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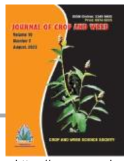

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Vermicomposted red mud- An up-and-coming approach towards soil fertility and crop quality

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ABSTRACT

In present study, the Eisenia foetida -mediated red mud-vermicompost was used for crop trial by taking Vigna radiata L. as tested crop. An improved soil quality with significant elevation (>60%) in NPK status in soil was resulted from the application of red mud vermicompost. Furthermore, significant reduction in bioavailability of toxic metals (Cr, Ni, Cd, Pb) and subsequent plant uptake which was in the order of root>shoot>grain was achieved (Root 0.1-13mg kg^{-1}; Shoot 2.23-9.45 mg kg^{-1}; Grain 1-9 mg kg^{-1}) Detected Cr (1.5-3.1 mg kg^{-1}) and Ni (2.4-3.1 mg kg^{-1}) in edible parts under organics intervention were within permissible limits. Free ion activity modelling (FIAM) based on soil pH, organic carbon, and labile metal (WS-EX) ensures insignificant grain uptake of toxic metal (Cr, Ni) including positive transport of micronutrient (Cu) in presence of increased organic C. Risk factor analysis confirmed intake of metal will be hazard free corresponding to hazard quotient (HQ) and cancer risk (CR) values. Moreover, the composite treatment ($VC_{1:2}$50%+NPK50%) attributed profuse yield of Vigna radiata L (15.90 g plant^{-1}). hence could be an alternative agronomic practice.

***Keywords*:** FIAM, crop uptake, metal, vermicompost, *Vigna radiata* L.

Rise of the population in India largely increases the demand for food. This has led to an increased usage of inorganic fertilizers and chemical supplements for rapid growth and high productivity of crops. Besides the quality of soil, precisely the red soil/lateritic zone of eastern India is extremely poor in terms of nutritional value and microbial activity. This large group of soil is generally gravelly and coarse-textured, have high acidity, deficient in soil organic matter (humus) and other macro and micronutrients viz. N, P, K, Ca, Zn, B etc. Cultivation practices mostly depend on rainwater in this semiarid region and nutrient loss is another limitation of agriculture practices. These contrasting issues led farmers to use more chemical fertilizers for greater productivity of crops (Ghosh, 2019). Extensive use of such inorganic fertilizer/supplements instantly boosts plant health by refurbishing the depleted nutrients however such high inputs directed to an increased accumulation in the grain which often exceeds the safety levels creating an alarming impact on the food chain (Bhattacharyya *et al.*, 2003). This necessitates the desirability of organic supplements as they are rich in microbial activity, carbon-nitrogen content, humic substance etc. Hence, agronomic practices that lift crop productivity as well as conserve the organic matter content in soil are highly appreciated. Such practices may take care of plants' health as well as protects the soil's health.

Vermitechnology is a classically recognized agricultural practice increasing crop yield by improving soil's physical, chemical, and biological properties. It is a non-thermophilic stabilized nutrient-rich end product of earthworm-microorganism interaction with high porosity, ventilation, and water retaining capacity. Vermicompost (VC hereafter) is a granular structure having a higher surface area for the strong retention of micronutrients and plays an effective role in plant growth and promotion. It contains nutrients like soluble potassium, nitrates, phosphates, exchangeable calcium, sodium, etc. They are rich in microbial activity viz. bacterial, fungal, or actinomycetes, and are considered nutritive organic fertilizers. Considering the origin of VC, it widely varies in chemical composition (Dume *et al.*, 2022). Declining of traditional natural resources and emergence of waste invites us to pay attention to an alternative strategy where the waste could be recycled and reutilized. Presently vermiconversion is quite a well-known process to convert different industrial wastes into a value-added product. In general, the major limitation of using industrial wastes directly for agricultural purposes is presence of hazardous toxicants (Cd, Cr, Pb, Ni, As) although sometimes being rich in micronutrients (Fe, Cu, Mn, Zn, Ca, K, Mg)

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(Bhattacharya *et al*., 2012). These toxicants are not volatile and mostly remain in labile pools thus threatening sustenance. Earthworms together with microorganisms synergistically act on it during vermicast processing and produce a nutrient-rich product. They unlock the micronutrients (Ca, K, Na, Mg, N, P, Fe, Zn, Mn) and make them into plant-available forms for easy uptake. Water soluble and exchangeable are the two most dicey fractions of any contaminants which enter into the food chain leading to bioaccumulation. Earthworm greatly sequesters them and shifts their availability state into a residual or organic bound fraction which lowers their toxicity (Goswami *et al*., 2017). The mineralization and humification process by earthworms and microorganisms is enhanced in presence of organic substrate (cow dung, cattle manure, rice straw, rice husk, etc). A mixture of natural organic substrate and waste serves as beneficial feedstock material for earthworm growth and productivity. The approach gives a twining benefit of effective disposal and resource recovery and unveils a new avenue of compost application for sustainable agriculture.

Pulses play a vital role in the Indian diet as a protein source, and green gram (*Vigna radiata* L.) holds the 3rd most important protein crop in India. It is also abundant in calcium, potassium, phosphorus, and vitamins A, B, and C, all of which are essential for human health. It was selected as the test crop in Giridih's depleted soil because of its brief lifespan (65–80 days), versatility in several intensive crop rotations, and ability to improve soil fertility by fixing atmospheric nitrogen through rhizobial symbiosis (Singh *et al*., 2017). Our study was aimed to investigate how vermi-sanitized industrial waste (Red mud) impacts soil-plant (pulse crop) system by preserving nutritional benefits, and also asses their relative toxicity towards human consumption. To comply with this objective a pot experiment was conducted for two consecutive years in the summer season on a green gram (*Vigna radiata* L.) at the agricultural farmhouse of the Indian Statistical Institute in Giridih, Jharkhand in the years 2020 and 2021. This study hypothesizes that a 50% substitution of inorganic fertilizer with vermicompost will give greater crop productivity by securing soil health.

MATERIAL AND METHODS

Processing of vermicompost (VC)

Vermicompost (VC) was prepared from industrial waste [red mud (RM)] using an epigeic earthworm species (*Eisenia foetida*) at the vermicompost unit of the Indian Statistical Institute (ISI), Giridih, Jharkhand. Red mud is alumina industry waste, collected from a local alumina plant in Jharkhand. It is alkaline (pH=11) with high sodicity. The chemical properties of RM are tabulated in Table S1. Cow dung (CD) was used as a bulking agent and collected from local cattle houses. Four different feedstock combinations were prepared, where, the ratios (RM: CD) were (1:1, 1:2, 1:3, and 1:4). 10 worms (healthy, non-ciliated, 35-40 days old) were employed per kg of substrate. At the end of November, the prepared VC was withdrawn from the composting unit, sieved, and preserved for crop trial.

Greenhouse pot experiment

A local summer variety of green gram (*Vigna radiata* L.) seed was bought from the local market (variety - IPM-02-03). The seeds were sown to a seedbed (30 x 50 cm) in the greenhouse for 10-12 days with 60% water holding condition. After 12 days the seedlings (6-7 cm) that were ready for the plantation, were uprooted, and transplanted in the experimental pot.

