Uttar Pradesh Journal of Zoology

Volume 44, Issue 24, Page 28-37, 2023; Article no.UPJOZ.3038
ISSN: 0256-971X (P)

Quantification of Humic Acid Produced in Worm-Activated Cast and Soil under Varied Land Use Types in Raebareli District

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Authors' contributions

This work was carried out in collaboration between both authors. Author TB conceptualized, designed, analysed and interpreted the data and prepared the first draft. Author GS was involved in the collection literature, collection of data (sampling and analysis) interpretation of data and manuscript typing and drafting. Both authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2023/v44i243806

Editor(s):
(1) Dr. Osama Anwer Saeed, University of Anbar Ramadi, Iraq.
Reviewers:
(1) Gilles Olive, Ecole Industrielle et Commerciale de la Province de Namur, Belgium.
(2) A. Avila Jerley, Holy Cross College (Autonomous) Affliated to Bharathidasan University, India.

Original Research Article

Received: 03/10/2023
Accepted: 08/12/2023
Published: 14/12/2023

ABSTRACT

The current study involves measuring the amount of humic acid produced by earthworms in their castings across five land uses, which were identified based on the level of anthropogenic activity along a disturbance gradient ranging from least disturbed protected forest to intensive agriculture to abandoned fallow to sodic ecosystem and then rehabilitated agroecosystem. Humic acids were recovered from typical soils and earthworm casts using the conventional alkali/acid fractionation method. According to the findings, humic acid obtained by casting has a significant advantage over humic acid derived from soil, which was directly impacted by the types of land use under consideration.

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Keywords: Humic acid; earthworm casting; organic matter; land uses.

1. INTRODUCTION

An essential part of the terrestrial ecosystem is humic compounds, which account for 60% of the soil's organic materials, and are crucial to several complex chemical reactions there [1]. According to Stevenson [2], it has 4% nitrogen and 33–36% oxygen. The humic acid structure's numerous functional groups enhance the soil's physicochemical characteristics [3]. Humic acid, a distinct and durable component of humus materials, acts similarly to auxin hormones and other growth-promoting substances to increase plant growth and performance. Because it is thought to be a rich source of humic acid and organic fertilizer that encourages plant development, earthworm casting is thought to be able to increase plant growth indices [4]. Humic acid physically supports a healthy soil structure and improves the soil's capacity to hold water and biologically, it fosters the establishment of beneficial soil organisms. It works well as a complex for adsorption and retention of inorganic plant nutrient availability increasing uptake and yield by enhancing the standard and output of crops [5]. Earthworms are widely acknowledged to have beneficial ecological, biological, and physiological effects on soils, and multiple studies have demonstrated that these benefits may boost plant growth and agricultural production in both natural and controlled situations and managed environments [6]. The earthworms break down the organic matter on their substrates, substantially promote the growth of microbes, and boost extraction rates, swiftly turning the residues into humus-like compounds with a more refined framework than compost but with a higher and more diverse microorganism action, known as vermicomposts. Humic acid synthesized from earthworm cast can rival commercially available products, usually formed of coal, leonhardite, and even indole phytohormones [7]. Vermicomposts have consistently increased seed emergence, seedling growth and proliferation, and plant productivity considerably more than the mere translation of nutrients from minerals into a more phyto-available condition could. The actions of earthworms improve the humification of organic materials and microbial richness [8]. This boosts the presence of auxins and gibberellins in worm castings even further [7]. The current study intends to determine the impact of soil nutrient absorption dynamics and soil physical state on humic acid availability depending on the patterns of land utilisation.

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2. METHODOLOGY

2.1 Study Sites and Landscape

The research sites are positioned between latitudes 80°41' and 81°34' East and 25°49' and 26°36' North. In the Raebareli district of the Indo-Gangetic plains, winter temperatures range from 6.89 °C to 26 °C (November to February). It is situated at an elevation between 8 and 10 meters. The Summer stretches from March to June, with a 43.4 °C high and 36 °C lowest temperature, respectively. 400 mm of precipitation is dispersed during the four months from July to October (Fig. 1).

