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Research Article

Effect of Vermicompost amendment to Goldmine Tailings on Growth of *Vetiveria zizanioides*

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ABSTRACT

Industrialization, on medical and agricultural practices, mining and smelting of metalliferous ores, disposal of sewage along with careless biodegradation methodologies had led to accumulation of harmful waste products, including heavy metal toxicity in soil. This environmental disturbance can be a threat to animal as well as human life on earth. Among the various bioremediation strategies , phytoremediation was accepted as an approved measure to remove toxic heavy metals making the remediated soil fit for agricultural purposes.

A pot culture experiment was designed to test the effects of vermicompost on plants growing in gold mine tailings. The addition of vermicompost to tailings increased the shoot growth of the selected species, Vetiveria zizanioides. The most significant increase in growth for the selected species was obtained at 1.25% of vermicompost amendment. These preliminary results suggest that vermicompost has potential as a tailings amendment for revegetation of the gold mine site.

With compost amendment, Vetiveria zizanioides has good potential as a native species for phytostabilization of mine tailings in semiarid environment.

Key words: Bioremediation, gold mine tailings, vermicompost, Vetiveria zizanioides.

INTRODUCTION

Kolar Gold Fields is a town in the Kolar District of Karnataka state, India. Kolar Gold Fields (KGF) was one of the major gold mines in India and was considered the world's second deepest gold mine. After almost 2,000 years of gold mining, and three centuries of great importance, the mines were closed a few years ago. Krishna and Gejji [1] have highlighted the environmental pollution being caused by the dumping of the mill tailing (sand) in the Kolar gold mines area. About 32 million tonnes of this sand, which makes up the 15 dumps spread out along 8-km long distance in the mine area. These sands have been causing considerable environmental health hazards to the people of the Kolar gold field. Major constituents of sand in the Kolar gold mines area are Calcium oxide, Silica, Aluminium oxide, Ferrous oxide ,Magnesium oxide etc along with heavy metals like Arsenic and lead. Parts of the tailings contain pyrite, which oxidizes creating acidity and a corresponding release of metals into the environment. Wind and water can physically move tailings off-site causing contamination of adjacent areas. Establishing a vegetation layer decreases oxidation and prevents erosion of the tailings. . It has been suggested that low nutrient levels, low water availability, metal toxicity, acidity and poor physical structure all prevent plant growth on this site[2] .

Phytoremediation has gained popularity in the past 10 years. This popularity is based in part on the relatively low cost of phytoremediation[3]. It is plant based technique which can be applied at the contaminated site. Biological processes by vermin-composting converts vegetable waste to useful organic fertilizer . Vermicomposting can be carried out of vegetable waste with earthworm (*Eisenia foetida*), which develops in to a natural fertilizer and the vermicompost contain high nutrient value, increases fertility of soil and maintains soil health[4,5]. Application of vermicompost in contaminated soil improves soil fertility and physical properties as well as helps in successful approach to phytoremediation which has been demonstrated by Zheljazkov and Warman[6]. Along with improving the quality of growing plants, more metal can be taken up from the contaminated growth media and the tolerance to the metal toxicity can also be improved[7]. The use of vermin-compost developed from vegetable waste by vermin-culture biotechnology with soil would provide natural environment for phytoremediation[8].

For phytoremediation the plant species used should be selected in such a way that they should be able to remove toxic elements such as heavy metals from soil[9]. It is important to select an appropriate plant species for successful site reclamation and in phytoremediation efforts efforts to ensure a self-sustainable vegetative cover. Vetiver grass is widely known for its effectiveness in erosion and sediment control. Vetiver can tolerate extreme climatic variations and soil conditions, including heavy metals,thus the concept of using vetiver for phytoremediation started. Many researchers reported the potential of utilizing vetiver to decontaminate heavy metals from soil [9,10] ,garbage leachate [11], wastewater[12], and mine tailings[13]. Application of vetiver for phytoremediation, however, depends upon various factors such as soil condition and agronomic practices. They should be carefully investigated and properly considered in applying for site specific conditions to achieve the desired goal.

The main objective of the present study was to evaluate the effects of vermicompost amendments on gold mine soil, using vetiver grass (V. zizanioides) in pot culture. The present investigation was aimed at establishing suitable dosage of vermicompost that could show maximum growth response of V. zizanioides.

MATERIALS AND METHODS

Soil Sampling and Site Description

The soil samples used were collected from dumpsites of KGF (Kolar Gold Fields, Karnataka, India). Many dumpsites are present in and around the mines. Robertsonpet- one of such areas has been considered for the present investigation. The dumping has practically denuded the local vegetation. However a few survivors have been recorded at the site. Soil samples collected were air-dried and sieved through a two mm mesh sieve.

