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Utilizing Three Different Biochars for Attenuation of Toxic Acidic Mine Spoils Reflected by Lixiviate Quality Vis-a-Vis Phyto-Toxicity on *Ocimum sanctum* and *Cassia angustifolia*

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ABSTRACT

Acidic mine rejects of pyretic sulphur coal mines were treated with biochar prepared from different feedstocks (distilled waste of *Cymbopogon flexuosus* (lemongrass) and *Cymbopogon winterianus* (Citronella) and the bark of *Eucalyptus citriodora*. The quality of lixiviate collected at three intervals (0, 15, and 30 days) from each treated and untreated mine waste was examined, and holy basil (*Ocimum sanctum*) and East Indian senna (*Cassia angustifolia*) bioassays were carried out. Results indicate that all three biochars improve the lixiviate quality, i.e., Enhanced the pH of lixiviate up to 3 units and reduces phytotoxicity. However, their magnitude of impact on lixiviates quality and phytotoxicity was different. The biochar prepared from the distilled waste of *Cymbopogon flexuosus* showed more promising results. The neutralizing capacity and dissolved organic carbon content were the significant factors for the discrimination of the amendments. The tolerance of *O. sanctum* and *C. angustifolia* was significantly different from each other for a particular treatment.

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1. Introduction

Coal mining activities have a significant negative impact on the environment, particularly on aquatic resources. Sulfide minerals, mainly pyrite and marcasite, present in coal and mine rejects are largely responsible for acid mine drainage (AMD) formation due to aqueous weathering [1]. The acidic mine spoils (mine rejects and overburdens) have high sulphur compounds and are strongly acidic in nature [2] which ultimately leads to metal leaching and phytotoxic effects on vegetation [3]. The areas affected by acidic mine spoils are characterized by scarcity of vegetation due to high acidity and metal content with low fertility. Revegetation in the acidic mine spoil affected area is the answer to this problem. However, the major limitations of the re-vegetation of these areas are the high acidic, compact texture of spoils and the presence of the toxic element.

Biochar recently has attracted tremendous research interest for the remediation of metal contaminated mine soils [4-6]. The capacities [7], liming properties, reduce fertilizer needs due to reducing of nutrient leaching loss [8], reducing N_2O emission from soil [9], diminishing disease incidence in crops [10], enhanced the availability of Ca, Mg, and Zn [11] and reduction in the bioavailability of toxic metals [12]. Biochars have a highly porous structure, contain various functional groups inserted, and have been shown to be effective in the adsorption of heavy metals (HMs), especially in aquatic systems [13, 14]. Due to its sorption characteristics, biochar can reduce mobility and phytotoxicity of certain organic (e.g. Phenols, polychlorinated biphenyls, trihalomethanes, pesticides and halogenated hydrocarbons) and inorganic pollutants in the soil and, therefore, able to eliminate the limitations of phytoremediation [15]. The availability of alkaline elements such as Ca, K, and Mg in the biochar can also neutralize the acid produced due to aqueous weathering of sulphide minerals as well as decreasing the exchangeable ions of aluminum and hydrogen by adsorption [16, 17]. Hence, biochar is considered as an HMs adsorbent as well as a promising agent for remediation of HMs polluted soil. It can play an important role to sequestration for HMs and neutralization of acidity of these spoils. Our recent studies on kinetic leaching of these mine rejects suggests that the biochar can be used to reduce the rate of acid production and alters the salt and carbon dissolution processes of mine rejects [18]. The acceleration in the alkali releasing rate was also observed after biochar addition.

The effectiveness of particular biochar for any application depends on its inherent propensities. Biochar with high recalcitrance could be used for carbon sequestration, while one with high alkaline elements acts as a good liming agent. Those with high cation exchange capacity (CEC), available nutrients, and high water holding capacity (WHC) could be better used as soil amendments to improve fertility. Feedstock with a different composition such as elemental composition; moisture content; and lignin, cellulose, and hemicellulose content affect the properties of the respective biochars after pyrolysis [19-21]. Biochars of the feedstock with the same category might show similar properties compared to those made from parent material of very different types. To optimize the benefits offered by biochar are to be maximized, it is important to develop an understanding of its physicochemical properties and their relations to functions in contaminated soil for a different range of biochar types [22].

The aims of this study were pre-screening of three biochars to identify their potential for the re-vegetation in the acidic mine spoil in the context of 1. potential neutralization of acidity, 2. the potential for leaching of HMs and nutrients, and 3. potential to reduce the eco-toxicological effects. Hence, the biochar prepared from the distilled waste of *Cymbopogon flexuosus* (lemongrass), *Cymbopogon winterianus (*Citronella) and the bark of *Eucalyptus citriodora* were used for the study. These biochars were varied in their pH, WHC, CEC, and bulk densities (BDs). The lixiviate water collected from mine rejects, and biochar amendments at the different intervals were evaluated for their physic-chemical properties. The phytotoxicity of the lixiviate was tested using seeds of *Tulsi (Ocimum sanctum)* and Sena (*Cassia angustifolia*). *O. sanctum* is an antioxidant herb, while *Cassia angustifolia* is a shrub for laxative use. These are the secondary agricultural crops and are industrially important for a significant source of income and could be a part of the strategy for land management in the future.

2. Experimental

2.1. Biochar Preparation

Biochars were prepared from *Cymbopogon flexuosus* (lemongrass),*Cymbopogon winterianus (*Citronella), and the bark of *Eucalyptus citriodora* (lemon-scented gum). The biomass samples were sieved for particle size of 0.211 mm and pyrolyzed in a programmed temperature fixed bed reactor (capacity: 300 g ± 60; tube diameter: 50 mm; sample bed: up to 300 mm) at 450◦C with a heating rate of 10◦C under inert conditions. Biochar physicochemical properties were determined through proximate, ultimate, and functional group chemistry analyses. Proximate analysis was done by ASTM method D3172. RSD [5] for replicates of moisture, volatile matter, and ash content were 0.36, 0.17, and 0.03, respectively. Total carbon, hydrogen, nitrogen, and sulphur were determined by Elemental Analyzer (Euro EA 3000 by Euro Vector)

2.2. Sample Collection and Preparation

Mine rejects (MRs) samples were collected from the coalfields of Jaintia Hills (Latitude: 25◦100 to 25◦280N; Longitude: 92◦080 and 92◦340E), Meghalaya, India. MR samples were obtained from the coal quality control laboratory, North East Institute of Science and Technology (NEIST), Jorhat, Assam. Samples were collected at the selected mine from a lateral and vertical representation of overburden materials. The composite samples of MR were obtained from the target seam of the mine area. The samples were ground to a mesh size of 72 BS (0.211 mm), preserved in desiccators, and used in all subsequent analyses.

Mine rejects were mixed with the biochar (40 g of biochar was added in 300 g of MR). The leaching experiments of pure MRs and MRs plus biochar were set as reported and optimized by [18]. All the experiments were performed in triplicates. The lixiviate water was collected separately from each experiment at different time points (0hr, 15days, and 30 days). At each time, 400 mL of deionized water was added and allowed to stand for a minute to saturate the sample column. The lixiviate collected were characterized and used for germination study following the protocol described by [23]. For statistical comparisons, the samples were divided into four groups based on lixiviate water from MR [treatments: T1 (0 days), T2 (15 days) and T3 (30 days)], MR + biochar from *C. flexuosus*, B_L [treatments: T4 (0 days), T5 (15 days) and T6 (30 days)], MR + biochar from *E. citriodora,* B_E [treatments: T7 (0 days), T8 (15 days) and T9 (30 days)] and MR + biochar from *C. winterianus,* B_C [treatments: T10 (0 days), T11 (15 days) and T12 (30 days)]. Statistical differences were confirmed by a two-sample t-test ($p= 0.05$) and by two-way repeated-measures ANOVA ($p= 0.05$) on transformations of the raw data.

