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Enrichment of vermicompost for improving soil quality and ensuring Zn and Fe bioavailability through rice grain

Sudip Sengupta, Siddhartha Mukherjee, Sanjay Halder and Parijat Bhattacharya

Abstract
Field experiment was conducted with rice (cv. IET-4786) with seven different combinations of regular and engineered vermicompost in Ghentugachhi village of Chakdah block, Nadia district, West Bengal, India (23°02'N latitude, 88°34'E longitude). Enriched vermicompost was prepared using Zn and Fe at three doses and two times of application. The application of these vermicomposted materials had been successful in creating a better soil environment for sustainability of crop production in terms of improvement in the water stable aggregates, water holding capacity, microbial biomass carbon and dehydrogenase activity of the soil. There was an increase in the rice grain yield (by 43.56%), as well as the content of zinc and iron accrued through the interventions. The antinutritional factor like phytic acid was curtailed resulting in lowering of phytic acid/Zn and phytic acid/Fe molar ratio, important from the bioavailability point of view, making the biofortification exercise for nutritional security a viable one.

Keywords: Enriched vermicompost, rice, bioavailability, nutritional security, soil quality, zinc and iron, phtytic acid

1. Introduction
The dietary dilemma of zinc and iron nutrition is profoundly influencing one third of the world's population (Saha *et al*., 2017; Bhattacharya, Sengupta and Halder, 2019) [60, 61, 62]. The prevalence is more accentuated in children under 5 years of age owing to a high demand to support growth and development (Wessells & Brown, 2012) [74]. and the deficiency culminates in mortality of about half a million annually (Krebs *et al*., 2014; Cakmak and Kutman, 2018) [40, 12]. The situation is far grave in the developing countries (Zhang *et al*., 2018) [78] like India where it has imposed massive health and economic burden, drop in financial productivity and hike in health care expenses which is impairing the poorer section of the society the basic amenities of sustenance (Sengupta *et al*., 2019) [61, 62].
Rice (*Oryza sativa* L.) is one of the major staple foods, contributing to half of the world population's dietary intake (Sunusi *et al*., 2019) [67]. It is grown in more than 100 countries, predominantly in Asia and contributes to about 21% of the global energy and 15% protein requirements (Maclean *et al*., 2002; Depar *et al*., 2011) [42, 17]. Rice productivity is often severely jeopardised by several abiotic hindrances of which Zn and Fe deficiency accrues primary significance (Rehman *et al*., 2012) [57].
Studies from scientific researchers have enunciated a strong positive relationship among Zn and Fe deficiency in human beings and its deficiency in soils, as the dietary pathways are jeopardized (Singh and Prasad, 2014) [64]. The coveted goal of converting subsistence to sustainable farming and deficiency in food grain production to its sufficiency (Sengupta and Dey, 2019) [61, 62] in the post green revolution era had the snag of imbalanced nutrition to the crops. The indiscriminate fertilization to augment the production hitherto had resulted large scale nutrient mining and robbed off the inherent fertility status of the soil (Majumdar *et al*., 2016) [43]. With every passing year, the soil fertility status evaluation in the country puts forward new cases of nutrient deficiency, especially the micronutrients, owing to their very narrow range of deficiency and toxicity.
The imbalance of fertilizer application mediated nutrient deficiency causes shifts in the composition of the soil microbial community (Eo and Park, 2016) [21]. Long-term imbalances in fertilizations can even adversely affect soil biological health (Bhatt *et al*., 2016). The biological component of the soil, living organisms and dead organic matter, is the major factor limiting fertility of dry-land soils. The use of diversified organic manure (Feng *et al*., 2017) [22] should thus be routinely practiced to improve the quality of the soil for better production, as the positive role of manure in soil health is well documented (Ahmed *et al*., 2017) [1].

Different studies have also proposed that vermicompost acts as miracle plant growth enhancer (Guerrero 2010; Rekha *et al*., 2018) [25, 59].

The application of vermicompost as source of nutrient as well as the application of inorganic fertilizers in the form of Zn and Fe as separate treatments has been widely practiced. However in many cases the bioavailability is hindered due to precipitation and/or sorption (Mortvedth *et al*., 1991) [48]. Enriching the vermicompost with Zn and Fe to avail the nutrients to the plants can be an option (Hashemimajd and Jamaati-e-Somarin, 2011) [28], although it has not been practiced in India widely. The estimate of the bioavailability of Zn and Fe through its content and interaction with phytic acid/phytate (Miller *et al*., 2007) [45] can eradicate a vital clog in human dietary needs and consumption.

