Transactions of the Illinois State Academy of Science
(2007), Volume 100, #1, pp. 3-11

received 8/8/06
accepted 11/29/06

Impact of Vermicompost on Growth of Two Native Illinois Prairie Plants in Biodiesel Contaminated Soil

A. L. Meteer, G.R. Bachman[1], B.R. Wiegand, and R.L. Rhykerd
Illinois State University, Department of Agriculture, Normal, IL 61790
[1]Corresponding Author: Office: 309-438-3275; Fax: 309-438-5653; Email: grbachm@ilstu.edu

ABSTRACT

The use of biofuel is rapidly increasing and is projected to have a positive impact on the economy of Illinois. With its increase in use, the likelihood of accidental spills also increases and methodologies to successfully re-vegetate biodiesel contaminated soils are needed. The objective of this study was to determine if vermicompost enhances growth of big blue stem (*Andropogon gerardii* Vitman) and partridge pea [*Chamaecrista fasiculate* (L.) Moench] in biodiesel contaminated soil. The experimental design was a completely randomized 2 x 4 factorial design with 3 replications per treatment. Seedlings of big bluestem and showy partridge pea were germinated in a commercial potting soil and grown for 42 days at which time plugs were transplanted. Plugs were transplanted into pots with dimensions of 10cm x 10cm x 9cm (l x w x h) containing loamy topsoil, loamy topsoil amended with 20% vermicompost (VC), loamy topsoil amended with 5g biodiesel kg^{-1}, or loamy topsoil amended with both 5g biodiesel kg^{-1} and 20% VC. Three plugs were transplanted per pot and were grown for an additional 49 days. Plant heights were measured on 49 days after transplanting (DAT) and destructively sampled to measure plant root and shoot biomass. Results show both big bluestem and showy partridge pea were able to survive being transplanted into biodiesel contaminated soil and differences in plant height between amended soils were not significant. Further research to determine if these plants and the added vermicompost can enhance remediation of biodiesel contaminated soils is recommended.

INTRODUCTION

Diesel fuel is an integral component of sustainable agriculture. Petroleum diesel is a mixture of hydrocarbons containing many toxic compounds including volatile low molecular weight alkanes, naphthalenes, and polycyclic aromatic hydrocarbons (PAH). Biodiesel fuel is composed mainly of triglycerides, making it a cleaner-burning, less toxic alternative to standard petroleum diesel. The increased use and production of biodiesel offers many benefits to the Illinois farmer, economy, and environment. Recent legislation offers sales tax exemptions up to 20% for biodiesel blends containing 1-10% biodiesel and a total tax exemption for diesel blends that contain above 10% biodiesel. This legislation could increase the demand for biodiesel fuels and result in price increases of 5 cents per bushel for Illinois soybeans, which would increase the state's economic output by more than $22.5 million (National Biodiesel Board, 2003).

Diesel or biodiesel fuel spills pose an environmental threat to soil and are toxic to plants. Soil contaminated with 5% diesel significantly reduced seedling germination (Adam and Duncan, 1999; Russell, 2005). Additionally, Rosa et al. (2005) showed that the rate of nitrogen fixation by soybean nodules was significantly reduced in soil contaminated with 1% biodiesel. Biodiesel spills on soil, while less damaging than petroleum diesel spills, still pose a threat to the environment and therefore, technologies to remediate biodiesel contaminated soils are needed.

Contaminated soils are frequently remediated by excavation and placing contaminated soils into landfills or incinerated. However, this technology is expensive and costs range from $100-400 per ton for land filling and $200 – 1,500 per ton for incineration (Schnoor, 1997). A low cost alternative to these technologies is phytoremediation, or using plants to naturally enhance remediation of the soil, for which the costs ranges from $10-35 per ton.

Phytoremediation may enhance remediation by several mechanisms (Cunningham et al., 1995). Plant roots can absorb contaminants, translocate them to vegetation which can be harvested and removed, thereby removing them from the environment. Absorbed contaminants can also be degraded by the plant into harmless metabolites. Roots can also enhance soil remediation by stimulating microbial activity in the rhizosphere and the increase in microbial activity may enhance decomposition of organic compounds.

Phytoremediation has been successfully used to enhance remediation of contaminated soils. For example, *Lolium perenne* L. (perennial ryegrass) significantly reduced the concentration of petroleum hydrocarbons compared to an unvegetated control (Günther et al., 1996) and in a field study phytoremediation significantly accelerated remediation of petroleum hydrocarbons compared to unvegetated controls (Schwab and Banks, 1999). However, phytoremediation is not always effective. In field study using *Sorghum halapense* (L.) Pers. (Johnsongrass), *Elymus Canadensis* L. (Canada wildrye), and a rotation of Johnsongrass-Canada wildrye did not enhance the disappearance of hydrocarbons from soil (Sung et al., 2002; Sung et al., 2003; and Sung et al., 2004).

