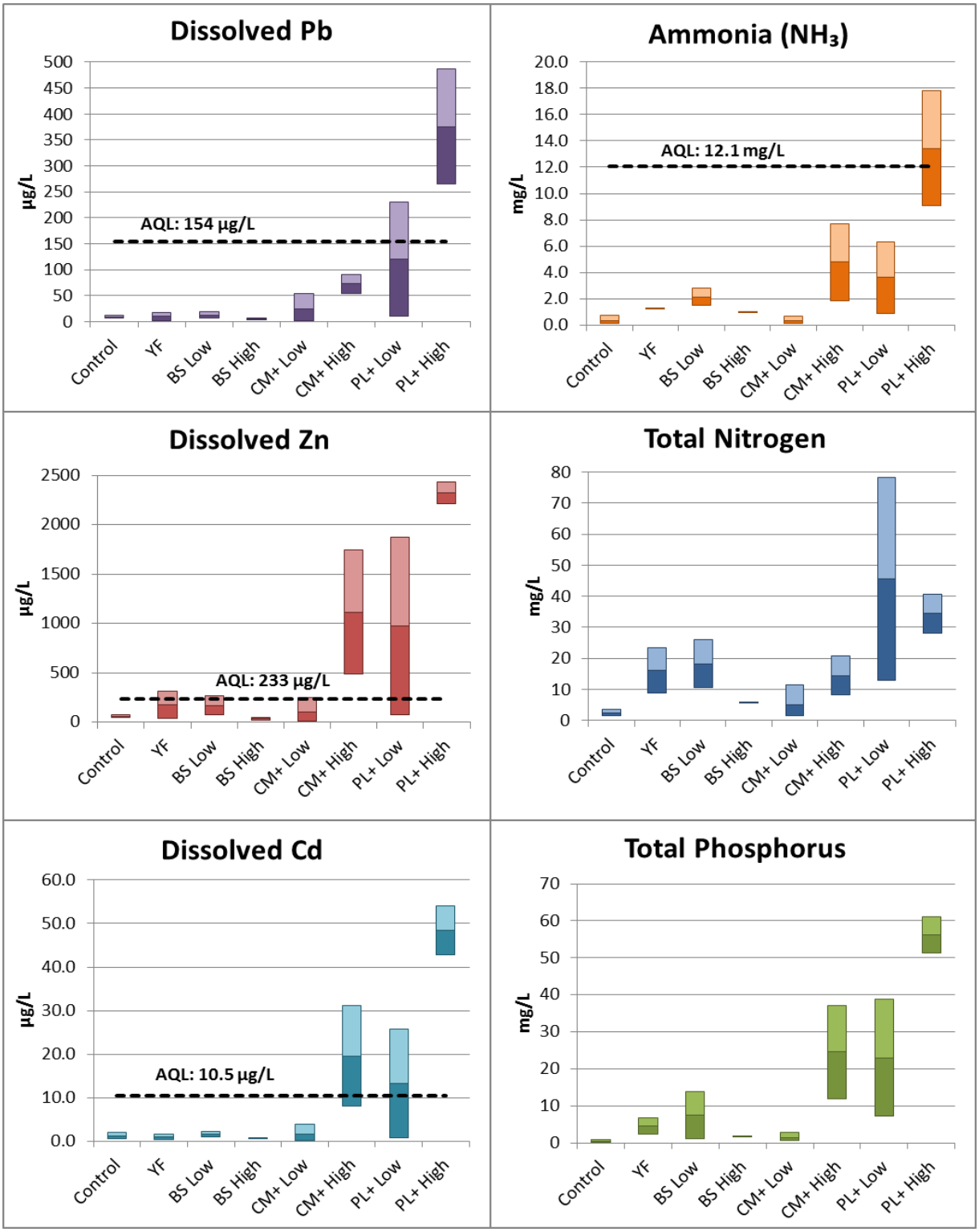

Figure 3. Dissolved metal and nutrient concentration ranges measured in runoff collected from experimental plots on December 12, 2014. Colored bars indicate maximum, minimum and mean concentrations reported. Chronic Aquatic Life Criteria (AQL), based on average parameters in Ben's Branch and Center Creek, are shown for comparison.

3.3.3 December 2014 PPCP Analysis

A total of 19 samples were analyzed for a suite of 71 PPCP analytes. Out of 1017 potential sample results for all treatments and analytical constituents, detection limits were exceeded 158 times. Reported values which did not exceed the detection limits were treated as non-detections, and are shown on graphs as 0 values. Detection limits were calculated by the laboratory separately for each sample, thus it is possible for a value reported for one sample as a non-detect to exceed a value reported for a different sample as being within the detection range. The laboratory produced detection limits for water samples less than 1 ppb, with the exception of 2 hydroxy-ibuprofen, bisphenol A, and minocycline, which ranged from 1.2 to 57 ppb. There were 47 different analytes that exceeded detection limits. The samples of the raw biosolids and wastewater treatment discharge contained the greatest number of analytes that exceeded the detection limits, 28 and 22, respectively. A description of all samples analyzed for PPCPs with their sample labels are presented in Appendix B.

The analyses and discussion in this report focus on those constituents found in raw or composted biosolids and manures, and those that exceed or approach an aquatic standard. Many of these contaminants have high soil adsorption coefficients (high Kd and/or Koc) values or relatively long degradation half- lives, which would tend to increase the persistence in ecosystems. A generalized exception to the characterization of high soil adsorption would be antibiotics that tend toward higher solubility. Analytes found in this study in raw municipal biosolids which are generally persistent in the environment are bisphenol A (1340 ppb), diphenhydramine (557 ppb), fluoxetine (298 ppb), ibubrofen (368 ppb), oflaxacin (4500 ppb), triclocarban (8480 ppb), and triclosan (22,300 ppb).

All PPCP concentrations which were measured above detection limits in raw organic materials, WWTP effluent, upland surface runoff samples, and wetland tub effluent samples are shown in Appendix B. The Raw Biosolids contained 28 detectable PPCP compounds while the Raw Cattle Manure contained 12 compounds and the Raw Poultry Litter contained only 1 detectable PPCP compound. Effluent from the WWTP contained detectable levels of 22 PPCP compounds. In total, 14 PPCP compounds were detected in upland surface runoff samples (see Table 6) and 18 PPCP compounds were detected in wetland tub effluent samples (see Table 15).

Most samples from the upland runoff experiment were well below (one order of magnitude) literature values indicating adverse effects to the aquatic ecosystem, with the exception of caffeine and triclosan found in the composite sample from the BS High plots. The caffeine concentration was 0.031 ug/l and triclosan concentration was 0.105 ug/l which approach the LOAECs of 0.05 ug/l (Bantle et al. 1994) and 0.15 ug/l (Wilson et al. 2003) respectively. The adverse effects determined by Bantle et al. (1994) were based on frog embryo malformations. The LOAEC determined by Wilson et al. (2003) for triclosan was based on alterations of algal assemblages in a stream, specifically a reduction of number of *Chlamydomonas* and increases in *Synedra* algae.

Table 6. Detectable PPCP concentrations in surface runoff samples collected from upland remediated plots treated with various organic soil amendments.

	Concentrations in micrograms per liter							
Constituent\Sampled media	Upland Biosolids High 1	Upland Cow/BS High 1	Upland Cow/BS Low 1	Upland Poultry/BS High 1	Upland Poultry/BS Low 1	Upland BS Low 1 = WetBS3N	Upland Cow/BS Low Dup= WetCBS3	LOAEC ug/L/Bio Endpoint
Caffeine	0.031							0.05/amphibian malformation
Diphenhydramine	0.00374							5.6 / behavioral
Erythromycin H2O		0.00477	0.00325	0.00321	0.0029			22700/shrimp morbidity
Fluoxetine	0.00291	0.00141						28/neurological/reproductive
Furosemide							0.0631	
Gembfiborzil	0.00835		0.00171	0.00799	0.00308	0.00773	0.00268	30400/crustacean
Miconazole			0.00572	0.0422				
Naproxen	0.00603						0.00407	
Sulfadimethoxine	0.0187		0.00351					
Sulfamerazine						0.0153		
Sufamethoxazole	0.0174							0.8/algal growth
Triclocarban	0.0127	0.0217		0.0256	0.00895	0.0183		0.2/reproduction
Triclosan	0.105							0.15 algae
Trimethroprim	0.00846		0.0112					bacterial inhibition

3.3.4 September 2015 Samples

Another simulated rainfall event was conducted in September 2015. Samples were collected from 20 out of 24 plots, although insufficient volume was collected from Plot 24 to analyze total nitrogen and total phosphorus. Only one sample was collected from the PL+ Low treatment, therefore statistical differences between the high and low PL+ treatments could not be calculated.

Average NH_3 concentrations ranged from 0.2 mg/L in the Control and CM Low plots to 0.53 mg/L in the Ag plots. Ammonia from the CM Low plots was significantly higher than from the CM High plots, and there was no difference between the BS application rates. Average TN ranged from 2.52 mg/L in the CM Low samples to 6.45 mg/L in the PL+ High samples. Average TP ranged from 0.56 mg/L in the Control plot samples to 3.91 mg/L in the PL Low samples. There were no significant differences in TN or TP concentrations between application rates among any of the treatments. The observed ranges of nutrient concentrations in surface runoff for each treatment are shown in Figure 4.

Average dissolved Pb concentrations ranged from 1.61 mg/L in the BS Low samples to 11.45 in the CM Low samples. Average dissolved Zn ranged from 25.9 mg/L in the BS Low samples to 89.1 mg/L in the CM High samples. In general, Cd concentrations showed a similar pattern among treatments to that of Zn, as seen in Figure 4. Average dissolved Cd concentrations ranged from 0.39 mg/L in the BS Low samples to 1.57 mg/L in the CM High treatment. There were no significant differences in Pb, Zn, or Cd concentrations between application rates among any of the treatments.

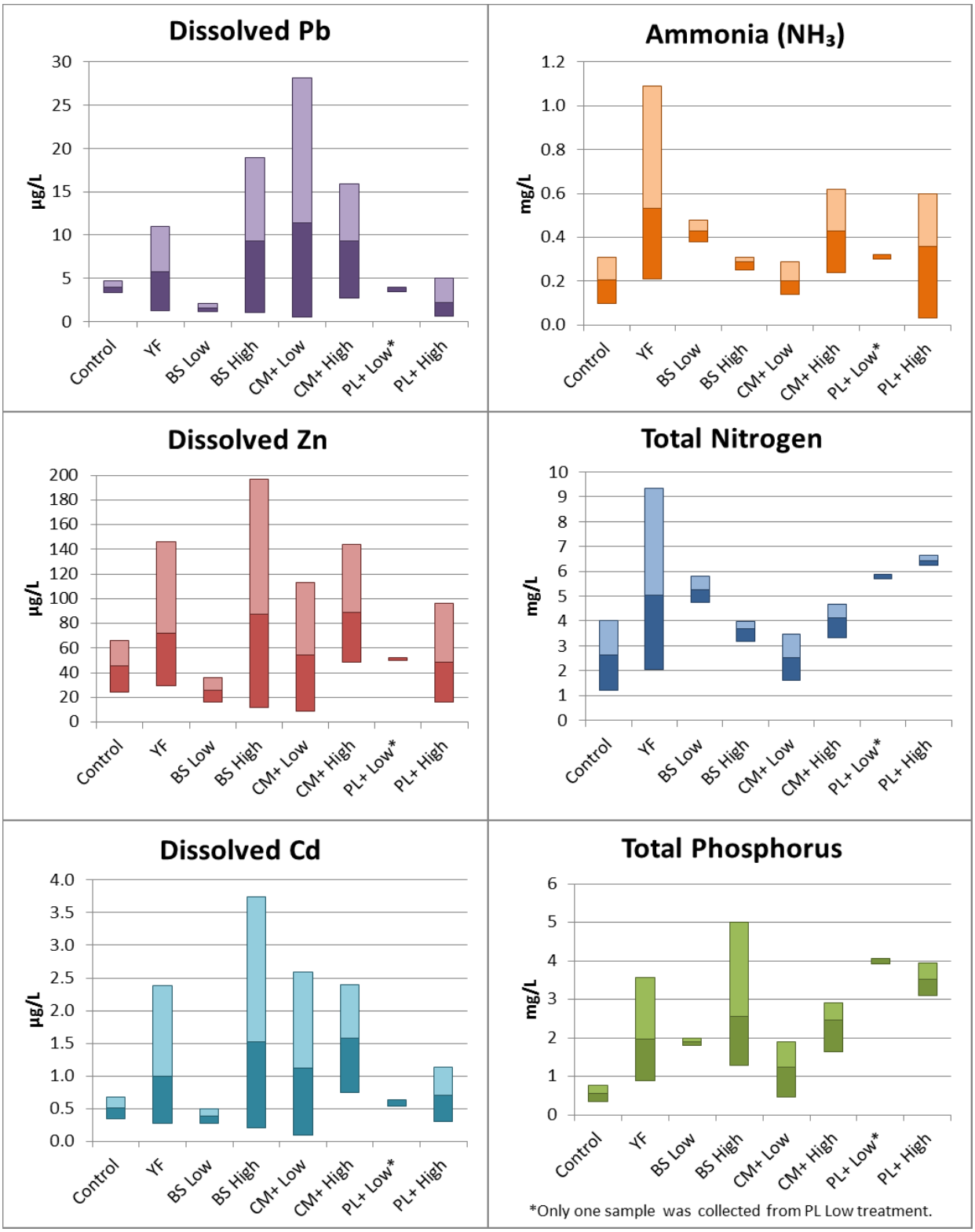

Figure 4. Dissolved metal and nutrient concentration ranges measured in runoff collected from experimental plots on September 9, 2015. Colored bars indicate maximum, minimum and mean concentrations reported. All sample concentrations are below AQLs.

3.4 Runoff Water Quality Discussion

All of the runoff samples collected from the Upland Remediated Soil plots contained concentrations of NH_3 lower than the WQS. Only one sample from a PL+ High treatment plot exceeded the Acute WQS for ammonia while four samples exceeded the Chronic WQS, including both of the PL+ High samples, one PL+ Low sample, and one CM+ High sample. All of the average concentrations, however, were greater than the average NH_3 concentration found in Center Creek, and most were above the maximum concentration as well. Only the Control and CM+ Low treatments had average NH_3 concentrations within the range measured in Center Creek.

For both sampling events, average TN concentrations from all of the experimental treatments exceeded the range of ambient values reported for Center Creek, while the average concentration in the control plot samples was below the average for Center Creek. Similarly, average TP concentrations from each of the experimental treatments during both sampling events exceeded the range of values for Center Creek during both sampling events, while the average control sample concentration was above the average Center Creek value but below the maximum.

Average dissolved Pb concentrations were below the WQS for all treatments except the PL+ High treatment. Both of the PL+ treatments exceeded the maximum Pb concentrations found in Center Creek and Ben's Branch. The Ag, Control, BS Low and both CM treatments exceeded the average Pb concentration of Center Creek but were below the maximum, while the average Pb concentration from the BS High treatment was below the average for Center Creek. Average concentrations from the Control, Ag, and BS High treatments were within the range of values measured in Ben's Branch as well.

Only the PL+ and the CM+ High treatments had average Zn values that exceeded any criteria or reference values. Average Zn concentrations from each of those treatments exceeded the WQS. Concentrations from the CM+ High and PL+ Low treatments exceeded the average concentration in Center Creek while the PL+ High treatment exceeded the maximum Zn concentration in Center Creek. Zinc concentrations in all the samples analyzed were below the average concentration in Ben's Branch.

While various water quality criteria and reference values were exceeded in some instances and PPCPs were detected in multiple samples, WET test results showed that runoff from the PL+ plots were the only treatment which was toxic to aquatic organisms. The CM+ and BS treatments did not show any measurable toxicity.

Temporally, the trends in runoff constituent concentrations over time were similar to results seen in the non-remediated plots. Concentrations declined over time for most treatments (Figure 5), with the exception of the BS High treatment. For the BS-High treatment, concentrations of Pb, Zn, Cd, and TP increased to varying degrees between the first and second sampling events, although the results were still within acceptable ranges and below the AQS and other reference values.

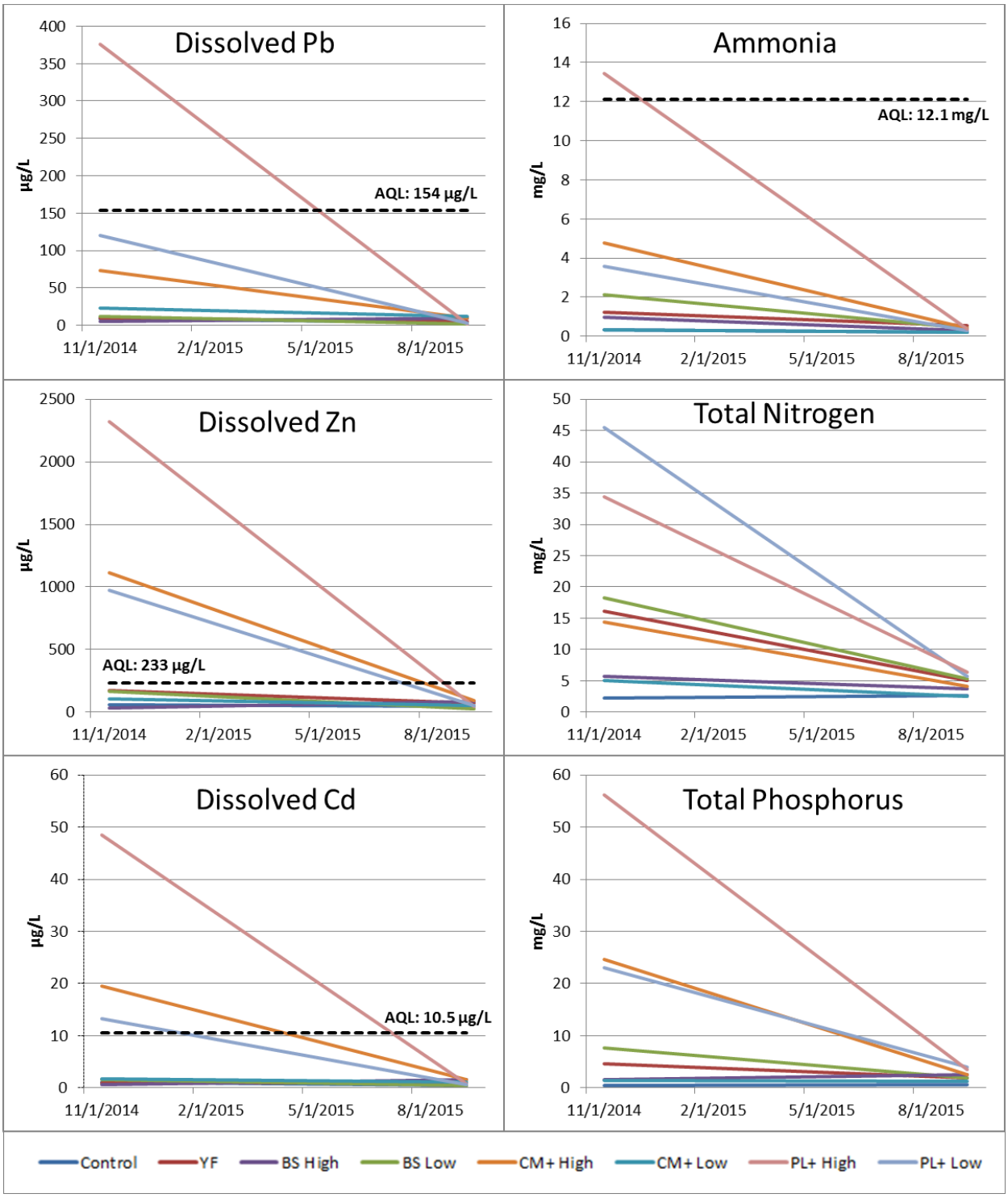

Figure 5. Temporal trends in dissolved metal and nutrient concentrations in runoff collected from compost treated plots. Chronic Aquatic Life Criteria (AQL), based on average parameters in Ben's Branch and Center Creek, are shown for comparison.

3.5 Plant Diversity and Metal Uptake Results

3.5.1 Metal Uptake

Ragweed shoot tissue was collected at the end of the growing season in 2015 to ascertain the uptake of metals within above-ground tissue. The soils within the root zones of the individual ragweed plants, as well as the plot averages, varied in their content of metals. The plant tissue metals concentrations were much lower across all treatments in the remediated soil plots as compared to the non-remediated soil plots. The average lead, zinc, and cadmium within plants on the remediated plots was 8.5, 396, and 12.5 ppm, compared to 18.5, 1905, and 41.8 ppm on the non-remediated soil plots. The average pre-amendment lead, zinc, and cadmium in soils were 597.7, 3100, and 50.4 ppm within the remediated plots, while the average lead, zinc, and cadmium in soils on the non-remediated plots was 835.7, 6111, and 50.9ppm.

To account for the difference in soil metal concentrations between plots and their potential impact on plant uptake, a bioaccumulation factor (BAF), expressed as the percent uptake, was calculated by dividing the metals within the plant stem by the average (3 XRF readings taken from the plot before amendment, averaged with the reading at the root zone) of metals present in the soil. This is the same methodology used for calculating plant uptake on the non-remediated areas. Using these similar methods for computing uptake, plants within the remediated soils had identical uptake of lead as the non-remediated plots (2%), while their uptake of zinc and cadmium were significantly ($P < 0.05$) lower (12% vs 29%) and (32 % vs 83%), respectively. Since the treatments are different in the remediated soils from the non-remediated soils, these are not identical datasets, but show a general trend of greater treatment in the remediated soils.

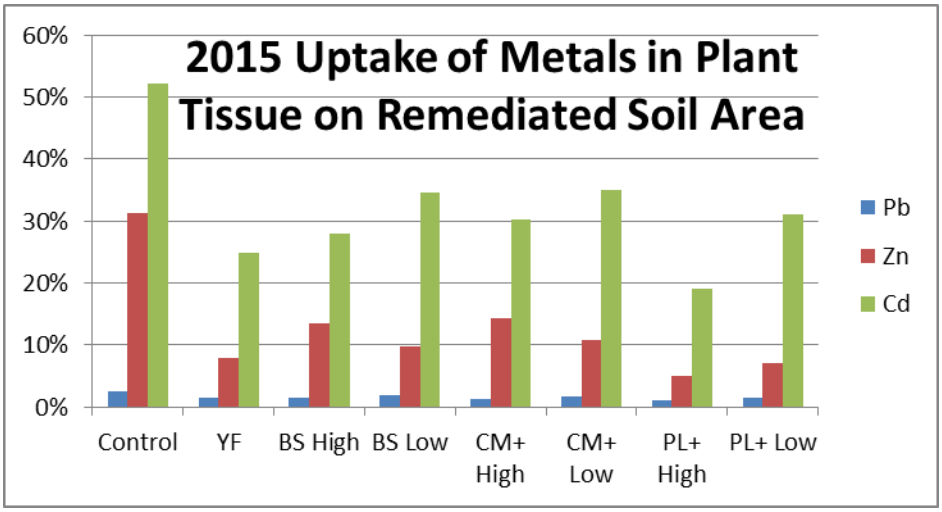

Figure 6. Plant uptake of metals using average of pre-amendment soils and rootzone soil readings.

Table 7. Soil metals (ppm) before and after incorporating amendments, and plant tissue metals (ppm).

Treatment	Pre-amendment Soil			Post-amendment soil			Plant tissue		
	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn	Cd
Control	491	2578.5	39.5	473.3	2373.5	12.9	10.3	760	13.9
YF	676	3688.3	56.7	314.1	1977.3	10.7	8.1	256	12.2
BS High	792	2919	54.7	120.6	837.8	5.0	9.4	422	12.3
BS Low	496.7	2626	39.3	77.1	580.5	4.7	7.1	212	11.8
CM+ High	759	4046.3	58.7	159.7	893.0	6.5	8.5	477	14.2
CM+ Low	504.7	2567.7	46.0	98.7	722.5	6.0	6.1	218	12.4
PL+ High	534	3634	68.0	403.4	2705	15.5	11.9	657	13.4
PL+ Low	528.3	2738.3	40.7	144.3	1068.1	10.4	6.5	166	9.8

3.5.2 Plant Diversity

Average plant species richness and diversity observed for each treatment in the remediated soils are shown in Figure 7. Treatment rates were not lumped for this part of the study because there were significant differences between rates in the seeded richness metrics. Of the soil amendment treatments, only the manure high and biosolids low grew a significantly greater richness of seeded species and a significantly higher diversity of seeded species than the control (p=0.05) for the 2015 season. The manure treatments were the only treatments to have significantly higher overall diversity than the control plots; the poultry high plot was significantly lower.