It is in the aforesaid background that the present study has been initiated to assess the potentiality of designer vermicompost through its enrichment of Fe and Zn for augmenting the soil quality as well as increasing the Zn and Fe bioavailability in the grain and ensure nutritional gain to the human populations.

2. Materials and Methods

2.1. Site selection

The experiment to explore the efficiencies of preparation of enriched vermicompost and its ability to improve the soil quality and increase the Zn and Fe load in rice grain was conducted in the farmer's field for two consecutive years. The selected site for the study was located in the Ghentugachhi village of Chakdah block in Nadia district of West Bengal, India (23°02'N latitude, 88°34'E longitude).

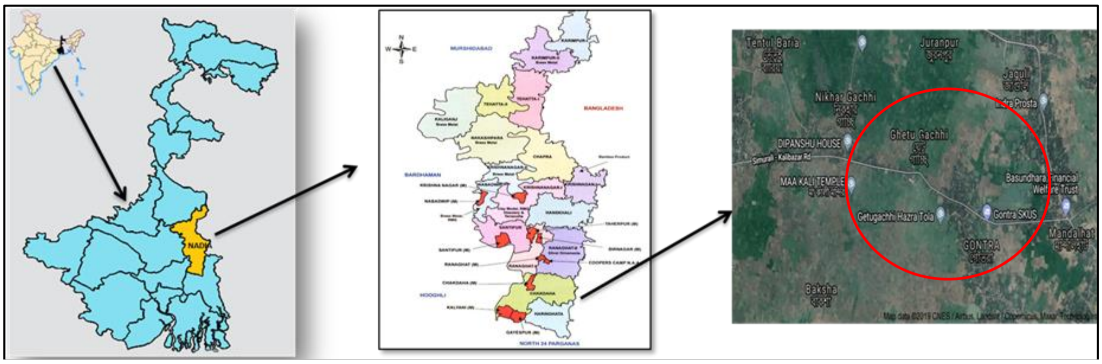

Fig 1: Location of Ghentugachi village in Chakdah block in Nadia district of West Bengal

2.2. Preparation of Zn and Fe enriched vermicompost

Fe and Zn enriched vermicompost was produced by fortification of pre-digested composting substrates with $FeSO_4$ (19% Fe) and $ZnSO_4$ (34% Zn) at 5%, 7.5% and 10% w/w; (Hashemimajd and Jamaati-e-Somarin, 2011) [28]. Normal vermicompost was produced with different residues, namely the plant residues (local farm residues), animal residues (cow-dung) etc. following standard production procedure in pits or trench. Thereafter, enrichment was carried out by applying requisite amount of $FeSO_4$ and $ZnSO_4$ with the pre-digested composting feed based on the hypothesis that Fe and Zn enriched compost will result in better complexation mediated slow release to avail nutrients without rendering them to be precipitated. $FeSO_4$ and $ZnSO_4$ have been incorporated to the mixture of composting substrates (cowdung:waste = 6:4) at two sets of time, one at the time of pre-decomposition (before vermiworm application) while the other set at the time of peak vermiworm multiplication. The Fe and Zn enriched materials was fed to surface dwelling earthworm *Eisinea foetida* in a cemented trench of 1 ft depth and appropriate length shaded with thatched roof for 3 months for 1st cycle and 35/45 days for 3rd cycle and onwards. Standard practices for watering, earthworm protection measures, harvesting and post-harvest processes have been followed.

2.3. Experimental details

The locally cultivated popular variety of rice (cv. IET-4786) was grown in a thrice replicated factorial randomized block design, where one factor was the different treatment combinations of vermicompost (seven levels) and the other factor was the dose of application of organic amendment (three levels). A control plot was employed where no vermicompost was applied and was used to compare the efficiency of vermicompost application. The recommended dose of fertilizer of the cultivated rice variety @ 130:65:65 kg/ha of N, P_2O_5 and K_2O was applied. Full dose of P and K and half amount of N were applied as basal and rest N in two splits at maximum tillering and panicle initiation stage. Standard water management practices were also employed. The details of the experimental details are furnished below in Table-1.

