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Impact of Iron Oxide Nanoparticles on the Growth, Vermicomposting Efficiency and Nutritional Status of Vermicompost through *Eisenia fetida*

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Abstract

The advent of nanotechnology has led to the presence of an enormous amount of nanoparticles (NPs) in the environment, especially in the soil where earthworms, the major megafaunal species, are continuously exposed to these NPs. The present study focuses on the impact of iron oxide NPs on the vermicomposting efficiency of *Eisenia fetida*. The worms were exposed to iron oxide NPs of sizes 30 nm and 100 nm at different concentrations (250, 500, 750, and 1000 mg kg^{-1} of soil) spiked in artificial soil. The maximum increase in earthworm's weight of 4.10% was observed at a concentration of 250 mg kg^{-1} of soil for the iron oxide NPs of 30 nm size and the highest increase in weight of 11.50% was observed in the case of 100 nm size at a concentration of 750 mg kg^{-1} of soil. In the treatment containing a combination of iron oxide NPs of both sizes, the highest gain in weight of 8.06% was observed at a concentration of 500 mg kg^{-1} of soil. The number of days for vermicompost preparation from the substrate was recorded to be 82, 89 and 92, respectively, for the control, treatments containing 30 nm and 100 nm iron oxide NPs. The maximum number of days (93 days) was recorded in the treatment containing both 30 nm and 100 nm iron oxide NPs. The nutrient analysis of the vermicompost from the substrates revealed a general trend of increasing levels of total nitrogen, potassium and phosphorus (%) along with decreasing levels of pH and total organic carbon content (%). It is inferred that iron oxide NPs caused morphological damage and colour change to *Eisenia fetida.* The NPs result in an increased duration for vermicomposting but better nutrient content as compared to the control. The nutrient content of vermicompost was found to be the highest for the treatment containing a combination of both sizes of iron oxide NPs. Therefore, the impact of iron oxide NPs in vermicomposting can potentially be explored by marginal farmers in developing countries to support farming practices.

Keywords: *Eisenia fetida*; Iron oxide; Nanoparticles; Artificial soil; Vermicompost

Introduction

Earthworms are small, segmented, and terrestrial Oligochaete worms that belong to the phylum Annelida. In terms of living biomass, earthworms are the most abundant organism in most soils which play a key role in providing agro-ecosystem sustainability (Pelosi et al., 2014). Earthworms are believed to be responsible for soil nutrient dynamics and the transformation of organic matter due to their ability to maintain soil fertility and structure (Bertrand et al., 2015). Almost 11% of the world's total earthworm diversity, a total of 509 species and subspecies representing 69 genera and 10 families have been identified from the Indian subcontinent (Lalthanzara et al., 2018; Mubeen and Hatti, 2018). Most Indian earthworms have a species-specific preference for natural habitats, but a few exotic species have successfully colonized in different agro-ecosystems (Deepthi and Kathireswari, 2016). In agricultural ecosystems, earthworms are popularly known as "natural tillers" because they are considered to increase the infiltration capacity, hydraulic

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conductivity, water-stable soil aggregates, and decrease bulk density (Maynard et al., 2002). Vermicomposting refers to the conversion of organic wastes to valuable humus-like material by the earthworm species, and the resulting product is used as a natural soil conditioner (Dominguez et al., 2000). This process involves 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 beneficial for vermicomposting because of their ease of handling, availability and sensitivity to changes in surroundings.

Nanomaterials are intentionally produced materials with at least one dimension in the 1 – 100 nm range. The use of nanoparticles (NPs) in various applications has increased significantly over the last 30 years (Conde et al., 2014). The key factors that make them unique and different from their bulk counterparts are their tiny size and the high surface-to-volume ratio (Joudeh and Linke, 2022). The nanomaterials are used in the agriculture sector as organic or inorganic NPs to reduce the damage due to pests and diseases, to maintain the nutritional contents and to enhance the food shelf-life. Due to their peculiar properties, nutrients are frequently supplied to the crops in the form of nanocomposites for controlled release and to enhance the efficiency of use, leading to significant improvement in plant crops with lower environmental impacts (Elmer and White, 2018). The group of the most important nanomaterials includes metal oxides such as 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) with zinc oxide and iron oxide NPs being the commonly used NPs (Chavali and Nikolova, 2019). Due to their widespread applications, NPs may intentionally or accidentally get discharged into the environment through activated sludge and their discharge into the wastewater stream. Eventually, these NPs get into the soil ecosystem through atmospheric deposition or when activated sludge is poured into fields to enhance soil fertility (Nowack and Buschelli, 2007; León-Silva et al., 2016). To date, very little information is available on the effects of the metallic NPs on soil megafauna. Therefore, this study was designed to study the effect of iron oxide NPs on the vermicomposting efficiency of *Eisenia fetida*.

