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Isolating and Quantifying DNA in Mine, Coal Wastes, Soil Amendments and Artificial Soils for Mine Rehabilitation

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Abstract:
Mining is an activity that has been practiced by humans for years. Abandoned mines, tailings and spoils may have significant impact on the neighboring environments. The derelict mines and waste generated are the major issues of concern, as they are capable of discharging acid waters that are often saturated with iron, metals and metalloids. The process of rehabilitation is vital, which should include neutralizing the acid generating potential of the spoils and support vegetation. This research focuses on creating artificial soil for mine rehabilitation process by rejuvenating the mine waste with amendments, so that they support microbial growth and thus support vegetation in abandoned mines. Evidence for the growth of microorganisms and its support to vegetation is established by quantifying the DNA and conducting growth test with grass.

Keywords: Amendments, coal wastes, mining, rehabilitation

1.0 Introduction:

Brown coal mining in general produces huge amount of wastes. Most of the waste generated during mining is disposed on to the surface, with very little care taken in adopting waste disposal options. Planning and control on waste disposal methods to minimize the environmental impacts requires utmost care. Improper planning may result in possible failure of waste embankments non-productive use of land and potential for air and water pollution.Currently the mining areas at Loy Yang mines include 800 hectares with a production of more than 30 million tonnes of coal every year (LYP, 2013).

The brown coal reserves in Gippsland basin are used to produce 80% of the electricity for the State of Victoria. At Loy Yang mine the present mining rate of about 30-35 million tonnes of coal per annum is coupled with high overburden ratios of around 1:3, results in the need for disposal of significant amounts of waste every year. The waste generated and disposed of, often comprises of overburden soil, brown coal and processed tailings. The development and management of such practices, in future can pose serious problems during mine rehabilitation.

The main objective of this paper is to investigate the possibility of creating artificial soils for mine rehabilitation purposes which support microbial growth. These soils, once proven safe for use, will be laid over the waste dumps in the Latrobe Valley brown coal mines for waste dump reclamations purposes. This study has quantified the amount of DNA present in the mine wastes, soil amendments and artificial soils for over a period of three months with fifteen days interval.

1.1 Microbiology Of Overburden Waste

The chemical characteristics, indigenous micro and macro flora are highly variable and differ from site to site. The biotic life that occurs in the acidic and ferruginous mine wastes and discharge water is explained in the following account. As evidenced the successful colonization of *Juncus bulbosus, Typha latifolia,Phragmites australis* and other macrophytes in the acidic mines of Germany, the microbiotic life cannot be excluded from such environments (Pietch, 1998). In many ways the most significant life-forms evidenced in this kind of environments are microbial. In acidic, metal-rich effluents such as mine spoils, tailings and AMD considerable diversity in microbial life is now recognized. Many of these are acidophilic

microorganisms which grow at pH greater than 4 (Johnson and Hallberg 2003). Table 1 provides the information related to different kinds of bacteria that mait be present in overburden waste.

1.1.1 Acidophilic Microorganisms

Mostly prokaryotic and eukaryotic microorganisms are acidophilic and the majority of known acidopillic are mesopillic (temperature range 20-40 ^{O}C), some are thermotolerant (temperature 20-40 ^{O}C) and others thermophillic (temperature >60 ^{O}C). The most significant of the acidophilies are those bacteria that oxidize ferrous iron and/or reduced forms of sulpher and accelerate the oxidation reaction of sulphide minerals. In biotechnological terms, often called as 'biomining', is a process of processing metal ores with these microorganisms (Rawlings and Johnson 2007). *Acidithiobacillus ferrooxidans* (formerly *Thiobacillus ferrooxidans*;(Olson, et al. 2003)) is the best known mineral degrading prokaryotes and first pyrite-oxidizing bacterium discovered.

Table 1: Sulfide mineral-oxidizing bacteria and archaea

	Iron-oxidizers	Iron/sulfur-oxidizers	Sulfur-oxidizers
Mesophiles	Leptospirillum ferrooxidans Ferroplasma spp. 'Ferrimicrobium acidophilum'	Acidithiobacillus ferrooxidans Thiobacillus prosperus 'Sulfobacillus montserratensis'	Acidithiobacillus thiooxidan Thiomonas Cuprina
Moderate Thermophiles	Acidimicrobium ferrooxidans Leptospirillum Thermoferrooxidan	Sulfobacillus thermosulfidooxidans Sulfobacillus acidophilus	Acidithiobacilus caldus
Thermophiles		Acidianus spp. Sulfolobus metallicus Sulfurococcus yellowstonensis	Metallosphaera spp.

