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Comparison of Soil Enzyme Activities as Biochemical Fingerprints of Soil Health: Effect of Vermicompost on Gold Mine Tailings

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ABSTRACT

Soil health can be defined as the ability of a specific kind of soil to provide a natural or artificial ecosystem, which helps to maintain balanced plant and animal life. Soil organisms act as the primary agents of nutrient cycling and regulate the dynamics of soil organic matter. Activities of soil enzymes have great potential to assess the health of soil biota, as soil enzymes control mineralization and therefore influence availability of N, C and P. Soil enzymes provide an easy, relatively rapid and low cost procedures to monitor soil health as they are considered to be indicators for measuring the degree of soil degradation. The present investigation was aimed at evaluating the basal soil respiration, total microbial activity and potential enzymatic activities of β-glucosidase, urease, phosphatase and dehydrogenase in gold mine tailings and was compared with fertile soil types like vermicompost, garden soil and control soil from mine area. Differences in the respective analyses reflect the changes in the N, C and P metabolism in the soil, which is the result of total microbial activity. Amending the mine tailings with vermicompost showed significant effect in restoring the soil quality, which is reflected as increased enzyme activity, with different levels of amendment on increasing time interval.

Keywords: gold mine tailings, soil amendment, soil enzymes, soil health, vermicompost
Abbreviations: CM, control soil near mine dump; **GS**, garden soil; **MT**, mine tailing; **VC**, vermicompost

INTRODUCTION

Soil is part of the terrestrial environment that supports all terrestrial life forms. Soil health is the result of continuous conservation and degradation processes and represents the capacity of soil to function as a vital living system. A unique balance of chemical, physical and biological (including microbial) components contribute to the maintenance of soil health. Evaluation of soil health therefore requires indicators of all these components. Nielsen and Winding (2002), in their work have emphasized on the important contribution of soil microorganisms to soil health.

Healthy soils are essential for the integrity of terrestrial ecosystems to remain intact or to recover from disturbances, such as drought, climate change, pest infestation, pollution, and human exploitation including agriculture and mining (Ellert *et al.* 1997). Protection of soil is therefore of high priority and a thorough understanding of ecosystem processes is a critical factor in assuring that soil remains healthy (Nielsen and Winding 2002). The global impact of mine tailings disposal sites is enormous, as unreclaimed mining sites generally remain unvegetated for tens to hundreds of years, and exposed tailings can spread over tens of hectares (Warhurst 2000; Morris *et al.* 2003; Gonzalez and Gonzales-Chavez 2006).

In the process of mining, the area is stripped of vegetation. Several changes occur in the physical, chemical and microbiological properties of soil as a result of mining. Eastern Coalfields Ltd (ECL) was investigated to assess the deterioration in soil quality of a large opencast coal project (Ghose and Kundu 2004). The study revealed a major qualitative deterioration in the excavated soil when dumped over a long period of time. Inability to preserve topsoil is one of the basic hindrances to restoration of mined land and overburdening dumps.

Rehabilitation of damaged environment calls for careful planning and implementation of sound ecological principles. One of the critical aspects of the rehabilitation process is the improvement of the tailings material to sustain plant growth through restoration of soil health.

Microbial activity is fundamental in the processes that make energy and nutrients available for recycling in the ecosystem and soil microorganisms play crucial role in the biogeochemical cycling of carbon (C), nitrogen (N), and phosphorus (P) (Bandick and Dick 1999; Schoenholtz *et al.* 2000). The presence and activity of enzymes is vital for all biochemical transformations in soil. Thus the study of soil enzymatic activities provides insight into microbial dynamics and populations (Riffaldi *et al.* 2002). Studies have shown that the enzymatic activities such as that of dehydrogenase, β-glucosidase, urease, and phosphatase show significant correlation with total organic carbon (TOC), total nitrogen (TN), water-filled pore space (WFPS), and heterotrophic bacterial and fungal biomass (Bandick and Dick 1999; Aon and Colaneri 2001). Specific enzymatic activities that are widely distributed in soil are given in **Table 1**

Table 1 Soil enzymes used as indicators of soil health.

Enzyme (E.C. number)	Reaction catalysed	Soil activity indicated
Dehydrogenase (1.1.1.95)	Electron transport system	Microbial activity
Beta-glucosidase (3.2.1.21)	Cellobiose hydrolysis	Carbon cycling
Urease (3.5.1.5)	Urea hydrolysis	Nitrogen cycling
Phosphatase (3.1.3.2)	Release of phosphate	Phosphorus cycling

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Table 2 Comparison of soil physical properties.

