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ORIGINAL RESEARCH

## Optimizing vermicompost and cobalt application for enhancing rice (*Oryza sativa* L.) yield in Inceptisol

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### Abstract

**Purpose:** Rice is an essential diet for more than half of the world's inhabitants and subjected to growth in heavy metal-contaminated soil. Rice accumulates heavy metals in the above and below ground parts; the magnitude of accumulation depends upon the plant's available form of heavy metals in the soil. One such heavy metal is cobalt, which its concentration has been manifolded in post-industrial activities, leading to a high soil Co concentration. Cobalt is a component of Vitamin B<sub>12</sub> and many enzymes. Humans can't synthesize these enzymes, and consumption of Co-enriched grain can lead to Co toxicity in humans. The present experiment aimed to evaluate the effect of vermicompost conjugated with an optimum dose of Co for rice plants grown in an Inceptisol.

**Method:** The experiment was conducted using a completely randomized design (CRD) comprising nine treatments. The treatment received vermicompost, Co in the form of cobalt chloride, and the recommended dose of fertilizer (RDF).

**Results:** The results indicated that the application of Co at lower concentration was beneficial for rice growth without any hyperaccumulation, resulting in a significant increase of 46% and 22% in grain and straw yield, respectively. A comparable trend was also observed for Nitrogen uptake, which increased by 2 and 1.5 times over RDF in grain and straw, respectively. In contrast, higher Co application rates, either alone or in combination with vermicompost, resulted in a 10–15% reduction in cobalt uptake in grains. These findings suggest that vermicompost plays a critical role in regulating and optimizing cobalt uptake under higher Co application levels.

**Conclusion:** Overall, vermicompost and the lower doses of Co provide a better yield in rice without any phytotoxicity.

**Keywords:** Hyperaccumulation, Phytotoxicity, Nitrogen, Uptake, Heavy metal

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## 1. Introduction

Rice as one of the most important cereals in the world, (*Oryza sativa* L.), occupies around 10% of arable land and comprises more than half of the world's population's primary diet. Additionally, it is essential to social and economic stability (Shukry et al., 2022). In addition to providing critical minerals, rice accounts for 30% of global energy use. Global rice demand is projected to reach 852 million tons by 2035, representing a 26% increase compared with 2010 levels (Javdan et al., 2021). Rice, a well-known semi-aquatic plant, mostly prefers wetland cultivation and has a varying degree of water requirement depending on growth stages. Rice productivity is constrained by several challenges such as soil degradation, nutrient imbalances, and climate stress, resulting in stagnant or declining yields. Excessive tillage and intensive cropping reduce soil health and water availability, while labour shortages and pest pressures further limit rice production. These factors threaten yield stability and necessitate sustainable management to enhance productivity. Oil moisture is one of the most critical factors influencing rice productivity and has significant implications for global food security. It affects rice plants, including leaf area, root length, plant height, and number of panicles. Rice is especially sensitive to moisture stress throughout its reproductive growth stage, which impacts the distribution and accumulation of the dry matter (Zhang et al., 2018; Alou et al., 2018).

Vermicomposting is widely understood to be the solid-phase breakdown of organic waste in an aerobic environment, utilizing the optimal biological activity of earthworms and microorganisms. Therefore, vermicomposting is an intriguing method for recycling the growing volume of organic waste while also reducing the need for fertilizer. Additionally, its application raises the amount of organic matter in the soil, which is essential for its long-term soil fertility (Dignac et al., 2017). The gut microbiota of earthworms and native microorganisms secrete a variety of hydrolytic enzymes, which greatly increase the availability of N, P, and K in vermicompost. Complex compounds are efficiently broken down by these enzymes, demineralizing vital nutrient elements (Chakraborty et al., 2022). By improving water and nutrient availability, reducing soil bulk density, and increasing organic content, vermicompost can significantly enhance soil health for rice production, which is highly dependent on fertile soil and efficient nutrient management. This results in improved growing circumstances for rice plants, which may boost yields and enhance plant health in general. Additionally, vermicompost's hormone-like activities can promote plant resilience and growth, which is especially helpful in addressing potential biotic and abiotic challenges for rice crops (Blouin et al., 2019). It plays a key role in managing the cobalt toxicity in the following ways: (i) improving the soil reaction, rendering cobalt unavailable to plant root, (ii) enhancing the water stable soil aggregates, which makes the cobalt immobilized, (iii) accumulating of cobalt in the body tissues of the earthworm, and (iv) forming stable complexes, that hinder the movement of cobalt toward plant roots (Dume et al., 2022). Through protein-assisted metal complexation process, earthworm-assisted vermicomposting successfully converts the exchangeable fraction of heavy metals into the refractory fraction, which is greatly decreased and immobilized and accumulated in their intestine. Earthworms use chloragosomes to absorb and hold



onto heavy metals in an insoluble state without interfering with the cytosolic process. The synthesis of fulvic acid and humic acid during vermicomposting is essential for the immobilization of heavy metals (Alam et al., 2024). Vermicompost enhances rice growth and yield by improving soil fertility and microbial activity. It reduces cobalt toxicity by lowering metal uptake in plants, promoting nutrient availability, and leading to sustainable production. Cobalt is an essential heavy metal for both animals and prokaryotes and is a constituent of many enzymes including Vitamin B12. Although its importance in plant physiological processes has not been established, it is regarded as a crucial but non-essential nutrient that may have a variety of effects on plant metabolism and growth (Awasthi et al., 2022). Some scientific work noted that Co, at optimum concentration, increases the water content of the leaf and reduces water stress. It also helps in water absorption, tightly binding water in leaf tissue, and increases cytoplasmic pressure (Kaura et al., 2015). Concentration is important since high soil concentrations can be detrimental to plant health. It can specifically harm seed germination, photosynthetic dynamics, plant growth, and metabolic processes (Wang et al., 2020). If the concentration is ideal, this element can boost plant development and is even required for certain taxa, like leguminous plants. However, it has significant effects at lower doses that are often overlooked because of its negative effects. The indiscriminate use of agrochemicals and agronomic practices leads to higher uptake of cobalt in crop plants (Panda et al., 2024). It is increasingly clear that for the groups that rely entirely on rice diets for subsistence, rice may be a significant source of Cobalt. In its inorganic form, Co is not regarded as a necessary element for humans. Overconsumption of Co can harm human and animal health in genotoxic, hepatotoxic, nephrotoxic, neurotoxic, and immunotoxin ways (Khanam et al., 2020).

Gaining a thorough understanding of the beneficial effects of Co on plants could be a useful strategy for accomplishing food security objectives and boost agricultural productivity to meet the need of the world's expanding population (Nunes da Silva et al., 2022; Tourky et al., 2023). Earlier research work was mostly focused on the foliar application of cobalt for mid-season correction of the micronutrient, and no significant work was reported regarding the soil application and along with the application of some organic material to study their interaction and how it is affecting soil properties and nutrient uptake. So, keeping all these research gaps in mind, the current experiment was designed with the following objective: the interaction effect of cobalt and vermicompost on the growth and yield attributes of rice, nutrient uptake, and post-harvest soil properties. But the broader goal was to determine the optimal cobalt level that boosts rice productivity and has a positive impact on plant development and growth.

