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Impact of Metallic Nanoparticles on *Eisenia fetida* Vermicomposting Efficiency, Growth and Nutrient Status

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Abstract

The proliferation of nanotechnology has led to a significant influx of nanoparticles (NPs) into the environment, with a particular focus on soil ecosystems where earthworms, a prominent megafaunal species, are continuously subjected to these NPs. The present investigation focuses on examining the impact of metal oxide NPs (namely iron and zinc) on the vermicomposting efficacy of *Eisenia fetida*. The earthworms were subjected to iron oxide NPs (30 nm and 100 nm) and zinc oxide NPs (20 nm and 240 nm), respectively, spiked in the artificial soil at various doses (250, 500, 750, and 1000 mg/kg of soil). The iron (zinc) oxide NPs with a diameter of 30 nm (240 nm) exhibited the most significant augmentation in the weight of earthworms, with a percentage increase of 4.10% (30.76%), when administered at a concentration of 250 (750) mg/kg in the soil. However, using 100 nm iron oxide NPs and 20 nm zinc oxide NPs, the weight gain reached its maximum value of 11.50% and 21.05%, respectively, at a concentration of 750 and 500 mg/kg of soil, respectively. The treatment that involved the administration of a blend of iron (zinc) oxide NPs with varying sizes exhibited the highest increase in weight, measuring 8.06% (16%) at the NPs concentration of 500 (750) mg/kg of soil. The shortest duration for converting the substrate (farm yard manure) into vermicompost of 84 days was observed in the treatment involving a combination of zinc oxide NPs of sizes 20 nm and 240 nm (@750 mg/kg of soil). In contrast, for iron oxide NPs treatment, the shortest duration of 82 days was observed for the control. The nutrient analysis conducted on the vermicompost derived from the substrate indicated a consistent pattern of elevated concentrations of total nitrogen, potassium, and phosphorus (%), accompanied by declining pH levels and total organic carbon content (%). The results suggest that the vermicompost produced by adding NPs exhibited higher nutrient content than the control, despite the prolonged duration (additional 2-13 days compared to the control) required for vermicomposting. The experimental results further indicated that using a combination of different sizes of iron and zinc oxide NPs resulted in the highest nutrient content in final vermicompost. Hence, it is plausible for marginal farmers in underdeveloped nations to investigate the possible effects of metal oxide NPs on vermicomposting as a mean to enhance agricultural practices.

Keywords: Earthworm; *Eisenia fetida;* Metallic Nanoparticles; Soil; Vermicompost

Introduction

Earthworms are diminutive, segmented, and terrestrial Oligochaete worms that are classified within the phylum Annelida. Earthworms are the predominant organisms in most soils in terms of living biomass, and their presence is crucial for the sustainability of agro-ecosystems (Pelosi et al., 2014).

Earthworms are considered to play a vital role in the modulation of soil nutrient dynamics and the conversion of organic matter (Bertrand et al., 2015 and Edwards and Arancon, 2022). This is attributed to their capacity to safeguard soil fertility and structure. The Indian subcontinent has been shown to harbor a significant proportion, specifically approximately 11%, of the global earthworm variety. This amounts to a total of 509 recognized species and subspecies, which are distributed among 69 genera and 10 families (Lalthanzara et al., 2018 and Kumar et al., 2020). While a limited number of non-native species have managed to establish themselves in different agro-ecosystems, the predominant preference among earthworm species in India is for natural environments (Deepthi and Kathireswari, 2016). Earthworms are commonly referred to be "natural tillers" in agricultural environments due to their perceived ability to enhance several soil properties. These properties include increased infiltration capacity, hydraulic conductivity, formation of water-stable soil aggregates, and reduction in soil bulk density (Hallam et al., 2020). Vermicomposting refers to the utilization of earthworms for the purpose of converting organic waste into a beneficial substance resembling humus. This resulting material is subsequently employed as a natural soil conditioner (Garg et al., 2006). This process entails the introduction of worms into organic debris, such as vegetable waste and leaf litter waste. The epigeic species of earthworm–*Eisenia fetida* is the most preferred for vermicomposting due to its convenient manageability, widespread availability, and responsiveness to environmental fluctuations.

