SENRA Academic Publishers, British Columbia
Vol. 7, No. 2, pp. 2387-2390, June 2013
Online ISSN: 1920-3853; Print ISSN: 1715-9997

Canadian Journal of pure & applied sciences

Short Communication

MICROBIAL CONTENT OF MANUFACTURED (FABRICATED) SOILS: 2002-2011

*Maria V Kalevitch[1] and Valentine I Kefeli[2]
[1]School of Engineering, Mathematics and Science, Robert Morris University
6001 University Blvd., Moon Township, PA 15108, USA
[2]Biomost, Inc, PA, USA

ABSTRACT

The main goal of this study was to examine the effects of bacterial activity on soil health. Fabricated soils (FS) have been used in the study of AMD damaged soils in Pennsylvania, USA. This study was also evaluated the bacterial activity in soil as the indicator of soil health.

Keywords: Fabricated soils, acid mine drainage (AMD), microbial content, soil health and sustainability.

INTRODUCTION

The structure and function of the soil foodweb has been suggested as a prime indicator of ecosystem health by several authors (Kalevitch *et al*., 2004). Measurement of disrupted soil processes and decreased bacterial or fungal activity along with other parameters can serve to indicate a problem long before the natural vegetation is lost or human health problems occur. Estimates of the loss of U.S. soil resources due to erosion to range from 2 billion to 6.8 billion tons annually (Beasley, 1972; Pimentel *et al*., 1976; USDA, 1980; Harlin *et al*., 1987; Pimentel *et al*., 1995). Worldwide estimates indicate that between 10-15 million hectares of arable land are rendered unproductive annually due to soil losses (Pimentel *et al*., 1995). Many researchers are concerned with the damage that AMD does to the soil, water, and biological communities. Honey and Kagle (2008) evaluated the impact of acid mine drainage (AMD) on bacterial populations in the Upper Tioga River Watershed, PA, USA. Their study confirmed that pH levels certainly affected the microbiological activity in soil. Authors concluded that both biodiversity and population size has been impacted in AMD affected sites. In our prior study Kalevitch (2006) the proposed recipes of fabricated soils are based on the concept of the carbon-nitrogen balance in the soil as well as on the transformation of carbon products such as glucose, phenolics, and plant polymers. The main objective of this study was to examine the effects of bacterial activity on soil health, while using a FS substrate.

MATERIALS AND METHODS

Study Site

The study site is located in Butler County, Pennsylvania, USA. The site was strip mined in the 1950's and has experienced acid mine drainage in the subsequent years. The resulting site has remained sparsely vegetated since the original mining. To remediate this site, a fabricated soil amendment, a natural mixture of decaying substrates rich in aluminosilicate, carbon, nitrogen, phosphorus and potassium sources, was added to test plots. Soil samples were collected from the reclaimed mining site; some samples were collected from test plots with fabricated soil and one from abandoned mining soil.

Sample Analysis and Data Analysis

Microbial analytics were done by US-Microsolutions, Inc. Soil samples were obtained from different locations named by the presence of certain trees grown on these specific plots. The weighted soil sample was placed in beaker containing deionized water, was allowed to settle at room temperature for 30min, vortexed vigorously, and dilutions were streaked onto TSA (tryptic soy agar) plates. Plates were incubated at 27^{o}C for 10 days. The bacterial colonies were enumerated, identified and the number of CFU-colony-forming unit/gram of material calculated. All data are statistically significant. 95% of confidence intervals exist for all points, a= 0.05.

RESULTS AND DISCUSSION

According to the soil legend, soils represented topsoil, manufactured soils, and mining soil plot. We placed a special emphasis on comparison of microbial composition of fabricated/manufactured soils in 2011, 2010, 2007 and 2002. Thus we compared analysis for manufactured soils that were fabricated this year, 1, 4 and 9 years ago. The bacterial and fungal composition of those soils is presented in table 1.

*Corresponding author email: kalevitch@rmu.edu

Table 1. Microbial Presence in Studied Soils.