2.3. Seed Germination, Root and Shoot Elongation

The incubation of seeds on filter paper soaked with lixiviate water was the method chosen due to the lower counter-effect of ions to particle interaction. The seeds of Holy basil *(Ocimum sanctum)*, East Indian senna (*Cassia angustifolia*) were assayed for germination mine rejects lixiviate with and without biochar. The effect of lixiviate water was studied on 20 seeds of each plant. The imbibitions of seeds were carried out by immersing them into 20 mL lixiviate in plant growth cabinets in the dark at 25°C for 4 h, the swelling period. Swollen seeds were then sown on Petri dishes with a double layer of filter paper (Whatman 1) wetted with 9 mL of solution in which seeds were immersed. The covers of the Petri dishes were closed and were kept at 25°C in the dark in growth cabinets for 5 days, determining the germination rate (GR) for every 24 h. A 1-mm radicle emergence from seeds was considered seed germination. The root and shoot lengths of germinated seeds were measured only at 5days of incubation. Our preliminary studies (unpublished) showed that this duration was required to determine the inhibitory effects of metals used. Each test was carried out in triplicate. The control group seeds were also treated the same way, using double distilled water (resistivity 18.0 MΩ) instead of lixiviates water**.** Inhibition percentage, germination energy (GR), and germination period were calculated using the formula cited by Czabator's index [24]

The seed germination energy (GE, %) = (number of germinating seeds/number of total seeds per treatment after germination for two days) ×100 was calculated according to the method described by Hu *et al*. [25]. Germination index (GI) was calculated following the method reported by Wang *et al*.[26].

2.4. Chemical and Metal Analyses

Carbon (C), hydrogen (H), and nitrogen (N) contents of the biochars were determined using a CHN Elemental Analyzer (Euro-Vector). Metals analysis (Ca, Co, Cr, Cu, Fe, K, Mg, Ni, Pb, and Zn) of lixiviate water and germinated seeds of Sena (metal accumulation) was done by inductively coupled plasma (ICP-OES, Perkin Elmer, Optima 5300 V) following the standard protocol (APHA, 2005). pH (biochar:water:: 1:2.5 ratio), Eh (biochar: water ratio::1:5), and total dissolved solids (TDS) of the lixiviate solutions at different time intervals were done by Mettler SevenGo DuoTMpH/Conductivity meter SG23. Sulfate ion concentrations were determined by standard gravimetric analysis. The forms of S in mine spoils were estimated by standard wet chemical methods [27].

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2.5. Chemometric Analysis

Chemometric techniques provide a combination of mathematical and statistical methods for handling and interpreting chemical data effectively. Correlation and Principal component analysis (PCA) and corresponding analysis are the methods frequently used for multivariate data analysis. In the present study, to assess the lixiviate quality and phytotoxicity, many factors such as MRs, biochar amendments, leaching time, neutralization, pH, toxic elements, micro, and macronutrients and two plant species were involved. Hence, correlation, principal component (PCA), and corresponding analyses were applied using IBM SPSS Statistics 21.0 software. The major goal of PCA is dimension reduction; it combines numerous variables involved in the seed germination process into a single or a few variables. Constant variables or highly correlating variables cause no problems for PCA [28]. For the determination of the effect of lixiviate water composition after three types of biochar amendments on seed germination of Holy basil *(O. sanctum)* and Sena (*C. angustifolia*), PCA was applied to the data. Corresponding analysis was used to assess the similarity/ dissimilarity between the different treatments on the basis of input parameters. All the data were subjected to ANOVA, and differences between means were determined using Tukey's test. Values in the text preceded by "±" indicate standard error (SE).

3. Results and Discussion

3.1. Physico-Chemical Characteristics of Biochars and Mine Reject (MR)

Biochars from the distilled waste of C. flexuosus (B_L), C. winterianus (B_C), and E. citriodora (B_E) were prepared and characterized (Table **1**). The examined biochars from the distilled waste of grasses (B_L and B_C) had lower total and organic carbon contents as compared to biochar from E. citriodora (B_E). These findings are similar to as reported by Zhao *et al*. [29] and opposite to that reported by Wang *et al*. [30], where wood-derived biochar had lower total carbon content. The organic carbon content was quite higher than reported for biochar prepared from Jatropha carcus [33]. The available phosphorus content in B_L was higher as compared to BC_E and B_C. All biochars exhibit ash content >10%. The biochars from grasses (B_L and B_C) have much higher ash content than B_E. This could be due to different inorganic elements of the original biomass as well as impurities and typically contains diverse species of carbonates, phosphates, silicates, and sulfates, as suggested by [31]. Wang *et al*. [30] also reported the lower ash content in wood-derived biochar as compared to straw residue due to low mineral content in the wood. No sulphur is detected in the biochar. All biochars were alkaline; the B_C (7.62±0.2) biochar had a lower pH as compared to B_L (10.5±0.5) and BC_E (9.2±0.6). Nanda *et al*. [32] reported that higher alkaline pH of prepared biochar due to its formation at elevated temperatures, which helps intensify the concentration of alkali metals, thus raising the biochar pH above 10. The CEC values of B_E, B_L, and B_C were 59.9± 0.54 meq kg^{-1}, 159± 1.61meq kg^{-1}, and 125 ± 1.4 meq kg^{-1}, respectively. Beside the low variations in the pH, the differences in the CEC values among the biochar were high. This may be explained by the fact that CEC is related to cations (e.g., K, Ca, Mg) present in biochar, which vary greatly with feedstock. A similar observation was also reported by Zhao *et al*. [29] with high variability in CEC values of biochar prepared from 12 waste biomasses (32.1 to 562 cmol/kg). The WHCs of prepared biochar materials were 50, 40, and 50% for B_E, B_L, and B_C, respectively. The water holding capacities of this biochar were similar to those reported for biochar prepared from coir peat (69%) and pine bark (45%) (Kaudal *et al*. 2016). However, WHC values of biochar understudies were lower than the biochar prepared from Jatropha carcus [33]. The bulk densities of these biochar materials were 0.29±0.002g cm^{-3} for B_E, 0.45± 0.001 g cm^{-3} for B_L, and 0.50 ± 0.002 g cm^{-3} for B_C. The bulk densities of these biochar were within the ranges as reported for biochar prepared from pine bark (0.31 g cm-3) [34], rice husk (0.27-0.37 g cm^{-3}) and maize cob (0.29-0.36 g cm^{-3}) [35]. All the prepared biochars have a high amount of Fe, Mg, Ni, Mn, Ca and Zn while Pb, Cr, Cu and Co were not detected.

Table 1: Physico-chemical properties of biochars (BC_L : biochar from C. flexuosus; BC_C: biochar from C. winterianus and BC_E: biochar from E. citriodora), mine rejects (MR) (Different letters within rows indicate significant differences at $p<0.05$)

Parameters	Unit	MR	BC_L	BC_C	BC_E
Ash	%	59.6+0.4	22±1.0	19±0.9	12±0.6
VM	%	-	29±0.8	27±0.5	33±0.9
M	%	-	3±0.2	5±0.1	5±0.2
C	%	29±0.9	51±1.1	59±1.2	76±1.4
H	%	3.6±0.1	3.6±0.1	4±0.1	8±0.4
N	%	1.2±0.1	1.2±0.1	1.8±0.1	1.4±0.1
S	%	3.3±0.7	ND*	ND	ND
Organic carbon	%	-	10±1.1	15±1.4	18±1.7
Total phosphorus	mg kg^{-1}	122±18	5520±123	2530±167	694±46
Available phosphorus	mg kg^{-1}	-	58±2.1	23±1.7	21±1.6
Ammoniacal nitrogen	mg kg^{-1}	-	99±9	108±12	78±5
pH		3.7±0.5	10.1±0.9	8.2±0.2	9.2±0.6
CEC	meq kg^{-1}	-	159± 1.61	125 ± 1.4	59.9 ± 1.54
Ca	mg kg^{-1}	122±11	52±7	52±4	68±5
Co	mg kg^{-1}	6.9±0.6	ND	ND	ND
Cr	mg kg^{-1}	7.3±0.5	ND	ND	ND
Cu	mg kg^{-1}	11.7±0.6	ND	ND	ND
Fe	mg kg^{-1}	17±0.3	3.7±0.8	1.4±0.3	1.2±0.5
Mg	mg kg^{-1}	130±13	824±29	818±32	433±45
Mn	mg kg^{-1}	83±4	80±3	56±2	66±3
Ni	mg kg^{-1}	30±1.6	6±0.6	5.5±0.2	6±0.3
Pb	mg kg^{-1}	7. 5±0.4	ND	ND	ND
Zn	mg kg^{-1}	8.2±0.5	21±1.4	58±1.6	68±1.6

*ND: Not detected.