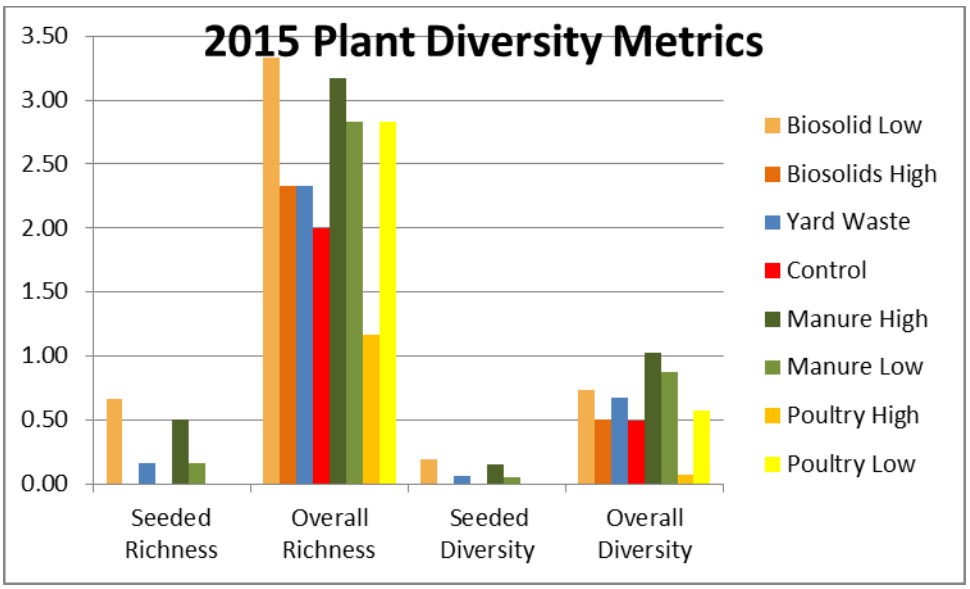

Figure 7. Plant diversity metrics for remediated soil plots, measured in September 2015. "Overall" refers to all plants identified, including species that grew from the existing soil seedbank as well as the species seeded.

3.6 Plant Diversity and Metal Uptake Discussion

3.6.1 Metal Uptake

The uptake of metals into plant tissue depends on the measurement of soils used, as this is the denominator for the calculation. When only readings from under the plant at the time of harvest are used, the uptake percentage is relatively high because the soil levels are already low from being "diluted" by the addition of soil amendments. In fact, soil metals measured after amendment application, on average, were only around 27% of the pre-amendment levels .Using the initial soil metals as the denominator for uptake best illustrates the whole treatment process envisioned for the restoration of remedial soils, and the uptake percentages are dramatically lower when this is considered.

Table 8. Metal uptake rates derived from ragweed plant tissue samples. Uptake rates were calculated using soil metal data collected prior to adding soil amendments.

Treatment	Pre-amendment Soil			Plant tissue			Uptake		
	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn	Cd
Control	491.0	2578.5	39.5	10.3	759.7	13.9	2.1%	29.5%	35.2%
YF	676.0	3688.3	56.7	8.1	255.6	12.2	1.2%	6.9%	21.5%
BS High	792.0	2919.0	54.7	9.4	422.1	12.3	1.2%	14.5%	22.5%
BS Low	496.7	2626.0	39.3	7.1	211.9	11.8	1.4%	8.1%	30.1%
CM+ High	759.0	4046.3	58.7	8.5	477.4	14.2	1.1%	11.8%	24.1%
CM+ Low	504.7	2567.7	46.0	6.1	217.9	12.4	1.2%	8.5%	27.0%
PL+ High	534.0	3634.0	68.0	11.9	657.0	13.4	2.2%	18.1%	19.7%
PL+ Low	528.3	2738.3	40.7	6.5	166.1	9.8	1.2%	6.1%	24.0%

The uptake of metals by plants is higher in the Control than the treatments (using initial soil metals as the denominator), which suggests that some treatment is taking place (see Table 9).

To determine if treatment (reduction) of plant metals uptake is occurring beyond simple dilution of the soil, we compared the plant tissue to the XRF scans of the root zone soil after the amendments had already been added. The plants should take up metals equally if there is no treatment effect from the soil amendments (see Table 9). Treatment of zinc is demonstrated as the majority of treatments show a reduction of metal uptake over the control. The uptake rates of lead in the various treatments are similar to the uptake of lead within the control, and the plant tissue metals are statistically similar for many of the treatments. The cadmium content of the soils and the cadmium content of the plant tissue treatments are statistically similar, and therefore it cannot be conclusively determined that the soil amendments are impacting treatment of cadmium.

Table 9. Plant tissue uptake of metals calculated using soil metal concentrations measured in soils within the root zone of the sampled plants.

Treatment	Soil from plant rootzone						Plant tissue						Uptake		
	Pb	Pb error	Zn	Zn error	Cd	Cd error	Pb	Pb error	Zn	Zn error	Cd	Cd error	Pb	Zn	Cd
Control	410.8	3.1	2348	10.8	13.6	3.8	10.3	1.0	760	5.3	13.9	4	3%	32%	102%
YF	322.3	2.4	2190	9.7	14.3	3.5	8.1	0.9	256	2.9	12.2	4	3%	12%	85%
BS High	315.6	2.5	1633	8.0	14.1	3.5	9.4	0.9	421	3.7	12.3	4	3%	26%	87%
BS Low	882.2	4.1	1076	6.1	5.3	3.9	7.1	0.9	212	2.7	11.8	4	1%	20%	223%
CM+ High	144.4	1.8	1130	6.3	7.8	3.7	8.5	0.9	477	4.0	14.2	4	6%	42%	182%
CM+ Low	188.6	2.0	998	6.3	7.6	3.8	6.1	0.9	218	2.8	12.4	4	3%	22%	163%
PL+ High	725.7	4.3	4514	18.1	29.1	3.7	11.9	1.0	657	4.3	13.4	4	2%	15%	46%
PL+ Low	176.1	1.9	1407	7.0	8.5	3.5	6.5	0.9	166	2.3	9.8	4	4%	12%	115%

The average lead concentrations in vegetation from unglaciated prairie and oak-hickory types within Missouri, according to EPA's Jasper County RI (EPA 2004), range from 28 to 30

ppm overall. Zinc levels for oak-hickory vegetation in Missouri ranges from 25-860 ppm, and cadmium plant tissue levels for these areas range from 2.8- 4.2 ppm according to the EPA RI. Other sources, such as the Jasper County Baseline Ecological Risk Assessment (Black and Veatch, 1998), have seemingly lower plant tissue metals concentrations in areas “distant” from mining (but within Designated Areas): 1.4 ppm cadmium, 3.6 ppm lead, and 173.2 ppm zinc (values are adjusted from wet weight). The lead and zinc within the plants from our study fall roughly within these background numbers, and thus our study is not causing any adverse environmental impacts beyond the existing conditions. The cadmium levels within the plant tissue, however, are higher than the reported backgrounds within the EPA documents, and therefore bear more toxicological scrutiny.

In 2013, Ford and Beyer published a study on wildlife exposure screening criteria from soil metals toxicity, using a variety of wildlife species as possible receptors. Of the species analyzed, the mourning dove was the most sensitive to Cd, having a soil screening limit of 9 ppm. The initial soils in our test plots were extremely high in Cd, having an average of 49 ppm, which is above EPA’s action level for remediation of 38ppm. Thus none of the soils would be suitable for the mourning dove habitat if no restoration were attempted. After adding amendments, the soil cadmium levels were much lower, averaging 9 ppm. Cadmium in the control plots (which had no soil amendments) however, was also greatly reduced (67% reduction, Table 10). A second set of samples from the root zone of the ragweed generally confirmed the lower numbers of cadmium in the soil, so it was assumed these lower numbers are accurate (note that the lead and zinc in the pre- and post- amendment control plots were relatively consistent). It is unknown whether the differences in Cd values between the control plot readings were due to an effect associated with tilling the control plots, or simply reflect a

difference in the instruments used to collect the various sets of readings. Unfortunately, because of the detection errors associated with such low levels of cadmium we are not statistically certain that there is a difference in the uptake of cadmium between the treatments.

Table 10. Metal concentrations measured in experimental soil plots before and after incorporating soil amendments, and post-amendment concentrations scaled proportionally to the Jasper County Remedial Action Objectives (RAO) or highest encountered zinc levels.

Treatment	Pre-amendment Soil			Post-amendment soil			RAO-adjusted post amendment soil		
	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn	Cd
Control	491	2578.5	39.5	473.3	2374	12.9	426.3	1359.1	12.6
YF	676	3688.3	56.7	314.1	1977	10.7	170.8	757.9	6.8
BS High	792	2919	54.7	120.6	837.8	5.0	47.1	445.8	3.6
BS Low	496.7	2626	39.3	77.1	580.5	4.7	67.6	333.3	4.5
CM+ High	759	4046.3	58.7	159.7	893.0	6.5	88.7	371.8	4.2
CM+ Low	504.7	2567.7	46.0	98.7	722.5	6.0	73.7	430.1	4.9
PL+ High	534	3634	68.0	403.4	2705	15.5	450.0	1571.9	17.0
PL+ Low	528.3	2738.3	40.7	144.3	1068.1	10.4	107.4	605.0	11.1
Mourning Dove		soil screening levels from Beyer					133.0	634.0	9.0
Sheep		soil screening levels from Beyer					1146.0	992.0	23.0
Horse		soil screening levels from Beyer					142.0	1674.0	21.0

Again, using Ford and Beyer's (2013) soil screening levels as a guide, the soils with amendments pose less adverse effects to less species than the soils without any treatment. Pre-amendment levels of soil zinc would adversely affect all 12 of the wildlife species assessed. Post amendment, there are adverse effects only to the mourning dove and sheep. The mourning dove would be adversely impacted by all of the soil treatments except biosolids low; the sheep would be adversely impacted by the control, YF, and PL+ treatments.

On average, the pre-amendment levels of lead would adversely affect 5 of the 12 animal species analyzed, while the average post-treatment soils would adversely impact 2 of the 12.

Again, the most sensitive species to lead is the mourning dove, which would show impaired health in all post- amendment treatments except the manure low and the two biosolids treatments.

As mentioned before, the levels of lead and cadmium within this pilot study are higher than the EPA's remedial action objective (RAO) levels, and the zinc levels are elevated, and thus the levels within our remediated plots are more contaminated than the conditions likely to be encountered in the restoration areas. Since our remediated plots are biased high, we can remove this bias by using the RAOs as a starting point, or pre-amendment soil, and then apply the treatment effect that was observed in our remediated plots. Mathematically, the RAOs are multiplied by the percent reduction (treatment) between pre-amendment soils and post amendment soils. Since the zinc RAO of 6400 is very high, and rarely encountered, the highest site zinc level encountered in the field (1500ppm) was used.

After adjusting the treatments for the more likely encountered soil metal concentrations, there would be no adverse impact to any species analyzed in Ford and Beyer's study for the biosolids and manure treatments, regardless of the metal. There would still be some adverse impacts to doves and horses if the YF treatment were used. The metals within the soil of the poultry treatments and control would negatively impact 3 or more species.

Metals toxicity can vary with target species, and it also varies with environmental conditions. For instance, quail and chickens can tolerate 200 mg/kg lead in their diet when their calcium intake is high, but only 1 mg/kg lead when their calcium intake is low (figures doubled for lead availability in biosolids as opposed to lead acetate, NRC 2005). In ruminants, 250 mg/kg lead in the diet can be tolerated for several months without significant effects on performance, while pigs show decreased growth with a 25 mg Pb/kg diet from lead acetate. Based on the plant

tissue information from our pilot study, it seems there would be minimal adverse effects from incidental ingestion of lead, particularly if there is adequate intake of calcium.

Within the *Mineral Tolerance of Animals 2nd ed*. (NRC 2005), a study very similar to ours reported "Ruminants including cattle, sheep, and goats have been fed crops grown in high-cadmium soils or diets supplemented with sewage sludge resulting in dietary cadmium levels of up to 13 mg/kg dry matter. In these studies, decreased performance was not reported and in some cases performance increased" (Combs et al. 1983). Indications of pathology in the kidney or other tissues occur at 5 mg/kg diet, and decreased growth rates occur at about 30 mg/kg diet in rats and mice. Chickens, ducks, and quail fed diets containing 10 mg/kg cadmium do not decrease gain or cause anemia, while mallards fed a 20 mg/kg diet did result in anemia. Cadmium uptake by plants has been shown in numerous studies to decrease with increasing pH (Efroymson et al. 2001). Interestingly, cadmium toxicity can be markedly (>80 percent) reduced by high levels of several minerals, especially calcium, phosphate, iron, and most interestingly, zinc (Groten et al., 1991). Cadmium becomes less active by increasing soil organic matter or cation exchange capacity (Munshower 1986). Since the plant tissue levels of cadmium within our study average around 12 ppm, there would be little risk from incidental ingestion of cadmium.

Zinc is relatively nontoxic to birds and mammals (NRC 2005). Rats, pigs, poultry, sheep, and cattle are tolerant to high intakes of zinc. Pigs can tolerate zinc at levels of 1,000 mg/kg of diet for many weeks, but 2,000 mg/kg diet resulted in signs of toxicosis. The maximum tolerable concentration of zinc for cattle and poultry is 500 mg/kg; for sheep, the maximum tolerable level is set at 300 mg/kg of diet. (NRC 2005) The plants within the high application treatments of biosolids, manure, and poultry litter do exceed 300ppm zinc. However, this is likely mitigated in the field by consumption of plants that are not hyperaccumulators of metals.

Common ragweed, *Ambrosia artemisiifolia*, is known to be a hyperaccumulator of metals (Pichtel et al. 2000) that has been used historically to prospect for metals (Robinson et al 1947). In their remedial investigation for Jasper County, EPA demonstrated similar results with a plant of the same genus, *Ambrosia psilostachya*. They reported that it is an "indicator and accumulator" and concentrations of lead in this plant were "a worst case scenario". Common ragweed is likely a maximal condition for the uptake of metals, as the stem tissue of grasses within the same plots as ragweed only accumulated 26% of the zinc and 33% of the cadmium (lead accumulated in grasses in roughly the same amount as ragweed).

The levels of zinc and lead in the plants within our treatment plots did not exceed background levels for this area. Cadmium levels within plant tissue did exceed background, but do not seem to pose a risk based on literature toxicological values except for the PL+ treatments, with respect to mourning doves. The study site's soils were inordinately high in metals. However, if adjusted for the amount of metals that are normally encountered in the field, the risk is largely removed. By adding in soil amendments, the soil metals were diluted within the topsoil, and in some cases showed reduced bioavailability as well. In general, the biosolids and manure treatments showed the least amount of risk associated with exposure to soil and ingestion of plants. Additional risk evaluation to migratory birds is performed using U.S. EPA's ecological risk assessment methodology and is reported in Section 6 of this report.

3.6.2 Plant Diversity

Compared to the non-remediated soil field plots, detailed in Appendix A, conditions for establishing vegetation in the remediated upland clay soil field plots were closer to an ideal situation. The soil metals were reduced, the compost had more time to break down, and the plots

were seeded in the fall, allowing for the cold stratification needed to stimulate germination. The site preparation, however, was inadequate to completely kill some of the existing grass and weeds in the area that was to become our plots. Competition is suspected to have contributed to the reduced diversity in our plots.

Phytotoxicity research by USGS indicates that metals concentrations are more critical determinants of floristic quality than are other soils factors, and that Zn and Pb concentrations negatively affect floristic quality (Struckhoff et. al. 2013). Species that are least tolerant of disturbance are most negatively affected by increasing metals content in the soils. It was surprising that the richness of the seeded species in our remediated plots (max= 0.67) was less than in the first year of our non-remediated plots (max= 3.0), or the second year of the non-remediated plots (max= 1.83). The species used in our remediated plots, however, did not include the most successful species of our non-remediated plots (because they had already proven to grow in a worst-case scenario), and therefore the two scenarios are not directly comparable. The two species excluded (vervain, *Verbena hastata* and partridge pea, *Chamaecrista fasciculate*) are able to grow quickly and complete their lifecycle in one year.

Figure 8. Annual ragweed growing in a remediated soil experimental plot treated with poultry litter compost (PL+) in the first growing season.

As noted in the non-remediated plots, annual ragweed was a vigorous competitor early in the season, completely blanketing some of the plots. The plots were mowed twice during the first season, but the mowing might have come too late to prevent the native seedlings from being shaded out initially (See Figure 8). At the end of the season, ragweed made up 14% of the total species inventoried. Other weedy species such as annual lespedeza, fescue, and crabgrass had equal or greater presence in the end of the year inventory. Certain prairie species can take years to establish, and prairie reconstruction undergoes developmental stages that can take decades (Smith et. al. 2010). This short-duration study cannot predict success decades in the future, it is hoped that the initial germination and survival of native species is indicative of future establishment success.

Initially, it was thought that the relative lack of success of the natives and the success of the weeds may lie in the difference in fertility between the two study areas. In a study by Blumenthal et al, 2003, they saw that nitrogen enrichment of soil hindered restoration of native tallgrass species and encouraged vigorous weed growth. The addition of carbon (in the form of sawdust) reversed this process. Data from our soil agronomy report contradicts this theory, as there is a greater carbon:nitrogen ratio in the remediated plots, which should be to the benefit of the natives. Though it is surprising that the native plants fared better in highly contaminated soil, this study was not designed to directly compare the remediated and non-remediated plots.

Ultimately, the goal of the pilot study was to evaluate different aspects of various soil amendment mixtures in order to learn more about the effects of applying these mixtures on remediated and non-remediated plots. Within the remediated plots, both the biosolids low and the manure high treatments grew a significantly greater richness and diversity of native plants. Given the good performance of the manure treatment in the non-remediated plots, it would seem that manure+biosolids or manure is the best amendment for any future restoration work.

4. Earthworm Exposure

4.1 Methods

Earthworms were exposed to upland remediated clay soils treated with the various soil amendments to analyze metals and PPCP uptake. The treatments used were the same amendments used in the upland remediated soils: Control, PL+ (low and high application rate), CM+ (low and high application rate), YF, and BS (low and high). Five-gallon buckets were filled with a 15 cm layer of sieved residual soil followed by a 5 cm soil/amendment mixture layer. The average Pb, Zn, and Cd concentrations in the 8 buckets of residual soil are listed in Table 11. Based on the surface size of the buckets, 1.1 pounds and 2.2 pounds of amendment were added to the buckets for low and high rates, respectively. Fifty-six worms were introduced to the buckets below the soil/amendment mixture layer and then sprayed with distilled water. The buckets were covered with window screen to prevent worms from escaping and placed in a climate controlled room with a light on at all times to maximize exposure to soil. Duplicate buckets were constructed as insurance against colony failure, but because there were no colony failures, only one bucket per treatment was sampled. Replicates were obtained by collecting duplicate subsamples of worms from the same bucket. Subsamples of worms were collected after 28 days of exposure, rinsed with distilled water and put in a ziploc bag for 24 hours to depurate. After depuration, the worms were freeze dried and analyzed using inductively coupled plasma-mass spectrometry (ICP-MS).

PPCP sample results were reported by AXYS Analytical Services laboratory as a wet weight concentration, and were then converted to dry weight concentrations for comparison to values and effects concentrations reported in other literature. These values were converted to dry

weight using average percent moisture data determined by USGS during metals analyses. The mean percent moisture by weight for worms was 86.7. Due to sample size limitations, no PPCP sample was collected from the CM+ Low treatment.

Table 11. Average soil metal concentrations in buckets of upland clay soil used to evaluate earthworm metal uptake.

Treatment	**Lead (Pb)**	**Zinc (Zn)**	**Cadmium (Cd)**
Control	188.4	2635.7	30.6
CM+ Low	179.3	2668.9	23.6
CM+ High	147.0	2162.6	25.8
BS Low	160.7	2222.4	21.1
BS High	184.8	2490.8	32.4
PL+ Low	144.1	2106.5	22.3
PL+ High	153.7	2310.6	29.7
YF	186.4	2414.1	27.7
Average	**168.1**	**2376.5**	**26.7**

4.2 Results

4.2.1 Heavy Metals

Earthworm tissue Cd, Pb, and Zn results are provided in Figures 9, through 11. A Tukey's test for significant difference was run on the earthworm tissue concentrations provided in Tables 12 through 14. The results for Pb indicate that worms in the Control soil had the highest average concentration (19.9 mg/kg), which was significantly higher than all other treatments except BS Low. The BS Low treatment was not significantly different from any of the other treatments. This demonstrates significant treatment effect for reduction of Pb uptake for all

amendments, except for the BS Low. The lowest Pb concentrations in earthworm tissues were observed in the two PL+ treatments followed by the CM+ High treatment.

The earthworm tissue results for Zn were similar to Pb in that the Control soil had the highest average concentration (423.7 mg/kg). The Control soil was not significantly higher than BS Low, BS High, or the YF treatments. Both CM+ and both PL+ treatments were similar to each other, but significantly lower than the Control soil. The CM+ High and PL+ Low treatments had the lowest mean tissue Zn followed by PL+ High and CM+ Low treatments. These results also indicate some treatment effect for all amendments, but significant treatment effects for Zn for both rates of PL+ and CM+ amendments.

Some of the soil Cd concentrations were below the quantification limit of the XRF. However, no more than one third of the readings in any treatment were below the detection limit. Values that incorporate concentrations below the quantification limit in the calculation of the mean are considered estimates. Non-detect values were estimates as half of the detection limit. The estimated values for Cd are for CM+ Low, BS Low, BS High, PL+ Low, and YF amendments.

The results for Cd uptake in worms did not follow a similar pattern as Pb and Zn. The only amendments that demonstrated any treatment effect were both rates of Cow Manure amendments. They were significantly different than any other treatments and similar to each other. No other treatments were significantly different from the Control soil. The CM+ Low amendment had the lowest average Cd concentration (4.3 mg/kg) in earthworm tissue, while the PL+ High treatment had the highest average Cd concentration 22.3 (mg/kg).

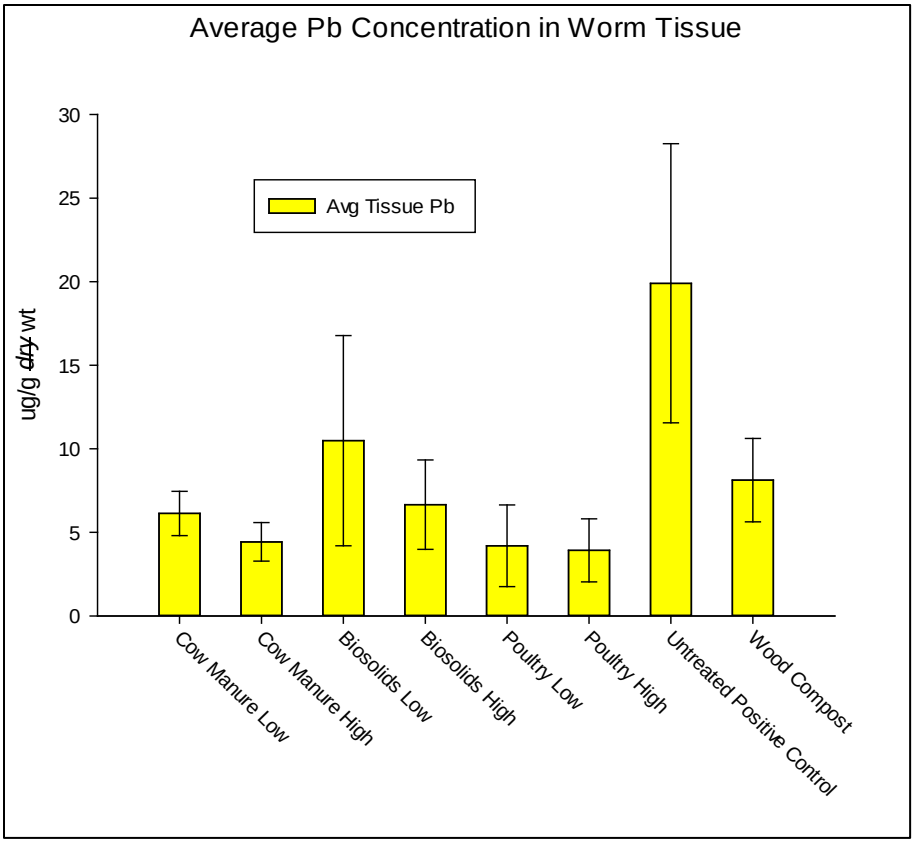

Figure 9. Average lead concentrations in worms following exposure to contaminated soils treated with various organic amendments.

Table 12. Average lead concentrations in worms following exposure to contaminated soils treated with various organic amendments.

	Avg Tissue Pb	n	Standard Deviation	Tukey's Test
Cow Manure Low	6.13	3	1.3262	b
Cow Manure High	4.43	3	1.151	b
Biosolids Low	10.4867	3	6.2919	a, b
Biosolids High	6.6533	3	2.6737	b
Poultry Low	4.1933	3	2.4437	b
Poultry High	3.9233	3	1.8864	b
Untreated Positive Control	19.9	3	8.354	a
Yard Waste & Fertilizer	8.12	3	2.4958	b

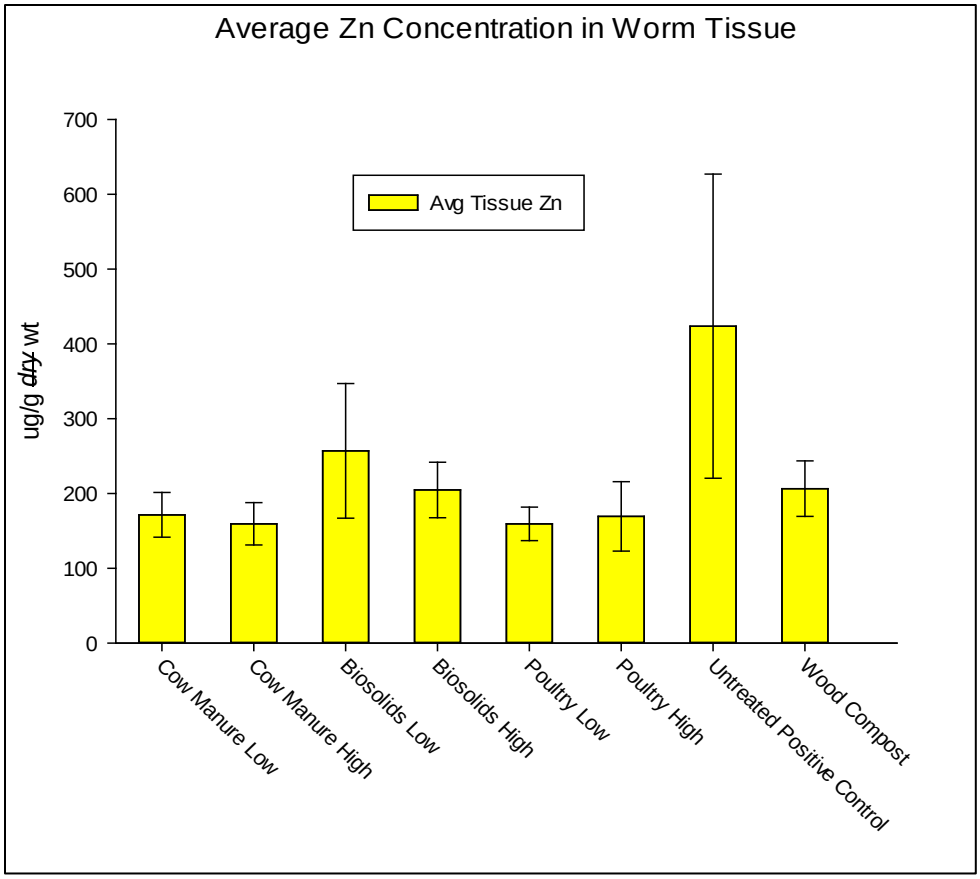

Figure 10. Average zinc concentrations in worms following exposure to contaminated soils treated with various organic amendments.