As it was easy to cultivate *At. ferrooxidans* at laboratory and because I is widely distributed in the natural and man-made acidic and iron rich environments, they were assumed to be most significant sulphide mineral-oxidizing bacteria. However there is another bacteria, *Leptospirillum ferrooxidans which* is more numerous and more active than *At. ferrooxidans* in many situations. *L. ferrooxidans* are less tolerant of low temperatures (<20 ^{O}C), and are reported to be less abundant than *At. ferrooxidans* (Johnson 1998; Johnson 2003).The significance of some of the more recently-described iron-oxidizing acidophiles (such as '*Ferrimicrobium*', and '*Ferroplasma spp'*.) has yet to be evaluated.

1.1.2 Iron- And Sulfate-Reducing Acidophiles

In anoxic environments oxidized (ferric) iron and sulfate can both act as alternative electron acceptors to oxygen as electron sinks. This condition hold true for neutral pH and acidic environments also. Indeed, because of the frequent elevated concentrations of iron in these environments, the solubility of ferric iron at pH < 2.3, and the high redox potential of the ferrous/ferric couple (+770 mV at pH 2), anaerobic respiration using ferric iron is both pragmatic and a thermodynamically-attractive alternative to aerobic metabolism in extremely acidic environments. Majority of acidophiles reduce ferric iron to ferrous, it has not been proved that the reaction is energy-transducing. However, it has been proved that under anoxic conditions some iron oxidizing acidophiles

such as *At. ferrooxidans* can couple oxidation of sulfur to the reduction of ferric iron. Iron oxidizers such as *Sulfobacillus* spp., *Acidimicrobium ferrooxidans* and *'Ferrimicrobium acidophilum'* which use organic electron donars can grow heterogenically in the absence of oxygenby ferric iron respiration (Bridge and Johnson 1998). All known species of the primarily heterotrophic genus *Acidiphilium* are also facultative anaerobes, coupling the oxidation of organic substrates to the reduction of ferric iron. This ability of oxidation of organic substrates to reduction of ferric iron is stable in some *Acidiphilium* and unstable in some (Toni A. M. Bridge 2000). In contrast to many acidophiles, some *Acidiphilium spp.* reduce iron even in the presence oxygen (up to about 60% of maximum ambient dissolved oxygen).

1.1.3 Other Acidophilic Microorganisms

Other acidophilic microorganisms do not have any direct impact on the geochemical cycle of sulpher or iron, but may have considerable ecological significance, occur in mine spoils, tailings and AMD. These include acidophilic eukaryotes and heterotropic prokaryotes (Rawlings and Johnson 2007). In AMD streams at coal and metal mines, photosynthetic eukaryote *Euglena mutabilis* has often been found contributing significantly to the carbon budgets of these ecosystems. From the AMD and acid streamers acidophilic protozoa has been isolated and studied under laboratory conditions. Phagotrophic flagellates, ciliates and amoebae have been described that, by grazing mineral-oxidizing bacteria, can control numbers and activities of the latter *in vitro* (Johnson, 1998b).

1.2 Microbiology of Compost

The addition of fresh organic matter to the soil should be avoided as it changes the ecosystem in which the plants are grown (de Bertoldi, et al. 1983). If the organic matter which is partially humified, is added to the soil, it will be degraded by the microbial flora resulting intermittent products which are not compatible with the plants growth (Zucconi, et al. 1981). The other disadvantage is the competition between micro-organisms and plant roots for nitrogen. Composting results in a stable product which is similar to that which occurs in soil naturally.

Sewage sludge, solid urban waste may contain very high quantities of health hazard causing pathogenic microorganisms (de Bertoldi, Vallini and Pera 1983; Raviv 2005). The pathogen (in particular fecal origin) contents in sludge are generally higher than in municipal solid wastes. The compost when used on the soil as an end-product should be very low in the pathogen concentration. The important issue is not to minimize the pathogen content but to avoid the regrowth of pathogens (de Bertoldi, Vallini and Pera 1983; Kawata, et al. 1977; Millner, et al. 1977; Pahren and Clark 1987). To produce a sufficiently hygenized end product by removing the pathogens, the pile should have a temperature of 70^0 C for 30 min (Kawata, Cramer and Burge 1977; Riffaldi, et al. 1986).

