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Impact Of Microbial Biomass And Basal Soil Respiration In Soil Enzyme Activities In Seven Different Chronosequence Mine Spoil

Samapika Dash[1], Arupa Kumar Nanda[2]. Nirius Jenan Ekka[3], Monty Kujur[4*]
[1, 2]School of Biotechnology, Gangadhar Meher University, Amruta Vihar, Sambalpur, Odisha, India, samapikadash13@gmail.com; jnarupa@gmail.com;
ORCID Id- 0000-0001-8098-6161
[3] School of Life Sciences, Sambalpur University, Jyoti Vihar, Burla, Sambalpur, Odisha , PIN- 768019. njekka@suniv.ac.in; ORCID Id - 0000-0001-7481-761X
[4]School of Biotechnology, Gangadhar Meher University, Amruta Vihar, Sambalpur, Odisha, India, mkujur@gmuniversity.ac.in ; ORCID Id 0000-0001-7973-3087

Abstract
The objective of the current study is to determine the potential of microbial biomass and basal soil respiration to improve the rate of soil enzyme activity on polluted land. The soil enzyme activities (amylase, invertase, protease, urease and dehydrogenase) were determined from the six different soil profiles collected from the Kanthar Manganese mine, Koira, Sundergarh, Odisha, and sampling was done in chronosequence, i.e., fresh mine spoil (MBO0), two-year-old mine spoil (MBO2), 4-year-old (MBO4), 6-year-old (MBO6), 8-year-old (MBO8), and 10-year-old (MBO10) mine spoil, and compared with nearby forest soil (FS). The activities of soil enzymes were increasing from fresh mine spoil (MBO0) to MBO 10, and the highest activity was seen in forest soil (FS), which may be due to improvement in the soil organic carbon and total nitrogen concentration that showed significant correlation at the $p < 0.01$ level of significance. Further, stepwise multiple regression analysis has shown positive regulation of soil pH, moisture, and clay content in the improvement of enzyme activities in disturbed areas. Additionally, the accumulation of microbial biomass carbon (MB-C), microbial biomass nitrogen (MB-N), microbial biomass phosphorus (MB-P) and basal soil respiration (BSR) concentration was increasing from fresh mine spoil to forest soil, which may facilitate the improvement of soil enzyme activities. Principal component analysis indicates the effect of physicochemical properties and microbial biomass on enzyme activities and is presented as seven independent clusters. Such studies indicate a reliable and comprehensive idea of any alteration in soil quality and modification that occurred due to exposure to heavy metal stress.

Key words: *Microbial biomass, Chronosequence, Mine spoil, soil organic carbon, Basal soil respiration*

1. INTRODUCTION

Enzymes are biocatalyst obtained from soil microbes to be present as extracellular /intracellular secretions and/or products and catalyses biochemical reactions during cycling of plant available nutrients (Agrawal et al., 2022). Moreover, easy access to well documented protocols for determination of enzymatic activities, makes it a selected tool to assess soil health. In addition, biochemical properties are more susceptible to minor changes in microbial activities. This is because of their active participation in organic matter mineralization process and cycling of minerals that influences stability of ecosystem and sustainability (Schoenholtz et al., 2000; Zhang et al., 2010). Enzyme activities are instantly respond to environmental shifts, due to their proactive role in cycling of C, N and P influencing microbial enrichment and community dynamics (Waldrop et al., 2000; Kizilkaya and Dengiz, 2010). The enzymatic studies on different chronosequence soil profiles contributes informations about origin, nature and catalytic efficiency of enzymes present in soil and hence reported as the 'biological fingerprints. Factors like soil reaction, soil organic matter content, total available nutrients, heavy metal accumulation, predominantly affecting activities of enzymes in soil (Yang et al., 2006; Zhang et al., 2014).