Amendment of Mine soil

Vermicompost was produced in Mount Carmel College premises, using Cow manure and leaf litter. Earthworm Eudrilus eugeniae and Perionyx excavatus were allowed to feed on the partially decomposed organic matter and castings of these earthworms was used as organic amendment in experimental soil.

Preparation of plant material and growth studies

A pot study was conducted to study growth performance of V.zizanioides in gold mine tailings amended with vermicompost. The experiment was carried out with seven treatments and control with three replications.

Soil weighing two Kg was added to each of the plastic pots (15cm in diameter, 27cm in height). Uniform-sized young culms of V. zizanioides collected from Kerala were used in this study. Plants were selected, pruned (shoots were 20 cm and roots were 5 cm in length) and then transplanted into the pots (1 plant/pot) containing various concentrations of vermicompost. (Table 1). There were three replicates for each treatment. The pots were placed under controlled conditions and the soil moisture content was maintained by adding deionized water once every two days. After 50 days, the plants were harvested. Plant samples were washed thoroughly with tap water, rinsed with deionized water and divided into shoots and roots. They were oven-dried at 60ºC for 48 h to a constant weight and the dry weight yield was recorded.

Recordings of plant growth

Plant growth and survival were determined as plant height and root/shoot ratio. The root/shoot ratio is an indicator of environmental stress that is encountered by plants (Chiu et al., 2006). All plant samples were dried at 60ºC for 48 h. Dry weight of roots and shoots was recorded and the ratio was calculated as follows:

Root/shoot ratio = Dry weight of root/Dry weight of shoot

Soil characteristics

Soil samples (Before plant growth and after 50 days of plant growth) were air-dried for 7 days and ground and sieved through a 200-mm mesh sieve. The following soil properties were tested:

a) Soil pH- Soil pH was measured in pH meter in soil and water suspension of 1:5

b) Electrical conductivity. The electrical conductivity (EC) of soil-water mixture was measured in conductivity meter.

c) Bulk density. Defined as the ratio of oven-dried soil (weight) to its bulk volume was

calculated using the formula:
Mass of dry soil (g)/Volume of dry soil (ml)= Bulk Density (g/cm3)

d) Soil respiration: Soil respiration was determined as substrate-induced respiration (Anderson, 1982). In the modified method, moist soil sample equivalent to one gram was amended with one gram of glucose. The CO_2 evolved was allowed for absorption by 0.1 N KOH and after 96 hours of incubation it was titrated against 0.1 N HCl

e) Water holding capacity (%): Water-holding capacity is dependent upon the organic matter in the soil and soil particle size .It was calculated using the formula:
Mass of the water contained in the saturated soil/Mass of the saturated soil x 100

Statistical analysis
One-way analysis of variance (ANOVA) was performed to find out the levels of significance of each of the parameters considered in the study.

RESULTS
Soil Properties
The general properties of the soil changed significantly. Soil pH showed great variation between unamended mine soil and others with amendment. The soil pH was near acidic (3.5) before planting vetiver, which changed towards neutral pH (6.3) with the addition of vermicompost and the growth of vetiver (Fig.3). The EC value also changed significantly (Fig.4). The soil texture was clayey with low organic matter content. The bulk density is a measurement of how tightly packed or dense is the soil. The bulk density also decreased with the enrichment of mine soil with vermicompost and growth of vetiver (Fig.5). The metabolic activities of soil microorganisms can be quantified by measuring basal soil respiration, assessed by the amount of CO_2 produced by soil microorganisms. Production of CO_2 in soil samples was determined in the laboratory based on alkaline CO_2 traps followed by chemical titration. Soil respiration in different soil samples reflected the amount of CO_2 produced by soil microbial activity (Fig 6).

The water holding capacity of soil is an important agronomic characteristic. Soils that have good water holding capacity are less subjected to leaching of nutrients. The amount of organic material in the soil also influences the water holding capacity. As the level of organic matter increased water holding capacity improved in vermicompost amended mine soils along with the growth of vetiver due to affinity of organic matter for water (Fig.7).

