

## Biodiversity Promotion in Restored Mine Land through Plant-Animal Interaction

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### Abstract

Ecological integrity includes enriched biodiversity, flourishing plant-animal interaction, and dynamic nutrient cycling. The present study examines the ecosystem structure and function of a newly developed ecosystem at a 24 year-old of restoration efforts in manganese mine area, Gumgaon, India. This study was initiated in the year 1988 through microbe assisted green technology (MAGT), which included application of top soil, site specific plantation, organic amendments and addition of soil microbes. The physico-chemical and biological properties of the reclaimed soil were gradually improved during the restoration programme with reference to soil pH, EC, bulk density, porosity, N, P, K, organic carbon, soil microbes, etc. Herbs occupied major ground cover with maximum density, followed by shrubs, trees and climbers. Regeneration capacity of the vegetation also followed the same pattern. An attempt was also made to analyse pollinator web with plant-pollinator interaction, influence of floral distribution on the composition of pollinator communities. The results of the study may help in understanding the linkage between plant and pollinator communities in ecorestoration programme. Four different groups of litter decomposing organisms, viz. microflora (bacteria, fungi, VAM), microfauna (Nematoda), mesofauna (Protura, Enchytraeidae, Acari, Collembola) and macrofauna (Diplura, Isopoda, Isoptera, Chilopoda, Diplopoda, Coleoptera, Earthworms) have been studied. The present study clearly illustrates the sequence of various successional stages of ecosystem development from hostile ecological conditions of mine spoil to fully developed ecosystem and the study demonstrated how a barren land could be converted into a flourishing carbon sink.

**Keywords:** Biodiversity promotion; Eco-restoration; Floral diversity; Faunal diversity; MAGT; Plant-pollinator interaction; Sustainable mine reclamation

### Introduction

Mining activity poses harsh environmental conditions with extreme physico-chemical and biological constraints like low cation-exchange capacity, low water holding capacity, low nutrient availability, poor organic matter and lack of soil organisms. Changes in plant species composition and disturbed functional ecosystem components like biotic and abiotic relationships are major impediments of mining activity [1]. Mine reclamation involves activities and amendments to restore ecological integrity through sustainable development of ecosystem [2]. The restoration of ecosystem in metal/ metalloids contaminated site is a major environmental task around the World. The existing techniques to enhance natural reclamation mechanisms in metal contaminated soils are by addition of top soil, organic amendments (biosludge, dairy waste, fly ash, pressmud, etc.), microbial amendments (*Azotobacter*, *Rhizobium*, VAM, etc.) along with site specific plantation [2].

Sustainable mine restoration programme requires establishment of self-sustainable ecosystem, by means of improved ecosystem structure and function with respect to ecological integrity [3]. Ecological integrity includes variability in biodiversity, nutrient cycle, ecological processes, and structural relationships like plant-pollinator interaction,

litter-decomposer organism interaction and nutrient release rates [4]. A satisfactory restoration of mine overburdens demand a permanent vegetation cover that will prevent soil erosion, permitting the long-term sustainable soil development.

Pollination is a major interaction in animal-plant relationship and it contributes to the reproduction in 70% - 90% of flowering plant species [5]. The diversity of pollinator species directly affects the plant species, each pollinator species having particular needs with respect to temperature, humidity, larval food and adult food sources. Pollinators have been shown to have a major role in reproductive stability [6] and community stability [7] of an ecosystem. The spatial distribution of plants and their interactions with animals provide critical information about community structure, plant-pollinator interaction, reproductive behaviour, mode of seed dispersal, seed germination, litter fall, litter-soil organism interaction, litter-nutrient cycling [8,9], which also determine the extent of success in ecorestoration programme.

The decomposition of leaf litter is essential in nutrient cycling and stabilization of soils. It is well established that without suitable soil organisms, there would be no organic matter breakdown and nutrient release. Soil organisms such as, annelids, nematodes, microarthropods, millipedes, centipedes, mites, collembolan, bacteria, and fungi are very mobile and develop populations rapidly once appropriate substrates are available [10]. So, analysis of the development of population structure and spatial distribution patterns of afforested forest on reclaimed mine spoils may provide information for examining the process of reclamation. Most studies have focused on techniques used

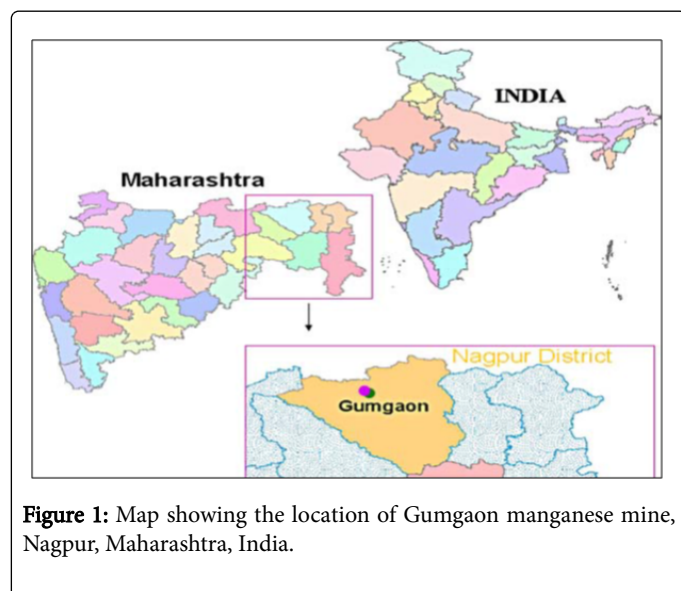
for vegetation establishment, phytoremediation and few researches have examined spontaneous revegetation in mine spoil through soil amendments [11-13]. However, little attention has been paid to the plant-animal interactions and spatial distributions of afforested plants in sustainable ecorestoration programme.

The present study was initiated by CSIR- National Environmental Engineering Research Institute (NEERI) in the year 1988 through microbe assisted green technology (MAGT) [2]. Present study examines the following parameters of newly formed post mine ecosystem after a period of 24 years: (i) changes in the available nutrients status (ii) floral biodiversity and its regeneration capacity (iii) monitor ecological interactions between plant-pollinator (iv) diversity studies of litter decomposers and (v) carbon sequestration capability of restored mine site.

