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Soil microbial enzyme activities in different age series sponge iron solid waste dumps with respect to reclamation

Bandana Kullu[1Ψ] and N. Behera[2]

[1] P.G. Department of Botany, Utkal University, Vani Vihar, Bhubaneswar-751 004, Odisha

[2] P.G. Department of Environmental Science, Sambalpur University-768 019, Odisha

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ABSTRACT

Soil enzymes are primarily derived from over all microbial population and activities of microorganism. Study of soil enzymatic activities provide an insight in to the microbial biotransformation process and soil enzymes could be used as the potential biochemical indicators of soil quality. In the present investigation soil microbial enzymatic activities of dehydrogrogenase, amylase, invertase, urease, protease and phosphtase were measured in different age series i.e. 0, 1, 3 and 5 years old sponge iron solid waste dumps to determine probable biotransformation of the waste. The study indicated that the soil microbial enzymatic activities were not detected in fresh dump (0 year old). But, there was an increasing trend in soil microbial enzymatic activities from 1 year to 5 year old waste dumps. The comparative analysis of the soil microbial enzymatic activities in different age series sponge iron solid waste dumps reflects the gradual improvement of the functionality in the older dumps.

1. Introduction

Land degradation due to the alteration and destruction of terrestrial habitat is a major environmental concern throughout the tropics and one of the major factors responsible for such degradation is rapid industrialization and spread of mining activity (Ezeaku and Davidson, 2008). Sponge iron or direct reduced iron (DRI) is mostly used for steel making through secondary sector and India continues to be the largest producer of coal based sponge iron in world (Dey *et al.*, 2016). The availability of principal raw materials like high grade iron ore and non-coking coal have created a favorable atmosphere for development of sponge iron industry in the central eastern belt of India including the states of Odisha, West Bengal, Jharkhand and Chhattisgarh (Patra *et al.,* 2008). From the coal based sponge iron industry huge amount of solid waste is generated in form of char, dust and accretion material (CPCB, 2007). Majority of the solid wastes are dumped on land which creates large areas of black calcareous derelict land that apart from reducing the productivity, also reduce aesthetic value of the region (Roy *et al*., 2002). In developing country like India, reclamation of such derelict land is an urgent necessity for restoration of the self sustaining capacity of the ecosystem and its delicate equilibrium.

Soil microorganisms and their activities contribute a wide range of essential services to the sustainable functioning of ecosystem (Langer and Mubarack, 2007). They are the driving force behind nutrient transformations and thus make an essential contribution to soil fertility and ecosystem functioning (Smith and Paul 1990; Shentu *et al.,* 2008). Thus, the soil microbiological parameters are considered as a sensitive indicator of soil quality, ecological stress and restoration process (Pascual *et al.*, 2000; Filip, 2002; Bending *et al.*, 2004).

Ψ *Corresponding author; Email:* bandkullu@gmail.com

Soil enzymes are essential for catalyzing reactions necessary for organic matter decomposition and its mobilization to different soil strata (Pavel *et al.*, 2004; Shi *et al.*, 2006) and are believed to be primarily originated from soil microbes (Ladd, 1978). They are usually associated with viable proliferating cells, but enzymes can be excreted from a living cell or be released into soil solution from dead cells (Tabatabai, 1994). The soil enzyme activities have often been used as indices of microbial activity and soil fertility (Kennedy and Papendick, 1995) because they are strictly related to nutrient transformations, they rapidly respond to the changes in soil properties and it is easy to measure their activities (Gianfreda and Bollag, 1996; Nannipieri *et al.*, 2002). Therefore in present study attempt is being made to study the soil microbial enzymatic activities in different age series sponge iron solid waste dumps with respect to reclamation.