Growth performance

The growth performance data of V. zizanioides are shown in Figures.1, 2a, 2b and 2c.They represent the growth pattern of vetiver observed in different levels of vermicompost amendment after 50 days of planting. V. zizanioides showed the best survival with treatment 3: Mine soil + 1.25% vermicompost (25g in 2 kg of MS) and the lowest survival with control (only mine soil). The height of the plants and biomass significantly increased with increasing vermicompost addition. The growth performance (root/shoot ratio and height) of V. zizanioides is presented in Figs 8 and 9. The results in terms of height and biomass of V. zizanioides showed that they significantly increased with the application of vermicompost in comparison to those grown in control i.e. mine soil.

Discussion
The biggest environmental concern in metal mining is disposal of mine tailings. Since mine tailings contain toxic chemicals used to extract valuable metals from the ore, it leads to large areas of unvegetated land. Using plants in insitu stabilisation of contaminated land is found to be an effective and feasible method of bioremediation called "phytostabilisation".
This study is a preliminary trial to emphasize the considerations for phytostabilization of mine tailings . We have restricted our study to find out the possible variations in soil characteristics due to soil amendment and phytostabilisation and the preliminary analysis of the selected plant grown in the contaminated and amended soil.
The choice of plants is a crucial aspect for the practical application of phytostabilization based techniques on mine-degraded soils[14,15,16,17]. V. zizanioides has massive finely structured, deep root system and it has been known for its effectiveness in erosion control and the ability to tolerate extreme soil conditions including heavy metal contamination[18]. Truong and Baker[19] have reported the successful use of V. zizanioides to stabilize mining overburden and highly saline, sodic, magnesic and alkaline (pH 9.5) tailings of coalmine and highly acidic (pH 2.7) and high arsenic tailings of goldmines. Vetiver efficiency for the remediation of soils contaminated by heavy metals was studied by Antiochia *et al* [20]. Vetiver

plants were tested for Cr, Cu, Pb and Zn. The concentrations of the heavy metals were determined in both experiments in shoot and root parts of vetiver plants . Their experiments showed that vetiver plant can be considered a quite good "hyperaccumulator" for Pb .

Solubility of soil minerals, the availability of plant nutrients, and the activity of microorganisms are greatly affected by soil pH. When these soils were initially drained, pH values were much lower. Most organic soils historically have pH values ranging from 4.5 to 5.5. In contrast to that pH range, the current typical range for the plant supporting soil of in this area range from 6.5 to 7.5[5], although pH is spatially variable. Soil can get acidic or alkaline based on the climate, agricultural practices and availability of water. This may occur either naturally or as a result of inappropriate soil use and management. Soils that are excessively acid or excessively alkaline affect productivity. Nutrients in soil are strongly affected by soil pH due to reactions with soil particles and other nutrients, so in fact the availability of many nutrients has been determined as a function of soil pH[21]. Vermicompost amendment changes the soil pH favorably. The production of NH_4^+, CO_2 and organic acids during microbial metabolism and their interaction with other soil elements may be contributed to the change in soil pH [22].

Plants vary in their ability to extract nutrients from the soil and, for this reason; some plants are able to perform better in acid soils, while others prefer higher pH. V. zizanioides is known to be able to cope up with a wide range of soil pH (3.0 to 10.5)[23]. (Truong and Baker 1998).). The soil pH of mine soil has improved with vermicompost amendment and by growing V. zizanioides (Fig. 3).

Soil electrical conductivity (EC) is a measurement that correlates with soil properties that affect crop productivity, including soil texture, cation exchange capacity (CEC), drainage conditions, organic matter level, salinity, and subsoil characteristics. The soil water seems central to evaluating the usefulness of EC mapping in precision agriculture. The quantity of dissolved solids is directly proportional to conductivity per unit volume[24]. (Chang et al, 1983). However the electrical conductivity (EC) varies not only to the concentration of salts present in the soil but also due to chemical composition of fertilizers or pesticides used. It is applicable to any sort of nutrient amendment. Amendment with vermicompost and on growing of vetiver the EC of mine soil has decreased tremendously (Fig.4) . The EC of vermicompost depends on the raw materials used for vermicomposting and their ion concentration[22]. Tharmaraj *et al* [25] reported that The physical properties such as the pH, electrical conductivity (EC), porosity, moisture content, water holding capacity and chemical properties like nitrogen, phosphorous, potassium, calcium and magnesium were found distinctly enhanced in vermicompost treated soil. Vetiver grass is a fast-growing plant that tolerates various extreme environments, including soil pH values between 3.0 and 10.5 and temperature from -10 to 48^oC[26]. In addition, the use of vermicompostalso resulted in effective management of EC and thus it may improve soil texture, cation exchange capacity , drainage conditions andvorganic matter level of mine tailings.