## 2. Materials and Methods

### 2.1. Study area

The experiment was carried out in the Department of Soil Science and Agricultural Chemistry's Net house at Institute of Agricultural Sciences of Banaras Hindu University, Varanasi, India (25°26' N, 82°99' E and 80 m above mean sea level), to study the influence of different cobalt concentrations and vermicompost application on rice growth and yield. The climatic condition of the study area was semi-arid to sub-humid climate,



featuring an average rainfall of 1100 mm and temperature fluctuations between 9°C (minimum) and 42°C (maximum) during the experiment period.

## 2.2. Experiment design

The pot experiment was carried out during the Kharif season of 2023-24, and Rice was taken as a test crop (variety Samba Mahsuri (BPT 5204). The treatment details are presented in Table 1. The soil was collected from the Agricultural Farm, BHU, and air dried, passed through a 2 mm sieve, and filled into the 10 kg earthen pots with polythene lining. Using standard techniques, the physicochemical characteristics of the soil were ascertained and are shown in Table 2. A total of nine treatments is allocated with three replications in a completely randomized design (CRD). The recommended dose of fertilizer (RDF) was 150:60:60:25 kg/ha N-P-K-Zn, and the sources of fertilizer are urea, Di Ammonium Phosphate (DAP), Muriate of Potash (MOP), and Zinc sulphate. Cobalt was applied in soil as a basal application, and the source was Cobalt chloride hexahydrate ( $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ), and the doses ranged from 5 to 15 ppm, as cobalt is an essential trace element for humans and plants. its uptake in the plant and average soil value is also low.

**Table 1.** Treatment details of the experiment

Treatments code	Treatments details
T1	RDF
T2	RDF + Co 5
T3	RDF + Co 10
T4	RDF + Co 12.5
T5	RDF + Co 15
T6	RDF + Co 5 + VC
T7	RDF + Co 10 + VC
T8	RDF + Co 12.5 + VC
T9	RDF + Co 15 + VC

(RDF: Recommended dose of fertilizer, VC: Vermicompost, Co: Cobalt, Co 5: 5 ppm of Co, similarly 10, 12.5 and 15 ppm)

**Table 2.** Physicochemical properties of the initial soil.

Parameters	Value	Reference method
pH	8.3	Jackson (1973)
EC ( $\text{dS m}^{-1}$ )	0.27	Jackson (1973)
Organic carbon (%)	0.52	Walkley & Black (1934)
Available N ( $\text{kg ha}^{-1}$ )	197.57	Subbiah & Asija (1956)
Available P ( $\text{kg ha}^{-1}$ )	23.6	Olsen (1954)
Available K ( $\text{kg ha}^{-1}$ )	147.8	Hanway & Heidel (1952)
Available Fe ( $\text{mg kg}^{-1}$ )	7.24	Lindsay & Norvell (1978)



Available Mn (mg kg <sup>-1</sup> )	5.56	Lindsay & Norvell (1978)
Available Cu (mg kg <sup>-1</sup> )	1.21	Lindsay & Norvell (1978)
Available Zn (mg kg <sup>-1</sup> )	0.46	Lindsay & Norvell (1978)
Available Co (mg kg <sup>-1</sup> )	0.15	Lindsay & Norvell (1978)

(pH: potential of hydrogen; EC: electrical conductivity)

Vermicompost was produced through the biodegradation of organic substrates by earthworms, using a mixture of cow dung and household organic waste in a 1:1 ratio at the University horticulture farm. The composting process was allowed to stabilize for 90 days, after which samples were collected for detailed physicochemical characterization. The prepared vermicompost exhibited favorable properties, including a neutral pH (7.2) (Jackson, 1973), high organic carbon content (28%) (Walkley & Black, 1934), and appreciable nutrient concentrations of nitrogen (2.1%) (Jackson, 1973), phosphorus (1.18%) (Olsen & Sommers, 1982), and potassium (1.65%) (Hanway & Heidel, 1952). Additionally, it showed a bulk density of 0.75 Mg m<sup>-3</sup> (Grossman & Reinsch, 2002) and a maximum water holding capacity of 21% (Black, 1965), indicating its suitability as a soil amendment. The amount of vermicompost application was 5 t ha<sup>-1</sup>. Four seedlings were maintained in each pot, and the pots were irrigated at regular intervals to maintain a saturated condition.

### 2.3. Growth and yield parameters

Seedling of each pot was used to observe for growth and yield attributes like plant height (cm) at 30, 60, 90 days after transplanting (DAT), chlorophyll content (SPAD value) at 30, 60 DAT, number of tillers, panicle length (cm), total number of grains per pot, grain yield, straw yield, 1000 grain weight, and Harvest index (%).

The harvest index was calculated using the formula:

$$HI(\%) = \frac{\text{Economic yield} \times 100}{\text{Biological yield}}$$

### 2.4. Post-harvest plant analysis

Plants from all three replications were collected separately and distinctly, tagging straw and grain samples from each pot. The mean value of the three replications was used for further data analysis. The collected plant samples were initially washed with 0.1N HCl and further rinsed with distilled water before drying them in the oven at 55°C. After drying, the plant samples were crushed to a fine powder. Further, it was treated with diacid (HNO<sub>3</sub>: HClO<sub>4</sub> in 9:4 (v/v) to digest the plant material (Page et al., 1982). Upon completion of digestion, the digestate volume was made up to 100 mL. The micronutrient (Fe, Mn, Zn, and Cu) content was determined using an atomic absorption spectrophotometer using Diethylene triamine penta-acetic acid (DTPA) extractant (Lindsay & Norvell, 1978). Total P in straw and grain was determined using Bartons reagent (Olsen and Sommers, 1982). A flame photometer was used to calculate the total K (Hanway & Heidel,



1952). Total nitrogen was measured by digesting straw and grain samples using triacid mixture and H<sub>2</sub>SO<sub>4</sub> in a block digester (Bremner, 1996).

## 2.5. Post-harvest soil analysis

Each pot's soil was gathered, allowed to air dry, crushed, and then sieved through a 2 mm screen before being used for additional examination. Available N was amount by Kjeldahl method (Subbiah & Asija, 1956), available P was measured by yellow colored hetero poly phosphor molybdic complex (Olsen, 1954), available K was determined by flame photometer (Hanway & Heidel, 1952) and available micronutrients were extracted using DTPA extractant (Lindsay & Norvell, 1978) and measured using AAS (Atomic Absorption Spectrophotometer).

## 2.6. Translocation factor (TF)

The translocation factor is defined as the concentration of Co in the grain to that in shoots (Xue et al., 2017).

$$TF = \frac{C_g}{C_s}$$

Where, C<sub>g</sub>: concentration of Cobalt, in grain and C<sub>s</sub>: concentration of Cobalt in straw.