Table 1: Experimental details of the factorial design employed

Treatment	Administration
Factor-I: Vermicompost Treatment *(level- seven)*	
V_1	Regular vermicompost (no enrichment)
V_2	Zn and Fe sulphate @ 5% w/w dry weight basis of composting substrates applied before application of vermiworms
V_3	Zn and Fe sulphate @ 7.5% w/w dry weight basis of composting substrates applied before application of vermiworms
V_4	Zn and Fe sulphate @ 10% w/w dry weight basis of composting substrates applied before application of vermiworms
V_5	Zn and Fe sulphate @ 5% w/w dry weight basis of composting substrates applied at peak stage of vermiworm multiplication
V_6	Zn and Fe sulphate @ 7.5% w/w dry weight basis of composting substrates applied at peak stage of vermiworm multiplication
V_7	Zn and Fe sulphate @ 10% w/w dry weight basis of composting substrates applied at peak stage of vermiworm multiplication
Factor- II: Doses of Application (*level-three)*	
D_1	Application @ 3 t/ha
D_2	Application @ 2.25 t/ha
D_3	Application @ 1.5 t/ha

2.4. Sample collection and preparation

The initial soil samples from the experimental sites were collected, dried and stored. Standard analytical processes were adopted for physico-chemical characterization. Post-harvest (PH) soil samples were also collected, dried, sieved and stored for analysis. The same process was also adopted for the different vermicompost samples, they were digested, filtered and analysed for different nutrient contents. The soil quality parameters were assessed primarily by percent water stable aggregate (wet sieving method of Kemper and Rosenau, 1984) [37] water holding capacity (Baruah and Barthakur, 1997) [8] microbial biomass carbon (fumigation extraction method of Vance *et al*., 1987) [71] and dehydrogenase activity (by Colorimetric estimation of triphenyl- formazan at 485 nm; following Casida *et al*., 1964) [8, 71, 13].

The plant (rice) samples were collected at harvest, washed initially by tap water followed by dilute hydrochloric acid and finally with double distilled water. The samples were appropriately labeled, chopped, separated into root, shoot and grain, and dried in an air-oven at 105°C for 24 hours. The dried samples were ground and digested with a mixture of acids *i.e.* HNO_3, $HClO_4$ and H_2SO_4 in a proportion of 10:4:1 (v/v) (Jackson, 1973) [34] and filtered using Whatman No. 42 filter paper.

2.5. Instrumental analysis

For physicochemical characterization of the experimental soils and plants different instruments have been employed, like precision electronic weighing machine (sensitivity ≅ 0.001g; Mettler Toledo, Model-Pl 602S & AB 54-S); centrifuge (Remi; Model: R 23 and R 24); microprocessor based pH- EC-Ion meter (Eutech made); muffle furnace; semi-automatic nitrogen estimation system (Pelican made; Model: Supra-LX); micro-processor based UV-VIS Spectrophotometer (Varian; CARY-50); micro-processor based flame photometer (Systronics; Model-128).

The Zn and Fe content of the soils, as well as the Zn and Fe load in the rice grains harvested were determined through Atomic Absorption Spectrophotometer (Perkin Elmer PinAAcle 900F). The instrument parameters were as follows: Wavelength, 213.9 nm and 248.3 nm for Zn and Fe, respectively; sit width, 1.3 nm for Zn and 0.2 nm for Fe; lamp current, 5 mA for Zn and 12.5 mA for Fe; photomultiplier tube negative high voltage, 349 V for Zn and 435 V for Fe. The analysis conditions were as follows: Type of flame, air–C_2H_2; gas flow rate, 1.8 L min^{-1}; gas pressure, 160 kPa; burner height, 7.5 mm; delay time, 2 s and data collection time, 1 s.

2.6. Estimation of Zn and Fe bioavailability

Apart from the indigenous method of determining the Zn and Fe content of the rice grain for the determination of bioavailability (Phattarakul *et al*., 2012) [55] another approach was catered to assess the same.

A second method of assessing biofortification is by determination of phytic acid content (Wheeler and Ferrel, 1971) [76]. 0.5 g finely ground samples was reacted with 3% Trichloro acetic acid (TCA) and phytate was precipitated by adding 1.5 M $FeCl_3$ solution as Fe-phytate and its concentration in the solution was determined colorimetrically (Saha *et al*., 2017) [60]. Phytic acid (PA) / Zn and PA/Fe molar ratio of the harvested grains can provide an insight to the biofortification process and thereby the bioavailability. Decrease in the phytate content in grains can increase the bioavailability of the mineral micronutrient (Zhao and Shewry, 2010; Tyagi *et al*., 2018) [70]. Considering the molecular weight of phytic acid = 660 Da; Fe = 56 Da and Zn = 65 Da, the formula for calculation is as follows:

$$\text{PA/Zn molar ratio} = \frac{(\text{Phytic acid content in mg/kg})/660}{(\text{Zinc content in mg/kg})/65}$$

$$\text{PA/Fe molar ratio} = \frac{(\text{Phytic acid content in mg/kg})/660}{(\text{Iron content in mg/kg})/56}$$

2.7. Statistical analyses

Statistical computations employed in the study to assess the effect of enriched vermicompost mediated Zn and Fe nutrition to rice plant and human bioavailability through grain like Duncan's multiple range test, simple descriptive statistics, etc. were performed using Microsoft Excel 2016 and SPSS version 23.0 (SPSS, Inc.).