Wide variety of saprophytic micro-organisms takes part in the process of composting (de Bertoldi, Vallini and Pera 1983). These micro-organisms can be considered as the microflora of the system of composting. Pathogens correspond to secondary microbial population representing numerically insignificant fraction of total microbial population. Real competition arises when the community is heterogeneous in nature and density of population is high, relative to the supply of limiting environment. The distinct competitive advantage for native saprophytic population over the other population is that the composting environment is not a natural environment for pathogens, therefore competition in this ecosystem eliminates the less fit rival (de Bertoldi, Vallini and Pera 1983). The other main feature of composting is, it reduces the proble of regrowth of pathogens preventing the recontamination through competition between microbes (L.Y.P 2012)

Table: 2 Principal pathogens of concern in domestic sewage and sewage sludge

Organism	Disease/Symptoms
Bacteria	
Salmonella sp.	Salmonellosis (food poisoning), typhoid fever
Shigella sp.	Bacillary dysentery
Yersinia sp.	Acute gastroenteritis (including diarrhea, abdominal pain)
Vibrio cholera	Cholera
Campylobacter jejuni	Gastroenteritis
Escherichia coli (pathogenic strains)	Gastroenteritis
Enteric viruses	
Hepatitis A virus	Infectious hepatitis
Norwalk and Norwalk-like viruses	Epidemic gastroenteritis with severe diarrhea
Rotaviruses	Acute gastroenteritis with severe diarrhea
Entero viruses	
Polioviruses	Poliomyelitis
Coxsackieviruses	Meningitis, pneumonia, hepatitis, fever, cold-like symptoms, etc.
Echoviruses	Meningitis, paralysis, encephalitis, fever, cold-like symptoms, diarrhea, etc.
Reovirus	Respiratory infections, gastroenteritis
Astroviruses	Epidemic gastroenteritis
Caliciviruses	Epidemic gastroenteritis
Protozoa	
Cryptosporidium	Gastroenteritis
Entamoeba histolytica	Acute enteritis
Giardia lamblia	Giardiasis (including diarrhea, abdominal cramps, weight loss)
Balantidium co/i	Diarrhea and dysentery
Toxoplasma gondii	Toxoplasmosis
Helminth Worms	
Ascaris lumbricoides	Digestive and nutritional disturbances,
	abdominal pain, vomiting, restlessness
Trichuris trichiura	Abdominal pain, diarrhea, anemia, weight loss
Toxocara canis	Fever, abdominal discomfort, muscle aches, neurological symptoms
Taenia saginafa	Nervousness, insomnia, anorexia, abdominal pain, digestive disturbances
Taenia solium	Nervousness, insomnia, anorexia, abdominal pain, digestive disturbances
Necator americanus	Hookworm
Hymenolepis nana	Taeniasis

1.3 Effect of Fly Ash on Microbes

In fly ash most of the elements are present in the mineral phase (Jala and Goyal 2006). Inferences are drawn expecting that the interaction between inorganic fly ash and organic matter may help tin enhancing the effects that benefit plant growth on problematic soils (Page, et al. 1979).The major factor that limits use of fly ash in agriculture is the toxicity of boron. The microbial respiration which is inhibited by boron can be reduced by the co-application of readily oxidizable organic substrate (Adriano, et al. 1979; Page, Elseevi and Straughan 1979). Adrino et al (1980) says that compost for soil treatment can be made by mixing highly carbonaceous acidic material with fly ash.

The amended of fly ash with swine manure increased the availability of Ca, Mg was increased balancing the ratio between monovalent and bivalent cations, which can be detrimental to soil (Jala and Goyal 2006). A significant rise in the rice crop yield, noticeable change in the soil physiochemical properties and a rise in pH was observed when the soil was treated with fly ash mixed with paper factory sludge and farm yard manure (Hill and Lamp 1980). Pot culture studies conducted on soil incorporated with fly ash had positive effect on soil pH, Ca, Mg and P content and reduction in translocation of Ni and Cd and enhanced growth and yield of corn, potatoes and beans (Rethman and Truter 2001). Not much of research is done n the effects of soil amendment of fly ash on biological properties (Schutter and Fuhrmann 2001).