The pot experiment was conducted for two consecutive years (2021 and 2022) from March to June to check the efficacy of prepared vermicompost. The experimentation was laid out into twelve different combinations of treatments depicted in Table 1. Recommended fertilizer dose opted for the trial was 20:40:40 (N_2:P_2O_5: K_2O)/ha. In a nutshell, four replicates of each treatment were made where the earthen pots were filled up with 10 kg of soil. Before transplantation of the plant, the organic amendments were mixed (both full dose and half dose) in soil and left for 7 to 10 days. Only inorganic amendment (fertilizer) was added one day before the transplantation. Urea, superphosphate, and muriate of potash were used as NPK sources. 6-7 cm long seedlings were transplanted in each pot (2 plants/pot). The organic amendment was used in two doses 10 t ha^{-1} (VC 100%) and 5 t ha^{-1} (50% VC). Other agronomic practices like watering, drainage of excess water, weeding, and application of pesticides were followed uniformly for all the treatments recommended by the Department of Agriculture and Ecology, ISI, Giridih, Jharkhand.

Soil sampling and analysis of soil and plant sample

The moist soil samples were collected at four different times i.e., transplanting time, flowering time, maturation time, and post-harvest time for microbial parameter analysis (microbial biomass C, soil respiration, enzymatic activities). Soil samples for physicochemical analysis were taken two times i.e., transplantation time, and post-harvest samples, which, were collected ten days after harvesting. The key attribute microbial biomass C was measured following

Alef (1995). It is the fumigation-extraction method followed by K_2SO_4 extractable C determination and calculated by multiplying Ec with 2.64, where Ec is the change between K_2SO_4 extractable C of the fumigated and unfumigated soils. Soil respiration was estimated by measuring CO_2 released at the time of incubation when soil was induced with 0.5% glucose in a closed system, and the trapped CO_2 in NaOH solution was subsequently titrated with HCl. FDA activity was measured following Tabatabai (1994) involving estimation of fluorescein released when moist soil incubated with phosphate buffer (pH 7.6) and fluorescein diacetate solution at 25^0C. Dehydrogenase was measured by taking 6g of moist soil incubated with 3% TTC at 37^0C for 24 h subsequently triphenyl formazan (TPF) was extracted and filtered with aid of methanol and finally measured spectrophotometrically at 485nm.

To assess the physicochemical parameters, viz. pH, EC, organic carbon (TOC), available nitrogen (N), phosphorus (P), and potassium (K) soil was air-dried and sieved through a 2 mm and 0.2 mm mesh. All the parameters were analyzed following Page *et al*. (1982). Available N was measured by using alkaline KMnO4 followed by distillation with standard H_2SO_4 (0.02N) for absorption of released NH_3 and subsequently titrated with NaOH, while total N was measured through kjeldahl digestion and distillation method**.** Available P was determined spectrophotometrically using Brays/ Olsen as extraction medium (based on pH) followed by the addition of chloromolybdic reagent and stannous chloride and finally measured at 660 nm by visible spectrophotometer. Available potassium (K) was estimated in neutral normal ammonium acetate (pH=7) (CH_3COONH_4) extract of soil and K was measured by using a flame photometer, Total K was estimated using acid-digested extract. Fractionation study was done following Tessier *et al*. (1979). Phytoaccumulated metal was checked by digesting the dried root, shoot, and grain in a 4:1 ratio (HNO_3:$HClO_4$) acid mixture. Concentrations of metal in the digests were measured using atomic absorption spectrophotometer (Systronics AA S-816) and expressed in terms of dry weight basis. Standard solutions of the metals (Cr, Ni, Cd, Cu, Pb) were made from the stock solution (1000 mg/L, Merck grade) in 1% (v/v) HNO_3 for calibration. For quality checking, certified reference materials 2710 and blank extract were used. Total nutrient uptake (P, K) in different parts of plants was analyzed using the same digest. Post-harvesting growth parameters and yield characteristics were recorded. Biochemical attributes like chlorophyll a, chlorophyll b, and total chlorophyll were extracted by acetone-hexane mixture in a 4:1 ratio. Protein content was quantified by Lowry et al. (1951). Total soluble sugar (TSS) was measured following the phenol-sulfuric acid method proposed by Dubois *et al*. (1951).

Nutrient benefit ratio and metal mobility assessment

The potential nutrient benefit of using organic amendment (here VC) can be calculated by the following Eq. 1 (Table S2). The nutrient benefit ratio (NBR) was computed for the variables TOC, available N, available K, and available P accountable for soil fertility (Sahariah *et al*., 2015). Additionally, the presence of heavy metals and their plausible movement toward plant systems were assessed and represented as bioconcentration factors (BCF), translocation factors (TF), and bioaccumulation factors (BAF) (Islam *et a*l., 2020). Respective equations (Eq.- 2, 3, 4) are presented in Table S2.

Prediction of metal content in mung bean grain

Metal content in the edible part was predicted by the solubility-free ion activity model (FIAM) without measuring metal solubility in real. The model describes that metal uptake could be controlled by free metal ion activity in soil solution. Transfer factor (TF) is the ratio of metal content in plant (M_{plant}) to metal ion activity in soil (M^{n-}) solution (Mirecki *et al*., 2015)

$$TF = log \frac{[Mplant]}{(M^{n-})} \quad \text{...} \quad (1)$$

A simple pH-dependent Freundlich equation was employed to predict the free ion activity of three metallic species (Cr, Ni, Cu) as follows

$$p(M^{n-})=\{p[Mc]+k_1+k_2\ pH\}/_{nF} \text{...............................}(2)$$

where M^{n-} denotes the free ion activity in soil solution and Mc denotes the humic content (mol/kg carbon) to which the labile metal be adsorbed, k1 and k2 stand for the metal coefficient and n_F is the power term of Freundlich equation. Predicted metal uptake was derived by combining equations 1 and 2 and depicted as follows

$$p[M_{plant}]= C+\beta1p[M_c]+\beta2[pH]\text{...........................}(3)$$

where C = k1/nF " log TF, â1 = 1/nF, â2 = k2/nF and C, â1 and â2 are empirical metal and plant-specific coefficients. Equation (3) was parameterized by nonlinear error minimization employing the "SOLVER" facilities in Microsoft Excel 2019.

Risk assessment

Estimated daily intake (EDI), carcinogenic and non-carcinogenic risk of the three metals (Cu, Ni, Cr) was computed following equations 5,6,7 (Table S2) (Rashid et al., 2022) and the derived value was compared with maximum tolerable daily intake set by regulatory bodies

JECFA (2003), WHO (2007), and RDA (1989). Similarly, non-carcinogenic (HQ) and carcinogenic risks were computed to assess possible health risks (USEPA, 2015). The CR is a dimensionless indicator that helps in assessing the incremental lifetime cancer risk for the intake of mung bean. CR was not calculated for Cu since they are not recorded to cause any carcinogenic effect.

Statistical analysis

Analysis of variance (ANOVA) followed by least significant difference (LSD) calculation was used to explain the consequence of different amendments on crop growth, yield characteristics, and soil fertility including the metal uptake by grains of green gram (*Vigna radiata* L.) using SPSS statistical package (IBM SPSS 25.0 statistics). The data presented and discussed were mean of two years. All the data were represented in mean ±SE form.