3. Results

3.1. Initial characterization of soils from experimental site

Physico-chemical characterization of the initial experimental soil sample was carried out through standard analytical approaches which have been reported in Table-3. The results indicate that the soil is neutral in reaction (6.94), has a low soluble salt content (EC- 0.35 dSm^{-1}), medium organic carbon content (0.55%), silty clay in texture with 48.5% clay, low in available nitrogen (258.03 $kgha^{-1}$) and available potassium (199.33 $kgha^{-1}$); while the available phosphorus content is high (33.46 $kgha^{-1}$). The DTPA extractable Zn (0.81 $mgkg^{-1}$), and Fe (5.10 $mgkg^{-1}$) are relatively on the lower side.

Table 3: Initial nutrient status of the experimental soil

Parameters	Methodologies	References	Value
pH	Soil-water suspension (1:2)	Datta *et al*., 1997 [16]	6.94
EC (dSm^{-1})	Soil-water suspension (1:2)	Jackson, 1973 [34]	0.35
Clay content (%)	Hydrometer method	Bouyoucos, 1962 [11]	48.5
Organic Carbon (%)	Wet oxidation method	Walkley and Black, 1934 [72]	0.55
Available N ($kgha^{-1}$)	Hot alkaline permanganate	Subbiah and Asija, 1956 [66]	258.03
Available P ($kgha^{-1}$)	0.5 M $NaHCO_3$ (pH 8.5)	Olsen and Sommers, 1982 [51]	33.46
Available K ($kgha^{-1}$)	Neutral normal ammonium acetate extraction	Knudsen, Peterson and Pratt, 1982 [39]	199.33
Available Zn ($mgkg^{-1}$)	DTPA extraction (pH 7.3)	Lindsay and Norvell, 1978 [41]	0.81
Available Fe ($mgkg^{-1}$)	DTPA extraction (pH 7.3)	Lindsay and Norvell, 1978 [41]	5.10

3.2. Chemical characterization of vermicompost samples

The regular as well as the enriched vermicompost prepared in the trenches were chemically analysed for assessing their nutrient supplying capacity and reported in Table-4. The total carbon was estimated by the wet oxidation method (Tiessen and Moir, 1993) [69] total nitrogen by digesting with concentrated sulphuric acid followed by distillation (Jackson, 1973) [34] C:N ratio was determined from the total C and N values; total phosphorus and potassium by HNO_3:$HClO_4$:H_2SO_4:: 10:4:1 (v/v) digestion (Jackson, 1973); while the total zinc and iron was determined by HNO_3:$HClO_4$:: 10:4 (v/v) digestion following the standard procedures as illustrated by Jackson (1967) [34, 35].

Table-4: Chemical parameters of the different vermicompost samples

Vermi-compost	Total C (%)	Total N (%)	C:N ratio	Total P (%)	Total K (%)	Total Zn ($mgkg^{-1}$)	Total Fe ($mgkg^{-1}$)
V_1	21.158	1.142	18.53	0.969	0.689	44.90	820.12
V_2	25.737	1.240	20.75	0.988	0.788	175.55	1335.22
V_3	27.711	1.394	19.88	1.004	0.948	216.50	1463.49
V_4	28.579	1.583	18.05	1.146	1.052	235.95	1628.12
V_5	24.316	1.212	20.06	0.990	0.797	177.00	1372.59
V_6	25.974	1.422	18.27	1.086	0.944	226.30	1529.14
V_7	26.914	1.415	19.02	1.127	0.962	232.34	1582.49

3.3. Effect of enriched vermicompost on augmenting soil quality

The soil health and quality parameters (in the form of percent water stable aggregate, percent water holding capacity, microbial biomass carbon and the dehydrogenase activity) as obtained from the post-harvest (PH) soil of the pooled data of the two years suggest that the application of vermicompost as a source of carbon can augment all the parameters over control (Table- 5). However, there was no significant difference in the parameters when different treatment combinations of enriched vermicompost were taken into consideration. The higher dose of application of the vermicomposts had an edge in establishing better soil quality and health.