2. Materials and methods

2.1 Study site

The study was carried out in solid waste dumping site of Scans Steels limited, Sundargrah, Odisha. Geographical location of the area is between 20°11' North Latitude and 84°19' East Longitude. Altitude of the area is about 213m above the mean sea level. The area experiences tropical climate with three distinct seasons i.e. summer, rainy and winter. The mean annual rainfall in the area is 1422mm and mean air temperature of the area varies from 10ºC to 45ºC. The relative humidity fluctuates from minimum of 40% to maximum of 83%. In the sponge iron solid waste dumping site, accumulation of solid waste over years resulted in formation of different age series of dumps. Dump age is expressed as time since the establishment of dump in the site. For the present study freshly laid dump (D_0), 1 year (D_1), 3 year (D_3) and 5 year (D_5) old dumps were selected. During dumping of the solid waste, when the dump attains sufficient height, soil of the adjacent area is covered over the dump for stabilization. Thus, D_1, D_3 and D_5 were with soil cover, where as D0 was without soil cover. A natural site adjacent to the waste dumping site was been taken as control site (C) for reference.

2.2 Sample collection

Sampling was done in accordance with the general methods for soil microbiological study (Parkinson *et al.,* 1971). Waste samples from different age series dump (D_0, D_1, D_3 & D_5) and soil from control site were collected by random sampling method from a depth of 0-15cm by digging pits (15 X 15 X 15 cm). For each site, waste sub-samples were collected from five locations. These sub- samples were brought to the laboratory in sterilized polythene bags and mixed thoroughly to form a composite sample. After sorting out larger pieces of materials, each of the samples was divided into three replicates for analysis.

2.3 Enumeration of microbial population

Microbial population of different age series waste dumps and control site was enumerated in terms of total bacterial and fungal colony forming units (CFUs), following the serial dilution plating technique (Parkinson *et al.,* 1971). For bacterial count diluted suspension was spread over Nutrient agar media by spread plate technique and incubated at 30°C for 24 hours. For total fungal count diluted suspension was spread over Rose Bengal agar media by spread plate technique and incubated at 30°C for 7 days. The numbers of colonies isolated were expressed as the CFU (Colony Forming Unit).

2.4 Estimation of soil microbial enzymatic activities

The soil enzymatic activities in different age series waste dumps and control site was analysed with respect to dehydrogrogenase, amylase, invertase, urease, protease and phosphtase by standardized protocols. Dehydrogenase activity was determined spectrophotometrically at 485nm by measuring reduction of 2, 3, 5 - triphenylotetrazolium chloride (TTC) to red-coloured triphenyl formazon (TPF) following Nannipieri *et al.* (1990) and enzyme activity was expressed as mg TPF/g soil/hr. Amylase activity was measured following the protocol of Somogyi (1952) and Roberge (1978) by taking starch as substrate and enzyme activity was expressed as µg glucose/g soil/hr. Invertase activity was determined by spectrophotometric method at 540nm (Ross, 1983) by taking sucrose as substrate and enzyme activity was expressed as µg glucose/g soil/hr. The urease activity was determined by following the method of Tabatabai and Bremner (1972), and enzyme activity was expressed as µg NH_4^+/g soil/hr. Protease activity was measured spectrophotometrically at 700nm (Ladd and Butler, 1972), by taking sodium caseinate as substrate and enzyme activity was expressed as µg tyrosine/g soil/hr. Phosphatase activity was estimated spectrophotometrically following the method of Tabatabai and Bremner (1969) and enzyme activity was expressed in µg p-nitrophenyl phosphate (PNP) /g soil/hr.

3. Results and discussion

Total bacterial and total fungal colony forming units (CFUs) of different age series waste dumps and control site were presented in Fig. 1a and 1b respectively. Among the waste dumps the bacterial and fungal CFUs could not be isolated from D_0. However, in rest of the waste dumps different numbers of bacterial and fungal CFUs were isolated,