According to some reports, amylases are a group of glycosidase hydrolase enzymes involved in various biochemical transformation reactions and are used as important indicators of microbial diversity and soil fertility. Invertase enzymes also belong to the glycosidase hydrolase category and play a significant role in the functioning of mine spoil (Mishra et al., 2010). Protease enzymes are hydrolases that break peptide bonds and facilitate carbon and nitrogen mineralization in soil. Additionally, phosphatase activity is

predominantly dependent on the metabolic state of the soil, while urease activity has a close relationship with the soil microbial population (Sarathchandra et al., 1984; Fernández-Calviño et al., 2010; Patel et al., 2018). The activities of dehydrogenase enzymes are closely related to the soil microbial population and the diversity of the microbial community structure (Zhang et al., 2015).
With raising anthropogenic activities such as surface mining, the modification of biochemical properties of soil microorganisms enhances the stress condition that leads to reduction in fertility of soil. Determination of soil quality status provides a partial interpretation about the status of the soil subsystem. The greatest concern is to monitor the manganese mine spoil restoration regularly over a period of time that opens the way to develop a clear understanding of the direction of improvement in soil fertility. The polyphasic approach is more suitable for assessing soil quality because it can effectively identify drastic changes in mine areas caused by anthropogenic activities, as each edaphic factor has its own constraints. The determination of enzyme activities represents active networking among factors like physico-chemical properties, available nutrients, structure of microbial communities, metabolic activities, and functioning of the ecosystem. Thus, the present study focuses on the contribution of various physico-chemical attributes towards variation in enzyme activities in chronosequence manganese mine spoil over time that can be assisted in the study of restoration from overburden spoil. The study also examined how microbial biomass and basal soil respiration affect enzyme activities, aiming to provide clear information about microbial diversity and their respiratory processes.

2. MATERIAL AND METHODS

2.1 Sampling: The current investigation was carried out at Kanther Managanese mines, Koira, located in the revenue district of Sundargarh, Odisha, India.The geographical location of sampling sites is between 85A° 20' 09.67 " east longitude and 21° 53' 45.34" north latitude, Sampling was carried out according to the standard methodology provided by soil microbiological protocol (Mishra 1968). Sampling site was splitted into 5 blocks, and from the depth of 0-15 cm five soil samples were assembled from the pit size of 15 x 15 x 15 cm3 in size.. After mixing above subsamples thoroughly form composite sample and subjected to further processing followed by characterization.. The sampling was done on the basis of age series in chronosequence manner such as Fresh mine spoil (MBO0), two year old dump (MBO2), 4 year old dump(MBO4),6 year old dump (MBO6), 8 year old dump (MBO8), 10 year old dump (MBO 10) which was compared with forest soil .

2.2 Soil enzyme activities

Amylase activities was determined by following the standard procedures given by Roberge (1978) and primary substrate used in estimation was starch and incubated for 24 hours at 30℃. Activities of invertase activity was determine by spectrophotometric methods (Ross, 1983). Here substrate used was sucrose and incubation period was 24 hours at 37℃. Protease activity was performed using methods of Ladd & Butler 1972. The urease activity from soil was measured as per the procedures of Tabatabai and Bremner (1971).Activity of dehydrogenase enzyme was estimated by standard procedure of spectrophotometric method (Casida, 1964; Nannipieri et al., 1990; Beyer et al,. 1992).

2.3. Physico-chemical characterization

Soil texture was estimated in percentage using general microbiological procedure. BD was quantified using TSBF Handbook (Anderson and Ingram, 1992). Procedures of Mishra 1968 were used to quantify moisture content and water holding capacity. Digital pH meter was used to measure soil acidity or alkalinity (1:2.5 ratio of soil: water). Soil organic carbon was estimated from different soil profiles was done using methods of Walkley and Black as mentioned by Mishra 1968. Kjeldahl method (Jackson, 1958) was used to calculate total nitrogen content.

2.4. Estimation of microbial biomass and basal soil respiration

A standard procedure of Vance et al., 1987 was used to determine microbial biomass carbon (MB-C) Whereas chloroform fumigation technique was used to quantified Microbial Biomass Nitrogen (MB-N) was quantified as per the procedures of Brookes et al., 1985. Standard procedures of Olsen et al., 1954 and Brookes et al, 1984 were used to estimate Microbial Biomass Phosphorous (MB-P). Basal soil respiration was measured by detailed methods of Witkamp, 1966 described in Ohya et al., 1988 calculating the amount of CO_2 removed from soil through alkali absorption technique.