RESULTS AND DISCUSSION

General characterization of RM-based vermicompost

Vermicompost produced from industrial waste (red mud) was found to contain a significant amount of nutrients (Table S3). The compost was rich in organic matter and other nutrients viz. nitrogen (N), phosphorus (P), potassium (K), etc. It not only contained nutrients but was also enriched with nitrogen-fixing and phosphate-solubilizing bacteria. The cow dung used in this study was having a neutral pH of 7.7, EC 1.1 mS/cm, available N 124 mg/kg, available P 35 mg/kg, and potassium 169 mg/kg.

Effect of amendments on crop growth, yield attributes, and biochemical quality

Overall, the beneficial effect of organic amendments was evidenced in growth and development of plants. A perusal of data (Table 2a and 2b) distinctively identified that application of VC50% + NPK50% logged maximum plant height, number of branches, nodule number, root and shoot length, and pod count. The organic + inorganic treatments were found to be significantly ($p<0.05$) superior to organic amendments alone. Significantly higher plant height was recorded in T12 (VC50%+NPK50%) followed by T6> T2> T8> T7> T11> T9> T10> T5> T4> T3 >T1 (LSD=1.25; p<0.0001; F= 37.50). The root length was in the order as follows T8> T10> T3> T11> T6> T12> T9> T5> T4> T2> T7> T1 (LSD=1.57; p=0.0309; F=2.47). On the other hand, highest shoot length was achieved in T12 (35.76 cm) followed by T2> T6> T7> T8> T11> T9> T10> T3> T5> T4 >T1 (LSD=1.84; P<0.0001; F=24.06). Root nodule formation is an important attribute that varied significantly ($p<0.05$). The higher number of nodules was found in T6 (28.00±1) and T8 (27.33±2.9), and had the order somewhat like T6> T8> T12> T2> T10> T9> T11=T7> T5> T4> T3>T1 (LSD=4.27; p=0.047; F=2.24). The addition of organic amendments triggered the soil's organic matter content which possibly reduced the bulk density thereby improving the pore spaces that directly increased fertility of amended soil and facilitated better growth of plants (Rahman *et al*., 2019; Dong *et al*., 2021). Considering the grain (seeds) number per pod T8 produced a maximum of 8.33±0.33. It was distributed and differed among the treatments as T8> T2=T4=T6=T10> T12> T11=T5> T7> T2=T9> T1 (LSD=0.577; P<0.0001; F=11.56). Following similar trend pod numbers per plant varied significantly and were noted highest in T6 (29.67±2.86) followed by T2> T8> T12> T10> T4> T11> T9> T3=T5> T7>T1 (LSD=1.38; p<0.0001; F=16.13). Significantly higher number of seeds per plant produced by T6 (50% VC+50% NPK) and T2(100%NPK) followed by T8> T4> T10> T12> T11> T9> T3> T5> T7> T1 (LSD=16.91; p<0.0001; F=11.95). Considering 100 seed weight which was also highest in 50%VC+50%NPK treatment (T6- 6.7g±0.12). Nearly all the treatments showed >5.5 cm pod length except T1 showed 4.5 cm of pod length, which was the lowest among all. The highest pod length was recorded in T2 (6.3 cm±0.05) supplemented with 100%NPK. Yield was highest in T6 (15.90±0.88 g/plant) followed by T2> T8> T12> T10> T4> T11> T9> T7> T5> T3> T1 (LSD=2.88). The notable performance of composite treatments may be from the application of vermicompost which contains a balanced amount of nutrients, along with beneficial microbial consortia like N_2-fixing, phosphate, and potassium solubilizers (Hussain et al., 2016). They enhance nitrogen fixation with aid of enzyme nitrogenase thereby triggering the nodule formation, as well as solubilizing more insoluble P and K, and also producing secondary metabolites viz. stimulants, growth regulators, and enzymes that support plant growth and development. The result of our observation is consistent with earlier studies (AL-Dulimi *et al*., 2017; Mahmoud et al., 2015; Kaysha *et al*., 2020, and Blouin *et al*., 2019).

Photosynthetic pigments like chlorophyll a and b were found to be highest in T6 (11.21±0.55; 4.68±0.19 µg g^{-1}) while an elevated concentration of total chlorophyll was detected in T12 (33±0.45 µg g^{-1}). The data that a higher quantity of pigments exists in organic+inorganic manure-treated soil (Fig. 1c) is in close agreement with the study of Manikandan and Thamizhiniyan (2016). Total soluble sugar (TSS) was highest in T10 (2908.20±7.3 µg g^{-1}) and lowest in T1(1155.93±5.93) while soluble protein content was highest in T12 (3030.81±0.81 µg g^{-1}) (Fig. 1d). A balanced NPK uptake of plants had a rewarding effect on plant nutrition that triggered photosynthesis for a suitable making of food. The results of having high protein content in compost-treated plants coincide with the study of Gowda et al. (2008) who detected higher protein in wheat plants treated with vermicompost.

Influence on soil physicochemical properties

Changes in the physicochemical parameter amid treatments are depicted in Fig. 2a-f. In general, addition of vermicompost raised the pH slightly due to release of alkaline humates from vermicompost (Das *et al*., 2018) hence, a small increment (average 0.06-fold) was observed in all the amended soil compared to control (Fig. 2a). After harvesting, soil pH was little altered among the treatments except in T9 which remained same ($LSD_{Treatment}$=0.16; p for time=0.012). Electrical conductivity (EC) was not much differed among treatments between pre-plantation and post-harvesting time ($LSD_{Treatment}$=0.07) (Fig. 2b). The present study evidenced an average of 30.09% increase in SOC content of soil after the addition of RM-vermicompost compared to control however there was no significant difference exists among the treatments. The post-harvest SOC content was as follows T4=T5=T10> T12> T2> T3> T6> T7> T11> T9> T8> T1 ($LSD_{Treatment}$=0.05; p for time=0.008). The findings are in accord with Antil and Singh (2007) and Dong *et al*. (2021). The mineralizable N content of amended soil increased considerably compared to control owing to microbial decomposition of the organic manure which in turn releases N thus increasing the plant available N in soil, supported by earlier studies (Laos *et al*., 2000; Abbasi *et al*., 2007). Mineralizable N in post-harvest soil was significantly high in treated soil compared to control nevertheless among the treatments it was insignificant ($LSD_{Treatment}$= 0.912; $p<0.05$; p for treatment × time=0.009). The N content was highest in T2 followed by T10 and T12 (Fig. 2f). Potassium (K) is another vital macronutrient for plant growth. Average 21.21% K content was elevated after the addition of NPK and VC, in which T2 gained maximum (Fig.2d). The difference between pre-plantation and post-harvesting K content between control and treatments was highly significant ($p<0.0001$, $LSD_{Treatment}$=2.07; p for treatment × time=0.0002). At the end of cropping season K was highest in T12 (277.61±2.83) and lowest in control soil (177.65±1.08). Potassium solubilizing bacteria (KSB) might be the major drivers of K availability in VC-treated soil. Our findings are supported by Bhattacharyya *et al*. (2007) and Warman and Termeer (2005). During vermicast processing earthworm converts insoluble Ca, Al bound P into soluble one. Consequently, application of VC in the soil helped to increase the availability of P. Incorporation of VC did not work immediately nevertheless a gradual increment resulted in an adequate amount of P at the post-harvesting time ($LSD_{Treatment}$=2.45). The change was significant between control and treatments ($p<0.0001$), as well as treatments and time. A significant build-up in P availability was evidenced in T2 while lowest in control soil (Fig. 2e). Overall advancement under VC-treated soil may be accredited from the steady release of NPK due to augmented microbial activity and the secondary metabolic enzyme in the vermicompost, that coincide with the study by Goswami et al. (2017). The higher nutrient-benefit ratio demonstrated the beneficiary role of vermicompost in enhancing soil fertility (Sahariah et al., 2015). Almost all the organically amended treatments exhibited a higher ratio except organic C. The benefit ratio was in the order of P> K=N> OC (Fig. 3e). Moreover, the incorporation of vermicompost increased soil fertility either by solubilizing the essential nutrients or liberating them from organic compounds upon decomposition.