The notable feature of MR was high sulphur content (3.3% sulphate) with high ash (59.6%) and carbon (29%) contents. A similar finding had been documented by [36] when a total of 18 coal cleaning reject samples from four regions (Lauro Müller, Treviso, Urusanga and Criciúma) in Santa Clara State and Brazil were analysed. It has a highly acidic pH (3.7). The concentrations of Ca, Fe, Cu, and Mn were higher in MR as compared to biochar, while Mg and Zn concentrations were higher in biochar as compared to MR.

3.2. Lixiviate Quality

The MR sample was subjected to leaching for different periods with and without the addition of biochar. Compositional parameters and the quality of lixiviates were determined over three different time periods (0, 15, and 30 days) (Table **2**). The pH values over different leaching time periods indicate that the lixiviates were highly acidic, showing an increase from 3.7 to 4.5 up to 15 days and then decreased to 4.1 after 30 days. The addition of biochar in MR promotes an increase in pH of the water; it exceeds the pH of lixiviate up to 2 to 3 units.

Table 2: Physico-chemical characteristic of lixiviate of different treatments (values are mean of three replications ±SE) (Different letters within rows indicate significant differences at $p<0.05$)

Variables	Treatments*											
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
pH	3.7±0.3	4.5±0.5	4.1±0.3	7.3±0.3	6.9±0.5	6.6±0.4	5.3±0.2	6.5±0.4	6.8±0.3	5.6±0.2	6.2±0.1	6.5±0.5
E_h (mV)	194±17.6	148±14.3	174±14.7	102±13.7	12±1.6	27±2.5	211±13.6	140±11.2	127±12.9	198±9.7	136±12.1	105±8.2
TDS (mg L^{-1})	799±54.8	88±32.1	67±24.1	3470±364	546±54	200±42	2310±231	97±51	80±24	2950±504	99±43	81±11
Ca (mg g^{-1})	103±13	23±4	32±5	430±14	44±8.5	46±2.3	364±12	13.5±10	18.7±6.1	312±19	5.5±0.3	5.9±0.4
Co (mg g^{-1})	1.49±0.4	0.22±0.02	0.08±0.01	0.19±0.1	0.07±0.01	0.04±0.01	0.33±0.06	0.02±0.01	0.08±0.01	0.28±0.05	0.03±0.01	0.06±0.02
Cr (mg g^{-1})	2.2±0.7	1.1±0.6	0.6±0.08	1.0±0.05	0.5±0.02	0.17±0.8	0.41±0.02	0.46±0.03	0.48±0.02	0.37±0.03	0.60±0.03	0.34±0.01
Cu (mg g^{-1})	3.3±1.2	0.2±0.4	0.3±0.7	0.3±1.2	0.1±0.9	0.2±0.6	0.64±0.05	0.35±0.06	0.19±0.04	0.29±0.03	0.15±0.02	0.10±0.02
Fe (mg g^{-1})	2085±136	16±4.8	20±2.9	12±2.1	12±3.2	83±5.5	13±2.5	53±3.1	40±2.8	10±1.1	30±1.6	11±0.9
K (mg g^{-1})	19±3.8	24±3.8	23±2.7	1295±145	216±13	102±12	1194±134	216±41	190±6	1171±161	270±25	210±14
Mg (mg g^{-1})	54±4.8	6±3.7	6±0.5	270±23	19±3.2	13±0.8	115±19	13±2	11±2	123±16	24±1	14±2
Ni (mg g^{-1})	1.7±0.8	0.6±.02	1.2±0.8	0.5±.01	0.4±.12	0.6±.15	1.1±.02	0.1±.01	0.08±.01	0.69±.01	0.1±.01	0.2±.01
Pb (mg g^{-1})	0.36±0.1	0.39±.14	0.41±.14	0.28±.05	0.27±.06	0.28±.08	0.31±.05	0.29±.07	0.28±.01	0.26±.05	0.18±.05	0.18±.01
Zn (mg g^{-1})	16.0±3.5	1.0±.44	1.9±.24	1.2±.09	1.0±.06	1.3±.04	1.9±.07	0.66±.01	1.8±037	1.21±.02	0.76±.02	1.11±.03
Sulphate (%)	0.30±.03	0.043±.02	0.031±.01	1.36±0.06	0.18±0.02	0.029±.01	0.76±0.06	0.14±0.03	0.15±0.01	0.98±0.01	0.19±0.01	0.16±0.01
Nitrate (mg g^{-1})	47±9.5	46.7±8.8	34.2±4.4	80.2±20.1	68.5±11.1	69.6±10.1	69±11	64±4.1	56±2.1	74.59±14.1	67.16±7.2	66.65±4.2
DOC (%)	18.1±2.8	0.1±0.98	9.1±1.7	11.3±4.5	19.5±4.8	12.1±3.2	21.4±2.2	24.3±2.3	15.3±1.2	18.7±2.1	28.6±2.4	14.3±2.0

*lixiviate water from MR [treatments: T1 (0 day), T2 (15 days) and T3 (30 days)], MR + biochar from *C.flexuosus*, B_L [treatments: T4 (0 day), T5 (15 days) and T6(30 days)], MR + biochar from *E. citriodora,* B_E [treatments: T7 (0 day), T8(15 days) and T9 (30 days)] and MR + biochar from *C. winterianu,* B_C [treatments: T10(0 day), T11(15 days) and T12 (30 days)].

Iron and alkaline elements (K, Ca, and Mg) are the dominating elements present in the MR lixiviate water. Martín *et al*. [37] reported that Fe and alkaline elements in MR are related to feldspar, oxyhydroxides, and hydrated sulfates (jarosite) of ferric iron, which is formed as the result of the oxidation of MR. The soluble micro-nutrients (Co, Cu, Ni, Zn) and toxic elements (Cr and Pb) were detected in the lixiviate water from MR and were higher in the initial flush (T1) (Table **2**). Among micro-nutrients, Zn concentration was more prevalent (1-16 μgmL^{-1}) followed by (0.3 to3.3 μgmL^{-1}), Ni (0.6 to 1.7 μgmL^{-1}) and Co (Not detectable, ND to 1.4 μgmL^{-1}). Among toxic elements, Cr (0.6 to 2.2 μgmL^{-1}) was more prevalent, followed by Pb (0.36 to 0.4 μgmL^{-1}). This pattern of leaching elements was similar to their concentration levels. This could be explained by MR-water interaction processes which firstly break down metal sulfides, then adsorb metals by the iron oxyhydroxide colloids and organic matter as suggested by [38]. Pb concentration was found to increase with the increment in the pH, while Cr concentration showed an opposite trend with pH. Lixiviate of biochar treatments had significant ($p<0.05$) reduced the concentration of Fe, alkaline elements (K, Ca and Mg), soluble micro-nutrients (Co, Ni, and Zn), and toxic elements

(Cr, Cu, and Pb) as compared to that of pure mine reject (T_1, T_2, and T_3). Also, the micro-nutrients and toxic element concentrations in lixiviate water of three biochar amendments were significantly different from each other ($p = 0.05$). At zero days, sulphate and nitrate concentrations in biochar amendments (T4, T7, and T10) were higher than mine rejects (T1). However, the reverse is true after 15 and 30 days. Similar data have been obtained in coal residues of abandoned mines of Santa Catarina state in Brazil [39]. Dissolved organic carbon (DOC) showed no trend with the time and biochar amendments. Σ DOC in 30 days was significantly ($p<0.05$) higher in the biochar amendments as compared to MR. Moreno-Barriga *et al*. (2017) concluded that biochars increased recalcitrant soil C to reclaim tailings mine residue at the Mining District of Cartagena-La Unión (SE Spain).

