

Heavy Metals and Micronutrient Bioaccumulation by Locally Isolated Earthworms From Temperate Kashmir in Final Vermicompost

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Abstract The experiment was laid in completely randomized design which comprised of 16 treatments of 4 waste combinations including cow dung and mixture of organic wastes (kitchen wastes, municipal waste, crop residues, sheep/poultry manure and apple pomace) cow dung (24 kg) and organic waste (60 kg). The ratio of waste combinations was maintained as: 0:1=(0% cow dung + 100% organic waste), 1:1 = (50% cow dung + 50% organic waste), 1:2 = (33% cow dung + 67% organic waste), 1:3 = (25% cow dung + 75% organic waste). The micronutrients contents like zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) were

significantly higher in vermicompost obtained from treatment T₆ (50% cow dung + 50% organic waste) by *Eisenia foetida* (Shalimar) as compared to other treatments. There was nonsignificant difference between different treatments regarding cadmium concentration in vermicompost, with treatment combination of *Eisenia foetida* (Shalimar) and cow dung + organic waste (1:1) having significantly higher lead content among the interactions. The results indicated that the *Eisenia foetida* isolated from Shalimar proved to be having the best vermicomposting potential due to its individual capability and better adaptability to the local temperature conditions as compared to standard *Eisenia foetida* and other local isolates.

Keywords *Eisenia foetida*, Micronutrients, Temperature, Vermicompost.

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Introduction

Vermicomposting is an eco-biotechnological process that transforms energy-rich and complex organic substances into stabilized humus-like product vermicompost. Increasing interest in composting as a waste management strategy has led to increase research efforts directed toward utilization of various organic wastes in agriculture. To reduce the environmental strain, it is essential to make organic wastes a resource rather than a by-product. Among various composting methods, vermicompost has gained wide acceptance and popularity because of its faster de-

Table 1. Details of treatments and waste combinations.

Treatments	Combinations	Composition
T ₁	S ₁ C ₁	S ₁ <i>Aporrectodea rosea</i> (Wadura isolate) C ₁ (0% cow dung + 100% organic waste)
T ₂	S ₁ C ₂	S ₁ <i>Aporrectodea rosea</i> (Wadura isolate) C ₂ (50% cow dung + 50% organic waste)
T ₃	S ₁ C ₃	S ₁ <i>Aporrectodea rosea</i> (Wadura isolate) C ₃ (33% cow dung + 67% organic waste)
T ₄	S ₁ C ₄	S ₁ <i>Aporrectodea rosea</i> (Wadura isolate) C ₄ (25% cow dung + 75% organic waste)
T ₅	S ₂ C ₁	S ₂ <i>Eisenia foetida</i> (Shalimar isolate) C ₁ (0% cow dung + 100% organic waste)
T ₆	S ₂ C ₂	S ₂ <i>Eisenia foetida</i> (Shalimar isolate) C ₂ (50% cow dung + 50% organic waste)
T ₇	S ₂ C ₃	S ₂ <i>Eisenia foetida</i> (Shalimar isolate) C ₃ (33% cow dung + 67% organic waste)
T ₈	S ₂ C ₄	S ₂ <i>Eisenia foetida</i> (Shalimar isolate) C ₄ (25% cow dung + 75% organic waste)
T ₉	S ₃ C ₁	S ₃ <i>Aporrectodea caliginosa</i> (Gulmarg isolate) C ₁ (0% cow dung + 100% organic waste)
T ₁₀	S ₃ C ₂	S ₃ <i>Aporrectodea caliginosa</i> (Gulmarg isolate) C ₂ (50% cow dung + 50% organic waste)
T ₁₁	S ₃ C ₃	S ₃ <i>Aporrectodea caliginosa</i> (Gulmarg isolate) C ₃ (33% cow dung + 67% organic waste)
T ₁₂	S ₃ C ₄	S ₃ <i>Aporrectodea caliginosa</i> (Gulmarg isolate) C ₄ (25% cow dung + 75% organic waste)
T ₁₃	S ₄ C ₁	S ₄ <i>Eisenia foetida</i> (standard isolate) C ₁ (0% cow dung + 100% organic waste)
T ₁₄	S ₄ C ₂	S ₄ <i>Eisenia foetida</i> (standard isolate) C ₂ (50% cow dung + 50% organic waste)
T ₁₅	S ₄ C ₃	S ₄ <i>Eisenia foetida</i> (standard isolate) C ₃ (33% cow dung + 67% organic waste)
T ₁₆	S ₄ C ₄	S ₄ <i>Eisenia foetida</i> (standard isolate) C ₄ (25% cow dung + 75% organic waste)

composition [1], superior quality [2—5] and positive effect on yields of crops in general [6].