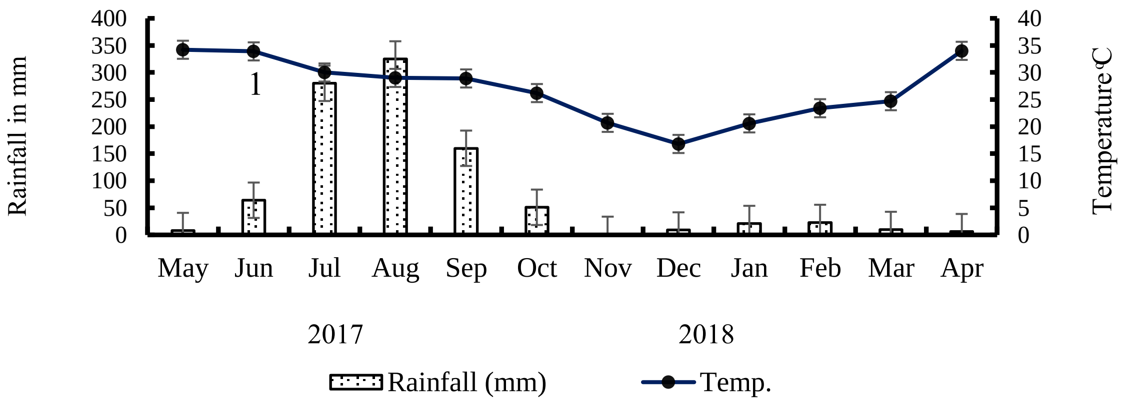

Fig. 1. Monthly fluctuations in average annual rainfall (mm) and temperature (° C) in the District Raebareli of Uttar Pradesh

There are five sites located in Lalganj and Salon of Raebareli District (Uttar Pradesh).

2.1.1 Protected forest (Fp)

The proportion of forest to the total area of India is 22, and in the state of Uttar Pradesh, it is about 17. While this is the case, just 4,802 hectares, or 1.04 percent of the district's total land area, are covered by forests in Raebareli. This includes 316 hectares of protected forests and 4,486 hectares of reserve forests. Protected Forest With very little human interference, the state forest department protects the Solone Rajapur Forest, which is home to a variety of native and exotic plants, including *the Fecus locor, Erithyrina suberosa, Acacia Arabic, Ficus religiosa, Terminalia chebula, and Tri Felis caracal, Herpestes auropunctatus, Hystrix indica, Gyps fulvus, Testndo elegans, Bungarus caerulens, Naja naja, Clarias mrigala,* and *Mystus singhala* were among the diverse species of animals that were also present.

2.1.2 Mechanized agroecosystem (Am)

Rice and wheat are two crops that are harvested annually during the summer and winter months from well-drained soils that have been added with mainly inorganic fertilizer and very little organic manure. In the district, rice is planted in 31.5% of the gross cropped area while wheat is grown in 36%. The same food grain is grown in Jowar and Bajra, albeit on a much smaller scale. Less than 2% of land is used for planting sugar and oil seeds, while less than 1% is utilized to grow potatoes. The production of pulses takes up about 13% of the total planted area.

2.1.3 Abandoned fallow (Fa)

A method of farming whereby arable land is not planted for varied vegetative cycles. It enables the recovery and storage of organic substances by the land. Here significant problem with entering the Raebareli area. To restore soil fertility, the formers are removed. On these fallow lands, cow grazing is a typical fallow-time activity.

2.1.4 Sodic agroecosystem (As)

A lack of an appropriate drainage system, a high rate of evaporation, and a concentration of soluble salts on the soil surface all contribute to sodic soil. Sodic soil has a very high pH, is very dense, is very hot, has fewer nutrients readily available, and has little to no flora. crop-unfit agroecosystem due to severe degradation.

2.1.5 Rehabilitated Agroecosystem (Ar)

The sodic agroecosystem was restored ten years ago using biodegradable materials like kitchen and garden waste, farmyard manure and dung from cattle, incorporating crop wastes into the soil, backlash of dry grasses, and roots caused by the application of biopesticides, and livestock, especially sheep and cows