## Materials and Methods

### Site description and plantation strategies

The selected manganese mine is under Manganese Ore India Limited (MOIL), which is partly opencast and partly underground. It is located in Gumgaon, 40 km away from Nagpur city, central India. It is situated at the geographic location of 21°24' N latitude and 078°59' E longitudes (Figure 1). It has the total recoverable reserve of 2.64 million tons with the production of 19,405 tons of manganese ore per annum. The total lease hold area is 53.6 ha, out of which about 6.3 ha, was covered under spoil dump. The average rain fall ranges from 1000 mm to 1200 mm, temperature ranges from 27°C to 48.1°C. The pre mining floristic topography of the area was full of forest comprising dry deciduous vegetation. Spoil was composed of mainly low grade manganese ore. It consisted of 61.0% stones, 5.8% gravel and 33.2% low grade manganese ore.



**Figure 1:** Map showing the location of Gumgaon manganese mine, Nagpur, Maharashtra, India.

Plantation pits of different sizes, 0.6 m<sup>3</sup> and 1 m<sup>3</sup> were prepared in post mining slopes and plain areas, respectively. Plantation was done in the year 1988 following microbe assisted green technology (MAGT) as explained in our earlier reports [2]. Top soil, pressum (soil amendment), nitrogen fixing bacteria (*Azotobacter* and *Rhizobium*) and vesicular arbuscular mycorrhiza (VAM) were applied to grow native plant species such as *Tectona grandis*, *Cassia siamea*, *Acacia*

*nilotica*, *Pongamia pinnata*, *Phyllanthus emblica*, *Ailanthus excelsa*, *Dendrocalamus strictus*, *Azadirachta indica*, *Gmelina arborea*, *Syzygium cumuni* etc.

### Physico-chemical properties of soil

Mine spoil and top soil samples were collected before plantation and reclaimed soil samples were collected once after every six years period during last 24 years. All the samples were ground, passed through 2 mm sieve and thoroughly mixed before for the determination of physico-chemical analysis. The pH and electrical conductivity (EC) were measured by using a pH meter and EC meter, respectively [14]. The percentage of organic carbon was determined with the method of Walkley and Black [15]. Soil samples from four different depths viz., 0–15 cm, 15–30 cm, and 30–60 cm and 60–90 cm were studied for total and available nitrogen (N), phosphorus (P), potassium (K) and left over manganese (Mn) in the soil. Diethylenetriamine penta- acetic acid (DTPA) was used to extract soil samples to analysis of available Mn concentration [16]. Total and available N, P, K were analysed using standard methods [17,18]. Trace elements such as sodium, calcium and magnesium were determined by inductively coupled plasma atomic emission spectrometry (ICP- AES).

### Vegetation cover, species diversity and biomass estimation

An inventory of vegetation cover was recorded for herbs, shrubs, climbers and trees by using random sampling quadrat method [19]. Three different sizes of plots were drawn, i.e. plots of one meter radius to observe annual vegetation and seedlings; three meter radius for shrubs and saplings, ten meter radius for the study of trees and woody climbers. Plant species were identified with the help of “The Flora of British India” [20], and “The Flora of Nagpur district” [21]. Vegetation cover was quantitatively analysed for number of individuals, density, frequency and abundance [22]. The species richness, diversity index (H) and concentration of dominance (CD) of the species under the vegetation cover were followed by Shannon and Wiener and Simpson [23,24], respectively.

Tree height (H), diameter at breast height (DBH) and percentage of foliage cover in the crown or canopy (Fc) were recorded in all studied vegetation samples. Total biomass of vegetation was analysed through non-destructive, morphometric measurements and algometric equations related to above ground biomass (AGB), below ground biomass (BGB) and canopy biomass [25]. AGB was calculated by using volume of the above ground plant and wood density, BGB was calculated with the formula given by MacDicken [26] and the biomass of foliage cover was determined with the help of crown volume calculation.

### Plant-pollinator interaction studies

Seasonal surveys were conducted for period of one year (January 2013 to February 2014) to study plant-pollinator interactions. Pollinator assemblage was surveyed on warm, dry days with moderate wind speed in day light. During each survey, observations were made to record the flower species, flower colour and the major class of pollinators randomly placed in one m<sup>2</sup> quadrats across the restored site. Insects were identified as pollinators if they were observed probing reproductive parts of the flowers. Pollinators were collected with nets and put into individual clean waxed paper envelopes and placed into a kill jar filled with ethyl acetate [27].

### Soil decomposers and microbial biomass

Soil samples were taken by line transect method from all around the reclamation site to observe population measurements viz., frequency, density and abundance. Extraction of soil samples was carried out by 'Expedition Funnel Apparatus' modified by Macfadyen [28]. Density of the species was calculated per 100 gm of the soil and was counted with the help of Expedition Funnel Apparatus. The species richness, diversity index (H) and concentration of dominance (CD) of the species in the forest were analysed. The identification of soil animals was followed by using Macfadyen [28], Choudhuri [29], Van der Drift [30] and Borrer et al. [31]. Soil microbial populations such as bacteria, fungi, actinomycetes, and nitrogen fixing strains of Rhizobium and Azotobacter were estimated as per the procedure given in the manual of land quality evaluation [32] and were expressed in terms of colony forming units (CFU/g).

### Carbon sequestration analysis

Carbon percentage of the wood was calculated by estimating total organic carbon (TOC) with the help of TOC analyser. Atmospheric carbon sequestration by plants was calculated through morphometric equations using total biomass of the plant and TOC. Soil carbon sequestration was estimated by using the equations as suggested by IPCC good practice guidance for land use, land- use change and forestry [33].

### Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SPSS software program and significant differences were calculated at  $p < 0.05$ . Species richness, diversity index (H) and concentration of dominance (CD) of the species under the vegetation were studied by using the following formulae: Diversity index (H) [23]  $= -\sum(N_i / N) \log_2 (N_i / N)$ ; Concentration of dominance (CD) (Simpson index)  $= \sum(N_i / N)^2$ , where,  $N_i$  = number of individual plant species in the quadrat;  $N$  = total number of all species present in all quadrats. Tree volume was analysed by the formula:  $Ab \times H \times K_c$ , where  $Ab$  is the basal area,  $H$  is the height, and  $K_c$  is a site-dependent constant. Crown volume [ $V$  ( $m^3$ )] was calculated by the equation,  $\pi \times d_b^2 \times H_c / 12$ ; where  $\pi = 3.141592$ ;  $D_b$  = the diameter of the crown;  $H_c$  = the height from the ground to the base of the crown.