Bulk density is an indicator of soil compaction. It is calculated as the dry weight of soil divided by its volume. Bulk density is dependent on soil texture and the densities of soil mineral (sand, silt, and clay) and organic matter particles, as well as their packing arrangement. Soil bulk densities range; in general, from < 1.0 (in organic soils) to 1.7 g/cm3and are dependent on the densities of the soil particles (sand, silt, clay, and organic matter) . Compacted soil layers have high bulk densities, restricted root growth, and inhibit the movement of air and water through the soil. Soils made of minerals (sand, silt and clay) have a different bulk density than soils made of organic matter. Soil bulk indicates how tightly soil particles are packed together and the way it affects the plant roots to grow through soil horizon[27]. Mine soil has higher bulk density indicating that it was difficult for vetiver growth as compared to that amended with vermicompost in which vetiver roots could grow well. (Fig.5) . Azarmi *et al* [22] showed that soils amended with vermincompost had significantly greater soil bulk density in comparison to control. Compost addition caused a significant increase of bulk density due to the more porosity added to the soil[28]. Due to its extensive, deep and penetrating root system, it is expected that some soil physical parameters such as water infiltration rates and soil bulk density would be improved under vetiver cultivation[29]. Thus vermicompost amendment and phytostabilisation by vetiver improves the soil characteristics for further vegetation in the improved soil.

The CO_2 evolution from soil is a good indicator of overall biological activity of the soil, and is often used when studying the carbon cycle. Soil respiration, represents the biological oxidation of organic matter to CO_2 by microorganisms. It provides the principle means by which photosynthetically fixed carbon is returned to the atmosphere. The metabolic activities of soil microorganisms can be quantified by measuring CO_2 production and/or O_2

consumption[30]. More fertile soils would be able to store more soil organic C (vermicompost, garden soil and control mine soil) and release more CO_2 to the atmosphere compared with less fertile soils (Mine soil) [5]. As shown in Fig.6 the results indicate that amendment of mine soil with vermicompost significantly enhances the amount of CO_2 release and it represents the increase in the soil organic Carbon.

Soil has the ability to store moisture which get affected by evaporation from soil surface, transpiration by plants and percolation through soil particles. Decrease in soil moisture content can be a cause for stress to the plants. The moisture storage capacity of a soil provides buffer, which determines its capacity to support the standing crop to withstand dry spells. The water holding capacity has increased significantly with vermicompost amendment (Fig.7) indicating better growth of vetiver. Aggregation of the soil becomes an important factor as far as the percolation of water and water-holding capacity of soil are concerned. The vermi-cast is rich in micro and macronutrients. Therefore, aggregation of the soil with vermi-cast also leads to storage of nutrients. The nutrients are released to the plants as and when required [31]. Sivaraj [32] has summarized his research results that use of vetiver grass improved the water holding capacity and moisture percentage of soil in wastelands.

Soil amendment is a major requirement for the successful establishment of vegetation in metal-contaminated soils. The addition of amendments such as vermicompost, is effective in lowering the metal toxicity of soil and provides a slow release of nutrient sources such as N, P, K to support plant growth[18,33]. Among the sources of organic manures, vermicompost has a special place because of the presence of readily available plant nutrients, growth enhancing substances, and number of beneficial microorganisms like N2 fixing, P solubilising and cellulose decomposing organisms[34]. Vermicomposting technology also enables the utilization/recycling of organic wastes for which no proper disposal mechanisms are available, or that the conventional techniques such as incineration may be hazardous. The root/shoot ratio is the indicator of environmental stress encountered by plants[33] . Plants have been documented to increase root/shoot ratio when stressed by harsh environments[35]. In the present study, vetiver grown in mine soil exhibited the highest root/shoot ratios indicating the greatest stress. Vetiver grass grown in gold mine soil amended with vermicompost showed lower root/shoot ratios and better growth performance than those grown in mine soil alone. (Fig.8)

The main objective of the present study was to evaluate the effects of vermicompost amendments on gold mine tailings, and response of vetiver grass (V. zizanioides) in pot culture studies. It was observed that the plants grew best with Treatment 3: Mine soil + 1.25% vermicompost (25g in 2 kg of MS). The present study demonstrated the optimum level of vermicompost needed for amendment to bring about maximum biomass of vetiver in gold mine soils. The study has highlighted that vermicompost amendment could provide enough nutrients for the growth of vetiver in gold mine soils and thereby amendment with appropriate selection of plant may improve its physical conditions. Soil enzymes, act as indicators of soil health. There was increase in their activity with increase in levels of vermicompost amendment to mine soil. This was also influenced by extended time intervals of amendment[5]. (Nair et al 2010ed). The present work is in par with the findings of Nair et al [5] and physicochemical properties of mine tailings can be further favorably improved, by planting of plants like vetiver and make it productive.