Vermicompost is believed to be a material that can enhance soil remediation. Vermicompost is produced through the degradation of organic wastes through the action of earthworms that results in the bio-oxidation and stabilization of the wastes. This is a different process than traditional composting which requires a thermophilic stage, while vermicompost undergoes a mesophilic stage. The resulting vermicompost material is a finely divided peat-like substance with excellent structure, porosity, aeration, drainage, and moisture holding capacity (Edwards, 1985; Atiyeh et al., 2000).

Vermicompost has been reported to have greater numbers of microorganisms than native soil (Daniel and Anderson, 1992) More than 50 species of bacteria have been isolated from vermicompost associated with *Lumbriscus terretris* (Parle, 1963). Anastasi et al. (2004) reported a wide biodiversity of fungal species in vermicompost when compared to traditional compost. Species count total of 139 fungal species with 74 species being unique to the vermicompost evaluated.

In limited studies and applications, vermicomposts have been shown to be effective in the remediation of soil contaminated with hydrocarbon compounds, such as biodiesel. The fungal and bacterial content of vermicompost has been implicated in the degradation of polycyclic aromatic hydrocarbons (PAHs). When used as a soil inoculant vermicompost may be well suited to promote growth of PAH degrading fungi and bacteria (Lowery and Hurley, 2005).

Clearly there is a need for more research to identify technologies that will enhance remediation of contaminated soils. The objective of this study was to examine the potential of using big blue stem (*Andropogon gerardii*), and partridge pea (*Chamaecrista fasiculate*), a grass and legume prairie species native to Illinois, and vermicompost to establish vegetation soil contaminated with biodiesel.

MATERIALS AND METHODS

The experimental design was a completely randomized 2 x 4 factorial design with 3 replications per treatment. Experiments were conducted in a greenhouse at Illinois State University. The daytime temperature was set at 24 °C and nighttime temperature at 18 °C. The day temperature was equivalent to the outside ambient temperature on days when the outside temperature was above the set point. On days when the outside temperature was at or below the set point, then the inside temperature was within 1-2 °C range of the set point. For night periods, the inside temperature was within 1-2 °C of the set point, except during those nights when the outside temperature remained above the set point.

Seedlings of big bluestem and showy partridge pea were germinated in peat-based potting media and grown for 42 days at which time plug height was recorded. Three plugs were transplanted into each container having dimensions of 10cm x 10cm x 9cm (L x W x H) containing either a loamy topsoil (control), a loamy topsoil amended with 20% VC (VC control), a loamy topsoil contaminated with 5g biodiesel kg^{-1} (B100), or a loamy topsoil amended with 20% VC and contaminated with 5 g biodiesel kg^{-1} (VC B100).

Properties of the loamy top soil are presented in Table 1. Soil texture was determined using the hydrometer method as described by Gee and Bauder (1986). Soil reaction (pH), organic matter (OM), potassium (K), cation exchange capacity (CEC), nitrate-nitrogen (NO_3-N), and phosphorus (P) were measured by the commercial laboratory Mowers Soil Testing Plus Inc. Toulon, IL following the methods presented in the Recommended Chemical Soil Test Procedures for the North Central Region (North Central Regional Publication No. 221, 1998). Soil pH was measured using a 1:1 soil:distilled water ratio. Soil OM was determined by chromic acid oxidation. Available K was determined by atomic absorption. Soil CEC was determined by summing the exchangeable bases, which were measured using atomic absorption. Soil NO_3-N was measured using a nitrate ion-selective electrode. Soil P was measured using the Bray P-1 method. The soil was selected to represent a typical highly productive Illinois soil that is subject to oil contamination.

These plants were grown for an additional 49 days after which data was collected. Plants were destructively sampled to measure fresh and dry root and shoot biomass. Roots and shoots were separated at the crown. Soil was washed from the roots under a gentle stream of water. Roots and shoots were then placed in separate paper bags and placed in an oven at 60 °C for 72 hours. Statistical analysis was performed using analysis of variance (ANOVA) and if significant by means separation using least significant difference (LSD), P=0.05.

