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Organic and Biofertilizer Interventions in Iron Mine Spoil for Nutrient Fortification with Facilitation of Microbial Exoenzyme Activity and Plant Growth

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ABSTRACT
Open cast iron ore mining generates considerable volume of spoil which is nutrient deficient and therefore does not support microbial and plant growth. Post mining landscape restoration needs nutrient enrichment of the spoil for revegetation. This study reports the effects of suitable organic and biofertilizer amendments on the chemical characteristics, bacterial-fungal load, activities of the exoenzymes, invertase, amylase, cellulase, protease and dehydrogenase along with growth of three subtropical plant species, *Cassia tora*, *Artocarpus heterophyllus*, and *Pistacia vera* in iron mine spoil amended with farm yard manure, poultry manure and *Rhizobium* biofertilizer in three suitable treatment combinations. All the treatments indicated significantly higher pH, electrical conductivity, organic carbon, nitrogen, phosphorous, and potassium levels with microbial population and exoenzyme activities relative to control over an experimental period of 180 days. Plants grown in treated spoils indicated significantly higher biomass, shoot length, leaf area index and total leaf chlorophyll. The study thus indicated that reclamation of iron mine spoil could be achieved with suitable organic and biofertilizer amendments for subsequent revegetation.

Key words: Iron mine spoil, nutrients, bacteria, fungi, exoenzymes, plant growth.

INTRODUCTION

Mineral production comes mostly from opencast mines is conventionally linked to land degradation (Bradshaw 1983, Miao and Marrs 2000, Li 2006, Chaturvedi and Singh 2017). During mining, the overlying top soil is removed and along with fragmented rock is heaped in the form of mine spoil (Ghosh 2002, Chaturvedi and Singh 2017). The spoil materials occupy a large area, exposed to natural precipitation, and lose nutrients over time due to leaching. The nutrient poor spoil dumps are thus converted into unsuitable habitats for microbial and vegetation growth and colonization of soil fauna (Barapanda et al. 2001, Chaturvedi and Singh 2017). Open cast mining might lead to drastic alteration in biological, physical and chemical properties of soil and therefore, natural recovery of these altered systems is generally a slow process (Bradshaw and Chadwick 1980, Roberts et al. 1981, Ahirwal et al. 2017). Several studies have indicated that the slow recovery of mine spoil is largely due to constraints in microbial proliferation (Lindermann et al. 1984, Smejkalova et al. 2003, Kavamura and Esposito 2010). Therefore, nutrient fortification of the spoil is necessary for the restoration of its chemical and biological quality (Cooke and Johnson 2002, Asensio et al. 2013).

Nutrient enrichment of degraded soil through organic inputs appears to be one of the most suitable and cost effective process of reclamation. Organic intervention of spoils is considered as an ideal method for revegetation with facilitation of the microbial colonization with consequent improvement in soil quality (Jha and Singh 1991). Beneficial effects of organic amendments include decreased soil bulk density and increased water holding capacity, aggregate stability, saturated hydraulic conductivity, water infiltration rate, and biochemical activity (Martens and Frankenberger 1992, Turner et al. 1994). Since organic matter plays a key role in soil productivity by affecting almost all physical, chemical, and biological properties, successful land reclamation depends on recreating a surface horizon with enough soil organic matter to sustain productivity (Akala and Lal 2000, Larney and Pan 2006).

Considerable quantity of spoil is generated during iron ore mining in Barbil block in Keonjhar district (22.12°N and 85.40°E), Odisha, India. These spoil materials are dumped near the mines for long period of time causing serious environmental problems (Rath et al. 2010). Therefore, this study was undertaken to evaluate the nutrient status of the mine spoil and its enrichment through amendment with various organic manures and bio-fertilizer combinations to restore its chemical and biological quality and make it suitable for microbial colonization and plant growth.

MATERIALS AND METHODS

Collection of spoil

The mone spoil samples were collected at random in from 5 years old dumps, transferred to gunny bags and transported to the campus of Odisha University of Agriculture and Technology, Bhubaneswar, India for the experiment. The samples were crushed mechanically with a mechanical grinder to make those finer. Farmyard manure (FYM), poultry manure (PM) and vermicompost (VC) were obtained, respectively, from the dairy, poultry farms and vermicomposting facility located in the University campus. *Rhizobium* biofertilizer (BF) was procured from the culture facility of the College of Agriculture, Bhubaneswar, India.