## 2.7. Statistical analysis

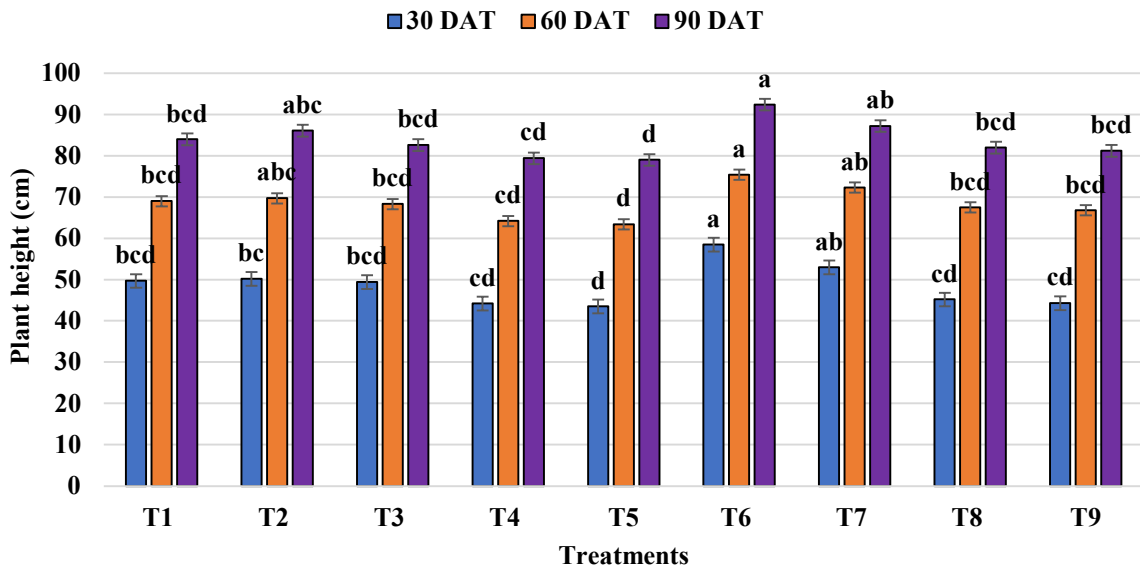
The test of significance between treatments was tested by ANOVA as follows by completely randomized design (CRD) at the 5% level of significance. SPSS 22.0 was used for all statistical evaluations. The Duncan multiple range test (DMRT) was used to determine the significance level between the treatment averages at the  $p \leq 0.05$  levels (Gomez & Gomez, 1984).

## 3. Results and Discussion

### 3.1. Growth and yield attributes of rice

The height of the rice plant varies from 43.5 to 58.5 cm, 63.4 to 75.4 cm, and 79 to 92.4 cm at 30, 60, and 90 DAT, respectively (Fig. 1). Treatment T6 recorded the highest height in all three observations, with a corresponding increase of 18%, 9%, and 10% over RDF (T1) at 30, 60, and 90 DAT, respectively. In contrast, T2, which only received the Co application, recorded increases of 1%, 1%, and 2.5%, which were non-significant. Treatment receiving 15 ppm of cobalt (T5) shows reductions of 12%, 8%, and 6% in plant height at 30, 60, and 90 DAT, respectively, compared to RDF (T1)



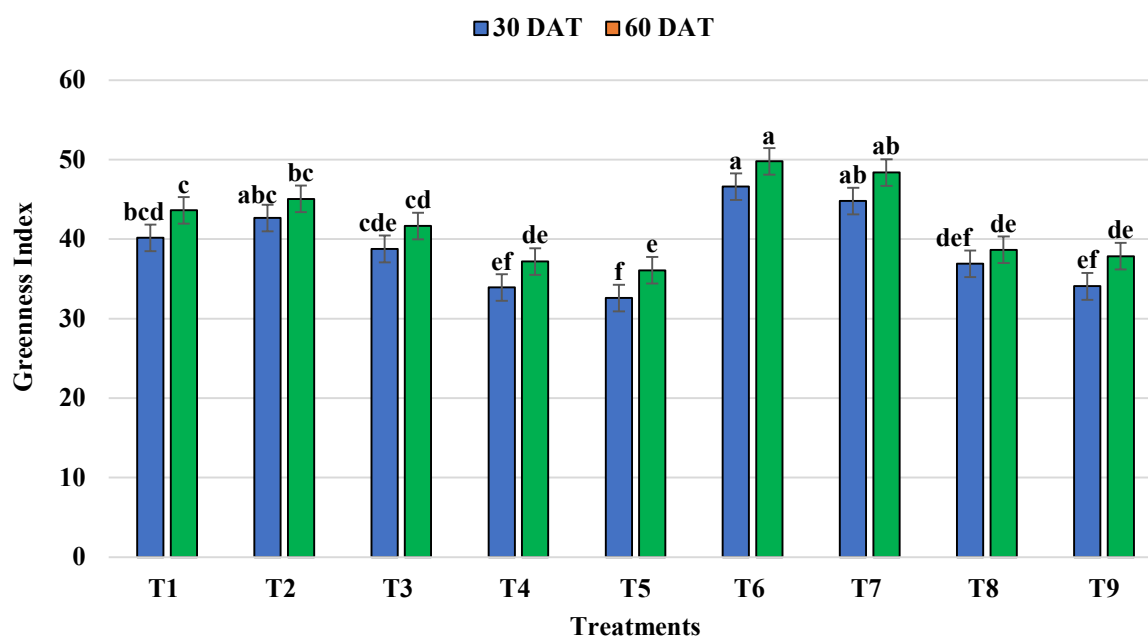


**Figure 1.** Effect of cobalt application along with vermicompost on the plant height of rice at 30, 60, and 90 days after transplanting (DAT)

(Different letters for each parameter show a significant difference at  $p \leq 0.05$  by Duncan's Multiple Range Test). (Data represents mean  $\pm$  SE of three independent replications and error bars identify standard errors of different treatments).

Among the treatments, T6 recorded the highest greenness index, at 46.62 and 49.8 at 30 and 60 DAT, corresponding to a 16% and 14% significant increase over RDF (Fig. 2). However, T2 had non-significant increases of 6% and 3% over RDF at 30 and 60 DAT, respectively. T5 showed a significant reduction in the greenness index compared to T1, with reductions of 19% and 17% at 30 and 60 DAT, respectively. The treatment, T7 was found to be statistically similar to T6, while T3, exhibited a significant reduction to T7, of 13% and 14% at 30 and 60 DAT, respectively.





**Figure 2.** Effect of cobalt application along with vermicompost on the greenness index (SPAD value) of rice at 30 and 60 days after transplanting (DAT)

(Different letters for each parameter show a significant difference at  $p \leq 0.05$  by Duncan's Multiple Range Test). (Data represents mean  $\pm$  SE of three independent replications and error bars identify standard errors of different treatments).