Material and Methods

The iron oxide NPs of sizes 30 & 100 nm with weakly antiferromagnetic properties were purchased from Sukhmani Enterprises, Ludhiana. The TEM analysis of iron oxide NPs was done in Electron Microscopy and Nanoscience Laboratory, Punjab Agricultural University, Ludhiana. The stock of the *Eisenia fetida* was obtained from Mahavir Organic Farm, Phillaur. The farm yard manure (FYM) was obtained from Punjab Agricultural University, Ludhiana. The work on the standardization of iron oxide NPs and the study on the impact of standardized dose on *Eisenia fetida* was carried out in the Department of Zoology, Punjab Agricultural University, Ludhiana. The nutrient analysis of the vermicompost was also performed at the Punjab Agricultural University, Ludhiana.

Maintenance of Stock

The stock of *Eisenia fetida* was maintained in the Department of Zoology, Punjab Agricultural University, Ludhiana. Prior to its use for the study, the dung was dried in the sunlight for 15 days. To use dung as a substrate, it was crumbled and sprayed with water to maintain the moisture content to a level of 70%. The stock was regularly sprayed with water to preserve the culture of earthworms. The substrate analysis of chemical properties is given in Table 1.

Table1. Initial physio-chemical composition of FYM used in the study.

Substrate	pH	N	P	K	C
		(%)			
Farm Yard Manure	8.87±0.18	1.42±0.02	0.46±0.05	0.58±0.03	0.50±0.02

Values are mean±S.E.

Preparation of artificial soil

The artificial soil prepared according to the OECD guideline no. 222 (OECD, 2016), composed of finely ground sphagnum peat (10%), Kaolin clay (30%) and air-dried quartz sand (70%). The sand was obtained from the local market, kaolin clay from the pottery supplier, and the sphagnum peat from the nearby nursery. All the soil contents were appropriately mixed, and the pH of the soil was adjusted to 6.2 by adding calcium carbonate. Adult clitellate worms weighing 200-300 mg were selected randomly from the stock of *Eisenia fetida* and placed in the artificial soil for seven days before the experiment so that earthworms could acclimatize to the artificial soil.

Characterization of iron oxide NPs

The iron oxide NPs morphologically characterized using Transmission Electron Microscopy (TEM) with Hitachi H-7500 TEM at Electron Microscopy and Nanoscience Laboratory, Punjab Agricultural University, Ludhiana. The iron oxide NPs were dispersed in acetone for 30 minutes inside a sonicator before fixing them on a carbon-coated copper grid for imaging. The TEM analysis (Fig. 1) revealed that the iron oxide NPs are of hexagonal shape and have average sizes of 30 nm and 100 nm, respectively.

Standardization of iron oxide NPs dose

Four doses of each NPs (30 nm and 100 nm) were applied to the artificial soil as 250 mg/kg (250 mg of iron oxide NPs in 1 kg of artificial soil); 500 mg/kg (500 mg of iron oxide NPs in 1 kg of artificial soil); 700 mg/kg (700 mg of iron oxide NPs in 1 kg of artificial soil); 1000 mg/kg (1000 mg of iron oxide NPs in 1 kg of artificial soil). For the treatment involving a combination of 30 nm and 100 nm iron oxide NPs, doses were prepared with equal ratios of both NPs. The experiment was run in triplicate for each treatment and compared with the control. Ten clitellate worms each weighing about 300 mg were introduced into each artificial soil inside plastic trays (54 cm x 45 cm x 22 cm) for 14 days. 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 concentrations that resulted in the highest gain in weight and no mortality were selected as optimum doses of three treatments and used for further testing of the physio-chemical characteristics of the vermicompost. Table 2 summarizes various treatments under study.