Formulation of treatments

The processed mine spoil was amended with different organic manures and biofertilizer for the experiment in suitable proportions. Three treatments were formulated, Control (C)- mine spoil without organic amendment, T1- mine spoil+FYM+BF, T2- mine spoil+PM+BF, T3- mine spoil+VC+BF. The major Nutrient content of organic manures was: FYM: N-0.5%, P-0.25%, K- 0.4%; PM: N- 0.9-1.5%, P- 0.4-0.5%, K-0.8% and VC: N- 1.5-2.10%, P- 1.0-1.5%, K- 0.6%.

Experimental set up

Triplicates of each combination were taken in rectangular plastic pots (30×30 cm). The control (C) set had 4 kg of unamended mine spoil, while the experimental treatment sets T1, T2 and T3 contained 4 kg of mine spoil and 20 g of BF in each pot along with 1 kg of FYM, 1 kg of PM and 100 g of VC, respectively. Healthy three months' old saplings of three native plant varieties *Cassia tora*, *Artocarpus heterophyllus*, and *Pistacia vera* were planted in the pots of all treatments to record their shoot length, biomass, leaf area index and total chlorophyll. Evaluation of chemical characteristics, microbiological and plant growth studies in the treatment sets were done at15 days interval for 180 days. The moisture level of 30-40% was maintained in each pot by intermittent sprinkling of distilled water and subsequent monitoring with a digital moisture meter.

Chemical parameters

The pH of the samples was measured by digital pH meter (Systronics). The EC was measured by a conductivity bridge (Elico), OC(%) by titrometric method (Walkey-Black 1934), nitrogen by Kjeldahl method (Subbiah and Asija 1956), phosphorus by Olsen's method (1982) and potassium with the help of flame photometer (Systronics) (Lu 1999).

Microbial population and exoenzymes

The bacterial and fungal populations in the samples were assessed by serial dilution and spread plate method (Johnson and Curl 1972, Doran 1980). The activities of amylase, invertase, cellulase were measured spectrophotometrically as per Mishra et al. (1979). The activities of protease and dehydrogenase were measured as per Speir and Ross (1975) and Casida et al. (1964), respectively.

Plant growth parameters

The shoot length of plants was measured with the help of a measuring tape. Leaf area index was estimated using portable leaf area meter (Weiber). The initial and final biomass of the plants was measured by a top pan balance before plantation and after harvest. Total chlorophyll content was assessed spectrophotometrically (Aron 1949)

RESULTS AND DISCUSSION

The initial and final values of chemical parameters of the amended mine spoils have been mentioned in Table 1 and the percent changes over the

Table 1. Change (%) in the chemical parameters in the control and amended iron mine spoil over the experimental period

Parameter	Treatment	Day 01	Day 180
pH	C	6.5± 0.01	6.69± 0.01
	T1	6.52± 0.001	6.89± 0.001
	T2	6.51± 0.001	6.90± 0.004
	T3	6.51± 0.005	6.89± 0.005
EC (dS/m)	C	0.0006± 0.0001	0.0022± 0.0008
	T1	0.0010± 0.0005	0.0060± 0.0012
	T2	0.0021± 0.0003	0.0099± 0.0011
	T3	0.0009± 0.0001	0.0047± 0.0005
OC (%)	C	1.22± 0.01	0.98± 0.01
	T1	1.46± 0.01	0.41± 0.01
	T2	4.02±0.008	1.01± 0.001
	T3	1.42± 0.01	0.43± 0.01
N (%)	C	0.0017± 0.0001	0.0014± 0.0001
	T1	0.0019± 0.0005	0.0015± 0.0001
	T2	0.0023± 0.0002	0.0017± 0.0001
	T3	0.0022± 0.0002	0.0015± 0.0001
P (%)	C	0.01±0	0.01±0
	T1	0.25± 0.001	0.23± 0.001
	T2	0.28± 0.001	0.24± 0.005
	T3	0.26± 0.002	0.24± 0.001
K (%)	C	0.022± 0.001	0.021± 0.002
	T1	0.028± 0.005	0.026± 0.002
	T2	0.032± 0.001	0.029± 0.005
	T3	0.028± 0.001	0.026± 0.002

experimental period have been depicted in Figure 1. The pH of the spoil in C, T1, T2 and T3 increased by 2.92, 5.67, 5.99, and 5.83%, respectively, over the experimental period. The EC indicated similar trend with 72.72% in C, 83.33, 78.78, 80.85% increase in T1, T2, T3, respectively. Decrease in OC in C, T1, T2, T3 was 19, 71.9, 74.8 and 69.71%, respectively. N content declined by 15.1, 31.81, 26.08 and 21.05% in C, T1, T2 and T3, respectively. Similarly P content also declined by 7.9, 14.2, 7.69% in T1, T2 and T3, respectively. Control set did not indicate any appreciable alteration in P. K content declined by 4.5, 7.14, 9.37 and 7.17% in C, T1, T2 and T3, respectively. One way ANOVA test indicated significant variation in pH and EC for all the treatments ($P<0.05$). Changes in OC, N, P, K were also found to be significant at ($P<0.01$).