Nanomaterials refer to deliberately fabricated materials that possess at least one dimension within the range of 1–100 nm. The utilization of NPs in diverse applications has shown a substantial growth during the past three decades (Conde et al., 2014). The distinguishing characteristics that set them apart from their larger counterparts are their tiny dimensions and the elevated ratio of surface area-to-volume (Mekuye and Abera, 2023). Nanomaterials are employed in the agriculture sector as either organic or inorganic NPs to mitigate the negative impacts caused by pests and diseases, preserve the nutritional composition of crops, and extend the shelf-life of food products. Nutrients are often administered to crops in the form of nanocomposites to achieve controlled release and enhance utilization efficiency, resulting in notable enhancements in plant crops while minimizing environmental repercussions (Elmer and White, 2018). The category of significant nanomaterials encompasses metal oxides, including titanium oxide (TiO_2), zinc oxide (ZnO), magnesium oxide (MgO), copper oxide (CuO), aluminium oxide (Al_2O_3), manganese oxide (MnO_2) and iron oxide (Fe_3O_4, γ-Fe_2O_3). Among these, zinc oxide and iron oxide NPs are frequently utilized (Chavali and Nikolova, 2019). As a result of their extensive range of applications, NPs have the potential to be intentionally or unintentionally released into the environment via activated sludge and subsequent discharge into wastewater streams. Over time, these NPs are introduced into the soil ecosystem either through atmospheric deposition or by applying activated sludge to agricultural areas as a means of improving soil fertility (León-Silva et al., 2016 and Rajput et al., 2020). Various earthworm species have exhibited varied responses to different types of NPs (Stewart et al., 2013 and Świątek et al., 2017). For the present study *Eisenia fetida* was chosen as the model earthworm species. Currently, there is a scarcity of information regarding the impact of metallic NPs on soil megafauna. Hence, the present study was conducted with the aim of investigating the impact of metal oxide NPs on the vermicomposting efficiency of *Eisenia fetida*.

Material and Methods

The iron oxide NPs measuring 30 and 100 nm, which exhibit weakly antiferromagnetic characteristics, and the zinc oxide NPs measuring 20 and 240 nm, were procured from Otto Chemie private limited, located in Mumbai, India. The examination of morphology and size of NPs was conducted using Transmission Electron Microscopy (TEM) at Electron Microscopy and Nanoscience Laboratory, Punjab Agricultural University, Ludhiana. The stock of the *Eisenia fetida* was acquired from Mahavir Organic Farm, Phillaur. The farm yard manure (FYM) used in this study was sourced from dairy farms of the School of Organic Farming, Punjab Agricultural University, Ludhiana. The work pertaining to the standardization of metal oxide NPs dose and the impact of the standardized dose on *Eisenia fetida* was conducted in the Department of

Zoology, Punjab Agricultural University, Ludhiana. The nutrient analysis of the vermicompost was performed at the Department of Soil Science, Punjab Agricultural University, Ludhiana.

Maintenance of Stock

The stock of *Eisenia fetida* was maintained in the animal dung substrate sourced from the dairy farms of the School of Organic Farming, Punjab Agricultural University, Ludhiana and stored at the Department of Zoology, Punjab Agricultural University, Ludhiana. The dung was subjected to a 15-day period of solar drying prior to its utilization for the purposes of the study. In the utilization of dung as a substrate, it was fragmented and moistened through the application of water to sustain the moisture level at approximately 70%. In order to maintain the earthworm culture, a consistent application of water was administered to the stock. The chemical characteristics of the substrate FYM were examined in accordance with the previous study (Goyal et al., 2023a).

Preparation of artificial soil

The artificial soil was developed in accordance with the OECD guideline no. 222 (OECD, 2016), using a mixture of finely ground sphagnum peat (10%), Kaolin clay (30%), and air-dried quartz sand (70%). The sand was procured from the local market, kaolin clay was sourced from the pottery supplier, and the sphagnum peat was obtained from the nearby nursery. The soil components were well blended, and the soil pH was modified to 6.2 with the addition of calcium carbonate. A random sample of adult clitellate worms, specifically *Eisenia fetida*, weighing between 200-300 mg, was chosen from the available stock. These worms were then introduced to an artificial soil environment for a period of seven days prior to the commencement of the experiment. This acclimatization period allowed the earthworms to adjust to the conditions of the artificial soil.