Table 3 represents yield attributes, grain yield, straw yield, and harvest index. Among treatments, T6 had the highest number of tillers (9 hill<sup>-1</sup>), followed by T7 (8 hill<sup>-1</sup>) and T2 (8 hill<sup>-1</sup>) with a significant increase of 29%, 14%, and 14% as compared to RDF, while T5 had 43% lowest number of tillers (4 hill<sup>-1</sup>). Treatments receiving the higher dose of cobalt show a significant reduction in tiller number as compared to RDF. The total number of grains per pot ranges from 740 to 1050. The highest number of grains observed was in T6 (1050), 19% more, and the lowest in T5 (740), 16% less than RDF, respectively. Moreover, T2 had 940 total of grains per pot, which is 7% increase over RDF but statistically at par. Treatment T7 was found to be statistically at par with T6, while T8, T9, T4, and T5, receiving higher doses of Cobalt, are statistically similar. The panicle length of rice varies from 13.43 to 22.97 cm in T5 and T6 treatments, which is 21% less and 35% more compared to RDF, respectively. Treatments, such as T6, T7, T1(RDF), and T2 are yielding no significant difference in panicle length, while treatments like T4, T5, T8, and T9 are yielding statistically reduced panicle length as compared to above mentioned treatments. The highest and lowest test weight was recorded in T6 and T5, comprising 23% increase and 19% decrease over RDF, respectively. Treatments T6, T7, T1, and T2 are statistically at par, and treatments like T4, T5, T8, and T9 are significantly below in test weight when compared with T6 i.e., 31%, 34%, 25%, and 25%, respectively. The highest grain (24.51) and straw yield (29.44) were reported in T6, which yielded 46% and 22% significant increases over RDF. Treatments like T7, T1, T2, and T3 were found to be statistically equal. All the treatments receiving the higher dose of Co above 10 ppm show a significant reduction in both grain and straw



yield. The lowest grain and straw yields were reported in T5, which had a corresponding reduction of 32% and 21% over RDF. The minimum and maximum harvest index (HI) were reported in T6 (45.43%) and T5 (37.27%). Treatments including T6, T7, T1, T2, and T3 are statistically equivalent. Treatments receiving higher doses of cobalt (T4, T5, T8, and T9) are statistically similar among themselves, and their comparison with RDF shows a reduction in HI value of 5%, 9%, 2%, and 2%, respectively.

**Table 3.** Effect of cobalt application along with vermicompost on the yield attributes and yield of rice

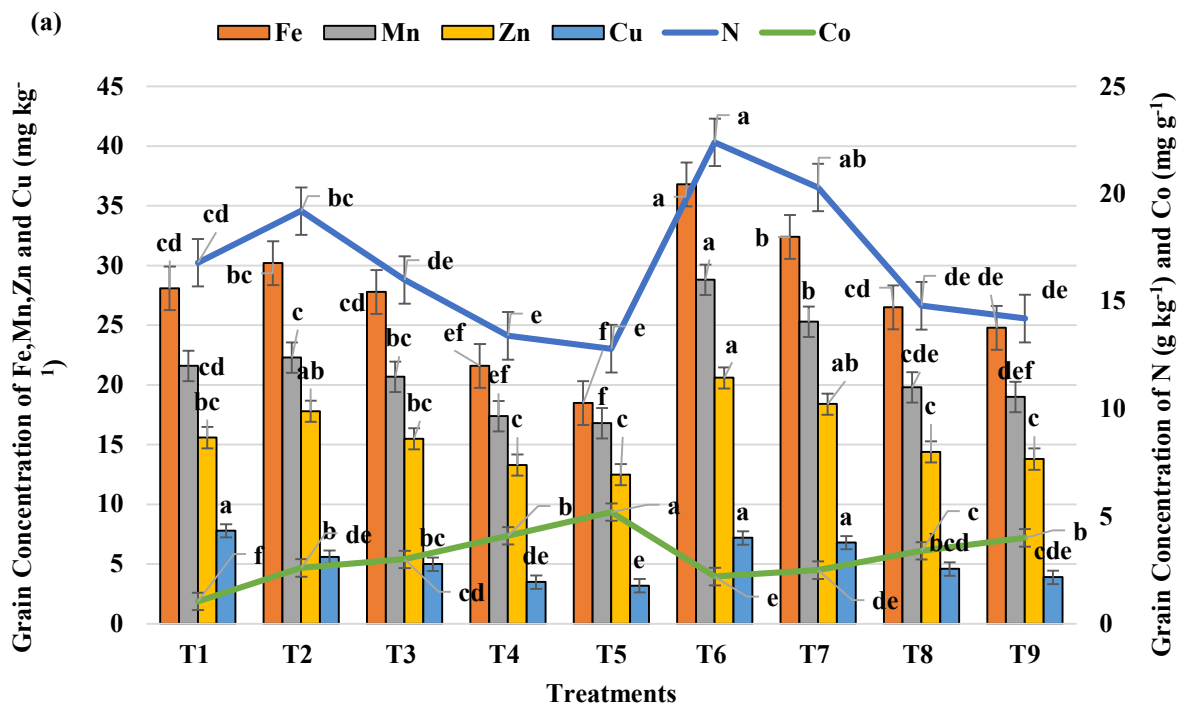
Treatments	No of Tiller hill <sup>1</sup>	Total Number of Grain pot <sup>-1</sup>	Panicle Length (cm)	1000 grain Weight (gram)	Grain Yield (gram pot <sup>-1</sup> )	Straw Yield (gram pot <sup>-1</sup> )	Harvest Index (%)
T1	7 ± 0.577 <sup>bc</sup>	880.00 ± 23.62 <sup>cd</sup>	16.97 ± 1.50 <sup>abc</sup>	19.03 ± 1.54 <sup>bc</sup>	16.75 ± 0.85 <sup>bc</sup>	24.07 ± 1.44 <sup>bcd</sup>	41.03 ± 0.57 <sup>abc</sup>
T2	8 ± 0.577 <sup>ab</sup>	940.00 ± 20.81 <sup>bc</sup>	18.62 ± 1.82 <sup>abc</sup>	19.37 ± 1.22 <sup>abc</sup>	18.21 ± 0.75 <sup>b</sup>	25.40 ± 1.77 <sup>abc</sup>	41.76 ± 1.52 <sup>abc</sup>
T3	6 ± 0.577 <sup>cd</sup>	870.00 ± 26.45 <sup>cde</sup>	16.20 ± 1.60 <sup>bc</sup>	18.88 ± 1.06 <sup>bc</sup>	16.43 ± 0.99 <sup>bc</sup>	22.87 ± 1.62 <sup>bcde</sup>	41.81 ± 1.07 <sup>abc</sup>
T4	5 ± 0.577 <sup>de</sup>	770.00 ± 35.11 <sup>f<sup>g</sup></sup>	14.00 ± 1.73 <sup>bc</sup>	16.21 ± 1.11 <sup>c</sup>	12.48 ± 0.81 <sup>de</sup>	19.70 ± 1.45 <sup>de</sup>	38.78 ± 1.34 <sup>c</sup>
T5	4 ± 0.0 <sup>c</sup>	740.00 ± 24.66 <sup>g</sup>	13.43 ± 2.33 <sup>c</sup>	15.34 ± 1.75 <sup>c</sup>	11.35 ± 0.72 <sup>e</sup>	19.10 ± 1.05 <sup>e</sup>	37.27 ± 1.18 <sup>c</sup>
T6	9 ± 0.577 <sup>a</sup>	1050.00 ± 15.27 <sup>a</sup>	22.97 ± 1.73 <sup>a</sup>	23.34 ± 1.45 <sup>a</sup>	24.51 ± 1.50 <sup>a</sup>	29.44 ± 1.44 <sup>a</sup>	45.43 ± 1.23 <sup>a</sup>
T7	8 ± 0.577 <sup>ab</sup>	990.00 ± 30.55 <sup>ab</sup>	19.93 ± 1.82 <sup>ab</sup>	21.86 ± 1.48 <sup>ab</sup>	21.64 ± 0.91 <sup>a</sup>	27.31 ± 1.19 <sup>ab</sup>	44.2 ± 2.23 <sup>ab</sup>
T8	6 ± 0.577 <sup>cd</sup>	830.00 ± 20.81 <sup>d<sup>ef</sup></sup>	15.20 ± 2.49 <sup>b</sup>	17.62 ± 1.31 <sup>bc</sup>	14.62 ± 0.87 <sup>cd</sup>	21.76 ± 1.46 <sup>cde</sup>	40.19 ± 1.06 <sup>bc</sup>
T9	6 ± 0.577 <sup>cd</sup>	800.00 ± 20.20 <sup>efg</sup>	14.80 ± 1.90 <sup>b</sup>	17.57 ± 0.86 <sup>bc</sup>	14.06 ± 1.20 <sup>cde</sup>	21.03 ± 1.38 <sup>cde</sup>	40.07 ± 1.73 <sup>bc</sup>
SEm ±	0.544	24.81	1.911	1.461	0.990	1.442	1.402
CD (p ≤ 0.05)	1.63	74.29	5.722	4.013	2.963	4.318	4.200

