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Fe(III) Reduction Potential in Rice Soil as Influenced by Microbial Communities Under Flooded Condition

Ritesh Pattnaik [1]*, A.K. Mishra [1] and B. Ramakrishnan [1, 2]

[1] Laboratory of Soil Microbiology, Division of Soil Science & Microbiology, Central Rice Research Institute, Cuttack, Odisha-753006, India
[2] Division of Microbiology, Indian Agricultural Research Institute, New Delhi-110012, India

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Abstract: In the present investigation, soil samples collected from different rice growing tracts were assessed for temporal changes in iron reduction and fractions of different iron pools under submergence condition. Results suggest that potential for Fe(III) reduction is largely due to biological means as the amounts of reduced or dissolved Fe(II) in the antibiotics-treated or steam sterilized soil samples were negligible compared to control. As the concentration of iron in a soil depends initially on the nature of its parent material, the crystalline forms of iron constituted about 67 to 89 %; non-crystalline forms of iron ranged from 11 to 33 % and microbially reducible iron ranged from 6-18 % of total chemically extractable iron. XRD analysis attributed the presence of quartz, muscovite, biotite, carbonates and fluorapatite in the analyzed samples. The population densities of iron reducing microorganisms were about 10^5 to 10^6 g^{-1} soil, as enumerated by the MPN, suggesting the addition of glucose and acetate extensively support iron reducers in soils under flooded condition.

Key words: Fe(III) reduction, rice soil, flooding, organic matter, microbially reducible iron.

Introduction

Being the fourth most abundant element in the earth's crust, reduction of ferric iron [Fe(III)] is considered as a dominant process within the redox sequence in various anaerobic systems [22]. In flooded rice soils, iron reduction contributes significantly to electron capture released from organic compounds [25]. Organic substrates of different origin can support microbial population and stimulate general microbial activity in soils. About 5-20 % of soil organic matter consists of carbohydrates [10], which are relatively low as compared to content of plant biomass, yet during decomposition can serve as one of the most vital carbon and energy sources for diverse microorganisms inhabiting soil. The formation of ferrous iron [Fe(II)] in a flooded soil is often associated largely with microbial functions, and moreover the addition of organic matter to it increases the iron reducing activity [2].

Extensive studies highlight that fractions of soil iron constitute mixtures of x-ray amorphous material such as goethite or hematite, of variable but low water solubility [9]. As kinetics of Fe(II) production in puddled soils follow a roughly asymptotic course [20], about 50 % of the free iron oxides in a soil may be reduced within a few weeks of submergence depending on temperature, organic matter content, and crystallinity of oxides [16]. The differences in reducibility among various iron oxides are attributed by differences in reactive surface area [21] and follow the order: amorphous iron > lepidocrocite > goethite > hematite. The wetland rice soils undergo periodic changes of oxic

*Corresponding author (Ritesh Pattnaik)
E-mail: < riteshpattnaik@gmail.com >

and anoxic conditions, thereby establishing as biogeochemical "hot spots" for iron cycling. Depending on the intensity of rice cropping, the nutrient mining from soils is often reported.

The main objective of the present study was to assess the microbial involvement during iron reduction in soil under flooded conditions and ferric reduction potential as influenced by addition of substrates, correlate fractions of different forms of iron in soil and the role of iron reducing microbial populations in Fe-cycling in soils under rice-rice cultivation.

Materials and Methods

Experimental site and soil sample

Soil samples after harvesting of crops were collected from different rice growing tracts (Table 1), were powdered with wooden mallet, sieved through 2 mm mesh, and then stored in plastic containers under room temperature before analysis.

Study of selected physico-chemical properties of soils

The physico-chemical characteristics of the soils, as presented in Table 2 were determined using the following methods: Soil pH was determined in 1:1.25 soil to water suspension using glass electrode pH meter [8]. The maximum water holding capacity (WHC) of soils was determined by the Keen-Raczkowski method [19]. Electrical conductivity (EC) was determined in supernatant of 1:1.25 soil to water suspension using conductivity bridge [8]. The organic carbon content of the soil samples was estimated by the Walkley-Black's wet-oxidation method [8]. All soil samples were analyzed for clay, silt, and sand fractions by employing Bouycous hydrometer method [19].

Determination of microbial role and reduced iron content in soil

The alluvial soil samples (50 g portions) from the experimental field plots were placed in Schott duran incubation bottles. The soil samples without steam-sterilization and antibiotic treatment served as control. In case of treatment for steam sterilization, soil samples were autoclaved for 30 min at 15 lb, consecutively for 3 days. For antibiotic treatment, both soil samples in incubation bottles were treated with chloramphenicol at 1mg g^{-1} soil. In all cases, deionized water at 1:1.25 soil to water ratio was used to simulate flooded conditions.

After mixing, soil samples were incubated at

Table 1. Soil samples collected from different rice growing tracts used in the experimental analysis

No.	Sample Index	Soil type	Collection site
1	(A-CRRI)	Alluvial	Central Rice Research Institute (CRRI), Cuttack, Odisha
2	(A-HZR)	Alluvial	Central Rainfed Upland Rice Research Station, Hazaribagh, Jharkhand
3	(A-GER)	Alluvial	Regional Rainfed Lowland Rice Research Station, Gerua, Bihar
4	(L-BBSR)	Laterite	Orissa University of Agriculture and Technology (OUAT), Bhubaneswar, Odisha
5	(L-HZR)	Laterite	Central Rainfed Upland Rice Research Station, Hazaribagh, Jharkhand
6	(L-HZR)	Laterite	Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu
7	(Zn-d-RAN)	Zinc-deficient	Regional Research Station, Ranital, Odisha
8	(S-CAN)	Alkaline	Central Soil Salinity Research Institute, Canning, West Bengal
9	(AS-KER)	Acid sulphate	Agricultural University, Vellayanikara, Kerela