Soil carbon sequestration was analysed by means of amount of carbon ( $ton\ ha^{-1}$ ) soil by the following:

SOC

$$= \sum_{\text{Horizon} = 1}^{\text{Horizon} = n} \text{SOC horizon} = \sum_{\text{Horizon} = 1}^{\text{Horizon} = n}$$

$$([\text{SOC}] * \text{Bulk density} * \text{depth} * (1 - C \text{ frag.}) * 100)$$

Where,  $\text{SOC}_{\text{horizon}}$  = Soil organic carbon content for a constituent soil horizon ( $ton\ carbon\ ha^{-1}$ )

[SOC] = Concentration of SOC in a given soil mass obtained from analysis,  $g\ C\ (kg\ soil)^{-1}$

Bulk density = Soil mass per sample volume,  $tonnes\ soil\ m^{-3}$  (equivalent to  $Mg\ m^{-3}$ )

C fragments = % volume of coarse fragments/100.

### Results

#### Physico-chemical properties of mine spoil

At initial stage, the manganese mine spoil at Gumgaon had very unfavorable physico-chemical conditions such as, high percentage of stones, gravels and high manganese concentration ( $215 \pm 38\ mg\ kg^{-1}$ ). Low porosity ( $32.1 \pm 5.2\%$ ), high bulk density ( $1.4 \pm 0.05\ g\ cm^{-3}$ ), low water holding capacity ( $29.8 \pm 2.8\%$ ), low electrical conductivity, poor nutritive capacity in terms of total and available N, P, K, and deprived exchangeable cations (Na, Ca, and Mg) were the characteristic features of the spoil at the initial time of reclamation programme (Table 1).

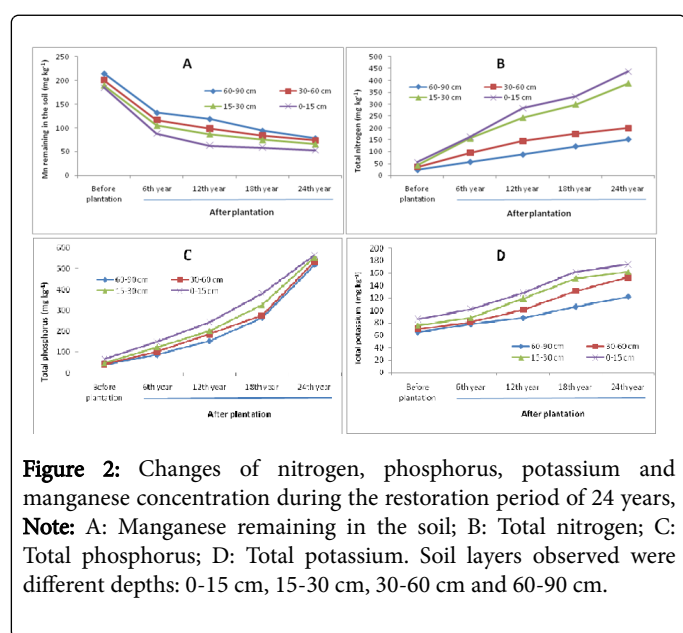
#### Changes in physico-chemical characteristics of reclaimed soil

Changes in physico-chemical characters of restored top soil for every six years duration are depicted in Table 1. Concentration of available manganese in the soil declined with respect to increase in time after reclamation (Figure 2). It was observed that the reclamation process improved soil nutrients to a greater extent with more than ten-fold increase in available NPK at ( $158.75 \pm 16.52\ mg\ kg^{-1}$ ;  $157.18 \pm 8.33\ mg\ kg^{-1}$ ;  $49.0 \pm 0.02\ mg\ kg^{-1}$ , respectively), organic carbon ( $1.78 \pm 0.18\ 52\ mg\ kg^{-1}$ ) and total N P K at ( $484.00 \pm 79.53\ mg\ kg^{-1}$ ;  $567.96 \pm 14.14\ mg\ kg^{-1}$ ;  $174.25 \pm 4.35\ mg\ kg^{-1}$ , respectively). Different layers of the soil showed variation in their total N, P and K concentrations, however top layer (0-15 cm depth) trapped more nutrients compared to other soil depths viz. 15-30 cm, 30-60 cm and 60-90 cm (Figure 2).

Parameters	Top soils	Changes of soil in regular interval period				
		Initial	6 <sup>th</sup> year	12 <sup>th</sup> year	18 <sup>th</sup> year	24 <sup>th</sup> year
Bulk density ( $g\ cm^{-3}$ )	$1.25 \pm 0.02$	$1.40 \pm 0.05$	$1.35 \pm 0.10$	$1.27 \pm 0.01$	$1.24 \pm 0.10$	$1.15 \pm 0.04$
Porosity (%)	$63.6 \pm 3.6$	$32.1 \pm 5.2$	$50.1 \pm 7.7$	$55.8 \pm 8.7$	$61.82 \pm 7.70$	$62.82 \pm 2.30$
Maximum water holding capacity (%)	$56.4 \pm 2.5$	$29.8 \pm 2.8$	$41.2 \pm 4.2$	$45.2 \pm 5.4$	$51.20 \pm 5.20$	$56.20 \pm 1.55$
Chemical parameter						
pH	$7.2 \pm 0.5$	$6.9 \pm 0.2$	$6.9 \pm 0.2$	$7.4 \pm 0.2$	$7.2 \pm 0.2$	$7.66 \pm 0.08$
Electrical conductivity ( $mS\ cm^{-1}$ )	$0.48 \pm 0.09$	$0.32 \pm 0.05$	$0.59 \pm 0.02$	$0.55 \pm 0.05$	$0.61 \pm 0.03$	$0.63 \pm 0.01$

