

## BACTERIAL AND MINERAL CONTENT OF FABRICATED SOIL (FS)

\*Maria Kalevitch<sup>1</sup>, Valentine Kefeli<sup>2</sup> and Margaret Dunn<sup>2</sup>

<sup>1</sup>Robert Morris University, 6001 University Boulevard Moon Township, PA 15108, USA

<sup>2</sup>Biomost, Inc. 3016 Unionville Road, Cranberry Twp., PA 16066, USA

### ABSTRACT

Fabricated soil was created for the rehabilitation of the damaged mining soil that is abundant in Western Pennsylvania. Our major goal was to evaluate mineral and microbial content of fabricated (manufactured) soil and to identify if this soil is a long-term or a short-term solution for maintenance of soil health. We also considered plant-soil-microbiota interactions as the contributors to ecological sustainability. The recipe of this soil contains parts of top soil, old mushroom compost, pond sediment, dry leaves and saw dust. This composition makes a favorable ratio of main nutrient elements, N, P, and K, as well as a sufficient ratio of carbon and nitrogen (1:5). The microbial composition of FS consists of fungi imperfecti and various bacteria species. During the five years of the experiment, trees of poplar and willows were grown on the fabricated soil. The level of fungi was diminished and the presence of bacteria increased or varied over the years of research. Five years of investigating FS showed a decrease in the nitrogen level, but phosphorus and potassium were not changed actively. The addition of clay in the form of pond sediment increased the level of Al and Si in the FS composition.

**Keywords:** Soil bacteria, fungi, mineral nutritive elements, carbon-nitrogen ratio, and biological tests of the soil samples.

### INTRODUCTION

Fabricated soil (FS) is a mixture of decaying substrates rich in alumino-silicate, carbon, nitrogen, phosphorus and potassium sources. This substrate usually is used for landscape rehabilitation (Kefeli *et al.*, 2004). Fabricated soils (FS) are developed from a mixture of materials, which encourage plant development. FS in this study were developed for use in the reclamation of drastically disturbed lands. One of the main components of native soil as well as fabricated soil is the aluminosilicate matrix provided by clays (illite, smectite, kaolinite, etc.) formed as weathering products of such minerals as orthoclase and other feldspars, and micas, such as muscovite (high potassium content), biotite and others (Jordahl *et al.*, 1996; Taylor *et al.*, 2003).

These minerals are necessary contributors of calcium, magnesium, sodium, and iron (Brady, 1990). The size consistency (soil texture) of the mineral fraction in native soil varies from clay-size to coarse sand-size. The carbon- and nitrogen-rich organic matter contains the monomers and polymers, the main constituents of the humus complex. Sources of cellulose are dry leaves and sawdust, which also provide lignin. These constituents are humus precursors. Humus is more or less a stable fraction of soil organic matter. It sorbs mineral nutritive elements, nitrogen, potassium, and phosphorus, which are important for plant growth and development. Natural soils are commonly described through soil profiles. Soil is a necessary intermediate substrate in the regulation of

the Biosphere activity. The loss of soil resources increased up to 10-15 million hectares in a year; therefore, rehabilitation of the soil cover is a global problem that could be solved in cooperation of such disciplines as mineralogy, soil science, biology, ecology, agrochemistry, and biochemistry. Developing public-private partnership efforts to utilize (recycle) local waste is promising both with economic and environmental benefits.

Numerous researchers are concerned with the damage that acid mine drainage 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. 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.

Janzen *et al.* (2008), described the impact of acid mine drainage on microbial community diversity and stream chemistry in the Shamokin Creek Watershed, PA. It is a well known fact that diatoms as the representatives of biodiversity indicate the health of a particular environment. Bacterial presence also showed the sustainable 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

\*Corresponding author email: kalevitch@rmu.edu

demonstrated to be involved in sulfur oxidation. Another bacterial species were closely related to *Sphingomonas* species.

In our research 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 the plant polymers, cellulose and lignin in the humus (polymer that is tightly connected to the aluminosilicate matrix of the soil micelle).

The role of microorganisms in the composting process is important. They combine their activity with plants in the transformation of plant organic substances (Kalevitch and Kefeli, 2006).