It has earlier been reported that addition of biosolids was effective in enhancing properties related to soil quality and fertility on reclaimed copper mine tailing sites (Gardner et al. 2010). The application of organic amendments to mine spoil is likely to improve its quality for revegetation (Asensio et al. 2013). A gradual improvement in pH in different age series of iron mine spoil dumps due to nutrient enrichment has been reported over time (Jha and

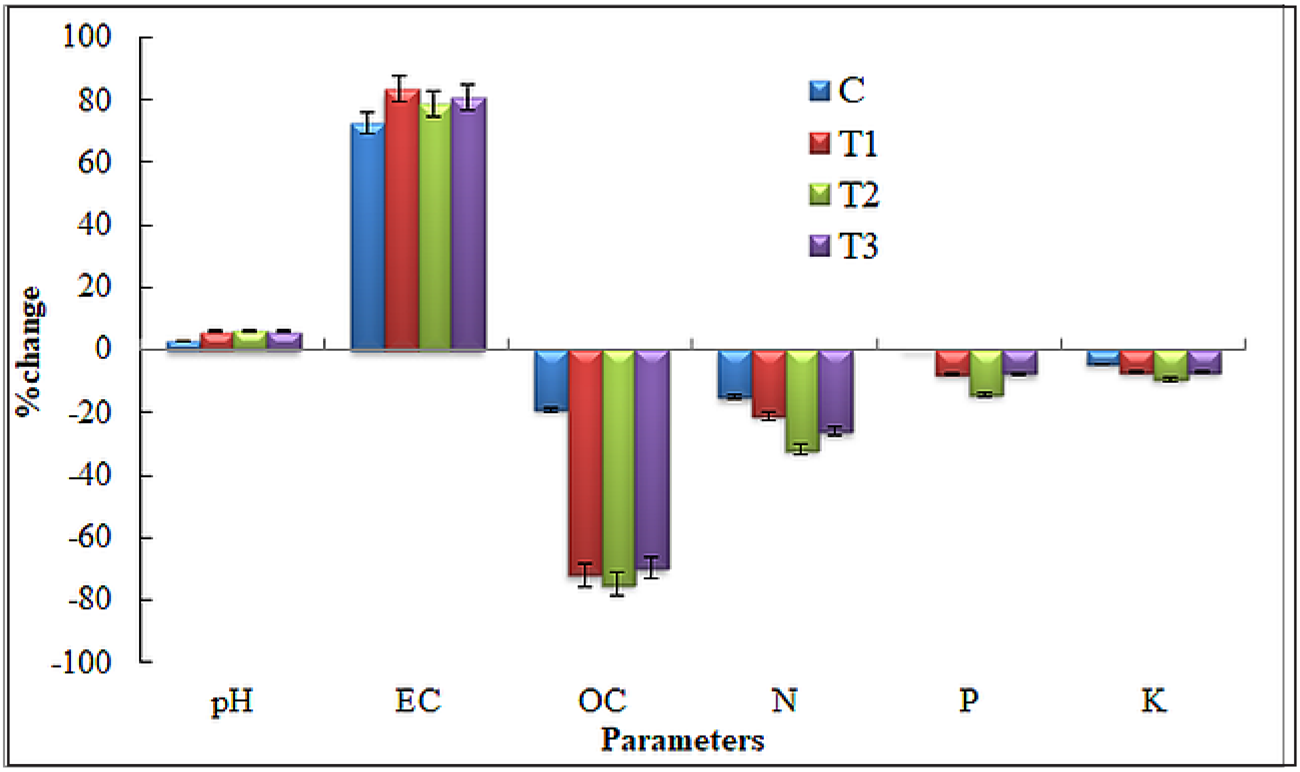

Figure 1. Change (%) in chemical parameters in amended iron mine spoil over the experimental period

Singh 1991, Dutta and Agrawal 2002, Bendfeldt et al. 2001). In the present study, the pH of the mine spoil achieved near neutral values in the treated sets which was likely to facilitate microbial colonization and exoenzyme secretion. It has also been reported that amendment with poultry droppings with paper mill sludge could improve the soil nutrient status of the mined soil and enhance N and P contents (Opafunso et al. 2008, Dere et al. 2008, Stehouwer et al. 2010). Addition of organics could improve C, N and P level in spoil significantly (Kucharik et al. 2001, Breuer et al. 2006, Sheoran et al. 2008). Our results corroborate these earlier reports and support the hypothesis that organic amendments in suitable combinations enhance the nutrient level in degraded soil.