2.5 Statistical analysis

Duncan's Multiple Range Test (DMRT) was performed to show significant variation among mean and standard deviation values ($p < 0.05$) using software SPSS 16.0. Simple correlation coefficients were performed within physicochemical properties, microbial biomass, basal soil respiration, and enzyme activities using software SPSS 16.0. Further confirmation was done using stepwise multiple regression analysis to know the participation of physicochemical properties, microbial biomass, and basal soil respiration in enhancing enzyme activities using software STATA 15.0× 64. An analysis of variance (ANOVA) was performed to show significant differences among values of enzyme activities, microbial biomass activities, and basal soil respiration using SPSS 17.0 software.

3. RESULTS

3.1 Quantification of enzyme activities

Amylase activities was calculated from chronosequence manganese mine spoil and expressed in µg glucose/g soil/hr. Amylase enzyme shown its activity with range from 1.475±0.565 to 12.107±0.109 µg glucose/g soil/hr in mine spoil which was further increases upto 20.537±0.514 µg glucose/g soil/hr in forest soil (FS). Invertase was found to be ranged from 9.179±0.172 to 715.879±0.562 µg glucose/g soil/hr from MBO0 to forest soil (FS). Variation in protease activity was found in range from 5.295±0.356 µg tyrosine/g soil/hr to 198.49±0.592 µg tyrosine/g soil/hr. Urease activity was shown in the range from 4.672±0.47 µg NH_4^+/g soil/hr to 47.855±0.535 µg NH_4^+/g soil/hr from MBO0 to FS. Dehydrogenase activity was shown in the range from 0.0029±0.0001 µgTPF/g soil/hr to 4.0976±0.0125 µgTPF/g soil/hr across all the sites of chronosequence manganese mine spoil and presented at table 3.1.

Table 3.1 Showing activities of soil enzymes (Amylase, invertase, protease, urease, and dehydrogenase) in different age series of manganese mine spoil (0-15cm)

	Soil enzyme activities (0-15cm) in soil				
SITES	**Amylase(µg glucose/g soil/hr)**	**Invertase(µg glucose/g soil/hr)**	**Protease (µg tyrosine/g soil/hr)**	**Urease (µg NH_4^+/ 4 g soil/hr)**	**Dehydrogenase (µgTPF/g soil/hr)**
MBO0	0	0	0	0	0.0029±0.0001[a]
MBO2	1.475±0.565[a]	9.179±0.172[a]	5.295±0.356[a]	4.672±0.47[a]	0.0093±0.00052[a]
MBO4	3.109±0.1102[b]	21.7103±0.690[b]	15.371±0.048[b]	9.218±0.0921[b]	0.1646±0.0135[b]
MBO6	6.131±0.118[c]	91.018±0.442[c]	21.71±0.615[c]	16.957±0.390[c]	0.6156±0.085[c]
MBO8	9.3303±0.4206[d]	131.583±0.474[d]	30.535±0.662[d]	21.992±0.7533[d]	0.983±0.0121[d]
MBO10	12.107±0.109[e]	245.818± 0.542[e]	42.124±0.988[e]	29.488±0.538[e]	1.5063±0.0765[e]
FS	20.537±0.514[f]	715.879±0.562[f]	198.49±0.592[f]	47.855±0.535[f]	4.0976±0.0125[f]

*Means with different letters are significantly different at $P< 0.05$ level of significance.