Soil pH is another important soil property that affects the availability of nutrients. Macronutrients tend to be less available in soils with low pH whereas micronutrients tend to be less available in soils with high pH. In the desired pH range of 6.0 to 6.5, nutrients are more readily available to plants and microbial populations in the soil increase. Microbes convert nitrogen and sulfur to forms that plants can use.

Macronutrients can be broken into two more groups: primary and secondary nutrients.

The primary nutrients are nitrogen (N), phosphorus (P), and potassium (K). These major nutrients are usually lacking from the soil first because plants use large amounts for their growth and survival. The secondary nutrients are calcium (Ca), magnesium (Mg), and sulfur (S). Usually there are enough of these nutrients in the soil so fertilization is not always needed. Also, large amounts of calcium and magnesium are added when lime is applied to acidic soils. Sulfur is usually found in sufficient amounts from the slow decomposition of soil organic matter, an important reason for not throwing out grass clippings and leaves.

Micronutrients are boron (B), copper (Cu), iron (Fe), chloride (Cl), manganese (Mn), molybdenum (Mo) and zinc (Zn). Recycling organic matter such as grass clippings and tree leaves is an excellent way of providing micronutrients (as well as macronutrients) to growing plants.

## MATERIALS AND METHODS

Soil sampling was done on the FS plots. The starting year FS, 2002, was used as a control. Other samples were used for the investigation of mineral composition, plant and microbiological activity of the FS (Table 1). Mineral elements were determined by Conti, Inc, USA. Microbial analytics were done by US-Microsolutions, Inc.

Table 1. Number of bacterial colonies found in soil samples (Kalevitch and Kefeli, 2006).

SOIL TYPE	*Total Bacterial Count,	
	CFU x 10 <sup>6</sup> / number of bacterial species	
	2004	2005
Mining soil	.0022/8	0.063
Top soil	.43/8	711
Fabricated Soil	27/9	711
Fabricated Soil 2002 Poplar	66/10	68.4
Fabricated Soil 2002 Willow	18/8	486
Fabricated Soil 2003 Poplar	114/14	495
Fabricated Soil 2003 Willow	30/8	882

\*All data are statistically significant. 95% of confidence intervals exist for all points,  $\alpha = 0.05$

**RESULTS**

Fabricated soil contains two forms of carbon sources: dry leaves and saw dust. Leaves were subjected to composting in 2 months and saw dust carbon transformed in 2-3 years. Fabricated soil (FS) contains more carbon than regular Grashem Soil (Fig. 1).

After one year the FS contained almost the same amount of carbon on the willows (Pussy and Red) and under poplar. The levels of mineral elements after one year of FS exposure on the mining soil were much higher than in Grashem soil. Grashem soil is considered as standard and is nearly level, very deep, and somewhat poorly drained. Typically the surface layer is dark grayish brown silt loam about 8 inches thick (Soil Survey of Butler County, 1989).