Total nitrogen (mg kg <sup>-1</sup> )	95.0 ± 15.2	41.00 ± 5.06	163.0 ± 1.5	283.0 ± 19.4	319.0 ± 12.4	484.00 ± 79.53
Available nitrogen (mg kg <sup>-1</sup> )	31.0 ± 5.3	12.0 ± 2.2	40.0 ± 5.2	84.0 ± 2.1	151.0 ± 15.6	158.75 ± 16.52
Total phosphorous (mg kg <sup>-1</sup> )	86.0 ± 11.2	46.0 ± 7.4	149.0 ± 6.6	169.0 ± 5.7	291.0 ± 5.4	567.96 ± 14.14
Available phosphorous (mg kg <sup>-1</sup> )	41.0 ± 6.3	9.2 ± 0.3	10.0 ± 0.9	19.0 ± 2.6	33.0 ± 2.5	157.18 ± 8.33
Total potassium (mg kg <sup>-1</sup> )	120.0 ± 20.3	86 ± 9.8	102.0 ± 7.4	128.0 ± 12.7	162.0 ± 2.3	174.25 ± 4.35
Available potassium (mg kg <sup>-1</sup> )	31.0 ± 2.2	1.8 ± 0.1	25.0 ± 0.2	31.0 ± 0.2	45.0 ± 0.1	49.00 ± 0.02
Organic carbon (%)	3.70 ± 0.04	0.099 ± 0.005	0.700 ± 0.013	1.30 ± 0.17	1.45 ± 0.14	1.78 ± 0.18
Organic matter (%)	6.36 ± 0.07	0.170 ± 0.008	1.204 ± 0.022	2.236 ± 0.290	2.49 ± 0.24	3.06 ± 0.31
<b>Exchangeable cations (c mol (+) kg<sup>-1</sup>)</b>						
Na	0.0300 ± 0.0001	0.012 ± 0.006	0.019 ± 0.004	0.029 ± 0.004	0.0440 ± 0.0001	0.045 ± 0.001
Ca	0.046 ± 0.005	0.021 ± 0.004	0.031 ± 0.004	0.042 ± 0.004	0.046 ± 0.005	0.0480 ± 0.0005
Mg	0.0160 ± 0.0001	0.0060 ± 0.0001	0.009 ± 0.002	0.015 ± 0.002	0.0160 ± 0.0001	0.0190 ± 0.0008

**Table 1:** Changes in physico-chemical characteristics of reclaimed mine soil during 24 years of plantation.



### Vegetation cover, species diversity and microbial counts

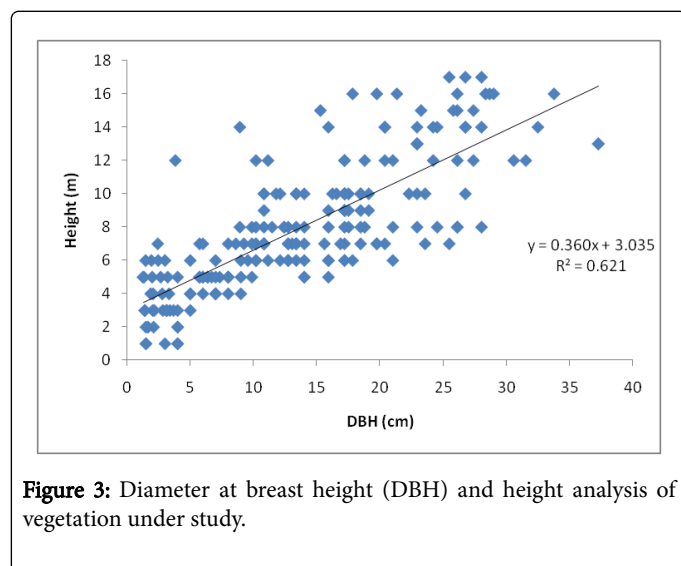
The colonization capacity of natural vegetation gradually progressed with the restoration time. During the first six years, the site had only planted tree species with few herbaceous foliage; in the next six years period, the area practically more of vegetation and regeneration of native plant species taking place. Now after 24 years the reclamation programme, the site is covered by dense plant cover of tropical dry deciduous forest type of vegetation. A total of 191 plant species

belonging 47 families were observed, out of which 162 taxa belong to dicots and 29 taxa belong to monocot. The dominant tree species of the area are *Tectona grandis*, *Cassia siamea*, *Azadirachta indica* and *Acacia nilotica*. The common climbers recorded in this area are *Dioscorea bulbifera*, *Rhynchosia minima*, while dominant herbaceous species include *Alternanthera sessilis*, *Oplismenus burmanii*, *Corchorous aestuans*, *Ocimum americanum*, *Cassia tora*, etc. The floristic details of the plant species of the area are listed in supplementary Table 1. The regeneration capacity of herbs was more (8967 ± 1265 individuals ha<sup>-1</sup>) when compared with other forms of vegetation. The value of species diversity in terms of Shannon–Wiener index was: 3.34 ± 0.08, 2.81 ± 0.10, 1.77 ± 0.11 and 2.12 ± 0.13 for herbs, shrubs, climbers and trees, respectively. The details of frequency, density, species evenness, species richness, basal area, crown cover etc. are given in Table 2. After a period of 24 years of reclamation programme, the colony forming units of soil microorganisms were multiplied at a faster rate. The microbial counts of *actinomycetes*, *Rhizobium* and *Azotobacter* are presented in Table 3.

Parameters	Trees	Shrubs	Herbs	Climbers
Regeneration density (individual sha <sup>-1</sup> )	340 ± 61	4792 ± 675	8967 ± 1265	40 ± 7
Species richness (Simpson index)	0.80 ± 0.05	0.84 ± 0.04	0.87 ± 0.06	0.79 ± 0.05
Species diversity (Shannon–Wiener index)	2.12 ± 0.13	2.81 ± 0.10	3.34 ± 0.08	1.77 ± 0.11

Frequency (%)	26.00 ± 0.01	16.00 ± 0.51	12.00 ± 0.93	7.00 ± 0.05
Density (individuals ha <sup>-1</sup> )	967 ± 25	14667 ± 250	790667 ± 1232	75 ± 9
Species evenness	0.73 ± 0.08	0.77 ± 0.06	0.67 ± 0.04	0.90 ± 0.08
Basal area (m <sup>2</sup> ha <sup>-1</sup> )	24.4 ± 1.2	8.4 ± 0.4	2.6 ± 0.1	1.3 ± 0.2
Litter (mm)	18 ± 4			
Crown cover (%)	64 ± 10			
Litter + humus depth (mm)	37 ± 9			