Soil type	pH	Conductivity (mV)
Garden soil	5.43 ± 0.13*	90 ± 12.49*
Mine tailings	3.96 ± 4.07	151 ± 5.03
Control soil (mine area)	4.72 ± 0.08	110 ± 1.3
Vermicompost	6.55 ± 0.015*	28 ± 1.188*

pH and conductivity of mine soil is compared with other types of fertile soils. Values were given as mean ± SEM. All data were analysed by ANOVA. The level of significance is indicated using * at P<0 01.

(Dick *et al.* 1996).

This study was undertaken to differentiate the soil health of gold mine tailings (MTs) and other normal soils, including vermicompost (VC), by enzymatic assays. Many reports suggest that soil enzyme activity can be used as an indicator of soil quality for assessing the sustainability of ecosystems (Ndour Ndèye Yacine Badiane *et al.* 2001; Roldan *et al.* 2005; Li *et al.* 2008). Thus, soil enzyme assays can be considered for the biochemical fingerprinting of soil quality. Laboratory-scale studies were conducted to find out whether enrichment using VC could replenish or improve soil quality in MT.

MATERIALS AND METHODS

Soil sampling and site description

The soil samples used were collected in December 2006, from dumpsites of KGF (Kolar Gold Fields, Karnataka, India). Many dumpsites are present in and around the gold mines. Robertsonpet, one of the areas, was considered for the present investigations. The MTs were collected from the site not more than 8 cm deep. The dumping is practically denuded of local vegetation. However a few survivors have been recorded at the site. The control mine soil (CM) was collected 5 km away from the mine dump where plants showed normal growth. MT was compared with fertile garden soil (GS) from Bangalore and VC, which is the heterogenous mixture of decomposing vegetable or food waste, produced during the course of normal vermiculture operations.

In order to assess the soil health of the region, some of the biochemical parameters along with microbial activity in general were considered. The pH and conductivity of the soils of the experimental site are reported in **Table 2**.

Amendment of mine tailing

VC was prepared using cow manure and leaf litter. The earthworm, *Eudrilus eugeniae*, was allowed to feed on the partially decomposed organic matter mix and collected excreta of this earthworm (VC) were used as organic amendment to experimental MT.

MT was enriched with 1, 2, 4 and 8% VC and incubated for 4 weeks. Soil respiration and microbial activity were assessed and soil enzyme assays were conducted after 2 and 4 weeks. pH and conductivity were also measured at the end of the incubation periods (**Table 3**).

Estimation of microbial activity

The consolidated soil samples collected from the site at random were passed through a 2 mm sieve for the following estimations:

a) Soil respiration: Soil respiration was determined as substrate-induced respiration (Anderson 1982). The modified method consists of moist soil sample equivalent to 1 g was amended with 1 g of glucose. The CO_2 evolved was allowed for absorption by 0.1 N KOH and after 96 hrs of incubation it was titrated against 0.1 N HCl.

b) Total microbial count: Fluorescein diacetate (FDA) hydrolysis rate was followed to estimate total microbial activity (Bandick and Dick 1999). FDA is the substrate for a wide variety of enzymes, including proteases, lipases, and esterases. Fluorescein diacetate (FDA) hydrolytic activity was determined by the enzymatic assay procedures described by Bandick and Dick (1999).

The determination of FDA hydrolysis has the advantage of being simple, rapid, and sensitive, and it has been proved useful, especially for comparative studies of microbial activity (Schnurer and Rosswall 1982).

c) Soil enzymes: Soil enzymes are the direct mediators for biological catabolism of soil organic components. Thus, they act as indicators of reaction rates for important soil processes. Soil enzyme activities are often closely related to soil organic matter, soil physical properties and microbial activity or biomass. Changes in these parameters provide an early indication of changes in soil health. **Table 1** gives enzymatic activities that are widely distributed in soil, and it also justifies the selection of specific enzymes for biochemical assessment of soil health (Dick *et al.* 1996).

For the determination of soil respiration and total microbial activities, soil was kept field moist in refrigerated condition, while air-dried samples were used for determination of dehydrogenase, β-glucosidase, urease and phosphatase activities (Bandick and Dick 1999). All analyses were carried out in triplicate. The procedures of Öhlinger (1996), Alef *et al.* (1998) and Bandick and Dick (1999) were followed for measuring the activities of dehydrogenase and urease. Measurement of β-glucosidase and phosphatase activities was based on *p*-nitrophenol release after cleavage of a synthetic substrate (*p*-nitrophenyl glucoside and *p*-nitrophenyl phosphate, respectively) (Alef *et al* 1998). Modified universal buffer (pH 9) was used to measure total phosphatase activity.