Characterization of metal oxide NPs

The morphological characterization of the metal oxide NPs was carried out using a Transmission Electron Microscope (Hitachi H-7650) at the Electron Microscopy and Nanoscience Laboratory, Punjab Agricultural University, Ludhiana. The metal (iron and zinc) oxide NPs were subjected to dispersion in acetone for 30 minutes using a sonicator, after which they were affixed onto a copper grid coated with carbon for imaging. The TEM analysis (Goyal et al., 2023a), revealed that the iron oxide NPs exhibited hexagonal morphology, with average dimensions of 30 nm and 100 nm, respectively. In contrast, the zinc oxide NPs displayed a tetragonal morphology (Goyal et al., 2023b), with average diameters of 20 nm and 240 nm, respectively.

Standardization of NPs dose

Table 1 presents a comprehensive overview of various treatments under investigation. Four different doses of each type of NPs were administered to the artificial soil as 250 mg/kg (250 mg of metal oxide NPs in 1 kg of artificial soil); 500 mg/kg (500 mg of metal oxide NPs in 1 kg of artificial soil); 750 mg/kg (750 mg of metal oxide NPs in 1 kg of artificial soil), and 1000 mg/kg (1000 mg of metal oxide NPs in 1 kg of artificial soil). For the treatment involving a combination of 30 nm and 100 nm iron oxide (20 nm and 240 nm for zinc oxide) NPs, doses were prepared with equal ratios of both NPs. A total of ten individuals of clitellate worms, with an approximate weight of 300 mg each, were introduced into different artificial soil environments for 14 days within plastic trays measuring 54 cm x 45 cmx 22 cm. Five grams of dried farmyard manure was added to the soil at an interval of 7 days. The final weight of the earthworms was observed. The experiment was conducted in triplicate for each treatment and subsequently compared with the control. The concentrations that yielded the greatest increase in weight and no instances of mortality were chosen as the optimal dosages for the treatments. These doses were then utilized in further investigations of the physio-chemical characteristics of the vermicompost. The standardized doses determined in this way for various treatments were 500 mg/kg (for 20 nm zinc oxide NPs), 750 mg/kg (each for 240 nm zinc oxide NPs and the combination of 20, 240 nm zinc oxide NPs), 250 mg/kg (for 30 nm iron oxide NPs), 750 mg/kg (for 100 nm iron oxide NPs), and 500 mg/kg (for the combination of 30,100 nm iron oxide NPs) of soil.

Table 1. Treatments applied to soil amended with increasing concentration of metal oxide NPs.

Treatment	Characteristics
Control	Artificial Soil + FYM + Earthworms
T_1	Artificial Soil + 240 nm zinc oxide NPs at concentrations 250, 500, 750, 1000 mg/kg of soil + FYM + Earthworms
T_2	Artificial Soil + 20 nm zinc oxide NPs at concentrations 250, 500, 750, 1000 mg/kg of soil + FYM + Earthworms
T_3	Artificial Soil + 20, 240 nm zinc oxide NPs in equal ratio at concentrations 250, 500, 750, 1000 mg/kg of soil + FYM + Earthworms
T_4	Artificial Soil + 100 nm iron oxide NPs at concentrations 250, 500, 750, 1000 mg/kg of soil + FYM + Earthworms
T_5	Artificial Soil + 30 nm iron oxide NPs at concentrations 250, 500, 750, 1000 mg/kg of soil + FYM + Earthworms
T_6	Artificial Soil + 30, 100 nm iron oxide NPs in equal ratio at concentrations 250, 500, 750, 1000 mg/kg of soil + FYM + Earthworms
T_7	Artificial Soil + 20, 240 nm zinc oxide NPs and 30, 100 nm iron oxide NPs in equal ratio at concentrations 250, 500, 750, 1000 mg/kg of soil + FYM + Earthworms