3.2 Quantification microbial biomass and basal soil respiration

Estimation of microbial biomass showed significant variation among different soil profiles from chronosequence manganese mine spoil (Table 3.2) expressed in terms of µg/g of soil. It was observed from the data collected from the analysis, microbial biomass carbon MB-C, confines a range of (53.519± 1.371) µg/g of soil to (646.969± 6.025) µg/g of soil including minimum MB-C shown in fresh mine spoil (MBO0) while maximum in forest soil (FS). The level of MB-N was found to be maximum in MBO10 (61.173± 0.3403) µg/g of soil as compare to fresh mine spoil (MBOO: 5.239±0.526) µg/g of soil that further shown highest level of MB-N in nearby forest soil (646.969± 6.025) µg/g of soil. MBO10 (27.256± 1.265 µg/g of soil) shown highest level of MB-P as compare to fresh mine soil (2.542± 0.394) µg/g of soil. Forest soil (30.366± 0.881 µg/g of soil) shown highest level of MB-P among all when compared with all mine spoil from MBO0 to MBO10. Rate of basal soil respiration was quantified from seven different soil profiles which was increasing from MBO0 (0.352 ± 0.007 µg CO_2-C/g soil/hr) to FS (0.958 ±0.014 µg CO_2-C/g soil/hr) in an increasing rate.

Table 3.2 Microbial Biomass C, N and P in seven different overburden spoil from chronosequence manganese mine

SITES	MB-C (µg/g of soil)	MB- N(µg/g of soil)	MB –P (µg/g of soil)	BSR (mg CO_2-C/g soil/hr)
MBO0	53.519± 1.371[a]	5.239±0.526[a]	2.542± 0.394[a]	0.352 ± 0.007[a]
MBO2	124.91±2.587[b]	9.659± 0.429[b]	5.743± 0.212[b]	0.392 ± 0.011[b]
MBO4	244.401± 5.392[c]	22.875± 1.503[c]	10.738± 0.390[c]	0.594 ±0.016[c]
MBO6	431.163± 8.378[d]	40.865± 1.603[d]	20.107±0.164[d]	0.842 ±0.013[d]
MBO8	498 ± 3.126[e]	54.041± 1.957[e]	22.256± 1.265[e]	0.914 ±0.015[e]
MBO10	540.942± 6.025[f]	61.173± 0.3403[f]	27.256± 1.265[f]	0.921 ±0.012[e]
FS	646.969± 6.025[g]	67.657± 1.769[g]	30.366± 0.881[g]	0.958 ±0.014[e]

*Means with different letters showed significance difference (P< 0.05)

4. DISSCUSSION

In the current investigation, different soil enzyme activities were shown increasing trend from fresh mine spoil to MBO10 which accelerates upto forest soil. The activities are ranged from amylase (1.475±0.565 - 20.537±0.514 µg glucose/g soil/hr); invertase (9.179±0.172 - 715.879±0.562 µg glucose/g soil/hr); protease (5.295±0.356 - 198.49±0.592 µg tyrosine/g soil/hr); urease (4.672±0.47 µg NH_4^+/g soil/hr - 47.855±0.535 µg NH_4^+/g soil/hr); dehydrogenase (0.0029±0.0001 - 4.0976±0.0125 µgTPF/g soil/hr).The activities of above soil enzymes are found to be lowest in fresh mine spoil because, all these enzymes catalyses hydrolytic substrate induced reactions, thus inadequacy of suitable nutrients may cause lack of activities in fresh mine spoil as nutrients including metal ions can directly affect the reaction catalysis mechanisms (Nannipieri et al., 1990; Beyer et al., 1992). Additionally, reduction of activities may be due to lack of microbial flora those are engaged in important metabolic reactions in soil and now negatively influenced by heavy metal contaminants such as manganese, cadmium and lead due to disruption of osmotic balance (Abdu et al., 2017).

4.1 Effect of physicochemical properties on enzyme activities

In the current investigation, physicochemical attributes such as organic matter show a high correlation with amylase activities ($r = 0.997$, $p < 0.01$) and help in the simplification of complex saccharides in soil and carry out the mineralization process (Meeti et al., 2020). Multiple regression analysis has shown a 99.38% contribution of OC to the acceleration of amylase activities, and total nitrogen explains 98.25% ($r=0.991$, $p<0.01$) of the variation of amylase activities. Clay fractions contribute 78.13% of amylase activity ($r = 0.884$, $p < 0.01$). Clay-enzyme positively influence sequestration of carbon in soil and help in improvement of soil health (Paul and Sahoo, 2022).