Among the sources of available organic manures, vermicompost is a potential source due to the presence of readily available plant nutrients, growth enhancing substances and a number of beneficial microorganisms. Vermicompost being a biofertilizer is enriched with all beneficial soil microbes and also contains all the essential plant nutrients. As per reports of Gupta, [2] vermicompost that is prepared through conventional method has standard values of total nitrogen: 1.94%, phosphorus: 0.47% and po-

tassium: 0.70% it is also enriched with various micronutrients such as Mg (0.46%), Fe (7563 ppm), Zn (278 ppm), Mn (475 ppm), Bo (34 ppm), Cu (27 ppm) thus eliminate usage of any further artificial chemical inputs. *Eisenia foetida*, a temperate species of earthworm commonly used for vermicomposting has adapted to the temperate conditions of Kashmir valley, but its vermicomposting ability gets reduced during cold winter months. So to look for local worms that can tolerate low temperature and perform better under such conditions is need of hour. Keeping in view these facts, the present study is done to see the micro and macronutrients of vermicompost produced by locally isolated earthworms from temperate Kashmir region.

Materials and Methods

Experimental details

Collection and identification of earthworms from various areas of Kashmir

Earthworms were collected from three different locations Wadura (altitude of 1589 meters) ASL with atmospheric temperature of (-05 to +35°C), Shalimar (altitude of 1680 meters) ASL with atmospheric temperature of (-10 to +35°C) and Gulmarg (altitude of 2650 meters) ASL with atmospheric temperature of (-20 to +35°C). The species *Eisenia foetida*, *Aporrectodea rosea* and *Aporrectodea caliginosa* were identified from Wadura; *Eisenia foetida* and *Aporrectodea caliginosa* were identified from Shalimar and *Eisenia foetida* and *Aporrectodea caliginosa* were identified from Gulmarg. Preliminary screening of the identified worms from each location was carried out on the basis of vermicomposting potential to select the best isolate. *Aporrectodea rosea*, *Eisenia foetida* and *Aporrectodea caliginosa* from Wadura, Shalimar and Gulmarg respectively were selected for vermicomposting process.

Details of layout

The experiment comprised of 16 treatments (Table 1) which included waste combinations and different earthworm species. Earthworm species used are S₁;

Aporrectodea rosea, S₂: *Eisenia foetida*, S₃: *Aporrectodea caliginosa* and S₄: *Eisenia foetida* (standard isolate). Waste combination used are C₁ = 0:1 (0% cowdung + 100% organic waste), C₂ = 1:1 (50% cowdung + 50% organic waste), C₃ = 1:2 (33% cowdung + 67% organic waste) and C₄ = 1:3 (25% cowdung + 75% organic waste). Organic wastes include a mixture of kitchen wastes, municipal waste, crop residues, sheep/poultry manure, apple pomace in equal proportions. The experiment was laid in completely randomized design comprised of 16 treatments and 3 replications each. All the organic wastes were shredded and chopped into small pieces and mixed with cow dung (one week old) in its required proportions. The mixed substrates were kept for three weeks for pre-decomposition under shade and rain proof shed.

The pre-decomposed material was put in their respective vermibeds labeled and designed in CRD. Vermicomposting technique was used as per the procedure outlined by Hopp [7]. Fifty earthworms were released on the substrate in each treatment combination. The temperature of the shed varied from 10 to 35°C and the moisture was maintained at 40 to 60% by sprinkling water every day to keep earthworms

active. The vermibeds were covered with a hessian cloth and the compost material was turned upside down after every 15 days for proper aeration.