Statistical analysis

All values were expressed as the mean of three measurements for each treatment. Data were subjected to analysis of variance (Lindman 1974). The mean values for each parameter (soil respiration and activities of β-glucosidase, urease, phosphatase and dehydrogenase) were compared for the different soil samples (MT, GS, CM and VC), at different percentages of VC amendment (1, 2, 4 and 8%) and different incubation times (2 and 4 weeks). Significant differences ($P < 0.05$, $P < 0.001$) were also determined.

RESULTS

Soil pH and electrical conductivity

Soil pH and conductivity measurements showed significant variation among MT and other fertile soil types (VC, GS and CM; **Table 2**). Moreover, enrichment of mine soil with VC produced significant change in pH and conductivity measurements (**Table 3**), which was influenced by the incubation period and percentage of VC amendment.

Soil quality is described as the status of soil related to agricultural productivity or fertility. The extent to which biological activity remains reflects the degree of soil health (Singer and Ewing 2000).

Comparison of soil quality among three fertile soil types (VC, GS and CM) and MT showed significant variation in all the parameters studied.

Table 3 Properties of mine tailings after vermicompost amendment.

Amendment levels	pH	Conductivity (mV)
Mine tailings	3.96 ± 4.07	151.33 ± 5.03
Mine tailings + 1% vermicompost	5.193 ± 0.152*	93 ± 8.548*
Mine tailings + 2% vermicompost	5.99 ± 0.111*	51.33 ± 5.85*
Mine tailings + 4% vermicompost	6.39 ± 0.015**	32 ± 3.01**
Mine tailings + 8% vermicompost	6.39 ± 0.105**	26.1 ± 2.64**

pH and conductivity of mine soil is compared with mine soil amended with different percentages of vermicompost. All the data were analysed by ANOVA. The level of significance is indicated using (*) for $P < 0$ 05 and (**) for $P < 0$ 001 compared to mine soil.

Soil metabolism

The metabolic activities of soil microorganisms can be quantified by measuring soil respiration, assessed by the amount of released CO_2. CO_2 production from soil samples

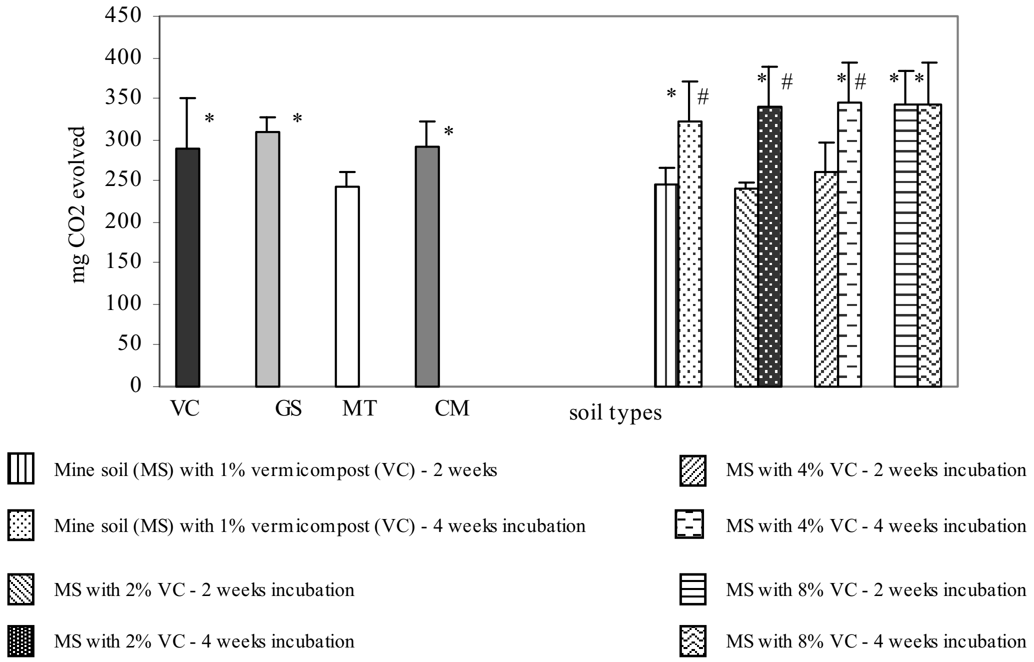

Fig. 1 Soil respiration in different soils compared with vermicompost (VC)-amended gold mine tailings (MTs). Values are given as mean ± SEM. All the data were analysed by ANOVA. The level of significance is indicated using * for $P < 0.01$ compared to mine soil, and (#) for $P < 0.01$ for 4 weeks of amendment compared to 2 weeks of incubation. CM, control mine soil; GS, garden soil.