Invertase activity shows a positive correlation with organic matter accumulation ($r= 0.944$, $p< 0.001$) and increases the degree of decomposition (Ciarkowska et al., 2014). The carbon and nitrogen concentration was also positively correlated by invertase activity (C: $r = 0.944$; N: $r = 0.958$; $p < 0.001$, table 4.1). This is because nitrogen concentration majorly contributes to the transformation of organic matter in soil. To further confirm the findings, a multiple regression analysis was conducted, demonstrating that invertase activity positively regulates organic matter accumulation, explaining 89.14% of the variability, while total nitrogen contributes an additional 2.61% to the enzyme activity as the second variable.

Protease activity in the current study explained positive regulation with OC and N ($r = 901$; $p < 0.001$). For further confirmation, multiple regression analysis was done, and protease enzyme activities were positively correlated with organic matter with 81.15% variability and associated with mineralization that facilitates mineral nutrients for the growth of plants. TN contributes 86.29% variation as the first variable and 6.51% as the second variable because protease activity was regulated by N (Geisseler and Horwath, 2008).

Urease activities in soil regulate the improvement in soil pH and loss of nitrogen into the atmosphere by nitrogen volatilization. The result was significant at the 0.01 level of significance with $r = 0.984$ for PH and $r = 0.987$ for available nitrogen. Multiple regression analysis has shown that the positive regulation between urease enzyme activity and available nitrogen in soil contributes 98.41% as the first variable. Because the synthesis and expression of the urease enzyme are highly regulated by nitrogen available in soil (Gianfred et al., 2005). Soil organic carbon positively affects urease activity ($r=0.996$; $p<0.01$) and acts

as a primary source of energy for the establishment of microbes (Ciarkowska et al., 2014; Kumari et al., 2017). Multiple regression analysis also showed a 99.25% contribution of organic carbon to the activities of urease enzymes.

Dehydrogenase reduces triphenyltetrazolium chloride (TTC) in the presence of soil attributes, and its activity was higher toward neutral pH($r = 0.897$; $p < 0.01$, table- 4.1). Multiple regression analysis showed an 80.46% contribution of P^H in the improvement of dehydrogenase activity. As moisture content acts as a major factor for the establishment of microbial diversity, dehydrogenase activity increases with increased moisture content of soil ($r= 0.830$; $0.830; p<0.01$).The organic matter content of soil is positively correlated to dehydrogenase activity (0.960; $p<0.01$) and contributes 92.23% as the first variable. Several reports are available that show the positive correlation of organic matter with dehydrogenase activity (Chodak & Niklińska, 2010; Rumero et al., 2010). Additionally, total nitrogen content positively regulates dehydrogenase activity in soil (0.992; $p<0.01$) and contributes 93.92% as the first variable which was represented at table 4.2.

Table 4.1 Pearson correlation coefficients of various physico-chemical properties with enzyme activities (amylase, invertase,protease, urease, and dehydrogenase) among seven age series of manganese mine spoil

	AMY	INR	PRO	URE	DHA	Clay	BD	WHC	MC	PH	OC	TN
AMY	1											
INR	.950**	1										
PRO	.906**	.987**	1									
URE	.998**	.941**	.896**	1								
DHA	.965**	.998**	.981**	.956**	1							
Clay	.884**	.709**	.642**	.896**	.743**	1						
BD	-.905**	-.741**	-.667**	-.920**	-.774**	-.969**	1					
WHC	.978**	.889**	.839**	.985**	.910**	.932**	-.955**	1				
MC	.929**	.805**	.736**	.936**	.830**	.937**	-.936**	.952**	1			
PH	.976**	.877**	.816**	.984**	.897**	.941**	-.957**	.984**	.952**	1		
OC	.997**	.944**	.901**	.996**	.960**	.891**	-.915**	.983**	.931**	.976**	1	
TN	.991**	.958**	.929**	.992**	.969**	.874**	-.883**	.971**	.907**	.967**	.989**	1

** Correlation significant at 0.01 level *Correlation significant at 0.05 level (AMY- Amylase, INR- Invertase, PRO- Protease, URE- Urease, DHA- Dehydrogenase, BD- Bulk density, WHC- Water holding capacity, MC- Moisture content, OC- Organic matter, TN- Total nitrogen)

Table 4.2 Showing Step wise multiple regression analysis of soil physicochemical properties with enzyme activities in different age series of manganese mine spoil