Results indicate that the lixiviate of MR was highly acidic, and biochar addition enhanced the pH towards the neutral. This confirms that biochar is an effective amendment for pH neutralization of MR, also observed by [40]. Furthermore, [41] reported that carbonate dissolution appears to be a key mechanism for acid neutralization in MR. The pH of MR lixiviate increases with the leaching time, though it remains acidic. The increase in pH of lixiviate of mine reject with leaching time was due to *in situ* neutralization. The presence of alkaline earth metals, nutrients, and toxic metals in the lixiviate of mine reject was due to the dissolution of minerals such as quartz, kaolinite, calcite, illite, sphalerite, and pyrite [1]. The possible reasons for this may be the dissolution of hosted minerals, solubility, and dilution effect. The solubility of some metals is strongly dependent on the pH dependents and decreased sharply with pH >6 [42]. This can be evidenced for Fe, Mg, Ni, Co, Cu, and Pb, which negatively correlate with pH.

Dissolve organic carbon (DOC) content in lixiviate has a vital contribution to the solubility of metal and other ions. The negative correlation between pH and DOC suggests that dissolved organic content in lixiviate is enhanced with an increase in its acidity. The acidic pH leads to the release of low molecular weight compounds from the coal fines present in the mine rejects. These compounds, in turn, formed soluble complexes with metals present in the mine rejects. They enhanced their dissolution by preventing the absorption of the solid phase. This theory could be applied for Fe, Mg, and Co as they showed a positive correlation with DOC. However, Ca, K, Zn, Cu, and Pb showing a negative correlation with DOC suggests that these elements formed stable complexes with organic matter, which adsorbed on solid phase and as an increase in the pH further reduced their solubility.

The positive correlation of Fe and SO_4^{2-} with each other and with Co, Ni, and Cr suggests the dissolution of their sulphides associated with pyrites minerals. In biochar amendments, the enhancement of the pH is consistent with the alkali nature of the biochar (Table **1**). Dissolution oxides, hydroxides, and carbonates (e.g., ash) of base cation (Ca, Mg, K and Na) present in the biochar act as a liming agent when applied to acidic mine soil [43]. However, the neutralization pattern in the lixiviate water collected in the biochar amendments was different. The B_L amendment showed a prevalent increase from an initial pH of 3.7 to 7.3 (at day 0), whereas B_C and B_E amended mine reject showed an increase in pH to a lesser extent (from3.7 to 5.6 and 5.3, respectively). The high pH and CEC of B_L could be the possible reason for its highest buffering capacity. However, the buffering capacity of B_C and B_E were similar despite the differences in their pH. This suggests that different phenomena are occurring during the leaching of different biochar amendments. Besides the pH, the CEC and organic carbon content of biochar could also be responsible for the release of the buffering elements such as Ca and Mg.

The correlation pattern of DOC and SO_4^{2-} with each other and alkaline metal was different in amendment of three different biochar. This demonstrates the difference in dissolution mechanism among the biochar treatments. In the biochar B_L and B_C, the pH increased due to more dissolution of neutralizing elements such as Ca, K, and Mg which are capable of buffering the acid produced. It was supported by the positive correlation of pH with the Ca, K, Mg, and SO_4^{2-}. While in B_E amendment, an increase in pH was due to lower production of acid and the neutralization elements. The negative correlation of pH with the Ca, K, Mg, and SO_4^{2-} support these phenomena.

The addition of biochar reduced the mineral and toxic metal content in lixiviate. The addition of biochar in the MR affects the concentration of individual elements in lixiviate water differently. The release pattern of mineral and toxic elements in the three biochar amendments was almost similar but had a significant ($p<0.05$) difference in magnitude. In biochar amendments, pH and dissolved organic carbon are crucial factors since they can significantly impact the solubility and distribution of ions in the soil solution. The increase in pH reduces the solubility of metal complexes. It may also be possible that at higher pH, precipitation of Fe and Al oxyhydroxide may also reduce the solubility of these metals. The decrease in the metal ion in biochar treatments may also be due to their sorption on the biochar surface. The adsorption of metal on particulate/biochar surface followed by the formation of stable complexes restricts their mobility in the lixiviates. On the other hand, metal-binding with DOC facilitates their release in the lixiviate [44]. Hence, this could be the reason for the variability in the mineral and toxic metal behavior in lixiviate. For example, the negative correlation of Zn with pH suggests that Zn concentration in lixiviate water was controlled by the dissolution-precipitation of oxide and hydroxide. This could also be the reason for the behavior of Co in lixiviate of B_L and B_C amendments. The positive correlation of Co with pH in B_E suggests that pH facilitates its mobility. The positive correlation of Pb in all treatments and Cu in B_E with DOC indicates they have an affinity to form the aqueous organo-metallic compounds and remain in the solution or absorbed in the particles. The electrostatic interaction and the precipitation on biochar surface might be the main Pb immobilization mechanism of biochar prepared at 500°C. Oxygen-containing functional groups on biochar surfaces may also be formed complexes with cationic Pb to some extent [45]. The negative correlation of Cu with DOC in B_L and B_C could be due to its distribution between the solid surface phase (adsorption) and the liquid solution.

Similarly, the correlations of Ni with pH and DOC were different in all treatments suggests the dominance of different processes in different treatments. Generally, Ni solubility decreases with increasing pH in inorganic systems due to the increased sorption of Ni(II) species on negatively charged mineral surfaces.

Also, Ni readily substitutes into the hematite present in the mine reject [46]. Hence, it is very unlikely that the Ni present in mine reject can be mobilized due to the presence of DOC in the solution.

3.4. Phytotoxicity

The seed germination could be considered a highly sensitive process during various stresses such as acidity and metal contamination, as in this period, seed coats become softer and more permeable to contaminants [47]. The present study indicates that 100% inhibitory effect of zero daylixiviate of mine reject (T1) on *O. sanctum* and 62.5% on *C. angustifolia* (Table **3**). However, inhibition of seeds of both the plants was significantly ($p<0.05$) lower in lixiviate collected at 15 and 30 days. Germination energy (GE), germination Index (GI), and germination rate (GR) of seeds were enhanced by increasing the time of lixiviate collection. Inhibition in *Ocimum sanctum* was highly reduced in T2 and T3 as compared to T1. The degree to which the lixiviate water quality inhibits germination differed with acidity, metal content, nutrient availability, and plant species. Results indicate that at high acidity (pH:3.7) and high Fe content, *C. angustifolia* appeared to be more tolerant as compared to *O. sanctum*. While at pH: 4.7 and lower Fe content, later one is more tolerant. Besides the highly acidic nature of mine reject lixiviate water, 0-75% and 38-50% germination occurred in *O. sanctum* and *C. angustifolia,* respectively. Probably, acidic conditions accelerated germination due to deterioration of the seed coat, which leads to hydration. Also, the germination process is very tolerant to acidification. The retardation of the growth rate of seedlings in mine reject lixiviate as compared to control (DW) was more prevalent to the most acidic conditions with the heavy metal load. However, seedlings were grown in more acidic (pH), and high heavy metals load were not healthy. They were yellowish in color due to the accumulation of high concentrations of iron hydroxide and sulphide. However, the seedling growth in MR lixiviates was improved gradually with the leaching time. This suggests that the metal tolerant capacity of these plants can improve in acidic mine sites with due course of time.

Biochar amendments reduced the inhibition of seed germination, which further decreases with the leaching time. This is attributed to the lower acidity, lower metal content, and higher nutrient availability. Among biochar amendments, inhibition is more in B_E treatments for *C. angustifolia*. The inhibition in *O. sanctum* was similar between all biochar treatments. However, inhibition in seed germination is lower in lixiviate collected at 30 days as compared to 0 and 15 days. The improvement in the germination rate after biochar addition might be due to the decline in acidity and metal concentration. Also, the carbon compounds released from biochar could act as humic-like supermolecular compounds. They can deliver bioactive molecules by adhering to seedlings. This enhanced bioactivity masks off the negative effect of the metal and favor coleoptiles growth [48]. This is corroborated with the correlation of roots and shoots lengths of *O. sanctum* and *C. angustifolia* with DOC of the lixiviate. The other priming effect of biochar addition was due to the enhanced water-soluble nitrogen content and nutrients such as P and K [49]. The good correlation of root and shoot lengths with Ca, K, Mg, and P in biochar treatments is validating this phenomenon. This is further supported by the strong negative correlation between the inhibition and potassium accumulation in the seedlings of C. *angustifolia*.