CONCLUSIONS

V. zizanioides is good choice for phytostabilization of gold mine soil. The applications of vermicompost to gold mine soil could provide needed nutrients for plant growth. V. zizanioides
showed the best growth when grown in gold mine soil amended with 1.25% vermicompost (25g
in 2 kg of MS). In addition, the use of vermicompost and growth of V. zizanioides resulted in
increase in pH and decrease in EC and bulk density. Increase in soil respiration was indicative of
establishment of microbial activity

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Table 1 : Various treatments of mine soil with vermicompost

Treatments	Mine soil + Vermicompost percentages
Treatment 1	Mine soil (MS) + 0.25% Vermicompost (5g in 2 kg of MS)
Treatment 2	Mine soil + 0.75% Vermicompost (15g in 2 kg of MS)
Treatment 3	Mine soil + 1.25% Vermicompost (25g in 2 kg of MS)
Treatment 4	Mine soil + 1.75% Vermicompost (35g in 2 kg of MS)
Treatment 5	Mine soil + 2.5% Vermicompost (50g in 2 kg of MS)
Treatment 6	Mine soil + 3.75% Vermicompost (75g in 2 kg of MS)
Treatment 7	Mine soil + 5.0 % Vermicompost (100g in 2 kg of MS)
Treatment 8	Soil without adding vermicompost (100g in 2kg of MS)

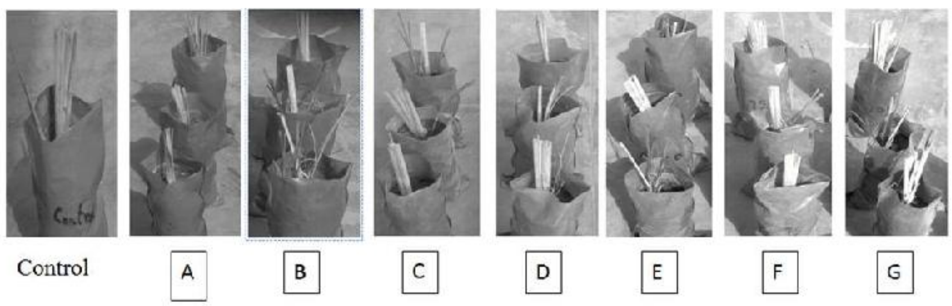

Figure. 1: Plants, pruned and then transplanted into the pots (1 plant/pot) containing various concentrations of vermicompost

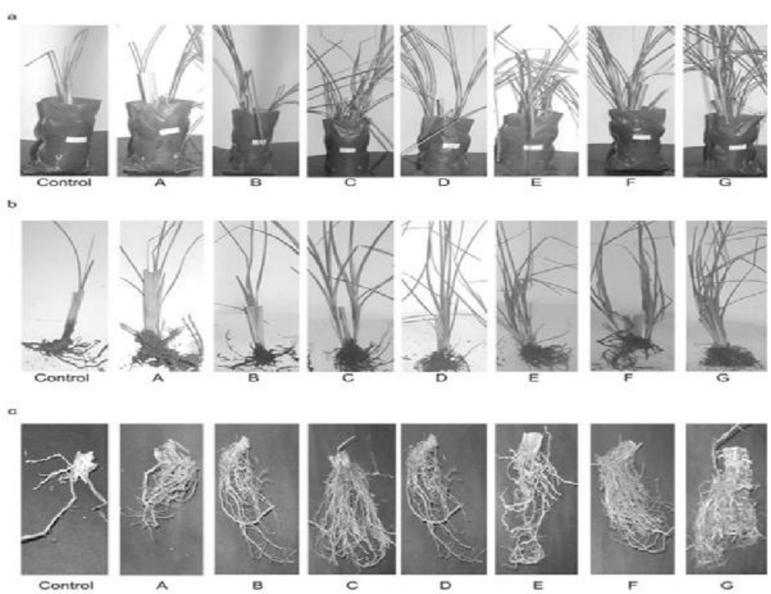

Figure.2

(a) Plants grown in vermicompost amended with mine soil for 50 days.