1.4 Microbiology of Brown Coal

The heterogeneous and hydrophobic nature of coal, existence of persistent compounds including polycyclic aromatic hydrocarbons made coal seem unlikely for microbial growth (Fakoussa and Hofrichter 1999). Fakoussa and Hofrichter (1999) in their studies observed that the presence of these compounds is a problem as the enzymes are highly specific. However there are many studies which report that microbes can use coal as a growth substrate (Fakoussa and Hofrichter 1999; Gokcay, et al. 2001; Scott and Lewis 1988; Scott, et al. 1986; Temp, et al. 1999). Despite of the hydrophobicity, the nutrients which are non volatile may dissolve in the water adhering to the coal surface. Micro organisms may live in the moist microenvironments within the coal (Kirby, et al. 2010).

The porosity in the low rank coals is high and thus they provide much surface area for the microbial colonization (Faison 1991). In a mine, the predominant source of energy for growth is the desulfurization process of coal. The desulfurization process may be initiated by spontaneous oxidation of sulphide minerals (Kirby, Vengadajellum, Burton and Cowan 2010). As the pH decreases the sulphide mineral oxidizing bacteria catalyzes the oxidation process, mainly by the acid tolerant micro organisms such as Gallionella ferruginea for which the optimal pH for growth is pH 4. With the time passage, as the pH decreases continuously the acid tolerant microbes are replaced by the acidophilic organisms for which the pH range should be 1.5-3 for good growth (Kirby, Vengadajellum, Burton and Cowan 2010).

The pH required for the optimum growth of acidophiles is 0 and 5.5 and at neutral pH they cannot have a good growth. High concentration of hydrogen ions is required by the cytoplasmic membrane of acidophiles to maintain membrane stability and at neutral stability the membrane begins to dissolve (Strandberg and Lewis 1988). Thermoplasma acidophilum is a thermophilic acidophilic archaeon that was isolated from a burning coal refuse pile. This strain grows from 45 to 62^0C, with optimal growth at 59^0 C and has a pH growth range of 0.96–3.5. The first reported T. acidophilium was mycoplasma (Kirby, Vengadajellum, Burton and Cowan 2010). Structurally similar strains, for example pleomorphic spheres (which lack a cell wall) were isolated from thermal spings at Yellostone Natioanla Park (Darland, et al. 1970) and another kind of acidophilic bacteria Thermoplasma volcanium was isolated from solfatara field (Darland, Brock, Samsonoff and Conti 1970; Segerer, et al. 1988).

2 Materials and Methods

2.1 Sampling of Study Materials

Overburden samples and fly ash samples were collected from different locations as shown in the figure 2. The samples were collected according to the procedures recommended by Sobek*et al*. (1978) and ASTM D4220 (ASTM 2007a). Each representative sample weighed about 3 kg. The samples were

properly sealed in plastic bags to avoid the loss of moisture.

2.2 Composition of Artificial Soils

The compositions of artificial soils were chosen in such a way that they should address two major problems at Loy Yang mine.

1. The soil laid on the dump should support and maintain the dump stability.
2. The artificial soils should neutralize the acid mine drainage at the overburden waste dump site.
3. The soil matrix should support plant growth and help in enhancing bacterial community which helps in plant growth.

For these reasons three artificial soil compositions were tested with varying compositions of brown coal and compost, maintaining a fixed ratio of overburden waste and fly ash.

Artificial Soil 1 (AS-1) → 97 g (OB+FA) + 2g of Compost + 1g of Brown Coal

Artificial Soil 2 (AS-2) →97 g (OB+FA) + 2.25 g of Compost + 0.75 g of Brown Coal

Artificial Soil 3 (AS-3) → 97 g (OB+FA) + 2.5g of Compost + 0.5g of Brown Coal

2.3 Isolating DNA From Study materials and Artificial Soils

The following step by step procedure was adopted to isolate DNA from the study materials.