Influence on temporal variation of soil microbial attributes

Considering the fact of securing soil quality, maintenance of OC from the microbial origin is highly important for sustenance. Addition of VC immediately augmented the microbial biomass (average 39%) compared to the control soil which further slowed down up to maturation time of the plant and thereafter increased at the end of cropping season (Fig. 3a). 100% NPK and 50%CD+50%NPK treated soil showed a maximum increase ($p<0.0001$). Periodically, the declining trend of microbial biomass content in all the treatments might result from plant-microbe interaction (Kuzyakov & Xu, 2013; Bhattacharyya *et al*., 2003). The initial increase in microbial activity helps plant growth promotion by providing nutrition to the plant while the contrasting phenomena of increased plant growth and decreased microbial biomass generated with time might result from the competition for nutrients between plants and microbes (Kuzyakov & Xu, 2013; Bhattacharyya et al., 2003). An increase in biomass C after cropping irrespective of treatments may be the result of plant residues left in the soil, that restored microbial activity ($LSD_{Treatment}$= 1.49, LSD_{time}=0.86). On a contrary note, dehydrogenase activity went higher up to flowering time (35 days) after the addition of the amendments and surprisingly got a reduction at maturation time (60 days) followed by an increase in post-harvesting soil (Fig. 3b). Although the initial increase was not significant among treatments nonetheless found significant ($p<0.05$) with control. The increase was an average of 37.62% (LSD_{time}= 0.36; $LSD_{Treatment}$=0.63, p for treatment × time =0.011). The improved microbial activity may be due to plant-microbe mutualism, where plant root exudates attracted the organism to colonize. The experimental results are supported by the findings of Pradhan and Sahoo (2012). Soil respiration (glucose-induced) is a direct measurement of active microbial population. It was increased up to flowering time (35 days) across the treatments followed by a substantial decrease during maturation time (at 60 days). The post-harvesting soil again produced good respiration owing to greater microbial activity (Fig. 3c). Addition of VC augmented cellular activity by more than 50% compared to control soil. Notably, soil amended with 50%VC+50%NPK and 50%CD+50%NPK exhibited a significant increase ($LSD_{Treatment}$=2.10, LSD_{time}=1.21, p for treatment x time=0.009). Likewise, fluorescein diacetate hydrolysing activity (FDA) elevated in the amended soil (average 48%; $p<0.0001$). Exceptionally FDA did not show much decrease during maturation time across the treatments (Fig 3d), however, post-harvest activity was more impressive compared to other attributes ($LSD_{Treatment}$=0.89; LSD_{time}=0.52; p for treatment × time =0.0008). Such contrasting behaviour of FDA could be associated with active enzymes within dead biomass, related cell fragments along with active cells (Bhattacharyya et al., 2003). Results of the overall microbial attributes promptly indicated that composite treatments (50% VC+ 50% NPK) were indeed a good supplementation for both soil and plant nutrition.

Plant uptake of nutrients

Nutrient uptake (P and K) by the plant in root, shoot, and grain was assessed and depicted in Fig. 1a-b. The findings showed that composite treatment (VC+NPK) causes more nutrient uptake than soil treated with VC alone, however, the comparison was significant with control ($P<0.0001$). The K uptake was in the order of grain>shoot >root while P was highest in shoot > root>grain. Across the treatments, grain uptake of K was highest at T6 (LSD_{grain}=7.32, p=0.0006). On a contrary note, P uptake was highest in shoot followed by root and grain. The highest transfer took place in shoot of T12 plants that subsequently got the maximum amount of P in their grain compared to others. Gross P uptake by the plant under various treatments was in the order of T11> T12> T8> T2> T6> T7> T10> T5> T4> T9> T3> T1 (LSD_{root}=17.99, p=0.0003, LSD_{shoot}=19.78, p=0.0012; LSD_{grain}=20.05, p=0.0061). Availability of P was greatly induced in VC-aided soil which was not only the result of insoluble P transformation but rather an augmentation of P-solubilizing microbes and their enzymatic activity. The findings concur with Mondal *et al*. (2020).

Metal distribution

Metal exists in the soil in six different fractions i.e., water-soluble, exchangeable, carbonate bound, Fe-oxide bound, organic bound, and residual fraction. The result (Fig. S1) showed that metals across VC-treated soil were most predominant in the organic bound phase and residual phase except in control soil. This might be due to the presence of humic content in VC which retained the metal through an organometallic complex (Hussain et al., 2016). Two metals Cd and Cu is widely distributed in carbonate phase while Pb was in Fe- oxide and residual phase. Considering the water-soluble and exchangeable fraction which was much higher in control soil for all the metal compared to treatments.

Phytoavailability and bioaccumulation of metal

In the present study, we assessed the availability of metal in roots and their possible translocation in shoot and grain (Fig. 4a-c). The uptake of five metals (Cr, Cu, Ni, Cd, and Pb) irrespective of plant parts (root, shoot, grain) was significantly lower compared to the control soil plants. Ni showed maximum accumulation in root followed by shoot and grain, while Cu showed a maximum grain uptake followed by root and shoot except for T1 and T4. Cr got its highest accumulation in root in almost all the treatments (LSD=0.70) followed by shoot (LSD=0.42) and grain (LSD=0.06). Pb was below detection limit in root, shoot, and grain whereas Cd was poorly accumulated (0.1-0.5 mg/kg) in the root of the plant. However, the studied metal concentration in the edible part was within safe limits (WHO, 2007). Concentration of metals (Cu, Cr, Ni) in grain was significantly low compared to the control ($p<0.0001$). Similar trend was observed in case of root (pCr<0.0001, F=63.80; pNi=0.0016, F=6.45; pCu<0.0001, F=19.57) and shoot (pCr<0.0001, F=61.56; pNi=0.0016, F=36.18; pCu=0.0010, F=7.95). The availability of metal was significantly lowered in treatments under the influence of VC. Such reduction in metal migration from soil to plants might be due to presence of increased organic matter which likely forms an organometallic complex thus lowering transfer (Mondal et al., 2020). However, a significant positive correlation at 5% and 1% levels, between water soluble, exchangeable phase and root, shoot, grain metal indicated that these two metal fractions were responsible for plant uptake, however, their concentration in the edible part within the safe limit is harmless. Root uptake was highly significant with Cr, Ni, and Cu (r-Cr=+0.65; r-Ni=+0.69; r-Cu= +0.91). For shoot it was as follows r-Cr= +0.66; r-Ni=+0.88; r-Cu= +0.90, while grain accumulation was highly