**Table 2:** Plant diversity at 24-year-old restored mine site at Gumgaon.



**Figure 3:** Diameter at breast height (DBH) and height analysis of vegetation under study.

### Biomass estimation and carbon sequestration studies

The height and diameter (DBH) analysis revealed that restored site has generated huge above ground biomass (Figure 3). The data obtained after 24 years of reclamation showed that *Tectona grandis* and *Dalbergia sissoo* grew faster than the other species and gained maximum DBH. However, visual observations at the site showed that broad leaved *Tectona grandis* added more litter to generate soil humus and runners of bamboo plants helped to conserve the soil. Carbon sequestration by vegetation was calculated through above and below ground plant biomass. As per our earlier studies [2], the area was a barren land and there was no vegetative biomass at initial stage at the site. After first six years of reclamation programme, it was found that the planted and naturally developed vegetation sequestered huge amount of carbon (21.46 Mg C ha<sup>-1</sup>). Carbon sequestration capacity of the vegetation was doubled (44.62 Mg C ha<sup>-1</sup>) in the next six years of reclamation, and thereafter it increased only marginally (Figure 4). After 24 years of reclamation, a thick layer of leaf litter (18 ± 4 mm) was observed with humus layer of 37 ± 9 mm thickness. It reveals how the biomass of vegetation contributed organic matter to the soil. Soil

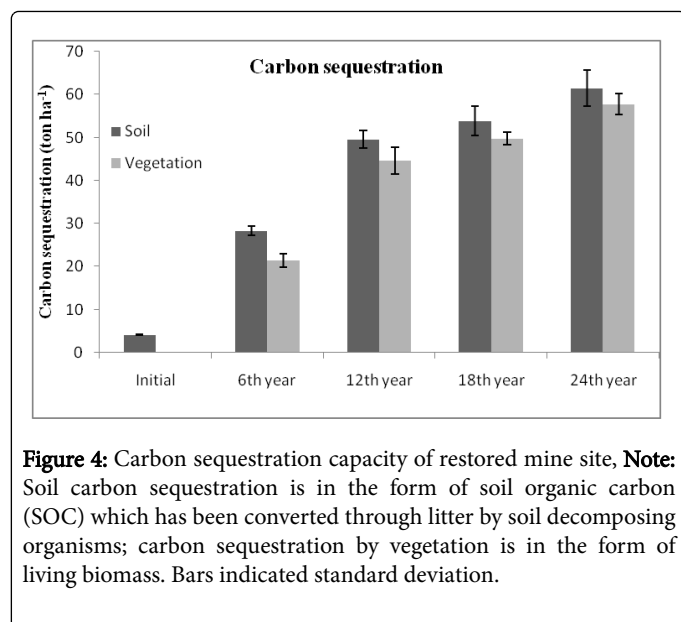
carbon sequestration was analysed in terms of conversion rate of soil organic matter (SOM) into soil organic carbon. Soil carbon sequestration capacity of newly formed soil during the process of reclamation is given in Figure 4.

### Plant-pollinator interaction and soil fauna

Seasonal observations were made for a period of one year to note down flower visitors/pollinators and their interaction with the plant species (Figure 5). The pollinator species observed at the site belong to the following major groups; Coleoptera, Diptera, Thysanoptera, Chiroptera, Hymenoptera and Aves. Honey bees (*Apis cerana*) were recorded all over the site and also lead to fruit setting of different species i.e. *Tectona grandis*, *Cassia siamea*, etc. Physical observations of honey combs and bird nests were also recorded at several places of the site. Colour is one of the major attractive components in plant-pollinator interaction, hence we observed flower colour to know-how these pollinators were attracted towards different plant species (Figure 5). Dominant flower colour on site was yellow (58 species, 32%), followed by white (35 species, 19%), red (21 species, 11%), green (19 species, 10%), blue (8 species, 4%), and others (50 species, 24%) represented by mixture of colours (Figure 5). Soil fauna under study was represented by microfauna (Nematoda), mesofauna (Protura, Enchytraeidae, Acari, Collembola) and macrofauna (Diplura, Isopoda, Isoptera, Chilopoda, Diplopoda, Coleoptera, Earthworms). Frequency, density, species richness and species diversity of soil fauna is presented in Table 4. The existing relation between ecosystem function and structure in the present eco-restoration programme is graphically explained in terms of interaction between plant-pollinator and decomposer-litter; vegetation forms, nutrient release rates in Figure 6.