The population of bacteria was found to be the highest in T2 (201±2×10u CFU) followed by T1 (173±2×10u CFU) and T3 (123±2×10u CFU), while C had the least bacterial count (95±1×10u CFU). The population of bacteria increased by 85.2, 89.5, 92.03 and 87.8% in C, T1, T2 and T3, respectively, during the study period (Fig. 2). However, the fungal population declined during this period by 85.29, 97, 98.9 and 91.6% in C, T1, T2 and T3, respectively (Fig. 3). The least fungal count was recorded in T2 (1±0.5×10u CFU) followed by T1 (5±2×10u CFU) and T3 (6±3×10u CFU), while the control had the highest fungal population (10×10u CFU). ANOVA indicated significant difference in the bacterial and fungal populations between treatments (*P*<0.01).

Soil microbes mediate the biochemical transformations of organic matter that underpin essential ecosystem functions, including decomposition, mineralization, nutrient retention, and consequent soil fertility (Drinkwater and Wagoner 1998 , Burger and Jackson 2003, Burger et al. 2005, Kramer et al. 2006, Gattinger et al. 2012, Jackson et al. 2012, Seufert et al. 2012, Syswerda et al. 2012, Cavigelli et al. 2013). The microbial activity of soil directly influences the ecosystem stability (Dick et al. 1993). It has been reported that the total count of soil bacteria were lower in copper mined soil in comparison to unmined soil (Long et al. 2003). Reports indicate lower microbial biomass in mine spoil due to low organic carbon (Pierzynski et al. 1994, Machulla et al. 2005). Significantly low bacterial and fungal population were observed in iron and chromite mine spoil in comparison to unmined soil (Rath et al. 2010). Organic amendments caused significant enhancement in the density of culturable bacteria in soil (Yuan et al. 2011, Li et al. 2013).