During 45–90 days, worms converted all the organic wastes into vermicompost. Watering was stopped 7 days prior to collection of excrements, then it was dumped on the ground as a heap and within 6 to 8 h, earthworms and cocoons moved down and settled at the bottom of the heap as a cluster and were separated and counted. The compost was dried in shade, mixed thoroughly, sieved and samples were drawn from each treatment in air tight poly bags for analysis of microbial composition.

Determination of macro and micronutrients of vermicompost

Total magnesium was determined from diacid digestion extract by use of AAS (Atomic Absorption Spectrophotometer) as described by Lindsay and Norwell [8]. Available magnesium was determined by the addition of carbamate crystals and Eriochrome Black-T indicator to the known volume of extract (sample +

Table 2. Effect of different waste combinations and earthworm isolates on Zn, Cu, Mn and Fe (%) of vermicompost. 0:1 = (0% cowdung + 100% organic waste), 1:1 = (50% cowdung + 50 organic waste), 1:2 = (33% cowdung + 67% organic waste), 1:3 = (25% cowdung + 75% organic waste).

Earthworm isolates (S)	Isolated from	Vermicompost									
		Zn (%)				Cu (%)					
		0:1	1:1	1:2	1:3	Mean	0:1	1:1	1:2	1:3	Mean
<i>Aporrectodea rosea</i>	Wadura	0.050	0.056	0.053	0.052	0.052	0.012	0.014	0.013	0.013	0.013
<i>Eisenia foetida</i>	Shalimar	0.048	0.056	0.054	0.051	0.052	0.014	0.016	0.015	0.015	0.015
<i>Aporrectodea caliginosa</i>	Gulmarg	0.040	0.046	0.043	0.043	0.043	0.013	0.015	0.015	0.14	0.015
<i>Eisenia foetida</i>	Standard	0.046	0.054	0.052	0.049	0.050	0.013	0.015	0.013	0.013	0.014
	Mean	0.046	0.053	0.050	0.048		0.013	0.016	0.014	0.014	
		CD ($p \leq 0.05$)					CD ($p \leq 0.05$)				
		Isolates (S)				=0.011	Isolates (S)				=0.001
		Combination (C)				=0.003	Combination (C)				=0.001
		S × C				=0.018	S × C				=0.003

Table 2. Continued.

Earthworm isolates (S)	Isolated from	Mn (%)					Vermicompost				
		0:1	1:1	1:2	1:3	Mean	0:1	1:1	1:2	1:3	Mean
<i>Apporectodea rosea</i>	Wadura	0.04	0.05	0.05	0.04	0.05	0.42	0.43	0.42	0.42	0.42
<i>Eisenia foetida</i>	Shalimar	0.05	0.05	0.05	0.05	0.05	0.43	0.43	0.43	0.43	0.43
<i>Apporectodea caliginosa</i>	Gulmarg	0.04	0.04	0.04	0.04	0.04	0.38	0.39	0.38	0.38	0.39
<i>Eisenia foetida</i>	Standard	0.05	0.05	0.05	0.05	0.05	0.38	0.38	0.38	0.38	0.38
	Mean	0.04	0.05	0.05	0.05		0.40	0.41	0.40	0.40	
		CD ($p \leq 0.05$)					CD ($p \leq 0.05$)				
		Isolates (S)				=0.080	Isolates (S)				=0.010
		Combination (C)				=0.001	Combination (C)				=0.001
		S × C				=0.011	S × C				=0.010

ammonium acetate solution) and the determination was carried out with the help of EDTA titration method described by Jackson [9]. Total sulfur was determined from diacid digestion sample with the addition of biffer solution, gum acacia and barium chloride and determination was carried out with the help of spectrophotometer at 420 nm wavelength described by Chesnien and Yien [10]. Available sulfur was determined from organic waste sample by the addition of 0.51% CaCl_2 solution by shaking followed by filtration with the addition of barium chloride and gum acacia and determined with the help of spectrophotometer at 660 nm (Transmittance) described by Chesnien and Yien [10]. Micronutrient cation were determining in samples by method given by Lindsay and Norwell [8]. These available micronutrients were determined by using DTPA (Diethylene Triamine Penta acetic acid) as an extractant which has been widely accepted for the simultaneous extraction of these micronutrients. The Zn, Cu, Fe, Mn were determined by Atomic Absorption spectrophotometer. The total micronutrients of organic waste samples were estimated by extraction with diacid mixture of HNO_3 and HClO_4 in ratio of 10:3 by Atomic Absorption spectrophotometer. Available content of heavy metals viz., Cd, Pb, Ni, in organic waste samples were assessed by extraction with 0.5 M diethylenetriamine penta acetic acid (pH 7.3) and determined by Atomic Absorption spectrophotometer (AAS-4141) as described by Lindsay and Norwell

[8]. The data collected on different observations were analyzed statistically using the standard procedures followed by Gomez and Gomez [11].