Sample	Year	Type	Bacterial Content	%	Fungal Content	%
FS	2011		*Stenotrophomonas maltophilia*	58%	*Rhizopus* spp.	N/A
			Gram-positive *Coryneform bacillus*	12%	*Penicillium* spp.	N/A
			Coagulase-negative *Staphylococcus* spp.	9%	Non-sporulating Derm. fungi	N/A
			Acinetobacter spp.	9%		
			Pseudomonas fluorescens	5%		
			Bacillus spp. 1	1%		
			Bacillus spp. 2	1%		
FS	2010		Gram-positive *Coryneform bacillus*	67%	*Hypodiscosia* spp.	88%
			Bacillus spp. 1	13%	Non-sporulating Derm. fungi	10%
			Pantoea spp.	13%	*Penicillium* spp.	1%
			Bacillus spp. 2	7%		
FS	2007	Chestnut	*Sphingomonas paucimobilis*	42%	Non-sporulating hyaline fungi	N/A
			Gram-positive *Coryneform bacillus* 1	15%	*Trichoderma* spp.	N/A
			Pseudomonas fluorescens	15%	*Penicillum* spp.	N/A
			Gram-positive *Coryneform bacillus* 2	12%	*Gliocladium* spp.	N/A
			Bacillus spp. 2	12%		
			Bacillus spp. 1	4%		
FS	2002	Poplar	*Stenotrophomonas maltophilia*	42%	*Fusarium* spp.	N/A
			Bacillus spp. 1	19%	Non-sporulating hyaline fungus	N/A
			Bacillus spp. 2	19%	*Cliocladium* spp.	N/A
			Burkholderia cepacia	19%	*Penicillium* spp.	N/A
FS	2002	RBW	Gram-positive *Coryneform bacillus*	55%	*Penicillium* spp.	N/A
			Bacillus spp. 1	14%	*Trichoderma* spp.	N/A
			Klebsiella pneumniae spp. Ozaenae	14%	*Alternaria* spp.	N/A
			Bacillus spp. 2	9%		
			Pantoea spp.	9%		
	2002	Pussy Willow	*Bacillus* spp. 1	43%	*Aspergillus versicolor*	40%
			Aeromonas spp.	30%	*Penicillium* spp.	33%
			Gram-positive *Coryneform bacillus* 2	19%	*Fusarium* spp.	13%
			Gram-positive *Coryneform bacillus* 1	4%	*Trichoderma* spp.	2%
			Bacillus spp. 2	1%	*Gliocladium* spp.	2%
			Pseudomonas fluorescens	1%		
Top Soil	2011		Gram-positive *Coryneform bacillus* 4	43%	*Penicillium* spp.	64%
			Gram-positive *Coryneform bacillus* 3	33%	*Fusarium* spp.	18%
			Gram-positive *Coryneform bacillus* 2	19%	*Papulaspora* spp.	18%
			Sphingomonas paucimobilis	3%		
			Bacillus spp.	1%		
			Gram-positive *Coryneform bacillus* 1	1%		
Mining Soil	N/A		Gram-positive *Coryneform bacillus* 1	73%	*Fusarium* spp.	63%
			Gram-positive *Coryneform bacillus* 2	12%	*Penicillium* spp.	23%
			Bacillus spp. 1	5%	*Trichoderma* spp.	5%
			Bacillus spp. 4	5%		
			Bacillus spp. 2	2%		
			Bacillus spp. 3	2%		

Fabricated soil, 2011 had 58% of *Stenotrophomonas maltophilia*, 12% of *gram-positive coryneform bacillus*, 9% of both coagulase-negative *Staphylococcus* spp and *Acinetobacter spp*, and 5% of *Pseudomanas fluorences. Bacillus* spp. was at 1%. Fungi population was represented by *Rhizopus* spp., *Penicillium* spp., and non-sporulating dematiaceous fungi. The percentage could not be calculated due to overgrowth of competing fungal flora.

S. maltophilia is the most abundant bacteria present in this sample. It is an aerobic, nonfermentative, Gram-negative bacterium. *S. maltophilia* are slightly smaller (0.7–1.8 × 0.4–0.7 micrometers) than other members of the genus. They are motile due to polar flagella and grow well on MacConkey agar producing pigmented colonies. It is catalase-positive, oxidase-negative (which distinguishes them from most other members of the genus) and positive for extracellular DNase. *S. maltophilia* is ubiquitous in aqueous environments, soil and plants; it has also been used in biotechnological application, Microwiki.