RESULTS

Both big bluestem and showy partridge pea were able to survive being transplanted into soil contaminated with 5g biodiesel kg^{-1} soil (Table 2). Plant growth responses were different for big bluestem than showy partridge pea and as such all comparisons are reported within each species. Plant height of plugs at transplant for big blue stem and partridge pea were 10.12 cm ± 0.14 and 1.60 cm ± 0.03, respectively.

Height and percent increase from transplant of the big bluestem at DAT 49 was 11.48 cm (13%), 11.93 cm (18%), 11.40 cm (12%), and 9.89 cm (-3%) for control, VC control, B100, and VC B100, respectively. Height and percent increase from transplant of the showy partridge pea at DAT 49 was 3.88 cm (142%), 2.19 cm (31%), 1.47 cm (-8%), and 2.13 cm (33%) for control, VC control, B100, and VC B100, respectively. Negative height measurements were an indication that little growth occurred after transplantation. Height of the big bluestem treatment amended with B100 was not significantly lower than the control; the VC B100 treatment was significantly lower than the VC control (Table 2). The height of the showy partridge pea grown in the control soil was 3.88 cm and decreased significantly to 2.19 cm with the addition of vermicompost and the VC B100 treatment at 2.13 cm. Comparing the control soil to the B100 shows a significant decrease in plant height from 3.88 cm to 1.47 cm, respectively (Table 2).

The addition of B100 tended to reduce shoot and root fresh and dry weights of big bluestem and showy partridge pea (Tables 3 and 4). The shoot dry weight of big bluestem decreased from 0.43 g in the control to 0.38 in the presence of B100 and it decreased from 0.50 g in the vermicompost amended control to 0.27 g in the B100 amended vermicompost treatment. The root dry weight of big bluestem was not significantly reduced in the presence of B100 (0.64 g) compared to the control (0.68 g), however, it did decrease significantly in the VC B100 treatment (0.51 g) compared to the VC control (0.70 g).

Showy partridge pea produced much less plant biomass than big bluestem (Tables 3 and 4). The fresh shoot and root weights of showy partridge pea tended to decrease significantly with the addition of B100 (Table 4). Shoot and root dry weights for the B100 treatment was significantly reduced compared to the controls. Showy partridge pea biomass was reduced by the addition of VC (Table 4). For example the dry weight of showy partridge pea decreased from 0.06 g in the control to 0.03 g in the VC control treatment. Showy partridge pea growing in the B100 amended VC treatment showed an increase in biomass.

DISCUSSION

Plant growth has been used to evaluate the suitability of plants for vegetating contaminated soils (Issoufi et al., 2006; Spiares et al., 2001a; and Spiares et al 2001b). Of the plant parameters measured in this study, Issoufi et al., (2006) and Spiares et al., (2001b) have stated that production of root biomass is the most critical when evaluating the potential of plants to enhance remediation. Increasing root biomass is a major factor because microbial populations in the rhizosphere are generally 10-100 times larger than that of non-rhizosphere soil (Pierzynski et al., 2005). Jordahl et al., (1997) found that population of selected hydrocarbon degraders were five times more abundant in rhizosphere soil than non-rhizosphere soil. Increasing microbial population in the rhizosphere leads to an increase in microbial activity, which may increase metabolic and cometabolic transformations of biodiesel into less toxic products and reduce the time required to remediate soil. Because big blue stem produced from approximately 7 to 32 times more roots on a dry weight basis than showy partridge pea, it appears to have a greater potential to enhance remediation than showy partridge pea.

The addition of vermicompost to biodiesel contaminated soil tended to reduce plant growth, especially with the partridge pea, when compared to plants grown in contaminated soil without added vermicompost. Perhaps the C:N or C:P ratio of the added vermicompost was too great, causing immobilization of N and P, resulting in a nutrient limitation, which limited plant growth. Chang et al., (1996) found that the optimal C:N and C:P ratios in hydrocarbon contaminated soils were 60:1 and 800:1 respectively and rates of remediation were slowed when either nutrient was limiting. Therefore, fertilizing the vermicompost amended treatments with N and P fertilizers may have enhanced plant growth. Another possible cause of reduced growth may have been the process of transplantation from the 128-cell plug trays to the treatments media and containers. Generally, native plant species do not transplant well due to root sensitivity or deep taproots and the natural soil conditions are hard to duplicate in containers. Further research is recommended to determine the mechanism that caused this less than optimum growth of both species.

CONCLUSIONS

Both big bluestem and showy partridge pea grew in soil contaminated with 5 g biodiesel kg^{-1} soil and may be useful at restoring vegetation of contaminated soils. Additional research to evaluate management practices to enhance the growth of these plants in contaminated soils and evaluate if they along with additions of vermicompost are capable of enhancing remediation are recommended.