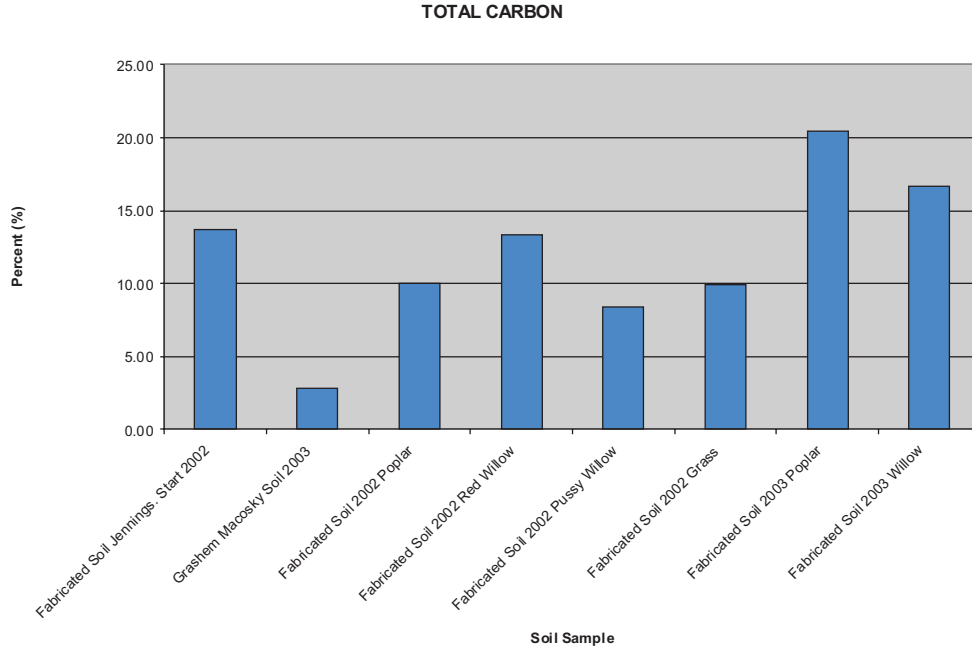


Fig. 1. Total Carbon in Soils.

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Composting processes that took place in manufactured soils. Definitely the combination of compost effects and the decay of leaf materials contributed to the increase of nitrogen concentration specifically within the first three months of soil exposure. At the same time the total bacterial count and number of bacterial species increased in FS during the first year of exposure. However, mining soil was very poor in microbial cenosis. In general it is important to mention that FS during a one-year exposure was still rich of nutritive elements, with the favorite carbon-nitrogen content on both types of plots willow and poplar. However, after five years of exposure on the same plots, FS components have been changed (Table 2).

Table 2. Change of Elemental Presence in % in FS after 5 years of Exposure on Willow Plot.

Elements	N	P	Ca	K	Mg	Mn	Fe	Cu
Change in %								
(2007 vs. 2002)	-90	-77	-88	64	25	25	34	25

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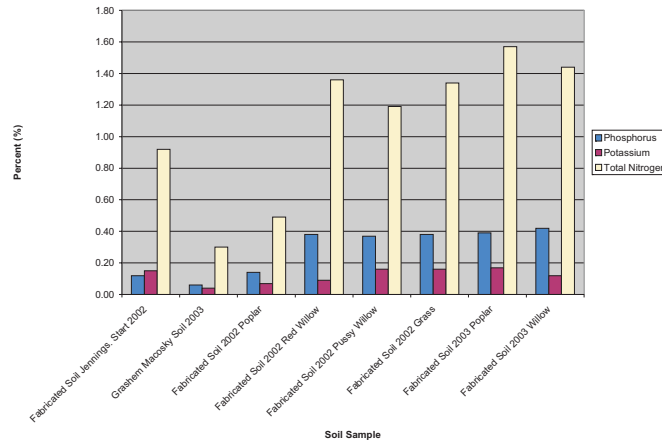


Fig. 2. Mineral Elements Phosphorus, Potassium, and Nitrogen in Soil Samples.

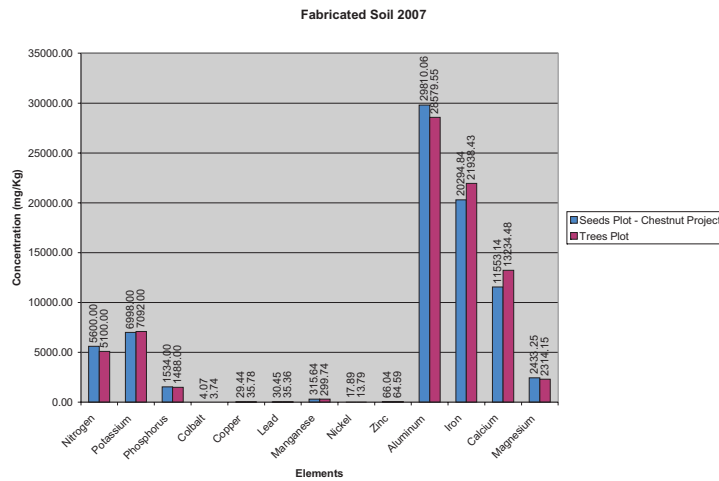


Fig. 3. Elemental Analysis of Seed Plot (Chestnut Project) and Trees Plot.