Table 13. Average zinc concentrations in worms following exposure to contaminated soils treated with various organic amendments.

	Avg Tissue Zn	n	Standard Deviation	Tukey's Test
Cow Manure Low	171.3333	3	29.8719	b
Cow Manure High	159.3333	3	28.3078	b
Biosolids Low	257	3	90.0722	a,b
Biosolids High	204.6667	3	37.072	a,b
Poultry Low	159.3333	3	22.3681	b
Poultry High	169.3333	3	46.3717	b
Untreated Positive Control	423.6667	3	203.3773	a
Wood Compost	206.3333	3	37.072	a,b

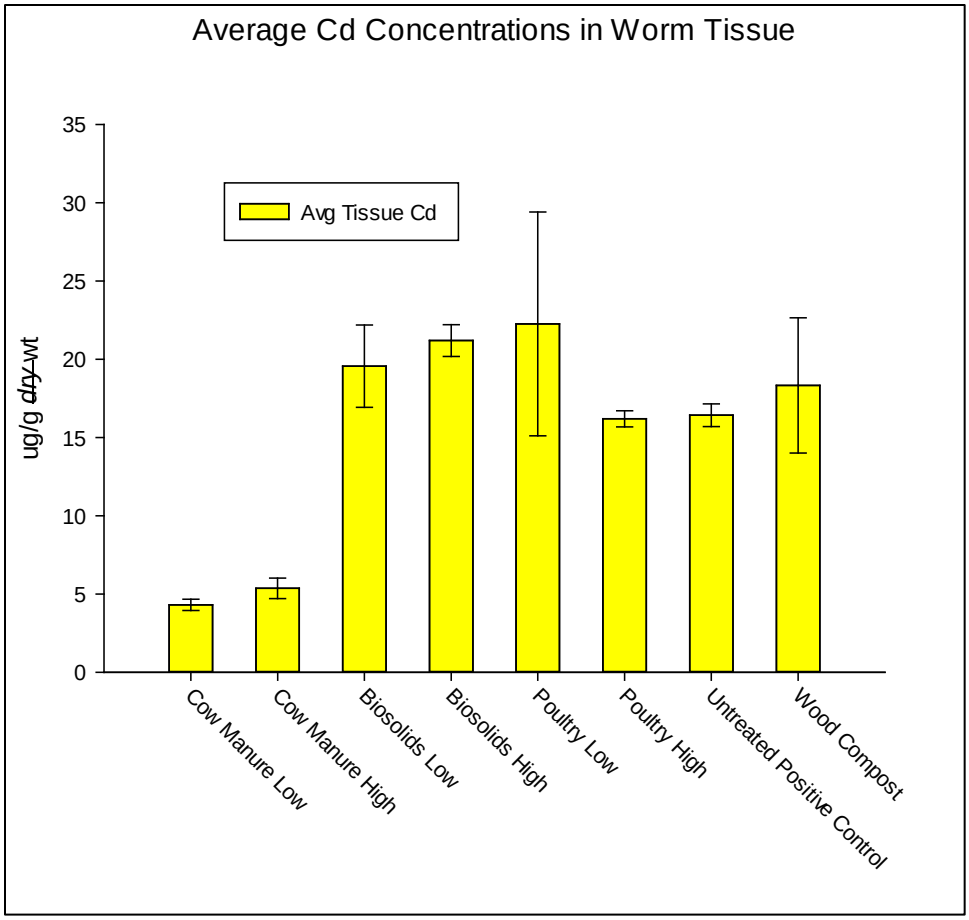

Figure 11. Average cadmium concentrations in worms following exposure to contaminated soils treated with various organic amendments.

Table 14. Average cadmium concentrations in worms following exposure to contaminated soils treated with various organic amendments.

	Avg Tissue Cd	n	Standard Deviation	Tukey's Test
Cow Manure Low	4.3067	3	0.3614	b
Cow Manure High	5.36	3	0.6578	b
Biosolids Low	19.5667	3	2.6312	a
Biosolids High	21.2	3	1.0149	a
Poultry Low	22.2667	3	7.1501	a
Poultry High	16.2	3	0.5196	a
Untreated Positive Control	16.4333	3	0.7234	a
Wood Compost	18.3333	3	4.3247	a

4.2.2 PPCP Results

PPCP concentrations in earthworms were above detection limits for ten constituents: bisphenol A, ofloxacin, oxolinic acid, erythromycin, fluoxetine, mycanozole, sulfamethazine, sulfathiazole, triclosan, triclocarban. All PPCPs detected in worm tissue samples are shown in Figures 12 through 15, or are reported in the text where appropriate.

PPCPs were evaluated for their potential for adverse terrestrial effects by evaluating worm tissue and/or amendment results compared to terrestrial literature values representing a Lowest Observed Adverse Effect Concentration (LOAEC) and a No Adverse Effects Concentration (NOAEC) where applicable. Effect levels could not be found for all constituents.

There were limited literature values available to evaluate potential terrestrial ecological or toxic effects of PPCPs. Most of the constituents were measured in concentrations less than one part per billion, which are not expected to have toxic effects. Figure 13 contains a comparison of the three antibiotics that were detected in worm tissue. Many of the samples were less than the detection limit. Very few of the antibiotics had terrestrial ecological risk data associated with them to use for comparisons.

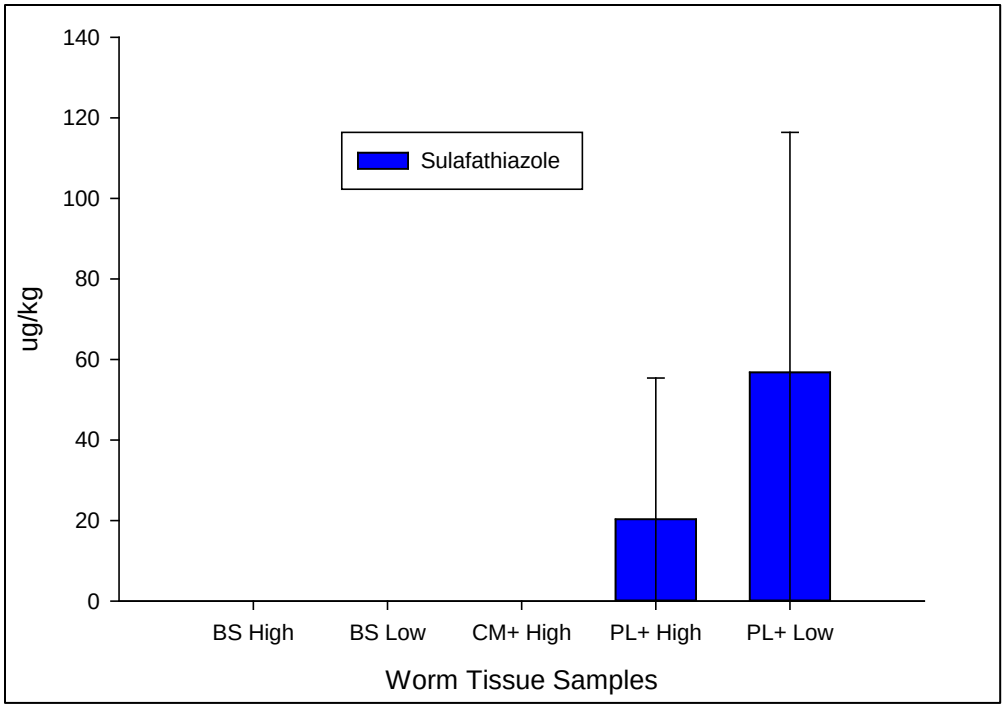

Figure 12. Sulfathiazole concentrations in earthworm tissue, per dry weight.

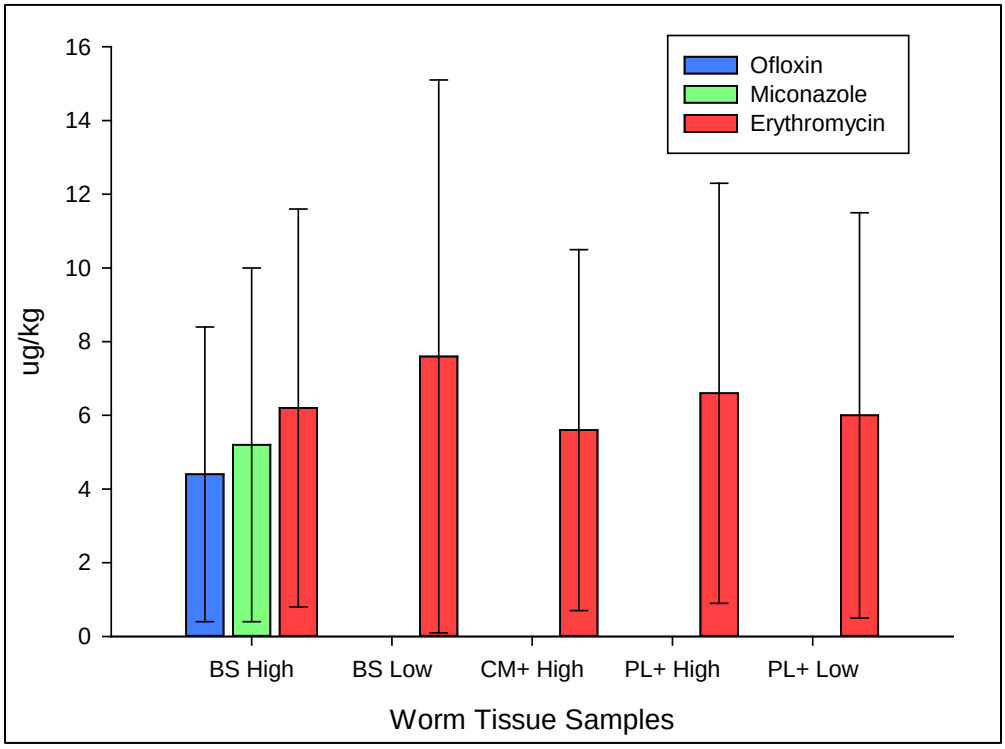

Figure 13. Antibiotic concentrations measured in the tissue, per dry weight, of worms raised in soils treated with organic soil amendments.

Fluoxetine is a frequently prescribed anti-depressant, more commonly known by its trade name Prozac. This contaminant was found in concentrations above detection limits in two worm tissue samples: BS High (Rep. 1, 3.68 µg/kg) and BS Low (Rep. 3, 4.1 µg/kg wet weight). The two worm concentrations of fluoxetine were almost an order of magnitude below concentrations (26.2 ug/kg worm tissue) that caused changes in starling feeding behavior according to Bean et al. (2014).

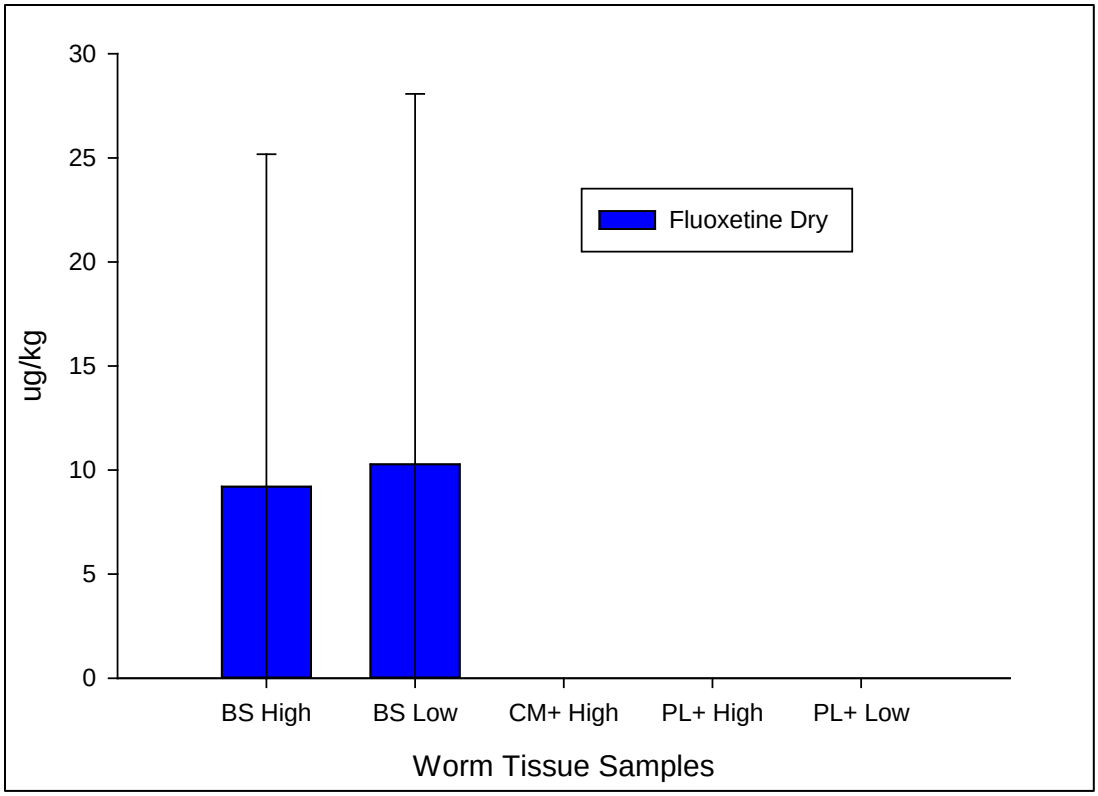

Figure 14. Fluoxetine concentrations in earthworm tissue, per dry weight.

The highest PPCP concentrations in worm tissues were the antimicrobials: triclosan and triclocarban. As expected the highest sample results for these constituents were in the biosolid samples as shown in Figure 15. However all of the concentrations were less than an order of magnitude of any adverse effects concentration for terrestrial receptors found in the literature. The only triclosan samples that were above detection limits were those associated with the BS treatments. All samples contained triclocarban above the detection limits except worms grown in the Control treatment. However, similar to triclosan, triclocarban concentrations in the biosolids treatments were at least one order of magnitude higher than other treatments containing animal wastes.

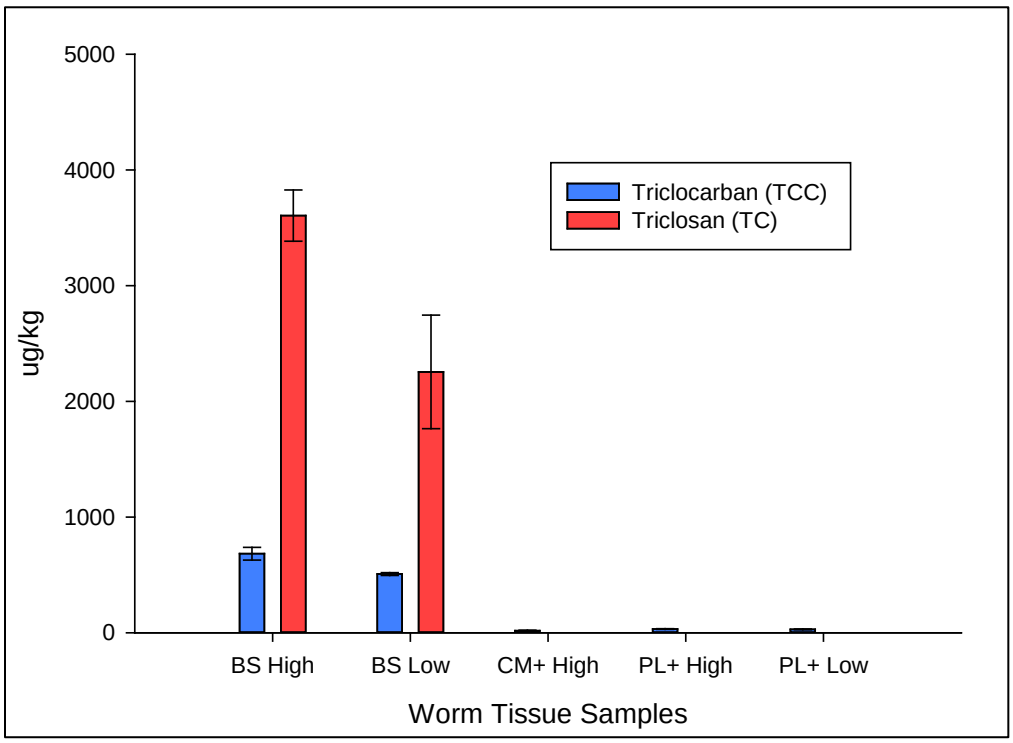

Figure 15. Concentrations of antimicrobial compounds in earthworm tissue, per dry weight.

Bisphenol A was found in all worm tissue samples and in the raw biosolids sample (at 1340 µg/kg), but not in any of the compost samples (Figure 19). Bisphenol A is a plasticizer that has been linked to a variety of detrimental ecological effects (Staples et al, 1998, Staples et al. 2002). The highest concentration was detected in the Control sample, which was almost double the next lowest sample concentration. The fact that Bisphenol A was not found in the compost in which the worms were grown; was relatively uniform in concentration other than the control; and was highest in the Control sample strongly suggests that the contaminant found in worms was derived from the plastic buckets in which the worms were grown and is an artifact of the experimental design. The ecological impacts of Bisphenol A were not evaluated due to the high concentrations found in the Control sample, and the absence of this compound in the organic amendments themselves.

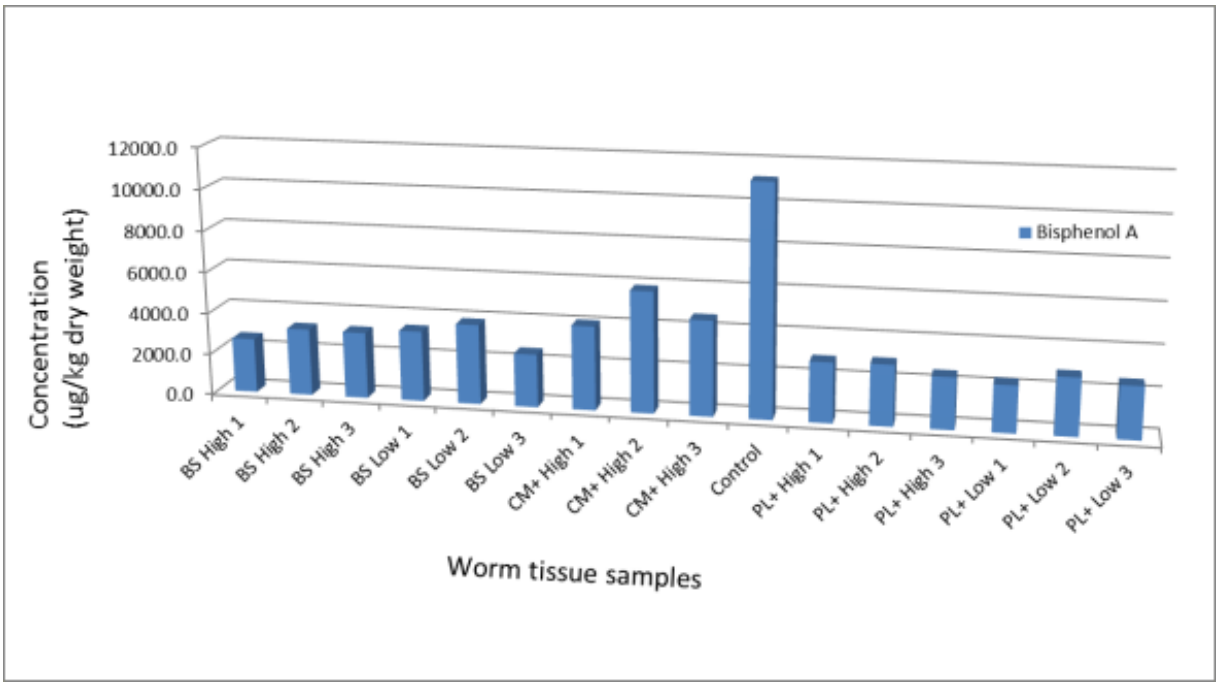

Figure 16. Concentrations of the plasticizer Bisphenol A in tissue from worms raised in soils treated with various organic amendments.

4.3 Discussion:

Bioaccumulation Factors (BAF) were calculated from mean earthworm metal concentrations as determined from ICP-MS and mean post-treatment soil concentration as determined by XRF. All soil Cd concentrations values, including those below the quantification limit were used in calculation of the mean concentrations used in the BAF values.

Consistent with literature values Pb had the lowest BAF and Cd had the highest compared across all treatments. The Control plots had the highest BAF for Pb and Zn, demonstrating a treatment effect for all amendments. However, comparing BAF for Zn across treatments is complicated by the ability of plants and most organisms to regulate Zn due to its role as an essential nutrient. All amendments had similar BAF for Pb and Zn, with the exception of the BS Low, which was appreciably higher than the other amendments. The PL+ Low amendment had the lowest BAF for Pb (0.024) and the CM+ Low amendment had the lowest BAF for Zn (0.060). See Figure 16 for Pb and Zn BAF.

Cd bioaccumulation factors, shown in Figure 17, were much more variable than those for Pb and Zn. Both CM+ amendments and the PL+ High amendment were lower than the other treatments and were appreciably lower than 1.0 (0.36 CM+ High to 0.65 PH). The BS High, PL+, and Ag Compost amendments were similar to control BAF. The BS Low amendment had significantly higher BAF than all other treatments.

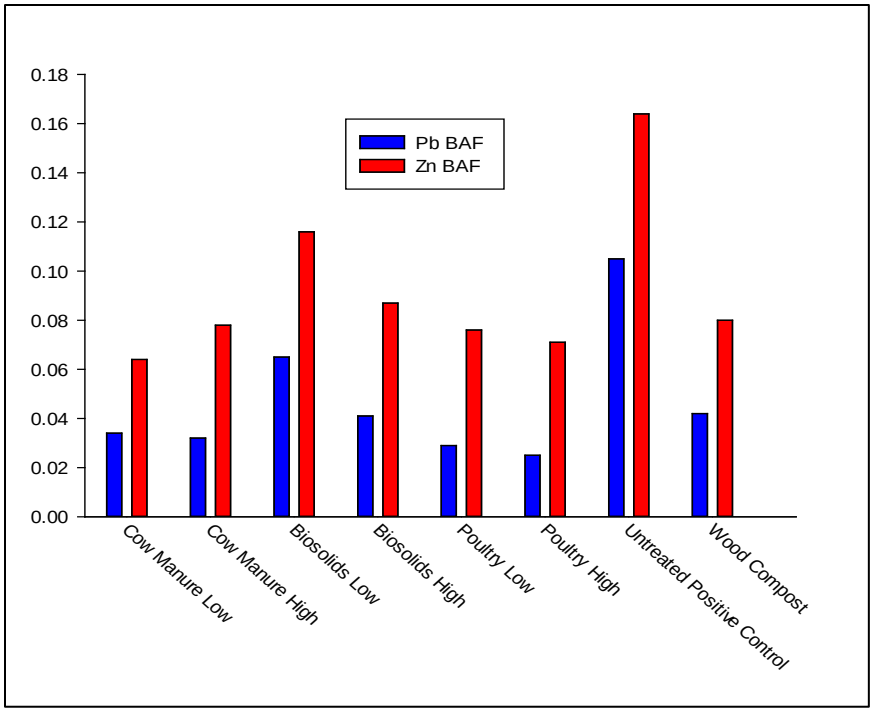

Figure 17. Lead and zinc bioaccumulation factors for worms following 28-day exposure to contaminated soils treated with various organic amendments.