Table 2. Physico-chemical properties of different soils

Soil	pH	EC (ds m^{-1})	Organic carbon (%)	Maximum water holding capacity (%)	Total N (%)	CEC [c mol (+) kg^{-1}]	Soil fraction (%)		
							Clay	Slit	Sand
Alluvial-CRRI (A-CRRI)	6.62	0.79	1.12	43.70	0.07	18.00	32.00	27.00	41.00
Alluvial-Hazaribagh (A-HZR)	5.78	0.85	1.01	42.78	0.06	17.56	33.00	29.00	38.00
Alluvial-Gerua (A-GER)	6.01	0.67	1.76	45.68	0.08	18.58	34.00	29.00	37.00
Laterite-Bhubaneswar (L-BBSR)	5.89	0.82	0.71	32.45	0.06	11.63	09.00	11.20	79.80
Laterite-Hazaribagh (L-HZR)	5.94	0.76	0.87	36.45	0.07	13.45	16.00	24.00	60.00
Laterite-Tamil Nadu (L-TN)	5.71	0.72	0.89	38.76	0.08	14.11	21.00	26.00	53.00
Zinc-deficient-Ranital (Zn-d-RAN)	5.81	0.12	0.60	32.51	0.08	10.25	05.20	10.00	84.80
Saline-Canning (S-CAN)	6.69	10.23	1.13	62.41	0.13	19.10	40.60	49.60	09.80
Acid sulphate-Kerala (AS-KER)	4.37	4.50	5.64	60.40	0.21	19.20	54.90	09.60	33.50

30 ±2°C. At a periodic interval of 5 days, the contents of triplicate soil incubation bottles were thoroughly mixed. The concentration of reduced iron [Fe(II)] in soil slurries, after using fixatives such as deionized water, 0.5 N HCl was analyzed colorimetrically using ferrozine reagent [13].

Estimation of reducible Fe (HCl - Fe)

The easily reducible-Fe fraction of soil was examined colorimeterically [12]. Approximately 0.1 ml of soil slurry sample was transferred to 5 ml of 0.5 M HCl in a glass test tube of known weight. At room temperature, a 0.1 ml sample of the extract was added to 5ml of ferrozine (0.1 %) in 50 mM of HEPES (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid) buffer at pH 7. The amount of Fe(II) was determined by measuring the A_{562} of the filtrate [24].

Determination of water soluble-Fe concentration

In order to quantify the dissolved-Fe concentration, the soil suspension was extracted with deionized water as described for HCl-Fe, and the Fe(II) contents in the extracts was recorded at 562 nm in a spectrophotometer (Spectronic $20D^{+}$, Spectronic Inc., USA).

Determination of amorphous, crystalline and total chemically extractable Fe in soil samples

The contents of total chemically extractable, amorphous and crystalline Fe in soil samples were quantified by absorption spectroscopy [4]. Soil sample (1g) was placed in a solution of 40 ml of 0.2 M ammonium oxalate (pH 3.0), agitated for 4 hours followed by centrifugation at 3400 rpm for 20 min. The supernatant was made to volume (100 ml) with deionized water. For estimating crystalline Fe, sample (1g portions) was agitated for 16 h in dark condition with 50 ml of 4:1 solution of 0.3 M $Na_3C_6H_5O_7.2H_2O$, 1M $NaHCO_3$ and supplemented with 0.8 g $Na_2S_2O_4$. The suspension was centrifuged at 3400 rpm for 20 min. The supernatant liquid was diluted using deionized water. For all samples, Fe was determined by atomic absorption spectrophotometry (Perkin Elmer, USA).

Determination of microbially reducible and total Fe in soils

The microbially reducible Fe fractions and total Fe of soils were estimated as described by Mandal [16] with some modifications. Briefly, soil sample (0.1 g portions of wet sediments) was transferred to 5 ml of 0.5 M HCl and of 0.25 M hydroxylamine hydrochloride in 0.25 M HCl separately in scintillation vials of known weight. The sediment and acid was mixed with gentle swirling for 30 sec and centrifuged at 3000 rpm and 0.1 ml of the aliquot was added to 5 ml of 0.1 % ferrozine (wt/v). The amount of Fe(II) was determined colorimetrically by measuring the absorbance at 562 nm. The amount of microbially reducible Fe was calculated as the difference between the Fe(II) measured in the hydroxylamine and HCl extractions.

Study of kinetics for iron reduction in different soils under flooded condition

Soil samples from different rice growing tracts was used to simulate flooded condition for quantitative determination of dissolved, reduced and amorphous [Fe(II)] iron content. Air-dried and sieved (2 mm) soil samples (in 50 g portions) were placed in 100 ml incubation bottles, mixed with sterile distilled water at 1:1.25 soil to water ratio. The incubation bottles were incubated anaerobically at room temperature 30 ± 2°C. Following an incubation interval of every 5 days, the contents of incubation bottles, in triplicates were mixed thoroughly and analyzed Fe(II) contents in the extracts was recorded at 562 nm in a spectrophotometer (Spectronic $20D^{+}$, Spectronic Inc., USA.

Microbial iron reduction potential of soils as influenced by the exogenous addition of carbon substrates

The soil samples (10 g portions) were mixed with glucose, acetate or lactate (on the basis of C at 4 mg C g^{-1} soil) and the soil slurry was prepared by adding 10 ml of distilled and sterilized water in a sterile 120 ml serum bottle. The bottles were then closed with sterile black rubber stoppers and incubated at 30°C. After 5 days of incubation, the concentration of Fe(II) was determined by the assays using ferrozine, 2,2'-dipyridyl and *O*-phenanthroline. All Concentrations are given in µmol g^{-1} dry soil.