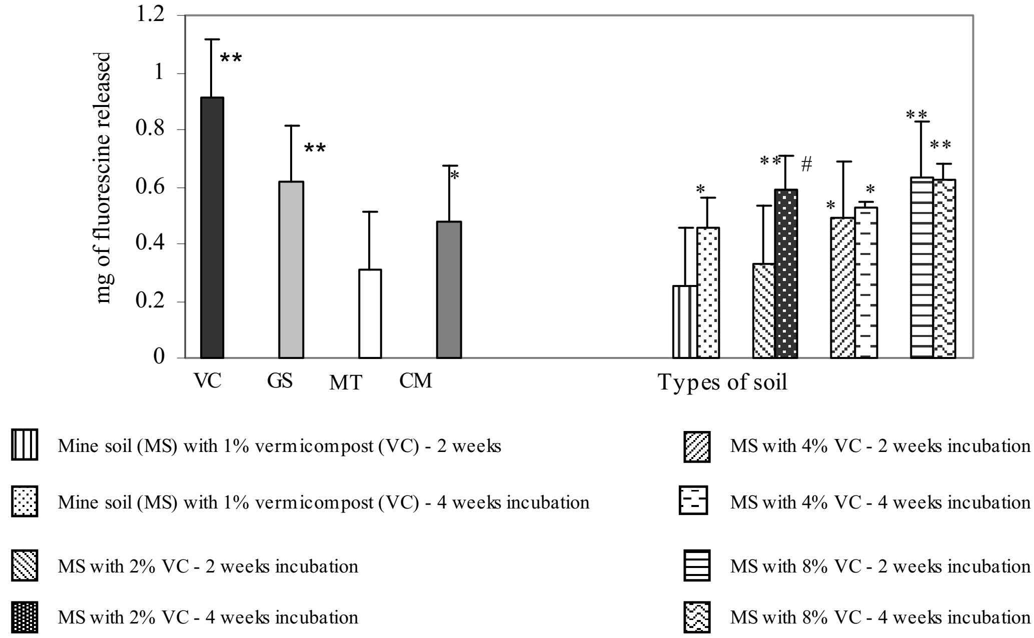

Fig. 2 Total microbial activity in different soils compared with vermicompost (VC)-amended gold mine tailings (MTs). Values are given as mean ± SEM. All the data were analysed by ANOVA. The level of significance is indicated using * for $P < 0.01$ compared to mine soil, and (#) for $P < 0.01$ for 4 weeks of amendment compared to 2 weeks of incubation. CM, control mine soil; GS, garden soil.

was determined in the laboratory based on alkaline traps for absorption of released CO_2 and measuring the remaining alkali against acid titration. Soil respiration in different types of soils and CO_2 produced by soil microbial activity is represented in **Fig. 1**.

Total microbial activity was measured as the hydrolysis of FDA (**Fig. 2**) and shows significant increase in fertile soils (CM < GS < VC) compared to mine soil. Enrichment with VC in mine soil enhanced the FDA hydrolysis and with an increase in incubation period, the FDA hydrolysis was higher.

Soil enzymes as indicators of soil health

Enzymatic activities in mine soil was very low compared to the fertile soils (VC > GS > CM). Amending mine soil with VC improved the soil enzyme characteristics depending on the percentage of VC (1 to 8%) and incubation period (2 to 4 weeks).

Mean enzyme activities of β-glucosidase, urease, dehydrogenase and phosphatase exhibited significant increase following VC amendment, which in turn depends on the percentage of amendment and incubation period (**Figs. 3-6**). Increased levels of enzymes present in fertile soils compared to the MT may be the reason.

Statistical analysis

Significant differences were obsered between fertile soils and MT in all the parameters ($P < 0.05$). VC-amendment MTs resulted in significant improvement in soil quality ($P < 0.001$). Enriching MT with VC resulted in restoration of β-