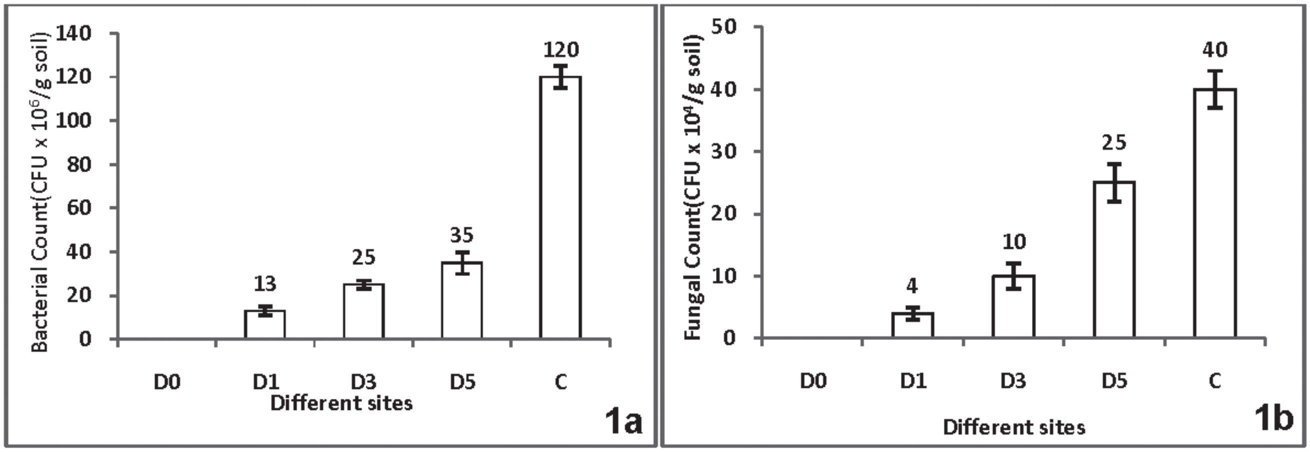

Fig 1a-b: Total bacterial (1a) & Total fungal (1b) Colony Forming Units (CFU) in different age series sponge iron solid waste dumps (D_0, D_1, D_3 and D_5) and control site (C).

which showed gradual increasing trend with increasing age of the waste dumps. Further analysis of variance (ANOVA) indicated that the total bacterial (F = 714.48) and fungal (F = 259.45) CFUs in different sites was observed to be statistically significant at $p< 0.001$.

Such findings can be correlated with fact that solid waste freshly released from the sponge iron industry was dumped on D_0. The microbial inoculums from the surrounding region might not have established them in the freshly laid waste dump, which might explain the present finding. Further, with increase in the age of the waste dumps there was gradual increase in the bacterial and fungal population. Several factors including physicochemical properties of soil (Sexstone *et al.*, 1985), soil organic carbon (He *et al.*, 2012), changes in pH (Lauber *et al.*, 2009), soil water content (Zhou *et al.*, 2002), plant diversity and composition (Wardle *et al.*, 2004; Carney and Matson, 2006) and soil mineral nutrient availability (MacKenzie and Quideau, 2010; Nannipieri *et al.*, 2003) have all been shown to influence soil microbial communities. The improvement in physicochemical properties (Kullu and Behera, 2015) and increase in plant diversity and composition (Kullu and Behera, 2011) of the different age series sponge iron waste dumps has already reported, which resulted in increased microbial population with increase in age of the waste dumps.

The soil microbial enzymatic activities in different age series waste dumps and control site was analysed with respect to dehydrogrogenase, amylase, invertase, urease, protease and phosphtase were presented in Figs. 2 (a-f). It was observed that none of the microbial enzymatic activities could be detected in freshly laid dump D_0. Dehydrogenase is an oxidoreductase group of enzyme is considered to be an index of microbial activity (Tabatabai, 1982; Dick, 1994, 1997). It is an intracellular enzyme and it is not active as extracellular enzymes in soil, hence considered as a good indicator of overall microbial activity (Garcia *et al.,* 1997; Taylor *et al.,* 2002). Absence of microbial population in D_0 resulted in non detection of dehydrogenase activity in the D_0. Amylase, invertase, urease, protease and phosphatase are substrate induced hydrolytic enzymes (Roberge, 1978; Ross, 1983; Nannipieri *et al.,* 1990; Beyer *et al.,* 1992). Therefore, absence or inadequacy of the substrate such as organic carbon, nitrogen and extractable phosphate (Kullu & Behera, 2015) may be the reasons for such non-detection of enzymatic activities in freshly laid waste dump (D_0).