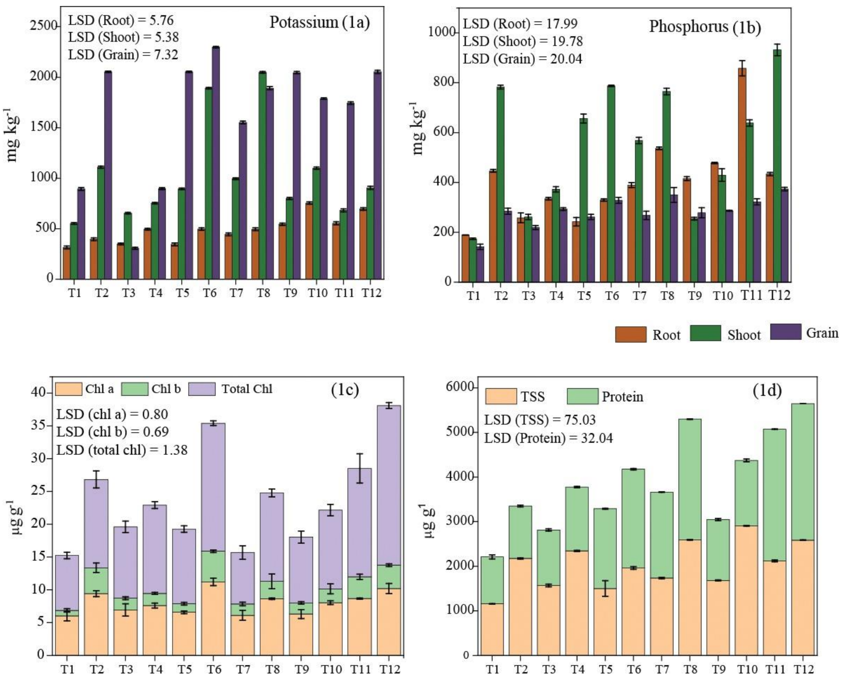

Fig. 1: (a-b) K and P uptake by plants; (c-d) Biochemical attributes Chlorophyll a, b and Total chlorophyll, protein and total soluble sugar (TSS)

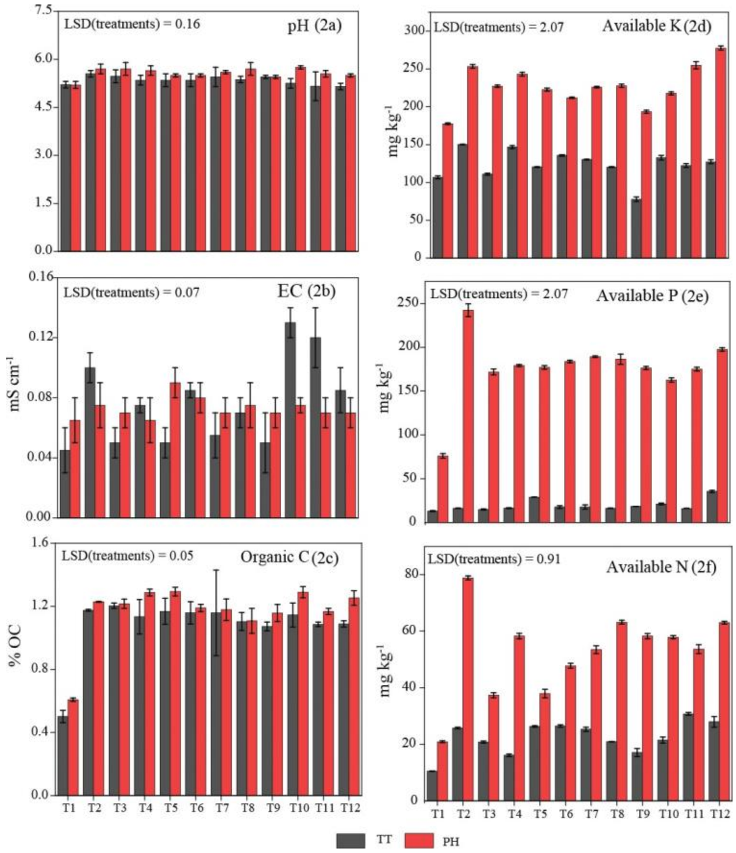

Fig. 2: Changes in the soil physicochemical properties and nutritional status before-transplantation (seedlings) and post-harvest soil

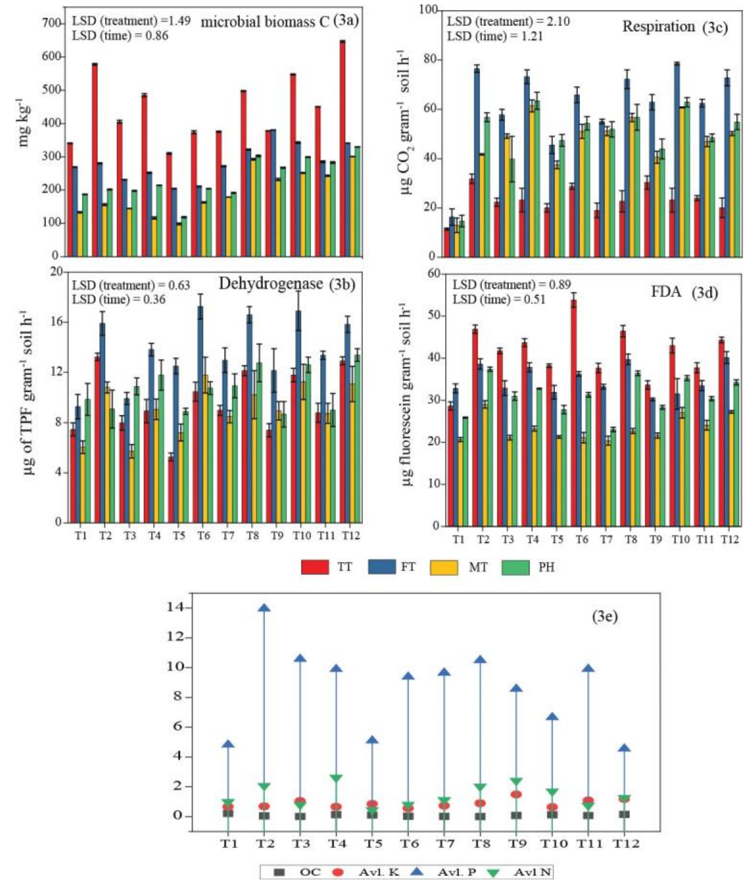

Fig. 3: (a-d) periodical changes in soil microbial properties during different stages of growth and development. TT- transplantation time; FT- flowering time; MT- maturation time, PH-post harvest (e) Nutrient benefit ratio