Table 5: Variation in the soil quality parameters under applied treatments

Treatment	Water Stable Aggregate (%)	Water holding capacity (%)	Microbial biomass carbon ($\mu g\ g^{-1}$ soil)	Dehydrogenase activity (μg TPF g^{-1} soil 24 h^{-1})
Control	49.54^{c}	32.16^{d}	47.52^{d}	6.36^{c}
V_1D_1	58.65^{ab}	39.52^{bc}	158.48^{b}	9.65^{b}
V_2D_1	60.36^{a}	36.41^{c}	142.56^{b}	10.23^{ab}
V_3D_1	61.68^{a}	34.56^{c}	106.92^{bc}	10.65^{ab}
V_4D_1	55.73^{b}	45.19^{a}	229.68^{a}	11.12^{a}
V_5D_1	59.64^{ab}	43.31^{ab}	194.04^{ab}	10.54^{ab}
V_6D_1	56.83^{b}	40.81^{b}	170.28^{ab}	10.62^{ab}
V_7D_1	54.62^{b}	41.61^{b}	213.84^{a}	10.63^{ab}
V_1D_2	59.44^{ab}	38.96^{bc}	178.22^{ab}	9.98^{b}
V_2D_2	57.85^{ab}	35.22^{c}	150.48^{b}	10.02^{b}
V_3D_2	53.61^{bc}	42.88^{ab}	229.68^{a}	10.31^{ab}
V_4D_2	56.23^{b}	41.36^{b}	186.12^{ab}	10.25^{ab}
V_5D_2	58.45^{ab}	39.68^{bc}	166.32^{ab}	10.29^{ab}
V_6D_2	62.66^{a}	43.26^{ab}	146.52^{b}	10.39^{ab}
V_7D_2	55.93^{b}	41.98^{b}	122.76^{bc}	10.65^{ab}
V_1D_3	61.96^{a}	40.23^{b}	91.08^{c}	9.31^{b}
V_2D_3	55.62^{b}	41.12^{b}	142.56^{b}	9.99^{b}
V_3D_3	56.25^{b}	39.85^{bc}	118.80^{bc}	10.35^{ab}
V_4D_3	56.63^{b}	36.54^{c}	99.01^{c}	10.21^{ab}
V_5D_3	52.59^{bc}	40.32^{b}	186.12^{ab}	10.25^{ab}
V_6D_3	57.64^{ab}	41.69^{b}	138.69^{b}	10.41^{ab}
V_7D_3	58.04^{ab}	36.87^{c}	136.62^{b}	10.16^{ab}

Where V_1 to V_7 indicates the types of vermicompost; D_1 to D_3 indicate the doses (as designated earlier) Means followed by a different letter are significantly different (otherwise statistically at par) at $P < 0.05$ by Duncan's multiple range test

3.4. Influence of enriched vermicompost on grain yield, Zn, Fe and phytic acid load

Application of regular vermicompost as well as the enriched vermicompost acts as an important source of organic carbon and other essential nutrients that play a pivotal role in plant growth and development. This can be attributed by the increase in the grain yield of rice which was found to statistically significant than soil unamended with organic matter. Although the variation in the yield increase for the different combinations of the enriched vermicompost was not conspicuous, yet it can be illustrated that the maximum yield obtained was for vermicompost enriched with 10% Fe and Zn (w/w) and applied at the rate of 3 t/ha. The increase is by a magnitude of 43.56%. Even the lower rate of application of vermicompost had resulted in the increase in the yield, which has a silver lining in terms of lower rate of recommendation and better economizing of the process of rice cultivation, as evident from Figure-2.

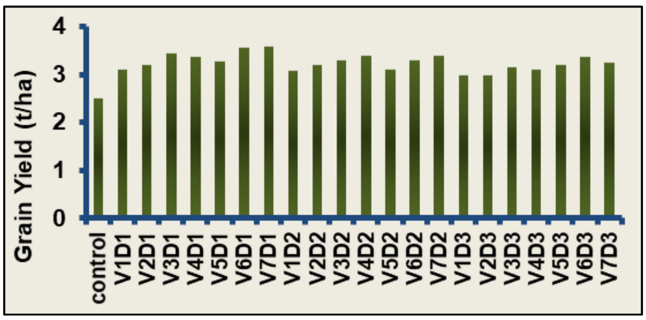

Fig 2: Variation in the grain yield (t/ha) under applied interventions

The enrichment of regular vermicompost with Zn and Fe at various doses and time of application resulted in variation in the load and its supplying capacity when applied in the soil for sustaining nutrition of the rice plants. The grain load of Zn and Fe both had an increase on application of enriched vermicompost. Although, there was a significant gain in the content of the nutrient on application of enriched vermicompost over the regular one, yet the variation among the six enriched treatment combinations were not significant. The effect of dose also brought very little changes in the load of the micronutrients.

Another interesting parameter that was taken into consideration in this study was the phytic acid content of the rice grain. It was observed from the study that the application of enriched vermicompost resulted in significant reduction in the content of the phytic acid of the grain, as evident from Table-6. This assumes a greater significance because of the negative relationship between the phytic acid content and Zn as well as Fe bioavailability. Thus the reduction of the phytic acid content under applied interventions can be important for effectuating the process of biofortification of rice grain.