3. METHOD AND MATERIALS

3.1 Collecting and Preserving Samples

A 40x50 m2 plot was set aside in each selected land used to collect soil samples. Three replicates of 25x25 cm2 earth monoliths were chosen at random from every single experimental site and the cast was collected at regular intervals. From 0 to 10 cm of depth, samples were collected in triplicate. Earthworm casts were collected weekly from a quadrate size of 25 x 25 cm^2. All soil samples and air-dried earthworm casts were run through a 2mm sieve [9]. The percentage of soil moisture was reported to be at a depth of 0-10 cm after oven drying at 105°C. Using a 1:5 (soil: water) solution, the soil pH was ascertained, and the soil texture was assessed using the method of hydrometer [10]. Organic Carbon was determined using the Walkey-Black technique [11]. A spectrophotometer was used to assess the quantity of phosphorus that was accessible after the nitrogen content of the soil was measured using the semi-micro Kjeldahl method and Olsen extractant. Cations were extracted from soil and cast samples using 1M ammonium acetate at pH 7, Exchangeable Ca and K were assessed using the EDTA titration method, Mg was measured using an atomic absorption spectrophotometer, and K was estimated using a flame photometer after [12].

3.2 Extraction of Humic Acid

Humic acids were extracted using the classic alkali/acid fractionation procedure from normal soils and earthworm casts [13]. 200 ml solution of 0.5N NaOH was introduced to an 8-oz. plastic centrifuge bottle that had been filled previously with a 40-g sample of acid-washed (0.1N HCl) soil and cast. The bottle was then tightly closed with a rubber stopper. To remove any floating plant matter, the mixture was mechanically stirred for 12 hours. After centrifuging the dark-coloured supernatant liquor, it was taken away, and put through glass wool filtering. The pH was subsequently adjusted down to roughly 1.0 with strong HCl. After adding 200 ml more of the 0.5 N NaOH solution to the soil, the centrifuging and decanting procedures were repeated, and the mixture was agitated for an additional hour. After combining the residue with distilled water (200 ml), the mixture was centrifuged once more, and the earlier extracts were then combined with the supernatant liquor. Humic acid was then allowed to settle after the resulting solution's pH was adjusted with concentrated HCl to 1.0 [13].

3.3 Analysis of Statistics

IBM SPSS Statistics 24 and Standard biostatistical techniques were used for statistical analysis [14]. One-way ANOVA was used to detect significant deviations in the soil's physicochemical properties across treatments. The sample standard error is calculated using the mean standard error values (SE).

4. RESULTS

4.1 Soil and Earthworm Cast Attributes

In comparison to sodic agroecosystems (As), the percentage of soil sand was considerably ($P< 0.05$) lower in mechanized agroecosystems (Am). The percentages of silt and clay in the Am and rehabilitated agroecosystems (Ar) were greater ($P <0.05$). In the Sodic agroecosystem (As), the soil had limited porosity and a relatively high bulk density, followed by fallow (Fa). The pH was as high as 9.94 (Alkaline) in As to a mildly acidic pH of 6.9 in Ar and Fp. Under all the land use types except in As the earthworm casting activity resulted in lowering the soil pH and increased the moisture percentage (Table 1).

The carbon percentage was much greater ($P< 0.05$) across all land uses, compared to the typical soil, in the casts, the trend towards growth in carbon percentage did not vary between different land use types (Fig. 2), The nitrogen percentage in worm casts was considerably higher ($P< 0.05$), compared to normal soil and this percentage increase was more prominent in Ar followed by Am and was lowest in Fa (Fig. 3). Organic matter percentage and phosphate phosphorus also had significantly higher ($P< 0.05$) ratios in the worm casts than in the surrounding soils. In terms of organic matter percentage, Am showed a greater growth than the other sites (Fig. 4). Whereas the casting activity by earthworms revealed a noticeably higher ($P< 0.05$) increase in phosphate phosphorus in the Am site in contrast to other land use types (Fig. 5). Ca Mg and K had increased concentration in cast in comparison to normal soil all different forms of land use and land management techniques did not change the increase in the concentration of these cations. Because there were no earthworm castings in As the concentrated amount of soil nutrients was low ($P < 0.05$), but the casting nutrient was insignificant (Figs. 6, 7, 8).

4.2 Humic Acid of Soil and Earthworm Cast

The earthworm enhances the availability of humic acid in the worm cast, compared to other types of land use, the Am has a higher concentration of this activity, and the increase in humic acid concentration was 6 times higher than the normal soil here. The concentration of the humic acid was higher in worm cast in Ar but the humic acid concentration was also higher in the surrounding soil too. That means in relative terms the increase in humic concentration in the cast was more significant ($P < 0.05$) in Am than in Ar (Fig. 9).