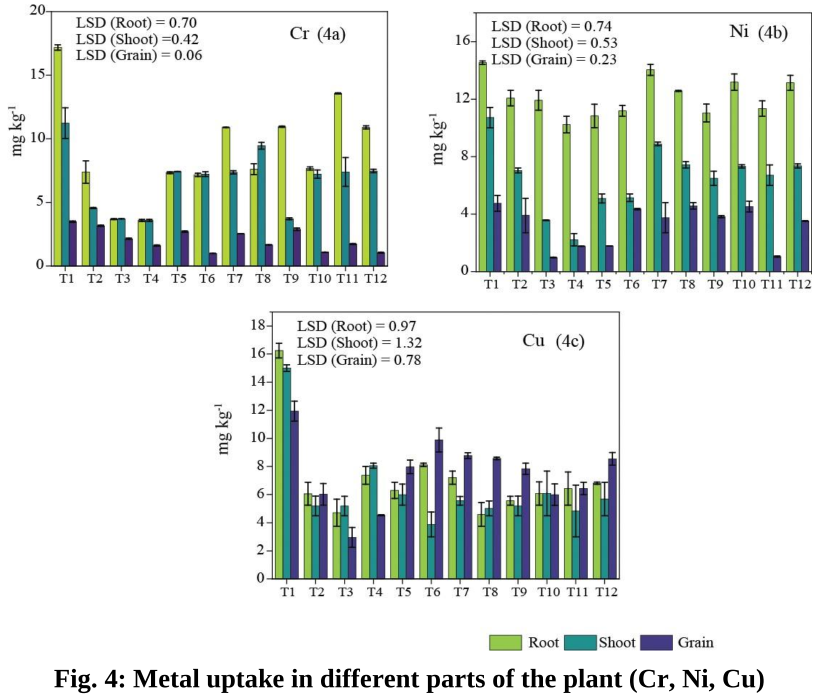

Fig. 4: Metal uptake in different parts of the plant (Cr, Ni, Cu)

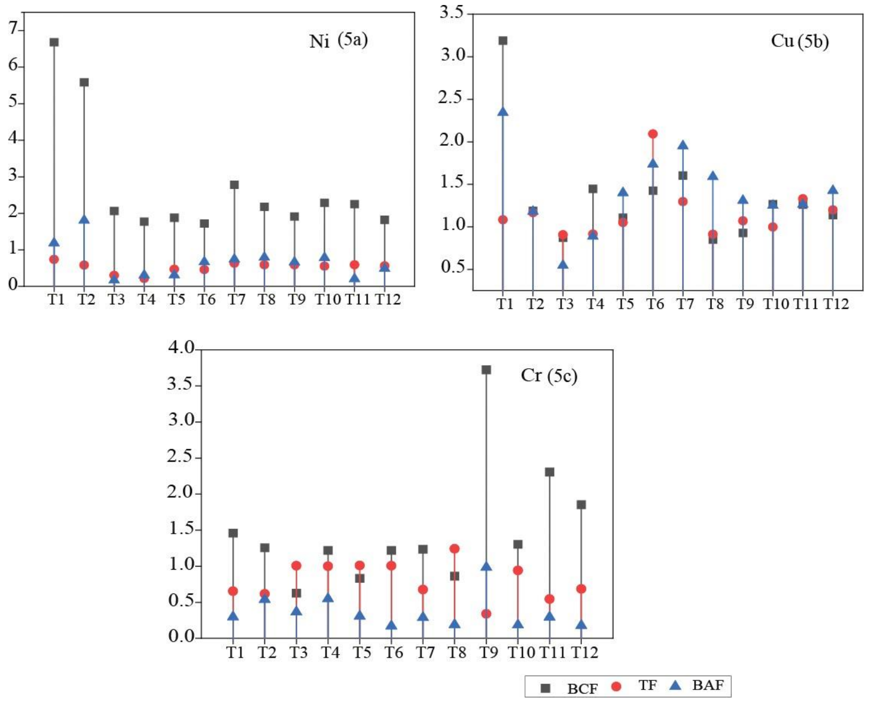

Fig. 5: BCF- bioconcentration factor; TF- translocation factor, BAF- bioaccumulation factor of Ni, Cu, and Cr

significant with Cu followed by Ni and Cr (r-Cu= +0.93; r-Ni= +0.75, r-Cr= +0.59).

Prediction of toxic metals in grain

Solubility-free ion activity model (FIAM) is a mechanistic approach through which grain metal content was predicted without actually measuring their solubility (Golui *et al*., 2014; Mandal et al., 2019). The values coefficient viz. C, â1, and â2 of FIAM for three metal Cr, Cu, and Ni was calculated and tabulated in Table 3. Results implied that approximate 88% variability of Cr, 95% variability of Cu, and 80% variability of Ni content was explainable by the model based upon pH, organic carbon, and extractable (labile pool) metal. These three measurements were used as input assuming that the organic matter adsorbs the extractable metal. From the result, it was well evident that pH and organic carbon had a negative effect on Cr and Ni solubility (â1 and â2 value positive) whereas organic carbon had a positive effect (â1 negative value) in Cu solubility. The negative effect explains that an increase in each organic carbon or pH will diminish the metal content (Cr, Ni) in mung bean grain, while, the negative â1 value demonstrated the positive impact of organic carbon in Cu uptake in grain. The uptake can be justified as Cu is a micronutrient required for many physiological processes. In addition, they affect membrane permeability. Since, humic substances contain both hydrophobic and hydrophilic sites they might have a strong interaction with phospholipid structure of cell membrane thereby reacting as a carrier of nutrients through them (David *et al*., 1994; Govindasmy and Chandrasekaran, 1992). The observation of our study is well associated with the findings of Golui *et al*. (2014) who found a similar trend of Cu uptake in spinach.

Bioconcentration, translocation, and bioaccumulation factors

Bioconcentration factor, translocation factor, and bioaccumulation factor are some calculative measures, used to ensure the metal distribution pattern among plant parts under different treatments (Fig. 5a-c). The bioconcentration factor (BCF) majorly deals with root metal uptake, where the trend was Ni> Cr> Cu. In general, high concentrations of Ni trigger an easy movement to the xylem and phloem for better translocation, however, the minimal concentration of plant available (approximately 2-7 mg/kg) metal across the treatments may retarded its migration that can be further validated by an inconsequential translocation rate (TF<1). Root accumulation of Cr depends on its oxidation state. Commonly, Cr (VI) is soluble and can easily absorb by roots nevertheless a lower translocation (TF<1) was a benefit for the tested crop. A high translocation factor (TF) i.e., >1 concludes greater mobility from root to aerial parts. TF varies from 0.9-2.09 for Cu, 0.3-0.8 for Cr, and 0.2-0.7 for Ni which indicated Cu had higher translocation rate than Ni and

Table 1: Treatment combinations for two-year crop trial (2021-2022)

Pot name	Treatment combination
T1	Control (No amendment)
T2	Fertilizer (NPK)100%
T3	Vermicompost (1:1) 100%
T4	Vermicompost (1:1) 50% + Fertilizer (NPK) 50%
T5	Vermicompost (1:2) 100%
T6	Vermicompost (1:2) 50%+ Fertilizer (NPK) 50%
T7	Vermicompost (1:3) 100%
T8	Vermicompost (1:3) 50% + Fertilizer (NPK) 50%
T9	Vermi compost (1:4) 100%
T10	Vermicompost (1:4) 50% + Fertilizer (NPK) 50%
T11	Cow dung 100%
T12	Cow dung 50% +Fertilizer (NPK) 50%

Table 2(a-b): Effect of different treatments on growth and yield attributes (mean±SE of two years)

(2a)