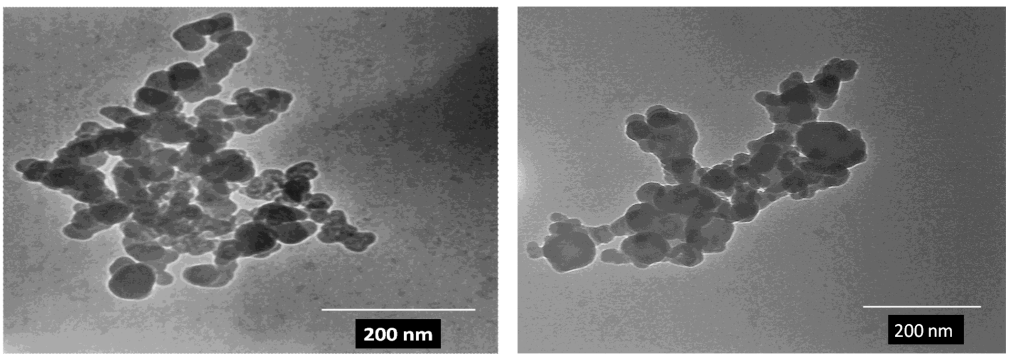

Fig. 1: TEM images of the samples of iron oxide NPs with average sizes of 30 nm (left) and 100 nm (right).

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 adjust the moisture content and the mixtures were turned over manually every day for 15 days to remove volatile gases that may be potentially toxic to the earthworms. After 15 days, ten adult *Eisenia fetida* individuals of known biomass were introduced into each container having substrate spiked with standardized doses of NPs treatments: Control (no NPs), T_1 (30 nm@250 mg/kg of soil), T_2 (100 nm@750 mg/kg of soil), and T_3 (30 nm+100 nm@500 mg/kg of soil). The substrate was covered with a moist jute cloth to retain moisture and avoid intrusion by the pests. The experiment was replicated thrice for control and each treatment and monitored regularly. The samples of the substrate were drawn at 1st, 30th, 60th, and 93rd day from each tub. The time taken by earthworms under different treatments to prepare vermicompost from the substrate was compared with the control.

Nutrient Analysis

Samples of the vermicompost prepared from farmyard manure were drawn every 30 days after the onset of the experiment till a maximum of 93 days. Table 3 presents the methodology adopted for analyzing the nutrient composition of the vermicompost.

Table 2. Treatments applied to soil amended with increasing concentration of iron oxide NPs.

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

Table 3. 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

Statistical analysis for earthworm reproductive parameters and estimated nutrient contents in vermicompost are represented as mean ± standard error. One way ANOVA in SPSS 16.00 was used to find the significant difference. A p-value of 0.05 was chosen as a standard for a statistically significant difference.

Results and Discussion

Growth of earthworms under different treatments and NP concentrations

The observed growth rates of earthworms under different treatments are presented in Tables 4-6 and Fig. 2. There was a significant increase in the body weight of earthworms at end of the experiment. For the treatment T_1 (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 change in colour of earthworms, lacerations, and swelling on the clitellum of earthworms was observed at the concentration of 250 mg/kg of soil. The primary damage observed was inflammation and explosions on the body.

For the treatment T_2 (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 (Table 5). At the same time, the lowest percentage change in the weight was observed for the control. No mortality was observed at any concentration. However, the colour of earthworms changed from light brown to dark brown. For the treatment T_3 (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 as depicted in Table 6. At NPs concentrations of 250 mg/kg and 1000 mg/kg of soil, a negative percentage change in the weight was observed. A graphical representation of the growth of *Eisenia fetida* (% change in weight) in soil for all the treatments and control at all concentrations is presented in Fig. 2.

Table 4. Growth of *Eisenia fetida* in soil treated with iron oxide NPs of 30 nm size.

NPs Concentration (mg/kg)	Initial weight (mg)	Final weight (mg)	Change in weight (mg)	% Change in weight
Control (0)	0.52±0.02	0.53±0.03	0.01	1.92
250	0.73±0.03	0.76±0.09	0.03	4.11*
500	0.62±0.02	0.63±0.04	0.01	1.61
750	0.60±0.01	0.62±0.02	0.02	3.33
1000	0.74±0.03	0.77±0.01	0.03	4.05*

Values are mean±S.E. of three replicates
* Indicates significant difference between values ($p<0.05$)

Table 5. Growth of *Eisenia fetida* in soil treated with iron oxide NPs of 100 nm size.

NPs Concentration (mg/kg)	Initial weight (mg)	Final weight (mg)	Change in weight (mg)	% Change in weight
Control (0)	0.52±0.02	0.53±0.03	0.01	1.92
250	0.51±0.02	0.54±0.03	0.03	5.88*
500	0.53±0.10	0.56±0.07	0.03	5.66*
750	0.52±0.01	0.58±0.02	0.06	11.54*
1000	0.53±0.08	0.55±0.04	0.02	3.77

Values are mean±S.E. of three replicates
* Indicates significant difference between values ($p<0.05$)

Table 6. Growth of *Eisenia fetida* in soil treated with a combination of iron oxide NPs of sizes 30 nm and 100 nm.