(b) Growth of V. zizanioides 50 days after planting

(c) Root proliferation of V. zizanioides observed after 50 days of planting in vermicompost amended gold mine tailings

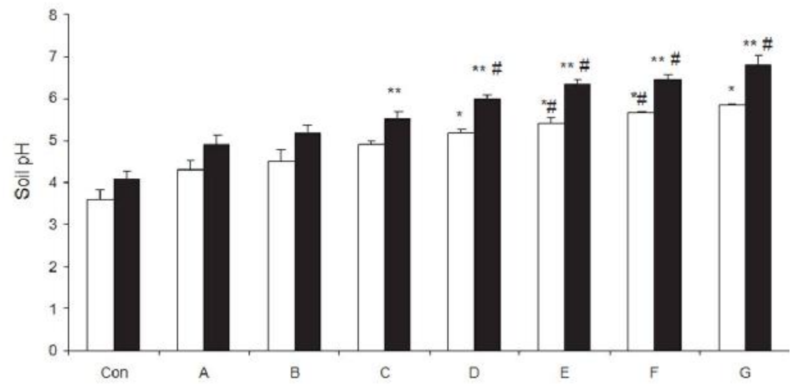

Figure.3. Changes in pH of vermicompost amended gold mine tailings before plant growth as compared to after 50 days of plant growth (V. zizanioides)

where Control - Mine soil (MS) MS with 0.25% Vermicompost (VC), B - MS with 0.75% VC, C - MS with 1.25% VC , D – MS with 1.75% VC, E - MS with 2.5% VC, F - MS with 3.75% VC, G - MS with 5.00% VC are indicated. All the data were analysed by ANOVA at *p< 0.001 for vermicompost amended mine soil (before plant growth) compared to mine soil; ** p< 0.001 for vermicompost amended mine soil (after 50 days of plant growth) compared to mine soil. # p< 0.001 for after plant growth compared to before plant growth(both are vermicompost amended mine soil).

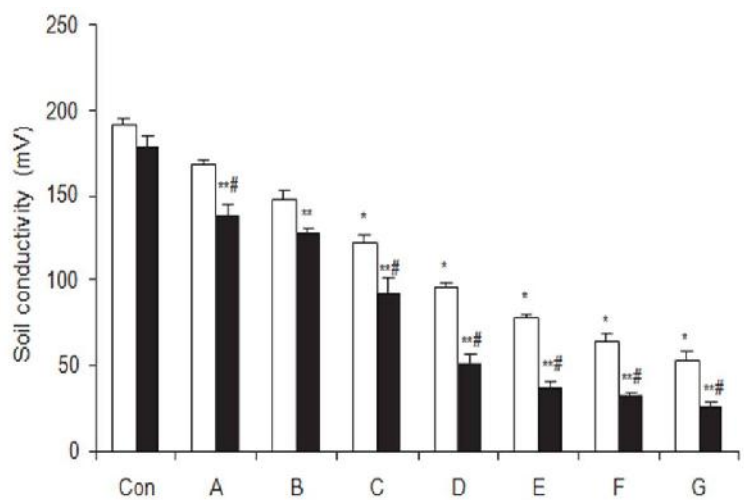

Figure.4 Changes in Soil Conductivity of vermicompost amended gold mine tailings before plant growth as compared to after 50 days of plant growth (V. zizanioides)

Where Control - Mine soil (MS), A- MS with 0.25% Vermicompost (VC), B - MS with 0.75% VC, C - MS with 1.25% VC ,D - MS with 1.75% VC, E - MS with 2.5% VC, F - MS with 3.75% VC, G - MS with 5.00% VC are indicated.

All the data were analysed by ANOVA at *p< 0.001 for vermicompost amended mine soil (before plant growth) compared to mine soil; ** p< 0.001 for vermicompost amended mine soil (after 50 days of plant growth) compared to mine soil, # p< 0.001 for after plant growth compared to before plant growth(both are vermicompost amended mine soil).

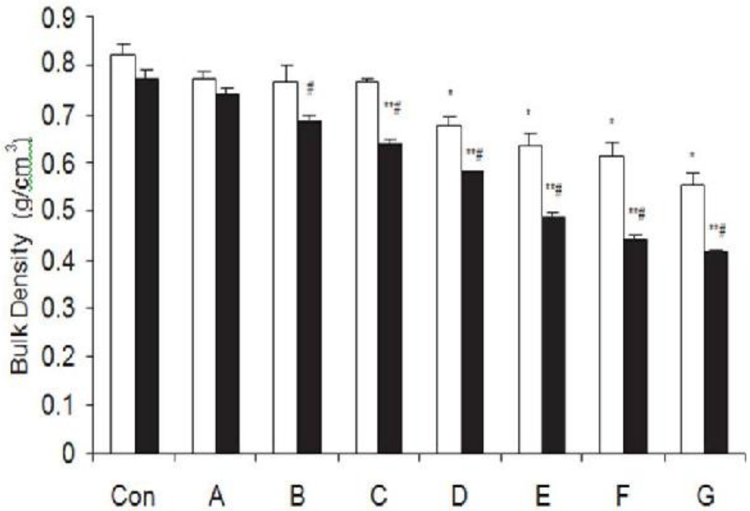

Figure.5 Changes in Soil Bulk Density of vermicompost amended gold mine tailings before plant growth as compared to after 50 days of plant growth (V. zizanioides)