Table 3: Germination parameters of Sena and Holy basil grown in different leachate water (values are mean of three replications ±SE)

Treatments	Sena				Holy basil			
	GEº	**GI^{s}**	**GR^{ss}**	**Inhibition**	**GE**	**GI**	**GR**	**Inhibition**
	(%)							
Control	100	27.6±5.9	3.68±1.5	40±5.5	60±8.6	16.66±6.5	4.46±1.5	30±5.5
T1	-	1.3±0.34	1.05±0.18	62±6.5	-	1.13±0.5	1.08±0.35	100
T2	5±0.5	3.4±1.3	1.24±0.45	56±7.8	5±0.55	2.37±1.5	1.54±0.45	20±6.0
T3	20±5.5	9.3±2.7	1.58±0.34	50±3.4	20±5.0	7.07±1.5	2.92±0.95	15±4.9
T4	5±0.3	10.5±3.2	4.18±0.23	36±1.4	30±4.2	10.20±3.5	73.85±6.75	30±3.6
T5	10±1.5	8.7±2.2	2.79±0.32	25±4.6	35±4.8	11.03±4.5	3.77±1.45	10±3.2
T6	30±2.7	15.5±5.5	2.47±0.64	19±3.6	20±7.3	7.40±3.5	2.85±0.34	15±7.3
T7	5±0.5	1.43±0.08	0.6±0.1	41±3.1	10±0.9	3.33±0.3	1.0±0.1	29±2.1
T8	20±2.5	6.33±1.2	2.0±0.4	32±1.3	25±1.1	9.03±1.3	3.2±0.1	15±1.3
T9	15±2.3	4.97±0.9	1.8±0.3	29±1.5	15±1.2	6.03±1.1	2.2±0.1	11±0.8
T10	5±0.4	2.17±0.7	1.0±0.1	29±2.4	15±1.0	4.90±0.5	1.4±0.1	26±0.9
T11	15±1.5	6.03±1.6	2.2±0.2	21±1.1	20±1.3	6.20±0.7	2.2±0.2	13±0.3
T12	15±0.6	4.77±0.9	1.6±0.1	20±1.9	20±1.4	6.40±0.7	2.4±0.2	11±0.2

*Distilled water,ºGE: germination energy; ^{s}GI; germination index; ^{ss}GR: germination rate.

The variations in the shoot, root, and total elongation of seedlings (Figure **1**) are the first visual effect of any stress on the plant. The lixiviate water of mine rejects enhanced the elongation in root and reduced the shoot length of seedlings of *O. sanctum* as compared to distilled water. It has no effect on the shoot length of *C. angustifolia*, but reduced the root length *C. angustifolia* as compared to distilled water. The addition of biochar in mine reject further enhanced the elongation in root and shoot of seedlings as compared to mine reject and control (distilled water). The elongation is more pronounced in the lixiviate water obtained from mine reject with B$_L$ (T4, T5, and T6) for both the plants. The diverse effects of acidic lixiviate water on root and shoot elongation in both the plant are probably due to the different distribution of heavy metals, nutrients, and plant tolerance. The difference in inhibition in the root and shoot part of the plant resulted from a different distribution of heavy metals in plants. The elongation is more pronounced in the lixiviate water obtained from mine reject with B$_L$ (T4, T5, and T6) for both the plants. This could be attributed to high pH and high content of Ca, Mg, SO_4^{2-} and nitrate in its lixiviate.

3.5. Metal Accumulation

The metal and mineral content accumulated in the seedlings can be another measure to evaluate the plant tolerance in acidic mine environments and biochar amendments. The dry mass weight of germinated seeds of Ocimum was insufficient for the metal analysis; therefore, further studies on metal accumulation were done in only *C. angustifolia* (Table **4**). Concentrations of K, Mg and carbon in seedling of *C. angustifolia* showed almost similar in all treatments. However, significant ($p<0.05$) reduction was observed in the concentration of Ca (117-69 μgg^{-1} to 50 to 92μgg^{-1}) and Fe (1.93-5.15 μgg^{-1} to 0.92-3.04 μgg^{-1}) in seedling germinated in lixiviate of biochar amendments as compared to that of mine reject. Toxic elements such as Co, Cr, Pb, Ni, and Zn were significantly less ($p<0.05$) accumulated in seedlings grown in lixiviate of biochar amendments (T4-T12) as compared to that of mine reject (T1-T3). Cr and Pb were found significantly ($p<0.05$) higher in seeds grown in T1. In the case of Cu, no significant ($p<0.05$) variation was observed in its accumulation in seedlings grown T1, T4, T7, and T11 (lixiviate water collected at zero days). However, the accumulation of Cu was higher in T2 and T3 as compared to respective biochar amendments (T5, T6, T8, T9, T11, and T12). The highest concentration of Fe and Zn was observed in T1.

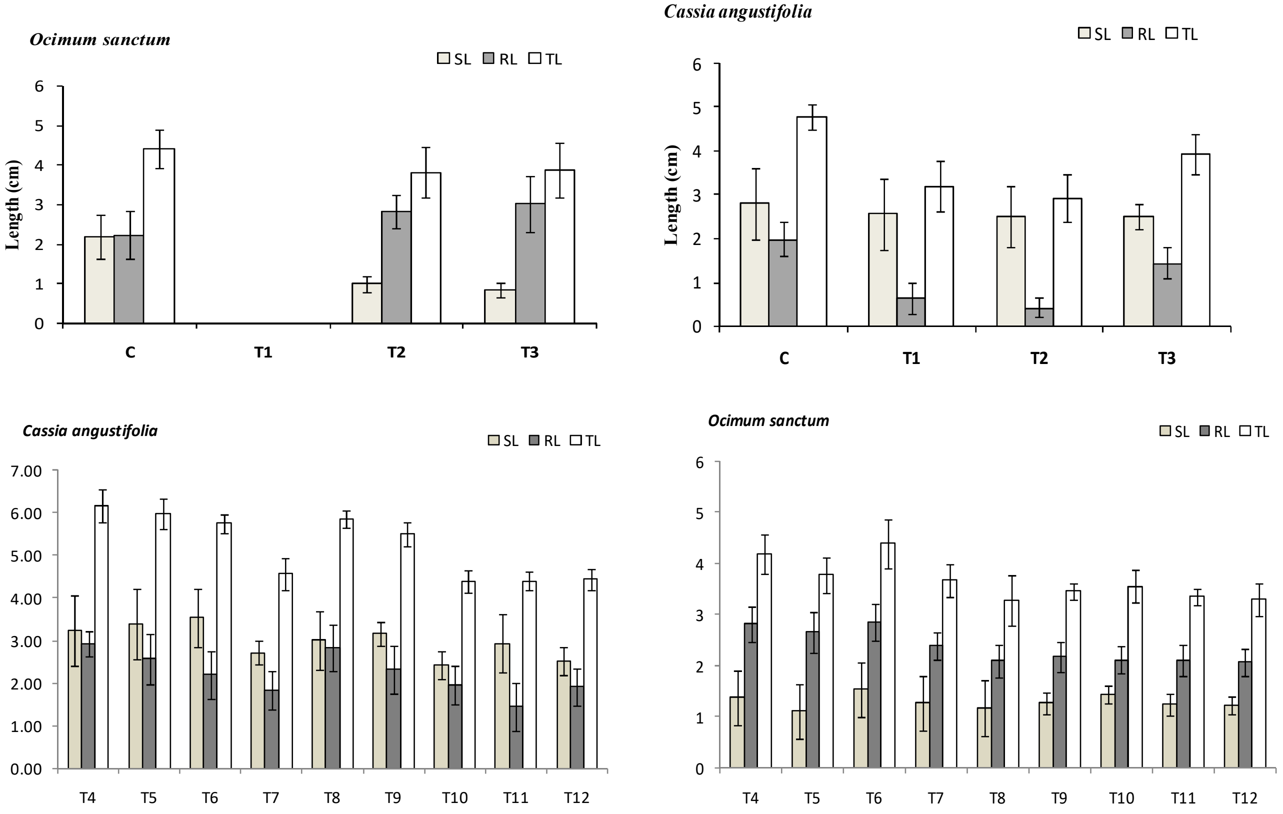

Figure 1: Root and shoot elongation of *O. sanctum* and *C. angustifolia* in different treatments (bars indicate mean of three replicates and the vertical bar indicates ± SE) [*SL: Shoot length; RL: Root length and TL: SL+RL].