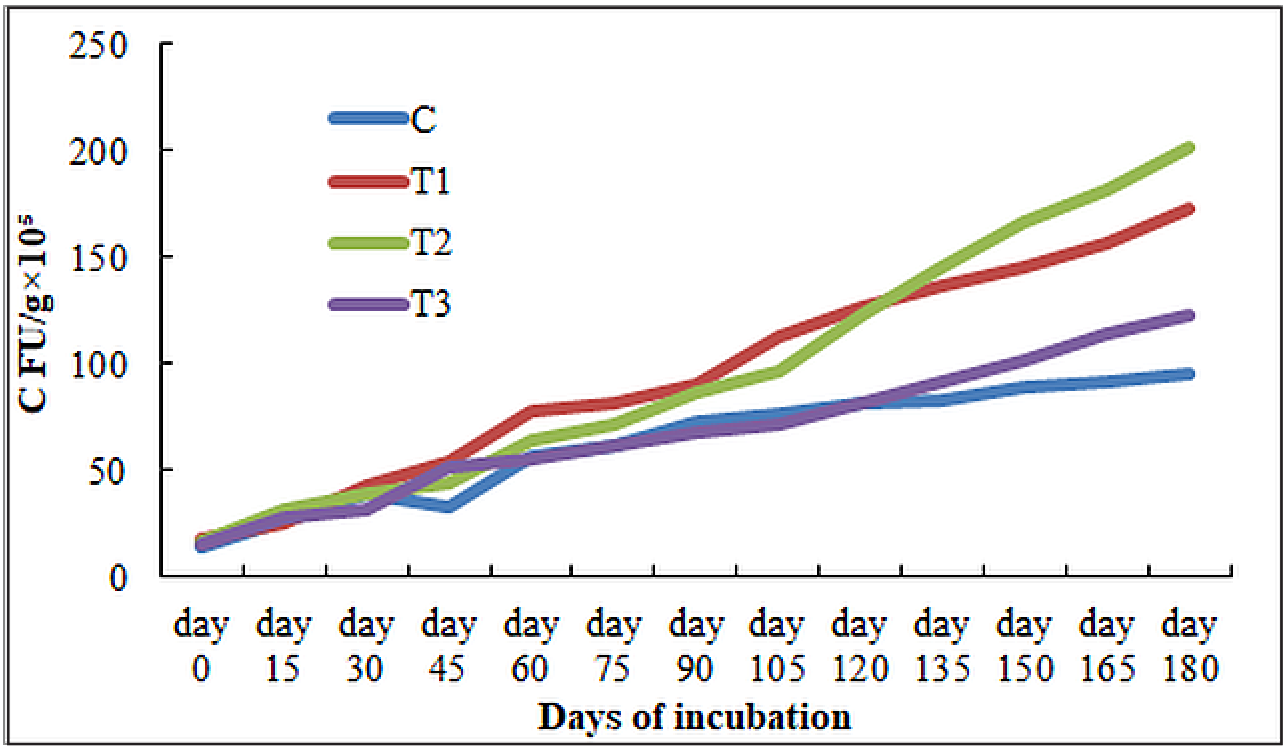

Figure 2. Bacterial population (CFU) during the period of incubation

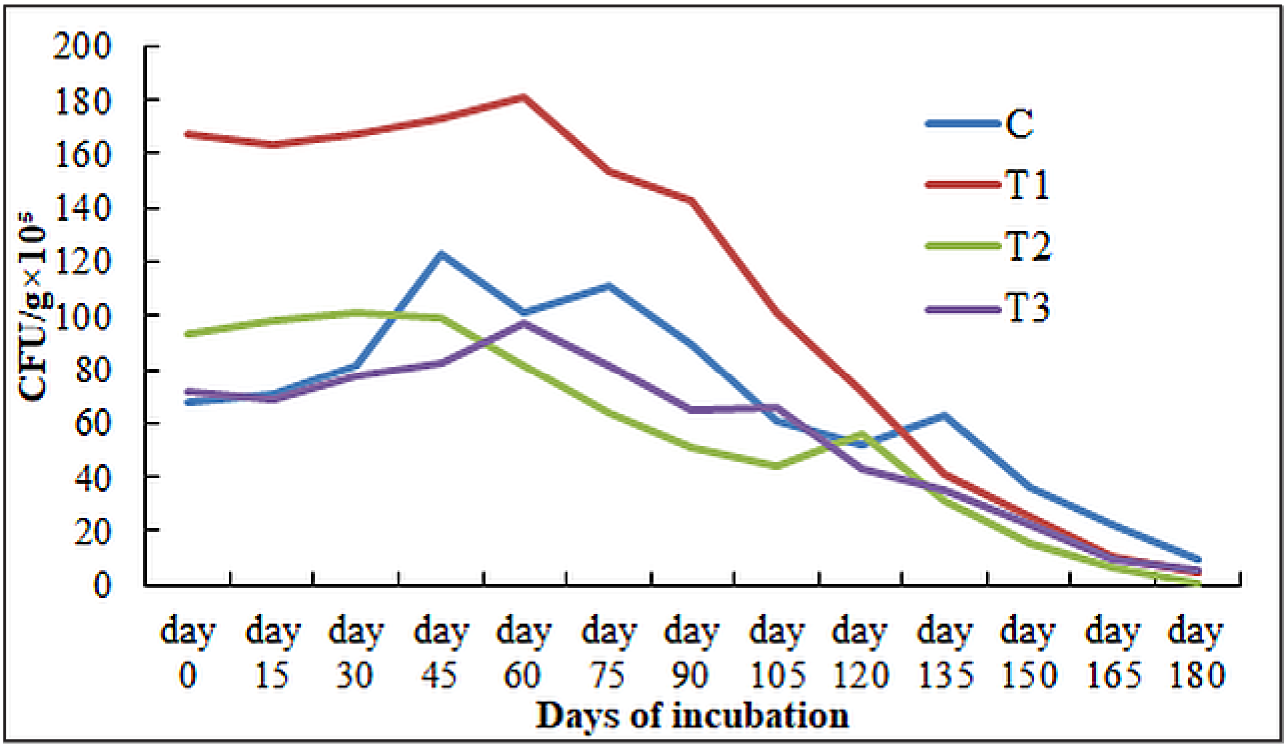

Figure 3. Fungal population (CFU) during the period of incubation

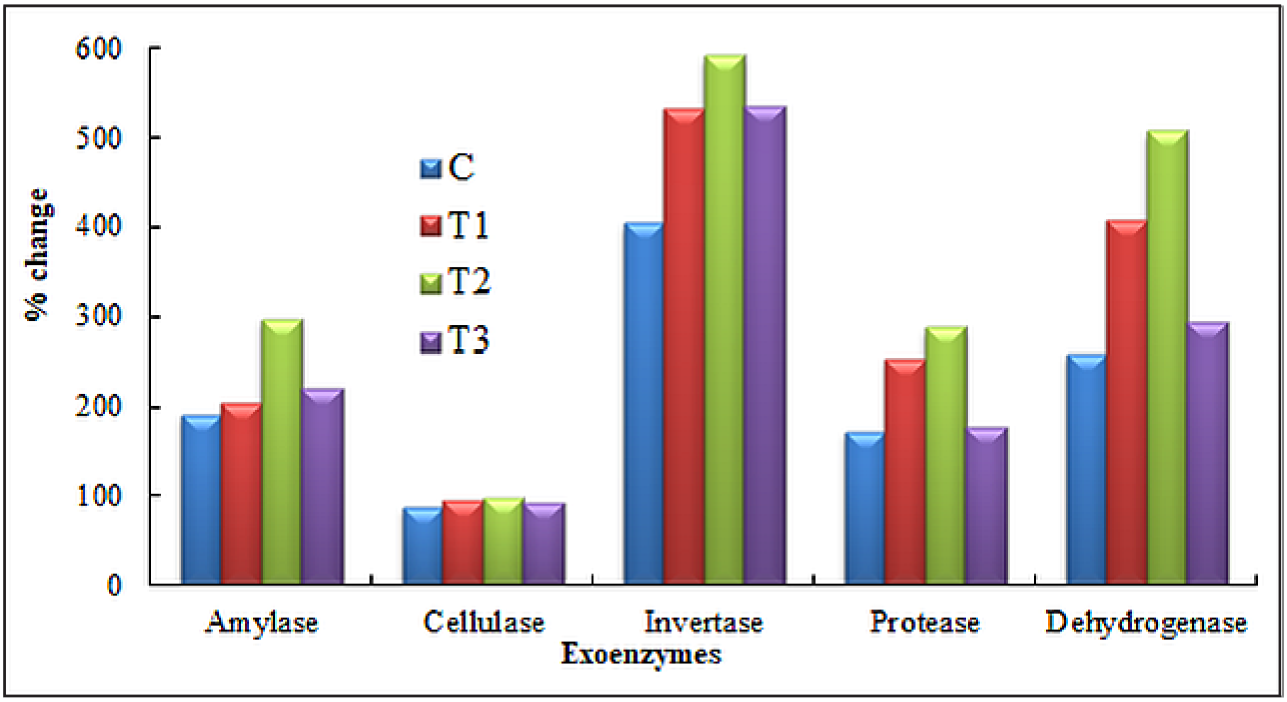

Figure 4. Change (%) in exoenzyme activities in the control and amended iron mine spoil

Significantly high bacterial population in soil with higher organic carbon and other nutrients have also been reported by Chhotaray et al. (2014). Mishra et al. (2011)demonstrated increased bacterial count in iron mine spoil amended with FYM and PM with inoculation of earthworms. Our results are consistent with these findings and indicate that lower bacterial counts in iron mine spoil are likely due to lower level of essential soil nutrients. Higher bacterial population in all the treated spoil samples might be attributed to higher levels of organic carbon, N, P and K relative to the control. Lower fungal population in the treatment sets might be attributed to antagonistic effects due to competitive inhibition by the bacteria over fungi.

Consistent increment in the exoenzyme activities in amended spoil have been observed in the present study over the incubation period (Fig. 4). Amylase activity increased by 190.3, 202.4, 295.7 and 219.0% in C, T1, T2 and T3, respectively. Similarly, cellulase activity increased by 87.12, 94.21, 96.62 and 91.17% in C, T1, T2 and T3, respectively. Invertase enzyme also showed similar trend with an increase of 403.8, 533.3, 591.04 and 534.2% in C, T1, T2 and T3, respectively. Protease activity increased by 253.3, 286.6 and 175.2% in C, T1, T2 and T3, respectively. Dehydrogenase activity also increased by 258, 408.3, 507.3 and 292.1% in C, T1, T2 and T3, respectively. One way ANOVA test indicated that the variations in activities of all the exoenzymes between treatments were significant (p<0.01).

Most current soil enzyme assays use bulk soils, which include enzymes released from active microorganisms in response to nutrient stress and availability (Caldwell et al. 1999, Nannipieri et al. 2002, Veres et al. 2013). By adding organic matter to the soil, enzyme activities intensify by providing available nutrients to microbes (Liang et al. 2005, Tejada et al. 2006). It has been reported that manure application increased soil dehydrogenase, acid and alkaline phosphatases, cellulase and protease activity significantly (Saha et al. 2008). The enhanced exoenzyme activities in organically amended mine spoil in the present study are consistent with the proposal that nutrient increment through organic interventions facilitate microbial growth and consequent enzyme secretions.