Analysis of soil mineralogy by X-Ray diffraction

Soil sample representing alluvial and laterite property were dried, sieved (< 200 mesh), and ground in an agate mortar for XRD. The processed samples were packed in the circular cavity of holder and then, the XRD pattern was obtained on a P3 Siemens instrument, equipped with a diffracted-beam monochromator in the range of 3-40° 2θ. Mineral composition of soil samples was determined.

Estimation of population density of iron reducing bacterial isolates in soil

The population densities of ferric iron reducing bacteria was enumerated in soil samples using glucose (20 gl^{-1}), acetate (10 mM) and lactate (10 mM) as the carbon source. The medium was prepared under anaerobic conditions and comprised of following constituents (g l^{-1} deionized water) : Fe_2O_3 (1.0) , K_2HPO_4 (0.8), KCl (0.2), NH_4Cl (1.0), $MgCl_2$ (0.2), $CaCl_2$ (0.1), yeast extract (0.05), 1 % mixture of vitamins and trace minerals in solution. The medium was sterilized after adjusting the pH to 7.2. The MPN tubes was inoculated with appropriate 10-fold dilutions of soil samples and incubated under N_2 atmosphere for a period of 30 days. The presence or absence of ferric iron reducing bacterial population in each MPN tube was tested after injecting a 0.2 ml 0.1 % ferrozine reagent .The development of purple color was considered positive .The population densities of ferric iron reducing bacteria in the soil samples were calculated using MPN statistical table [1].

Fe(III) reduction potential of enriched cultures

Enrichment media using ferric oxide was prepared for cultivating ferric reducing microorganisms as describe earlier. The subculturing of iron reducing microorganisms was performed under strict anoxic conditions. At an interval of every 24 hrs, an aliquot of 500 µl bacterial suspension

was fixed in 4.5 ml of 0.5 M HCl. The Fe(II) iron content was estimated colorimetrically using ferrozine reagent.

Statistical analyses

All analyses were carried out on basis of three replicates. The data were analyzed statistically using analysis of variance (ANOVA) procedure. Duncan's new multiple range test (DMRT) was employed to assess the differences between the treatment means. The treatment effects were declared as significant at 5 % probability levels.

Result and discussion

Role of soil microorganisms in iron reduction process

To ascertain the involvement of microorganisms in iron reduction in soils, an experiment was carried out by monitoring different iron pools as influenced by steam-sterilization, and treatment with antibiotic. The concentration of Fe(II) in dissolved iron was less than 10 ìmol g^{-1} in the sampled soils (Fig. 1). The inhibitory effect of antibiotic application was generally higher than the effect of steam sterilization. The concentration of reduced iron [Fe(II)], as extracted by 0.5 N HCl, was 10-fold higher than concentration of Fe(II) in dissolved iron as attributed previously by Chao and Zhou [3]. Both steam sterilization and antibiotic application inhibited the production of reduced iron [Fe(II)]. However, the steam sterilization appeared to increase the extractability of Fe(II) in amorphous iron oxides, which might be due to the chemical changes occurred during autoclaving and partial effects of moist heating on microbial members. Lower amounts of reduced iron [Fe(II)] in the antibiotics treated as well as the steam sterilized soils compared to that of control soil indicate of iron reduction by biological means in soils as earlier discussed earlier by Hart and Brookes [6].

Relative fractions of reduced iron present in soil sample

For determining Fe(II) in the present study, the diverse extractants as well as the reagents were employed. Although the coefficient of variation for all assays was less than 20 % except that of 2,2'-dipyridyl assay, the differences in the concentrations Fe(II) among different soils were not so distinct as that of ferrozine as corroborated by Stookey [24]. Since the procedure using BPDS involved five extraction steps, the assays using ferrozine, 2,2'-dipyridyl or O-phenanthroline were preferred in the subsequent studies. Among the soils incubated for 5 days, the laterite soil of Hazaribagh (L-HZR) had the maximum Fe(II) as determined by the ferrozine assay, followed by Zinc-deficient soil (Zn-d-RAN) and saline soil (S-CAN) (Table 3). As compared to results obtained by Lovley and Phillips [14], determination of Fe(II) in soil slurries by 2,2'-dipyridyl or *O*-phenanthroline against ferrozine as highlighted by

Table 3. Concentration of reduced iron [Fe(II)] in different soils as measured by different methods

Soil	Reagents used in different methods for measuring Fe(II) concentration			
	Ferrozine [1]	BPDS [2]	Dipyridyl [3]	O-phenantroline [4]
Alluvial-CRRI (A-CRRI)	1.59^{e}	0.74^{de}	0.64^{c}	1.78^{ab}
Alluvial-Hazaribagh (A-HZR)	4.03^{c}	2.88^{b}	2.97^{a}	2.24^{a}
Alluvial-Gerua (A-GER)	3.14^{d}	1.79^{c}	1.79^{b}	2.19^{a}
Laterite-Bhubaneswar (L-BBSR)	2.14^{e}	0.48^{e}	1.23^{bc}	1.46^{b}
Laterite-Hazaribagh (L-HZR)	6.45^{a}	0.49^{e}	1.29^{bc}	2.17^{a}
Zinc-deficient-Ranital (Zn-d-RAN)	5.11^{b}	1.18^{cd}	1.48^{b}	1.75^{ab}
Saline-Canning (S-CAN)	5.04^{b}	5.31^{a}	2.47^{a}	1.23^{b}

In a column, means followed by a common letter are not significantly different at the 5 % level by DMRT (Duncan's Multiple Range Test)