Vermicompost preparation

One kg of FYM was taken as substrate in circular plastic tubs (54 cm x 45 cm x 22 cm). Adequate quantities of water were sprayed to regulate the moisture levels, and the mixtures were manually agitated on a daily basis for a duration of 15 days in order to eliminate volatile gases that could be potentially toxic to the earthworms. After a period of 15 days, a total of ten adult *Eisenia fetida* specimens with known biomass were introduced into individual containers containing substrate spiked with standardized doses of NPs treatments: Control (no NPs), T_1 (240 nm@750 mg/kg of soil), T_2 (20 nm@500 mg/kg of soil), T_3 (20 nm+240 nm@750 mg/kg of soil), T_4 (100 nm@750 mg/kg of soil), T_5 (30 nm@250 mg/kg of soil), T_6(30 nm+100 nm@500 mg/kg of soil), and T_7 (20 nm+240 nm+30 nm+100 nm@500 mg/kg of soil). To maintain moisture and prevent pest infestation, a damp jute cloth was placed over the substrate. The experiment was replicated thrice for both the control and each treatment and regular monitoring was conducted. The samples of the substrate were drawn at 1^{st}, 30^{th}, 60^{th}, and 90^{th} day from each tub. A comparative analysis was conducted to assess the duration required by earthworms, under various experimental conditions, to produce vermicompost from the substrate in comparison to a control group.

Nutrient Analysis

Samples of vermicompost derived from farmyard manure were collected at 30-day intervals starting with the initiation of the experiment and continued for a total duration of 90 days. The methodology employed for assessing the nutritional composition of the vermicompost is presented in Table 2.

Table 2. Various methods used for determining the nutrient composition of vermicompost.

Sr. No.	Parameter	Method
1.	pH	Potentiometric method (Jackson, 1973)
2.	Total Nitrogen Content	Micro- Kjeldahl method (Jackson, 1973)
3.	Total Phosphorus Content	Vanadomolybdo-phosphoric yellow colour method (Jackson, 1967)
4.	Total Potassium Content	Flame photometer (Jackson, 1967)
5.	Total Organic Carbon	Rapid titration method (Walkley and Black, 1934)

Statistical Analysis

The statistical analysis for the reproductive parameters of earthworms and the estimated nutrient contents in vermicompost are reported as the mean value together with the standard error. The statistical software SPSS version 16.00 was utilized to conduct a One-way Analysis of

Variance (ANOVA) to determine the presence of a statistically significant difference. A p-value of 0.05 was used as the threshold for determining statistical significance.

Results and Discussion

Growth of earthworms under different treatments and NP concentrations

Figures 1(a)-(b) depict a graphical illustration of the percent increase in weight of *Eisenia fetida* in soil across all treatments and control groups, encompassing all concentrations. By the end of the experiment, there was a notable augmentation in the body mass of the earthworms. The treatment labelled as T_1, which involved the application of 240 nm zinc oxide NPs at the concentration of 750 mg/kg of soil, resulted in the most significant weight gain of 30.76%. The treatment T_2, which utilized 20 nm zinc oxide NPs, had the most substantial weight gain of 21.05% when administered at a concentration of 500 mg/kg of soil. For the treatment T_3 (20 nm + 240 nm zinc oxide NPs), the highest increase in weight of 16% was observed at the concentration of 750 mg/kg of soil. In contrast, the lowest gain in weight (1.92%) was observed for the control for each of these treatments.

For the treatment T_4 (100 nm iron oxide NPs), the highest increase in the weight of 11.54% was observed at the concentration of 750 mg/kg of soil. However, the lowest percentage change in the weight of 1.92% was observed for the control. No mortality was observed at any concentration.

For the treatment T_5 (30 nm iron oxide NPs), the highest increase in weight of 4.11% was observed at the concentration of 250 mg/kg of soil. In comparison, the lowest change in the weight of 1.61% was observed at the concentration of 500 mg/kg of soil. After 14 days, no mortality was observed at any concentration. However, the colour of earthworms changed from light brown to dark brown.

For the treatment T_6 (30 nm + 100 nm iron oxide NPs combined in equal ratio), the highest increase in weight of 8.06% was observed at the concentration of 500 mg/kg of soil. At NPs concentrations of 250 mg/kg and 1000 mg/kg of soil, a negative percentage change in the weight was observed.

For the treatment T_7 (20 nm + 240 nm zinc oxide NPs and 30 nm + 100 nm iron oxide NPs combined in equal ratios), the highest increase in weight of 8% was observed at the concentration of 500 mg/kg of soil. In contrast, the highest negative gain in weight (-9.25%) was observed at the concentration of 750 mg/kg of soil.