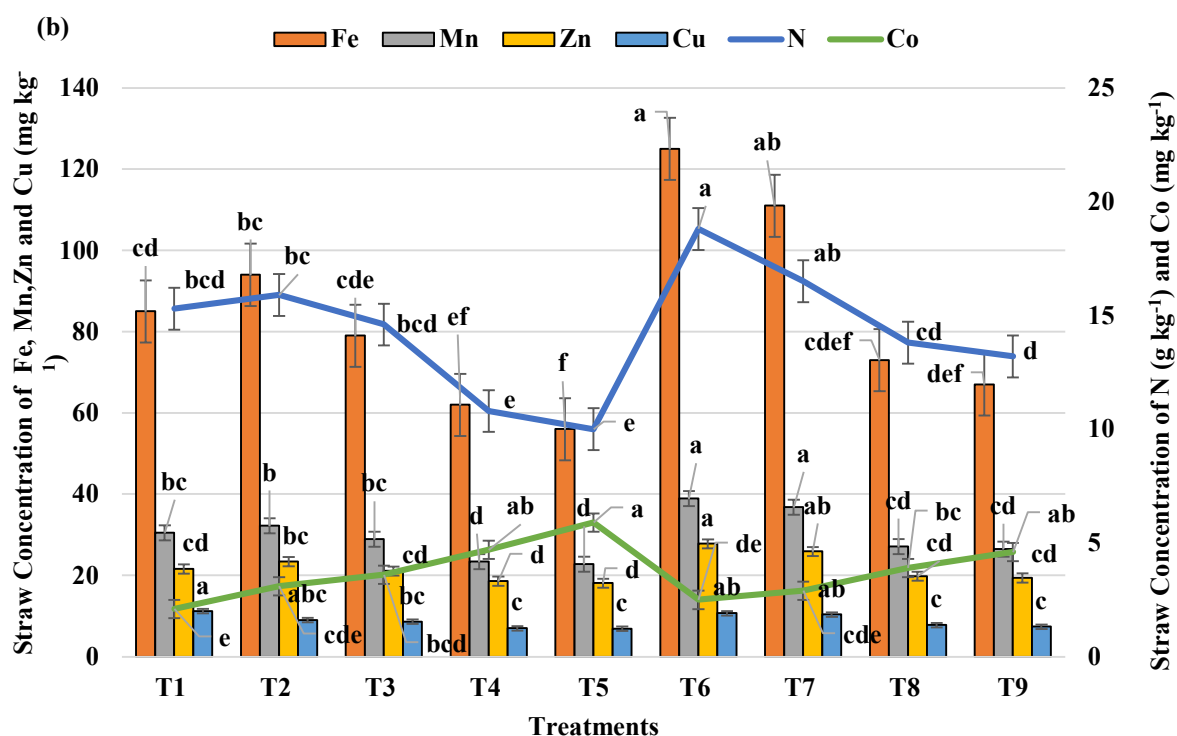
(Different letters for each parameter show significant difference at  $p \leq 0.05$  by Duncan's Multiple Range Test) (CD: critical difference; SEm: standard error of mean)



## 3.2. Nutrient concentration and uptake in grain and straw

Fig. 3 represents the result of the application of cobalt along with vermicompost on nutrient content (N, Fe, Mn, Zn, Cu, and Co) of grain and straw. The grain and straw nitrogen ranged from 1.28-2.24% and 1.0-1.88%, respectively. The highest grain and straw N reported in T6, which is 33% and 23% higher than RDF, respectively. T2 receiving only Co-5 ppm had a grain and straw N content, 14% and 4% over RDF, respectively. The lowest grain and straw N were reported in T5, 24% and 35% less than RDF, respectively. The grain micronutrients (Fe, Mn, Zn, Cu, and Co) content stretched from 18.5-36.8, 16.8-28.8, 12.5-20.6, 3.2-7.8, and 2-5.2 mg kg<sup>-1</sup>, and correspondingly, in straw, the contents were varied from 56-125, 22.8-38.9, 18.1-27.8, 6.9- 11.2, and 2.1- 5.9 mg kg<sup>-1</sup>. The maximum content of Fe, Mn, and Zn was found in T6, with an increment of 31%, 33%, and 32%, while in Cu, it is T1 (RDF), and in Co, T5 (160%) over RDF, respectively. A similar trend was reported in the case of straw with a corresponding increase of 47% (Fe), 28% (Mn), 29% (Zn), and Co (180%) over RDF. The lowest content of micronutrient in grain and straw was reported in T5 for Fe (34%, 34%) Mn (22%, 25%), Zn (20%, 16%), Cu (59%, 38%) less over RDF, respectively and T1 (RDF).