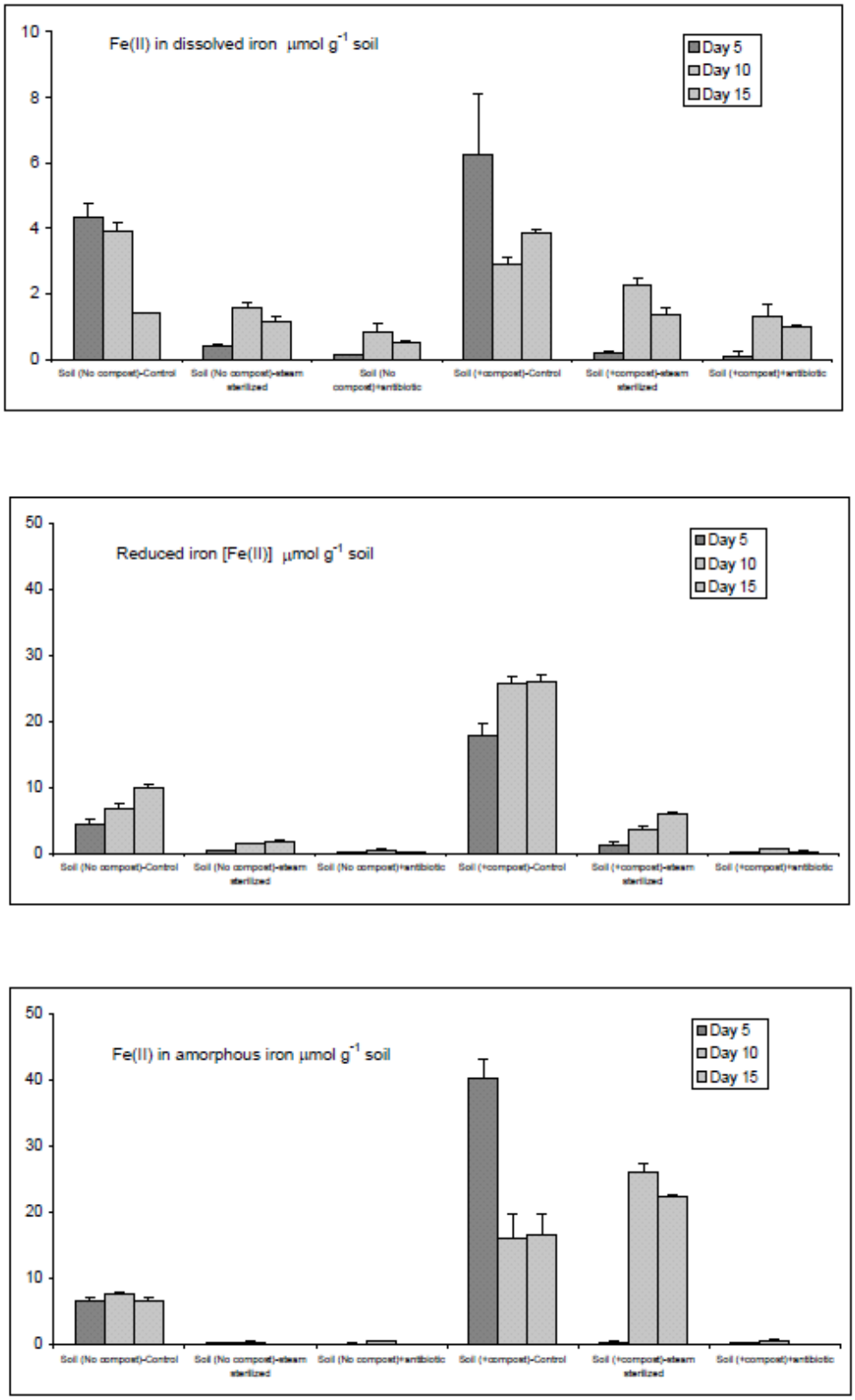

Figure 1. Concentrations of different extractable Fe(II) as influenced by antibiotic and steam sterilization treatments

Stookey [24] was probably interfered by other metal ions.

Microbial iron reduction in flooded condition

When the soils were flooded, Fe(II) in the dissolved iron increased by 15 days of incubation and thereafter declined (Table 4 & 5). Among the soils examined, higher concentrations of Fe(II) in the dissolved iron were observed with the alluvial soil of CRRI (A-CRRI) and the laterite soil of Hazaribagh (L-HZR). Generally, the Fe(II) concentrations in dissolved iron increased at slower rates, peaked around 15 days and declined thereafter in soils under flooded conditions. Similar results were obtained in laboratory incubation studies carried out by Patrick and Jugsujinda [18] confirming a significant drop in redox potential values as low as 10-fold aided by abiotic factors.

Influence of substrates on potential for iron reduction in soil sample

Typically, the soils collected from different regions within India contain about less than 2 % organic carbon, except the acid sulphate soil from Kerala which has about 5.64 %. Addition of carbon at 4 mg g^{-1} soil in terms of glucose, acetate and lactate led to increased iron reduction in most cases (Table 6 & 7). Interestingly, the microbial activities involved in the Fe(II) production due to the addition of glucose were higher compared to

Table 4. Concentration of Fe(II) in the dissolved iron in different soils under flooded condition

Soil	Days of incubation					
	5	10	15	20	25	30
Alluvial-CRRI (A-CRRI)	1.10^{a}	1.37^{a}	2.90^{a}	0.83^{a}	0.62^{a}	0.29^{a}
Alluvial-Hazaribagh (A-HZR-A)	0.30^{b}	0.58^{b}	1.53^{c}	0.77^{ab}	0.33^{a}	0.29^{a}
Alluvial-Gerua (A-GER)	0.21^{b}	0.28^{b}	0.31^{e}	0.32^{c}	0.29^{a}	0.31^{a}
Laterite-Bhubaneswar (L-BBSR)	0.12^{b}	0.24^{b}	0.94^{d}	0.67^{abc}	0.55^{a}	0.39^{a}
Laterite-Hazaribagh (L-HZR)	0.35^{b}	0.63^{b}	2.18^{b}	0.56^{abc}	0.42^{a}	0.41^{a}
Zinc-deficient-Ranital (Zn-d-RAN)	0.23^{b}	0.33^{b}	0.34^{e}	0.64^{abc}	0.35^{a}	0.30^{a}
Saline-Canning (S-CAN)	0.36^{b}	0.51^{b}	1.25^{cd}	0.35^{bc}	0.34^{a}	0.40^{a}

In a column, means followed by a common letter are not significantly different at the 5 % level by DMRT (Duncan's Multiple Range Test).