1. 0.25 grams of soil sample was added to the PowerBead tubes provided in the isolation kit.
2. The tubes were gently vortexed, to mix the sample.
3. Unprecipitated C1 solution, 60 ml in quantity was added to the tube and tubes were gently vortexed.
4. Tubes were properly secured on the tube holder of the vortex adapter and were vortexed at maximum speed for 10 minutes.
5. Centrifuge the tubes at 10,000 x *g* for 30 seconds at room temperature.
6. The supernatant was transferred to a 2 ml collection tube and was observed the supernatant still had some soil particles in it.
7. 250 µl of C2 solution was added and the tubes were vortexed for 5 seconds and incubated at 4 ^{o}C for 5 minutes.
8. The tubes were centrifuged at room temperature for 1 minute at 10,000 x g.
9. Avoiding pellets, the supernatant, not more than 600 µl was transferred to a 2 ml collection tubes.
10. 200 µl of solution C3 was added to the tubes and vortexed gently. The tubes were then incubated at 4 ^{o}C for 5 minutes.
11. The tubes were centrifuged at room temperature for 1 minute at 10,000 x *g.*
12. Avoiding pellets, the supernatant, not more than 750 µl was transferred to a clean 2 ml collection tubes.
13. 1200 µl of solution C4 was added to the supernatant in the tubes and vortexed gently for 5 seconds.
14. Load approximately 675 µl onto a Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the flow through and add an additional 675 µl of supernatant to the Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. Load the remaining supernatant onto the Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature.

Note: A total of three loads for each sample processed are required.

15. 500 µl of solution C5 was added to the supernatant in the tubes and centrifuged at room temperature for 30 seconds at 10,000 x g.
16. The flow through was discarded.
17. Tubes were centrifuged for 1 minute at 10,000 x *g*.
18. The spin filter was transferred to a clean 2 ml collection tube carefully, avoiding splashing any C5 solution on to the spin filter.
19. 100 µl of solution C6 was added to the centre of the white filter membrane.
20. Centrifuge at room temperature for 30 seconds at 10,000 x *g*.
21. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application.
22. The final volume of eluted DNA will be 100 ?l. The DNA may be concentrated by adding 4 µl of 5 M NaCl and inverting 3-5 times to mix. Next, add 200 µl of 100% cold ethanol and invert 3-5 times to mix.
23. Centrifuge at 10,000 x *g* for 5 minutes at room temperature. Decant all liquid. Remove residual ethanol in a dessicator, or air dry.
24. The concentrated DNA is quantified using spectrophotometer. If providing evidence for the presence of a particular bacteria is the area of interest, the obtained DNA is analyzed for

PCR reactions (In this study, focus is laid only in providing an evidence for the growth of microbial community which is directly proportional to the increase in the content of DNA in the samples)

3.0 Results and Discussion:

The overburden wastes, fly ash, compost, brown coal and artificial soils A.S.1, A.S.2 and A.S.3 were tested for the presence of microbial DNA. The reason for the microbial analysis is to find out the presence of microbial community. Microbes play a very important role in nitrogen cycle, which in turn help the plant growth on the artificial soils. Table 3 provides and evidence to the presence of microbial community in all the materials. The amount of DNA that was initially present in the samples is provided in the Table 3. It was observed that fly ash which was obtained on burning coal at very high temperatures, contain DNA in it. The amount of DNA that was noticed in fly ash was 1.04 μgml^{-1}. This mait be because the samples were collected from tha fly ash pond into which the ash was disposed and laid for a long period. Due to the interaction of fly ash with atmosphere for a significant period, chances for microbial growth cannot be neglected. The highest concentration of DNA was found in compost and was quantified as 274 μgml^{-1}. The amount of DNA in overburden waste and brown coal was 0.792 μgml^{-1} and 0.804 μgml^{-1} respectively.

The same procedure for extracting DNA was repeated in triplicate for the artificial soil matrices. It was observed that the artificial soil 3 (AS-3) had high amount of DNA in it. The primary reason for this may be the presence of high amount of compost in it. The amount of DNA that was found in AS-3 was 0.992 μgml^{-1}. The amount of DNA hat was found in artificial soil 1 and artificial soil 2 was 0.572 μgml^{-1} and 0.756 μgml^{-1}. The quantity that was found in AS-1 and AS-2 was less when compared to AS-1because of the varying composition of compost in them. AS-1 contains more compost that other two artificial sol matrices.

Table 3: Initial concentration of DNA in OB waste, fly ash, brown coal, compost and Artificial soils

Materials	qbit reading μgml^{-1}	Dilution Factor	Concentration μgml^{-1}
OBW	0.0198	40	0.792
F.A	0.026	40	1.04
B.C	0.0201	40	0.804
Compost	1.37	200	274
AS-1	0.0248	40	0.572
AS-2	0.0189	40	0.756
AS-3	0.0143	40	0.992

The Table 4 presents an evidence for an increase in microbial community in the artificial soils after 30 days of the initial analysis. It is clearly evident that there was an increase in the amount of DNA in the three artificial soil compositions. It is also evident that the artificial soil in which the content of compost is high showed the presence of high amount of DNA than the other two soils. The amount of DNA that was found in artificial soil 3 (in which the compost content is high) was 1.056 μgml^{-1} followed by AS-2 and AS-1 with 0.78 μgml^{-1} and 0.596 μgml^{-1} respectively.

