Discovery, The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences

Volume 9 Article 10

Fall 2008

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ABSTRACT

While the plant rhizosphere and associated microbial processes have been shown to amplify the degradation rate of chemical residues in soils, phytoremediation can be a slow process. The objective of this greenhouse study was to determine if the addition of biosolids as an organic soil amendment would enhance growth of plants in oil-contaminated soil and thus potentially increase effectiveness of phytoremediation. Pearl millet (*Pennisetum glaucum* (L.) R. Br.) or sudangrass (*Sorghum sudanense* (Piper) Stapf (Piper)) was grown in a Captina silt loam (fine-silty, siliceous, mesic Typic Fragiudults) contaminated with 5% crude oil (v/w) and amended with 24 g biosolids/kg soil. Addition of biosolids enhanced oil degradation after 10 weeks as indicated by the lower carbon (C) content in the oil-contaminated soil that was amended with biosolids compared to the C content of the oil-contaminated soil only. The addition of biosolids to the oil-contaminated soil resulted in a significant increase in plant shoot biomass. Pearl millet plus biosolids produced more root biomass, root length, root surface area, and root diameter than sudangrass plus biosolids in the oil-contaminated soil. The addition of biosolids also increased the amount of nitrogen (N) and phosphorus (P) in the soil. The results suggest that the addition of biosolids could increase potential for remediation of oil-contaminated soil.

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MEET THE STUDENT-AUTHOR

I am from Saddle, Ark., but graduated from the Arkansas School for Mathematics, Sciences and the Arts in Hot Springs in May 2003. That fall I enrolled in the University of Arkansas as an undecided major in the Fulbright College of Arts and Sciences. In fall 2004 I declared my major in environmental, soil and water sciences in the Department of Crop, Soil, and Environmental Sciences and joined the AFLS Honors Program.

As part of my honors program requirements, I worked with my honors mentor, Dr. Duane Wolf, on a greenhouse project to evaluate the use of biosolids for phytoremediation. My research project was funded by the U of A Honors College as well as by the Carroll Walls AFLS Undergraduate Research Grant through the Dale Bumpers College of Agricultural, Food and Life Sciences.

After completing my honors research project, being able to study for a semester at the Scottish Agricultural College in Edinburgh, Scotland, and getting to participate in the Belize Service Project (all as part of my B.S. degree), I graduated in August 2007. I look forward to continuing my studies in graduate school and eventually working as an environmental liaison between developing and developed countries.

INTRODUCTION

Terrestrial oil spills cause many problems within ecosystems due to environmental and health issues posed by oil-contaminated soil (Cunningham et al., 1996). Oil contamination comes from many sources, including tankers, holding tanks, oil-water separators, dissolved air floatation units, and drilling operations (Manning and Thompson, 1995). According to Anderson et al. (1993), oil spills adversely impact the environment in multiple dimensions. Biologically, oil can be detrimental to both plant and microbial life present in the area of a spill. At high oil concentration, most, if not all, plants originally in the area of an oil spill die. Chemically, numerous organic compounds including polycyclic aromatic hydrocarbons (PAHs) increase in oil-contaminated soil and can reduce plant growth (Baker, 1970). Physically, since oil is hydrophobic, petroleum creates a water-impermeable layer in the soil.

Common soil remediation options for oil-contaminated soils are excavating soil and either hauling it to a landfill or to an incinerator. Both options are costly but are common techniques in areas where it is important to clean up the contaminated site quickly because of human health and land-use concerns (Ward et al., 2003). For spills that occur in more remote areas where money is a more important commodity than time and space, it is desirable to find an economically and environmentally acceptable way of remediating contaminated soils.

Phytoremediation is defined as the use of green plants to remove, contain, or render harmless environmental contaminants (Cunningham and Lee, 1995). Plant rhizospheres, plant roots in conjunction with their associated microbial communities, have been shown to amplify the microbial degradation rate of chemical residues in soils (Anderson et al., 1993). Growing plants in contaminated soil can be a cheaper alternative or a supplement to more expensive soil remediation options. Phytoremediation costs to clean up oil-contaminated soil have been estimated at $162/m3 as compared to removal and incineration at an estimated $810/$m^3$ (Rock and Sayre, 1998). Phytoremediation can be not only cost-effective but also low-maintenance and environmentally friendly (Cunningham et al., 1996). While phytoremediation is much less costly than traditional remediation, it is also a slow process. In some cases it takes years for plants and their associated microorganisms to degrade contaminants to a safe level (Boopathy, 2000). Therefore it is important to develop strategies to speed up the degradation process.