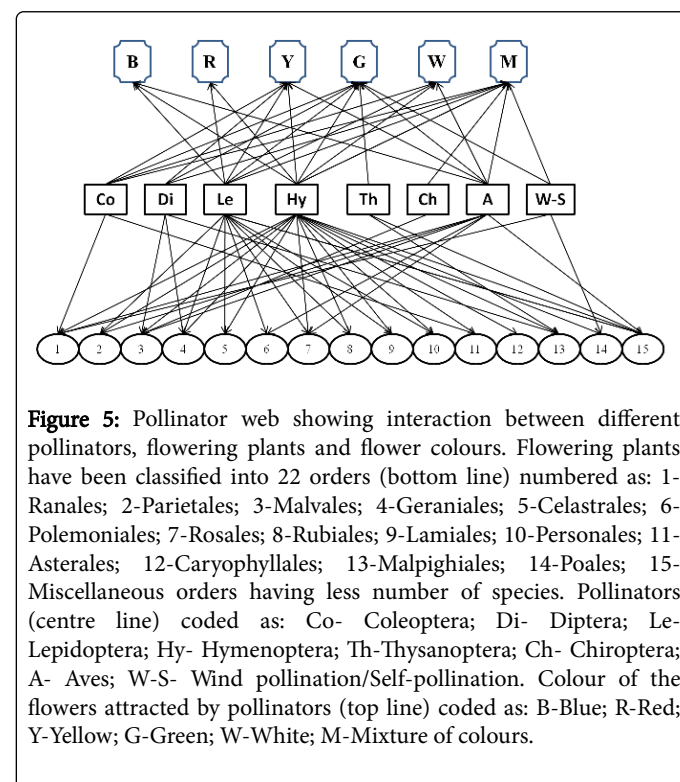
### Discussion

Successful land reclamation in disturbed areas after mining activity needs restoration of both biotic and abiotic components, which requires several years of intensive rehabilitation efforts with different approaches. During the reclamation process, the changes in physico-chemical properties of soil mainly depend on the type of amendments used [25]. In the present study, press mud was used as an amendment supplement source of organic matter and influence the nutrient supply to the planted tree species. Pressmud is a by-product of the sugar industry, it has high organic carbon (370 g kg<sup>-1</sup>), is rich in bioavailable nutrients like nitrogen, phosphorus and potassium [2], and it has been reported to increase soil fertility [34,35]. Soil pH, bulk density, EC, organic carbon and NPK are important indicators for soil physico-chemical characteristics that are strongly related to restoration [13]. In the present study, the physico-chemical properties of the restored soil were gradually improved during the course of reclamation time (Table 1). The pH of the newly formed soil was 7.4 ± 0.1. There was a multifold increase in NPK and organic carbon. Roots and soil organisms can alter soil physico-chemical properties through several processes such as assimilation/production of anions/cations, release of organic acids, CO<sub>2</sub>, O<sub>2</sub> consumption and redox reactions [36,37]. In the present study, soil nutrients viz. N, P and K were increased with reclamation period at four different soil depths i.e. 0-15 cm, 15-30 cm, 30-60 cm and 60-90 cm (Figure 2). However, the levels of these nutrients were inversely proportional to their soil depth. Recent studies on rehabilitated sites also support the changes in soil chemical properties encountered in the present study [38].



Spatial distribution pattern of vegetation is commonly observed to know-how eco-restoration programmes succeed at disturbed sites [9]. Tree species belonging to small DBH class (saplings) the represented the regeneration capacity and also reflected on the reproduction ability of the ecosystem. Large DBH class (>10 cm) was a reflection of survival capacity in particular ecosystem. In Gumgaon afforested site, spatial distribution of trees can be classified in various DBH sizes (from 5 cm to 40 cm) with different heights (5 to 20 m) (Figure 3). A total of 191 plant species naturally colonized by seed dispersal in the reclaimed mine site and interaction of pollinator species indicating that the present reclamation programme has achieved a wide diversity of plant species (Table 1). The study also provides evidence of mixed vegetation with different habit forms, viz. herbs (137), shrubs (16), climbers (7) and trees (31). However only ten tree species were planted in the beginning of the programme. These results are in conformity accord with the findings of Zhao et al. [39]. The study evidently shows a number of significant associations between the DBH classes of planted trees and patterns of colonization by primary forest species. Moreover, many successional herbaceous species were found on reclaimed mine site in the first decade of the reclamation, e.g., *Cynodon dactylon*, *Dactyloctenium aegypticum*, *Dichanthium annulatum*, *Iseilema laxum*, *Heteropogon contortus*, *Apluda mutica*, *Aristida adscensionis*, *Bothriochloa pertusa*, *Brachiaria ramosa*, *Chrysopogon fulvus*, *Alysicarpus monilifer*, *Alysicarpus vaginalis*, *Crotalaria linifolia*, *Crotalaria pusilla*, *Crotalaria retusa* etc. The outcome of eco-restoration depends on the nature and extent of the plant distribution after reclamation of the site. Therefore, the record of vegetation cover, species diversity, and processes of ecosystem development is important to prove the success of eco-restoration [40]. In addition, eco-restoration process depends on specific characteristics of a particular ecosystem with respect to mine site, plantation species, and climatic conditions, hence the restoration process of different sites around the world is not alike [41]. In the present investigation, herbs occupied ground cover with maximum density of  $790667 \pm 1232 \text{ ha}^{-1}$  followed by shrubs, trees and climbers in decreasing order. The regeneration density was highest with maximum number of short-lived herb species ( $8967 \pm 1265 \text{ ha}^{-1}$ ), followed by early successional shrubs ( $4792 \pm 675 \text{ ha}^{-1}$ ), trees ( $340 \pm 61 \text{ ha}^{-1}$ ) and climbers ( $40 \pm 7 \text{ ha}^{-1}$ ), which were

altogether absent at beginning of reclamation. Plant diversity, vegetation structure and ecological processes were analysed in the study as ecological succession measures. The present study reveals that the migration capacity of natural vegetation had been gradually improved by seed dispersals/ pollinating agents (Table 2). Our earlier reports also revealed the planted species gained thick crown canopy during the first decade of the restoration and became good shelter for several pollinators [42]. These results are in line with the earlier reclamation studies [43,44]. These studies also suggest that older reclaimed mine sites host a wide variety of the plant species from the neighbouring ecosystem [45]. Earlier eco-restoration studies also reported that the selection of species for restoration programme appears to influence regeneration capacity of the plant species [42].



Mining operations may eliminate many of animal species or entire taxonomic group of animals from an area. Most of the animals have strong association with vegetation structure (particular shape, size and patchiness). However when vegetation of the area is altered due to mining activities, animals of the area disappear as they cannot find food, shelter, predation or closely associated animal groups [45]. In any of newly formed ecosystems, animals play a significant role as “ecological engineers” in restoration programme as plant pollinators, seed dispersers and litter decomposers [46]. Our previous studies reported that at the time of the start of reclamation in the year 1988, there were few or no pollinators found at the site [42]. Moreover in the present study on 24 years old reclaimed mine site, we have analysed pollinator web with interaction of major groups of plants and pollinators, hence this study will provide an understanding of the linkage between plant and pollinator communities in ecorestoration programme. Reports of Ghazoul [47] and Carvalheiro et al. [48] also claims that pollinator richness increases with vegetation and decreases with distance from high-quality habitat [48].

In this study, we evaluated interaction of plant-pollinators, influence of floral distribution on the composition of pollinator communities. We have observed few key pollinator groups such as Coleoptera, Diptera, Lepidoptera, Hymenoptera, Thysanoptera, Chiroptera and Aves (Figure 5). It is well known that pollinator invasion is markedly influenced by site-specific, plant-specific and physical environment-specific variations [49]. The spatial distribution of floral resources can subsequently affect subsets of pollinators. In the present study, Hymenoptera is an important group of pollinators as it pollinates almost all the plant groups, followed by Lepidoptera, Aves and others. Bees (Hymenoptera) are ecologically diverse group, they play main role in pollination with different flight ranges of various forage habitats [50]. Butterflies (Lepidoptera) are specialised group, each species have particular needs with respect to forage source, climatic conditions and floral specification. Hence, the distribution of Lepidoptera is different from Hymenoptera [2,47,51]. Birds (Aves) visit a broad range of flowering plant species, interact with them for nectar and mean while facilitate the transportation of pollen among the vegetation [52]. Further, pollinator richness improves network stability and positive effects on network structure [53]. The study also helps in widening the knowledge base on taxonomic limits of species-specific approaches for the development of ecosystem structure and function.