Simultaneously plant growth was also tested on the three artificial soils and is reported as shown in the figures. There was no much difference in terms of growth in both artificial soilsAS-1 and AS-2, but there was slight growth of grass in the artificial soil 3, which was a positive sighn.

Table 4: Concentration of DNA in OB waste, fly ash, brown coal, compost and Artificial soils after 30days

Materials	**qbit reading μgml^{-1}**	**Dilution Factor**	**Concentration μgml^{-1}**
A.S.1	0.0264	40	0.596
A.S.2	0.0195	40	0.78
A.S.3	0.0149	40	1.056

Figure 1: Plant growth in artificial soils **a**: AS-1, **b**: AS-2, **c**: AS-3 after 30 days

The analysis of the artificial soils for the third time was done forty five days after the initial analysis. The trend was same as it was in the second analysis. The artificial soil in which the compost content was high had high amount of DNA in it when compared to the other two soils. Table 5 gives the concentration of DNA in the samples after twenty day to the initial analysis. The artificial soil 3 had high amount of DNA in it, which was quantified as 1.156 μgml^{-1} and the artificial soils AS-1 and AS-2 had 0.672 μgml^{-1} and 0.796 μgml^{-1} respectively.

Table: 5 Concentration of DNA in OB waste, fly ash, brown coal, compost and Artificial soils after 45days

Materials	qbit reading μgml^{-1}	Dilution Factor	Concentration μgml^{-1}
A.S.1	0.0289	40	0.672
A.S.2	0.0199	40	0.796
A.S.3	0.0168	40	1.156

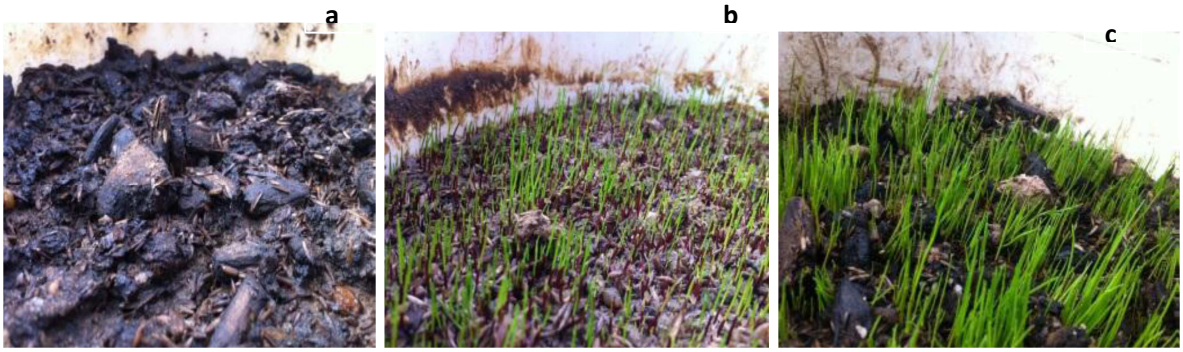

Figure 2: Plant growth in artificial soils **a**: AS-1, **b**: AS-2, **c**: AS-3 after 45 days

The plant growth as also monitored on the artificial soils and was filmed at the end of 45th day and is shown in the Figure 2. By the end of 45th day there was a slight growth in the AS-2 and reasonable grass growth in AS-3 but there was no growth in AS-1.

The amount of DNA in the artificial soils was quantified at the end of 60 days and is reported in the Table 6. It was observed that the concentration of DNA in the AS -1 at the end of 60th day was less when compared to that at the end of the 45th day. The pattern with the other two soil matrices remained same and the soil in which the compost content was high had high amount of DNA when compared with the other two. The amount of DNA in AS-1 was 0.432 μgml^{-1}, in AS-2 was 1.657 μgml^{-1} and in AS-3 was 2.734 μgml^{-1}.