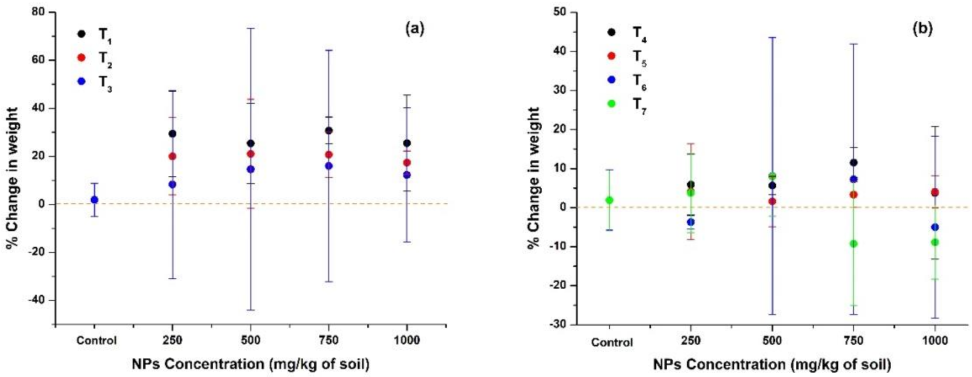

Fig. 1. Growth of *Eisenia fetida* (% change in weight) in soil with different treatments of metal oxide NPs.

Pacheco et al. (2022) reported studies on the toxicity of silver sulfide nanoparticles on the earthworm species *Eisenia Andrei*. The studies revealed that after two weeks of exposure silver sulfide nanoparticles were engulfed by *E. Andrei* cells. However, phenoloxidase activity and lipid peroxidation of the earthworms remained similar to the untreated control group. No mortality was observed, but exposure to silver sulfide nanoparticles resulted in weight loss in the earthworms. Moreover, silver sulfide nanoparticles caused an imbalance in the nitric oxide metabolism. Similar results were reported by Samrot et al. (2017) in their studies on the evaluation of possible effects of chemically synthesized magnetic NPs on the earthworm *Eudrilus Eugeniae* at different concentrations of 100,200, and 400 mg/ml of de-ionized water. The study revealed that the impact caused on the earthworms was proportionate to the concentration of NPs. The colour of the earthworm's skin turned black from brown with the increased NPs concentration.

The study by Liang et al. (2017) to observe the acute and sub-acute toxicity of nanoscale zerovalent iron at different concentrations of 100 mg/kg, 500 mg/kg, and 1000 mg/kg of dry natural soil on *Eisenia fetida* reported that nanoscalezerovalent iron at 500 mg/kg and 1000 mg/kg perturbed the activities of enzymes like superoxide dismutase, and malondialdehyde content. The study also revealed that nanoscalezerovalent iron influenced the growth, survival, and reproduction patterns of earthworms as well as the enzyme activities such as catalase and malondialdehyde. The study highlighted that changes in biochemical parameters are the early indicators of the appearance of sublethal effects.

Number of days taken by earthworms for vermicompost preparation

Figure 2 shows the number of days taken by the earthworms to prepare the vermicompost under different treatments. The vermicompost produced from FYM was harvested and observed to be much darker than the initial substrate's colour. No mortality was observed in any animal waste during the study period. The fastest substrate conversion into vermicompost was recorded in the control, whereas the maximum number of days (95 days) was recorded in the treatment T_7. This reveals that metal oxide NPs influence the vermicomposting efficiency of earthworms, which may be caused by the impact of these NPs on their metabolic processes.

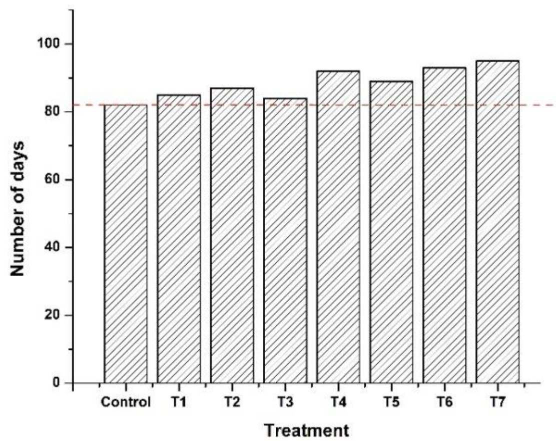

Fig. 2. Number of days taken by earthworms to prepare vermicompost under different treatments.