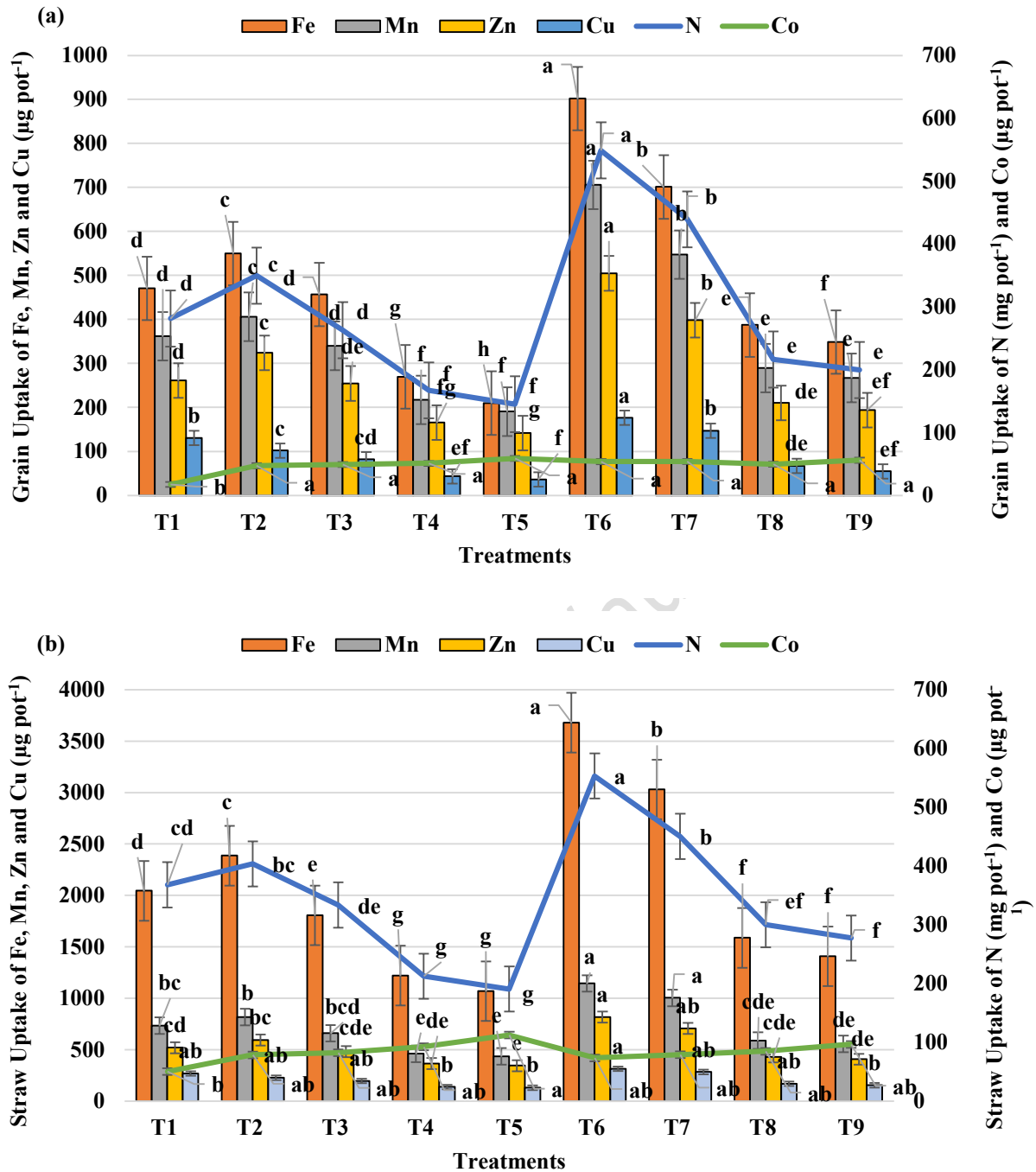


**Figure 3.** Effect of cobalt application along with vermicompost on the concentration of N ( $\text{g kg}^{-1}$ ) and micronutrient ( $\text{mg kg}^{-1}$ ) in (a) grain and (b) straw

(Different letters for each parameter show significant difference at  $p \leq 0.05$  by Duncan's Multiple Range Test). (Data represents mean  $\pm$  SE of three independent replications and error bars identify standard errors of different treatments).

The values of the uptake of N and micronutrients (Fe, Mn, Zn, Cu, and Co) in grain and straw were presented in Fig. 4. The range of N uptake in grain and straw varied from 145-549 and 191- 553  $\text{mg pot}^{-1}$ , respectively. The highest uptake of N, both in grain and straw, was recorded in T6, which is 1.95 and 1.5 times of RDF, and the lowest N uptake was recorded in T5, at 0.51 and 0.52 times of RDF. The uptake of micronutrients (Fe, Mn, Zn, Cu, and Co) in grain and straw stretched from (209-901, 191-706, 141-505, 36-176, 34-59) and (1069-3680, 435-1145, 345-818, 132-315, 50-112)  $\mu\text{g pot}^{-1}$ , respectively. The highest uptake in grain and straw was recorded in T6 for Fe (1.91 & 1.8 times), Mn (1.95 & 1.55 times), Zn (1.93 & 1.57 times), and Cu (1.35 & 1.17 times), and T5 for Co (1.78 & 2.24 times) over RDF, respectively. The lowest uptake in grain and straw was recorded in T5 for Fe (0.44 & 0.52 times), Mn (0.52 & 0.59 times), Zn (0.54 & 0.66 times), Cu (0.27 & 0.48 times) of RDF, respectively, and for Co, T1 (RDF).





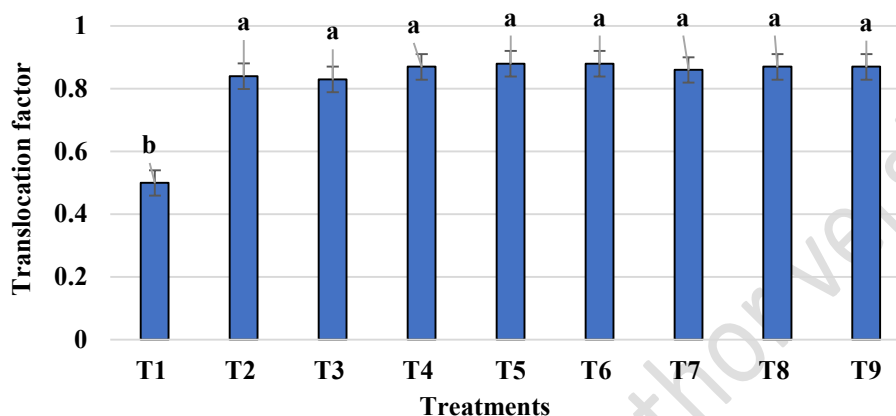
**Figure 4.** Effect of cobalt application along with vermicompost on the uptake of N ( $\text{mg pot}^{-1}$ ) and micronutrient ( $\mu\text{g pot}^{-1}$ ) in (a) grain and (b) straw.

(Different letters for each parameter show a significant difference at  $p \leq 0.05$  by Duncan's Multiple Range Test). (Data represent mean  $\pm$  SE of three independent replications and error bars identify standard errors of different treatments).



### 3.3. Translocation factor

The translocation factor (TF) of Co from straw to grain is presented in Fig. 5. T5 has the highest TF, while T1 has the lowest. Among the treatments, treatments (T2-T9) are at par with each other and statistically significance differences are observed between T1 and (T2-T9). The TF of each treatment is less than 1, denoting no hyperaccumulation of Co.



**Figure 5.** Translocation factor of cobalt from straw to grain.

(Different letters for each parameter show a significant difference at  $p \leq 0.05$  by Duncan's Multiple Range Test). (Data represents mean  $\pm$  SE of three independent replications and error bars identify standard errors of different treatments).