Table 5. Temporal changes in the concentration of reduced iron [Fe(II)] in different soils under flooded conditions

Soil	Days of incubation					
	5	10	15	20	25	30
Alluvial-CRRI (A-CRRI)	0.44^{c}	0.84^{b}	0.65^{d}	0.34c	0.14^{c}	0.15^{c}
Alluvial-Hazaribagh (A-HZR)	5.63^{a}	10.50^{a}	14.96^{b}	12.42^{b}	6.29^{b}	0.88^{c}
Alluvial-Gerua (A-GER)	3.35^{bc}	7.98^{a}	15.87^{b}	16.44^{b}	14.56^{a}	6.91^{b}
Laterite-Bhubaneswar (L-BBSR)	0.35^{c}	1.48^{b}	2.65^{d}	0.94^{c}	0.42^{c}	0.40^{c}
Laterite-Hazaribagh (L-HZR)	0.68^{c}	1.23^{b}	3.11^{d}	0.36^{c}	0.24^{c}	0.33^{a}
Zinc-deficient-Ranital (Zn-d-RAN)	6.18^{b}	11.05^{a}	21.49^{a}	8.29^{a}	3.58^{bc}	0.54^{c}
Saline-Canning (S-CAN)	0.38^{c}	2.91^{b}	7.35^{c}	23.64^{a}	17.78^{a}	13.84^{a}

In a column, means followed by a common letter are not significantly different at the 5 % level by DMRT (Duncan's Multiple Range Test)

Table 6. Iron reduction potential[1] of different soils as influenced by the exogenous addition of different substrates

Soil	Substrates used			
	Control	Glucose	Acetate	Lactate
Alluvial-CRRI (A-CRRI)	1.43^{b}	64.38^{a}	1.00^{bc}	2.84^{abc}
Alluvial-Hazaribagh (A-HZR)	5.72^{a}	51.78^{ab}	0.80^{bc}	4.18^{abc}
Alluvial-Gerua (A-GER)	4.67^{a}	45.79^{b}	1.79^{ab}	4.04^{abc}
Laterite-Bhubaneswar (L-BBSR)	0.46^{b}	49.57^{ab}	0.70^{bc}	5.28^{abc}
Laterite-Hazaribagh (L-HZR)	0.93^{b}	26.79^{c}	0.84^{bc}	2.64^{bc}
Laterite-Tamil Nadu (L-TN)	1.12^{b}	56.31^{ab}	0.69^{bc}	0.82^{c}
Zinc-deficient-Ranital (Zn-d-RAN)	1.39^{b}	51.23^{ab}	1.21^{abc}	4.44^{abc}
Saline-Canning (S-CAN)	1.80^{b}	15.37^{c}	2.22^{a}	7.23^{a}
Acid sulphate-Kerala (AS-KER)	0.46^{b}	49.41^{ab}	0.50^{c}	5.45^{ab}

[1] Reduced iron in the soil samples were estimated using 2,2'-dipyridyl

In a column, means followed by a common letter are not significantly different at the 5 % level by DMRT (Duncan's Multiple Range Test).

that of acetate or lactate. Among the soils examined using *O*-phenanthroline assay, the alluvial soil of CRRI (A-CRRI), laterite soil of Tamil Nadu (L-TN) and acid sulphate soil of Kerala (AS-KER) showed about 20-27 fold increases in Fe(II) concentration after amendment with different carbon substrates. The least response was observed with the laterite of Hazaribagh (L-HZR). But, the addition of acetate led to increases in the range of 0.7 to 6.6 fold only in these soils. Earlier studies by Lovley [11] suggest the involvement of heterotrophic or facultative anaerobic microorganisms in the reduction of iron. Besides, the decomposability of carbon substrates may be one of the determinants for iron reduction, in addition to the crystalline or amorphous status of iron minerals [2].

Composition of iron in different soils

The concentration of iron in a soil depends initially on the nature of its parent material. The total chemically extractable iron in these soils ranged from 144 to 427 µmol g^{-1} soil; the least concentration was observed with the saline soil from Canning (S-CAN) while highest amount was in the alluvial soil of Hazaribagh (AHZR). The crystalline forms of iron constituted about 67 to 89 % while the noncrystalline forms of iron ranged from 11 to 33 % of total chemically extractable iron (Table 8). The amounts of microbially reducible iron in these soils were only about 6 to 18 % of total chemically extractable iron. Reports by Lovley and Phillips [14] suggest probable fraction of lower content of microbially reducible iron in comparison to other fractions, as Fe(II) is measured as potential rate rather than in situ rate.

Presence of soil mineralogy by X-Ray diffraction

The data obtained through standardized procedures as highlighted by Kahle [15] can reasonably be interpreted to show the characteristic distribution pattern of minerals in soils. The alluvial soil of CRRI (A-CRRI) and (L-HZR) contains phases of quartz, muscovite, sodium aluminum silicate gallium and sanidine. The major phases contain quartz and sodium aluminum silicate gallium, followed by phases of muscovite and sanidine (Fig. 2 & 3).