where Control – Mine soil (MS), A- MS with 0.25% Vermicompost (VC), B - MS with 0.75% VC, C - MS with 1.25% VC ,D - MS with 1.75% VC, E - MS with 2.5% VC, F - MS with 3.75% VC, G - MS with 5.00% VC are indicated.

All the data were analysed by ANOVA at *p< 0.001 for vermicompost amended mine soil (before plant growth) compared to mine soil; ** p< 0.001 for vermicompost amended mine soil (after 50 days of plant growth) compared to mine soil; # p< 0.001 for after plant growth compared to before plant growth(both are vermicompost amended mine soil).

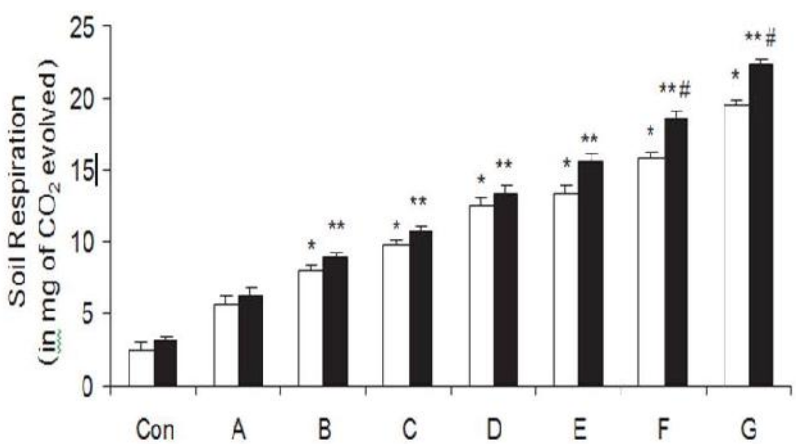

Figure.6 Changes in Soil Respiration of vermicompost amended gold mine tailings before plant growth as compared to after 50 days of plant growth (V. zizanioides)

where Control - Mine soil (MS), A- MS with 0.25% Vermicompost (VC), B - MS with 0.75% VC, C - MS with 1.25% VC ,D - MS with 1.75% VC, E - MS with 2.5% VC, F - MS with 3.75% VC, G - MS with 5.00% VC are indicated.

All the data were analysed by ANOVA at *p< 0.001 for vermicompost amended mine soil (before plant growth) compared to mine soil; ** p< 0.001 for vermicompost amended mine soil (after 50 days of plant growth) compared to mine soil; # p< 0.001 for after plant growth compared to before plant growth(both are vermicompost amended mine soil).

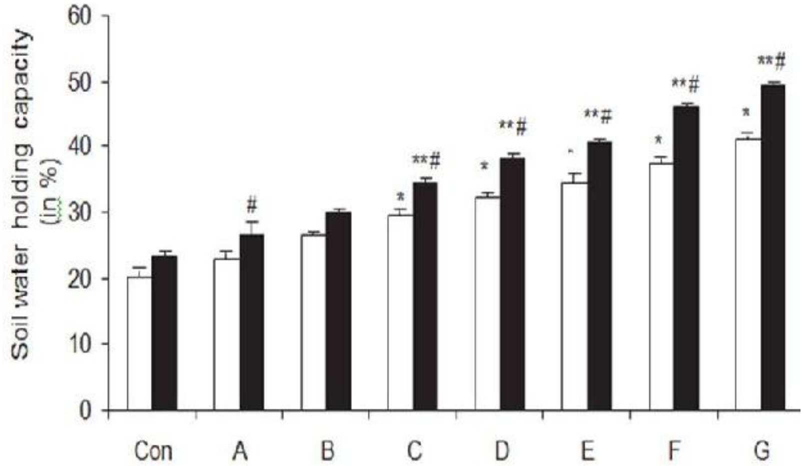

Figure.7 Changes in Water Holding Capacity of vermicompost amended gold mine tailings before plant growth as compared to after 50 days of plant growth (V. zizanioides)

Where Control - Mine soil (MS), A- MS with 0.25% Vermicompost (VC), B - MS with 0.75% VC, C -MS with 1.25% VC ,D - MS with 1.75% VC, E - MS with 2.5% VC, F - MS with 3.75% VC, G- MS with 5.00% VC are indicated.
All the data were analysed by ANOVA at *p< 0.001 for vermicompost amended mine soil (before plant growth) compared to mine soil; ** p< 0.001 for vermicompost amended mine soil (after 50 days of plant growth) compared to mine soil; # p< 0.001 for after plant growth compared to before plant growth(both are vermicompost amended mine soil).