Table 4: Accumulation of different metals as well as carbon and hydrogen contents (μg g^{-1}) in seedlings of Sena (values are mean of three replication ±SE)

	Ca	Co	Cr	Cu	Fe	K	Mg	Ni	Pb	Zn	C	H
T1	68.7±2.1	0.08±0.07	1.26±0.2	0.52±0.12	5.15±0.98	728.3±42.7	11.96±3.7	0.26±0.02	0.32±.01	2.82±0.9	42.8±4.9	6.53±1.5
T2	104.6±22..7	0.06±0.03	1.22±0.3	0.82±0.02	4.58±0.24	847.5±54.7	8.22±2.7	0.22±0.01	0.59±0.01	7.81±1.8	44.3±2.0	6.58±1.3
T3	117.6±25.1	0.05±0.02	0.92±0.12	0.63±0.07	1.93±0.32	743.3±36.7	12.88±5.7	0.17±0.03	0.33±0.04	11.12±2.9	50.0±3.9	7.42±3.2
T4	82.3±12.3	0.04±0.02	0.82±0.11	0.53±0.03	1.41±0.12	864.8±42.7	70.35±7.5	0.15±0.01	0.01±0.0	1.96±0.6	42.8±5.8	6.53±0.8
T5	45.3±24.4	0.07±0.03	0.55±0.11	0.46±0.05	0.94±0.77	792.0±63.7	13.72±3.6	0.01±0.0	0.14±0.02	1.80±0.2	44.2±3.9	6.58±1.4
T6	50.6±12.6	0.07±0.02	0.73±0.07	0.48±0.02	2.54±0.20	787.1±63.7	12.26±2.9	0.10±0.01	0.13±0.05	1.93±0.3	46.7±2.9	7.10±2.0
T7	92.1±10.3	0.09±0.01	1.02±0.10	0.43±0.03	2.01±0.42	894.8±35.1	89.45±3.1	0.16±0.01	0.11±0.01	2.19±0.8	45.8±5.8	6.03±1.1
T8	65.1±14.1	0.08±0.01	0.85±0.13	0.46±0.05	1.54±0.62	762.0±41.2	21.44±4.6	0.05±0.00	0.19±0.01	1.91±0.3	47.2±3.9	6.18±1.2
T9	59.1±12.7	0.08±0.02	0.73±0.14	0.41±0.02	3.04±0.60	757.1±43.2	18.12±1.7	0.11±0.01	0.13±0.02	1.90±0.2	49.3±2.1	6.28±2.1
T10	84.3±13.7	0.08±0.02	0.86±0.12	0.51±0.03	1.68±0.19	884.8±43.0	74.35±8.5	0.15±0.01	0.01±0.01	1.90±0.4	43.8±2.1	6.40±0.6
T11	55.3±14.2	0.07±0.01	0.55±0.11	0.47±0.05	1.02±0.87	751.0±31.2	15.72±2.2	0.03±0.01	0.13±0.01	1.88±0.1	44.2±1.3	6.52±1.2
T12	50.6±12.7	0.07±0.02	0.63±0.07	0.46±0.02	2.68±0.31	741.1±23.2	13.56±1.9	0.07±0.01	0.13±0.02	1.91±0.1	49.1±1.4	7.00±1.3

The correlation between metal accumulation and their impact on germination parameters gave a clear picture of phytotoxicity (Table **5**). The inhibition of *C. angustifolia* was correlated with Ca (r = 0.71), Ni (r = 0.86), Zn (r = 0.61), Cr (r = 0.92), Cu (r = 0.63), Pb (r = 0.71) and Fe (r = 0.66). Shoot length of *C. angustifolia* was correlated with Ca (r = -0.61) while the root length was correlated with Ni (r = -0.65), Cr (r = -0.64), Cu (r = -0.63) and Pb (r = -0.76). The negative correlation of root length with Ca, Cr, Cu, Fe, Ni, and Zn accumulated in the seed suggests that these elements were responsible for the reduction in the root. Shoot length had negatively correlated with Cr, Cu, Fe, Pb, and Zn indicating their inhibitory effect on its growth. The positive correlation of inhibition with Cr, Cu, Fe, Ni, Pb, and Zn indicates that these elements are responsible for the reduction in germination. Lower accumulation was observed in the seedling grown in the biochar amendments. Metal accumulation pattern demonstrates that the biochar has the potential to reduce the metal bioavailability in the AMD-affected areas.

Table 5: Results of principal component analysis for lixiviate water and germination parameters

	MR		**MR+BL**		**ME+BE**		**MR+BC**	
	P1	**P2**	**P1**	**P2**	**P1**	**P2**	**P1**	**P2**
pH	**0.803**	-0.595	**0.998**	0.002	**0.950**	0.050	0.382	**0.618**
TDS	**-0.996**	0.089	**0.914**	0.086	**0.842**	0.158	**0.906**	0.094
DOC	**-0.840**	0.542	0.117	**0.883**	0.142	**0.858**	0.249	**0.751**
SO4	**-0.997**	0.075	**0.918**	0.082	**0.826**	0.174	**0.921**	0.079
Fe	**-0.993**	0.116	**0.631**	0.369	**0.545**	0.455	0.062	**0.938**
Ger(S)	**0.841**	0.541	**0.953**	0.047	**0.728**	0.272	0.445	0.555
In(s)	**-0.917**	-0.398	**-0.998**	0.002	**-0.970**	0.030	**0.954**	0.046
Ger(O)	**0.771**	**0.637**	**0.860**	0.140	0.399	**0.601**	**0.984**	0.016
In(O)	**-0.998**	0.062	**-0.646**	0.354	**-0.960**	0.040	**0.963**	0.037
SL(S)	**-0.993**	0.114	**0.994**	0.006	**0.996**	0.004	0.121	**0.879**
RL(S)	0.418	**0.908**	**0.978**	0.022	0.327	**0.673**	0.076	**0.924**
SL(O)	**0.965**	-0.261	0.065	**0.935**	0.012	**0.988**	**0.926**	0.074
RL(O)	**0.998**	-0.055	0.004	**0.996**	**0.608**	0.392	0.555	0.445
Ca	**0.989**	0.145	**0.746**	0.254	**0.941**	0.059	**0.965**	0.035
K	0.501	**-0.865**	**0.890**	0.110	**0.859**	0.141	**0.936**	0.064
Mg	-0.220	**0.975**	**0.867**	0.133	**0.866**	0.134	**0.920**	0.080
Co	**-0.976**	-0.218	**0.852**	0.148	**0.837**	0.163	**0.903**	0.097
Ni	**-0.890**	-0.456	0.221	**0.779**	0.299	**0.701**	**0.633**	0.367
Zn	**0.957**	0.289	0.091	**0.909**	**0.859**	0.141	0.016	**0.984**
Cr	**-0.678**	**-0.735**	0.199	**0.801**	**1.000**	0.000	**0.710**	0.290
Cu	**0.704**	**-0.711**	**0.609**	0.391	0.088	**0.912**	**0.984**	0.016
Pb	**0.980**	-0.231	**0.800**	0.200	0.118	**0.882**	**0.903**	0.097
Variance (%)	61.000	31.000	68.000	29.000	60.000	32.000	65.000	30.000

Significant clustering of the variable is shown in bold.