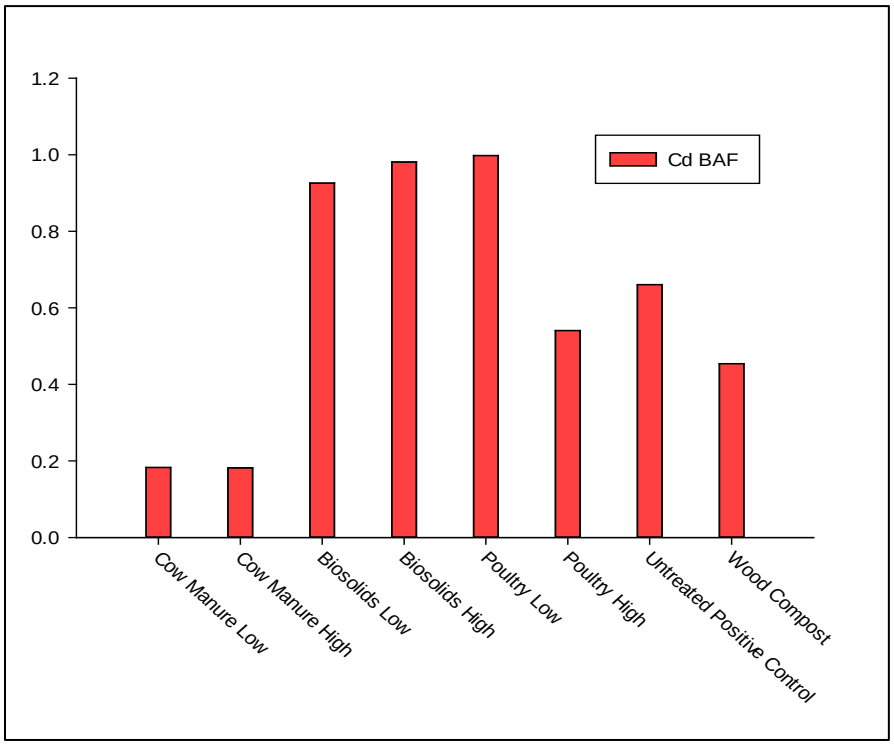

Figure 18. Cadmium bioaccumulation factors for worms following 28-day exposure to contaminated soils treated with various organic amendments.

Bioaccumulation factors (BAF) calculated for triclocarban from the mean dry weight values for worms tested in amended soils ranged from 3.47 to 1.94, which were generally well below those BAF values found in the literature. Snyder et al. (2011) measured BAF in *E. foetida* earthworms in fine sand and silty clay loam at 18+/- 3.5 and 20+/- 2.1, respectively. No PPCPs were measured in plant tissues in this study. Previous studies (Wu et al. 2010, and Snyder et al. 2010) of bioaccumulation in plants showed very low BAF (<0.03).

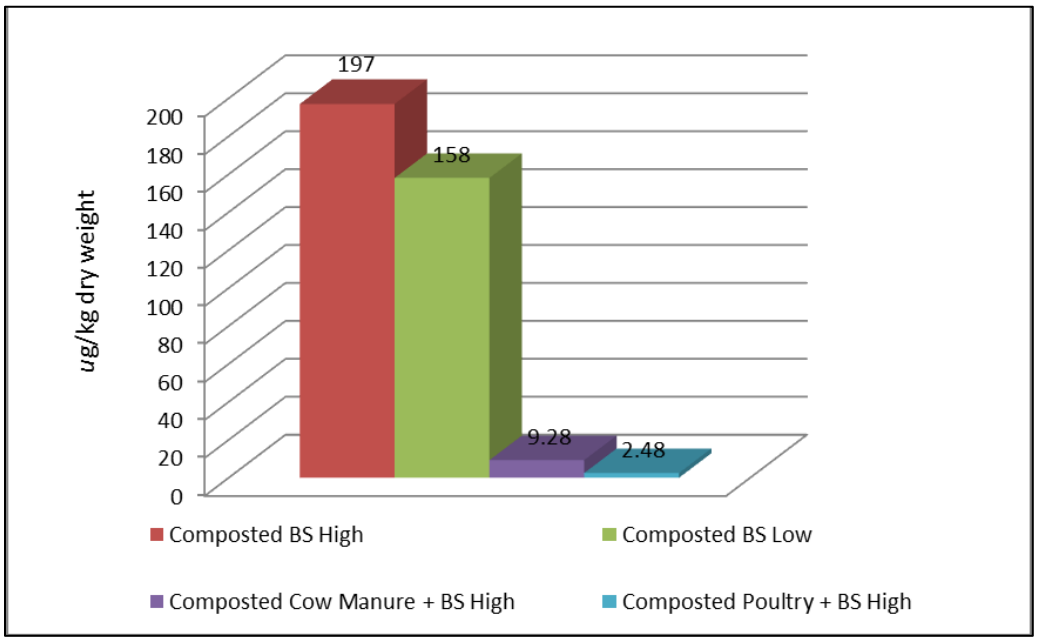

Figure 19. Triclocarban concentrations in worms measured in composted organic soil amendments.

A BAF of 26 for Triclosan was calculated for the BS High Rep. 3 sample, which was one of only two samples for which the results were above the quantification limit. This value is

more consistent with those found in the literature. Kinney et al. (2008) found earthworm BAFs from 10.8 to 27 in a soybean field amended with agronomic rates of biosolids.

The other antimicrobial constituent found above detection limits in worm tissue samples was sulfathiazole (Figure 12). Sulfathiazole was relatively elevated at three of six samples of the PL+ samples. The highest concentration was in one of the low application rate samples as shown in Figure 12. No terrestrial risk information was found for sulfathiazole. It has been shown by Ji et al. (2012) to effect reproduction in aquatic invertebrates and has toxic effects on algae, but at concentrations two to three orders of magnitude higher than observed in earthworm tissues in this study.

5. Remediated Wetland Soil Tubs

5.1 Methods

The suitability of the soil amendments in saturated conditions was evaluated using simulated wetlands in 45” (1.1m) diameter (11.04 ft^2, or 0.95 m^2) plastic tubs. The bottom of the tubs was filled with native contaminated floodplain soil to a depth of 9 cm, followed by the lower rate of soil amendment mixture. The soil and amendment were hand mixed. The 3 original amendment treatments (BS, CM, and AG), plus a no-amendment control, were replicated 3 times, for a total of 12 tubs with a total area of 132.5 ft^2, or 12.3 m^2. Twelve more tubs were eventually added using the 2nd generation amendments used in the upland remediated scenario: poultry + biosolids (PL+), manure + biosolids (CM+), composted yard waste and fertilizer (YF) , and a fresh mixture of the BS amendment, all replicated 3 times. These newer tubs were added at the end of the 1st growing season and thus no plant data was recorded.

Native wetland plants were seeded into the tubs after the soil was saturated with treated effluent. The seeding rate was approximately 12 lbs/ac. Following seedling establishment, the soil was allowed to be submerged periodically as rain filled the tubs. The tubs were fitted with overflow drains to allow a maximum water level of 1 cm above the soil level. When rainfall was insufficient to maintain proper water levels for plant survival, the tubs were watered with effluent from the WWTP clarifying pond. Effluent from the WWTP is usually discharged to a nearby creek and is the planned source of water for our restoration wetlands.

Water samples were collected at the end of the growing season and analyzed for metals, nutrients and PPCPs. Prior to sampling, treated effluent water was held in a bench-scale “biochemical reactor”, a container filled with composted poultry litter, woodchips and limestone

rock, for approximately 1 month, to simulate a hypothetical treatment cell anticipated in the large scale wetland. Drain holes were plugged and the treated effluent was allowed to inundate the wetland tubs for approximately 1 day prior to collecting water samples for analysis. Three lists of PPCPs were collected representing the most persistent and toxic PPCPs as per McClellan and Halden (2010), who characterized persistence and decay curves for 72 PPCPs sampled from 32 states in the U.S. PPCP analysis included Acid Extraction in Positive Ionization, Tetracyclines in Positive Ionization and Acid Extraction in Negative Ionization. Only the biosolids and composted manure treatments were tested for PPCPs.

Samples were collected from a PVC pipe placed horizontally at the bottom of the tubs (See Figure 20). These pipes have holes to allow seepage of water, and are lined with filter material sufficient to screen out anything larger than soil colloids.

Figure 20. Wetland tubs and the collection pipe apparatus.

Due to the limited volume and the difficulty in collecting consistent samples from the initial wetland tubs, larger tubs were used the second year (summer 2015). The sediment from the smaller tubs was combined, by treatment, into seven larger tubs. These treatments were: Biosolids 1st generation, Biosolids 2nd generation, Manure 1st generation, Manure 2nd generation, Poultry litter, Compost, and Control. Three obligate wetland species (cattail, *Typha latifolia*; pickerel weed, *Pontedaria cordata*; and arrowhead, *Sagittaria latifolia)* were planted in each tub. The plants were collected from clean sites early in the growing season, so the leaf tissue later collected was grown entirely when the plant was exposed to the soil amendments within the tubs. Leaf tissue was collected from all of the plants within a tub, separated by species, dried, and analyzed with XRF. Surface sediment was collected from throughout the tubs, dried, and analyzed with XRF.

5.2 Results

5.2.1 Water Quality

Water samples were collected on October 30, 2014. Due to problems with leaking tubs, a sufficient volume of water could not be collected from every tub, so the manure and biosolids treatments were consolidated in order to collect one representative sample from each treatment. Most of the original 12 tubs leaked enough to prevent collection of samples, with the exception of the Control tubs, which yielded a sufficient amount. The Control had the highest levels of dissolved Zn (2273.7u/L) and Cd (14 ug/L), while the poultry litter treatment had the highest dissolved lead (317.6 ug/L).

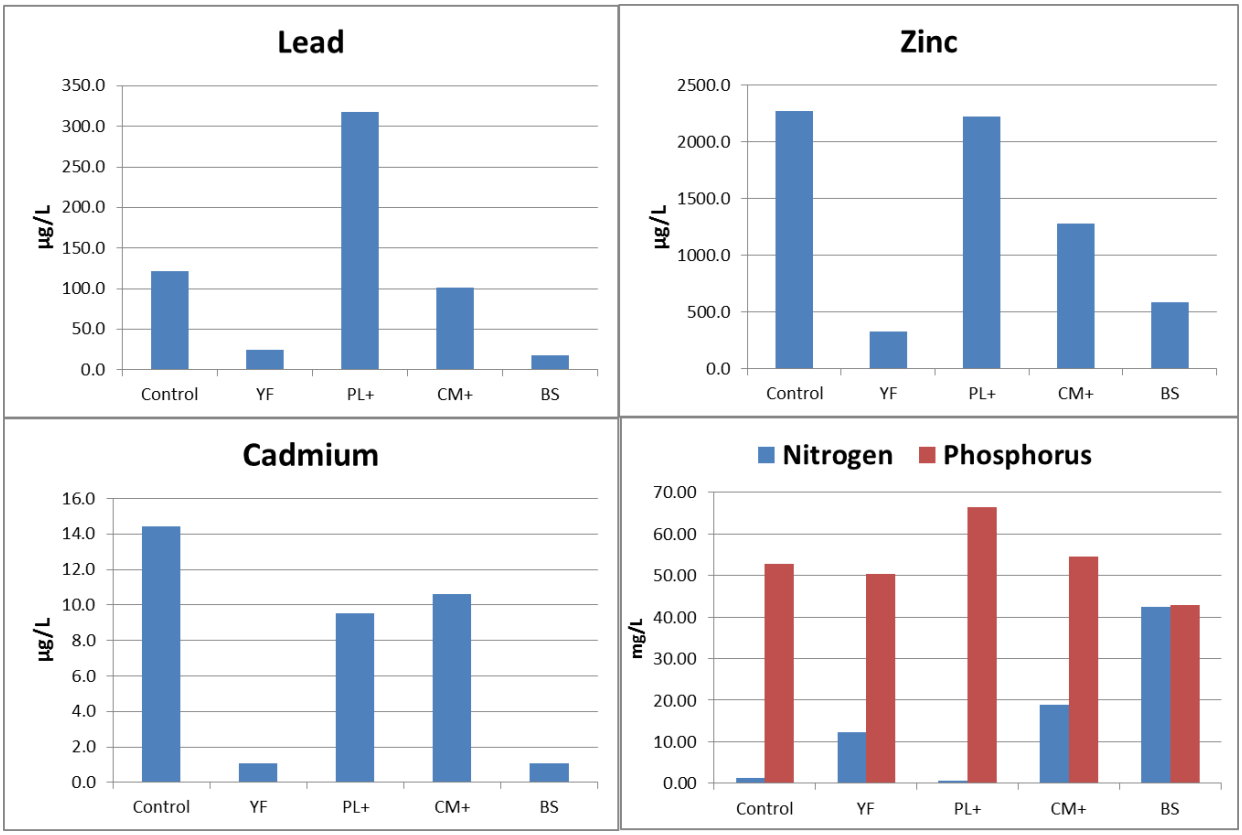

Figure 21. Concentrations of metals and nutrients in samples of water from simulated wetland tubs.

All of the treatments and the Control had relatively high levels of phosphorus, most likely due to the high levels of phosphorus within the biochemical reactor, which was inoculated with poultry litter. The nitrogen levels were much more varied among the treatments, with the BS having the highest level of 42.4 ug/L. Because of the low numbers of samples evaluated, their statistical relevance was limited.

5.2.2 PPCP Concentrations in Wetland Waters

The water samples from the Wetland BS tub, Upland BS High plot, two Wetland PL+ tub samples, and one Wetland Control sample had a moderate number (as compared to raw amendments and WWTF effluent discussed in Section 4.2.2) of analytes that exceeded detection limits at 14, 10, 10, and 9 analytes, respectively. The remainder of the samples ranged from seven analytes detected in the duplicate Wetland Control sample to three analytes detected in the Cow Manure + BS duplicate sample. Sample results that exceeded detection limits are presented in Table 15 below.

Only the Biosolids sample from the wetland tubs was above toxicity values found in the literature. The sample exceeded LOAECs for three constituents (shown in red in Table 15); the antimicrobials, triclosan and triclocarban, and the stimulant, caffeine. An additional sample from a Poultry + BS tub exceeded a No Effect Concentration of triclocarban. The Wetland BS New and the Wetland Poultry/BS2 tub contained triclocarban at 0.535 and 0.103µg/l, respectively. The triclocarban NOAEC was derived from a study of *Potomopyrgs antipodarum*, an aquatic mudsnail, which was 0.05 micrograms/liter (µg/l) found by Giudice and Young (2010). The LOAEC in the same study was 0.2 µg/l, which was found to cause endocrine disruption that resulted in an increase in embryo production. The Wetland BS sample contained 1.02 µg/l of triclosan, which was almost an order of magnitude over the LOAEC based on effects on algal assemblage found by Wilson et al. (2003).

Table 15. Detected PPCP concentrations in simulated wetland effluent samples.

Concentrations in micrograms per liter									
PPCP Analyte\ Sampled Media	**BS (2nd Mix)**	**CM+ (composite)**	**CM+ (duplicate)**	**Control Rep 1**	**Control Rep 2**	**Control Rep 3**	**PL+ Rep 1**	**PL+ Rep 2**	**LOAEC (ug/L) Biological Endpoint**
4 Epianhydrochlortetracycline							0.271	0.397	
Acetaminophen	1.52			1.44	1.16	1.34		1.45	
Azitrhomycin	0.0195	0.0106	0.0124	0.0204	0.0205		0.0139	0.0109	
Caffeine	0.105								0.05/amphibian malformation
Carbadox							0.0583	0.0635	
Carbemazepine	0.045			0.0407	0.0272	0.04	0.0427	0.02	100/algal growth
Clarithromycin	0.0116			0.0107		0.0107	0.00635		5/bacterial growth
Diphenhydramine	0.0118						0.00253	0.00388	5.6 / behavioral
Erythromycin H2O	0.0189		0.0226	0.0254		0.0312	0.029	0.0258	22700/shrimp morbidity
Gemfibrozil	0.0405		0.00997	0.0169	0.0134				30400/ crustacean
Licomycin	0.0394								
Miconazole	0.055							0.0573	
Naproxen				0.0853					
Oxolinic Acid	0.0517	0.035	0.073	0.0481	0.0642	0.056	0.0806		
Sulfamethizole					0.0348				
Sulafathiazole	0.0901	0.0161	0.0546	0.0596	0.0426	0.0519	0.0293	0.0594	
Triclocarban	0.535						0.0105	0.103	reproduction (NOAEC)
Triclosan	1.02								0.15/ algal assemblage
Exceeds adverse effects concentration									
Concentrations greater (>15%) than highest Control concentration									

The majority of the analytes detected in the treated wetland tubs were also detected in the control wetland tubs in similar concentrations. This indicates that the majority of these constituents were likely derived from the wastewater treatment effluent used to flood the tubs and not the biosolids, cattle, or poultry compost mixtures. The exceptions to this trend (shown in yellow in Table 15) are 4 epianhydrochlortetracycline, carbadox, diphenhydramine, miconazole, oxolonic acid, and triclocarban found in the PL+ tubs, and caffeine, diphenhydramine, gemfibrozil, licomycin, miconazole, sulfathiazole, triclocarban, and triclosan found in the BS

wetland tub. None of the analytes found in the Cow + BS wetland tubs exceeded or were higher than 15% of the control samples.

5.2.3 Wetland Plant Growth and Metal Uptake

Plants grown within the wetland tubs in 2014 showed dramatic differences between soil amendment treatments. Due to time constraints, species composition was only qualitatively described, and only cover percentages were quantified. In July, the manure treatments had an average cover of 37%, compared to 8%, 3%, and 0% for the BS, Control, and AG treatments, respectively. In September, the manure treatment had an average of 80% plant cover, while BS, Control, and AG treatments had 23%, 7%, and 8% cover, respectively. The majority of the plants grown within the manure treatments were the seeded natives, while the biosolids tended to grow unseeded grasses. The AG treatments were largely barren except exotic spurge that grew late in the season. The control tubs had a sparse cover of a few native plants and spurge. The results from the 2014 growing season indicate that the manure treatment performs the best as a plant growth medium.

During the 2015 growing season, no mortality was observed in any of the planted wetland species (arrowhead, pickerel weed, and cattails) at the time of leaf collection, although some wilting and browning of leaf margins was observed in plants from the Control tub. Metals accumulated in the leaf tissue of the three species similarly, except cattails accumulated roughly twice the amount of lead than other species. The tissue data for the three species was lumped for purposes of comparison between treatments. To account for the difference in sediment metal concentrations between tubs and their potential impact on plant uptake, a bioaccumulation factor

(BAF), expressed as the percent uptake, was calculated by dividing the metals within the plant leaf tissue by the average metals present in the sediment (see Table 16).

5.3 Discussion

5.3.1 Water Quality

Metal and nutrient concentrations in the wetland tub samples were highly variable. Total phosphorus concentrations were highly elevated in all samples including the control tubs, although the poultry litter tubs had the highest average concentration. The concentrations in all treatments were orders of magnitude greater than anything measured in Center Creek. This indicates a significant influence from the effluent of the BCR, which used poultry litter as an organic feedstock. Cattle manure or biosolids, which have been shown to produce lower levels of nutrients in runoff from the upland plots, would be a better option for a BCR feedstock material.

Total nitrogen concentrations from the YF, CM+ and BS tubs were well above the values reported for Center Creek, while concentrations from the Control and PL+ tubs were below even the minimum value reported for Center Creek. The concentrations found from the PL+ wetland tubs contrast sharply with the results of the terrestrial plots, where the PL+ plots had the highest TN concentrations and were more than twice as high as the next highest treatment.

Dissolved Zn exceeded the WQS in all treatments, although only the Control tubs had an average concentration which exceeded those measured in Center Creek. All of the treatments were below the average Zn concentration measured in Ben's Branch. The PL+ tubs were the only treatment that exceeded the WQS for Pb. Compared to Center Creek, dissolved Pb in the PL+ and the Control were both higher than the maximum value. All samples exceeded the range of Pb found in Ben's Branch.

Figure 22. Simulated wetland tubs with wetland plants established, 2015.

Table 16. Metal concentrations and bioaccumulation factors (BAF) of wetland plants grown in contaminated soil treated with organic amendments.

Treatment	Sediment Pb ave (ppm)	error +/-	Sediment Zn ave (ppm)	error +/-	All species plant tissue Pb (ppm)	error +/-	All species plant tissue Zn (ppm)	error +/-	Overall plant uptake Pb (%)	Overall plant uptake Zn (%)
Biosolids 1st gen	211.1	21.3	1590.7	68.9	5.4	0.6	199.0	1.8	3%	13%
Biosolids 2nd gen	128.5	16.0	804.4	46.3	5.0	0.6	100.0	1.3	4%	12%
Manure 1st gen	200.0	20.6	1913.5	74.5	5.0	0.5	110.2	1.4	3%	6%
Manure 2nd gen	68.9	16.5	730.1	55.8	5.5	0.5	54.1	1.0	8%	7%
Control	366.2	26.1	3159.0	91.3	4.8	0.6	216.6	1.7	1%	7%
Poultry	256.3	21.9	1724.5	67.4	4.7	0.6	77.8	1.1	2%	5%
Compost	121.7	15.8	890.1	48.6	5.1	0.6	98.3	1.3	4%	11%

5.3.2 Wetland Plant Growth and Metal Uptake

Wetland plants grown in mine-waste contaminated sediment did not accumulate metals in concentrations above average plant tissue concentrations for unglaciated regions of Missouri, as discussed previously in Section 3.6.1 (EPA RI pg 8-43). The sediment used for this study exceeded the Threshold Effects Concentration for lead, zinc, and cadmium, and exceeded the Probable Effects Concentration for zinc and cadmium. The sediment originated in wetlands that were being excavated due to high levels of metals, and therefore represent a field condition scenario at the more contaminated end of the spectrum. Despite the sediment metals concentrations varying at least fourfold between the highest (control) and lowest treatments, the plant tissue concentrations of lead and cadmium were remarkably similar among those treatments. The difference in soil concentrations was likely due in part to the dilution of the contaminated wetland soil with the various soil amendments.

When taking into account the detection limitations of the XRF apparatus, the cadmium and lead plant tissue concentrations are statistically the same amongst their respective treatments. The same can be said for the cadmium levels within the sediment: only the control was different from some of the treatments, all of the other treatments were similar to each other. The majority of the plant tissue cadmium data was below detection limits and was therefore not reported. The various soil amendments did not seem to “treat” the lead bioavailability of mine waste in the wetland setting, since the plant BAF was lowest in the control for lead (and second lowest to the poultry litter treatment in the case of zinc). Since the sediment metals are highest in the control tub, and the sediment all was excavated from the same place, the results suggest that metal concentrations in the soils are being diluted by the addition of the amendments.

However, metals from the soil or wetland sediment were not the only source of plant available metals. The wetland tubs were saturated with wastewater treatment effluent which contains dissolved metals. Dissolved metals are, in effect 100% bioavailable to the plant. Therefore, treatment effects from wetland soils based on plant data are complicated by the dissolved metal contribution of the irrigation water.

There was mixed support for increased treatment of metals (reduced BAF) over time. Both the 1st generation of manure and biosolids were amended in June 2014, and plants grown in these substrates had reduced rates of lead uptake compared to the sediment amended in October 2014 (the newer generation of the manure treatment also included 10% biosolids, so the treatments are not strictly identical). The lead uptake was reduced over time; the zinc uptake decreased over time in the manure treatments but increased over time in the biosolids.

The plant uptake of metals was lower than reported by Ford and Beyer (2013). In our study, the average uptake of lead, and zinc was 3%, and 9%, respectively, while Ford and Beyer (2013) reported 3.9% and 37%. Ford and Beyer (2013) study reports wildlife soil criteria for metal toxicity associated with the feeding habits of various animals. Inputs into their equation include assumptions on plant metals uptake, feeding rate, incidental soil ingestion, and species-specific toxicology data. Of the species used in their study, the mallard and Canada goose would be the most relevant receptors for a wetland scenario. The wildlife soil criteria is exceeded for both species in the control soil because of the elevated levels of zinc (and cadmium as well, for the mallard). By simply adding the soil amendments and thereby diluting the metals content of the sediment, all of the treatment soils are below the screening criteria.

Specific wildlife receptors for lead are discussed in more detail within the remediated soils discussion. Barring any calcium deficiency, the lead levels in the wetland plants in this

study should not pose a risk to wildlife. Similarly, the cadmium levels detected in the wetland vegetation, the average of which was 3.3ppm, do not pose a risk to wildlife. Though increased blood pressure has been observed at levels as low as 1 mg/kg cadmium diet in rats and mice, most studies do not report changes at this level. More pertinent to our wetland scenario, chickens, ducks, and quail fed diets containing 10 mg/kg cadmium do not decrease gain or cause anemia, while mallards fed a 20 mg/kg diet did result in anemia. Pasture herbage of zinc content ranges from 17 to 60 mg/kg dry weight, though environmental pollution can increase the zinc content of grass from 5- to 50-fold (Mills and Dalgarno, 1972, in NRC 2005). The zinc concentration in plants usually falls with advancing maturity, and leguminous plants usually have higher zinc levels than grasses grown under the same conditions (Hambidge et al., 1986). Heavy dressings with lime and to a lesser extent, superphosphate, can greatly reduce pasture zinc levels. From the levels observed in our study, there is no risk to wildlife from zinc in the tissue of wetland plants.

5.3.3 PPCP Analysis

The PPCP concentrations detected suggest there is some potential for adverse effects from various PPCP constituents, particularly triclocarban and triclosan, to occur from the application of raw, un-composted amendments. However, the composting process and the addition of wood chip wastes, greatly reduced concentrations of PPCPs to levels which are below available adverse effects literature values, with two exceptions. Triclocarban was detected in waters from all the composted amendments, but one runoff sample of BS amendments was the

only concentration that approached adverse effects levels (0.2 μg/L) as determined by reproductive changes in aquatic mudsnails. One sample of PL+ contained a concentration that was between a NOAEC and LOAEC concentration for mudsnails and other aquatic receptors.

Figures 23 and 24 show comparisons between raw and composted amendments. The concentrations of most constituents, with the exception of erythromycin and miconazole, have lower concentrations in the composted amendments than the raw material than would be expected from simple dilution due to the addition of wood chip waste. Wood waste was added at a rate of approximately 50% by weight. The concentrations of compounds in the composted amendments compared to the raw parent material range from 2.3% for Triclocarban to 0.62% for Triclosan. This suggests that photodegradation and/or biodegradation during the composting process reduced the concentrations of many of these contaminants. Walters et al. (2010) calculated degradation curves for 72 PPCP contaminants derived from biosolids. Triclocarban was amongst the most persistent contaminants, with no discernable consistent degradation after three years, which is consistent with our findings. However, it must be mentioned that the degradation byproducts themselves were not measured by this study. Miconazole concentrations decreased to 27% of the raw biosolid concentration. Erythromycin increased by 180%; although the concentrations measured were near the limits of detection; 4.45 and 8.06, for raw and composted biosolids, respectively. A reasonable conclusion from our results is that the low threat of adverse effects found from triclocarban, and the majority of the measured PPCPs would decrease further over time due to continued photochemical and biochemical processes.