Soil organisms are important in ecosystem function in the development of soil organic matter and subsequent nutrient cycling in

any restoration programme [54]. Soil organic matter is measured to be an important indicator for any of restored ecosystem. Present analysis after 24 years of restoration programme, produced an appreciably enhanced soil organic matter from 0.17% to 3.06%. It shows how the restored land can become carbon sink with adopted technology. Litter decomposition is an important biological process, which includes the interaction of vegetation structure, availability of soil nutrients, soil fauna and soil microbial populations [55,56]. Soil organic matter is directly proportional to type of soil organisms and their feeding habits. In the present study four different groups of litter decomposing organisms have been studied, i.e. microflora (bacteria, fungi, VAM), microfauna (Nematoda), mesofauna (Protura, Enchytraeidae, Acari, Collembola) and macrofauna (Diplura, Isopoda, Isoptera, Chilopoda, Diplopoda, Coleoptera, Earthworms). At initial stage of reclamation, the counts of soil microbes were very less number of colony forming units (CFU/g), however the reclamation process through microbe assisted green technology improved the quality of the soil in terms of microbial biomass (Table 3). In addition, soil microbial counts closely correlated with the changes in vegetation structure. Soil microbes help in mineralization of organic substrates form litter and release nutrients as a result of the heterotrophic activity [57]. Hence the microbial biomass is an important factor for maintaining soil quality, nutrient cycling and soil reclamation [58].

S. No.	Microorganisms	Initial stage of mine spoil	Top soil	Time period after reclamation			
				6 <sup>th</sup> year	12 <sup>th</sup> year	18 <sup>th</sup> year	24 <sup>th</sup> year
1	Actinomycetes (CFU/g)	0	4.5×10 <sup>4</sup>	8.1×10 <sup>2</sup>	2.1×10 <sup>4</sup>	8.5×10 <sup>5</sup>	9.5×10 <sup>5</sup>
2	Fungi (CFU/g)	0.1×10 <sup>1</sup>	5×10 <sup>2</sup>	3.8×10 <sup>2</sup>	5.6×10 <sup>3</sup>	9×10 <sup>4</sup>	6.2×10 <sup>5</sup>
3	Total bacteria (CFU/g)	0.6×10 <sup>1</sup>	1.9×10 <sup>4</sup>	7.9×10 <sup>4</sup>	1×10 <sup>7</sup>	5×10 <sup>9</sup>	8.6×10 <sup>9</sup>
4	Rhizobium (CFU/g)	0	5×10 <sup>1</sup>	5.4×10 <sup>2</sup>	2.2× 10 <sup>3</sup>	5×10 <sup>5</sup>	5×10 <sup>5</sup>
5	Azotobacter (CFUg <sup>-1</sup> )	0	2×10 <sup>1</sup>	7×10 <sup>2</sup>	3.6×10 <sup>5</sup>	4.1×10 <sup>7</sup>	4.0×10 <sup>7</sup>
6	VAM/10 g	0	9	12	28	48	51

**Table 3:** Microbial properties of 24 year old restored mine site at Gumgaon.

In any ecosystem the soil fauna ranges from nematodes to arthropoda, however arthropoda are among the most evident and diverse group of the fauna. Macro-arthropods (millipedes, centipedes, insect larvae, termites, ants and others) have the capacity to alter soil structure by minimizing bulk density, increasing soil pore size, rearrangement of the soil profiles and improving aggregate structure by ingestion and egestion mechanism [59]. Microarthropods (mites and collembolans) have the capacity to reduce litter mass and to

increase rates of nutrient release [60]. In the study, soil nematodes were highly frequent and dense among the soil animals, these organisms are smaller in sizes compared to the other biota, and hence the individual counts per m<sup>2</sup> were observed more. Shannon–Wiener index and Simpson index (diversity indices) of the soil fauna revealed that these faunal compositions were diversely distributed in the Gumgaon manganese post mine reclaimed site (Table 4).

Soil fauna	Frequency (%)	Density (individuals/m <sup>2</sup> )	Species richness (Simpson index)	Species diversity (Shannon–Wiener index)
<i>Nematoda</i>	64.13 ± 5.50	409.0 ± 51.2	0.42 ± 0.04	1.05 ± 0.21
<i>Acari</i>	10.84 ± 3.80	73.0 ± 12.3		
<i>Collembola</i>	9.38 ± 2.50	63.0 ± 5.1		

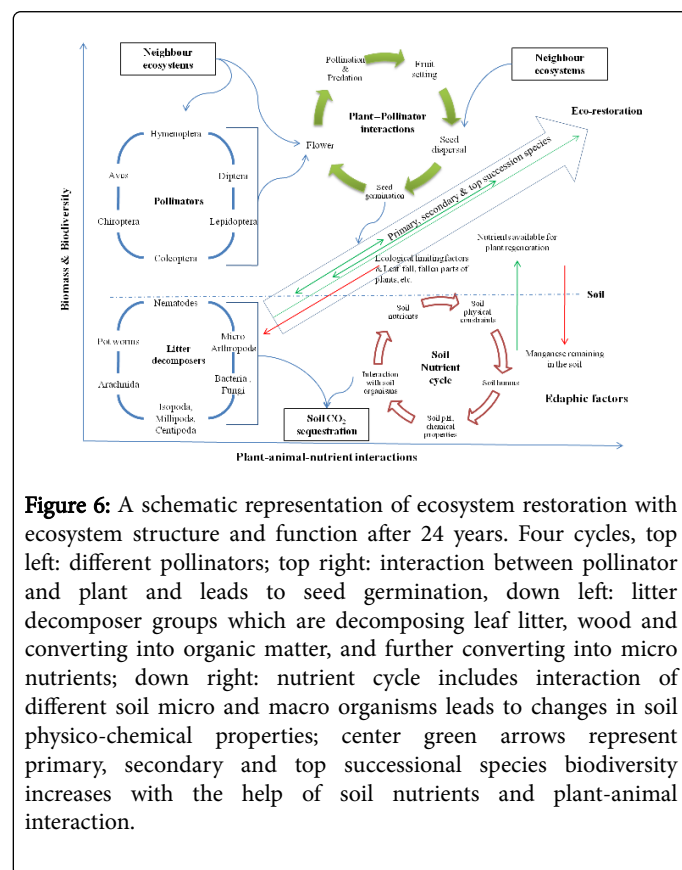
<i>Protura</i>	0.87 ± 0.08	5.0 ± 0.8
<i>Diplura</i>	0.43 ± 0.05	3.0 ± 0.5
<i>Enchytraeidae</i>	2.01 ± 0.10	14.0 ± 1.4
<i>Isoptera</i>	1.24 ± 0.09	7.0 ± 1.0
<i>Isopoda</i>	1.26 ± 0.11	7.0 ± 0.9
<i>Chilopoda</i>	0.17 ± 0.01	1.00 ± 0.01
<i>Diplopoda</i>	1.51 ± 0.05	9.00 ± 0.06
<i>Coleoptera</i>	2.82 ± 0.08	19.0 ± 1.2
<i>Earthworms</i>	5.36 ± 0.21	3.0 ± 0.6