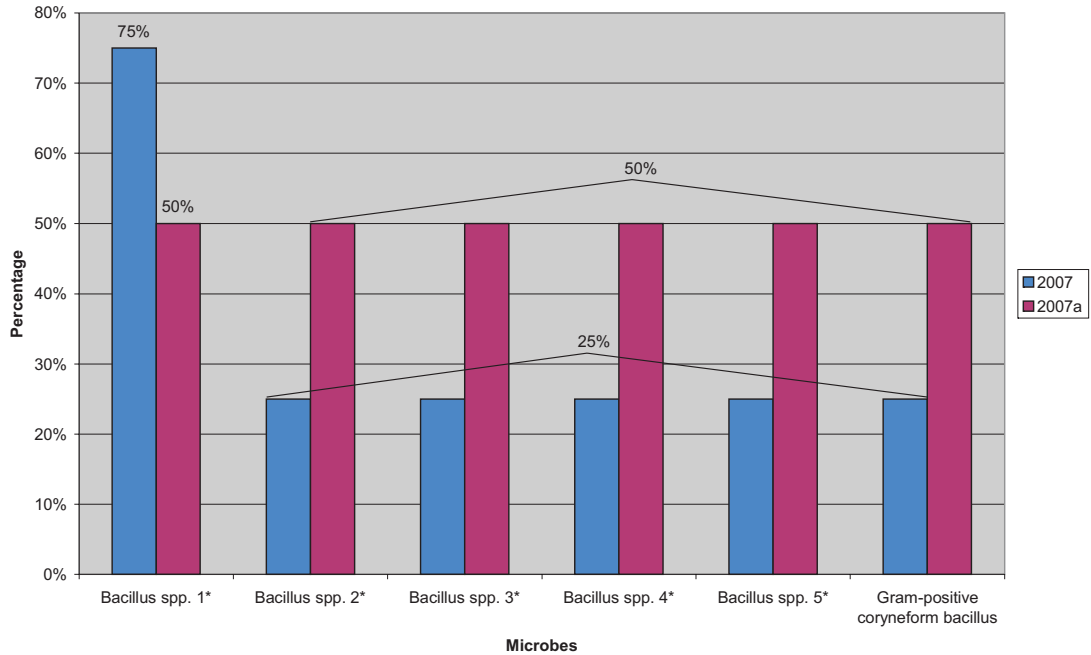
The mineral content correlated with soil biota presence and varied for different plots including Chestnut Project (Fig. 3) and De Sale Plot. Fabricated soil and Mineral soil samples were

used more for control comparison to demonstrate nutrients levels in newly developed plots vs plots that were under bioremediation for several years (see figures below).

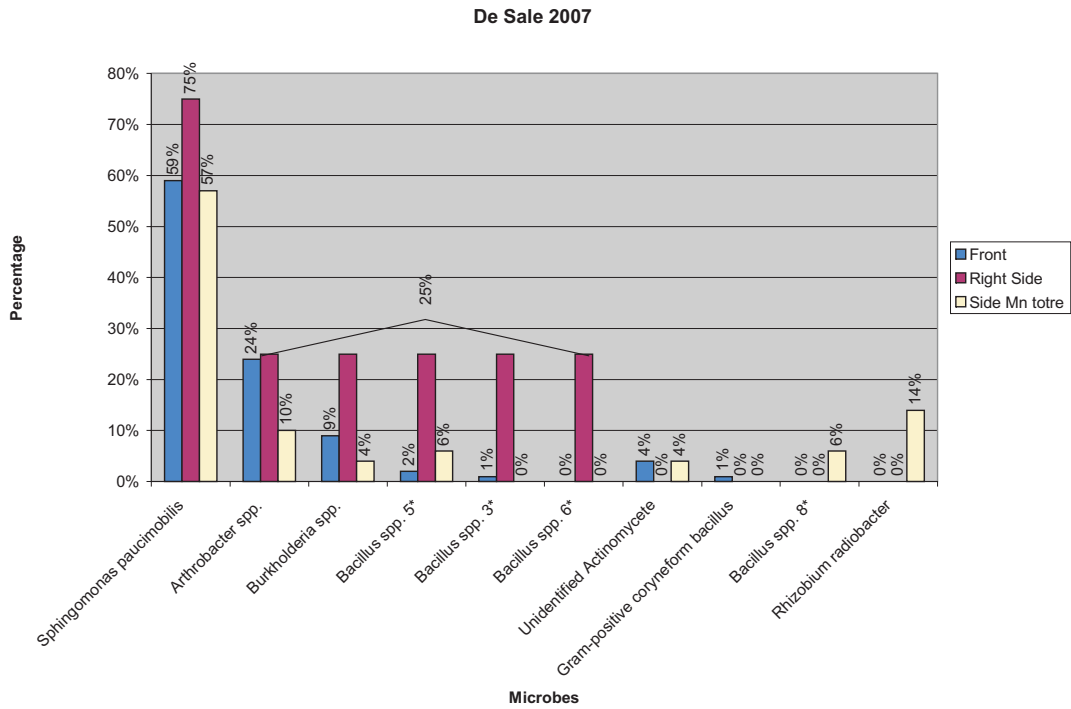
Table 3. Microbial content in FS (seed plot, Chestnut project and willow). Mining soil contains mostly actinomycets and the amount of bacterial species was more than 2 times reduced.