The biomass of the plants showed significant increment in all the treatments except control over the experimental period. It changed by -45.5, 480.9, 1652.2 and 8.6% for *Cassia tora*; 152.06, 125.72, 976.3 and 409.79% for *Artocarpus heterophyllus* and -188.3, 360.46, 606.4 and 154.9% for *Pistacia vera* in C, T1, T2 and T3, respectively. The highest plant biomass was achieved in T2 followed by T1 and T3 irrespective of the species, whereas in the control it noticeably decreased which is indicative of the fact that nutrient deficient soil adversely impacts plant growth. Similarly, the plants achieved their highest shoot length in T2 followed by T1, T3 and C. The

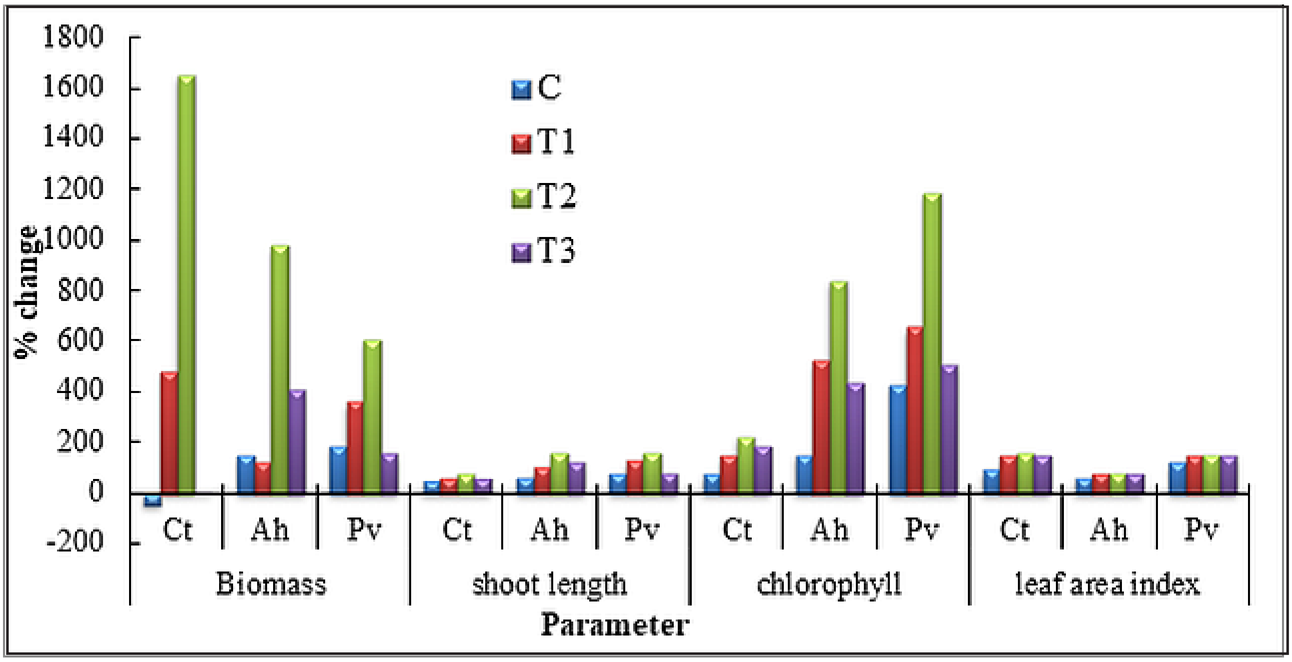

Figure 5. Change (%) in the biomass, shoot length, chlorophyll content and leaf area index of plants Ct: *Cassia tora*, Ah: *Artocarpus heterophyllus*, Pv: *Pistacia vera* over the experimental period

Table 2. Initial and final values (mean±SD) of plant growth parameters in control and amended iron mine spoil over the experimental period