### 3.4. Post-harvest soil properties

The result of post-harvest soil (PHS) analysis is depicted in Table 4. The pH of the PHS ranged from 7.91 to 8.28. The highest pH was reported in T1 (RDF), and the lowest in T9 (Co-15 ppm + Vermicompost). The EC and OC ranged from 0.27 to 0.39 ( $\text{dS m}^{-1}$ ) and 4.48 to 5.84 ( $\text{g kg}^{-1}$ ). The highest EC was reported in T5 (Co-15 ppm), statistically significant over RDF, and the lowest T6 (Co-5 ppm + vermicompost). The highest OC was documented in T6 and T7, and the lowest in T1, T3, T4, and T5. Treatments receiving vermicompost (T6, T7, T8, T9) had statistically significantly more OC content compared to RDF. The available N content in PHS ranged from 213 (lowest: T5) to 310  $\text{kg ha}^{-1}$  (highest: T6). Treatment, T6, and T7 are statistically similar, with 1.16 and 1.10 times more than RDF, respectively, while T2 had N content of 281, i.e., 1.05 times and statistically equivalent to RDF. The DTPA extractable micronutrients, Fe, Mn, Zn, Cu, and Co, varied from 1.3-2.2, 2.38- 2.6, 0.22-0.32, 0.4 4 to 0.55, and 0.07 to 0.88  $\text{mg kg}^{-1}$ , respectively. DTPA extractable Zn and Cu content among treatments are statistically similar. The maximum Fe and Mn content in PHS is documented in T6, 29% and 5% over than RDF, but statistically insignificant. The lowest Co content in PHS was found in T1 (RDF), and the maximum in T9 (enhanced by 12% of RDF).



**Table 4.** Effect of cobalt application along with vermicompost on the physico-chemical properties of the post-harvest soil

Treatment	pH	EC (dS m <sup>-1</sup> )	OC (g kg <sup>-1</sup> )	Avail able N (kg ha <sup>-1</sup> )	Availabl e Fe (mg kg <sup>-1</sup> )	Availabl e Mn (mg kg <sup>-1</sup> )	Availabl e Zn (mg kg <sup>-1</sup> )	Availabl e Cu (mg kg <sup>-1</sup> )	Availabl e Co (mg kg <sup>-1</sup> )
T1	8.28 ± 0.05 <sup>a</sup>	0.31 ± 0.02 <sup>de</sup>	4.48 ± 0.03 <sup>b</sup>	267 ± 7.57 <sup>bc</sup>	1.7 ± 0.02 <sup>ab</sup>	2.47 ± 0.05 <sup>ab</sup>	0.28 ± 0.02 <sup>a</sup>	0.55 ± 0.03 <sup>a</sup>	0.07 ± 0.01 <sup>f</sup>
T2	8.15 ± 0.04 <sup>ab</sup>	0.33 ± 0.03 <sup>cde</sup>	4.49 ± 0.04 <sup>b</sup>	281 ± 4.35 <sup>b</sup>	1.8 ± 0.02 <sup>ab</sup>	2.49 ± 0.05 <sup>ab</sup>	0.29 ± 0.02 <sup>a</sup>	0.51 ± 0.03 <sup>a</sup>	0.22 ± 0.02 <sup>c</sup>
T3	8.1 ± 0.04 <sup>abc</sup> d	0.37 ± 0.02 <sup>bcd</sup>	4.48 ± 0.03 <sup>b</sup>	253 ± 7.21 <sup>cd</sup>	1.6 ± 0.02 <sup>ab</sup>	2.44 ± 0.04 <sup>ab</sup>	0.28 ± 0.02 <sup>a</sup>	0.47 ± 0.02 <sup>a</sup>	0.39 ± 0.03 <sup>cd</sup>
T4	7.98 ± 0.03 <sup>bcd</sup>	0.41 ± 0.02 <sup>ab</sup>	4.48 ± 0.03 <sup>b</sup>	219 ± 7.1 <sup>e</sup>	1.4 ± 0.02 <sup>b</sup>	2.39 ± 0.06 <sup>b</sup>	0.23 ± 0.02 <sup>a</sup>	0.44 ± 0.02 <sup>a</sup>	0.63 ± 0.04 <sup>b</sup>
T5	7.92 ± 0.02 <sup>cd</sup>	0.45 ± 0.02 <sup>a</sup>	4.48 ± 0.04 <sup>b</sup>	213 ± 9.07 <sup>c</sup>	1.3 ± 0.02 <sup>b</sup>	2.38 ± 0.05 <sup>b</sup>	0.22 ±0.02 <sup>a</sup>	0.44 ± 0.02 <sup>a</sup>	0.72 ± 0.04 <sup>b</sup>
T6	8.12 ± 0.04 <sup>abc</sup>	0.27 ± 0.02 <sup>e</sup>	5.84 ± 0.03 <sup>a</sup>	310 ± 6.65 <sup>a</sup>	2.2 ± 0.03 <sup>a</sup>	2.6 ± 0.04 <sup>a</sup>	0.32 ± 0.03 <sup>a</sup>	0.54 ± 0.03 <sup>a</sup>	0.32 ± 0.03 <sup>de</sup>
T7	8.09 ± 0.04 <sup>abc</sup> d	0.32 ± 0.03 <sup>cde</sup>	5.84 ± 0.03 <sup>a</sup>	294 ± 10.21 <sup>a</sup> b	2.0 ± 0.01 <sup>ab</sup>	2.55 ± 0.05 <sup>ab</sup>	0.31 ± 0.03 <sup>a</sup>	0.52 ± 0.03 <sup>a</sup>	0.48 ± 0.03 <sup>c</sup>
T8	7.97 ± 0.03 <sup>bcd</sup>	0.37 ± 0.03 <sup>bcd</sup>	5.83 ± 0.04 <sup>a</sup>	238 ± 14.46 <sup>d</sup> e	1.5 ± 0.01 <sup>b</sup>	2.42 ± 0.04 <sup>b</sup>	0.26 ± 0.03 <sup>a</sup>	0.46 ± 0.03 <sup>a</sup>	0.75 ± 0.04 <sup>b</sup>
T9	7.91 ± 0.03 <sup>d</sup>	0.39 ± 0.03 <sup>abc</sup>	5.83 ± 0.03 <sup>a</sup>	231 ± 8.00 <sup>de</sup>	1.5 ± 0.01 <sup>b</sup>	2.42 ± 0.06 <sup>b</sup>	0.25 ± 0.03 <sup>a</sup>	0.46 ± 0.03 <sup>a</sup>	0.88 ± 0.04 <sup>a</sup>
SEm ±	0.062	0.024	0.032	8.711	0.034	0.067	0.034	0.032	0.042
CD (p ≤ 0.05)	0.185	0.071	0.096	26.08 3	0.06	0.14	NS	NS	0.123

(Different letters for each parameter show significant difference at  $p \leq 0.05$  by Duncan's Multiple Range Test)

(pH: potential of hydrogen; EC: electrical conductivity; OC: organic carbon; CD: critical difference; SEM: standard error of mean)

### 3.5. Comparison of research results with other studies

It is illustrated from the result that application of cobalt (at a lower dose) along with vermicompost enhances the yield, yield attributes, harvest index, nutrient concentration, and nutrient uptake in rice, compared to the sole



application of cobalt at a lower dose. The higher dose of cobalt significantly reduced the yield and nutrient uptake in rice as compared to RDF, exhibiting toxicity symptoms, while application of vermicompost along with this higher dose of cobalt shows a reduction in toxicity. Application of vermicompost improves soil physical properties (water holding capacity, soil structure, porosity), chemical properties (soil pH, nutrient concentration, organic matter content), and biological properties (provides essential microorganisms, plant growth hormones, and improves enzyme activities). It also takes care of the plant's antioxidant and physio-biochemical defensive system (Liang et al., 2017). Vermicompost application improves the soil pH due to the release of OH<sup>-</sup> ion from carboxylic, hydroxyl, and phenolic compounds from the organic sources, and the brings the pH to near neutral value, which in turn improves the nutrient uptake (Iqbal et al., 2024). Bejbaruah et al. (2013) reported that vermicompost improves the NH<sub>4</sub><sup>+</sup>-N in soil as compared to NO<sub>3</sub><sup>-</sup>-N, which is preferred by rice plants.