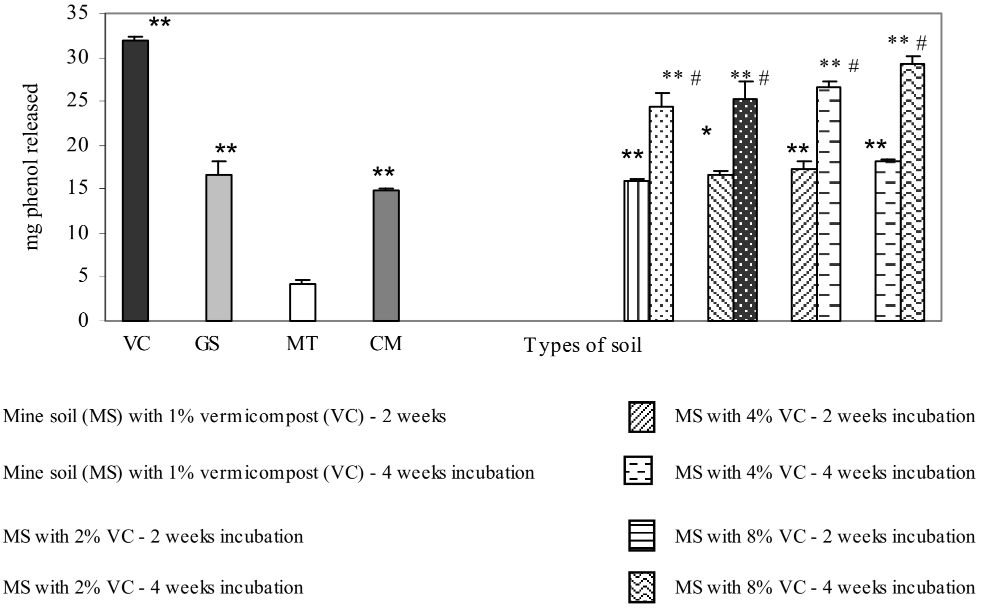

Fig. 3 Soil β-Glucosidase activity in different soils compared with vermicompost (VC)-amended gold mine tailings (MTs). Values are given as mean ± SEM. All the data were analysed by ANOVA. The level of significance is indicated using * for $P < 0.01$ compared to mine soil, and (#) for $P < 0.01$ for 4 weeks of amendment compared to 2 weeks of incubation. CM, control mine soil; GS, garden soil.

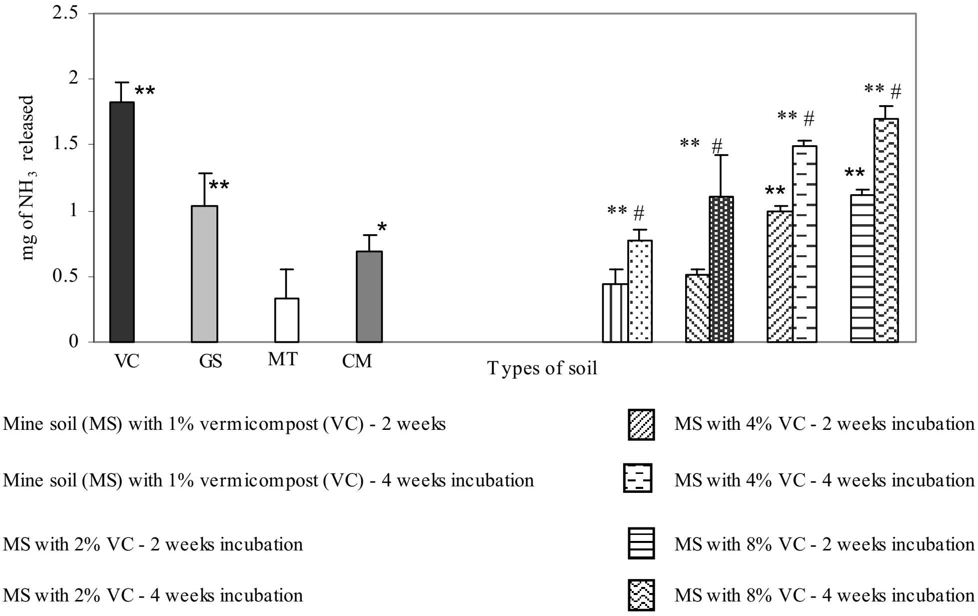

Fig. 4 Soil urease activity in different soils compared with vermicompost (VC)-amended gold mine tailings (MTs). Values are given as mean ± SEM. All the data were analysed by ANOVA. The level of significance is indicated using * for $P < 0.01$ compared to mine soil, and (#) for $P < 0.01$ 4 weeks of amendment compared to 2 weeks of incubation. CM, control mine soil; GS, garden soil.

glucosidase, urease, dehydrogenase and phosphatase activities (**Figs. 3-6**). The first three enzymes showed significant enhancement with VC enrichment, which dependended on the percentage of VC added and on the extension of incubation period (P < 0.001). Even though phosphatase was is restored by VC amendment, no significant increase was observed when the incubation period was extended from 2 to 4 weeks. Total microbial activity and soil respiration were also significantly enhanced when enriched with VC at low levels (1, 2 or 4%) while extending the incubation period resulted in a significant improvement of soil quality (**Figs. 1, 2**).

DISCUSSION

MTs remain without normal soil structure and represent a severely stressed heterotrophic microbial community (Mendez *et al*. 2007). Hence, the microbial community is extremely low in species richness and C utilization diversity compared with uncontaminated soil (Moyanahan *et al*. 2002).

Soil pH and electrical conductivity

Among the variables proposed to assess soil health, physical indicators are of prime importance, their site-specific interpretation with respect to soil quality in many instances depend on specific land use and crop tolerance.