ACKNOWLEDGEMENT

Funding for this research was provided by the Illinois Council on Food and Agricultural Research and the Illinois State University, University Research Grants Program.

REFERENCES

Adam, G. and H.J. Duncan. 1999. Effect of diesel fuel on growth of selected plant species. Environ. Geochem. and Health, 21:353-357.

Anastasi, Antonella, Giovanna C. Varese, Samule Voyron, Silvano Scannerini, and Valeria Filipello Marchisio. 2004. Characterization of fungal biodiversity in compost and vermicompost. Compost Science & Utilization 12(2):185-191.

Atiyeh, R.M., S. Subler, C.A. Edwards, G. Bachman, J.D. Metzger. 2000. Effects of vermicompost and composts on plant growth in horticultural container media and soil. Pedobiologia 44:579-590.

Chang, Z.Z., R.W. Weaver, and R.L. Rhykerd. 1996. Effect of fertilization on bioremediation of crude oil. Journal of Soil Contamination 5:215-224.

Daniel, O and J.M Anderson. 1992. Microbial biomass and activity in contrasting soil material after passage through the gut of the earthworm *Lumbriscus rubellus* Hoffmeister. Soil Biol. Biochem. 24:465-470.

Edwards, C.A. 1985. The use of earthworms for management of organic wastes. In: International Symposium on Earthworms, Bologna-Capri, Italy, 31 March-5 April, 1985, Collana U.Z.I.

Gee, G.W., and J.W. Bauder. 1986. Particle size analysis. *In* Methods of Soil Analysis, Part I, ed. A. Klute. Am. Soc. Agron., Madison, WI. Pp. 387-409.

Ghaly, R.A., J.B. Pyke, A.E. Ghaly, and V.I. Ugursal, 1999. Remediation of Diesel-Oil-Contaminated Soil Using Peat. Energy Sources, 21:785-799.

Günther, T., U. Dornberger, and W. Fritsche. 1996. Effects of Ryegrass on Biodegradation of Hydrocarbons in Soil. Chemosphere 33:203-215.

Issoufi, I., R.L. Rhykerd, and K.D. Smiciklas. 2006. Seedling growth of Agronomic Crops in Crude Oil Contaminated Soil. Journal of Agronomy and Crop Science. 192:310-317.

Jordahl, J.L., L. Foster, J.L. Schnoor, P.J.J. Alvarez. 1997. Effect of Hybrid Poplar Trees on Microbial Population Important to Hazardous Waste Bioremediation. Environmental Toxicology and Chemistry 16:1318-1321.

Lowery, David and Chad Hurley. 2005. Vermicompost is successfully used in bioremediation pilot on soil hydrocarbons. Biocycle 46(8):6.

National Biodiesel Board. June 12, 2003. http://www.biodiesel.org/resources/pressreleases/gen/20030612_IL_legislation.pdf

North Central Regional Publication No. 221. 1998. Recommended Chemical and Soil Test Procedures for the North Central Region. Missouri Agricultural Experiment Station SB 1001.

Parle, J.N. 1963. Microorganisms in the intestines of earthworms. J. Gen. Microbiol. 31:1-13.

Pierzynski, G.M., J.T. Sims, G.F. Vance. 2005. Soils and Environmental Quality, 3rd ed. CRC Press. Boca Raton, Florida.

Rosa, I, R.L. Rhykerd, and K.D. Smiciklas. 2005 Impact of Petroleum Diesel and Biodiesel Fuels on Acetylene Reduction in *Glycine max*. 2005 Agronomy Abstracts. American Society of Agronomy International Meetings, Salt Lake City, UT, USA.

Russell, S. 2005. Phytoremediation of Petroleum Diesel and Biodiesel Contaminated soil using Soybean (*Glycine max*) and Corn (*Zea mays*). Thesis. Illinois State University.

Schnoor, J.L. 1997. Phytoremediation. Ground Water Technology Analysis Center: Technology Evaluation Report TE-98-01.

Schwab, P. and K. Banks. 1999. Phytoremediation of Petroleum-Contaminated Soils. In D.C. Adraino (ed.) Bioremediation of Contaminated Soils. American Society of Agronomy. Madison, WI. Pp. 783-795.

Spiares, J.D., K.E. Kenworthy, and R.L. Rhykerd. 2001. Emergence and height of plants seeded in crude oil contaminated soil. Texas Journal of Agriculture and Natural Resources 14:37-46.