Physio-chemical parameters of FYM

pH

The pH of the substrate changed to nearly neutral after vermicomposting (Figure 3). The values with superscript (a, b, c, d) indicate significant differences between treated and untreated soil samples ($p<0.05$), whereas the values with superscript (A, B, C, D) indicate significant differences between values at different days ($p<0.05$). It was observed that the pH of the final vermicompost was lower than that of the initial substrate (control) in all experimental conditions. Elevated mineralization of nitrogen and phosphorus into nitrites or nitrates and orthophosphates, respectively, could potentially account for the pH-shift. The pH-shift may also result from the formation of intermediate organic acid species during the bioconversion of organic matter. The nature of this pH shift is dynamic, and it is substrate type-dependent (Ndegwa et al., 2000).

Bhat et al. (2015) observed comparable patterns of pH decline throughout the vermicomposting process involving a range of substrates, including sugarcane bagasse and cattle manure. An additional potential factor contributing to pH-shift is the generation of acids, including fulvic and humic acids, throughout the composting process, according to the study. Furthermore, the pH shift may be attributed to the generation of carbon dioxide and other organic compounds as a result of microbial metabolism (Haimi and Huhta, 1987).

Dominguez and Edwards (2011) reported nearly neutral values of the vermicompost pH when it was ready to harvest. The range of pH was 5-9 and the production NO_3 could be the possible reason behind observed pH trends. Several studies conducted by different researchers worldwide also revealed that most of the earthworm species prefer near neutral pH close to 7.0 (Pagaria and Totwat, 2007; Suthar, 2008, and Panday and Yadav, 2009). The present findings contradict the findings of Tripathi and Bhardwaj (2004), Loh et al. (2005), and Pattnaik and Reddy (2010), who reported higher pH of the vermicompost. According to this study, the possible reason behind the difference between pH of the final vermicompost made from control and treatments can be the elevated metabolic activities of the earthworms due to exposure to the metal oxide NPs. The production of organic acids during decomposition may reduce pH.

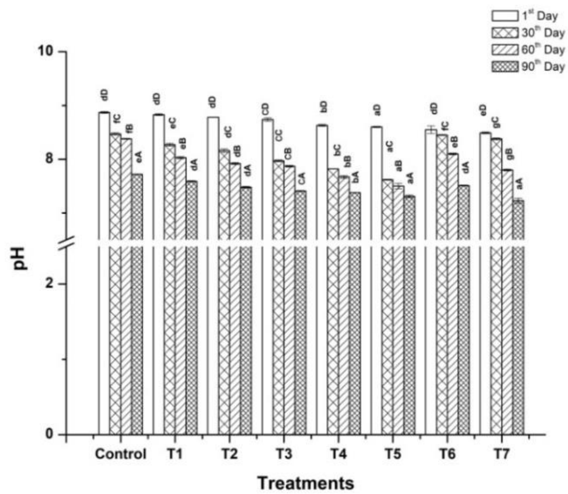

Fig. 3. Effect of different treatments of NPs on the pH of FYM.

Total Organic Carbon

The total organic carbon decreased over time during vermicomposting processes in control and other treatments. The maximum lowering in the value of total organic carbon content was observed in treatment T_7 and the minimum in control (Figure 4). The values with superscript (a, b, c, d) indicate significant differences between treated and untreated soil samples ($p<0.05$), whereas the values with superscript (A, B, C, D) indicate significant differences between values at different days ($p<0.05$). These results align with the findings of Tognetti et al. (2005) and Garg and Kaushik (2005). Carbon is a fundamental constituent of organic molecules and the structural element of all biological entities. Composting necessitates it as its principal energy source (Ansari and Jaikishun, 2011; Ansari and Rajpersaud, 2012). The microbial degradation of organic matter at the time of the vermicomposting and action of earthworms lead to a lowering of the total organic carbon values.

An additional factor contributing to the decline in organic carbon value trends could be the emission of CO_2 from microbial respiration and mineralization of organic matter, resulting in an increase in total nitrogen. A fraction of the carbon present in the decomposing residues is assimilated by the microbial biomass and is released as CO_2 (Fang et al., 2001 and Cabrera et al., 2005). Microorganisms derive their energy from carbon in order to decompose organic matter. Numerous studies have substantiated the notion that vermicomposting processes frequently induce a greater reduction in organic carbon content than alternative composting methods. The rationale for this is that earthworms possess a greater capacity for assimilation. In their study, Elvira et al. (1996) reported a reduction of 1.7-folds in the overall organic carbon content of vermicompost extracted after 40 days from paper pulp and mill sewage sludge substrates. Garg et al. (2006) observed a reduction in the overall organic content of agricultural residues, domestic wastes, and industrial wastes by 3.0, 2.2, and 1.7 times, respectively, as a result of vermicomposting.