Cobalt is a component of cobalamin, a vital vitamin (B12), and is also part of various proteins and enzymes (Odaka & Kobayashi, 2013). Rhizobia and other nitrogen-fixing bacteria need cobalt and cobalamin to convert atmospheric dinitrogen (N<sub>2</sub>) into ammonia (NH<sub>3</sub>), thus supplying plants with the essential macronutrient nitrogen. The role of cobalt in enhancing rice growth and productivity includes (i) reducing abiotic stress, (ii) activating antioxidant enzymes, (iii) replacing metals and alleviating metal stress, and (iv) maintaining hormonal balance (Hu et al., 2021). Cobalt is crucial in water uptake by stimulating aquaporin proteins and ABA hormone, which modify root hydraulics by elongating root cells, positively impacting rice water uptake, and subsequently improving yield and yield attributes (Kudoyarova et al., 2011). Cobalt also helps in micronutrient uptake, but is not essential. Its interaction with Zn is beneficial for cell homeostasis and the supplementation of nitrogen fixation. But the higher dose of Co also reduces the Zn uptake (Liu, 1999). Cationic micronutrients (Fe, Mn, Cu, and Zn) are the main competitors of Co, as they are placed in cation trap sites at the soil matrix, hindering Co binding. Only a higher dose of Co can replace them in the binding sites (Khrustalev et al., 2019). This indicates that higher doses of Co not only reduce micronutrient uptake but also induce phytotoxicity. Elshamly & Nassar (2024) also reported that in the presence of Co, there is reduced uptake of Fe, Mn, and Cu, and there exists a positive interaction between Co and Zn. They also figured out that due to the presence of Zn, the uptake of Fe, Mn, and Cu enhanced up to a certain lower concentration. This is the reason that, before starting the experiment, we go for the soil application of Zn.

Cobalt chloride undergoes hydrolysis in soil and produces free H<sup>+</sup>, which reduces the soil pH, but is buffered by vermicompost application. The application of cobalt also increases the electrolyte concentration in the soil solution pool, also buffered by vermicompost due to absorption and complexation of ions. As vermicompost enhances the soil reaction, it hinders the Co mobility to plant roots by the formation of oxide, hydroxide, and carbonates, which are less soluble (Panda et al., 2024). At low pH, cobalt tends to associate with soil particles via outer-sphere (electrostatic) complexation, forming relatively weak bonds. Under neutral to high pH conditions, it forms stronger inner-sphere (covalent) complexes, especially with mineral surfaces. In the present study, it was also found that treatment receiving higher doses of cobalt showed a greater uptake, and their post-harvest soil reaction value also supports the above-mentioned mechanism. There are also observations regarding minute changes in the post-harvest soil reaction value of the sole application of cobalt and its counterpart, along with



vermicompost is attributed to soil type, vermicompost quality and its application rate, and quality of irrigation water (Yadav & Chauhan, 2024)

Luo et al. (2020) reported that amending vermicompost to soil supplies plants with available nutrients to grow faster in the initial period and gradually slows in the entire crop growth period, facilitating a better photosynthetic rate, which in turn improves the yield. Vermicompost also activates the antioxidative enzyme system to counter the oxidative damage posed by cobalt toxicity. Application of vermicompost in the present experiment was found to reduce cobalt uptake compared to non-vermicompost treatments. The possible reasons may be (i) the chelation of Co by the binding site of organic colloid, (ii) microbial transformation, (iii) dilution effect, and (iv) improving the buffering capacity of soil (Charan et al., 2024). Cobalt complexation in soil refers to the binding of cobalt ions to soil minerals and organic matter, strongly influenced by soil pH, clay, organic carbon, and redox status. Cobalt exists mainly as Co (II) ions, easily forming complexes with clay minerals, iron/manganese oxides, and organic ligands, affecting its mobility and plant availability. High clay or organic matter increases cobalt retention, while acidic, anaerobic, or high organic soils enhance solubility and availability. Thus, cobalt complexation mediates its environmental fate and bioavailability in soils. The addition of the vermicompost improves the soil organic status, which promotes the cobalt complexation. Microorganisms immobilise cobalt primarily through biosorption to cell wall functional groups (carboxyl, hydroxyl, phosphate), bioaccumulation, biomineralisation, and biofilm formation. These processes reduce cobalt mobility and toxicity by forming stable surface complexes and precipitating cobalt as insoluble minerals, thereby mitigating its bioavailability in soil (Pathma & Sakthivel, 2012; Vyas et al., 2022)

The findings of our study are parallel with Gad et al. (2011), who found similar results in barley up to 10 ppm of cobalt application, and also mentioned the toxic effect of cobalt on increasing the concentration. It was reported that under salinity stress, application of Co certainly improves the plant growth in maize (Gad & El-Metwally, 2015), tomato (Gad et al., 2017), and onion (Gad et al., 2020) by improving physiological responses, such as ion and reactive oxygen species homeostasis, and modulating levels of phytohormone, including cytokinin and auxins. Ali et al. (2020) reported that amending vermicompost in soil not only modulates the cobalt toxicity but also improves the rice yield by improving soil fertility status and activating rice plants' physio-biochemical pathways to combat biotic and abiotic stresses.

## 4. Conclusion

The conclusion drawn from the experimental findings suggests that the Cobalt application at a lower concentration, along with vermicompost (T6), enhances the rice yield and its attributes, affecting the yield, and no hyperaccumulation was reported. Increasing the succeeding Co concentration up to 15 ppm leads to greater uptake of Co both in grain and straw, indicating a phytotoxic effect on the plant. But, comparing the sole application of cobalt to the application of vermicompost along with cobalt, the latter yields a redundancy in phytotoxic effect on the rice plant. Application of vermicompost improves the soil organic carbon status, as well as microbial diversity, and this process helps in averting the cobalt uptake to rice plant by complexation and immobilisation mechanism. The application of cobalt at lower concentrations synergistically enhances nitrogen



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uptake, whereas” higher doses of cobalt inhibit microbial activity, resulting in reduced nitrogen uptake in rice plants.” Soil application of Zn along with Co was found beneficial at lower concentrations, proving more micronutrient uptake. The inference drawn from the work is that application of Nitrogen (RDF) along with Zn, vermicompost, and cobalt (at lower concentrations) gives better yields and nutrient uptake in rice plants due to synergistic interaction among them.

## Author contributions

Conceptualization: DP and PKS; Soil sampling and analysis: PKS, SS, HP and MKS; Data analysis: DP, AP and PKS; Writing-original draft preparation: DP, PKS and AP; writing review and editing: RJB, DP and AP. All authors have read and agreed to the published version of the manuscript.

## Conflict of interest statement

The authors declare that they are no conflict of interest associated with this study.

## Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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