Results of Microbial Analysis			
Bulk Samples	Microbes	2007	2007a
		Percentages	
Forest Soil Chestnut Project	Total Bacterial Count	47.7x10 <sup>6</sup> CFU/gram	48.6x10 <sup>6</sup> CFU/gram
	Bacillus spp. 1*	75%	50%
	Bacillus spp. 2*	25%	50%
	Bacillus spp. 3*		
	Bacillus spp. 4*		
	Bacillus spp. 5*		
	Gram-positive coryneform bacillus		

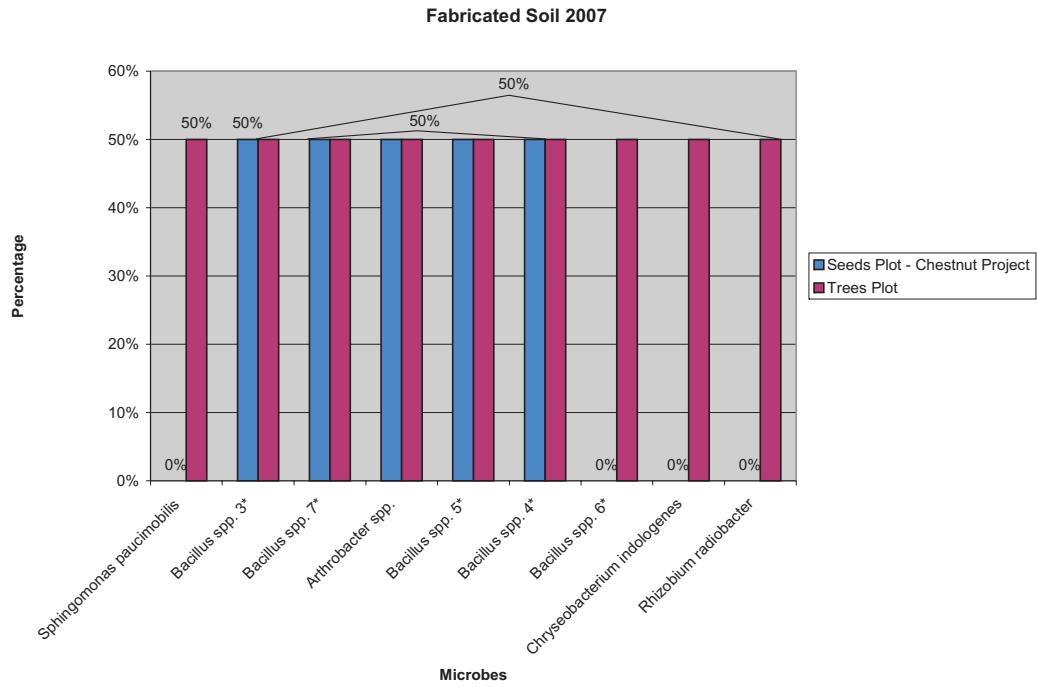
Forest Soil 2007 - Chestnut Project



Bulk Samples	Microbes	Front	Right Side	Side Mn totre
		Percentages		
De Sale	Total Bacterial Count	171.9x10 <sup>6</sup> CFU/gram	122.4x10 <sup>6</sup> CFU/gram	45.9x10 <sup>6</sup> CFU/gram
	Sphingomonas paucimobilis	59%	75%	57%
	Arthrobacter spp.	24%	25%	10%
	Burkholderia spp.	9%		4%
	Bacillus spp. 5*	2%		0%
	Bacillus spp. 3*	1%		0%
	Bacillus spp. 6*	0%	0%	0%
	Unidentified Actinomycete	4%	0%	4%
	Gram-positive coryneform bacillus	1%	0%	0%
	Bacillus spp. 8*	0%	0%	6%
	Rhizobium radiobacter	0%	0%	14%