Treatments	Plant height (cm)	No. of branches plant^{-1}	No. of nodules plant^{-1}	No. of pods plant^{-1}	Total no of seeds plant^{-1}
T1	28.33±1.10	5.33±0.33	13.33±2.60	7.00±0.71	61.00±5.86
T2	39.74±0.70	11.67±0.33	22.67±2.91	29.00±2.12	237.33±8.84
T3	28.76±1.09	7.67±0.33	15.33±2.33	13.33±3.49	165.67±10.84
T4	28.81±0.67	10.33±0.88	16.00±3.00	17.67±3.49	196.33±4.18
T5	29.65±0.78	7.67±0.67	17.00±1.53	13.33±3.49	159.33±17.33
T6	39.98±0.50	11.33±0.67	28.00±1.00	29.67±2.86	239.00±5.77
T7	35.61±0.93	7.33±0.88	19.00±3.21	13.33±1.47	138.33±6.12
T8	38.19±0.54	8.33±0.88	27.33±2.91	26.33±1.78	206.00±8.33
T9	34.05±1.62	8.33±0.88	21.00±6.08	15.67±2.86	169.00±5.86
T10	33.58±0.72	9.33±0.88	21.67±3.18	19.00±0.71	192.67±8.97
T11	34.96±1.13	9.00±0.00	19.00±0.58	16.67±1.78	173.33±15.19
T12	45.92±0.49	10.67±0.67	22.00±3.21	24.00±2.12	177.33±16.83
LSD	**1.25**	**0.96**	**4.27**	**1.38**	**1.3**
p	**<0.05**	**<0.05**	**0.047**	**<0.05**	**<0.05**

(2b)

Treatment	Root length (cm)	Shoot length (cm)	Pod length (cm)	No. of seeds pod^{-1}	100 seed weight (gram)
T1	6.97±0.12	20.63±0.82	4.50±0.60	3.00±0.58	3.07±0.36
T2	7.53±0.54	30.93±1.82	6.30±0.06	8.00±0.00	6.20±0.37
T3	9.70±0.56	20.93±0.79	5.13±0.13	6.67±0.33	4.67±0.08
T4	8.13±1.64	17.53±1.28	6.17±0.03	8.00±0.00	5.73±0.15
T5	9.07±1.49	19.13±0.81	5.90±0.06	7.33±0.67	5.13±0.29
T6	9.57±1.07	29.60±0.68	6.30±0.15	8.00±0.00	6.70±0.12
T7	7.40±0.45	29.03±1.47	5.93±0.29	7.00±0.58	5.47±0.43
T8	11.80±1.81	27.47±0.62	6.17±0.15	8.33±0.33	6.33±0.27
T9	9.10±1.63	27.07±1.56	5.97±0.12	6.67±0.33	5.33±0.27
T10	11.23±0.64	20.97±1.52	6.17±0.09	8.00±0.00	6.50±0.07
T11	9.60±0.10	27.17±1.27	6.00±0.15	7.33±0.67	5.87±0.04
T12	9.30±0.90	35.77±2.02	6.27±0.18	7.67±0.33	6.37±0.29
LSD	**1.57**	**1.84**	**0.31**	**0.57**	**0.29**
p	**0.25**	**<0.05**	**<0.05**	**<0.05**	**<0.05**

Table 3: Prediction of metal (Cr, Ni, Cu) content in green-gram grains by solubility-free ion activity model

Metal	Model parameters			
	C	**β1**	**β2**	**R^2**
Cr	-2.25	0.29	0.18	0.88
Ni	-2.53	0.33	0.11	0.83
Cu	-1.79	-0.39	1.49	0.96

*Values of R^2 are significant at 5% probability level.

Supplementary Tables

Table S1: General characteristics of red mud (RM) (values±standard error)

Attributes	Results
pH	11.2±0.9
EC (mS/cm)	5.02±1.2
TOC (%)	0.30±1.2
Total N (%)	0.028±0.4
Available Na (%)	6.5±2.6
Available K (%)	0.04±0.9
Available P (%)	0.0015±0.2
N_2 fixing microbial load	nil
Phosphate solubilizing microbial load	nil

Table S2: Formulae for different indices

1.	$Nutrient\ benifit\ ratio = \frac{Average\ concentration\ 60D - Average\ concentration\ 0D}{Average\ concentration\ 0D}$
2.	$Bioconcentration\ factors\ (BCF) = \frac{Croot}{Csoil}$
3.	$Translocation\ factor\ (TF) = \frac{Cshoot}{Croot}$
4.	$Bioaccumulation\ factor\ (BAF) = \frac{Cgrain}{Csoil}$
5.	$EDI = \frac{IngR\ X\ C}{BW}$ where EDI = estimated daily intake of metal from consumed crop (mg/d/ BW (kg), IngR = ingestion rate (kg) of, C = concentration of metal in the sample (mg/kg), and BW = body weight (kg). The average pulses consumption rate worldwide (0.021 kg/person/ day) and BW values for adults (70 kg) respectively (Rashid *et al*., 2022).
6.	$HQ = \frac{EDI}{RfD}$ HQ=Hazard quotient; If the HQ > 1, the average daily dose (ADD; mg kg/body weight/ day) of metal exceeds the reference dose (RfD; mg kg/body weight/day), demonstrating that there is a possible risk related with metal intake. The oral reference doses used for the calculation were 1.5, 0.02 and 0.04 mg/kg/day for Cr, Ni, and Cu respectively (USEPA 2010). Total hazard quotient is ?HQ.
7.	CR= EDI X CSF Where CSF is the cancer slope factor. The Carcinogenic slope (CSF) value of Ni and Cr is 0.84, and 0.5 respectively (USEPA; United State Environmental Protection Agency 2015).

Table S3: General characterization of applied red mud vermicompost (mean ±SE data)

Attributes	Results
pH	7.6±0.9
EC (mS/cm)	1.02 ±1.2
TOC (%)	2.8±0.9
Total N (%)	0.25±0.48
Total P (mg kg^{-1})	1908±3.8
Total K (mg kg^{-1})	3261±5.5
Available N (mg kg^{-1})	920±6.9
Available P (mg kg^{-1})	286±4.9
Available K (mg kg^{-1})	1023±6.7
N_2 fixing microbial load (CFU/g)	1.4 x 10^5
Phosphate solubilizing microbial load (CFU/g)	1.2 x 10^5

Table S4: Estimated daily intake of heavy metals (Cr, Ni, Cu) on consumption of *Vigna radiata* L.