However all the enzyme activity were recorded in D_1, D_3 and D_5, showing an increasing trend with increasing age of the waste dumps. The increase in the microbial population with increasing age of the waste dump has resulted in increased dehydrogenase activity which can be considered as an indication of the recovery of soil functionality (Harris and Birch, 1989; Fiedler *et al.,* 2004). Amylase is complex enzymes that hydrolyze starch to reducing sugar. Change in amylase activity related to increase in the soil organic carbon, microbial biomass fluctuations and diversity of soil microbiota (Pati and Sahu, 2004; Anjaneyulu *et al.,* 2011). The increase in invertase activities might also be related to increase in the soil organic carbon, microbial biomass over time (Tscherko and Kandeler, 2000; Luxhfi *et al.,* 2002; Bol *et al.,* 2003). Soil invertase activity is significantly correlated with the soil organic matter (Shi *et al.,* 2008). Generally, protease activity depends upon distribution of proteolyitc bacteria and the amount of proteinaceous substrate availability in soil organic matter (Subrahmanyan *et al.,* 2011). The increase in the protease activity is closely related to gradual accumulation of soil organic carbon, NH_4-N accumulation and microbial biomass content (Sardans *et*

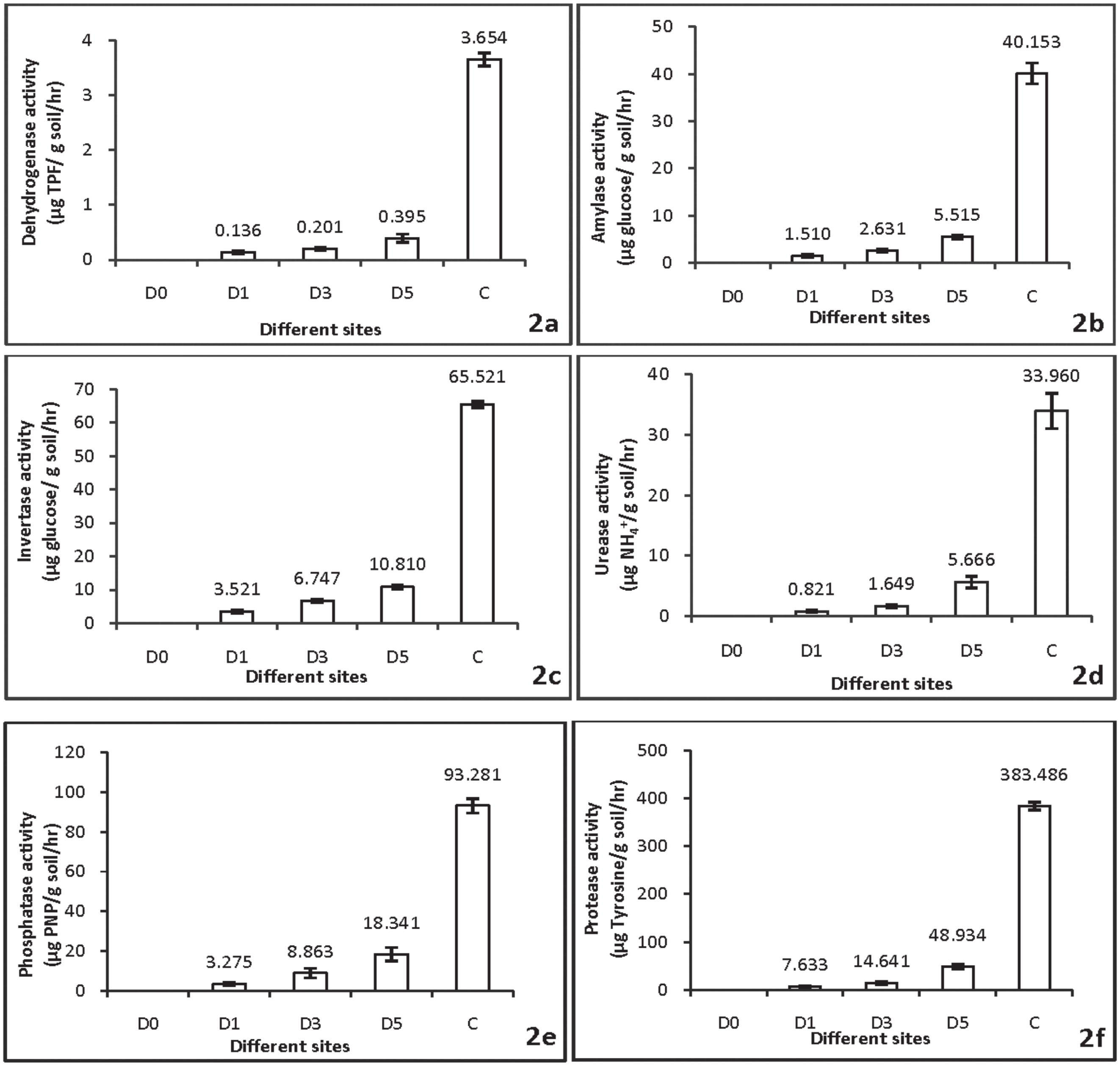

Fig. 2a-f. Soil microbial enzyme Dehydrogenase (2a), Amylase (2b), Invertase (2c), Urease (2d), Phosphatase (2e) & Protease (2f) activities in different age series sponge iron solid waste dumps (D_0, D_1, D_3 and D_5) and control site (C).

al., 2008; Anjaneyulu *et al.,* 2011). Urease activity has been reported to be closely associated with nitrogen mineralization, specifically the conversion of organic nitrogen to inorganic form (Pascual *et al.,* 1998, Giridhara *et al.,* 2003; Yang *et al.,* 2006). Phosphatase acts as an intermediary enzyme for the transformation of organic phosphorous to inorganic form and has a role in the soil phosphorous cycling (Appiah and Thomas, 1982; Kramer and Green, 2000). Thus in general, increase in the respective substrate level with increasing age of the waste dump, the different hydrolytic enzymes showed increasing activity.