Enzymes	Equations	R^2
	-0.7739+6.1062OC	99.38
	-8.71709+0.19567TN	98.29
Amylase	-2.8688+0.0507 TN+ 4.5501 OC	99.53
	-10.3060+1.8153 CLAY	78.13
	-0.50272-0.4366 CLAY+6.222 OC	99.39
	-23.6413+3.4789MC	86.22
	15.543+44.8675 EC	80.85
	-43.4688+9.4814CEC	97.86
	-45.6199+10.3486CEC-4.888EC	98.00
Invertase	-105.228+205.0986 OC	89.14
	-383.0166+6.7048 TN	91.75
	-418.3648+ 7.5809 TN-27.5018OC	91.78

	-334.0777+51.6781 CLAY	50.34
	184.069+328.8717OC-46.57701CLAY	97.57
	-504.3905+1318.556EC	55.51
	-1983.985+509.1003 CEC-1129.201EC	88.51
	-2024.671-1013.752 EC+543.6174CEC-22.8064MC	88.71
Protease	-27.6819+53.30909OC	81.15
	-102.2643+1.7713TN	86.29
	-161.6418-46.1973 OC+3.2431 TN	87.66
	-80.2734+12.7307 CLAY	41.16
	68.6546-16.5102 CLAY+94.525 OC +3.2431TN	87.66
	-80.2734+12.7307CLAY	41.16
	68.6546- 15.5105CLAY +94.525OC	93.74
Urease	-0.39658+13.9716 OC	99.25
	-18.6169+ 0.4483 TN	98.41
	136.0451-84.2147BD	84.68
	-103.5023+21.767BD+2.284WHC	97.56
	-194.5087+33.0424 BD+1.3027 WHC+17.368PH	98.76
	-35.224+104.672EC	83.93
	-49.0599+39.3788EC+5.29101MC	89.32
Dehydrogenase	-0.5656+1.191OC	92.23
	-2.1623+0.0387 TN	93.92
	0.6888+0.08813AP	96.77
	-1.3523+0.15905TN+0.05491 AP	98.85
	-1.980+0.3089CLAY	55.14
	-1.226+0.076003 CLAY+0.775 AP	98.71
	-2.1251-0.00036 CLAY+0.6723AP+0.0461WHC	98.95
	-12.9695+2.1232PH	80.46

*All R2- values are significant at $p < 0.001$

4.3 Effect of microbial biomass and Basal soil respiration on soil enzyme activities

In fresh mine spoil, accumulation of microbial biomass carbon decreases with increasing acidification of soil and reduction in the concentration of soil organic carbon in soil. Acidification of soil due to metal stress can negatively influence soil enzymes and their activities. In our study, amylase activities were increased with increasing MB-C (0.928; $p < 0.01$, table 4.3) and contributed 86.16% as the first variable (table 4.4). This may be observed because amylases readily hydrolyze polysaccharides and increase organic carbon, ultimately increasing microbial biomass carbon in soil and associated diversity (Venkatesan & Senthurpandian, 2006). Basal soil respiration contributes 12.25% as the second variable and is positively correlated with the regulation of amylase activities. However, basal soil respiration contributes 7.6% to amylase activities as the second variable next to MBN as the first variable. MBP showed less contribution to enzymatic activities (45.08%) as the first variable as compared to MBN (86.04%). Variation in amylase activities with respect to MB-C and BSR showed significant differences using $p<0.001$.

According to previous reports, C and N concentrations in soil are positively correlated with invertases (Ciarkowska et al., 2014).In our study, C- and N-rich MB-C and MB-N are positively impacted by the activities of invertases (MB-C: $r = 0.778$; MB-N: $r = 0.769$; $p < 0.05$). Stepwise multiple regression analysis showed more contribution of MB-C (60.59%) as compared to MB-N (59.21%) towards invertase activities. Additionally, basal soil respiration individually contributes 43.47% as the first variable; however, it contributes 28.23% as the second variable next to the first variable, MB-C, and contributes 34.85% as the second variable next to MB-N. Thus, N acts as a principal nutrient in the transformation of organic matter that accelerates noticeable changes in invertase activities. Similarly, BSR observed a 9.13% contribution to the amylase activities as the second variable in table 6, while MB-P contributes 43.48% individually.