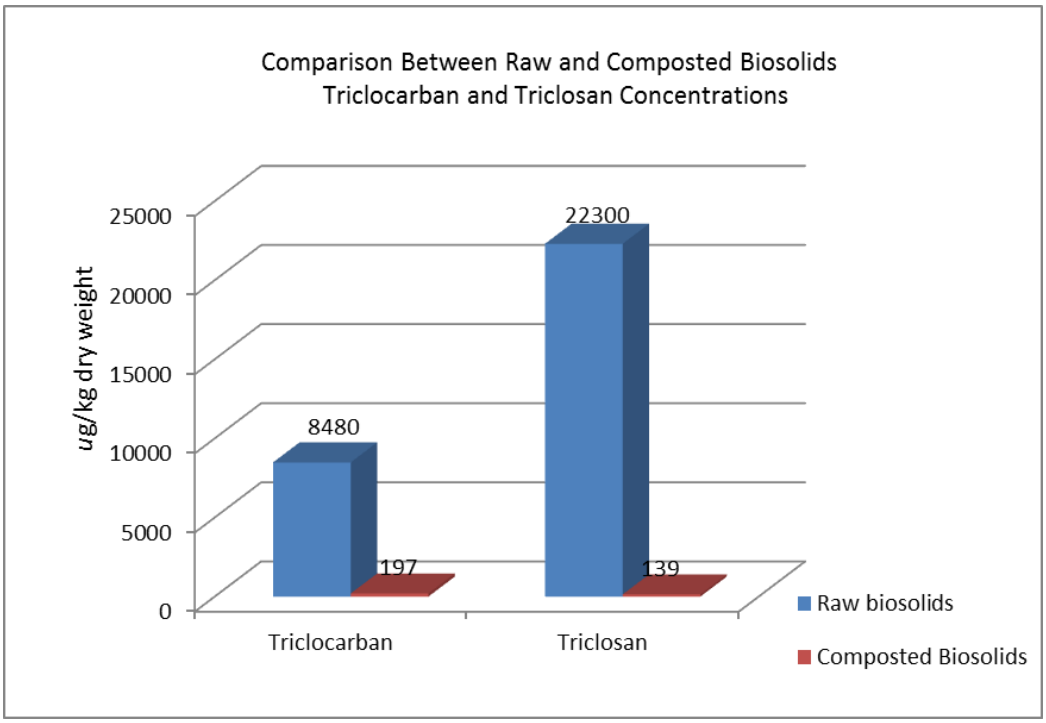

Figure 23. A comparison of Triclosan and Triclocarban concentrations measured in raw and composted municipal biosolids.

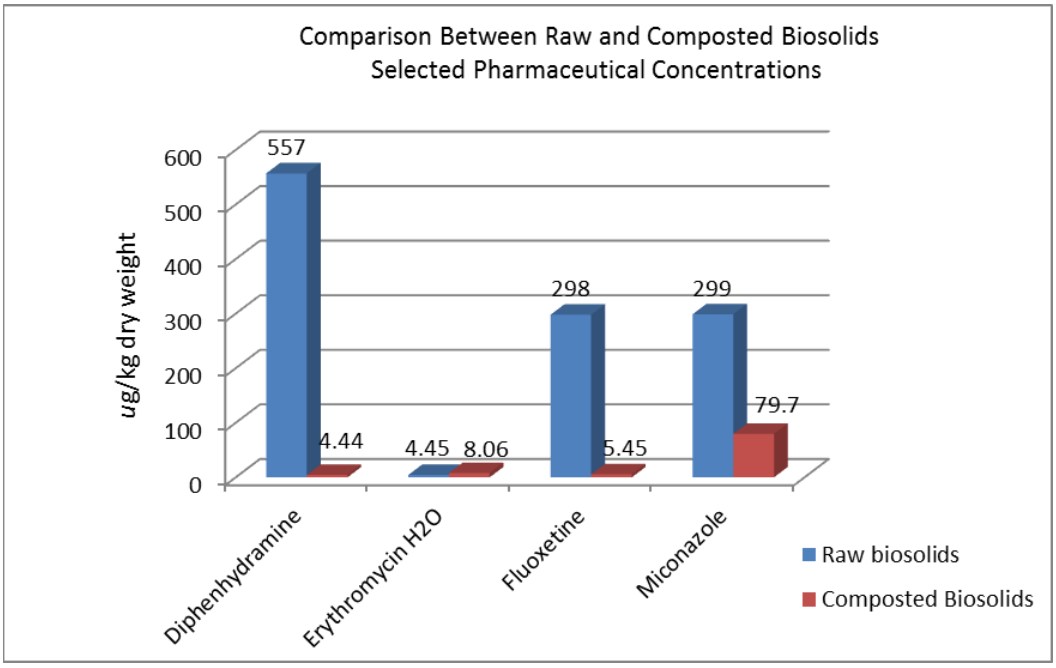

Figure 24. A comparison of concentrations of various PPCP compounds in raw and composted municipal biosolids.

Few studies have been conducted on the ecological effects from antibiotics, especially for terrestrial receptors. One primary concern of PPCPs in the environment would be changes in the microbial communities themselves. However, researchers found that the effects of antimicrobials and antibiotics were variable on microbe populations (Fuchsman et al. 2010, and Halling-Sorenen 2001). Park et al. (2013) found increases in microbial populations likely due to increase nutrients and other favorable conditions provided by the biosolids overwhelmed any potential antimicrobial effects.

The wetland exposure scenario has the highest potential to create adverse ecological effects due to potential introduction of amendments that would be submerged in the water during wetland saturation and terrestrial exposure during dry periods. In addition, the wetland scenario evaluated in this study was saturated with water from wastewater treatment effluent. However, adverse aquatic or terrestrial effects should not be anticipated based on runoff and wetland results from cow and poultry manure applications. Potential low-level chronic adverse effects may exist from triclocarban exposure due to direct application of biosolids into wetlands. The composting process appears to significantly reduce PPCP concentration to below detection for the majority of the constituents. However, the results of this study should be viewed with caution due to the limited number of replicates that exceeded detection limits and due to the limited ecotoxicological benchmarks in the literature for PPCPs.

6. Ecological Risk Assessment Discussion

Black and Veatch (1998) completed the Baseline Ecological Risk Assessment (BERA) for EPA using EPA's guidance and standard methodologies. Newfields and Black and Veatch (2001) subsequently submitted a Technical Memorandum that calculated soil concentrations that posed risks to vermivores. Vermivores are worm-eating vertebrates that tend to receive the highest dose from soil contaminated with non or low biomagnifying metals such as Pb, Cd, and Zn. The Technical Memorandum compared earthworm metal concentrations to Toxicity Reference Values (TRV) that were determined to pose risks to vermivores in the BERA. The Technical Memorandum went on to use the ratio of worm metal concentrations to soil metal concentrations to calculate soil concentrations that pose unacceptable risks to vermivores. The Technical Memorandum determined that soil concentrations that pose risk to terrestrial vermivores are 804mg/kg Pb, 41 mg/kg Cd, and 6424 mg/kg Zn. Remediated soil concentrations are less than these concentrations by definition.

This paper provides an additional evaluation of risk using avian species typically used for modeling exposure to wetland and upland environments. A more recent BERA produced by EPA ecological risk assessors using standard risk assessment guidance for a similar mining-impacted site in Missouri (Big River Mine Tailings Site) was used to evaluate risk to the woodcock (a vermivore) and the Canada goose (predominant herbivore) of amended soils. Other avian species modeled in the Big River Mine Tailings BERA included belted kingfisher, red-tailed hawk, and great blue heron. These receptors primary prey species as modeled in the BERA are fish, mice, and frogs, respectively. This study produced no data to support these inputs and

previous modeling did not indicate adverse effects to these receptors. Concentrations of metals from plant and worm tissues and amended soil concentrations from this study were used as inputs into EPA's model. Site specific water concentrations from the Jasper County Remedial Investigation were additional inputs. The BERA model calculates an average daily dose (ADD) from site specific prey and media concentrations for specific receptors based on data on food and water ingestion rates, incidental ingestion of soil, feeding behavior, and an area use factor (AUF). The ADD is divided by a TRV derived from the literature to arrive at a Hazard Quotient (HQ). An HQ above 1.0 indicates the potential for risk and EPA generally uses this threshold to consider remedial actions. The avian Lowest Observed Adverse Effects Levels (LOAEL) TRV for Pb used in this model was based on reduced egg production from Japanese quail found by Edens and Garlich (1983). The avian LOAELTRV for Cd used in this model was based on reduced egg production in mallard ducks (White and Finley, 1978). The LOAEL TRV for Zn used in this model was based on reduced hatch rate in leghorn chickens determined by Stahl et al. (1990). The concentrations of these TRVs are:

- Pb - 11.3 mg/kg body weight/day,
- Cd – 20.0 mg/kg body weight/day, and
- Zn – 131 mg/kg body weight/day.

Initial risk calculations (provided in Appendix D) were made using the highest concentrations of amended soil, plants, and earth worms. Ragweed concentrations from the upland plots were used as plant tissue concentrations. An AUF of 1.0 was applied to the Canada goose, since it is feasible that 100% of the time a resident goose could spend on the habitat created by amended upland and wetland soils. An AUF of 0.5 was used for the woodcock since

these birds are migratory and spend half their time annually off site. The HQs for Cd were well below 1.0 (0.09 for Canada goose and 0.45 for the woodcock). The HQs for Pb were 2.96 for the Canada goose and 2.21 for the woodcock. The HQs for Zn were 1.35 for the Canada goose and 1.54 for the woodcock. Since the Pb and Zn HQs were above 1.0, further evaluation of site data was conducted.

The first scenario evaluated the maximum exposure scenario for amended soils. The first scenario did not consider differences in metal uptake and exposure provided by the various treatments. The cow manure amended plots tended to yield lower concentrations of heavy metals in plants and earthworms. In addition, ragweed tissue concentrations from the upland represent concentrations from a hyperaccumulating plant. It is unlikely that an herbivore such as a Canada goose would only select ragweed in its diet. Therefore, the data from the wetland plants was added to the plant concentrations evaluated in a second scenario. Hazard Quotients for Pb and Zn dropped only marginally from evaluation of the second scenario and remained above 1.0.

A third scenario was evaluated that considers the soil bioavailability as measured by depurated worms. The first two scenarios assume 100% metal bioavailability, which is known to be significantly overly conservative. Metal bioavailability as determined by earthworms from amended soil was 33% for Pb and 40% for Zn. When the bioavailability factor was applied in the third scenario, HQs for both Pb and Zn declined below 1.0 indicating low potential for risks. The HQs for Pb were 0.47 for the Canada goose and 0.93 for the woodcock. The HQs for Zn were 0.55 for the Canada goose and 0.82 for the woodcock.

Although the BERA did not consider the effects of PPCPs, several researchers have evaluated triclocarban or triclosan to estimate ecological risk (Fuchsman, et al. 2010, Reiss et al.

2009, and Snyder et al. 2013). Reiss et al. (2009) modeled multiple exposure pathways using physiochemical and fate properties of triclosan compared to toxicity literature for earthworms, mammals, birds, fish, plants, and soil microorganisms. None of the scenarios evaluated were determined to exceed toxicological benchmarks. For example the lowest LC50 determined for bobwhite quail was 862 mg/kg-body weight. The highest concentrations found in earthworms in this study were approximately two orders of magnitude below this dose. Fuchsman et al. (2010) conducted a similar modeling exercise to evaluate terrestrial ecological risk from triclosan found in land-applied biosolids. The most likely risk from worst-case application rates and triclosan contamination was potential disruption of nitrogen cycling bacteria. However, Ji et al. (2012) found any negative impacts from triclosan on bacterial populations was mitigated by increased population and activity of diverse bacteria communities due to added nutrients and beneficial conditions created by the biosolids. Snyder et al. (2013) conducted a similar risk assessment of triclocarban using modeled exposure compared to toxicological literature. Snyder et al. (2013) did model one scenario that resulted in adverse risk based on earthworm exposure to the American woodcock, a vermivorous bird. However, the highest earthworm results from this study found in biosolids amended soils, were less than half of those determined to be a potential risk to the woodcock by Snyder et al. (2013).

7. Conclusions

7.1 Runoff

The run-off from our non-remediated plots was collected immediately at the downhill end of the soil amendment application (remediated plots had a 1 foot sod buffer), making this a worst-case scenario for contaminant concentrations. The purpose of the plots was to compare the run-off from the various treatments, it was not expected that the run off from every plot would achieve compliance with the Missouri Water Quality Standards (WQS) or be on par with Center Creek. These values are included merely for reference.

Rates of application had no detectable impact on the run-off of metals. There seems to be an initial flush in metals following the application of the amendments, but that effect tapers off with time. In the samples collected in the non-remediated plots in May, dissolved Pb concentrations were above the WQS acute criteria in all treatments except the Control and BS Low. Two weeks later, however, only the AG High and CM High samples exceeded the acute criteria. By September, samples from all treatments were below the acute criteria for Pb, and all of the experimental treatments had lower concentrations than the control plot. These findings are consistent with patterns over time of Pb in runoff from phosphate-treated soils reported by Weber et al. (2015). Similarly, dissolved zinc concentrations in non-remediated plots were higher in all of the experimental treatments compared to the control sample in the earlier samples, but only the AG treatments and BS high were significantly higher in the September samples. Only the AG high and BS High had significantly higher levels of dissolved cadmium than the control by the time of the September sampling event, and Cattle Manure low was significantly lower.

The remediated soil runoff showed the same trend as the non-remediated soil plots. The dissolved Pb concentrations were below the WQS for all treatments except the PL+ High

treatment in the initial sampling event in November 2014. The samples collected one year after the initial samples showed a metals reduction of 99% for the PL+ high treatment, and 96% for the PL+ low treatment, which is less than the control run-off concentration for that period. Only the PL+ and the CM+ High treatments had average Zn values that exceeded any criteria or reference values, and those were measured during the first run-off event. Zinc concentrations in all the samples analyzed were below the average concentration in Ben's Branch. Dissolved cadmium followed the same pattern of reduction over time as the other analytes, with BS low and the PL+ treatments having the lowest concentrations after the control.

In the non-remediated plots, nutrients in the runoff from the manure treatment plots were not elevated significantly over the Control plots, while the TN and TP concentrations from the other treatments were well above that of the Control. Total nitrogen from the cattle manure plots were at similar levels as those measured in Center Creek, while the other experimental treatments were above. Only the cattle manure treatments and the BS low were within the range of Center Creek phosphorus levels. In general, the cattle manure amendment had less nutrients in its runoff than the other treatments (other than the control).

All of the runoff samples collected from the remediated soil plots had lower concentrations of ammonia than the WQS. All of the treatment average concentrations, however, were greater than the average ammonia concentration found in Center Creek. The TN and TP concentrations from all of the experimental treatments except the control generally exceeded the average values reported for Center Creek. The cattle manure low rate treatment was below the Center Creek average, and had the least nutrient run-off of the experimental treatments. The control unsurprisingly had the least amount of nutrients within its run-off. There was an overall reduction of nutrients run-off (ranging from 50-75%) from the initial run-off results collected a

year earlier. The data show that the level of nutrients in run-off is high initially, and then diminishes over time for all treatments except the control.

The difference in run-off metals concentrations between the non-remediated plots and the remediated plots is noteworthy. The average lead concentration in the run-off from the non-remediated plots was 20.5 ppb, while the remediated plots' run-off was 6.1 ppb. The average dissolved zinc and cadmium run-off from non-remediated plots were two orders and one order of magnitude greater (respectively) than the average run-off from the remediated plots. Given that the soil metals recently encountered in the field are less than in the experimental plots, run-off concentrations would be even lower for the potential restoration sites.

Since WQS are meant to measure stream values rather than run-off, we decided to conduct a direct test on target organisms to see the impact of plot run-off. Two Whole Effluent Toxicity (WET) tests were conducted with run-off from the remediated plots. The test organisms, *Ceriodaphnia* and fathead minnows, survived 97.5%-100% in all of the treatments as well as the control when the run-off was diluted to simulate applying treatment to an 1/8 of the watershed. The second time a 50% dilution was used, and minnow survival was only impacted (77.5%) by the high rate of Poultry Litter and the bare ground control. The highest minnow growth rate was produced from the Cattle Manure treatment. None of the treatments had a significant impact on the survival of the Ceriodaphnia, but there were significant reductions in reproduction of Ceriodaphnia in the Poultry Litter and bare ground control.

Metal and nutrient concentrations in the wetland tub samples were highly variable, and due to low sample numbers, statistical differences between treatments could not be analyzed. Total phosphorus concentrations were highly elevated in all samples including the control tubs, almost certainly because the BCR used poultry litter as an organic feedstock. Dissolved Zn

exceeded the WQS in all treatments, though all of the treatments were below the average Zn concentration measured in Ben's Branch. All samples exceeded the range of Pb found in Ben's Branch.

7.2 Plant metal uptake

The levels of zinc and lead in the plants within our treatment plots did not exceed background levels for this area, though the cadmium levels did exceed background. To account for the difference in soil metal concentrations between plots and their potential impact on plant uptake, a bioaccumulation factor (BAF), expressed as the percent uptake, was calculated by dividing the metals within the plant shoots by the metals present in the soil. Within the non-remediated plots the various treatments had no effect on the uptake of lead, and only the AG treatment showed a significantly higher uptake of zinc than the control. Cadmium uptake varied among the treatments. Plants within the less-contaminated remediated soils had an identical uptake percentage of lead as the non-remediated plots, and the uptake of zinc and cadmium were significantly lower.

Soil metals measured after amendments were mixed into the remediated soil plots were only around 27% of the original levels. By adding in soil amendments, the soil metals were diluted within the topsoil, and in some cases showed reduced bioavailability as well.

The study site's soils were inordinately high in metals, and could adversely affect some animal species. After adjusting the treatments for the bioavailable soil metal concentrations, the only adverse impact to any species analyzed in Ford and Beyer (2013) would come from the poultry high treatment because of elevated cadmium levels. The use of common ragweed in our study to assess the accumulation of metals likely inflated the uptake of metals, since ragweed is a

known hyperaccumulator of metals. The stem tissue of grasses within the same plots as ragweed only accumulated 26% of the zinc and 33% of the cadmium (lead accumulated the same amount).

Similar to the upland scenarios, simply adding the soil amendments to the simulated wetlands diluted the metals content of the sediment, so that all of the treatment soils are below the screening criteria. This highlights the importance of “capping” an area to reduce exposure, via soil ingestion, to underlying contaminated soil.

In general, the biosolids and cattle manure treatments showed the least amount of risk associated with exposure to soil and ingestion of plants.

7.3 Plant diversity

One of our objectives of this study is to evaluate the suitability of amended soils to germinate and support native vegetation, so that the areas devastated by mining and the remedial excavation of soils can once again grow native tallgrass prairie species. A variety of potential landscapes were tested: wetlands, non-remediated soils, and remediated soils.

The initial simulated wetlands showed that the composted manure was able to grow the greatest quantity and diversity of native species, with minimal weedy species invading. Later, in the second version of the wetland tubs, all individuals of all three wetland species (arrowhead, pickerel weed, and cattails) were able to survive in inundated conditions in all soil amendments.

Of the non-remediated soil amendment treatments, only the cattle manure grew a significantly greater richness of seeded species and a significantly higher diversity of seeded species than the control for both the 2014 and 2015 seasons. It is clear that the cattle manure

treatment of these highly contaminated soils performed the best as a substrate for plant germination and survival. The initial AG treatment used in the non-remediated soil field plots performed poorly in every respect. The biosolids-based compost grew a lot of biomass, but not much of it was native species. Because biosolids from the Webb City WWTP are one of the most readily available sources or organic material within the potential restoration project area, the second phase of this pilot study, the upland clay soil plots, combined a relatively small amount of biosolids with cow and poultry manure to see if the plant performance was affected.

Surprisingly, the richness of the seeded species in our remediated plots was much less than in the non-remediated plots. Within these less contaminated plots, both the biosolids low and the manure-biosolids high treatments grew a significantly greater richness and diversity of native plants. This study indicates that cattle manure-based composted soil amendments perform the best in establishing a diverse stand of native vegetation.

7.4 Worm Exposure

Worms in the Control soil had the highest average Pb concentration, which was significantly higher than all other treatments except BS Low. This demonstrates a treatment effect for reduction of Pb uptake for all amendments. The lowest Pb concentrations in earthworm tissues were observed in the two PL+ treatments. The earthworm tissue results for Zn were similar to Pb in that the Control soil had the highest average concentration, and the CM+ High and PL+ Low treatments had the lowest mean tissue Zn. The only amendments that demonstrated any treatment effect on the uptake of cadmium were the Cow Manure amendments. The CM+ treatment demonstrated reduced metal uptake and, therefore, the lowest risk from metals within the terrestrial food chain.

7.5 PPCPs

The results of this study indicate that any quantifiable threat of adverse ecological effects from PPCPs from the selected application rates of composted biosolids and manures are low. Potential adverse aquatic effects of PPCPs were evaluated by comparing sample concentrations in surface runoff, worm tissue, and wetland effluent. There was only one scenario where concentrations were above literature values that demonstrated some adverse effects due to composted biosolids application: triclocarban in a wetland setting.

None of the upland runoff samples exceeded literature values that indicated adverse effects. Two of the samples from wetland tubs contained triclocarban above the No Effect Concentration for aquatic mudsnails, and a crustacean, *Mysidopsis bahia.*

The highest PPCP concentrations in worm tissues were the antimicrobials: triclosan and triclocarban. However all of the concentrations were less than an order of magnitude of any adverse effects concentration for terrestrial receptors found in the literature. Overall, the results of this study indicate that the quantifiable threat from PPCPs of adverse ecological effects from the composted biosolids and manures are low.

Photodegradation and/or biodegradation during the composting process reduced the concentrations of many of these contaminants. A reasonable conclusion from our results is that the low threat of adverse effects found from triclocarban, and the majority of the measured PPCPs would decrease further over time due to continued photochemical and biochemical processes. Adverse aquatic or terrestrial effects should not be anticipated based on runoff and wetland results from cow and poultry manure applications. Potential low-level chronic adverse

effects exist from triclocarban exposure due to direct application of composted biosolids into wetlands.

7.6 Conclusion

Overall, the results of this study indicate that cattle manure-based composted soil amendments tend to perform better than the other tested amendments in terms of providing the best growth medium for the seeded native vegetation and producing relatively low levels of excess nutrients and metals in surface runoff. For most performance variables tested, there were few differences in effects between the high (80 tons/ac) and low (40 tons/ac) application rates. While it is difficult to infer recommendations for application rates based on these results, it can be concluded that application rates, within the range tested here, could be scaled according to site specific soil conditions without a significant increase in potential negative environmental impacts.

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APPENDIX A: Non-Remediated Soil Field Plots.

Methods:

Study Area

The study plots are located adjacent to the Webb City wastewater treatment plant, within the Oronogo-Duenweg Designated Area of the Jasper County, Missouri, Superfund Site. The un-remediated soil at the study site has concentrations of metals above EPA's action levels: 7960 mg/kg mean Zn, 1053 mg/kg mean Pb, and 80 mg/kg mean Cd. The un-remediated soils represent a worst-case scenario with respect to the possible metals concentrations that might be found in potential restoration sites.

Prior to installation of study plots, whole metal concentrations throughout the non-remediated study area were measured with a handheld X-ray fluorescence instrument (XRF, Olympus NDT Inc., Waltham, MA). XRF readings were taken at three locations within the plot spaced evenly along a line running down the center of the plot lengthwise. Additionally, soil samples were collected from each study plot prior to the addition of amendments to verify the XRF results and analyze nutrient concentrations, agronomic properties, and soil carbon content. Each soil sample consists of a composite of five subsamples taken throughout the plot.

Experimental Soil Amendments

Three soil amendment mixtures were initially selected for evaluation in this study: 1) a municipal biosolids based mixture (BS), a cattle manure based mixture (CM) and yard waste applied along with conventional agricultural fertilizer (AG).

Biosolids (BS)

The biosolids produced from the Webb City WWTP routinely contain elevated levels of zinc (> 7500 mg/kg) due to sewer line inflow and infiltration from contaminated runoff and groundwater. In order to dilute the Zn concentration, biosolids from the nearby city of Springfield, MO were mixed with the Webb City biosolids at a rate of roughly 1 part Webb City material to 5 parts Springfield material. The biosolids were limed to a circum-neutral pH, and mixed at an approximate 2:1 ratio, by weight, with wood chips and leaf yard waste to add bulk, increase the C:N ratio to an ideal 20-40:1 ratio, and to further dilute the overall concentration of zinc to near a target level of 1000 mg/kg. The biosolids were applied at a low rate of 40 dry tons/acre and a high rate of 80 dry tons/acre, not including the additional carbon material.

Cattle Manure (CM)

Cattle manure sourced from the Joplin Regional Stockyard, was mixed with woodchips and leaves to increase the C:N ratio. Generally, cattle manure has nutrient concentrations as follows: 9 to 21 lb/ton Nitrogen, 4 to 18 lb/ton phosphate, and 8 to 26 lb/ton potassium. Cattle manure was applied at a low rate of 40 dry tons/acre and a high rate of 80 dry tons/acre, not including the additional carbon material.