Table 1. Physical properties of soil (±SE, n = 5) and earthworm cast across different land use types in District Raebareli

Site	B.D.%	Sand%	Silt%	Clay %	pH		Moisture (%)	
					Soil	Cast	Soil	Cast
Protected forest	1.65	75±6.9	19±1.2	6±.48	6.9	6.3	21	25
Mechanized agroecosystem	1.69	57±4.9	31±2.7	12±.87	7.3	6.9	19.5	22.5
Abandoned fallow	1.84	73±6.3	24±1.9	3±.19	7.9	7.1	16.5	18.5
Sodic agroecosystem	1.92	57±4.9	37±2.9	6±.48	9.4	-	10.5	-
Rehabilitated agroecosystem	1.62	65±5.8	26± 2.1	9±.27	6.9	5.8	20.5	23.5

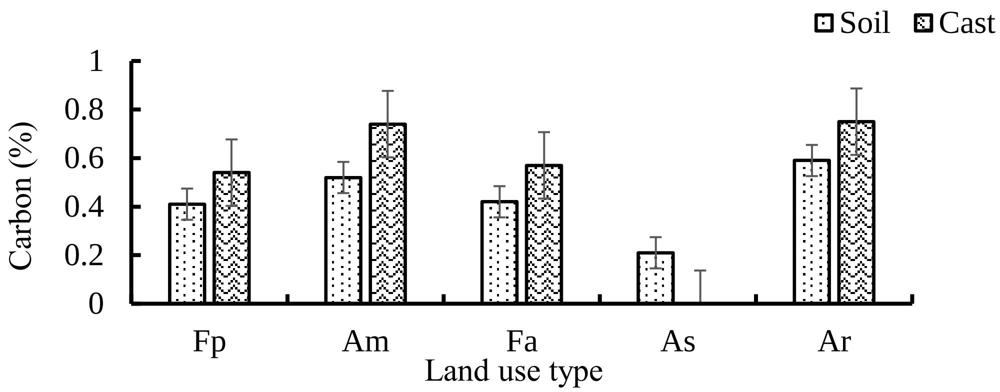

Fig. 2. Carbon concentration (%) of worm cast and surrounding soil under different land use types

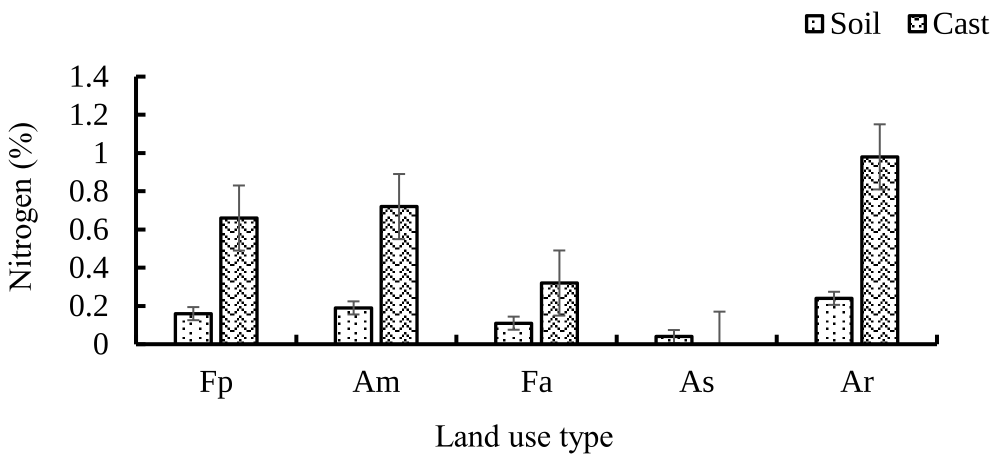

Fig. 3. Nitrogen concentration (%) of worm cast and surrounding soil under different land use types