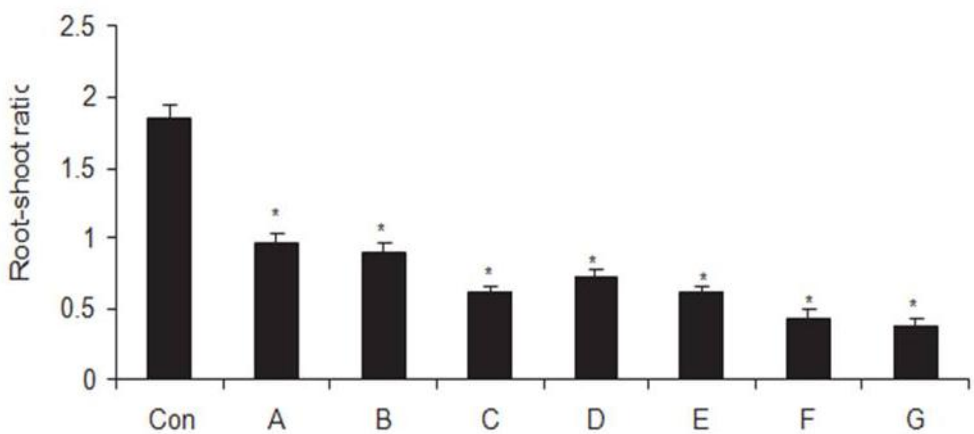

Figure.8 Root-Shoot Ratio in the V. zizanioides crop at the time of harvest (50 days) in vermicompost amended gold mine tailings .

where Control - Mine soil (MS), A- MS with 0.25% Vermicompost (VC), B - MS with 0.75% VC, C - MS with 1.25% VC ,D - MS with 1.75% VC, E - MS with 2.5% VC, F - MS with 3.75% VC, G - MS with 5.00% VC are indicated.
All the data were analysed by ANOVA at *p< 0.01 for V.zizanioides grow n in vermicompost amended mine soil (after 50 days of plant growth) compared to mine soil.

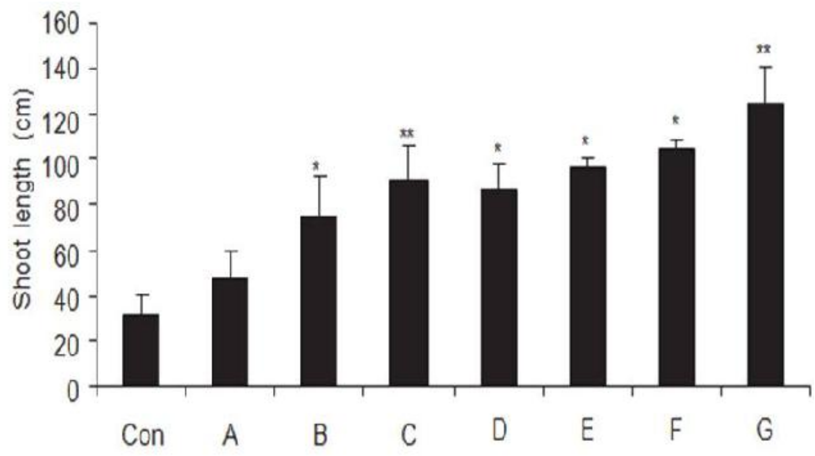

Figure.9 Plant height above the ground level of vermicompost amended gold mine tailings after 50 days of plant growth (V. zizanioides).

where Control - Mine soil (MS), A- MS with 0.25% Vermicompost (VC), B - MS with 0.75% VC, C - MS with 1.25% VC ,D - MS with 1.75% VC, E - MS with 2.5% VC, F - MS with 3.75% VC, G - MS with 5.00% VC are indicated.

All the data were analysed by ANOVA at *p< 0.01 for V.zizanioides grow n in vermicompost amended mine soil (after 50 days of plant growth) compared to mine soil.

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