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Proteolytic enzymes are extracellular and release plant-available nitrogen from soil organic matter by depolymerizing proteinaceous compounds (Brzostek and Finzi, 2011). Thus MB-N contributes 48.01% to the activities of proteases, which also significantly influence microbial respiration by contributing 18.1% as the second variable next to MB-N in soil. MB-C also positively correlated with protease activity by contributing 50.76% individually as the first variable; however, BSR contributes 38.06% as the second variable along with MB-C as the first variable. Additionally, MB-P also contributes 50.17% and influences protease activity positively across different manganese mine spoils. The variations are significant at the $p<0.01$ level of significance.

According to the Pearson correlation coefficient, the urease enzyme showed a positive correlation with MB-C and MB-N ($r = 0.941$ and 0.936 for both MB-C and MB-N, respectively). Thus, improvement in soil nitrogen causes higher microbial metabolism and enzyme activities (Qian et al., 2014; Ye et al., 2014). Further, confirmation was shown by stepwise multiple regression analysis by MB-C and MB-N by contribution of 88.48% and 87.65% as the first variable, respectively. In addition to this, urease enzyme also positively correlated to basal soil respiration ($R2= 0.1160$: MB-C; $R2= 5.59$: MB-N, $P<0.01$) as a second variable along with MB-C and MB-N.

As dehydrogenase exists in living cells, their activity represents real-time metabolic activities in soil microbes across different overburden spoil. In our current study, dehydrogenase showed a positive correlation with MB-C ($r= 0.811$, $p<0.05$, table 4.3) and MB-N ($r= 0.804$, $p<0.05$). Stepwise multiple regression analysis showed contributions of 65.77% and 64.58% towards DHA activities. Additionally BSR shows 28.07% and 16.29 % correlation DHA as second variable along with MB-C and MB-N as first variable (table 4.4). Li et al. (2009) reported the positive influence of microbial biomass on soil enzyme activities, and the accumulation of microbial biomass significantly affects enzyme activities at the 0.001 level of significance from the one-way ANOVA analysis.

Table 4.3 Showing correlation between impact of MB-C, MB-N, MB-P and BSR on enzyme activities of seven chronosequence manganese mine spoil (0-15 cm)

	MBC	MBN	MBP	BSR	AMY	INV	PRO	URE	DHA
MBC	1								
MBN	.994**	1							
MBP	.678	.618	1						
BSR	.983**	.977**	.653	1					
AMY	.928**	.928**	.671	.848*	1				
INV	.778*	.769*	.659	.659	.951**	1			
PRO	.712	.693	.708	.587	.906**	.987**	1		
URE	.941**	.936**	.675	.865*	.998**	.942**	.896**	1	
DHA	.811*	.804*	.679	.700	.966**	.998**	.981**	.957**	1

** Correlation significant at 0.01 level *Correlation significant at 0.05 level

*MB-C -Microbial biomass carbon, MB-N- Microbial biomass nitrogen, MB-P- Microbial biomass phosphorus,

BSR- Basal soil respiration, AMY- Amylase, INV- Invertase, PRO- Protease, URE- Urease, DHA- Dehydrogenase

Table 4.4 Showing stepwise multiple regression of MB-C, MB-N ,MB-P and BSR on soil enzyme activities

	Equations	R^2
Amylase	-3.2376+0.0296MBC	86.16
	12.24907+0.0894MBC-52.3597 BSR	98.41
	-2.3622+0.2647MBN	86.04
	9.4876+0.6274MBN-35.7534BSR	93.64
	1.6186+0.4397MBP	45.08
	-8.22583+0.1343 MBP+19.632BSR	74.34