Parameter	Treatment	*Cassia tora*		*Artocarpus heterophyllus*		*Pistacia vera*	
		Day1	Day 180	Day1	Day 180	Day1	Day 180
Biomass (g)	**C**	2.24±0.015	1.228± 0.015	2.42±0.01	6.10±0.015	5.31±0.012	15.31±0.011
	T1	2.1±0.011	12.2±0.015	2.41±0.017	5.44±0.016	4.3±0.01	19.8±0.015
	T2	2.26±0.015	39.66±0.01	2.42±0.012	26.12±0.015	5.31±0.017	37.15±0.012
	T3	2.32±0.01	2.81±0.011	2.45±0.015	12.49±0.012	5.2±0.015	13.26±0.017
Shoot length (cm)	**C**	18.75±0.4	27.8±0.21	17.42±0.15	38.5±0.45	15.8±0.2	28.2±0.45
	T1	18.6±0.42	29.5±0.36	17.07±0.21	34.6±0.51	14.8±0.35	33.6±0.33
	T2	19.42±0.45	34.02±0.42	20.17±0.45	51.2±0.4	12.8±0.45	32.5±0.21
	T3	21.22±0.31	30.12±0.45	19.2±0.36	31.45±0.21	14.8±0.4	26.8±0.4
Chlorophyll (µg/ml)	**C**	0.802±0.002	4.243±0.001	0.757±0.005	1.91±0.001	0.995±0.001	1.741±0.005
	T1	0.127±0.002	0.959±0.005	0.462±0.001	2.889±0.002	0.724±0.001	1.785±0.001
	T2	0.351±0.001	4.524±0.002	0.154±0.002	1.443±0.002	0.837±0.002	2.682±0.001
	T3	0.612±0.005	3.725±0.001	0.199±0.001	1.065±0.005	0.554±0.001	1.593±0.002
Leaf area index (cm^2)	**C**	16.35±0.04	31.6±0.8	71.2±0.25	115.4±1.36	46.7±0.17	102.7±1.65
	T1	16.42±0.04	40.8±0.81	71±0.21	125.1±1.31	46.8±0.15	115.7±1.65
	T2	16.01±0.01	41.5±0.7	71.5±0.25	127.3±1.33	46.5±0.17	117.3±1.5
	T3	16.33±0.04	39.9±08	71.6±0.25	124.3±1.36	46.5±0.11	114±1.61

increment in shoot length was 48.2, 58.6, 75.18 and 56.87% for *C. tora,* 63.8, 102.6, 153.8 and 121.0% for *A. heterophyllus* and 77.3, 127.1, 154.2 and 81.5% for *P. vera* in C, T1, T2 and T3, respectively. Total leaf chlorophyll content was maximum in the plants was 74.9, 146.5, 220.4 and 187.4% in *C. tora*,152.3, 523.37, 837.0 and 435.1% in *A. heterophyllus* and 429.05, 655.1, 1188.8 and 508.6% in *P. vera* in C, T1, T2 and T3, respectively. Leaf area index of the plants increased consistently up to 180^{th} day. It was 93.8, 148.7, 157.4 and 144.7% for *C. tora*, 62.07, 76.1, 78.04.0 and 74.3% for *A. heterophyllus* and 119.9, 147.2, 152.2 and 145.1% for *P. vera* in C, T1, T2 and T3, respectively (Table 2, Fig. 5).

The results from the present study indicated that amendments of iron mine spoil with FYM and PM along with BF not only enhanced the microbial growth and enzyme activity but also enriched the spoil with nutrient supplements there by creating suitable environment for plant growth. Similar results have been obtained by workers on degraded soil (Larney et al. 2011). Biomass and primary productivity of *Acacia auriculiformis* increased significantly in coal mine spoil with suitable organic input (Dutta and Agarwal 2003). The growth and foliar nutrient content of *Macaranga peltata* (Roxb.) were facilitated when grown in iron ore mine spoil amended with farmyard manure, vermicompost with arbuscular mycorrhizal species (Rodrigues and Rodrigues 2014). Nutrient deficient sand mine spoil showed very positive results for nutrient levels and growth of grass and legumes when amended with organics like coco peat, sewage sludge and sphagnum peat moss (Aschenbach and Poling 2015). All these findings are consistent with our results on the three plant species tested with amended spoil.

CONCLUSION

Organic and biofertilizer amendments of iron mine spoil have shown encouraging results to transform the otherwise nutrient deficient spoil to fertile soil facilitating microbial proliferation and subsequent vegetation growth. The most suitable amendment combinations have been identified to achieve maximal enzyme activity and plant biomass in the spoil. Since all the three plants used in this study are native to the mining area, these could be useful species in field trials for revegetation of spoil dumps after nutrient enrichment. Revegetation consequently would minimize erosion and contamination problems caused due to open disposal of spoils.

Author's contribution: Both the authors have contributed equally.

Conflict of interest: Authors declare no conflict of interest.

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