Table 6: Effect of applied interventions on rice grain yield, Zn, Fe and phytic acid content

Treatment	Grain Yield (t/ha)	Grain Zn (mg/kg)	Grain Fe (mg/kg)	Grain Phytic acid (mg/g)
Control	2.516^{c}	13.12^{c}	39.78^{c}	22.33^{a}
V_1D_1	3.112^{b}	18.69^{b}	49.85^{bc}	20.12^{ab}
V_2D_1	3.219^{ab}	21.12^{ab}	53.69^{a}	19.36^{b}
V_3D_1	3.465^{a}	23.23^{a}	54.12^{a}	19.41^{b}
V_4D_1	3.398^{ab}	22.34^{a}	56.94^{a}	19.63^{b}
V_5D_1	3.294^{ab}	20.83^{ab}	52.63^{ab}	19.24^{b}
V_6D_1	3.591^{a}	22.12^{a}	54.12^{a}	18.98^{b}
V_7D_1	3.612^{a}	21.54^{ab}	55.74^{a}	18.95^{b}
V_1D_2	3.101^{b}	17.69^{b}	48.36^{bc}	20.32^{ab}
V_2D_2	3.214^{ab}	20.69^{ab}	50.12^{b}	19.63^{b}
V_3D_2	3.312^{ab}	22.15^{a}	51.36^{ab}	19.45^{b}
V_4D_2	3.412^{a}	23.86^{a}	52.31^{ab}	19.51^{b}
V_5D_2	3.125^{b}	21.68^{ab}	50.12^{b}	19.32^{b}
V_6D_2	3.328^{ab}	23.45^{a}	53.01^{a}	19.35^{b}
V_7D_2	3.419^{a}	24.82^{a}	52.14^{a}	19.26^{b}
V_1D_3	2.998^{bc}	18.01^{b}	48.63^{bc}	20.34^{ab}
V_2D_3	3.002^{bc}	20.28^{ab}	49.36^{bc}	20.01^{ab}
V_3D_3	3.165^{b}	21.09^{ab}	51.2^{ab}	19.87^{b}
V_4D_3	3.112^{b}	23.12^{a}	50.69^{b}	19.63^{b}
V_5D_3	3.229^{ab}	23.85^{a}	53.21^{a}	19.78^{b}
V_6D_3	3.381^{ab}	23.65^{a}	54.23^{a}	19.54^{b}
V_7D_3	3.265^{ab}	22.98^{a}	53.26^{a}	19.46^{b}

Where V_1 to V_7 indicates the types of vermicompost; D_1 to D_3 indicate the doses (as designated earlier) Means followed by a different letter are significantly different (otherwise statistically at par) at $P < 0.05$ by Duncan's multiple range test

3.5. Bioavailability of Zn and Fe as influenced by the phytic acid/nutrient molar ratio

As evident from earlier studies, inspite of the application of Zn and Fe through different organic and inorganic sources, the bioavailability for consumption through grains is greatly influenced by anti-nutritional factors such as grain phytic acid content. The study reported that there is a concomitant increase in the Zn and Fe load of the grain along with reduction in phytic acid content as envisaged in Fig-3.

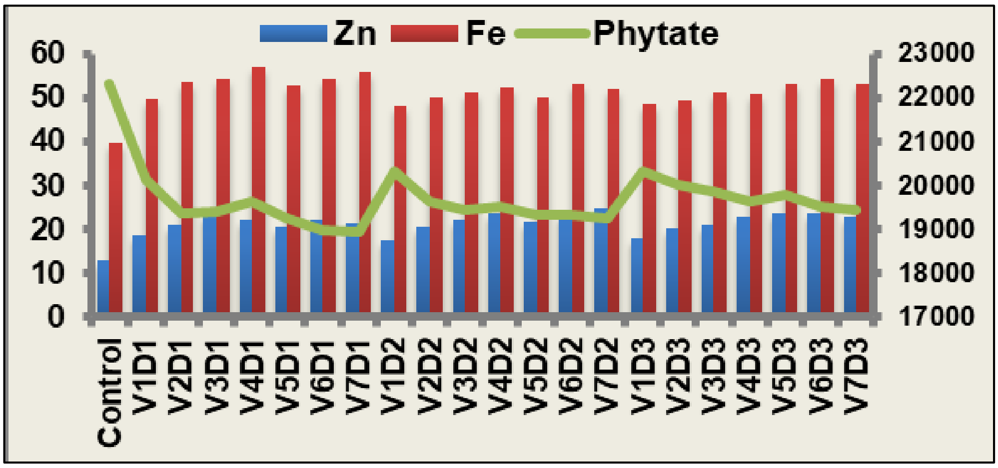

Fig 3: Pattern of change of the grain Zn, Fe and phytic acid content under applied interventions