NPs Concentration (mg/kg)	Initial weight (mg)	Final weight (mg)	Change in weight (mg)	% Change in weight
Control (0)	0.52±0.02	0.53±0.03	0.01	1.92
250	0.54±0.003	0.52±0.01	-0.02	-3.70
500	0.62±0.01	0.67±0.22	0.05	8.06*
750	0.55±0.17	0.59±0.08	0.04	7.27*
1000	0.60±0.14	0.57±0.01	-0.03	-5.00*

Values are mean±S.E. of three replicates
* Indicates significant difference between values ($p<0.05$)

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 mg/ml, 200 mg/ml, 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.

Another 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 nanoscale zerovalent iron at 500 mg/kg and 1000 mg/kg perturbed the activities of enzymes like superoxide dismutase, malondialdehyde content. The study also revealed that nanoscale zerovalent 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.

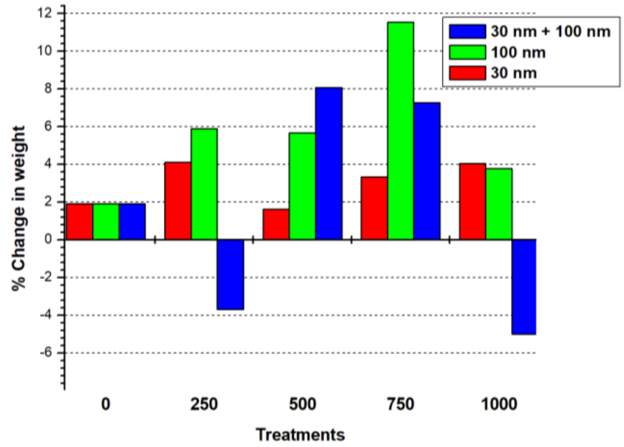

Fig. 2. Growth of *Eisenia fetida* (% change in weight) in soil with different treatments of iron oxide NPs.

Number of days taken by earthworms for vermicompost preparation

The vermicompost produced from FYM was harvested after 93 days (Table 7). It was observed to be much darker compared to the initial colour. No mortality was observed in any animal waste during the study period. Table 7 shows the number of days taken by the earthworms to prepare the vermicompost under different treatments. The fastest substrate conversion into vermicompost was recorded in the control, whereas the maximum number of days (93 days) was recorded in the treatment T3 (30 nm + 100 nm iron oxide NPs). This reveals that iron oxide NPs influenced the vermicomposting which may be caused by the impact of these NPs on the metabolic processes of the earthworms.

Table 7. Number of days taken by earthworms to prepare vermicompost under different treatments.

Treatment	Time taken to prepare vermicompost (Days)
Control	82±0.014
T1 (30 nm@250 mg/kg of soil)	89±0.090
T2 (100 nm@750 mg/kg of soil)	92±0.025
T3 (30nm+100nm@500 mg/kg of soil)	93±0.032

Physio-chemical parameters of FYM

pH

The pH of the substrate changed to nearly neutral after vermicomposting (Table 8). The final vermicompost's pH was observed to be lower than the initial substrate (control) for all the treatments. The possible reason behind pH-shift is the higher mineralization of nitrogen and phosphorus into nitrites or nitrates and orthophosphates, respectively. The formation of intermediate organic acid species while bioconversion of organic matter may also cause pH-shift. This pH-shift is dynamic in nature and depends on the type of substrate used (Ndegwa et al., 2000).

Similar trends of reduction in pH were reported by Bhat et al. (2015) during vermicomposting of various substrates such as sugarcane bagasse and cattle dung. The study revealed that another possible reason of pH-shift can be the production of acids such as humic and fulvic during the composting process. Moreover, the production of carbon dioxide and other organic acids during microbial metabolism can be another reason behind the pH shift (Haimi and Huhta, 1987).

Earlier study (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; the production NO_3 was could be the possible reason behind pH trends. Several studies conducted by different researchers worldwide also revealed that most of the earthworm species prefer near neutral pH, i.e. close to 7.0 (Pagaria and Totwat, 2007; Suthar, 2008; Panday and Yadav, 2009). The present findings contradict the findings of Tripathi and Bhardwaj, 2004; Loh et al., 2005; Pattnaik and Reddy, 2010, who reported higher pH. 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 iron oxide NPs. It may be because of the production of organic acids during decomposition, which reduces pH.