Grasses with their fibrous root systems can support greater microbial numbers and activity than taproot

plants (Anderson et al., 1993), and therefore have been used in many phytoremediation projects (White et al., 2003; Dickinson and Rutherford, 2006). Addition of organic amendments such as poultry litter, inorganic fertilizer, hardwood sawdust, or biosolids has been found to enhance plant growth in oil contaminated-soil (White et al., 2003). Biosolids are nutrient-rich organic material resulting from the treatment of wastewater and are commonly used as agricultural amendments to increase plant growth (EPA, 2007). Due to net N mineralization of organic-N, biosolids provide plants with a steady supply of N over the growing season. Increased root growth would increase potential rhizosphere microbial activity, resulting in a higher rate of oil degradation and more effective phytoremediation. Biosolids also improve soil structure by decreasing soil bulk density and increasing porosity and thus increasing the ability of soil to absorb and hold water (EPA, 2007; Dickinson and Rutherford, 2006).

Juteau et al. (2003) found that addition of biosolids to non-vegetated soils enhanced degradation of alkanes. Dickinson and Rutherford (2006) used biosolids to enhance degradation of diesel hydrocarbons in contaminated soils; they concluded that addition of biosolids to contaminated soil increased the soil and plant N contents. Soil physical and chemical properties such as water-holding capacity, cation-exchange capacity, and pH were also increased.

The objective of the 10-week greenhouse study was to determine the influence of biosolids addition on pearl millet or sudangrass growth and soil chemical properties in crude oil-contaminated soil.

MATERIALS AND METHODS

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) or sudangrass (*Sorghum sudanense* (Piper) Stapf (Piper)) was grown in oil-contaminated soil 1) with treatments of biosolids or 2) unamended to determine the influence of biosolids addition on plant growth. A non-vegetated treatment was also included. There were two treatments (biosolids or unamended), three vegetations (pearl millet, sudangrass, or no plant), and four replications, for a total of 24 individual sample units.

Soil. Soil used for the study was a Captina silt loam (fine-silty, siliceous, mesic Typic Fragiudults) collected from the Arkansas Agricultural Research and Extension Center, Fayetteville, and passed through a 2-mm stainless-steel sieve. On a dry-weight basis, 500 g of Captina silt loam soil (0.97% total C) were contaminated to 5% crude oil (v/w) with 25 mL (22.4 g) of crude oil collected from a drilling site near El Dorado, Ark. Oil and soil were thoroughly mixed by hand in plastic bags. After allowing the bags to sit for four days, the amount of oil volatilization was measured by reweighing the bags, and a mean of 3.0 g of volatile organic compounds were lost. The biosolids were aerobically digested sewage sludge obtained from the Springdale Wastewater Facility and analyzed by the Arkansas Agricultural Diagnostics Laboratory (Table 1). Assuming 85% C in crude oil, the amount of C remaining in the soil following volatilization losses was 33g C/kg soil. To attain a 20:1 C:N ratio for optimal microbial activity, 11.8 g biosolids dry weight/pot were added based upon the necessary addition of 1.65 g N/kg soil. The wet biosolids were weighed and mixed into the appropriate soil-oil mixture bags. The soil-oil amendment mixture was transferred into a Cone-tainer® (Stuewe & Sons, Inc., Corvallis, Ore.) (25-cm high x 6.4 cm in diameter) and the soil was adjusted to a water potential of –33 kPa (17.5% Θ_w).

Plants. The sample units for this experiment were placed in a randomized complete block design and grown in the University of Arkansas Greenhouse 3.2 for 10 wks. Ten seeds of pearl millet or sudangrass were planted at a depth of approximately 1 cm in the appropriate Cone-tainers®, except for the non-vegetated samples. At 2 wks, the plants were thinned to 3 plants/pot, and at 3 wks, plants were thinned to 1 plant/pot. Soil moisture was maintained by daily watering with deionized water.

Sample processing. After 10 wks, the plants were harvested by cutting the shoots at the soil surface, rinsed with deionized water, dried to a constant weight at 65°C, and weighed to determine shoot biomass. The roots were separated from the soil by gently shaking the soil cores onto a tinfoil-lined tray, breaking the soil up, and using tweezers to remove the roots. The roots were then rinsed with deionized water on a 500 μm stainless-steel sieve and stained with a solution containing 0.1 g methylene blue/L 10% ethanol. After letting the stain set overnight, the staining solution was discarded and the roots were placed in a layer of water in the scanning dish of the WinRHIZO Digital Imagery System® (Regent Instruments, Inc., Quebec, Canada). The roots were scanned by the imagery system, and the root length, average diameter, and surface area for each sample were determined from the image and an associated computer program (White et al., 2003). After scanning, root biomass was determined by drying roots to a constant weight at 65°C and weighing.