The one year old -2010 fabricated soil sample, had 67% of gram-positive *coryneform bacillus* vs 12% in fresh 2011 sample. It completely inhibited *S. maltophilia* that was present in fresh FS.

Bacillus spp increased to 7-13% vs only 1% in freshly prepared sample. Fungal count contained *Hypodiscosia* spp., *Penicillium* spp., and non-sporulating dematiaceous fungi. *Rhizopus* spp was not present as in fresh sample.

The 4 year old fabricated soil sample with American Chestnut growing on the plot had 42% of *Sphingomonas paucimobilis*. It is a gram-negative rod that exists in environmental niches such as water, including hospital water systems. It is not the part of normal human flora. This organism was not present in any of the recent samples, 2011 or 2010. The rest of the species were: gram-positive *Coryneform bacillus*-12%, *Pseudomanas fluorences*-15%, and different *bacillus* spp, 4-12%. Fungi population was represented by *Penicillium* spp., and non-sporulating hyaline fungi, including *Trichoderma* and *Cliocladium* spp. Total fungal count was 6.3x10.[6]

Fabricated soils manufactured 9 years ago had different types of trees grown on the plots: **Poplar, Red-Branched Willow and Pussy Willow.**

Poplar plot had 42% of *S. maltophilia*, 19% of *bacillus* spp. and *Burkholderia cepacia*-19%. *B. cepacia complex* (BCC) is a new group of organisms present in the soil sample. BCC is of catalase-producing, non-lactose-fermenting Gram-negative bacteria composed of at least seventeen different species. BCC organisms are typically found in water and soil and can survive for prolonged periods in moist environments. Fungi population was represented by *Fusarium* spp., *Penicillium* spp., and non-sporulating hyaline fungi. Also including *Gliocladium* spp. Total fungal count was 2.83x10.[6]

Red-Branched Willow Plot had gram-positive coryneform *Bacillus*-55%, *Bacillus* spp. 9-14%, *Pantoea* spp, 9% and *Klebsiella pneumonia* spp., *Ozaenae* at 14%. *Pantoea agglomerans* (formerly *Enterobacter agglomerans*) is a gram-negative aerobic bacillus in the family Enterobacteriaceae. All species of the genus *Pantoea* can be isolated from feculent material, plants, and soil, where they can be either pathogens or commensals.

The genus *Klebsiella* belongs to the tribe Klebsiellae, a member of the family Enterobacteriaceae. It is non-motile, rod-shaped, gram-negative bacteria with a prominent polysaccharide capsule. This capsule encases the entire cell surface, accounts for the large appearance of the organism on gram stain, and provides resistance against many host defense mechanisms.

Fungi population was represented by *Penicillium* spp., *Trichoderma* spp, and *Alternaria* spp. Total fungal count was 1.4x10.[6]

Pussy Willow Plot had 43% of *Bacillus* spp, 30% of *Aeromonas* spp, and 19% of gram-positive *coryneform bacillus.* ***Aeromonas*** are gram-negative facultative anaerobes that are straight rods or coccoid cells. They are inhabitants of aquatic ecosystems worldwide. These include groundwater and drinking water at treatment plants and in distribution systems and reservoirs as well as clean or polluted lakes and rivers.

Gram-positive *Coryneform bacillus* – Many species of corynebacteria are part of the normal flora of the skin & mucous membranes in human and mammals. Several species of *corynebacteria* have been found in the inanimate environment (e.g.) dairy products, plants, soil and activated sludge.

Fungi population was represented by *Aspergillus versicolor*-40%, *Penicillium* spp.-33***%***, and *Fusarium* spp.-13%, *Trichoderma* and C*liocladium* spp of 2% each.

Aspergillus versicolor is a widely distributed fungus being detected in very cold regions, unlike most other species of aspergilla which prefer warmer regions. It may be commonly found in soil, hay, cotton, dairy products, dried cereals, nuts, and especially spices. Total fungal count was 0.54x10.[6]

In case of mining soil, the majority of microflora were various *bacillus* spp. and gram-positive *Coryneform bacillus* that were present at 85%. The fungal species had *Fusarium*-63%, *Peniccilium*-23% and *Trichoderma*-5%.