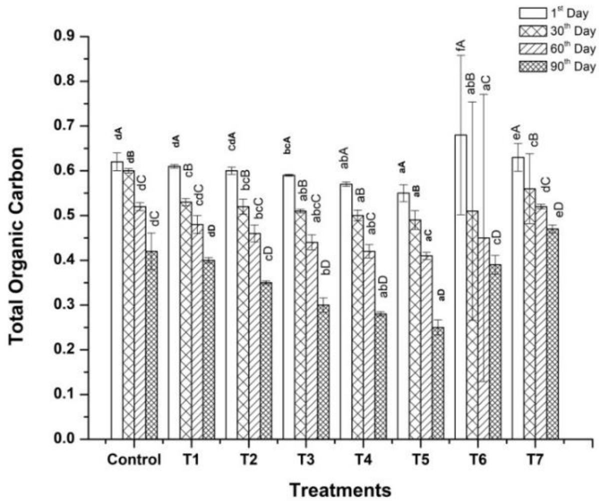

Fig. 4. Effect of different treatments of NPs on the total organic carbon of FYM.

Nitrogen, Phosphorus and Potassium

There was an increase in total nitrogen, phosphorus, and potassium levels in the final vermicompost, which might be due to the mineralization of the organic matter (Figures 5-7). The values with superscript (a, b, c, d) indicate significant differences between treated and untreated soil samples ($p<0.05$), whereas the values with superscript (A, B, C, D) indicate significant differences between values at different days ($p<0.05$). The maximum increase in total nitrogen, total potassium and total phosphorus contents was recorded in the treatment T_7 (combination zinc oxide and iron oxide NPs) whereas the minimum increase was observed in the control. According to Atiyeh et al. (2002), the conversion of ammonium-nitrogen into nitrate leads to an increase in nitrogen content. The possible reason behind the increase in total P and K levels is the biological grinding of the organic matter while passing through the earthworm's gut leading to the physical decomposition by the enzymatic activities (Rao and Pathak, 1996 and Goswami et al., 2013). The results of the present study are consistent with the findings of Manna et al. (2003), Suthar (2007), and Suthar (2008). The percentage changes in the pH, organic carbon, N, P, and K content are presented in Figure 8.

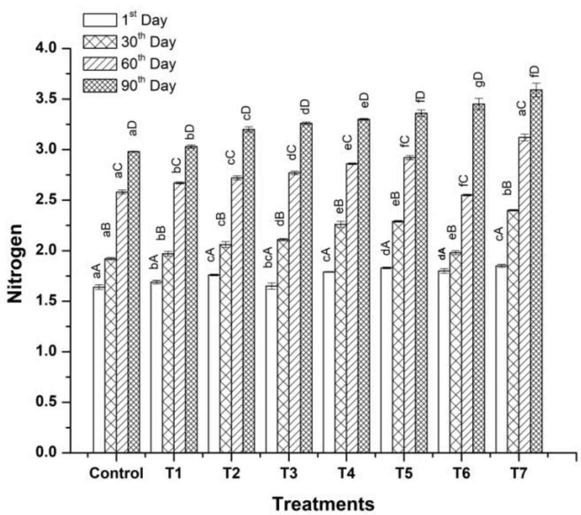

Fig. 5. Effect of different treatments of NPs on the nitrogen content of FYM.