Soil samples were sent to the Arkansas Agricultural Diagnostics Laboratory to determine the Mehlich 3-extractable nutrient contents and total C and N levels. Semi-micro Kjeldahl steam distillation was used to determine the NH_4-N and NO_3-N concentrations in the soils (Keeney and Nelson, 1982).

Statistical analyses were completed by the Agricultural Statistics Laboratory of the University of Arkansas using the GLM Procedure with the means separated by the LSD at $P \leq 0.05$ using SAS® software, version 9 (SAS Institute, Cary, N.C.).

RESULTS AND DISCUSSION

Influence of biosolids on plant growth. With the addition of biosolids to the oil-contaminated soil, shoot biomass production after 10 wks was significantly greater than in the oil-contaminated soil with no amendment, with mean values of 2.59 and 0.02 g/plant, respectively. There was not a difference between pearl millet and sudangrass shoot production. The addition of biosolids resulted in a significant plant species-by-amendment interaction with the greatest root biomass, root length, root surface area, and mean root diameter for pearl millet grown in the biosolids-amended oil-contaminated soil (Table 2). Pearl millet and sudangrass grown in the unamended oil-contaminated soil exhibited minimal growth and were not different for the four parameters evaluated.

Addition of biosolids to the oil-contaminated soil stimulated plant growth with the increase in soil N and P levels. Oil-contaminated soils generally have low concentrations of soil N because of net immobilization by microorganisms breaking down the C from the contaminant (Xu et al., 1995). When additional N is introduced into the system, plant growth increases. Pearl millet had a significantly larger root-growth response to the addition of the biosolids than did the sudangrass, indicating that the pearl millet would have a greater rhizosphere and thus could facilitate greater degradation of the contaminant. These findings are consistent with Kirkpatrick et al. (2006), who reported that pearl millet had significantly greater root length and surface area than sudangrass in oil-contaminated soil amended with 425 to 1275 mg N/kg soil.

Influence of biosolids on soil chemical properties. Total C in the crude oil-contaminated soil was significantly less in the treatments with added biosolids than in those without added biosolids, with values of 3.155% and 3.573%, respectively, regardless of vegetation treatment. Total N and NH_4-N contents were significantly higher in the soils amended with biosolids than in the non-amended samples, with the highest being the no plant-biosolids treatments, which were significantly higher than the vegetated treatments (Table 3). The NO_3-N concentration of the biosolids-amended pearl millet treatment was not significantly different from the non-amended treatments, while the sudangrass-biosolids treatment was significantly greater than the no amendment treatments (Table 3). The no plant-biosolids amendment treatment had the highest levels of NO_3-N. With addition of biosolids, the amounts of P, Ca, Zn, and Cu were significantly higher than in the unamended treatments regardless of vegetation treatment (Table 4).

The most important finding following the 10-week study was that C content of the biosolids-amended oil-contaminated soil was significantly less than oil-contaminated soil without biosolids. Even with the 4.2 g C/pot added with the biosolids, there was less C in the biosolids-amended treatments at the end of the 10-wk study, suggesting that N was limiting the degradation of oil in the unamended samples. The amount of C from the oil addition was the same across the experiment before the addition of biosolids to the amended samples. In order for the amended samples to have less C at the end of the study, the data show that the biosolids stimulated degradation of the oil with or without plants. In other studies, addition of organic amendments to oil-contaminated soils has been shown to decrease Total Petroleum Hydrocarbons (TPH) over time. White et al. (2003) found that addition of broiler litter resulted in reduction of gravimetric TPH levels across six plant treatments.

The high amount of total N, NH_4-N, and NO_3-N in the no plant-biosolids amended treatment indicated that N added to the system exceeded the microbial requirements and without plant uptake, the additional N remained in the soil. The NO_3-N levels in the pearl millet amended and all non-amended treatments were not significantly different, suggesting that the high levels of growth of the pearl millet in the biosolids-amended treatment used the NO_3-N as soon as it was produced. These results were consistent with findings by Dickinson and Rutherford (2006) where they tested use of biosolids during phytoremediation of hydrocarbon-contaminated soils. The sudangrass-amended treatments had significantly more NO_3-N than did the pearl millet because, with about half the root growth, the sudangrass would have utilized less NO_3-N for growth processes and rhizosphere activity.