Soil pH affects the solubility of soil minerals, the availability of plant nutrients, and the activity of microorganisms. Acidity is generally associated with leached soils, whereas alkalinity generally occurs in drier regions. However, agricultural practices, such as liming or the addition of ammonium fertilizers, can alter soil pH (Zong *et al.* 2007). In general, pH values between 6 and 7 are optimal for crop growth. Electrical conductivity of a soil-water mixture is an

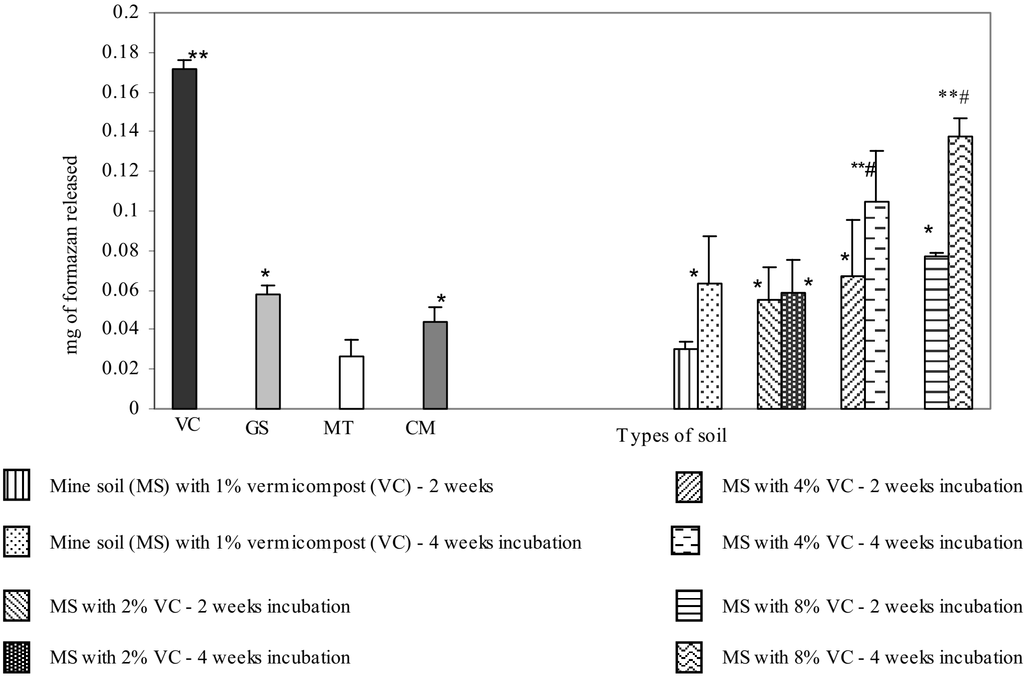

Fig. 5 Soil dehydrogenase activity in different soils compared with vermicompost (VC)-amended gold mine tailings (MTs). Values are given as mean ± SEM. All the data were analysed by ANOVA. The level of significance is indicated using * for $P < 0.01$ compared to mine soil, and (#) for $P < 0.01$ 4 weeks of amendment compared to 2 weeks of incubation. CM, control mine soil; GS, garden soil.