		Seeds Plot Chestnut Project	Trees Plot
Bulk Samples	Microbes	Percentages	
Fabricated Soil	Total Bacterial Count	76.5x10 <sup>6</sup> CFU/gram	82.8x10 <sup>6</sup> CFU/gram
	Sphingomonas paucimobilis	0%	50%
	Bacillus spp. 3*	50%	
	Bacillus spp. 7*		
	Arthrobacter spp.	50%	
	Bacillus spp. 5*		
	Bacillus spp. 4*		50%
	Bacillus spp. 6*	0%	
	Chryseobacterium indologenes	0%	
Rhizobium radiobacter	0%		



		2007
Bulk Samples	Microbes	Percentages
Mining Soil	Total Bacterial Count	3,600 CFU/gram
	Unidentified Actinomycete	50%
	<i>Bacillus spp. 3*</i>	25%
	<i>Bacillus spp. 7*</i>	25%

\* More than one species were recovered from sample

Biological activity of different soil components was also determined by using four plant crops sensitive to the presence of bioactive compounds (Table 4).

FOUR-CROP TEST IN KOCH DISHES					
No. SAMPLES	pH	CROPS ( shoots length in cm )			
		Turnip	Lettuce	Rye	Clover
1 Forest Soil Chestnut Project One	6.5	5.2	4.1	8.1	4.7
2 Forest Soil Chestnut Project Two	6.4	4.6	3.5	7.9	4
Avg. Forest Soil		4.9	3.8	8.0	4.4
		100%	100%	100%	100%
3 De Sale Front	6.6	5.0	3.1	8.9	4.3
		102%	98%	111%	97%
4 De Sale Right	6.7	4.6	3.3	7.6	5.0
		94%	87%	95%	114%
5 FS Seed Plot Chestnut Project	6.8	4.2	3.0	8.2	3.6
		86%	79%	102%	82%
6 FS Trees plot	6.8	4.9	2.9	7.3	4.5
		100%	76%	91%	102%
7 De Sale Mn	6.7	4.8	3	7.4	4.8
		98%	79%	93%	109%

All tested forms of the soils, Forest soil (Grashem type) and De Sale soil (Ernest series), had a neutral pH and more or less high biological activity. This form of testing should be considered in conjunction with wood plant (tree) growth as an integrative test for soil characteristics.

## CONCLUSION

Based on our findings, FS was used for landscape rehabilitation and the growth of hard wood plants: Willow, Poplar, and Chestnut trees. Willow plants excreted such allelopathic substances as salicylic acid that had anti-inflammatory activity and protected Chestnuts from blight. Chestnut trees are prone to blight infection.

The complex investigation of the FS showed that the mineral elements composition and biological characteristics of the soil samples, including microbacterial analysis, showed the important ecological role of FS for landscape restoration. However, we are yet far from the conclusion about long-term vs short-term effects of manufactured soils and their benefits to the environment.

## REFERENCES

Brady, NC. 1990. The nature and properties of soils. 3 edition. MacMillan Publishing, New York, USA. pp.604.

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 108<sup>th</sup> Annual ASM Meeting, Boston, USA. P.95.

Janzen, C., Halke III, D., Evanoski, 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 108<sup>th</sup> Annual ASM Meeting, Boston, USA. p.95.

Jordahl, JL., Foster, L., Schnoor, JL. and Alvarez, PJ. 1996. Effect of Poplar Trees (*Populus* species) on Microbial Population Important to Hazardous Waste Bioremediation. Environmental Toxicology and Chemistry. 16(6): 1318-1321.

Maria, VK., Valentine, I., Kefeli, BB., 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.

Kalevitch, M. and Kefeli, V. 2006. Bacterial Presence in Manufactured Soils. Journal of Agricultural, Food, and Environmental Sciences. Scientific Journals International. 1.1.

Taylor, W., Dunn, M. and Bustler, S. 2003. Accepting the challenge. SchiffPrint, Pittsburgh. pp.78.

Soil survey of Butler County PA. 1989. USDA, NRCS.