Population dynamics of iron reducing microorganism in soil

Iron reducing microorganisms use Fe(III) as the terminal electron acceptor for the oxidation of organic compounds to carbon dioxide or other oxidized metabolites. These organisms conserve energy for growth, at least partially by electron transport phosphorylation [17]. The carbon sources for these organisms include acetate, lactate, sug-

Table 7. Iron reduction potential of different soils as influenced by the exogenous addition of different substrates under flooded conditions

Soil	Substrates used			
	Control	Glucose	Acetate	Lactate
Alluvial-CRRI (A-CRRI)	3.42^{d}(8.19^{c})	55.56^{a}(93.02^{a})	17.34^{bc}(28.67^{ab})	24.40^{b}(31.14^{b})
Alluvial-Hazaribagh (A-HZR)	8.85^{ab}(12.77^{ab})	62.68^{a}(92.73^{a})	19.97^{c}(20.37^{b})	25.94^{b}(32.01^{b})
Alluvial-Gerua (A-GER)	9.90^{a}(13.02^{a})	84.15^{a}(96.22^{a})	32.35^{a}(38.79^{a})	45.81^{a}(63.92^{b})
Laterite-Bhubaneswar (L-BBSR)	4.25^{cd}(8.71^{bc})	87.65^{a}(99.20^{a})	16.09^{bc}(32.79^{ab})	35.33^{b}(46.54^{b})
Laterite-Hazaribagh (L-HZR)	8.03^{ab}(11.69^{abc})	80.36^{a}(120.20^{a})	28.79^{abc}(33.16^{ab})	31.24^{b}(38.75^{b})
Laterite-Tamil Nadu (L-TN)	4.33^{cd}(17.29^{abc})	47.40^{a}(55.31^{a})	22.38^{abc}(26.97^{ab})	20.69^{b}(49.03^{b})
Zinc-deficient-Ranital (Zn-d-RAN)	8.28^{ab}(11.33^{abc})	91.60^{a}(104.74^{a})	26.58^{ab}(35.93^{a})	28.39^{b}(43.70^{b})
Saline-Canning (S-CAN)	6.94^{abc}(10.64^{abc})	56.28^{a}(98.22^{a})	22.23^{bc}(40.35^{ab})	31.91^{b}(58.37^{b})
Acid sulphate-Kerala (AS-KER)	5.62^{bcd}(15.64^{abc})	59.55^{a}(78.63^{a})	19.35^{bc}(31.28^{ab})	30.43^{b}(54.30^{b})

ars, amino acids, long-chain fatty acids, and aromatic compounds. To enumerate the population density of iron-reducing microorganisms in different soils, three substrates, i.e. glucose, acetate, and lactate, were used individually as sole carbon sources and ferric oxide served as a sole electron acceptor. The population densities of iron reducing microorganisms were about 10^5 to 10^6 g^{-1} soil, as enumerated by the MPN method and after an incubation of 30 days (Table 9). Among the different carbon sources used for enumeration, the use of glucose supported highest population in the alluvial soil of Hazaribagh (A-HZR) whereas acetate supported the highest in the alluvial soil of Gerua (A-GER).

Fe(III) reduction potential of enriched cultures

The enrichment cultures from different soils showed that their potential for iron reduction which changed with repeated culturing and varied among the soils used for isolation of iron reducing microorganisms. The enrichment cultures obtained after initial transfer of soils had a peak for iron reduction potential, which declined thereafter. The results obtained can be easily correlated to findings by Roden [23], summarizing the probable availability of immediate ferric iron for reduction, coupled to organic matter mineralization. Among all, cultures obtained from the alluvial soil of Gerua (A-GER), the zinc deficient soil of Ranital (Zn-d-RAN) and the saline soil of Canning (S-CAN) had higher potentials compared to others (Fig 4 & 5).

Conclusion

Though microbiology of iron reduction has been extensively investigated using the pure cultures isolated from the freshwater and marine ecosystems, yet little is known about the iron cycling in rice soils of India. In the present study, the speciation analysis constitutes an important approach to understand the complex chemistry and behavior of iron in soil and biological systems. Although there are several methods employed, use of ferrozine in acidic medium apparently considered as an effective process to quantify accumulation of Fe(II) over time. In paddy soil subjected to

Table 8. Concentration of crystalline, non-crystalline, microbially reducible- and total free iron in different soils

Soil	Concentration of different pools of iron				
	Crystalline	Non-crystalline	Total chemically extractable	Microbially reducible	Total Free
Alluvial-CRRI (A-CRRI)	125.7^{de}	42.2^{b}	167.9^{de}	13.1^{a}	14.5^{f}
Alluvial-Hazaribagh (A-HZR)	167.5^{c}	65.4^{ab}	427.0^{a}	26.6^{bc}	32.8^{cde}
Alluvial-Gerua (A-GER)	156.4^{cd}	37.9^{b}	194.3^{cde}	35.8^{cd}	44.9^{abc}
Laterite-Bhubaneswar (L-BBSR)	165.4c	45.4^{b}	210.8^{cd}	36.3^{cd}	46.3^{abc}
Laterite-Hazaribagh (L-HZR)	340.0^{a}	87.1^{a}	232.9^{c}	32.7^{cd}	38.2^{bcd}
Zinc-deficient-Ranital (Zn-d-RAN)	294.9^{b}	37.8^{b}	332.7^{b}	43.9^{d}	52.0^{ab}
Saline-Canning (S-CAN)	96.1^{e}	47.4^{b}	143.5^{e}	40.1^{d}	60.8^{a}

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT (Duncan's Multiple Range Test)

Table 9. Population density of iron reducing microorganisms in different soils

Soil	Iron reducing microbial population density		
	Glucose utilizing-	Acetate utilizing-	Lactate utilizing
Alluvial-CRRI (A-CRRI)	1.2	2.1	1.0
Alluvial-Hazaribagh (A-HZR)	6.9	2.4	1.2
Alluvial-Gerua (A-GER)	2.2	3.9	2.5
Laterite-Bhubaneswar (L-BBSR)	0.2	0.2	0.5
Laterite-Hazaribagh (L-HZR)	1.4	1.0	0.9
Zinc-deficient-Ranital (Zn-d-RAN)	2.9	2.0	1.7
Saline-Canning (S-CAN)	2.1	1.0	1.4

periodic puddling, the reduction of ferric iron is predominantly mediated biologically in comparison to chemical transformations. Additionally, potential of ferric reduction are influenced by substrates and complexity of the iron fractions, through active involvement of diverse group of heterotrophic group of microbes.