Plants utilize both the NO_3-N and NH_4-N for growth processes. With available O_2 and CO_2, along with an abundance of Ca ions, the NH_4-N should have rapidly undergone nitrification, being quickly converted by *Nitrosomonas* sp. bacteria to NO_2-N and then converted by *Nitrobactor* sp. bacteria to NO_3-N. In most soils, NO_3-N is generally the predominant mineral form of N (Brady, 2002). However, the data show that high amounts of NH_4-N remained in the biosolids-amended soil, which indicated that the oil inhibited the first step of nitrification by *Nitrosomonas* sp. Plants significantly

decreased the amount of NH_4-N in the biosolids-amended soil compared to the soil of the no plant-biosolids treatment because NH_4-N was easily taken up by the plants for growth processes.

Biosolids addition to the soil resulted in increased levels of P, Ca, Zn, and Cu compared to the unamended treatments (Table 4). The addition of the essential nutrients for plant growth could also result in enhanced phytoremediation. The P was not significantly different between the biosolids-amended vegetative and non-vegetative treatments, suggesting that P was not limiting before the addition of biosolids. The increase in Zn and Cu with the addition of biosolids is noteworthy because of the concerns associated with the accumulation of trace elements in soils amended with organic waste materials such as biosolids. When considering use of biosolids as an amendment to an oil-contaminated site, the addition of trace elements might warrant additional consideration.

The C data indicated that addition of biosolids stimulated the degradation of crude oil in contaminated soil. Not surprisingly, the addition of biosolids increased the plant-available nutrients in the soil, including the NH_4-N, NO_3-N, P, and Ca, which, in turn, increased pearl millet and sudangrass growth as measured by shoot and root biomass, root length, root surface area, and mean root diameter. The high NH_4-N concentrations in biosolids-amended soil suggested that the oil was inhibiting the nitrification process. This study indicated that amending oil-contaminated soil with biosolids can enhance plant growth, which has the potential to increase the effectiveness of phytoremediation.

ACKNOWLEDGMENTS

Financial support for this project was provided by an Honors College Undergraduate Research Grant and a Carroll Walls AFLS Undergraduate Research Fellowship.

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Table 1. The C and N concentrations in the aerobically digested biosolids used in the greenhouse study.

Parameter	Units	Concentration (dry wt.)
Total C	%	35.8[1]
Total N	%	7.0
C/N Ratio		5.1/1
NH_4-N	mg/kg	1,144
NO_3-N	mg/kg	166

[1] The values are a mean of two samples.

Table 2. Interaction of soil amendment and plant species on root biomass, length, surface area, and mean diameter in crude oil-contaminated soil following a 10-wk greenhouse study.

Soil Amendment	Plant Species	
	Pearl Millet	Sudangrass
	Root Biomass	
	------ g/plant ------	
Biosolids	0.90 a[1]	0.47 b
No Amendment	0.01 c	0.02 c
LSD = 0.27		
	Root Length	
	------ cm/plant ------	
Biosolids	5049 a	2151 b
No Amendment	111 c	162 c
LSD = 1427		
	Root Surface Area	
	------ cm^2/plant ------	
Biosolids	806 a	332 b
No Amendment	11 c	19 c
LSD = 259		
	Mean Root Diameter	
	--------mm--------	
Biosolids	1.24 a	0.74 b
No Amendment	0.32 c	0.38 c
LSD = 0.31		

[1] Means for a given plant parameter in the table followed by the same letter are not significantly different at $P \leq 0.05$

Table 3. Interaction of soil amendment and plant species on total N, NH_4-N, and NO_3-N in crude oil-contaminated soil following a 10-wk greenhouse study.

Soil Amendment	Vegetation Treatment: Pearl Millet	Sudangrass	No Plant
		Total N	
		------ % ------	
Biosolids	0.202 b[1]	0.212 b	0.226 a
No Amendment	0.096 c	0.096 c	0.093 c
LSD = 0.011			
		NH_4-N	
		------ μg N/g dry soil ------	
Biosolids	248.1 b	291.5 b	413.2 a
No Amendment	1.8 c	1.4 c	1.7 c
LSD = 72.7			
		NO_3-N	
		------ μg N/g dry soil ------	
Biosolids	19.1 c	83.3 b	156.5 a
No Amendment	0.9 c	0.7 c	1.5 c
LSD = 40.0			

[1] Means for a given N form followed by the same letter are not significantly different at P ≤ 0.05.

Table 4. Main effect of biosolids amendment on plant available P, Ca, Zn, and Cu concentrations in crude oil-contaminated soil following a 10-wk greenhouse study.

Soil Amendment	P	Ca	Zn	Cu
		------ mg/kg ------		
Biosolids	503.2 a[1]	979.3 a	5.5 a	1.6 a
No Amendment	26.9 b	278.3 b	0.8 b	0.5 b
LSD	29.1	69.6	0.4	0.1

[1] Means in column followed by the same letter are not significantly different at P ≤ 0.05.