The analysis of variance (ANOVA) indicated that the dehydrogrogenase, amylase, invertase, urease, protease and phosphtase enzyme activities in different sites was observed to be statistically significant at $p<0.001$.The relationships between different soil enzyme activities and the age of the waste dumps were expressed in form of linear equations followed by coefficient of determinant (R^2), correlation coefficient (r) and their level of significance (Table -1). There was a positive correlation between the different soil microbial enzyme activities and age of the waste dumps, indicating the process of reclamation.

Table 1

Relationship between different soil enzymatic activity and age of the waste dump (year).

Parameters x	Parameters y	Equation	Coefficient of determinant (R^2)	Correlation coefficient(r)	Level of significance
Age of waste Dump (Year)	Dehydrogenase activity (µg TPF/g soil/hr)	y = 0.072x + 0.020	R^2 = 0.953	0.976	P >0.001
Age of waste Dump (Year)	Amylase activity (µg glucose/g soil/hr)	y = 1.001x + 0.214	R^2 = 0.939	0.980	P >0.001
Age of waste Dump (Year)	Invertase activity (µg glucose/g soil/hr)	y = 1.791x + 2.068	R^2 = 0.784	0.885	P >0.001
Age of waste Dump (Year)	Urease activity (µg NH_4^+/g soil/hr)	y = 1.070x - 0.374	R^2 = 0.892	0.944	P >0.01
Age of waste Dump (Year)	Protease activity (µg Tyrosine/g soil/hr)	y = 9.220x - 2.944	R^2 = 0.896	0.945	P >0.01
Age of waste Dump (Year)	Phosphatase activity (µg PNP/g soil/hr)	y = 3.592x - 0.466	R^2 = 0.984	0.992	P >0.001

4. Conclusion

Sponge iron solid waste dumps represent a distressed habitat and posses scare in the natural landscape which need urgent attention for their reclamation. To understand the natural process of reclamation, among the various features, study of microbiological feature of the sponge iron waste dumps is necessary, so that reclamation process can be favorably manipulated. The findings of the present study clearly revealed that with increasing age of the waste dump there is improvement in the microbiological status of waste dumps with respect to increase in the bacterial and fungal population and microbial enzyme induced operation of nutrient cycling, consequently paving the path way for reclamation.

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