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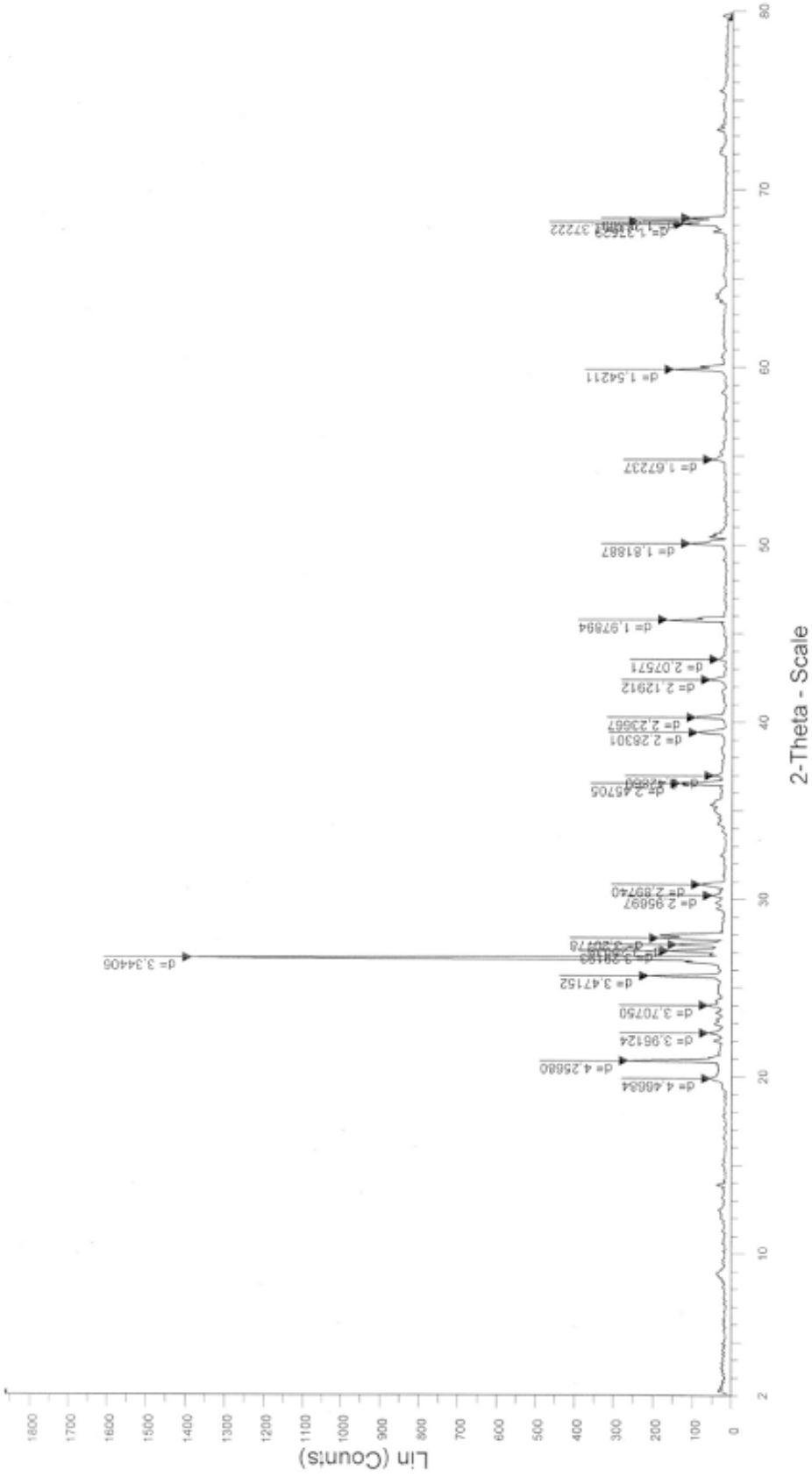

Figure 2. Diffractogram of alluvial soil from CRRI (A-CRRI)