Yard Waste and Fertilizer (AG)

The third amendment consists of high phosphate fertilizer 10-20-10 NPK and composted yard waste. The phosphate fertilizer was applied directly to the soil at a low rate of 12 tons/acre and a high rate of 23 tons/acre to the soil. This application rate is significantly higher than a typical agricultural application, but was calculated to treat the amount of lead in the soil based on

recommended P:Pb ratios from Yang et al (2001). The composted mulch and leaf litter was obtained from the Webb City yard waste collection area located adjacent to the WWTP. The application rate of composted yard waste was similar in volume to the volume of material used in the biosolids application treatment.

Plot Design

The biosolids (BS), manure (CM), and yard waste (AG) soil amendment mixtures were applied at a high and a low application rate, creating a total of 6 experimental treatments. Each experimental treatment, in addition to a control treatment with no soil amendments, was replicated 3 times in a randomized block design, making a total of 21 plots. The plots measure 2 m wide by 4 m long (8 m^2) and are situated on a slight grade oriented parallel to the slope. The total area for the upland plots was 168 m^2 (21 plots x 8m^2 plot size) or 1815 ft^2, or 0.04 ac. The soil amendments were applied to the experimental plots and incorporated 3-6" into the soil. After a period of approximately 1 week, a mixture of native prairie plant species, including warm-season grasses, forbs, and legumes, were sown by hand to lower 7.7 m of each plot. The upper 0.3 m of each plot were sown with tall fescue (*Festuca arundinacea*) seed. Approximately 6 lbs/ac of grasses and 7 lbs/ac of forbs was used. This rate was relatively high in order to compensate for the clumping of the seed and damp sawdust mixture used when cold-stratifying the seeds. The individual species used and the seeding rate are listed in Table A-1.

Table A-1. Native prairie plant species used in seed mixture.

Species	Common name	Plant Type	Seeding rate
Andropogon scoparius	Little bluestem	grass	1.5 lbs/ ac
Sorghastrum nutans	Yellow Indian grass	grass	1.5 lbs/ ac
Chasmanthium latifolium	Broad-leaf wood-oat	grass	1.5 lbs/ ac
Elymus virginicus	Virginia wildrye	grass	1.5 lbs/ ac
Strophostyles leiosperma	slick-seed wildbean	legume	0.75 lbs/ ac
Verbena hastata	Blue verbena	forb	0.75 lbs/ ac
Chamaecrista fasciculata	Showy partridge pea	legume	0.75 lbs/ ac
Symphyotrichum novae-angliae	New England aster	forb	0.75 lbs/ ac
Rudbeckia hirta	Black-eyed-Susan	forb	0.75 lbs/ ac
Dalea purpurea	Purple Prairie Clover	legume	0.75 lbs/ ac
Coreopsis lanceolata	Lanceleaf coreopsis	forb	0.75 lbs/ ac
Penstemon digitalis	Foxglove Penstemon	forb	0.75 lbs/ ac
Echinacea pallida	Pale Coneflower	forb	0.75 lbs/ ac
Liatris pycnostachya	Thick-spike gayfeather	forb	0.75 lbs/ ac

Sample Collection and Analysis

At the lower end of each plot, landscape edging was installed as a weir to direct runoff into a standard 5-gallon plastic bucket with a notch at the top rim to allow for run-off entry. Samples were collected periodically following rainfall events throughout the growing season. Initially, grab samples were collected from the collection basins of the non-remediated plots following a runoff event and split into separate bottles for each type of analysis. However, during sample collection, varying amounts of soil deposition and resuspension were observed in the collection basins. Due to concerns that the suspended sediment may not be representative of actual runoff, future samples were collected using modified “Wisconsin” type 1 L siphon samplers (Diehl 2007) installed inside each bucket (see Figure A-1). The samples captured by the siphon samplers were split and preserved according to the type of analysis. A 250 ml sample preserved with nitric acid was analyzed for total recoverable metals and another 250 ml sample

was collected and filtered through a 0.45 µm mesh filter and analyzed for dissolved metals as per EPA method SW 846 6010B. A 500 ml sample preserved with sulfuric acid was analyzed for total phosphorus, total nitrogen as per USGS method I-2650-03 and ammonia nitrogen as per EPA method 350.1. The bottles were packaged with ice for transport to the laboratory.

Figure A-1. Example of passive sampler set-up with bucket (prior to notching).

Agronomic properties of soil plots were measured from samples collected before and after amendments. Agronomic properties include total organic carbon, nutrients, bulk density, neutralizable acidity, and soil pH. Results are shown in Appendix C. Soil samples were collected as a 5 aliquot composite to a depth of 10cm. Soil pH was measured in the field with a pH meter

by analyzing a 10:1 deionized water:soil mixture from samples collected from each plot. No lime was used in these plots due to a neutral pH.

Species composition and diversity of native plants in each plot were measured using 50 cm by 50 cm (0.25 m^2) subplots. Each plot was divided into 0.25 m^2 grids and 2 randomly selected squares from the grid were sampled. The two random location "coordinates" were consistent throughout all plots to remove positional bias from possible seed movement during rain events. In the fall, a stem count was conducted within each square identifying all species found, including non-seeded plants. The results were reported as species richness and Shannon Diversity Index (Shannon and Weaver 1963).

At the end of the 1st growing season, samples of plant tissue were collected to measure biomass in below ground (root) and above ground (shoot) tissues. Each plot was subsampled using 20 cm by 20 cm quadrats (400 cm^2), from two random locations within each treatment plot. The two random location "coordinates" were consistent throughout all plots to remove positional bias from possible seed movement during rain events. Mid-way through the growing season, the vegetation in the plots was clipped to a height of 6" once during the 1st growing season. This was done because typically in prairie restorations weedy vegetation is mowed in the first couple years to deter shading of native perennials. The clippings from the quadrats were dried, weighed, and recorded. At the end of the growing season, all plant material within each quadrat, including roots, was dug up and placed into a paper bag for transport to the lab. In the lab, the plants were rinsed to remove any attached soil. The samples were air dried until no change in mass was detected (due to moisture loss) and then weighed. This mass data was added to the data from material collected from those same quadrats mid-year.

Common ragweed (*Ambrosia artemisifolia*) is a common weed that grows extensively throughout the Tri-State area, and has considerable tolerance for metal-contaminated soil. Since these plants grew in nearly all of the plots, this species was selected for measuring the uptake of metals from the soil. At the end of the year, a ragweed plant from each plot was randomly selected for plant tissue metals content analysis. After drying, the plant material was finely ground and scanned in triplicate using an X-Ray Fluorescence device similar to the method used by Gutierrez-Gines et al. (2013). Additionally, six samples, representing the full range of observed Pb concentrations, were analyzed using laboratory digestion methods (EPA 3052) in order to confirm the XRF results. The soil within the root zone of the ragweed plant was scanned with the XRF to determine the metal content of the soils within the root zone.

Data Analysis

Metals, nutrients, plant biomass and diversity, toxicity testing and PPCP concentration data were used to evaluate relative risks and benefits of various amendments versus untreated soils. Metals and nutrient runoff data were compared to the Missouri Aquatic Life Criteria (10 CSR 20-7). The water quality criteria used are listed in Table A-2. Sample concentrations were also compared to ambient conditions in two receiving streams in the potential restoration area, Center Creek and Ben's Branch. Ambient concentrations were estimated by finding the average of data reported in the Missouri Water Quality Assessment Database for a 10 year period from January 1, 2005 to December 31, 2014. Ambient concentrations are listed in Table A-3. Literature values for PPCPs were used to evaluate potential ecological concerns with PPCPs.

Table A-2. Selected water quality criteria for ammonia and dissolved metals.

Contaminant	Criteria Value	Units	Source
Ammonia			
- Acute	10.1 - 46.8	mg/L	MO WQS[1]
- Chronic	2.2 - 5.9	mg/L	MO WQS[1]
Dissolved Zinc			
- Acute	65 - 255	µg/L	MO WQS[2]
- Chronic	65 - 225	µg/L	MO WQS[2]
Dissolved Lead			
- Acute	30 - 172	µg/L	MO WQS[2]
- Chronic	4 - 7	µg/L	MO WQS[2]
Dissolved Cadmium			
- Acute	2.4 – 11.6	µg/L	MO WQS[2]
- Chronic	0.2 – 0.5	µg/L	MO WQS[2]

1: WQS for Ammonia are dependent on water temperature and pH.

2: WQS for metals are dependent on hardness.

Table A-3. Ambient nutrient and metal concentrations in Center Creek and Bens Branch, from samples collected during the period January 1, 2005 to December 31, 2014.

Analyte	Units	Center Creek			Bens Branch		
		Average	Max	Min	Average	Max	Min
Total Nitrogen	mg/L	2.58	4.2	1.35	No Data		
Total Phosphorus	mg/L	0.15	1.26	0.03			
Ammonia	mg/L	0.04	0.38	0.01			
Dissolved Lead	µg/L	4.9	103.7	0.17	2.03	10	0.5
Dissolved Zinc	µg/L	234.47	2260	2.01	2776.54	9230	572
Dissolved Cadmium	µg/L	1.95	13.35	0.06	11.73	61.2	0.12
Hardness	mg/L	180.53	240	74.1	503.82	750	290
pH		7.79	8.6	6.4	7.51	7.9	6.77

Run-off Water Quality

Results:

May 2014 Samples

A larger than average rainfall event between May 24th and 26th (3.11 in), resulted in the displacement of many of the collection basins and thus samples were only available from a limited number of plots. Complete samples were collected from plots 1 (AG High), 3 (CM Low), 4 (Control), and 7 (BS Low). Composite samples were collected from multiple collection basins within each of the CM High and BS High treatments, and no sample was collected from the AG Low treatment. Results are shown in Figure A-2.

Total Nitrogen concentrations ranged from 4.17 mg/L in the CM Low sample to 186.9 mg/L in the BS High sample. Both of the CM samples had lower N concentrations than the Control sample (8.31 mg/L). The AG High sample had a similarly high concentration (184.2) to the BS High treatment, while the BS Low sample had a concentration 43% lower than the BS High sample. Nitrate and Nitrite made up only 0.1% of total N in the Ag High sample but made up 20.6% of the Control sample. Nitrate and Nitrite made up 0.7% and 1.1% of the CM Low and CM High samples respectively, and 8.6% and 8.0% of the BS Low and High samples respectively. Phosphorus concentrations in most samples ranged between 1.05 mg/L in the Control sample to 7.55 mg/L in the CM High sample, with the exception of the AG High sample which had a concentration of 181 mg/L.

Total and dissolved lead and zinc concentrations were higher in all of the experimental treatments compared to the control sample. In the control sample, total Pb and Zn were 133 and 1010 µg/L while dissolved Pb and Zn were 5.35 and 169 µg/L respectively. Total Lead concentrations in the experimental treatments ranged from 1120 µg/L in the BS High sample to

1860 mg/L in the CM High sample, while dissolved lead ranged from 44.1 µg/L in the BS Low sample to 826 µg/L in the CM High sample. Total Zn concentrations ranged from 7850 µg/L in both the AG high and CM Low samples to 8740 µg/L in the CM High sample, while dissolved Zn ranged from 634 µg/L in the BS Low sample to 2020 µg/L in the CM High sample. Most dissolved cadmium concentrations were between 25.6 µg/L (BS High) and 29.9 µg/L (BS Low, Ag High), with the exception of the CM High and Low samples which had 99.3 and 61.1 µg/L respectively.

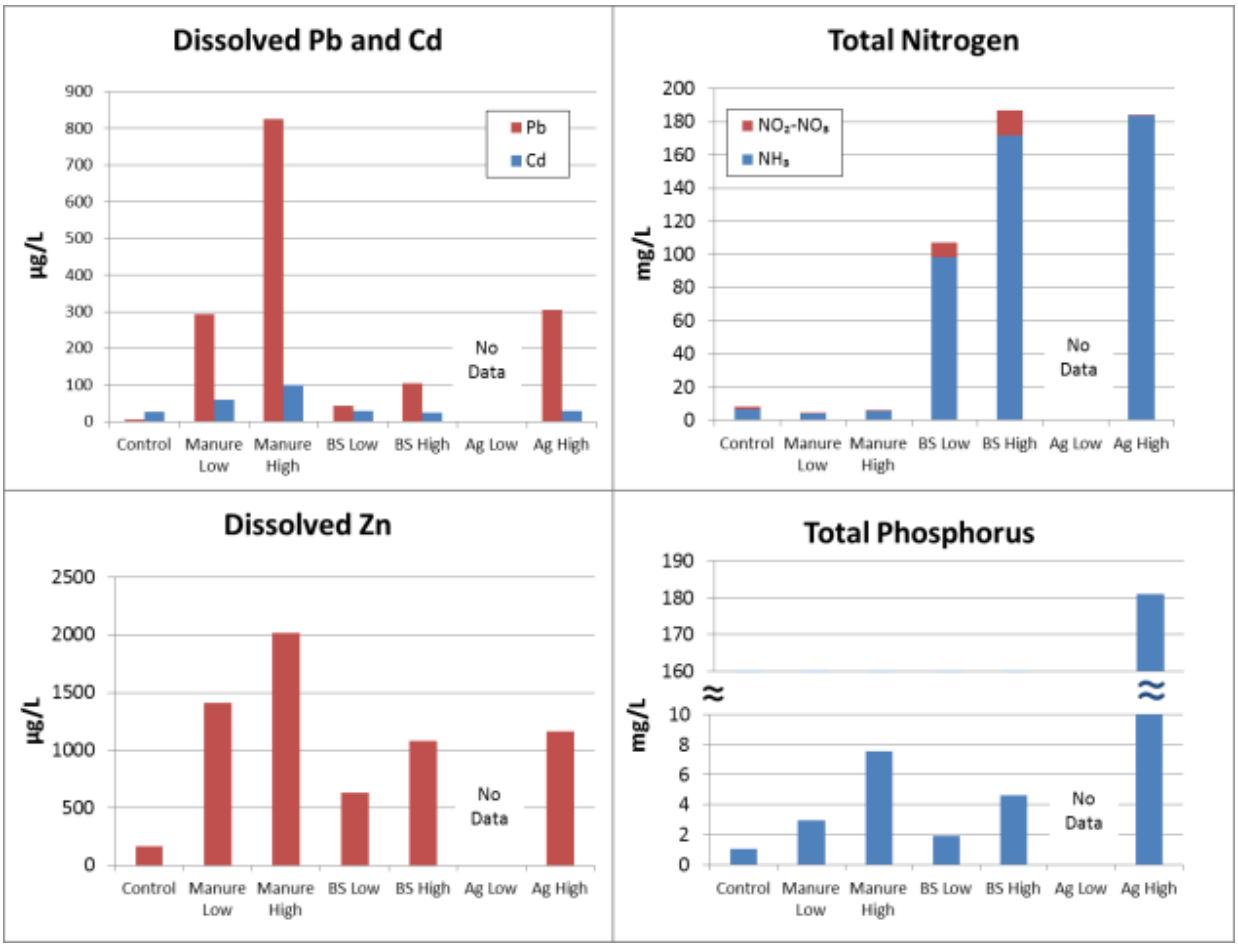

Figure A-2. Nutrient and dissolved metal concentrations in runoff samples collected on May 27, 2014.

June 2014 Samples

A rainfall event in June produced a better yield of samples than the previous sampling attempt in May. The collection basin below Plot 17 contained insufficient water to collect a sample, and the collection basins below Plots 14 and 21 were dislodged by the runoff. Samples were collected from the voids below the collection basins at those two sites and the samples were flagged as conditional due to the extremely turbid water and unusual conditions. The data from these samples showed extreme concentrations of many metals, thus these two samples were removed from statistical analysis.

Samples from the Control treatment had the lowest average TN concentration (1.8 mg/L) while the Ag Low treatment had the highest concentration (82.6 mg/L). Though the Ag Low treatment had the highest average concentration, an outlying sample (over 80% lower than the other two samples) resulted in a finding of no statistical difference with any other treatments. When the outlying sample is removed, the resulting average (115.5 mg/L) is statistically higher than all other treatments. Average TN concentrations in both of the BS treatments and the Ag high treatments were statistically higher than the Control. There were no significant differences between application rates among treatments, except when the outlying Ag Low sample was removed. Average TP concentrations ranged from 0.14 mg/L in the Control treatment to 51.8 mg/L in the Ag High treatment. There were no significant differences between application rates among treatments.

Total lead and zinc concentrations were highly variable and there were no significant differences between treatments or application rates. For example, Total Pb concentrations among Control samples varied from 154 ug/L, the lowest concentration measured, to 840 ug/L, while total Zn concentrations ranged from 1120 ug/L to 5360 ug/L, in each case spanning almost the

entire range of observed concentrations. This observed variability is likely a result of varying levels of suspended sediment in the collection basins. Due to this variability in the results of Total Metals analysis, the focus of the discussion is on dissolved metals.

The Control plot samples had the lowest average dissolved Pb, Zn, and Cd concentrations. The ranges of concentrations measured in each treatment are shown in Figure A-3. Average dissolved Pb ranged from 10.3 μg/L in the Control samples to 230.1 μg/L in the CM High samples. There were no significant differences between the High and Low application rates within any treatment types, or between the Control samples and any of the experimental treatments. Average dissolved Zn ranged from 80.8 μg/L in the Control samples to 1906.7 μg/L in the Ag Low samples. Dissolved Zn concentrations in the Ag High, Ag Low, and BS Low plots were significantly lower than the Control plots, while there were no significant differences between application rates among any on the treatments. Average dissolved Cd concentrations ranged from 14.0 μg/L in the Control samples to 50.3 μg/L in the BS Low samples. There were no significant differences among dissolved Cd concentrations.

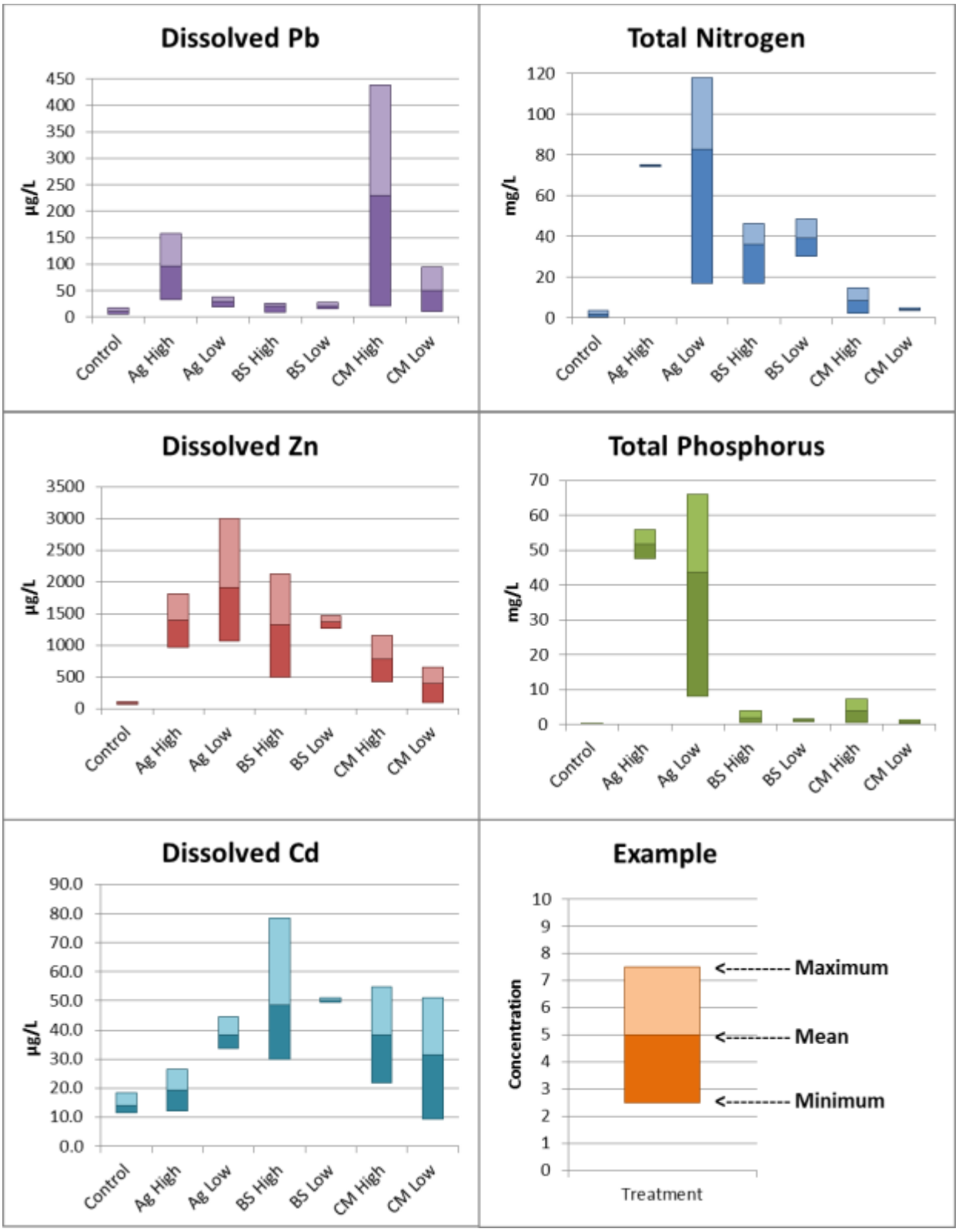

Figure A-3. Box plots showing the range of dissolved metal and nutrient concentrations in runoff samples collected from experimental plots, June 10, 2014.

September 2014 Samples

Average TN concentrations among treatments varied from 1.67 mg/L in the CM Low samples, to 96.53 mg/L in the BS High samples. Although TN concentrations from the high application plots were generally higher than that of the low application plots, there were no significant differences between application rates among CM or Ag treatments. In general, there was a high degree of variation within treatments with Coefficients of Variation (CV) ranging from 0.12 to 0.74. As with previous sampling events, TP concentrations from the Ag High plots were much higher than the other treatments. Most average TP concentrations ranged between 0.15 mg/L from the Control plots and 3.6 mg/L from the Ag Low plots while the Ag High treatment had an average concentration of 14.5 mg/L, which was significantly higher than all other treatments. There were no significant differences between application rates among either the BS or CM treatments.

Contrary to previous sampling events, average dissolved lead concentration was highest among the control samples compared to the experimental treatments. Average dissolved lead concentrations ranged from 10.42 ug/L in the Ag High treatment, to 32.37 ug/L in the Control treatment. There were no significant differences in dissolved lead concentrations between application rates among any of the treatments. Dissolved lead concentrations in the Ag High and BS Low samples were significantly lower than that of the Control treatment. Average dissolved zinc concentrations ranged from 291.7 ug/L in the CM Low treatment to 9290 ug/L in the Ag high treatment with the Control samples having an average of 416.7 ug/L. Concentrations in samples from both of the Ag treatments and the BS High treatment were significantly higher than the Control treatment. There were no significant differences in dissolved zinc concentrations between application rates among any of the treatments. Dissolved Cd

concentrations ranged from a low of 13.1 μg/L in the CM Low treatment, which was significantly lower than the Control treatment (22.7 μg/L), to a high of 76.4 μg/L in the Ag High treatment, which was significantly higher than the Control treatment. There were no significant differences between application rates within treatments.

Discussion:

Missouri Water Quality Standards for nutrients in streams are based on NH_3. Only the first round of samples from May was analyzed for NH_3. Concentrations from the Control and Manure treatments were below the Acute Criteria and the Manure treatments were within the range of the Chronic Criteria. The AG and BS treatments exceeded both the Chronic and Acute criteria. All of the NH_3 concentrations measured in the May samples were well above the range of values reported for Center Creek.

Total nitrogen from all of the AG and BS samples exceeded the range of concentrations measured in Center Creek. Average TN concentrations from the CM Low plots during all three sampling events were within the range of concentrations measured in Center Creek and the average from the September samples was 35% lower than the average from Center Creek. The September average TN from the CM High plots was also within the range of values reported for Center Creek.