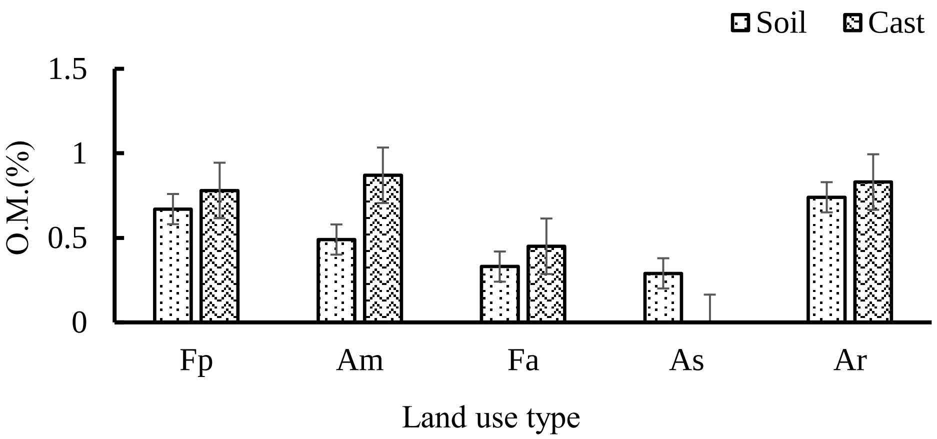

Fig. 4. Organic Matter concentration (%) of worm cast and surrounding soil under different land use types

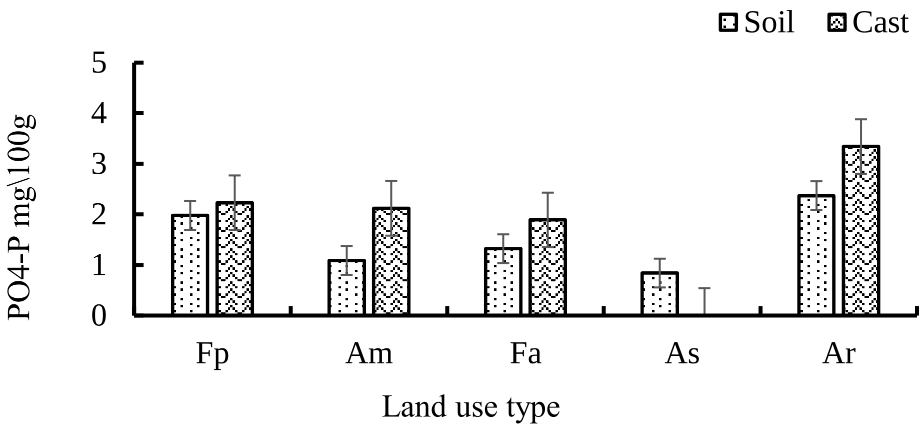

Fig. 5. Phosphorous-Phosphate concentration (Meq/100g) of worm cast and surrounding soil under different land use types

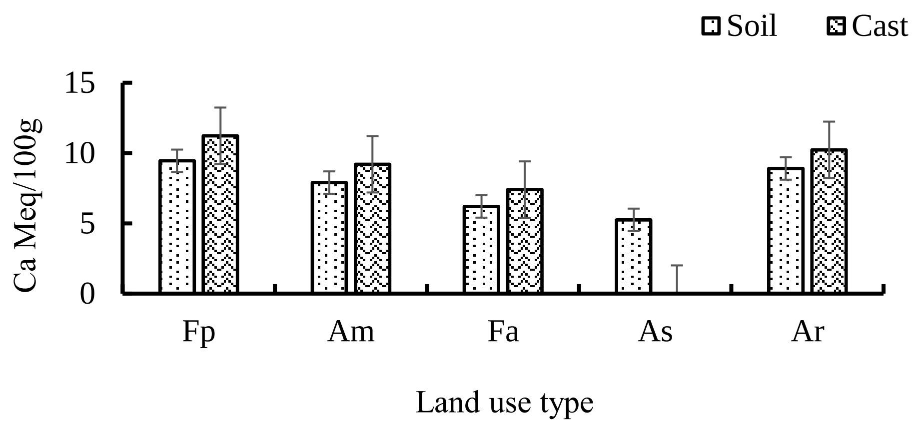

Fig. 6. Calcium concentration (Meq/100g) of worm cast and surrounding soil under different land use types