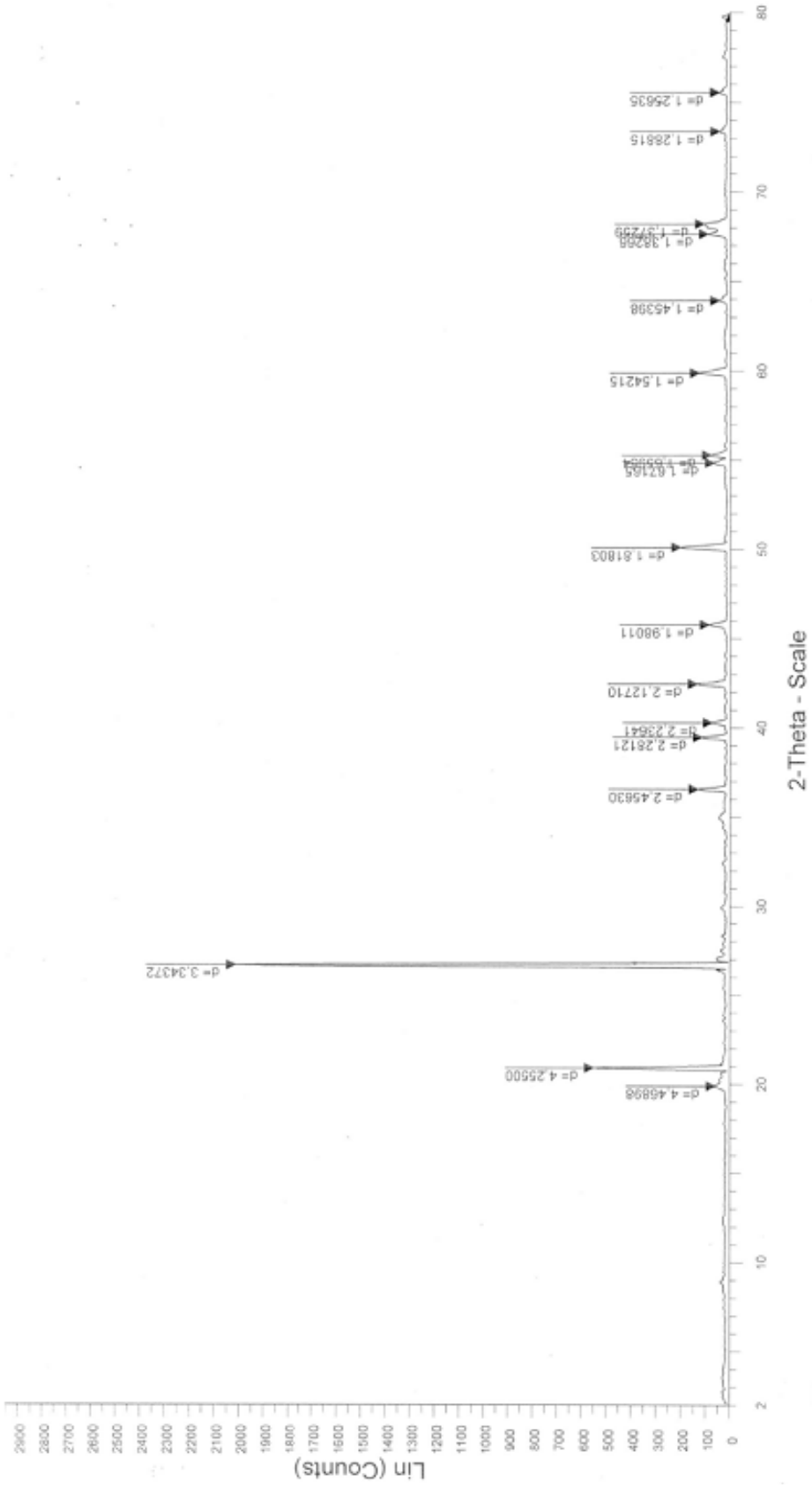

Figure 3. Diffractogram of laterite soil from Hazaribagh (L-HZR)

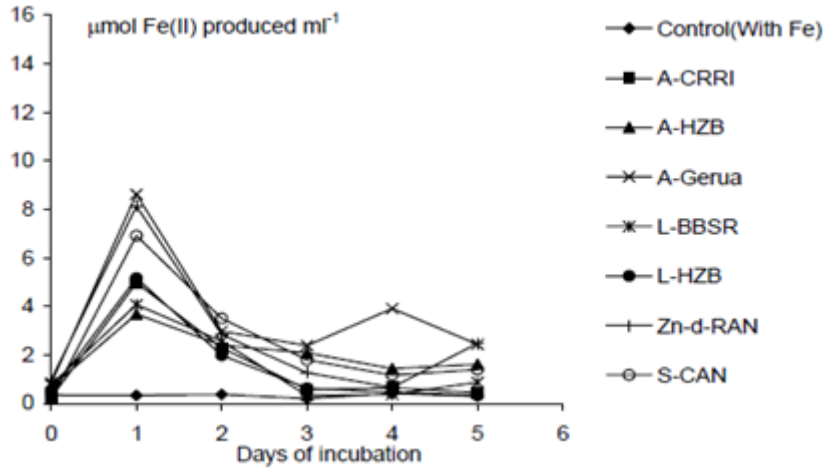

Figure 4. Fe(III) reduction potential of enrichment culture (I) obtained from different soils as source of inoculum

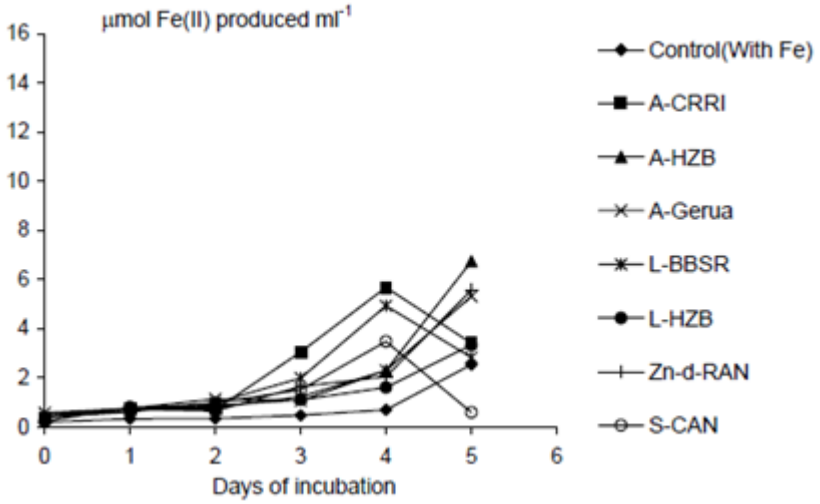

Figure 5. Fe(III) reduction potential of enrichment culture (II) obtained from different soils as source of inoculum

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