Table: 6 Concentration of DNA in OB waste, fly ash, brown coal, compost and Artificial soils after 60days

Materials	qbit reading μgml^{-1}	Dilution Factor	Concentration μgml^{-1}
A.S.1	0.0289	40	0.432
A.S.2	0.0199	40	1.657
A.S.3	0.0168	40	2.734

Figure 3: Plant growth in artificial soils **a**: AS-1, **b**: AS-2, **c**: AS-3 after 60 days

The plant growth at the end of 60th day on artificial soils appeared as shown in figure. It was observed that growth started in the artificial soil 1 and artificial soils 2 and 3 had good grass growth.

The DNA from the three artificial soil matrices was extracted following same procedure and at the end of 75th day the concentrations appear as shown in Table 7. The DNA concentration in AS-1 had an increase when compared with that on 60th day, and was reported to be 0.742 μgml^{-1}. In the artificial soils AS-2 and AS-3 the trend was as usual with the concentration in AS-2 to be 1.947 μgml^{-1} and in AS-3 to be 3.254 μgml^{-1}.

Table: 7 Concentration of DNA in OB waste, fly ash, brown coal, compost and Artificial soils after 75days

Materials	qbit reading μgml^{-1}	Dilution Factor	Concentration μgml^{-1}
A.S.1	0.0289	40	0.742
A.S.2	0.0199	40	1.947
A.S.3	0.0168	40	3.254

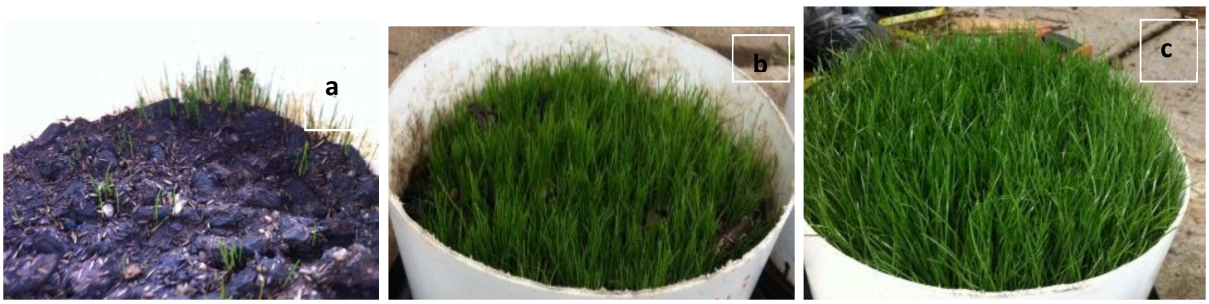

Figure 4: Plant growth in artificial soils **a**: AS-1, **b**: AS-2, **c**: AS-3 after 75 days

Plant growth in the three artificial soils was filmed and reported in the Figure 4. It was observed that AS-1 had very little growth when compared with AS-2 and AS-3. It was observed that AS-3 supported the plant growth to great extent when compared with AS-1 and AS-2. The details of the quantified DNA at the end of 90th day are provided in the Table 8. There was a slight increase in the DNA concentration in all the three artificial soils. The amount of DNA extracted from the artificial soil 3was 3.934μgml^{-1}, from As-2 was 2.212 μgml^{-1} and from AS-1 was 1.302 μgml^{-1}.

Table: 8 Concentration of DNA in OB waste, fly ash, brown coal, compost and Artificial soils after 90days

Materials	qbit reading µgml¬¹	Dilution Factor	Concentration µgml¬¹
A.S.1	0.0289	40	1.302
A.S.2	0.0199	40	2.212
A.S.3	0.0168	40	3.934

The plant growth in all the three artificial soils was photographed at the end of 90th day and produce in the Figure 5. It can be observed that the growth in As-3 was spectacular when compared to other two soils. The growth in artificial soil 1 was very poor and there was good growth noticed in the artificial soil 2.

Figure5: Plant growth in artificial soils **a**: AS-1, **b**: AS-2, **c**: AS-3 after 90 days

4.0 Conclusions:

This study is based on simple fact that presence of DNA provides an evidence for the existence of life in the soils.

1. It was noticed that there was significant increase in the amount of DNA extracted from the artificial soils in different periods. Artificial soil 1 provided no support to the plant growth even though it had microbial growth in it, artificial soil 2 supported plant growth reasonable well and artificial soil 3 had substantial increase in the quantity of DNA at the end of 3 month period and supported the plant growth extraordinarily. The growth on As-3 was substantial.
2. It can be concluded that even though there is very little variation in the composition of materials used to construct the soils, they played a vital role in supporting the plant growth.

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