Thus a lower content of the phytic acid accompanied by a higher load of Zn and Fe in the grain, or in other words a reduction in the phytic acid/Zn and phytic acid/Fe molar ratio will be the key for effective biofortification process. In the present study, we estimated the grain Zn, Fe and phytic acid, and thereby considering their molecular weight in Da, estimated their molar ratio (Table-7).

Table 7 Phytic acid/Zn and Phytic acid/Fe molar ratio determination

Treatment	Phytic acid/Zn molar ratio	Phytic acid/Fe molar ratio
Control	167.62^{a}	47.63^{a}
V_1D_1	106.02^{b}	34.25^{b}
V_2D_1	90.28^{bc}	30.60^{c}
V_3D_1	82.29^{c}	30.43^{c}
V_4D_1	86.54^{c}	29.25^{d}
V_5D_1	90.97^{bc}	31.02^{c}
V_6D_1	84.50^{c}	29.76^{d}
V_7D_1	86.64^{c}	28.85^{d}
V_1D_2	113.13^{b}	35.65^{b}
V_2D_2	93.44^{bc}	33.23^{b}
V_3D_2	86.48^{c}	32.13^{c}
V_4D_2	80.53^{cd}	31.65^{c}
V_5D_2	87.76^{c}	32.71^{c}
V_6D_2	81.27^{cd}	30.97^{c}
V_7D_2	76.42^{d}	31.34^{c}
V_1D_3	111.23^{b}	35.49^{b}
V_2D_3	97.17^{bc}	34.40^{b}
V_3D_3	92.79^{bc}	32.93^{c}
V_4D_3	83.62^{c}	32.86^{c}
V_5D_3	81.68^{c}	31.54^{c}
V_6D_3	81.37^{c}	30.57^{c}
V_7D_3	83.40^{c}	31.00^{c}

Where V_1 to V_7 indicates the types of vermicompost; D_1 to D_3 indicate the doses (as designated earlier) Means followed by a different letter are significantly different (otherwise statistically at par) at $P< 0.05$ by Duncan's multiple range test

The applied treatments in the form of enriched vermicompost containing Zn and Fe in different compositions resulted in a significant reduction in the phytic acid/Zn and phytic acid/Fe molar ratio over unamended soils or soils amended with regular vermicompost. Although the intra treatment combinations of the enrichment was not significant, yet the primary objective of grain biofortification and serving better nutrient bioavailability and ensuring the nutritional security to the country can assume a great fortitude in this way.

Discussion

The experimental site had been put into research for long times particularly emphasizing in the study of the effect of Zn and Fe as well as the influence of organic amendment in sustaining rice nutrition along with mitigating the problem of arsenic contamination in the said area (ICAR, 2011; Giri *et al*., 2011; ICAR-NAIP, 2012; Sinha and Bhattacharyya, 2014; Halder *et al*., 2019) [30, 24, 31, 65, 26]. The sole application of organic amendments in the form of vermicompost or inorganic sources of Zn and Fe have been in vogue, but engineering these two as a combined application have proven successful in several countries (Hashemimajd and Golchin, 2009; Hashemimajd and Jamaati-e-Somarin, 2011; Geiklooi and Shirmohammadi, 2013) [27, 28, 23] but is still not proclaimed in India. Nutrient contents of vermicomposts are much higher than those of commercial horticultural composts (Dickerson, 1999) [18] and interestingly the enrichment does not impair the C:N ratio, vital for microbial degradation (Khan *et al*., 2016; Mao *et al*., 2019) [38, 44] rather augment the mineralizable load of Zn and Fe to serve their deficiencies in plants (Barness and Chen, 1991; Hashemimajd and Jamaati-e-Somarin, 2011) [7, 28].