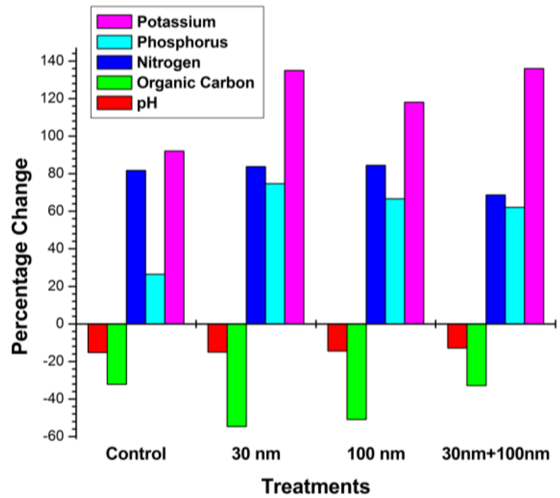

Fig. 3. Percentage change in chemical parameters of the FYM after formation of final vermicompost

Total Organic Content

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_1 and the minimum in control (Table 9). These findings are consistent with those of earlier authors (Garg and Kaushik, 2005; Tognetti et al., 2005). The microbial degradation of organic matter at the time of the vermicomposting and action of earthworms leads to a lowering of the total organic carbon values. Carbon is the building block of all living organisms and also a major component of organic molecules. It is needed as the primary source of energy for the process of composting (Ansari and Jaikishun, 2011; Ansari and Rajpersaud, 2012). Another reason behind the reduction in the trends of organic carbon values includes the loss of organic carbon amount as CO_2 through microbial respiration and mineralization of organic matter, causing an increase in total nitrogen. A part of the carbon in the decomposing residues is released as CO_2, and a part is assimilated by the microbial biomass (Fang et al., 2001; Cabrera et al., 2005). Microorganisms use carbon as a source of energy for decomposing organic matter. The various studies confirmed that the reduction in the organic carbon content in vermicomposting processes is often higher than the other composting processes. The reason behind this is the higher assimilating capacity of the earthworms. Elvira et al. (1996) reported a decrease of 1.7 folds in total organic carbon content in the harvested vermicompost made from the substrates - paper pulp and mill sewage sludge after 40 days. Garg et al. (2006) observed 3.0, 2.2, and 1.7 folds decrease in total organic content of vermicomposting of agricultural residues, kitchen wastes, and industrial wastes, respectively.

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 (Tables 10-12). The maximum increase of total nitrogen was recorded in treatment containing a combination of 30 nm and 100 nm of iron oxide NPs. According to Atiyeh et al. (2002), the conversion of ammonium-nitrogen into nitrate leads to an increase in nitrogen content.

Table 8. Effect of different NPs concentrations on pH of vermicompost

Treatment	1st Day	30th Day	60th Day	90th Day
Control	8.87±0.02^{cD}	8.47±0.02^{cC}	8.38±0.01^{dB}	7.72±0.01^{cA}
T1 (30 nm@250 mg/kg of soil)	8.60±0.01^{bD}	7.62±0.01^{aC}	7.50±0.05^{aB}	7.31±0.02^{aA}
T2 (100 nm@750 mg/kg of soil)	8.63±0.02^{bD}	7.82±0.004^{bC}	7.67±0.02^{bB}	7.38±0.004^{bA}
T3 (30 nm+100 nm@500 mg/kg of soil)	8.55±0.07^{aD}	8.45±0.01^{cC}	8.10±0.02^{cB}	7.51±0.01^{dA}

Values are mean±S.E. of three replicates
Values with superscript (a, b, c, d) indicate significant difference between treated and untreated soil samples (p<0.05)
Values with superscript (A, B, C, D) indicate significant difference between values at different days (p<0.05)

Table 9. Effect of different NPs concentrations on total organic content of vermicompost

Treatment	1st Day	30th Day	60th Day	90th Day
Control	0.62±0.02^{bD}	0.60 ±0.005^{cC}	0.52±0.01^{cB}	0.42±0.04^{cA}
T1 (30 nm@250 mg/kg of soil)	0.55±0.02^{aD}	0.49±0.02^{aC}	0.41±0.01^{aB}	0.25±0.02^{aA}
T2 (100 nm@750 mg/kg of soil)	0.57±0.005^{aD}	0.50±0.01^{aC}	0.42±0.02^{aB}	0.28±0.005^{aA}
T3 (30 nm+100 nm@500 mg/kg of soil)	0.68±0.18^{cD}	0.51±0.24^{bC}	0.45±0.32^{bB}	0.39±0.02^{bA}