Invertase	-146.743+0.8827MBC	60.59
	-283.3544+643.207BSR	43.47
	228.7335+1.22392MBC-891.9421BSR	88.82
	-117.5154+7.7924MBN	59.21
	549.199+28.1998MBN-2011.602BSR	78.32
	-32.3192+15.32403MBP	43.48
	-227.4271+9.272 MBP+389.0938 BSR	52.61
Protease	-66.0202+155.977BSR	34.45
	-35.0807+0.22009MBC	50.76
	228.7335+1.2392MBC-891.942BSR	88.82
	-26.6183+1.9114MBN	48.01
	150.1598+7.3223MBN-533.368BSR	66.11
	-15.4637+3.5869MBP	50.17
Urease	-6.3859+0.6889MBC	88.48
	26.6015+0.1962MBC-111.529BSR	99.08
	-4.2615+0.6118MBN	87.65
	19.0236+1.3246MBN-70.255BSR	93.24
	4.9969+1.0121MBP	45.54
	-18.3455+0.2881MBP+46.5508BSR	76.90
Dehydrogenase	-1.7144+3.897BSR	48.97
	-0.8513+0.00525MBC	65.77
	3.8979+0.235MBC-16.0571BSR	93.85
	-0.6816+0.0464MBN	64.58
	2.833-10.6054BSR+0.15405MBN	80.87

*All R^2- values are significant at $p < 0.001$

Principal component analysis was performed to acquire clear understanding about variations among different manganese overburden spoil differentiated from FS on the basis of factors such as enzyme activities and how its influenced from microbial biomass and basal soil respiration (Ludwig and Reynolds, 1988). The analysis shown the Z1 and Z2 components including maximum variance contributes maximum cumulative variance percentage about 99.93% , that differentiates seven different soil profiles into independent cluster (Fig:4.1).

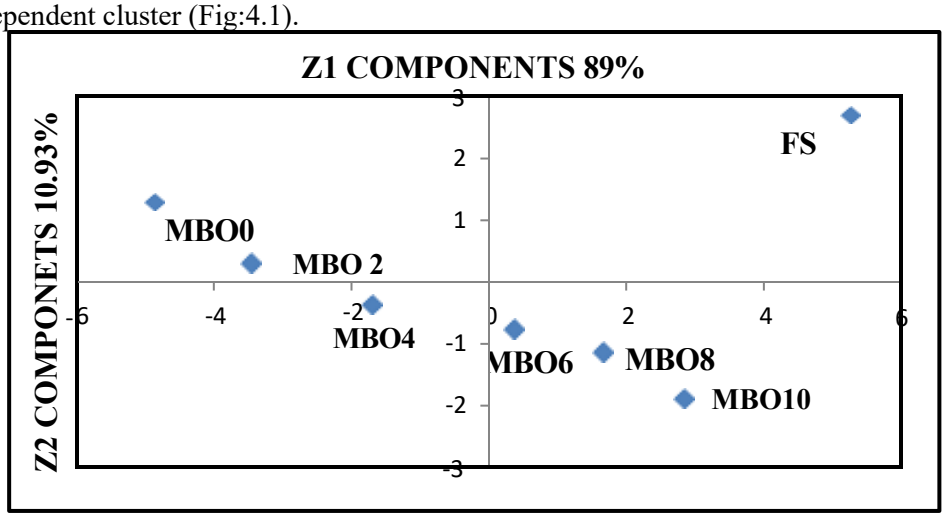

Fig 4.1 Principal component analysis based on MB-C, MB-N, MB-P and BSR on different chronosequence manganese mine spoil

5. CONCLUSION

The variations in physicochemical properties such as organic carbon, moisture content, total available nutrients, etc. within the landscape in the chronosequence manganese mine spoil have a major impact on the microbial dispersion and diversity. The objective of the current study was to focus on the impact of variation in physicochemical properties on soil enzyme activities. Factors like soil organic matter, total

nitrogen, and clay content of old overburden dump sites primarily contribute towards improvement of enzyme activities. The microbial biomass and enzyme activities are significantly reduced at fresh mine spoil due to lack of organic matter and other available nutrients. The contamination of heavy metals due to mining activities negatively affects microbial biomass and significantly lowers the nutrient status of soil. However, as an intracellular enzyme, dehydrogenase determines the overall metabolic activities of microbes and has shown reproducible results in degraded land and acts as a sensitive indicator of soil quality.

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