The tremendous role played by the earthworms in recycling the soil wastes and rendering them to be viable and productive had been put forth as early as 1881 by Darwin as 'it may be doubted if there are any other animals which have played such an important part in the history of the world as these lowly organized creatures'. Their role in regulating soil processes, maintaining soil fertility and in bringing about nutrient cycling (Ismail, 1997) [33] is of paramount agricultural significance (Ansari and Sukhraj, 2010) [5]. According to Shirani *et al*. (2002) [63] application of organic materials (manure and/or crop residues) can increase soil organic matter concentration and decrease bulk density. Vermicompost being a stable fine granular organic matter, when added to clay soil loosens the soil and improves the passage for the entry of air (Ansari and Sukhraj, 2010) [5]. The mucus associated with the cast being hydroscopic absorbs water and prevents water logging and improves water-holding capacity (Tharmaraj *et al*., 2011) [68]. The organic carbon in vermicompost results in improved soil aggregation that persist when soils are wetted or subjected to mechanical stress and influence soil's susceptibility to erosion, crust formation etc (Whalen *et al*., 2003) [75]. The most beneficial influence of earthworms is stimulation of microbial activity in casting (Parle, 1963; Dkhar and Mishra, 1983) [53, 19] during the composting process. Microbial population (Chowdappa *et al*., 1999) [15] and microbial biomass (Moore *et al*, 2000) [46] were found considerably higher under vermicomposting as compared with normal composting. This improvement of the content of microbial biomass carbon (Chaudhari *et al*., 2004) [14] along with the dehydrogenase activity (Arancon *et al*., 2006) [6] can be the flag bearer to improved soil quality.

Enrichment of vermicompost with iron and zinc increased total and available content of the nutrients in soil-plant system similar to the findings of Hashemimajd and Jamaati-e-Somarin (2011) [28]. The increase in the content of Zn and Fe along with other nutrients like P from vermicompost can bear a positive synchrony with the chlorophyll content (Zarrouk *et al*., 2005) [77] which can improve photosynthetic efficiency, assimilate partitioning and increase growth and yield (Anitha *et al*. 2004; Rehman *et al*., 2013) [58, 4] of different crops.

The enrichment of Zn and Fe at the time of vermicomposting and its application in soil can invariably improve the availability of the micronutrients in the plant parts upon absorption (Patra *et al*., 2000) [54]. However, the occurance of phytic acid can result in somewhat antagonism in the behavior of Zn and Fe in the plants (Saha *et al*., 2017) [60] through i) absorption by roots in soils (Dutta *et al*., 1989) [20] ii) loading into the xylem (Alloway, 2008) [3] iii) chelation for translocation (Kabata-Pendias, 2001) [36] and iv) cross membrane transport by particular carrier proteins (ZIP family proteins) (Palmgren *et al*., 2008) [52]. The lowering of the phytic acid content along with increasing in the Zn and Fe load can thus assume far greater significance (Imran *et al*., 2015) [32]. The phytic acid: Zn (PA:Zn) and phytic acid: Fe (PA:Fe) molar ratios are considered to be the indicators for Zn and Fe bioavailability in food (Morris and Ellis, 1989; Hussain *et al*., 2012) [47, 29]. This phenomenon of reduced availability of zinc and iron in presence of phytic acid can be attributed to the phenomenon of chelation of several mineral elements, especially Zn, Fe, Ca, Mg and Mo that interfere with their absorption and utilization (Ologhobo, 1980; Akond *et al*., 2011) [50, 2] or in other words, phytic acid (PA) as a substance often couples with cations such as Zn and Fe, forming insoluble phytates, which affects the bioavailability (Zhao *et al*., 2008) [80]. Moreover, if the soil is Zn deficient, then the plant root uptakes higher quantities of P, augmenting the PA content (Wang *et al*., 2015) [73]. Increased PA/Zn or PA/Fe ratio reveals decreased bioavailability of Zn and Fe, either through reduction in uptake by root and root-to-shoot translocation, or binding with phytate in grains (Rakshit *et al*., 2016) [56]. In our experiment the applied interventions reduced the molar ratios significantly, thereby having an edge regarding the bioavailability. This can also ensure successful biofortification and make the process viable for ensuring nutritional security in developing countries like India along with betterment of soil quality for sustaining agricultural production from the arable lands for long periods of time.

Conclusion

Rice is the major staple diet of a large population in South, South East and East Asia, thus low Zn creates turmoil in the form of widespread Zn deficiency in this population. The associated deficiency of Fe is also cropping up on a large scale owing to their meager availability through the rice-based diet. It is now becoming increasingly pertinent to take each and every step so as to augment the bioavailability of the nutrient to humans; the most common being biofortification and fertifortification. Moreover, wide scale crop productivity has robbed off the nutrient supplies of the crop transforming many lands into a state that they are not amenable to agriculture anymore. The concept of enrichment of vermicompost with Zn and Fe can thus serve two basic requirements; firstly improve the soil health and quality and secondly, serve adequate quantities of Zn and Fe in a slow release form, thus there is no scope of making them insoluble. The reduction of phytate can serve as another added benefit by making the nutrient bioavailable, thereby can be a potential solution to curb the malnutrition problems throughout the world.

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