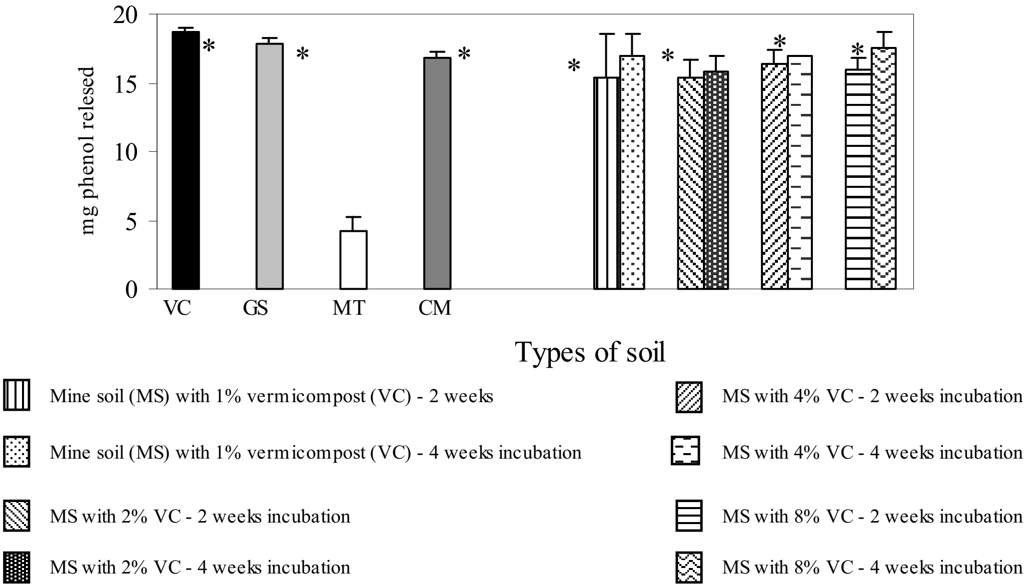

Fig. 6 Soil Phosphatase activity in different soils compared with vermicompost (VC)-amended gold mine tailings (MTs). Values are given as mean ± SEM. All the data were analysed by ANOVA. The level of significance is indicated using * for $P < 0.01$ compared to mine soil, and (#) for $P < 0.01$ 4 weeks of amendment compared to 2 weeks of incubation. CM, control mine soil; GS, garden soil.

indication of the amount of ions (dissolved salts) present in the soil solution. Excess salt content seriously affects plant growth and soil-water balance (Fitter and Hay 1987). This may occur either naturally or as a result of inappropriate soil use and management as in mining. The iron- and sulfur-oxidizing bacteria dominate the microbial community in mine tailings and are associated with plant death in acidic tailings (Schippers *et al*. 2000). Addition of topsoil amended with organic matter is generally used as a substitute for damaged soil. Organic amendments help to immediately decrease metal bioavailability, provide a slow-release fertilizer, and serve as a microbial inoculum. Organic matter improves soil structure, reduces erosion, and increases infiltration. The organic matter may be composed of wood chips, straw, biosolids, composted municipal waste, or manure (Munshower 1994).

Biosolids have also been used as an amendment to ameliorate the harsh conditions of MTs. For example, biosolids successfully increased plant growth in gold MTs in a field trial in New Zealand (Mains *et al*. 2006). From **Tables 1** and **2**, it is clear that there is variation in pH and conductivity among the fertile soil types and MTs and it can be amended to a certain extent by VC. The ability of VC to decrease soil pH, titratable acidity and increased soluble and insoluble solid was reported (Gutierrez-Miceli *et al*. 2007).

Soil metabolism

The biological activity in soil is largely concentrated in the topsoil, the depth of which may vary from a few to 30 cm. In topsoil, the biological components occupy a very small fraction of total soil volume and almost 10% of the total organic matter in soil. These biological components consist mainly of soil organisms, especially microorganisms, which have a major role in N, P and S cycling, and the decomposition of organic residues (Pankhurst *et al.* 1997). The energy input into soil ecosystems is derived from the microbial decomposition of dead plant and animal organic matter. Organic residues are converted into biomass or mineralized to C, oxygen, N, P, and other nutrients (Bloem *et al.* 1997; Barbhuiya *et al.* 2004). The enzymatic activity in soil is mainly of microbial origin (Yang *et al.* 2007). Only enzymatic activity of free enzymes is used to determine the diversity of enzyme patterns in soil extracts.

Comparison of soil quality among three fertile soil types (VC, GS and CM) and of MTs has shown negligible microbial activity in mine soil. As a result the C, N and P metabolism was significantly low in MTs. It is also clear that the poor quality of the mine soil can be improved by amendment with VC (Gutierrez-Miceli *et al.* 2007). With time the VC enhances the quality of soil. This was clearly evident by comparing the data obtained from 4 weeks' incubation with that of 2 weeks.

Soil respiration

Soil respiration, representing the biological oxidation of organic matter to CO_2 by microorganisms, occupies a major role in the C cycle of terrestrial ecosystems. It provides the principle means by which photosynthetically-fixed C is returned to the atmosphere. The metabolic activities of soil microorganisms can be quantified by measuring CO_2 production and/or O_2 consumption (Alef 1995; Steiner 2006).

Soil organic C is thought to be the dominant factor controlling *in situ* respiration, since it is a substrate for heterotrophic activity. Additional plant residue C produced under more fertile soils would be partitioned into labile (i.e., microbially accessible and transformed into CO_2) and stable (i.e., microbially resistant and transformed into soil organic C) fraction (Parton *et al.* 1988; Knorr *et al.* 2005). Therefore, more fertile soils would be able to store more soil organic C (VC, GS and CM) and release more CO_2 to the atmosphere compared with less fertile soils (MTs), as shown in **Fig. 1**. The results also indicate that amendment of MTs with VC significantly enhanced the amount of CO_2 release and it represents the increase in the soil organic C. Increase in the incubation time from 2 to 4 weeks produced a concordant increase in the release of CO_2.

Hydrolysis of the fluorescent fluorescein diacetate is thought to broadly represent soil enzyme activity, because it is hydrolysed by a number of different enzymes, such as proteases, lipases and esterases (Schnürer and Rosswall 1982; Wirth *et al.* 2008).