Values are mean±S.E. of three replicates
Values with superscript (a, b, c, d) indicate significant difference between treated and untreated soil samples (p<0.05)
Values with superscript (A, B, C, D) indicate significant difference between values at different days (p<0.05)

Table 10. Effect of different NPs concentrations on nitrogen content of vermicompost

Treatment	1st Day	30th Day	60th Day	90th Day
Control	1.64±0.02^{aA}	1.92±0.01^{aB}	2.58±0.02^{bC}	2.98±0.004^{aD}
T1 (30 nm@250 mg/kg of soil)	1.83±0.01^{cA}	2.29±0.01^{cB}	2.92±0.02^{cC}	3.36±0.03^{cD}
T2 (100 nm@750 mg/kg of soil)	1.79±0.004^{bA}	2.26±0.03^{cB}	2.86±0.01^{dC}	3.30 ±0.01^{bD}
T3 (30 nm+100 nm@500 mg/kg of soil)	1.80±0.02^{bA}	1.98±0.02^{bB}	2.55±0.01^{aC}	3.45±0.06^{dD}

Values are mean±S.E. of three replicates
Values with superscript (a, b, c, d) indicate significant difference between treated and untreated soil samples (p<0.05)
Values with superscript (A, B, C, D) indicate significant difference between values at different days (p<0.05)

Table 11. Effect of different NPs concentrations on phosphorus content of vermicompost

Treatment	1st Day	30th Day	60th Day	90th Day
Control	0.72±0.02^{aA}	0.76±0.02^{aB}	0.83±0.01^{aC}	0.91±0.01^{aD}
T1 (30 nm@250 mg/kg of soil)	0.71±0.03^{aA}	0.96±0.02^{cB}	1.07±0.03^{cC}	1.24±0.02^{cD}
T2 (100 nm@750 mg/kg of soil)	0.72±0.03^{aA}	0.91±0.05^{bB}	1.03±0.02^{bC}	1.20 ±0.03^{bD}
T3 (30 nm+100 nm@500 mg/kg of soil)	0.74±0.31^{bA}	0.95±0.16^{cB}	1.04±0.01^{bC}	1.20±0.04^{bD}

Values are mean±S.E. of three replicates
Values with superscript (a, b, c, d) indicate significant difference between treated and untreated soil samples (p<0.05)
Values with superscript (A, B, C, D) indicate significant difference between values at different days (p<0.05)

Table 12. Effect of different NPs concentrations on potassium content of vermicompost

Treatment	1st Day	30th Day	60th Day	90th Day
Control	0.88±0.01^{aA}	0.96±0.01^{aB}	1.30 ±0.01^{aC}	1.69±0.01^{aD}
T1 (30 nm@250 mg/kg of soil)	1.13±0.01^{cA}	1.21±0.02^{cB}	1.59±0.04^{dC}	2.65±0.02^{dD}
T2 (100 nm@750 mg/kg of soil)	1.16±0.02^{dA}	1.17±0.01^{bB}	1.52±0.07^{cC}	2.53±0.02^{bD}
T3 (30 nm+100 nm@500 mg/kg of soil)	1.10±0.16^{bA}	1.25±0.04^{dB}	1.48±0.02^{bC}	2.60±0.01^{cD}

Values are mean±S.E. of three replicates
Values with superscript (a, b, c, d) indicate significant difference between treated and untreated soil samples (p<0.05)
Values with superscript (A, B, C, D) indicate significant difference between values at different days (p<0.05)

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; 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 Fig. 3.

Conclusion

It is concluded that iron oxide NPs cause morphological damage and colour change to *Eisenia fetida*, while the untreated earthworms are grown without any damage to their body. The treatment involving a combination of the two sizes of iron oxide NPs significantly decreased the earthworm growth compared to the control and the earthworms took the highest time preparing vermicompost under this treatment. The nutrient content of the final vermicompost obtained from the treatment containing a combination of two sizes of iron oxide NPs was higher than the control and other treatments containing 30 nm and 100 nm size iron oxide NPs separately. The study also revealed that earthworm growth and vermicomposting efficiency depend on the concentration of NPs; therefore, it is crucial to develop further field and laboratory research for assessing ecological and environmental damage caused by the use and release of NPs.

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Author Contributions

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

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