Spiares, J.D., K.E. Kenworthy, and R.L. Rhykerd. 2001. Root and shoot biomass of plants seeded in crude oil contaminated soil. Texas Journal of Agriculture and Natural Resources 14:117-124.

Sung, K., C.L. Munster, M. Y. Corapcioglu, M.C. Drew, S. Park, and R. Rhykerd. 2004. Phytoremediation and modeling of contaminated soil using Eastern Gamagrass and Annual Ryegrass. J. Water, Air and Soil Pollution 159:175-195.

Sung, K., C. L. Munster, R. Rhykerd, M. C. Drew and M. Y. Corapcioglu. 2003. The use of vegetation to remediate soil freshly contaminated by recalcitrant contaminates. Water Research 37:2408-2418.

Sung, K, C.L. Munster, R. Rhykerd, M.C. Drew, and M.Y. Corapcioglu. 2002. The use of box lysimeters with freshly contaminated soil to study the phytoremediation of recalcitrant organic contaminants. Environ. Science and Technology 36:2249-2255.

Table 1. Selected physical and chemical properties of soil and vermicompost amendment used to evaluate survival of Big Bluestem and Showy Partridge Pea in biodiesel contaminated soil. Values are averages of 3 samples.

	Sand (%)[a]	Silt (%)[a]	Clay (%)[a]	pH (in water)[b]	OM (%)[b]	CEC ($cmol_c$ kg^{-1})[b]	NO_3-N	P	K
								($mg\ kg^{-1}$)[b]	
Sand	48	42	10	7.8	3.5	23	21.3	205.7	1725.3
VC				7.0		45	6200	458.0	659.5

[a] Hydrometer method (Gee and Bauder, 1986)
[b] Analyzed by Mowers Soil Testing Plus, Inc. Toulon, IL

Table 2. Plant height of big bluestem and showy partridge pea amended with vermicompost and contaminated with biodiesel 49 days after being transplanted.

Treatment	Big Bluestem (cm)[a]	Showy Partridge Pea (cm)[a]
Control	11.48 a	3.88 a
VC Control[b]	11.93 a	2.19 a
B100[c]	11.40 a	1.47 a
VC B100[d]	9.89 a	2.13 a
	LSD 2.49	LSD 1.55

[a] Means separation within column by least significant difference (LSD), P=0.05.
[b] Loamy topsoil amended with 20% VC
[c] Loamy topsoil amended with 5g biodiesel kg^{-1} soil
[d] Loamy topsoil amended with 20% VC and 5g biodiesel kg^{-1} soil

Table 3. Root and shoot biomass of big bluestem amended with vermicompost and contaminated with biodiesel 49 days after being transplanted.

	Shoot weight		Root weight	
Treatment	Fresh (g)[a]	Dry (g)[a]	Fresh (g)[a]	Dry (g)[a]
Control	1.24 ab	0.43 ab	2.48 a	0.68 a
VC Control[b]	1.53 a	0.50 a	2.48 a	0.70 a
B100[c]	1.10 ab	0.38 ab	2.44 a	0.64 a
VC B100[d]	0.85 b	0.27 b	1.73 a	0.51 a
	LSD 0.59	LSD 0.18	LSD 1.21	LSD 0.27

[a] Means separation within column by least significant difference (LSD), P=0.05.
[b] Loamy topsoil amended with 20% VC
[c] Loamy topsoil amended with 5g biodiesel kg^{-1} soil
[d] Loamy topsoil amended with 20% VC and 5g biodiesel kg^{-1} soil

Table 4. Root and shoot biomass of showy partridge pea amended with vermicompost and contaminated with biodiesel 49 days after being transplanted.

	Shoot weight		Root weight	
Treatment	Fresh (g)[a]	Dry (g)[a]	Fresh (g)[a]	Dry (g)[a]
Control	0.25 a	0.07 a	0.22 ab	0.06 a
VC Control[b]	0.35 a	0.03 a	0.24 a	0.03 a
B100[c]	0.09 a	0.03 a	0.08 b	0.02 a
VC B100[d]	0.11 a	0.07 a	0.13 ab	0.07 a
	LSD 0.12	LSD 0.04	LSD 0.15	LSD 0.04

[a] Means separation within column by least significant difference (LSD), P=0.05.
[b] Loamy topsoil amended with 20% VC
[c] Loamy topsoil amended with 5g biodiesel kg^{-1} soil
[d] Loamy topsoil amended with 20% VC and 5g biodiesel kg^{-1} soil