**Table 4:** Soil faunal diversity in 24-year-old restored mine site.

In the present study, mine spoil was found to have very less organic carbon ( $1.8 \pm 0.1\%$ ). The lower SOC pools of mine spoil indicates that there will be large scope to enhance the rates of SOC sequestration with proper reclamation practices through improvement of vegetation and soil organic matter. After 24 years of reclamation programme, both organic matter (OM) and organic carbon (SOC) were increased. Akala and Lal [61,62] reported that mine soils can sequester carbon at a rate of  $47\text{--}79 \text{ Mg C ha}^{-1}$  over a period of 21- 25 years after mine reclamation. In the present study, soil carbon sequestration was initially increased from 5 to  $50 \text{ ton C ha}^{-1}$  ( $45 \text{ ton C ha}^{-1}$ ) within a period of 12 years (1988-2000). In the due course of time, the soil carbon sequestration capacity was increased at a slow rate from 50 to  $60 \text{ ton C ha}^{-1}$  ( $10 \text{ ton C ha}^{-1}$ ) over a period of later 12 years (2000-2012) of reclamation (Figure 4). Soil carbon sequestration takes place at a fast rate in the initial stages of any reclamation programme, whereas in mature ecosystems, the soil carbon sequestration rate may sometimes drop to 0 or maintained same [63]. Conversion of mine soils into atmospheric carbon sinks takes place through accumulation of above and below ground biomass via photosynthesis. In the Gumgaon manganese mine reclamation site, the vegetation gained height, higher DBH, thick canopy, dense crown cover to increased vegetation biomass and finally led to a carbon sequestration capacity of  $54 \text{ ton C ha}^{-1}$ . Based on the above- and below ground biomass conversions in temperate forests, there will be an estimated increment of carbon at  $4.1 \text{ Mg C ha}^{-1} \text{ yr}$  [64].

The present study further explains various stages of ecosystem development recognized in the restoration programme (Figure 6). At initial stage of programme, the environment was characterized by hostile ecological conditions with barren land. In the first stage of development, biomass of planted trees gained thick crown canopy and inoculated soil organisms were also multiplied. In the second stage, increased biomass of trees became good forage and shelter for many pollinators, hence immigration of animals also started. These pollinators helped to get increase the process of pollination within the habitat and seed dispersal also took place from neighbouring ecosystems to the reclamation site. Hence several herbs, shrubs, climbers and trees grew by natural selection. In the third stage of development, some of the primary successional plant species developed into climax community; leaf/litter fall, fallen branches became a part of humus with increased activity of the soil decomposers. In the fourth stage, soil organic matter was further

converted into soil nutrients and soil organic carbon, which could be available for nutrient cycling (Figure 6).



**Figure 6:** A schematic representation of ecosystem restoration with ecosystem structure and function after 24 years. Four cycles, top left: different pollinators; top right: interaction between pollinator and plant and leads to seed germination, down left: litter decomposer groups which are decomposing leaf litter, wood and converting into organic matter, and further converting into micro nutrients; down right: nutrient cycle includes interaction of different soil micro and macro organisms leads to changes in soil physico-chemical properties; center green arrows represent primary, secondary and top successional species biodiversity increases with the help of soil nutrients and plant-animal interaction.

## Conclusions and Implications

The overall aim of eco-restoration programme is to promote eco-friendly environment through ecosystem interactions, species diversity and improvement in soil nutrient cycling. Results of this study suggest that manganese mining sites can be recovered and converted into carbon sinks, if appropriate management strategies are adopted. Reclamation programme by plant-animal interaction is more likely to support and strengthen ecosystem resilience [2,48]. Revegetation



facilitated the development of microbial population, N-fixing bacteria and mycorrhizal association, which are fundamental for maintaining the soil quality by mediating the processes of organic matter turnover and nutrient cycling [65].

Results from this study indicated that plantation alone is not always sufficient, and should be supplemented with application of organic amendments indicating addition of top soil and soil organisms in any eco-restoration programme, which are the integral part of the present technology i.e. MAGT (Microbe assisted green technology). The MAGT probably provided an opportunity for successful growth of existing plant individual's species through enhanced seed germination, expansion of vegetation cover and improved soil biota like micro-fauna, meso-fauna and macro-fauna [42]. The study also confirmed that plant-animal interactions improved the ecosystem structure with respect to frequency, density and diversity of both the communities. The DBH, height and crown cover analysis confirmed that the biomass production was increased along with time. Successful restoration of floristic diversity in manganese mine spoil dump not only facilitated the natural process of speciation but also became a source of germplasm of various species. It improved the environmental conditions of the local area, including the economical aspects.

Reclamation must go beyond planting a new landscape by considering the land as an integrated system that functions above and below the ground. Ecological restoration provides an insight of the ecological processes and helps to understand many structural aspects and function of ecology viz., dominance, invasion, conservation, succession, competition, ecosystem dynamics and equilibrium, etc.

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