		EDI	
Treatments	**Cr**	**Ni**	**Cu**
T1	0.001032	0.001591	0.003582
T2	0.000936	0.001531	0.001805
T3	0.00066	0.000287	0.000886
T4	0.00047	0.000534	0.001359
T5	0.00083	0.000537	0.002391
T6	0.000296	0.001321	0.002966
T7	0.000763	0.000813	0.002632
T8	0.000495	0.001306	0.002572
T9	0.000896	0.001126	0.002353
T10	0.000329	0.001246	0.001799
T11	0.000507	0.0003	0.001931
T12	0.000304	0.001066	0.002563

➢ MTDI Maximum tolerable daily intake Cu 30*, Ni 0.30**, Cr 0.20***
*(JECFA 2003)
**(WHO 1996)
***(RDA 1989

Table S5: Non-carcinogenic and Carcinogenic risk on consumption of *Vigna radiata L.*

Treatment	HQ	CR			
	Cr	**Ni**	**Cu**	**Cr**	**Ni**
T1	0.000803	0.079541	0.071647	3.89E-05	0.000101
T2	0.000728	0.076541	0.036097	3.53E-05	9.69E-05
T3	0.000513	0.014332	0.017713	2.49E-05	1.82E-05
T4	0.000365	0.026714	0.027184	1.77E-05	3.38E-05
T5	0.000645	0.026864	0.047822	3.13E-05	3.4E-05
T6	0.00023	0.066034	0.059322	1.12E-05	8.36E-05
T7	0.000593	0.04067	0.052635	2.88E-05	5.15E-05
T8	0.000385	0.065284	0.051441	1.87E-05	8.27E-05
T9	0.000697	0.056277	0.047069	3.38E-05	7.13E-05
T10	0.000256	0.062291	0.035977	1.24E-05	7.89E-05
T11	0.000394	0.015007	0.038618	1.91E-05	1.9E-05
T12	0.000236	0.053277	0.051266	1.14E-05	6.75E-05

*CR risk limit according to USEPA $1x10^{-4}$ to $1x10^{-6}$

Supplementary Figure:

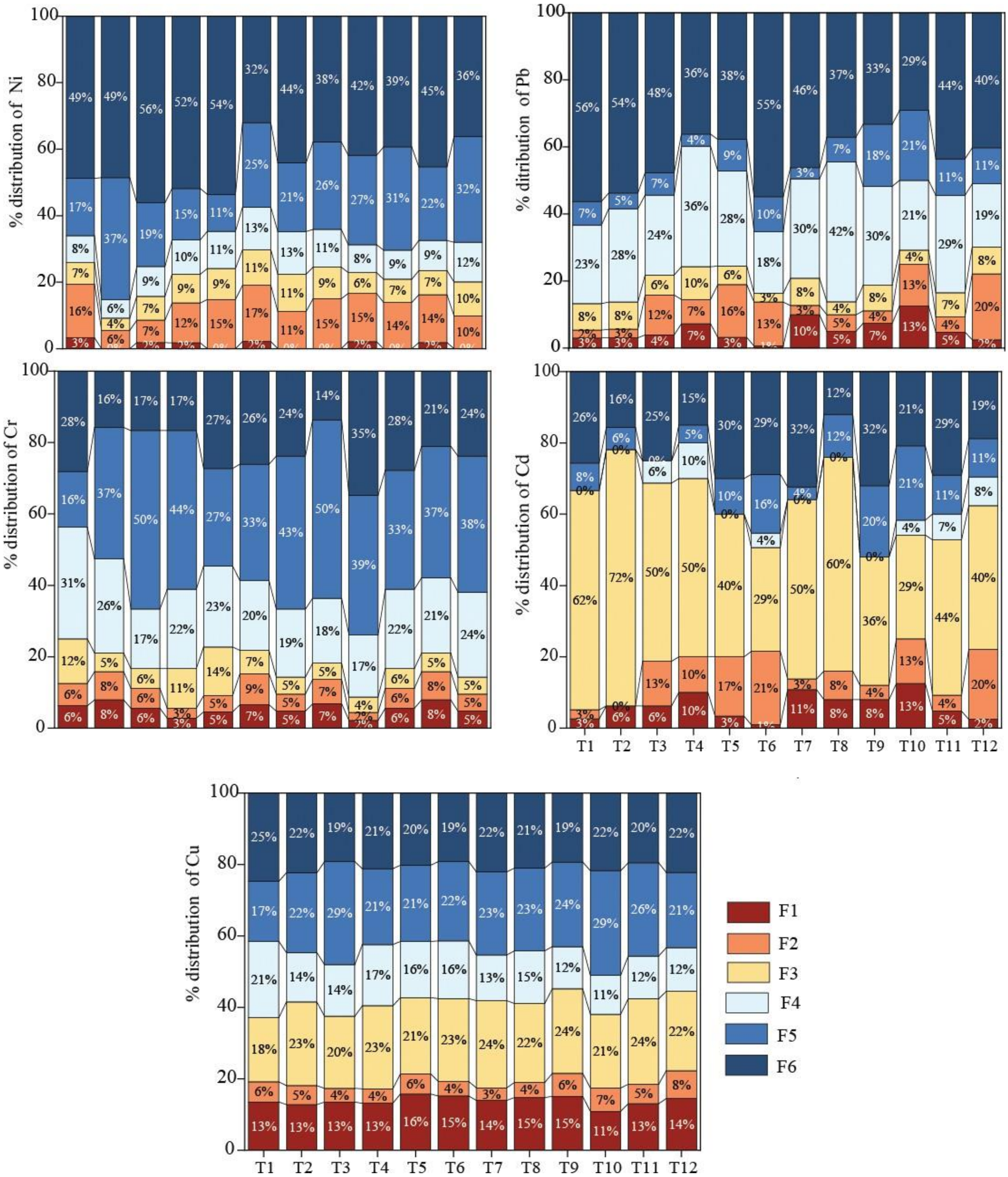

Fig. S1: Distribution of different metals (Cr, Ni, Cu, Pb, Cd) in experimental soil after harvesting

Cr across the treatments. Cu use symplastic pathway for its translocation additionally it forms a metal complex with nicotianamine that get translocated toward developing organ and matured seeds (Islam et al., 2020; Pasricha et al., 2021). Metal mobilization turns crucial when it comes to bioaccumulation which was found to be lower for Cr (0.17-0.8) and Ni (0.2-1.2) except Cu (0.5-2.3) for the tested crop.

Dietary exposure

The estimated daily risk (EDI) of the studied metal was found to be remarkedly lower across the treatments including control soil for all three metals (Table S4). EDI for Cu ranged between 0.002-0.0008, Ni varied between 0.001-0.0003 and Cr ranged between 0.001-0.0003, which is significantly lower than the suggested values. Health risks associated with metal intake through grain feeding are presented in the corresponding HQ (Table S5). Results demonstrated that the HQ values were far less than 1 for all the metals amid the treatments, out of which Cr showed lowest HQ indicating that intake of VC and VC+NPK-fertilizer-treated mung bean will be safe for human consumption. Yet a brief comparison among treatments exhibited control soil had a higher HQ compared to the amended one. Our findings concur with Mandal *et al*. (2019), who found a reduced HQ for As in organically amended soil. On other hand, the calculated CR of Cr ranged between 0.00003 to 3.8E-05 while Ni was from 0.0001 to 9.6E-05 which comes within limits (Table S5). Thus, from a health perspective the cultivated crop receiving organic or composite (50%VC+50%NPK) treatments, was found to be hazard free for consumption.

Application of two different dosages (100% organic; 50%NPK+ 50% organics) for two consecutive years provided a twining benefit to plant growth and soil fertility. Nutrient solubilization (N, P, K) and soil microbial activity was enhanced while migration of toxic metals got restricted determined by FIAM. Grain metal uptake within permissible limits ensures safe consumption. Composite treatments provided an improved pulse yield. Inclusively, vermitechnology could be a beneficial tool for turning poorly fertile soil into productive one, and recycled red mud could be an alternative nutritional source for plants. However, thorough washing of RM should be done to remove excess sodium content cautiously before earthworm employment, and additionally, regular monitoring during the vermiconversion process is required. To ensure the suitability of RM-vermicompost, it should be tested under field conditions with staple food like rice for its long-term applicability.

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