In top soil 2011 the presence of Bacterial spp. was prevalent at 90+%, and only 3% of *S. paucimobilis* present. *S. paucimobilis* is an aerobic Gram-negative soil bacillus that has a single polar flagellum with slow motility.

Fungal content had *Penicillium* spp. at 64% and *Fusarium* spp., 18%. *Papulaspora* spp. also was at 18%.

In case of mining soil, the majority of microflora were various Bacillus spp. and gram-positive *Coryneform bacillus* that were present at 85%. The fungal species had *Fusarium*-63%, *Peniccilium*-23% and *Trichoderma*-5%.

In top soil 2011 the presence of bacterial spp was prevalent at 90+%, and only 3% of *S. paucimobilis* present. Fungal content had *Penicillium* spp. at 64% and *Fusarium* spp., 18%. *Papulaspora* spp. also was at 18%.

Janzen *et al*. (2008), described the impact of AMD on diversity of microbial community and stream chemistry in the Shamokin Creek Watershed, PA. It is a well - known fact that diatoms as representatives of biodiversity indicate the health of a particular environment. Bacterial presence also indicates the level of ecological balance. The authors concluded that in AMD where the concentrations of iron are high, the predominant bacteria will be from phylum *Bacteroidetes*, and were closely related to known biofilm community members from acidic environments where they have been demonstrated to be involved in sulfur oxidation. Other bacterial species were closely related to *Sphingomonas* species. Soil ecology damage due to AMD has the potential to have a cascade of negative effects including the loss of vegetation leading to the loss of topsoil due to erosion.

As the environmental damage caused by AMD and surface mining operations increases, additional methods must be developed to repair or replace the topsoil in order to support normal ecological development. As soil is a necessary intermediate substrate in the regulation of the Biosphere activity, it is important to understand the long term effects of microbial change loss and to monitor attempts to amend soils to improve sustainability and viability of the soil.

As the research shown we have been able to maintain a healthy microbial presence in fabricated soils over 9 year period thus contributing to answer whether fabricated soils are a long-term or short-term solution. Future work will compare the mineral content of soil with microbial biomass and diversity.

REFERENCES

Beasley, R. 1972. Erosion and Sediment Control. Iowa State University Press, USA.

Harlin, J. and Barardi, G. 1987. Agricultural Soil Loss. West- view, Boulder, CO., USA.

Honey, W. and Kagle, J. 2008. An analysis of the impact of acid mine drainage on bacterial populations in the Upper Tioga River Watershed. Proceedings 108th Annual ASM Meeting, Boston, USA. pp95.

Janzen, C., Halke III, D., Evanovski, A., Heath, S., Raithore, S., Holt, J. and Tobin-Janzen, T. 2008. The impact of acid mine drainage mitigation on microbial community diversity and stream chemistry in the Shamokin Creek Watershed. Proceedings 108th Annual ASM Meeting, Boston, USA. pp95.

Kalevitch, M., Kefeli, V., Johnson, D. and Taylor, W. 2006. Plant Biodiversity in The Fabricated Soil Experiment. Journal of Sustainable Agriculture. 29(3):101-114.

Kalevitch, M., Kefeli, VI., Bruno, B., Jasmin, D. and Greg, B. 2004. Chemical Signaling During Organism's Growth and Development. Journal of Cell and Molecular Biology. Halic University. Faculty of Arts and Sciences. Turkey. 3:95-102.

Pimentel, D., Harvey, CP., Resoudarmo, K., Sinclair, D., Kurz, M., McNair, S., Christ, L., Shpritz, L., Fitton, RS. and Blair, R. 1995. Policy Forum : USA Soil Erosion Rates: Myth and Reality Science. 269, 461.

Pimentel, D., McNair, SJ., Janecka, J., Wightman, CS., C. O'Connell, E., Wong, L., Russel, J., Zern, TA. and Tsomondo, T. 1976. Environnemental and Economic Costs of Soil Erosion and Conservation Benefits. Science. 194,149.

USDA. 1980. Soil and Water Resources Conservation Act, 1980 Appraisal, Part I: Soil, Water, and Related Resources in the United States: Status, Conditions and Trends, and Appraisal; Part II: Soil, Water, and Related Resources in the United States, Analysis of Resources Trends (USDA, Washington, DC, 1981).

Received: March 13, 2013; Accepted: April 27, 2013