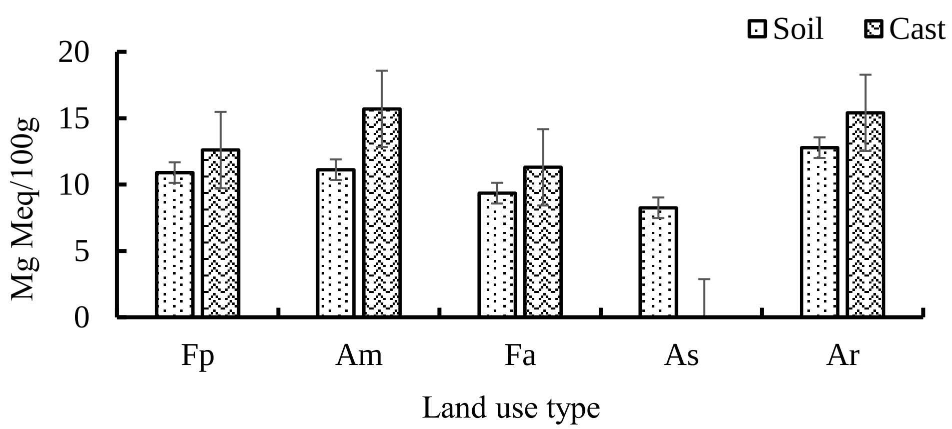

Fig. 7. Magnesium concentration (Meq/100 g) of worm cast and surrounding soil under different land use types

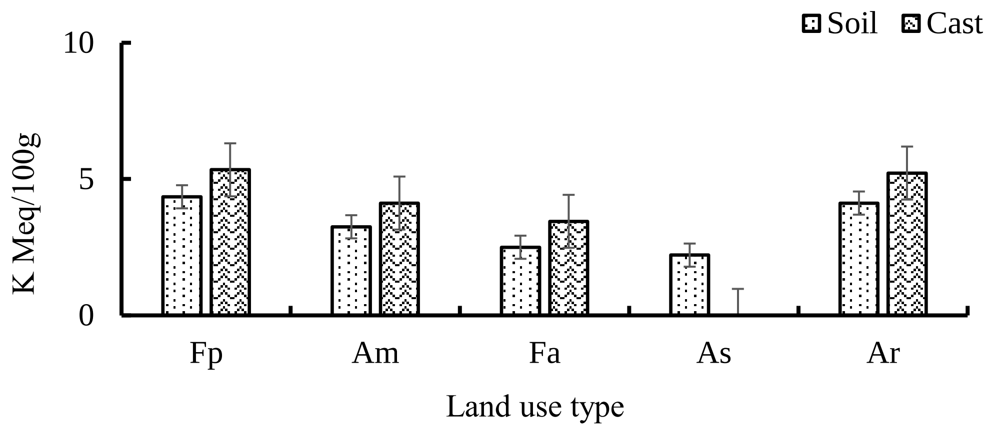

Fig. 8. Potassium concentration (Meq/100 g) of worm cast and surrounding soil under different land use types

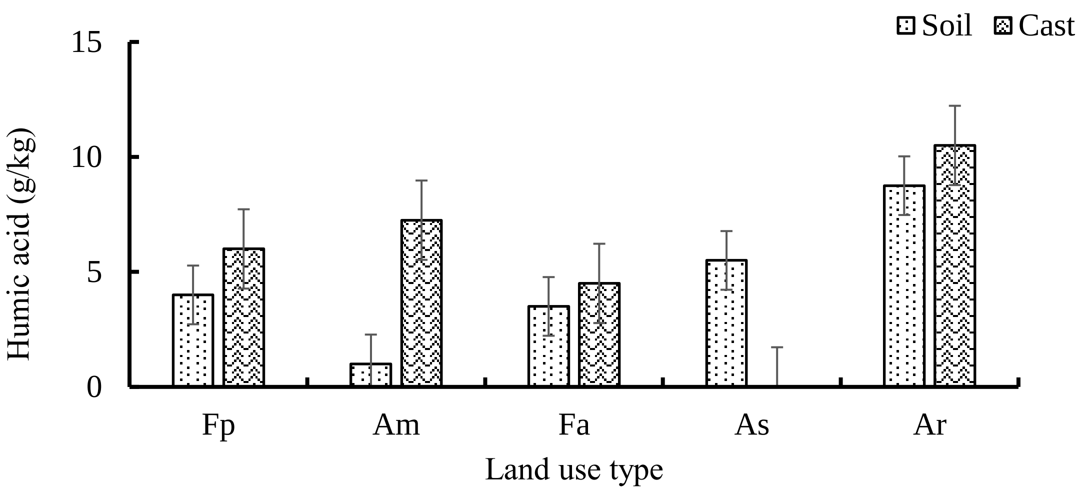

Fig. 9. Humic acid (g/kg) present in worm cast and surrounding soil under different land use types