Results and Discussion

Micronutrients

Eisenia foetida (Shalimar) and *Apporectodea rosea* on waste combination of cow dung + organic waste (1:1) recorded maximum total and available Zn, Cu and Mn content and maximum available Zn content in vermicompost and was significantly more than other worms. Minimum of total and available Zn, Cu and Mn content was recorded in *Apporectodea caliginosa* in waste combination of cow dung + organic waste 0:1 (Table 6 and 7). Use of *Eisenia foetida* (Shalimar) on waste combination of cow dung + organic waste 1:1 recorded significantly maximum total and available Fe content in vermicompost and minimum of total Fe and available Fe was recorded in *Apporectodea caliginosa* in waste combination of cow dung + organic waste 0:1, 1:1 and 1:3 (Table 7). The increasing trend micronutrients in vermicompost might be due to the additional supplement of organic wastes. Similar findings have been obtained by Rama Lakshmi et al. [12] evaluated the compositing with chemical additives and vermicomposting with *Eisenia*

Table 3. Effect of different waste combinations and earthworm isolates on heavy metals (%) of vermicompost. 0:1 = (0% cowdung + 100% organic waste), 1:1 = (50% cowdung + 50 organic waste), 1:2 = (33% cowdung + 67% organic waste), 1:3 = (25% cowdung + 75% organic waste).

Earthworm isolates (S)	Isolated from	Vermicompost									
		Cadmium (%)				Mean	Lead (%)				Mean
		0:1	1:1	1:2	1:3		0:1	1:1	1:2	1:3	
<i>Apporectode a rosea</i>	Wadura	0.0002	0.0002	0.0002	0.0002	0.0002	0.010	0.001	0.001	0.010	0.005
<i>Eisenia foetida</i>	Shalimar	0.0002	0.0002	0.0002	0.0002	0.0002	0.001	0.009	0.008	0.001	0.002
<i>Apporectode a caliginosa</i>	Gulmarg	0.0001	0.0001	0.0001	0.0001	0.0001	0.002	0.010	0.009	0.009	0.009
<i>Eisenia foetida</i>	Standard	0.0001	0.0002	0.0002	0.0002	0.0002	0.001	0.002	0.002	0.001	0.002
	Mean	0.0001	0.0002	0.0002	0.0002		0.005	0.004	0.004	0.005	
		CD ($p \leq 0.05$)					CD ($p \leq 0.05$)				
		Isolates (S)		= NS			Isolates (S)		= 0.003		
		Combination (C)		= NS			Combination (C)		= 0.003		
		S × C		= NS			S × C		= 0.003		

foetida in different organic residues i.e sugarcane trash, weeds, vegetable market waste and paddy straw mixed with cattle dung. Vermicomposting resulted in significant increase in Zn, Fe, Mn and Cu content after 65 days of composting over normal composting uninoculated with earthworms after 110 days.

Heavy metals (cadmium and lead)

There was nonsignificant difference between different treatments and among the interactions regarding cadmium concentration in vermicompost. However, treatment combination of *Eisenia foetida* (Shalimar) and cow dung + organic waste (1:1) produced maximum lead content among the interactions (Table 8). Reduced heavy metal content might be due to the longer duration of vermicomposting using earthworms enhance the quality of end product. The decrease in heavy metal concentrations in the final vermicompost indicates the capability of Iranian and Australian *E foetida* in accumulating heavy metals in their body tissues [13]. The results are also supported by

Jamaludin and Mahamood, [14] who reported that certain feed materials like coffee grounds at specific ratio and suitable condition will increase the nutrient elements (N, P and K) while reducing the heavy metal contents.

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