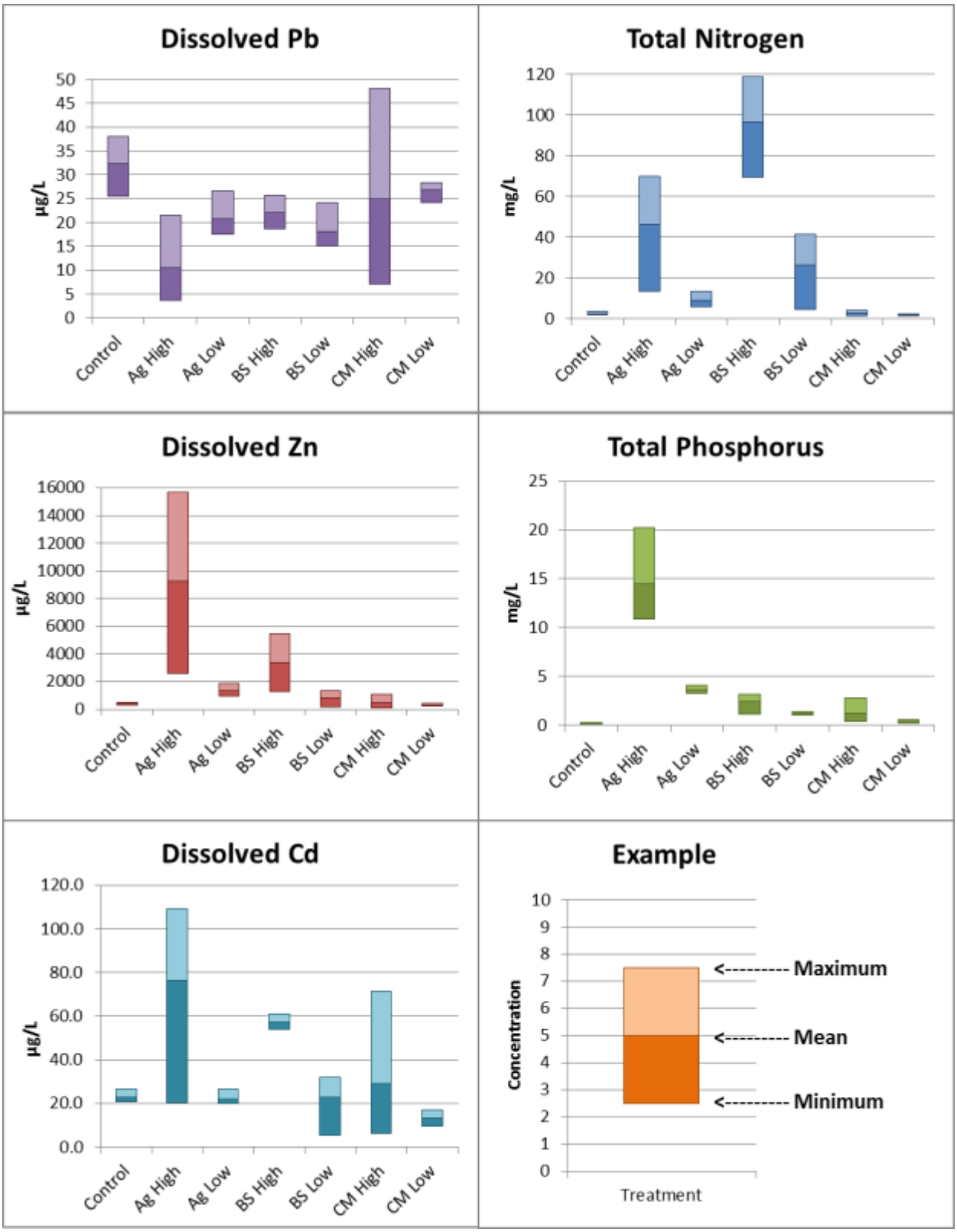

Figure A-4. Box plots showing the range of dissolved metal and nutrient concentrations in runoff samples collected from experimental plots, September 18, 2014.

In May, TP concentrations from all of the experimental treatments exceeded the range of values measured in Center Creek. In June, average TP from the BS Low and CM Low treatments was within the range of Center Creek values, and by September the average TP from the CM High treatment was as well. Only the Control treatment samples from June and September had average TP concentrations which were equal to or below the average TP measured in Center Creek.

In the samples collected in May, dissolved Pb concentrations were above the WQS in all treatments except the Control (5.35 µg/L), and BS Low (44.1 µg/L) which were below the acute criteria. Two weeks later, however, only the Ag High and CM High samples exceeded the acute criteria. By September, samples from all treatments were below the acute criteria for Pb, and all of the experimental treatments had lower concentrations than the control plot. Throughout the sampling, average concentrations from all treatments exceeded the chronic criteria, although individual samples from various treatments were occasionally below the criteria. These results indicate that the application and incorporation of the amendments causes an initial flush of dissolved Pb in the runoff, followed by an overall reduction in dissolved lead relative to the control plots. These findings are consistent with patterns over time of Pb in runoff from P treated soils reported by Weber et al. (2015).

Average dissolved Zn concentrations exceeded the WQS in all treatments except the control samples from May and June. Consequently, most samples exceeded the average Zn concentrations measured in Center Creek, as that value (234.5 ug/L) is less than the maximum WQS (255 ug/L). However, most treatments had Zn concentrations lower than the average measured in Ben’s Branch, and lower than the maximum concentration measured in Center

Creek. Only the average AG High treatment had an average Zn concentration that exceeded the maximum value measured in Ben's Branch.

In general, nutrients in the runoff from the manure treatment plots were not elevated significantly over the control plots, which indicates that applying manure at these rates does not pose a risk to the receiving streams. Runoff from the AG plots contained unacceptably high levels of nutrients. There seems to be an initial flush in nutrients and metals following the application of the amendments, but that effect tapers off with time as seen in Figure A-5. Certain treatments, notably the BS High and Ag High samples, showed an increase in concentrations of certain analytes between subsequent samples (Figure A-5). Those results are inconsistent with the majority of the treatments which show consistent decrease in most metal and nutrient concentrations over time. It is unclear what caused concentrations to increase in certain treatments, however those could be influenced by changes in pH over time, which was not measured, or differences in the intensity or timing of the rain events.

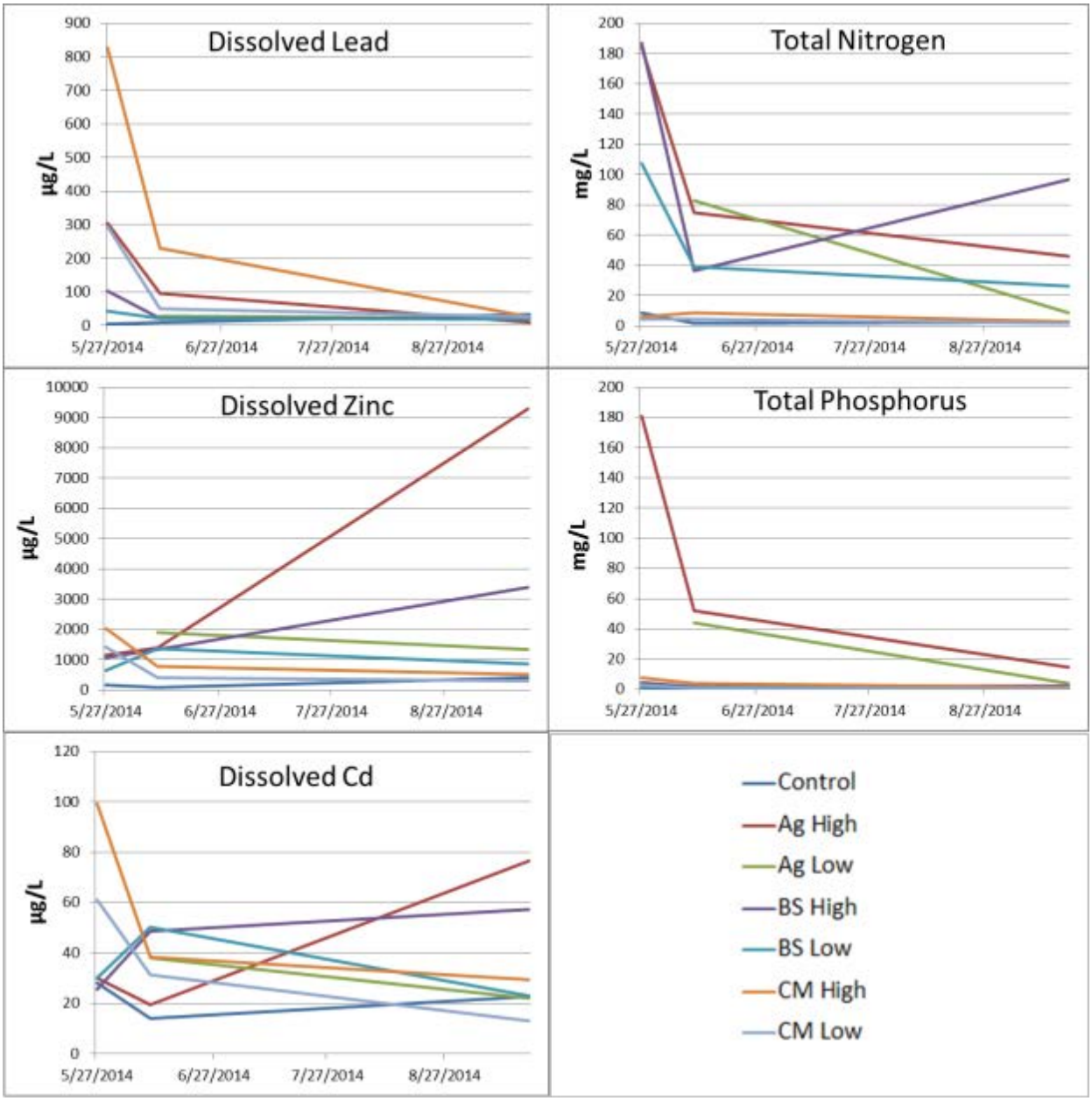

Figure A-5. Temporal trends in dissolved metal and nutrient concentrations in runoff collected from experimental plots.

Plant Diversity and Metal Uptake

Results:

Metal Uptake

In 2014,the ragweed plants in the AG High rate of application had significantly (p=0.05) more zinc than control, which averaged 972 ppm. The plants growing in the AG Low rate of application had significantly less zinc than control. The zinc levels of plants within the biosolids and manure treatments were similar to the plants in the control. There was a significantly (p=0.05) higher amount of lead in control plant tissue (45.6 ppm) than plant tissue from the manure, biosolids, and the AG High treatment. Cadmium levels within plants from the CM and BS treatment plots were no higher than the Control (26.1 ppm cadmium), but the plants from the AG plots had higher cadmium levels than Control. These differences were borne out in both XRF results and lab analyses.

Table A-4 Average plant tissue concentrations from ragweed harvested in 2014.

Plant tissue metal concentrations (ppm)			
	Pb	Zn	Cd
Control	45.6	972.6	26.1
Ag High	15.0	3389.8	35.0
Ag Low	56.9	1605.4	32.6
BS High	27.1	804.6	28.1
BS Low	24.2	1114.8	23.6
CM High	23.2	1391.1	23.3
CM Low	30.3	923.3	25.7

The soils within the root zones of the ragweed plants, as well as the plot averages, varied in their content of metals. To account for the difference in soil metal concentrations between

plots and their potential impact on plant uptake, a bioaccumulation factor (BAF), expressed as the percent uptake, was calculated by dividing the metals within the plant shoots by the average (3 XRF readings taken from the plot and 1 reading at the root zone) of metals present in the soil (see Figure A-6). The control plot ragweed plants had an average uptake of 4% of the soil lead, 14% of the soil zinc, and 31% of the soil cadmium. Only the Ag treatments had significantly different (higher) zinc BAFs than the control. Average BAFs for lead ranged between 2.1% and 7.9% and there were no significant differences between the Control plots and any of the experimental plots. The cadmium BAF of the AG, BS, and CM low treatment plants was significantly higher than Control, plants in the high rate treatment of manure had significantly less uptake of cadmium (24%) than the Control.

The rate of uptake was not the same for all treatments, and this indicates that the plant tissue metals uptake can be affected by soil amendments. Overall, the AG treatment plants tended to accumulate more metals than the control, while the other treatments' plants tended to accumulate about the same rate of metals, on average, as the control.

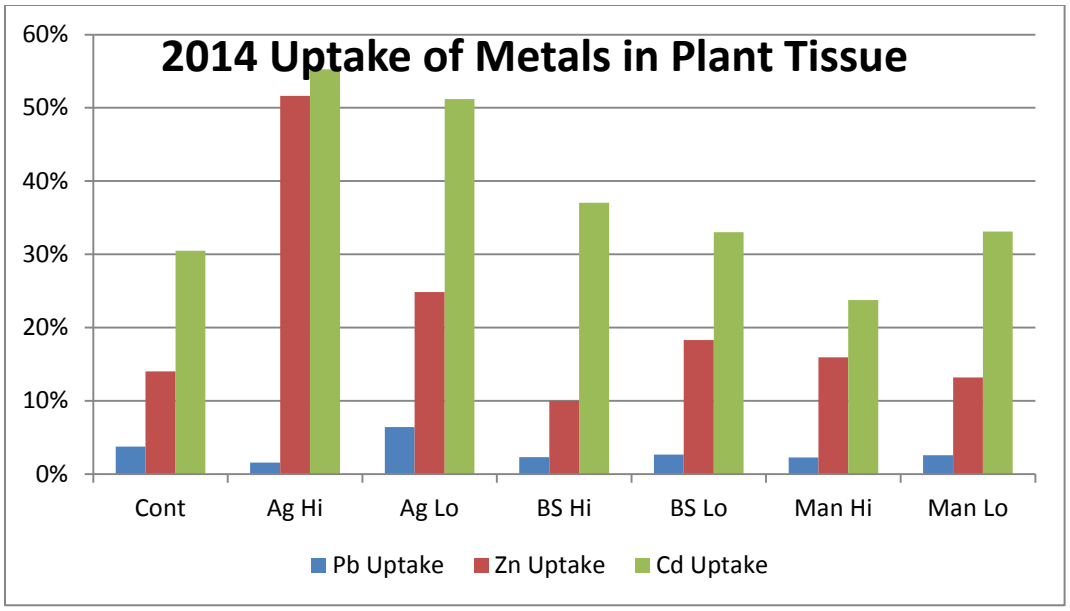

Figure A-6. Plant uptake of metals expressed as a percentage of metal concentrations in the soils.

In 2015, ragweed plants were again harvested at the end of the season and analyzed for metal content within the shoots (See Figure A-7). In general, there was a reduction of lead uptake within plant tissue (including control) in 2015, an increase in cadmium tissue uptake (including control), and the zinc uptake varied. Since the Control plots varied in step with the other treatments from year to year, this points to an overall change unrelated to the treatments being tested. Because of this, no further statistical analysis was conducted on year to year comparisons.

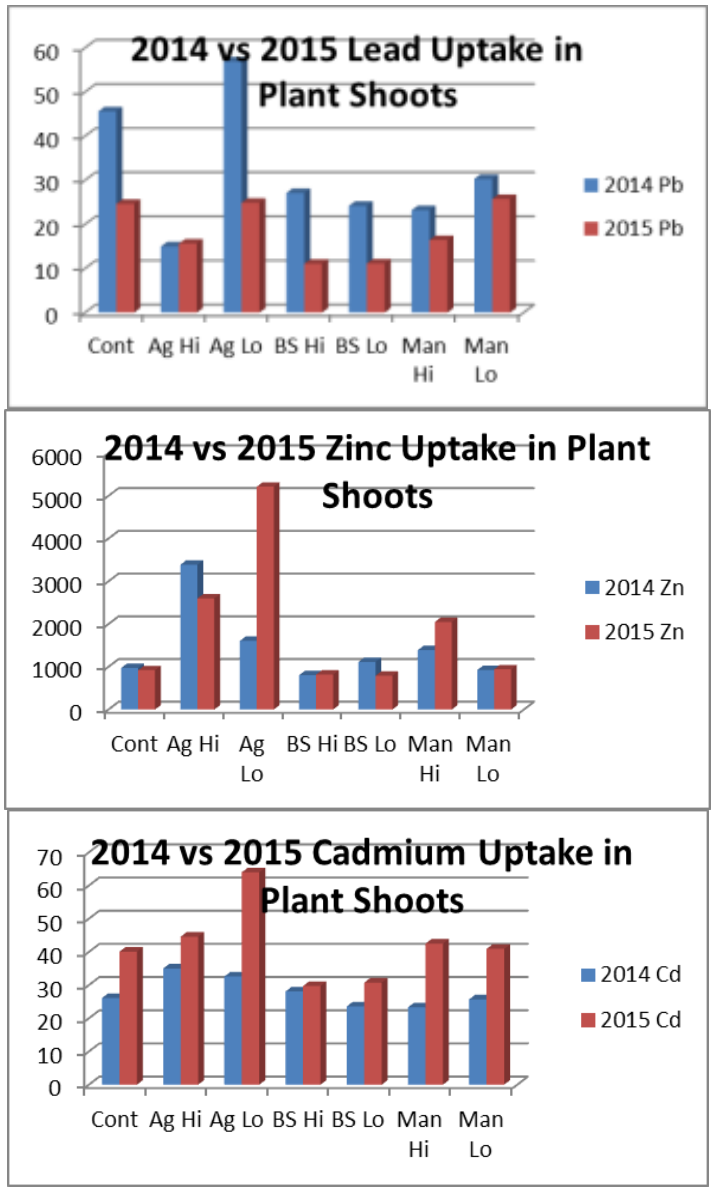

Figure A-7. A Comparison of Plant Tissue Metals Uptake Between the 2014 and 2015 seasons.

Plant Biomass

The control plots produced an average of 6.2 grams of dry plant material per 400 cm^2 quadrat. This translates to 0.16 kg/m^2. Because there was no significant difference between the rates of application within both the AG and CM treatments, the data from the two rates within these treatments was combined to compare with the control data. The CM treatments produced

significantly more plant biomass (0.23 kg/m^2) than the control, while the AG treatment was not significantly different. The biosolids treatment rates were clearly significantly different, and each was significantly higher (0.56 kg/m^2 and 0.98 kg/m^2, high and low) than the control.

Plant Diversity

Average plant species richness and diversity observed for each treatment in the non-remediated soils are shown in Figure A-8. Of the soil amendment treatments, only the manure grew a significantly greater richness of seeded species and a significantly higher diversity of seeded species than the control (p=0.05) for both the 2014 and 2015 seasons. The other treatments were either significantly lower or not significantly different than control in terms of richness (seeded and total) and diversity (seeded and total) in both 2014 and 2015. The manure treatment (rates were combined since they were statistically similar) performed best in growing the selected native seed mix. The manure treatment also grew a significantly greater total species richness (total richness includes volunteer species that sprouted from the soil/amendments) than the Control in 2014, but this difference was not significant for 2015.

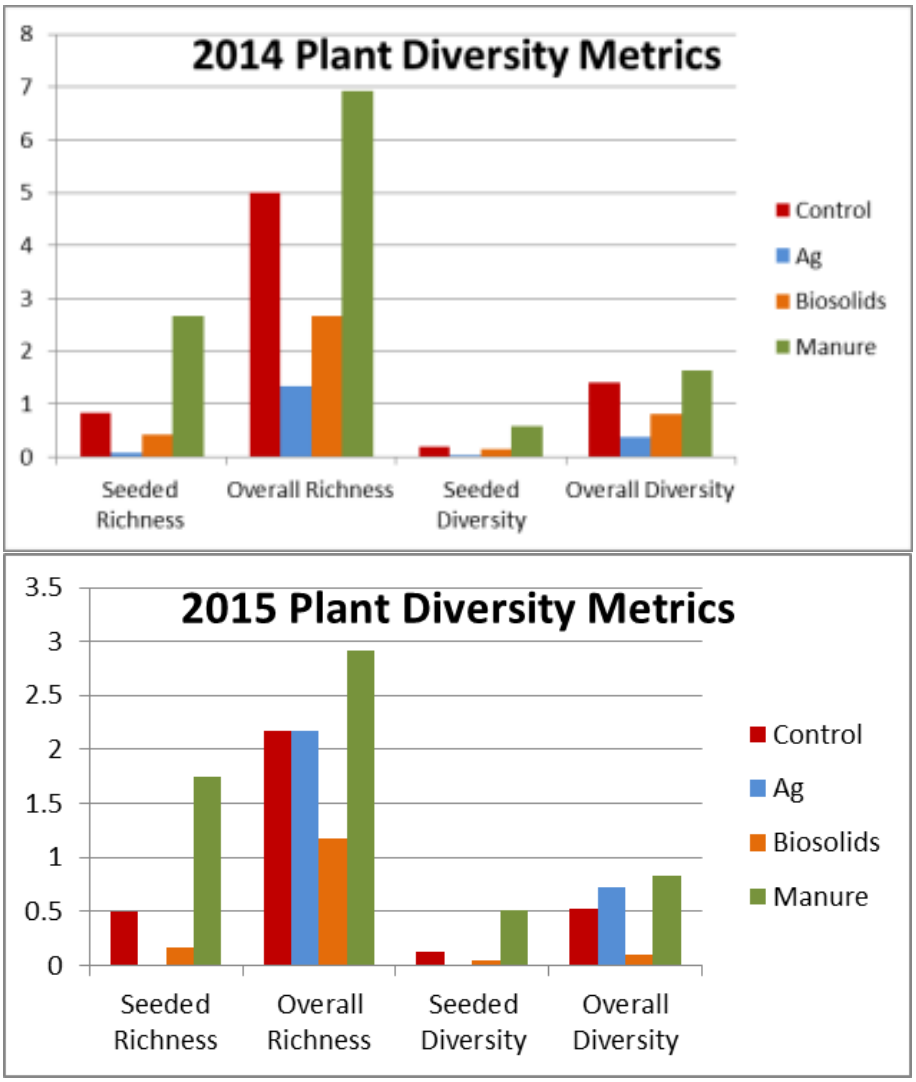

Figure A-8. Average plant species richness and diversity in experimental soil treatment plots.

Discussion:

Our initial plots, the "Non-Remediated Soil" plots, were a worst-case scenario in many ways. The soil zinc concentrations were double the expected concentrations in the remediated areas. In order to accommodate a minimum time for amendment composting, seeds were planted at the extreme end of the planting season, which likely affected germination rates. The composting process was minimal, and therefore affected texture as well as the chemical

properties of the amendments. Despite all of these factors, we did see the germination of a number of native species, and were able to discern significant differences between the treatments.

Plant Diversity

The manure treatment proved the best stand-alone treatment, prevailing in terms of highest seeded diversity and richness, while the other treatments were significantly worse than control (AG), or statistically the same (BS). Because of its ability to grow a diversity of native plants on contaminated soil, cow manure seemed ideal for use in large scale restoration. The AG treatment performed poorly in every respect, and is not a feasible amendment option. Because the biosolids are a free amendment, the second phase of this pilot study, on remediated upland clay soils, combined the biosolids with two types of manure to see if the plant performance was affected.

In the 2015 season , there was a decline in the diversity and richness across nearly all the treatments from the 2014 season (the AG treatment showed improved overall diversity and richness). The reason for this decline is not clear, though the study-wide decline, including in the Control plots, points to large-scale environmental factors. It could be that the metal contaminated soil has taken its toll on the plants, or that weather was not as conducive to a variety of plants this year. Interestingly, the number of ragweed plants in the quadrats increased nearly six-fold from 2014, while the overall number of plants within the quadrats declined. We did not clip plants to simulate mowing in 2015, and this allowed the ragweed plants to dominate the canopy in some plots. The increase in ragweed may have caused the overall declines of other species due to its quick growth rate and allelopathic qualities.

Metal Uptake

Initially, we had intended to test fescue stems for their accumulation of metals. Unfortunately, the fescue died out, and the only plant species growing in nearly all plots was common ragweed, a known hyperaccumulator of metals. See the Discussion section of the main text for details.

The risk to herbivores by zinc in plants is minimal, the main concern is the shoot content of lead and cadmium. The uptake of lead by the ragweed plant stems was consistently around 3% of the soil lead level, the average of which was approximately 1000 ppm. Toxicity from plant herbivory is discussed in detail within the section on remediated soils. When applying the soil amendments to the non-remediated plots, no lime was added to the soil. This was because the only data available at the time was the pH of the soil, and this was near neutral when tested. Literature suggests that liming the soil in conjunction with sludge application will lower the metal concentrations in plants. In a similar situation, concentrations of Cd in leaves and roots and concentrations of Pb and Zn in roots of *Ambrosia trifida* were significantly lower in plants collected from limed compared to unlimed sludge-treated areas (Peles, et al.1998). Our non-remediated plot plants had higher metals concentrations than what we would expect when restoring areas with lime and soil amendments. We revised out methods and added lime at the outset to the remediated plots that were started subsequently.

APPENDIX B: Pharmaceutical and Personal Care Product Analysis Supplemental Tables.

Table B.1. Description of material sampled for pharmaceuticals and personal care products (PPCPs) in organic amendments and wastewater treatment plant (WWTP) effluent.

PPCP Samples of Amendments and WWTP Effluent

Sample Media	Sample Label
Uncomposted Webb City and Springfield Biosolids	Raw Biosolids
Uncomposted Cow Manure from Joplin Stockyard	Raw Cow Manure
Uncomposted Poultry Litter from Opal Foods	Raw Poultry Litter
Webb City Waste Water Treatment Facility Outfall Discharge	WWTF Effluent
Composted Springfield and Webb City Biosolids + woodchips high application rate	SBSH1
Composted Springfield and Webb City Biosolids + woodchips low application rate	SBSL1
Composted Poultry litter+ 10% Webb City biosolids + woodchips high application rate	SPBSH1
Composted Cow Manure + 10% Webb City biosolids+woodchips high application rate	SPBSL1

Table B.2. Description of water samples from upland runoff and wetland tub effluent analyzed for pharmaceuticals and personal care products.

PPCP Samples of Runoff and Wetland Water

Sample Media	Sample Label
Composite of upland runoff sample of composted biosolids high application rate	BS High
Composite of upland runoff sample of high application rate composted cow manure and 10% biosolids	CM+ High
Composite of upland runoff sample of low application rate composted cow manure and 10% biosolids	CM+ Low
Composite of upland runoff sample of high application rate composted poultry litter and 10% biosolids	PL+ High
Composite of upland runoff sample of low application rate composted poultry litter and 10% biosolids	PL+ Low
Composite of upland runoff sample of composted biosolids low application rate	BS Low
Composite of upland runoff sample of composted cow manure and 10% biosolids low application rate	CM+ Low Dup
Wetland tub water sample with composted biosolids amendment	BS
Wetland tub water sample with cow manure/10% composted biosolids amendment 1st replicate	CM+ Rep1
Wetland tub water sample with cow manure/10% composted biosolids amendment 1st replicate duplicate	CM+ Rep2
Wetland tub water sample with no soil amendments 1st replicate	Control Rep1
Wetland tub water sample with no soil amendments 2nd replicate	Control Rep2
Wetland tub water sample with poultry litter/10% composted biosolids amendment 1st replicate	PL+ Rep1
Wetland tub water sample with poultry litter/10% composted biosolids amendment 2nd replicate	PL+ Rep2

Table B.3 Pharmaceutical and personal care compounds assayed.