Total microbial activity is a good measure of organic matter turnover in natural habitats, as most of the energy flow passes through microbial decomposers. VC amendment increases the total microbial activity in MTs and it also shows a time-dependent improvement (4 weeks > 2 weeks). Moreover a good relationship was found between FDA hydrolysis and soil respiration. The differences among the soil types reflect the decomposer activity and the low value obtained for MTs represents lower amounts of organic soil (**Fig. 2**).

Soil enzymes

Studies of enzyme activities in soil are important as they indicate the potential of the soil to support biochemical processes, which are essential for the maintenance of soil fertility. The enzyme activity in soil is strongly associated with microbial activity because the microbial biomass is considered as the primary source of enzymes of soils (Klose *et al*. 1999; Hu and Cao 2007). Preliminary studies with different soil types showed that the enzymatic activities are significantly low in mine soil compared to the fertile soils. Extension of this study was carried out by amending mine soil with VC. The percentage of VC (1 to 8%) and increase in incubation period (2-4 weeks) strongly influenced the improvement of enzyme activities (**Figs. 3-5**). This indirectly indicates that the addition of VC activated C, N and P metabolism of the MTs.

β-Glucosidase activity is related to the carbon cycle, fulfills a central role in the cycling of organic matter and is the most abundant of the enzymes involved in cellulose degradation (Turner *et al*. 2002; Martínez-Iñigo *et al*. 2009). The fertile soils are characterized by high β-glucosidase activity (**Fig. 3**). It may be due to the release of decomposable plant components such as low molecular weight cellulolytic substances into soil. They act as a substrate for β-glucosidase and other cellulolytic enzymes (Sagar 1988). β-glucosidase activity was positively correlated with soil health. Relative to the non-amended mine soil, the activity of β-glucosidase increased rapidly as the percentage of VC and incubation time increased (**Fig. 3**). The differences in enzyme activity may be due to the differences in soil microbial community composition, which must have been elevated by VC amendment.

Urease and phosphatase are often measured because of their importance in the N and P cycles, respectively (Aon and Colaneri 2001). Enzymatic activities in relation to the cycling of N (ammonification, nitrification, denitrification) or P (release of inorganic P) in soil have been used to evaluate the fertility of the soil or to describe the functioning of the ecosystem (Aon and Colaneri 2001; Brohon *et al*. 2001).

Urease, an enzyme that catalyzes the hydrolysis of urea, is abundant in soils. Large numbers of bacteria, fungi, and actinomycetes in soils possess urease. Urease activity increases with the size of the soil microbial population. Urea hydrolysis proceeds rapidly in warm, moist soils, with most of the urea transformed to NH_4^+ in several days. Correlation analyses of urease activity with soil properties indicate that it is associated with total N and organic C (Sahrawat 1983; Chaperon and Sauvé 2008). Thus, the data (**Fig. 4**) obtained in this study clearly indicates that fertile soil possesses significantly high levels of available N compared to MTs. Again, VC amendment contributed to replenishing soil N.

Soil phosphatases catalyze the hydrolysis of organic phosphate esters to ortho-phosphate, and thus constitute an important link between biologically unavailable and bioavailable P pools in the soil (Speir and Ross 1978; Carreira *et al*. 2000). Soil phosphatase, the enzyme that transforms organic to inorganic P is mostly of plant and microbial origin and consists of alkaline and acid phosphatases. Because phosphatase activity is sensitive to environmental perturbations such as organic amendments, water logging, compaction, addition of fertilizer, tillage, heavy metal inputs as in mining and pesticides, it is often used as an environmental indicator of soil quality in ecosystems. In this study the low levels of phosphatase activity observed in MTs indicates the poor soil quality compared to fertile soils and VC amendment considerably restores the soil quality in the MTs (**Fig. 6**).

Dehydrogenases represent a class of enzymes that provide information about the influence of natural environmental conditions of the microbial activities of the soil. Their activity appears to be more related to the metabolic state of microbial population of the soil than to the activity of specific free enzymes acting on a particular substrate. Dehydrogenases conduct a broad range of oxidative activities that are responsible for degradation, i.e., dehydrogenation of organic matter by transferring hydrogen and electrons from substrates to acceptors. They also provide correlative information on the biological activity and microbial populations in the soil. Measurement of dehydrogenase activity represents immediate metabolic activities of the soil microorganisms at the time of test (Rossel and Tarradellas 2006; Chaperon and Sauvé 2008). The results showed that the lack of microbial metabolic activities in MTs could be restored by VC amendment (**Fig. 5**).

CONCLUSION

To conclude, it is evident that the characterization of the soil enzyme assays is sufficiently sensitive to differentiate between the soil in the mine area and other fertile areas. The same factors can be used for assessment of VC amendment in the restoration of soil health where mining material is discarded.

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