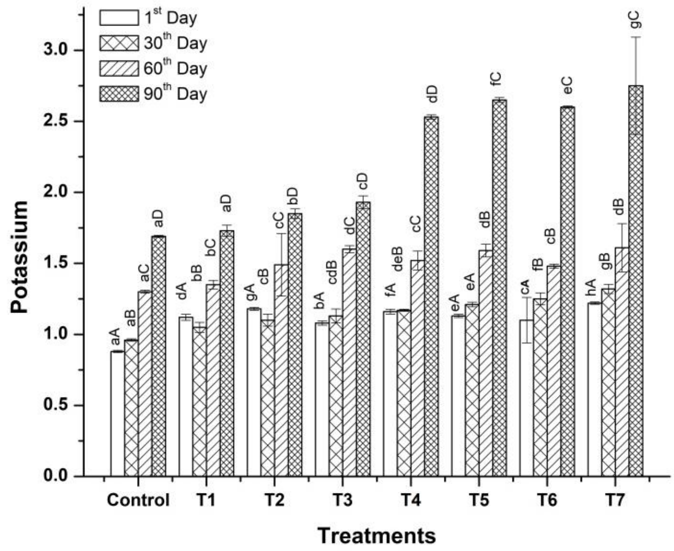

Fig. 6. Effect of different treatments of NPs on the potassium content of FYM.

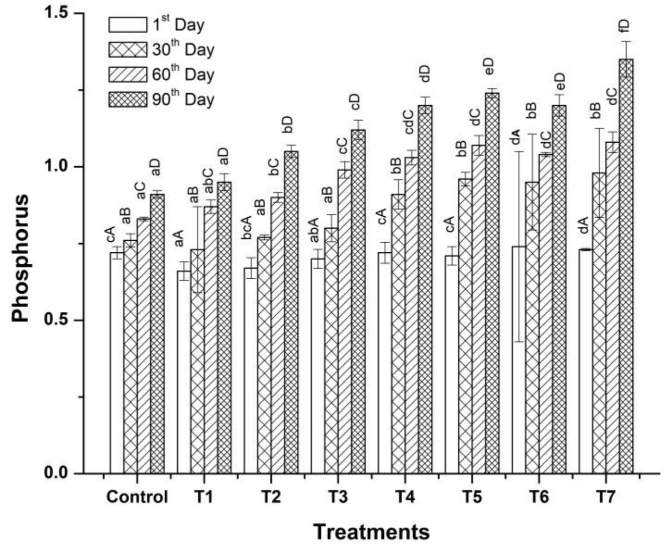

Fig. 7. Effect of different treatments of NPs on the phosphorus content of FYM.

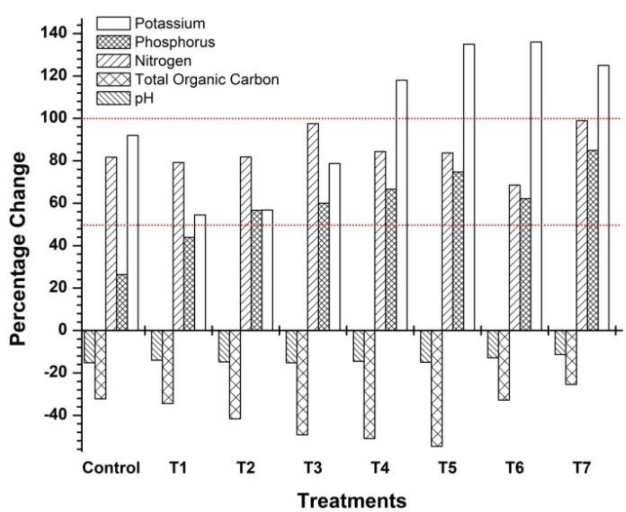

Fig. 8. Percentage change in chemical parameters of the FYM after the formation of final vermicompost.

Conclusions

The findings suggest that iron oxide NPs cause morphological damage and colour change to *Eisenia fetida*, whereas the untreated earthworms and those exposed to zinc oxide NPs are grown without any evident damage to their body. In contrast to the treatments incorporating zinc oxide NPs, the earthworm growth was considerably inhibited in the treatments incorporating iron oxide NPs. The nutrient content of the final vermicompost obtained from the treatment T_7 (combination of iron oxide NPs and zinc oxide NPs) was higher than the control and other treatments, however, the earthworms took the longest time to prepare vermicompost under this treatment. Additionally, the study unveiled that the growth of earthworms and the efficiency of vermicomposting are contingent upon the concentration of NPs. Consequently, it is imperative to advance field and laboratory investigations aimed at evaluating the ecological and environmental harm inflicted by the discharge and utilization of these NPs.

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Author Contributions
SSH and NR conceived the concept. AG, SSH executed the field experiments and data collection. AG, SSH and NR performed investigation, analysis of data and interpretation. ND and NR prepared and edited the manuscript. All authors approved the final version of the manuscript.

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