3.6. Discrimination Among the Treatments Using Correspondence Analysis

The associations among the treatments and the interactions among the lixiviate water parameters and seed germination parameters were examined using correspondence analysis. Figure (**2a**) shows the correspondence analysis of the entire data set (treatments, lixiviate water compositions, and germination parameters of all the treatments). We found that the T_1 was not associated with the other treatments. T_1 is associated with the Fe content present in the lixiviate water. T_1 has a very low pH, high Fe content, and high mortality in the seed. Also, it grouped with the germination of *O. sanctum*. The lixiviate water collected from biochar treatments at zero hours (T_4, T_7, and T_{10}) was also segregated from other treatments and clustered with Mg, SO_4^{2-} and TDS and germination rate of *O. sanctum* (Figure **2**). The lixiviate collected at 15 and 30 days (T2, T3, T5, T6, T8, T9, T11, and T12) showed little distinction, clustering together on the plot along with the other physiochemical and germination parameters. Corresponding analysis showed a clear distinction among the initial lixiviate of MR, biochar type, and lixiviate collection time. The correspondence analysis clearly showed distinction of T1 from other treatments was probably

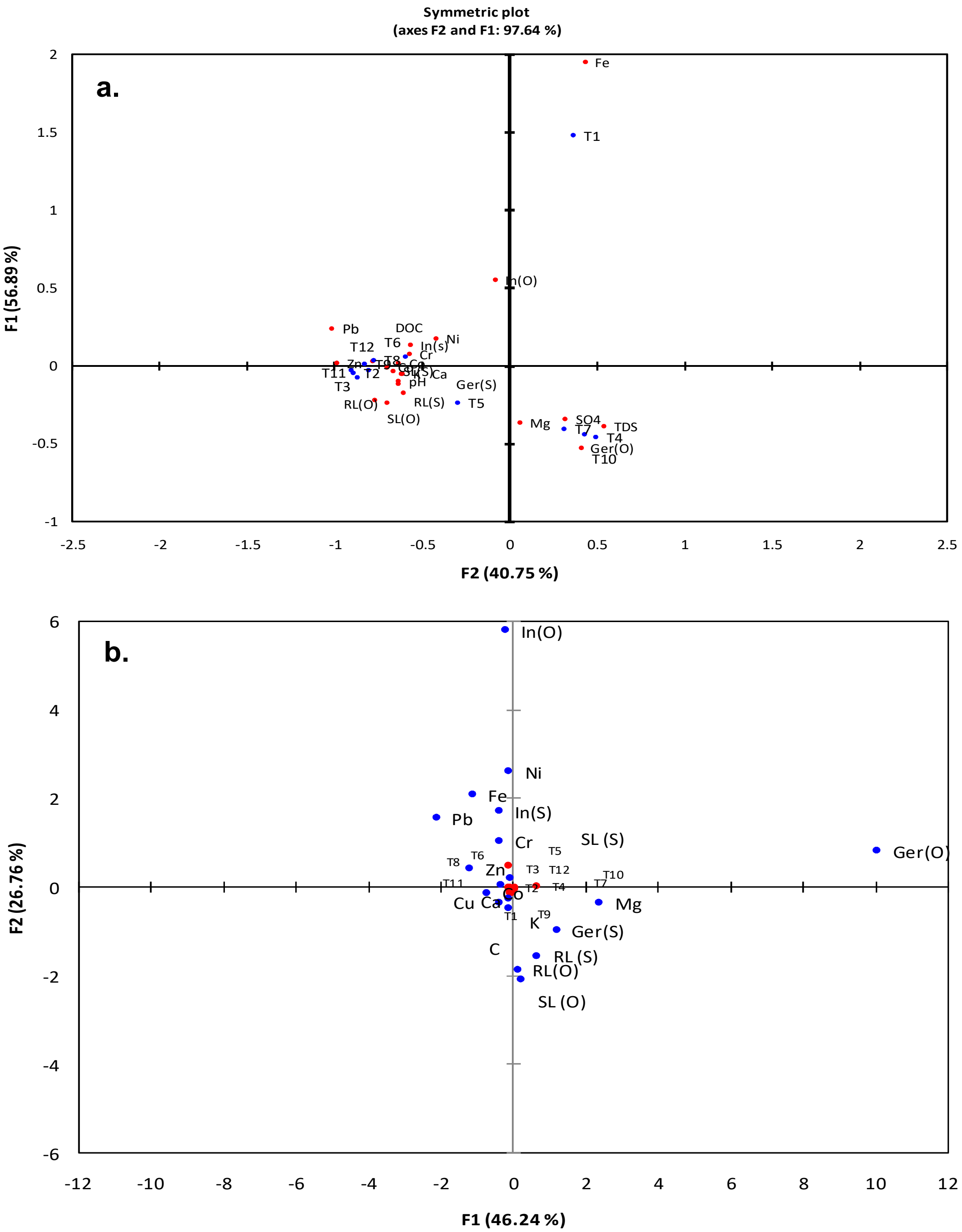

Figure 2: Correspondence analysis of a. lixiviate water quality and germination parameters b metal accumulated in *C. angustifolia* seedlings and germination. [Gen (O) and Gen (S): Germination of *O. sanctum* and *C. angustifolia* ; In (O) and In(S): inhibition of *O. sanctum* and *C. angustifolia;* RL and SL: root and shoot length of the respective plant].

due to highly acidic pH and high Fe content. The closeness of T1 with Fe is corroborating with releasing of the high amount of Fe at acidic pH. The segregation of T4, T7, and T10 (lixiviate collected at zero hrs from biochar amendments) was due to the high content of Mg, SO42- and TDS contents compared to other biochar treatments. However, lixiviates collected at 15 and 30 days were clustered together besides the difference in the pH and other components.

Figure (**2b**) shows the correspondence analysis of metal accumulated in the seedlings and germination parameters of all the treatments. All the treatments (T1 to T12) were clustered together suggests less variability in metal accumulation. However, four groups were formed by other parameters. Inhibition and germination of *O. sanctum* were segregated from the other parameters (Group 1 and Group 2). Inhibition of *C. angustifolia* was clustered with Fe and Ni (group 3), while germination of *C. angustifolia* and root and shoot length of both plant seedlings were clustered with the Ca, DOC, Ca, K and Mg.

3.7. Determination of the Contribution of Lixiviate Quality on Germination Using PCA

PCA analysis was performed separately for each set of treatments to evaluate the impact of lixiviate quality on phytotoxicity. Two groups concerned with lixiviate water composition were formed according to the PCA analysis in each case (Table **5** and Figure **3**). This illustrates that two factors can explain the relationship between germination parameters and lixiviate water composition. Variables that have loaded more than 0.6 are only considered to explain each factor.

For mine rejects, a large group of diverse data samples was explained in 61% and 31% as the first and second principal components (PC_1andPC_2) (Figure **3a**). Two components were extracted, describing approximately 92% of the common variance. Factor 1 is mostly associated with pH, TDS, DOC, SO_4^{2-}, Fe, Co, Ni, Zn, Cr, Cu, and Pb along with germination, inhibition, and shoot length of both the plant seedlings and root length of *O. sanctum*. It suggests that an increase in pH improves germination. Factors 2 is associated with the root length of *C. angustifolia* and germination of *O. sanctum* along with K, Mg, Cr, and Cu. The biplot of PC1 and PC2 demonstrates that the concentrations of pH, Pb, Cu, Ca, and Zn was associated in one group together with the shoot length and root length of *O. sanctum*, which indicates that the leaching behavior of these elements is dependent on pH and they influence the root and shoot elongation of *O. sanctum*. The correlation data also support this.

On the other hand, Ni, DOC, Co, Fe, TDS, and SO_4^{2-} were together along with inhibition of both plant seedlings and shoot length of *C. angustifolia* in another cluster and clearly differentiated from the first-mentioned cluster demonstrating relative similar dissolution behavior of Ni, Fe, and SO_4^{2-}. This is plausible because the mobility of Ni, Co, Fe, and SO_4^{2-} in lixiviate depends upon the formation of stable complexes with organic matter, as discussed previously. They are the major constituents of lixiviate and are responsible for the inhibition of seed germination. Mg along was clustered with germination of both plant and root length of *C. angustifolia*. This is reasonable because Mg is an important element for plant growth and is needed for the synthesis and function of adenosine triphosphate and nucleic acids [50].