PPCP Analytes			
Antibiotics	**Antimicrobials**	**Medicinal Non-antibiotics**	**Other Non-Medicinal**
4 Epianhydrochlortetracycline	Sulfathiazole	2 Hydroxy ibuprofen	bisphenol A
4 Epichlortetracycline (ECTC)	Sulfadimethoxine	Acetaminophen	caffeine
4 epioxytetracycline (EOTC)	Sulfamerazine	Dehydronifedipine	Thiabendazole
4 Epitetracycline (ETC)	Sulfamethazine	Diltiazem	
Anhyrdrochlortetracycline (ACTC)	Triclocarban	Diphenhydramine	
Anhydrotetracycline (ATC)	Triclosan	Carbemazepine	
Azitrhomycin		Fluoxetine	
Carbadox		Furosemide	
Chlortetracycline (CTC)		Gembfiborzil	
Ciprofloxacin		Hydrochlorothiazide	
Clarithromycin		Ibuprofen	
Doxycycline		Naproxen	
Enrofloxacin			
Erythromycin H2O			
Isochortetracycline			
Licomycin			
Miconazole			
Minocycline			
Ofloxacin			
Oxolinic Acid			
Oxytetracycline (OTC)			
Sulfadiazine			
Sulfamethizole			
Sufamethoxazole			
Tetracycline (TC)			
Trimethroprim			

Table B.4. List of PPCP compounds detected in raw amendments and WWTP effluent. Raw amendment or effluent samples that exceed a relevant LOAEC or NOAEC are highlighted in yellow.

PPCP Detects in Raw Amendments and Effluent Samples

	Dry Weight in µg/kg			Aqueous in µg/L	
Constituent \ Sampled Media	**Raw biosolids**	**Raw cow manure**	**Poultry litter**	**WWTF Effluent**	**LOAEC/Aquatic Bio Endpoint**
2 Hydroxy ibuprofen	167			0.104	
4 Epichlortetracycline (ECTC)	35.4	272			
4 epioxytetracycline (EOTC)		16.5			
4 Epitetracycline (ETC)	27.9	35.5			
Anhyrdrochlortetracycline (ACTC)				0.0149	
Anhydrotetracycline (ATC)	36.5				
Azitrhomycin	118			0.209	
Bisphenol A	1340				0.1/ gene expression
Caffeine				0.0186	0.05/amphibian malformation
Carbadox					
Carbemazepine	25.2			0.0933	100/algal growth
Chlortetracycline (CTC)	37.2	200	16.2		36/algal assemblage
Ciprofloxacin	4.74				
Clarithromycin				0.0246	5/bacterial growth
Dehydronifedipine				0.00314	
Diltiazem	2.86			0.0552	
Diphenhydramine	557			0.122	5.6 / behavioral
Doxycycline	439			0.0116	
Enrofloxacin	12.3				18.9/ penaeid shrimp
Erythromycin H2O	4.45	4.44		0.0515	22700/ penaeid shrimp
Fluoxetine	298			0.0199	28/neurological/reproductive
Furosemide	292			0.0434	
Gemfibrozil	160			0.0331	30400/crustacean
Hydrocholorthiazide				0.0854	
Ibuprofen	368				21/hormonal
Isochortetracycline		182			

Licomycin	12.6	41			
Miconazole	299				
Minocycline	138				
Naproxen	30.6	9.33		0.0421	
Ofloxacin	4500	20.5		0.243	10/NOAEC Growth
Oxolinic Acid					10000/bacterial
Oxytetracycline (OTC)	11.9	43.4			50/growth macrophytes
Sulfadiazine				0.00516	17000/ bacterial growth
Sulfadimethoxine					
Sulfamerazine					
Sulfamethazine		14.1			8/ algal growth
Sulfamethizole					
Sufamethoxazole				0.519	0.8/algae growth
Sulafathiazole					
Tetracycline (TC)	29.9	35.2			1/algal growth
Thiabendazole	14.2			0.0043	310/crustacean
Triclocarban	8480			0.0211	0.05/reproduction
Triclosan	22300				0.15/algal assemblage, 12.8 reproduction
Trimethroprim				0.392	180/ NOAEC bacteria
Total Detects	28	12	1	22	

APPENDIX C: Soil Chemistry Test Results

Table C-1: Soil sample results from non-remediated soils test plots, prior to adding soil amendments.

		milliequivalents per 100 grams									
		NH_4Cl Extractable Bases					Extractable	Sum of		% Base Saturation	
Treatment	Plot	Ca	Mg	Na	K	Sum	Acidity	Cations	CEC	Sum	CEC
Control	Plot 2	17.0	1.0	0.0	0.2	18.2	2.0	20.2	11.3	90	161
CM Low	Plot 5	18.0	0.7	0.0	0.2	18.9	1.3	20.2	10.0	94	189
CM High	Plot 9	24.5	0.7	0.0	0.2	25.4	1.0	26.4	9.2	96	276
BS Low	Plot 11	19.7	0.5	0.0	0.2	20.4	4.5	24.9	8.9	82	229
BS High	Plot 12	20.4	0.6	0.0	0.2	21.2	0.9	22.1	9.0	96	236
Ag High	Plot 1	14.1	0.7	0.0	0.2	15.0	1.7	16.7	9.5	90	158
Ag Low	Plot 8	23.9	0.7	0.0	0.2	24.8	1.4	26.2	9.9	95	251

		% Organic	pH		Bray 1 P	% Total	Mineralizable Nitrogen	Active Carbon	% Water Stable
Treatment	Plot	Carbon	$CaCl_2$	H_2O	(ppm)	Nitrogen	(ppm)	mg C/kg soil	Aggregates
Control	Plot 2	0.59	7.2	7.6	4.2	0.051	10.0	112.3	17
CM Low	Plot 5	0.72	7.4	7.8	4.3	0.045	9.0	172.8	14
CM High	Plot 9	0.88	7.5	8.0	4.5	0.071	15.5	187.9	7
BS Low	Plot 11	0.86	7.5	8.0	4.8	0.050	15.0	187.9	8
BS High	Plot 12	0.72	7.5	7.9	3.0	0.056	10.0	146.9	10
Ag High	Plot 1	0.69	7.2	7.6	4.3	0.048	11.5	138.2	15
Ag Low	Plot 8	0.71	7.5	8.1	4.3	0.048	8.0	152.6	16

Table C-2: Soil sample results from non-remediated soils test plots, after adding soil amendments.

		milliequivalents per 100 grams									
		NH_4Cl Extractable Bases					Extractable	Sum of		% Base Saturation	
Treatment	Plot	Ca	Mg	Na	K	Sum	Acidity	Cations	CEC	Sum	CEC
Control	Plot 2	11.8	1.0	0.0	0.2	13.0	1.6	14.6	10.1	89	129
CM Low	Plot 5	13.2	1.8	0.5	2.4	17.9	1.6	19.5	10.5	92	170
CM High	Plot 9	16.4	2.1	0.7	3.7	22.9	1.0	23.9	11.8	96	194
BS Low	Plot 11	21.1	1.2	0.2	0.6	23.1	3.2	26.3	12.5	88	185
BS High	Plot 12	22.0	1.1	0.1	0.6	23.8	3.1	26.9	11.4	88	209
Ag High	Plot 1	4.0	2.2	0.4	13.2	19.8	7.9	27.7	27.7	71	71
Ag Low	Plot 8	6.3	1.2	0.3	8.2	16.0	5.0	21.0	21.5	76	74

		% Organic	pH		Bray 1 P	% Total	Mineralizable Nitrogen	Active Carbon	% Water Stable
Treatment	Plot	Carbon	$CaCl_2$	H_2O	(ppm)	Nitrogen	(ppm)	mg C/kg soil	Aggregates
Control	Plot 2	0.61	7.4	7.6	8.2	0.052	19.0	123.8	8
CM Low	Plot 5	2.13	7.6	7.8	32.6	0.170	107.5	396.7	8
CM High	Plot 9	2.36	7.7	7.9	58.0	0.211	124.0	422.6	12
BS Low	Plot 11	2.05	7.2	7.2	75.0	0.376	655.0	295.2	7
BS High	Plot 12	2.78	7.2	7.2	65.2	0.395	643.5	275.8	10
Ag High	Plot 1	1.69	6.8	7.0	2120.0	0.695	2635.0	311.8	33
Ag Low	Plot 8	1.61	6.8	7.1	1820.0	0.462	2515.0	220.3	44

Table C-3. Soil sample results from remediated upland clay soil plots, prior to adding soil amendments.

		milliequivalents per 100 grams									
		NH_4Cl Extractable Bases					Extractable	Sum of		% Base Saturation	
Treatment	Plot	Ca	Mg	Na	K	Sum	Acidity	Cations	CEC	Sum	CEC
Control	Plot 8	26.0	0.9	0.1	0.4	27.4	3.4	30.8	13.2	89	208
Control	Plot 14	19.4	0.9	0.0	0.2	20.5	2.2	22.7	9.8	90	209
YF	Plot 5	25.3	0.9	0.0	0.2	26.4	2.4	28.8	11.1	92	238
YF	Plot 12	18.6	0.9	0.0	0.2	19.7	2.3	22.0	8.6	90	229
YF	Plot 20	19.8	0.8	0.0	0.2	20.8	0.5	21.3	8.3	98	251
CM+ Low	Plot 2	30.6	1.4	0.0	0.3	32.3	2.5	34.8	13.3	93	243
CM+ Low	Plot 7	24.3	0.9	0.0	0.3	25.5	2.7	28.2	11.6	90	220
CM+ Low	Plot 16	18.4	1.2	0.0	0.3	19.9	2.8	22.7	9.7	88	205
CM+ High	Plot 11	19.8	0.8	0.0	0.2	20.8	2.9	23.7	11.2	88	186
CM+ High	Plot 13	21.1	1.0	0.0	0.2	22.3	2.2	24.5	10.2	91	219
CM+ High	Plot 18	15.0	0.8	0.0	0.2	16.0	3.2	19.2	8.5	83	188
BS Low	Plot 4	30.5	1.2	0.0	0.3	32.0	2.1	34.1	13.4	94	239
BS Low	Plot 10	23.7	0.7	0.0	0.2	24.6	2.8	27.4	11.5	90	214
BS Low	Plot 15	27.3	1.3	0.0	0.4	29.0	2.1	31.1	11.3	93	257
BS High	Plot 1	28.8	1.4	0.0	0.3	30.5	2.3	32.8	13.7	93	223
BS High	Plot 6	24.7	0.9	0.0	0.2	25.8	2.6	28.4	11.2	91	230
BS High	Plot 17	17.9	0.9	0.0	0.2	19.0	3.3	22.3	8.7	85	218
PL+ Low	Plot 9	11.4	0.8	0.0	0.2	12.4	4.8	17.2	11.2	72	111
PL+ Low	Plot 3	32.6	1.5	0.0	0.3	34.4	2.6	37.0	15.8	93	218
PL+ Low	Plot 21	18.7	0.9	0.0	0.2	19.8	2.0	21.8	10.5	91	189
PL+ High	Plot 19	14.6	0.9	0.0	0.2	15.7	2.8	18.5	8.6	85	183

		%			Bray 1	%	Mineralizable	Active	% Water
		Organic	pH		P	Total	Nitrogen	Carbon	Stable
Treatment	Plot	Carbon	$CaCl_2$	H_2O	(ppm)	Nitrogen	(ppm)	mg C/kg soil	Aggregates
Control	Plot 8	1.36	7.2	7.7	16.4	0.137	260.0	345.6	6
Control	Plot 14	0.69	7.3	7.8	10.8	0.062	112.5	230.4	6
YF	Plot 5	0.68	7.4	7.9	6.3	0.065	142.5	223.2	5
YF	Plot 12	0.78	7.1	7.6	33.9	0.063	127.5	208.8	6
YF	Plot 20	0.61	7.3	7.8	12.3	0.065	75.0	172.8	4
CM+ Low	Plot 2	1.21	7.4	7.9	8.4	0.116	260.0	374.4	16
CM+ Low	Plot 7	1.01	7.3	7.8	15.0	0.097	172.5	280.8	5
CM+ Low	Plot 16	0.75	7.3	7.8	20.8	0.080	130.0	223.2	7
CM+ High	Plot 11	0.91	7.1	7.6	7.7	0.089	120.0	302.4	4
CM+ High	Plot 13	0.93	7.2	7.7	24.1	0.096	187.5	288.0	10
CM+ High	Plot 18	0.77	6.8	7.2	27.6	0.072	42.5	194.4	3
BS Low	Plot 4	0.96	7.4	7.9	17.7	0.091	272.5	288.0	9
BS Low	Plot 10	1.00	7.3	7.7	10.9	0.102	200.0	295.2	4
BS Low	Plot 15	1.33	7.3	7.7	29.3	0.130	332.5	374.4	11
BS High	Plot 1	1.17	7.4	7.8	11.0	0.130	302.5	324.0	14
BS High	Plot 6	0.88	7.3	7.8	9.6	0.090	177.5	280.8	6
BS High	Plot 17	0.91	6.7	7.4	35.4	0.069	55.0	223.2	5
PL+ Low	Plot 9	0.71	6.6	7.1	3.0	0.078	60.0	187.2	2
PL+ Low	Plot 3	1.30	7.4	7.8	11.8	0.138	252.5	367.2	17
PL+ Low	Plot 21	1.14	7.1	7.6	17	0.112	182.5	338.4	3
PL+ High	Plot 19	0.59	7.0	7.5	14.2	0.050	42.5	158.4	2

Appendix C-4. Soil sample results from remediated upland clay soil plots, after adding soil amendments.

		milliequivalents per 100 grams								
		NH_4Cl Extractable Bases					Extractable		% Base Saturation	
Treatment	Plot	Ca	Mg	Na	K	Sum	Acidity	CEC	Sum	CEC
Control	Plot 8	27.2	1.0	0.0	0.5	28.7	3.0	13.5	91	213
Control	Plot 14	20.9	1.1	0.0	0.3	22.3	2.8	12.0	89	186
Control	Plot 23	21.5	1.0	0.0	0.3	22.8	2.5	11.1	90	205
YF	Plot 5	23.6	2.4	0.0	1.7	27.7	6.8	20.0	80	139
YF	Plot 12	15.2	1.8	0.0	1.0	18.0	5.8	14.6	76	123
YF	Plot 20	20.2	2.0	0.4	1.7	24.3	5.2	14.7	82	165
CM+ Low	Plot 2	25.0	4.6	0.1	3.6	33.3	3.8	19.7	90	169
CM+ Low	Plot 7	29.2	6.3	0.4	5.8	41.7	6.1	23.3	87	179
CM+ Low	Plot 16	23.1	4.4	0.0	3.8	31.3	3.0	16.5	91	190
CM+ High	Plot 11	28.3	6.6	0.7	6.8	42.4	6.0	22.7	88	187
CM+ High	Plot 13	29.9	7.6	0.9	8.6	47.0	3.7	26.7	93	176
CM+ High	Plot 18	24.1	6.1	0.6	6.4	37.2	2.6	22.4	93	166
BS Low	Plot 4	42.5	4.8	0.0	2.1	49.4	12.7	34.4	80	144
BS Low	Plot 10	27.5	3.2	0.0	1.3	32.0	7.4	20.9	81	153
BS Low	Plot 15	30.6	3.6	0.0	1.3	35.5	7.8	23.9	82	149
BS High	Plot 1	41.4	5.7	0.0	2.2	49.3	13.3	35.5	79	139
BS High	Plot 6	51.5	8.2	0.0	2.8	62.5	17.4	49.4	78	127
BS High	Plot 17	21.0	3.0	0.0	2.1	26.1	6.6	19.3	80	135
PL+ Low	Plot 3	27.2	14.4	0.6	5.1	47.3	6.8	19.6	87	241
PL+ Low	Plot 9	19.1	9.9	1.0	7.8	37.8	4.7	17.8	89	212
PL+ Low	Plot 21	19.8	9.8	1.2	7.1	37.9	6.8	16.8	85	226
PL+ High	Plot 19	13.2	7.3	1.5	5.9	27.9	3.1	12.3	90	227
PL+ High	Plot 22	21.8	14.5	2.2	10.2	48.7	6.2	17.1	89	285
PL+ High	Plot 24	17.5	8.7	2.1	11.1	39.4	1.8	20.8	96	189

		%			Bray 1	%	Mineralizable	Active	% Water	
		Organic	pH		P	Total	Nitrogen	Carbon	Stable	
Treatment	Plot	Carbon	$CaCl_2$	H_2O	(ppm)	Nitrogen	(ppm)	mg C/kg soil	Aggregates	C:N ratio
Control	Plot 8	1.6	7.2	7.3	47.6	0.189	32.0	335.5	10	10.48438
Control	Plot 14	1.2	7.1	7.2	40.8	0.127	27.5	279.4	10	10.16
Control	Plot 23	1.1	7.2	7.3	26.8	0.115	18.0	240.5	13	13.36111
YF	Plot 5	4.7	6.6	6.8	354.0	0.300	90.0	1149.1	19	12.76778
YF	Plot 12	2.8	6.6	6.9	292.0	0.242	50.5	601.9	17	11.91881
YF	Plot 20	4.1	6.8	7.2	304.0	0.280	86.0	878.4	45	10.21395
CM+ Low	Plot 2	4.3	7.3	7.4	212.0	0.374	93.0	1088.6	56	11.70538
CM+ Low	Plot 7	6.0	7.0	7.1	292.0	0.675	87.0	2062.1	58	23.7023
CM+ Low	Plot 16	4.0	7.3	7.4	177.0	0.407	78.0	1036.8	39	13.29231
CM+ High	Plot 11	6.4	6.8	6.9	292.0	0.556	69.0	2592.0	45	37.56522
CM+ High	Plot 13	7.5	7.7	7.9	170.0	0.741	107.5	1561.0	77	14.52093
CM+ High	Plot 18	5.4	7.6	7.8	220.0	0.501	97.5	1069.9	69	10.97333
BS Low	Plot 4	9.0	6.4	6.4	707.0	0.823	222.5	2318.4	51	10.41978
BS Low	Plot 10	4.6	6.7	6.8	414.0	0.117	107.5	806.4	32	7.501395
BS Low	Plot 15	6.0	6.7	6.8	512.0	0.555	152.0	933.1	48	6.138816
BS High	Plot 1	8.6	6.7	6.7	453.0	0.894	257.5	1759.7	73	6.833786
BS High	Plot 6	11.1	6.5	6.5	618.0	1.077	338.0	2592.0	76	7.668639
BS High	Plot 17	3.9	6.8	6.9	430.0	0.387	96.0	616.3	41	6.419792
PL+ Low	Plot 3	5.6	6.9	6.9	960.0	0.668	155.5	1448.6	65	9.315756
PL+ Low	Plot 9	3.8	7.5	7.6	1216.0	0.472	89.0	1111.7	58	12.49101
PL+ Low	Plot 21	4.1	6.9	6.9	1261.0	0.515	100.0	933.1	66	9.331
PL+ High	Plot 19	2.1	7.3	7.3	950.0	0.273	46.5	516.2	38	11.10108
PL+ High	Plot 22	4.8	6.9	7.0	1363.0	0.573	78.0	1635.8	59	20.97179
PL+ High	Plot 24	4.6	8.3	8.4	118.0	0.483	107.5	1097.3	66	10.20744

Yellow fill indicates that the highlighted samples consisted of ≥ 50% sand-sized particles.
The Soil Survey Laboratory Methods Manual recommends not reporting determinations on such samples.
Base saturation results above 100% indicate sample contains free carbonate that is dissolving in the extracting medium.

APPENDIX D: Avian Ecological Risk Calculations

Highest Concentrations from Amended Upland Plots

C_{sw}	NIR_w	NIR_d	NIR_{soil}	C_{soil}		C_{sed}		FD_{veg}	C_{veg}	FD_{ew}	C_{ew}	FD_{sm}	C_{sm}	FD_F	C_F	FD_{BI}	C_{BI}	C_{frog}	Fd_{frog}	AUF	ADD	TRV_{NOAEL}	TRV_{LOAEL}	HQ_{NOAEL}	HQ_{LOAEL}
0.0098	0.053	0.033	0.082	16		9.1		0.918	14	0	87.61	0	0.632	0	0.23	0	1.14	0.27	0	1.00	1.74	1.45	20.00	1.20	0.09
0.0098	0.1	0.77	0.104	16		9.1		0	14	1	21	0	0.632	0	0.23	0	1.14	0.27	0	0.50	8.92	1.45	20.00	6.15	0.45

C_{sw}	NIR_w	NIR_d	NIR_{soil}	C_{soil}	RBA	C_{sed}		FD_{veg}	C_{veg}	FD_{ew}	C_{ew}	FD_{sm}	C_{sm}	FD_F	C_F	FD_{BI}	C_{BI}	C_{frog}	Fd_{frog}	AUF	ADD	TRV_{NOAEL}	TRV_{LOAEL}	HQ_{NOAEL}	HQ_{LOAEL}
0.0288	0.053	0.033	0.082	403		418.3		0.918	12	0	10.5	0	12.81	0	1.49	0	9.34	7.57	0	1.00	33.41	1.42	11.30	23.53	2.96
0.0288	0.1	0.77	0.104	403		418.3		0	12	1	10.5	0	12.81	0	1.49	0	9.34	7.57	0	0.50	25.00	1.42	11.30	17.61	2.21

C_{sw}	NIR_w	NIR_d	NIR_{soil}	C_{soil}		C_{sed}		FD_{veg}	C_{veg}	FD_{ew}	C_{ew}	FD_{sm}	C_{sm}	FD_F	C_F	FD_{BI}	C_{BI}	C_{frog}	Fd_{frog}	AUF	ADD	TRV_{NOAEL}	TRV_{LOAEL}	HQ_{NOAEL}	HQ_{LOAEL}
1.4684	0.053	0.033	0.082	1977		1914		0.918	657	0	257	0	41.8	0	51.8	0	136	37	0	1.00	176.93	14.50	131.00	12.20	1.35
1.4684	0.1	0.77	0.104	1977		1914		0	657	1	257	0	41.8	0	51.8	0	136	37	0	0.50	201.82	14.50	131.00	13.92	1.54

Worm, Plant, Sediment, and Soil Concentrations from Cow Manure Amended Upland and Wetland Remediated Soil Plots

C_{sw}	NIR_w	NIR_d	NIR_{soil}	C_{soil}	RBA	C_{sed}		FD_{veg}	C_{veg}	FD_{ew}	C_{ew}	FD_{sm}	C_{sm}	FD_F	C_F	FD_{BI}	C_{BI}	C_{frog}	Fd_{frog}	AUF	ADD	TRV_{NOAEL}	TRV_{LOAEL}	HQ_{NOAEL}	HQ_{LOAEL}
0.0288	0.053	0.033	0.082	403		418.3		0.918	7	0	10.5	0	12.81	0	1.49	0	9.34	7.57	0	1.00	33.26	1.42	11.30	23.42	2.94
0.0288	0.1	0.77	0.104	403		418.3		0	7	1	10.5	0	12.81	0	1.49	0	9.34	7.57	0	0.50	25.00	1.42	11.30	17.61	2.21

C_{sw}	NIR_w	NIR_d	NIR_{soil}	C_{soil}		C_{sed}		FD_{veg}	C_{veg}	FD_{ew}	C_{ew}	FD_{sm}	C_{sm}	FD_F	C_F	FD_{BI}	C_{BI}	C_{frog}	Fd_{frog}	AUF	ADD	TRV_{NOAEL}	TRV_{LOAEL}	HQ_{NOAEL}	HQ_{LOAEL}
1.4684	0.053	0.033	0.082	1977		1913		0.918	293	0	171	0	41.8	0	51.8	0	136	37	0	1.00	165.82	14.50	131.00	11.44	1.27
1.4684	0.1	0.77	0.104	1977		1913		0	293	1	171	0	41.8	0	51.8	0	136	37	0	0.50	168.71	14.50	131.00	11.64	1.29

Worm, Plant, Soil, and Sediment Concentrations from Cow Manure Amended Remediated Upland and Wetland I Soil:Earthworm Bioavailability Factor Applied

C_{sw}	NIR_w	NIR_d	NIR_{soil}	C_{soil}	RBA	C_{sed}		FD_{veg}	C_{veg}	FD_{ew}	C_{ew}	FD_{sm}	C_{sm}	FD_F	C_F	FD_{BI}	C_{BI}	C_{frog}	Fd_{frog}	AUF	ADD	TRV_{NOAEL}	TRV_{LOAEL}	HQ_{NOAEL}	HQ_{LOAEL}
0.0288	0.053	0.033	0.082	403	0.31	200		0.918	7	0	10.5	0	12.81	0	1.49	0	9.34	7.57	0	1.00	5.27	1.42	11.30	3.71	0.47
0.0288	0.1	0.77	0.104	403	0.31	200		0	7	1	10.5	0	12.81	0	1.49	0	9.34	7.57	0	0.50	10.50	1.42	11.30	7.39	0.93

C_{sw}	NIR_w	NIR_d	NIR_{soil}	C_{soil}	RBA	C_{sed}		FD_{veg}	C_{veg}	FD_{ew}	C_{ew}	FD_{sm}	C_{sm}	FD_F	C_F	FD_{BI}	C_{BI}	C_{frog}	Fd_{frog}	AUF	ADD	TRV_{NOAEL}	TRV_{LOAEL}	HQ_{NOAEL}	HQ_{LOAEL}
1.4684	0.053	0.033	0.082	1977	0.40	1913		0.918	293.5	0	171	0	41.8	0	51.8	0	136	37	0	1.00	72.39	14.50	131.00	4.99	0.55
1.4684	0.1	0.77	0.104	1977	0.40	1913		0	293.5	1	171	0	41.8	0	51.8	0	136	37	0	0.50	107.47	14.50	131.00	7.41	0.82