InMR+B_L (Figure **3b**), factor 1 and factor 2 accounts for 68% and 29%. Factor 1 is associated with the pH, TDS, SO_4^{2-}, Fe, Ca, K, Mg, Co, Cu, and Pb along with germination and inhibition of both the plant seedlings, shoot, and root length of *C. angustifolia.* While factor 2 comprises the shoot and root length of *O. sanctum* along with DOC, Ni, Zn, and Cr. The bi-plot showed that the constituents of lixiviate behave differently with different controlling factors and impact on phytotoxicity. Zn, Ni, Cr, Cu, Ca, Mg, K, Ca, SO_4, TDS, and pH as well as root length, inhibition, and germination of both the plants were clustered together, while shoot length of *O. sanctum* was relatively close to this cluster. The DOC, Co, and Pb are separated from the first group demonstrating a different dissolution pattern. It demonstrates that the dissolution of the elements present in the first factor is related to the pH, while that in the second factor is controlled by the formation of the complexes with the organic molecules.

For MR-B_E (Figure **3c**), factor 1 and factor 2 accounts for 60% and 32%, respectively. Factor 1 is associated with pH, TDS, Fe, Ca, K, Mg, Co, Zn, and Cr along with inhibition of both plant seedlings, germination of *C. angustifolia*, root length of *C. angustifolia,* and shoot length of O. *sanctum*. Factors 2 are associated with germination and shoot length of *Ocimum sanctum* and root length of *C. angustifolia* along with DOC, Ni, Cu, and Pb. In bi-plot, the association of pH, Pb, Cu, Fe, and DOC together with germination of O. *sanctum* and *C. angustifolia* and shoot length of *C. angustifolia* in one group shows that the release of Pb, Cu, and Fe is relatively different for both MR-B_C and MR-B_E. This might mean that the properties of biochar seem to have an effect on the dynamics of these elements. It might be possible that an increase in pH likely increased negative charges on the soil surface, resulting in the higher affinity of the element to the biochar-amended soil surface [51, 52]. Also, they further altered the germination of the seeds. The separation of Cr, Ca, Mg, Co, K, Zn, Ni, and SO_4^{2-} in a different group indicates that similar dissolution processes control their lixiviate mobility.

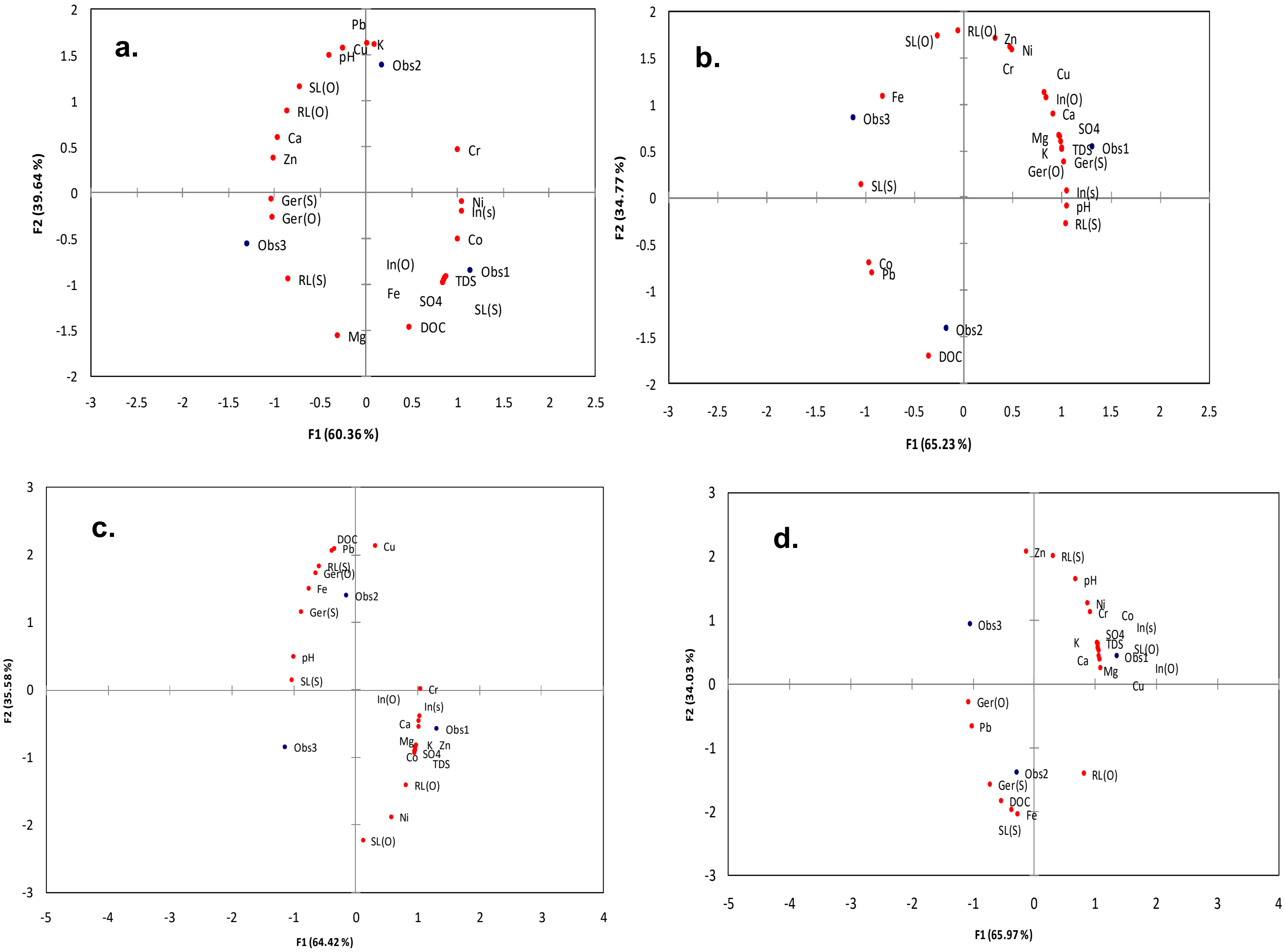

Figure 3: Biplot of PCA analysis of a. mine reject b mine reject with BL c. mine reject with BE d. mine reject with BC. [Gen(O) and Gen (S): Germination of *O. sanctum* and *C. angustifolia* ; In (O) and In(S): inhibition of *O. sanctum* and *C. angustifolia;* RL and SL: root and shoot length of the respective plant].

For MR+B_C (Figure **3d**), factor1 and factor 2 accounts for 65% and 30%, respectively. The association pattern on this is quite different. Factor 1 is associated with TDS, SO_4^{2-}, Ca, K, Mg, Co, Ni, Cr, Cu, and Pb along with inhibition of plant seedlings, germination, and shoot length of *O. sanctum*. Factors 2 are associated with the shoot and root length of *C. angustifolia* along with pH and DOC. In bi-plot, pH, Zn, Ni, Cr, Co, K, Ca, Mg, Cu, TDS, and SO_4^{2-} as well as inhibition of both plants and shoot length of *O. sanctum* and root length of *C. angustifolia* were clustered in one group. It demonstrates that pH is responsible for the dissolution of these elements here. The DOC, Pb, and Fe are separated from the first group along with germination of both plant and shoot length of *C. angustifolia*, while the shoot length of *O. sanctum* was relatively close to this group. Four groups illustrate that there are significant (p<0.05) differences between all four treatments. The inhibitory effect on the root and shoot elongation depended on a particular lixiviate and plant type component. For example, in B_L and B_E treatments, root and shoot elongation of *O. sanctum* was linked with DOC, Ni, and Zn. While, the root and shoot elongation of *C. angustifolia* in BC treatments was linked with DOC, Fe, and Zn.

4. Conclusion

We concluded that the biochar amendments showed the potential applicability for the remediation and revegetation of the acidic mine-affected areas. The biochar amendments improve the lixiviate quality, germination, and root elongation of *O. sanctum* and *C. angustifolia.* The behavior of each type of biochar was different and governed by the presence of neutralizing elements and dissolved organic content, which altered the distribution of toxic metals between the solid surface phase (adsorption) and the liquid solution. As time progressed, in-situ improvement in the lixiviate quality and phytotoxicity was also observed in mine reject. However, biochar accelerates the process. Also, biochar amendments can provide a solution to the leaching of acid and metal in the groundwater near the mine reject dumping sites.

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