5. DISCUSSION

In the majority of humid tropical habitats, earthworms play a significant part in the ecosystem of soil fauna and constitute an important portion of the macrofauna biomass. High concentrations of nutrients (N, P, K, and Ca) that are simple to absorb in fresh cast deposition have a more significant influence on earthworms [15]. Due to soil compaction and limited soil porosity, the sodic agroecosystem was characterized by high sodicity, a high pH of 9.94, a higher bulk density, and reduced soil nutrient availability [16]. This prevented vegetation from growing on this land use due to the lower concentration of carbon and nitrogen that prevented root formation and the movement of air and water through the soil and hampered earthworm colonization in As. The pH of the earthworm castings produced under all land use types was lower than the surrounding soils, and variation in concentration of organic matter between each land use type influenced the process through which nitrogen and phosphorus are mineralized to form nitrites, nitrates, and orthophosphates, [17]. The hydrophilic groups in humic acid, which enhanced hydration and hence increased water retention capacity in worm castings [18], contributed to humic acid's acidic character, which may play a role in decreasing pH under varied land uses in comparison to normal soil.

All land use types showed an increase in nitrogen content between the soil’s surroundings and casts which could occur from increased nitrogen mineralization. This could happen both directly via their metabolic byproducts (casts, urine, NH4+-containing mucus, urea, and uric acid), which release N [19], and through humic acid which increases the urease activity of the soil [20] therefore the variations in N mineralization were caused by variation in humic acids following gut transit in Ar. By forming a coating on soil particles, humic acid consumption enhances the ability to exchange cations and aeration and promotes root growth. The earthworm enhances the availability of humic acid in the worm cast [21]. In the Am, this activity was more prevalent, than in other land use categories; the increase in humic acid content was six times more than in the normal soil here, this along with organic matter improved the physicochemical properties of soil, and enzyme activity. Thus, the combination of humic acid and inorganic fertilizer resulted in significantly higher nitrogen concentration in Am as compared to other land use types [22] Castings from earthworms contain more exchangeable phosphorus. Similar observations have been made by others [23]. In terms of soil phosphorus status, changes in pH in earthworms and the enhanced microbial activity inside were probably the main causes of increases in accessible phosphorous [24]. In all land use categories, except the sodic agroecosystem, earthworm activity increased the amount of exchangeable potassium in comparison to the surrounding soil, in earthworm castings. As previously demonstrated in temperate [25] and tropical [26] environments, earthworm casting included much more organic carbon than adjacent soil. Humic acid and soil organic matter are positively associated, demonstrating that humic acid has a higher organic carbon content when there is more soil organic matter [27]. According to Bhadauria and Saxena [19], the presence of humic acid combined with the mucus associated with water output in the earthworm stomach enhances microbial activity. This increased microbial activity, together with the formation of micro-macro aggregates by earthworms and microorganisms, may be related to worm castings' capacity to raise humic acid content in cast and retain organic carbon at greater quantities than conventional cast [28]. Furthermore, differences in the content of humic acids and soil organic matter in worm castings between different land-use sites may vary with the dynamics of biological matter [29], the kind of substrate utilised, and the functional categories of earthworms (epigees or endogeics) that favour distinct food sources. Any modifications in humic acid in the casting of worms might influence the soil's characteristics and the diversity of microbial life in the soil, thereby affecting the levels of vital nutrients in the soils surrounding it and the worm cast. Therefore, humic acid concentrations might be used as useful indicators for alterations in soil and land quality [30]. This was also seen in our investigation, wherein earthworm activities increased the content of humic acids in castings when compared with nearby soils. This might explain the differences in humic acid levels caused by different forms of land use. These changes are likely to affect nutrient mineralization. The addition of considerable volumes of sheep dung, cow dung, and kitchen waste to reclaimed lands resulted in soil and casting humic acid levels that were significantly higher than at other locations.

6. CONCLUSION

Thus, it may be inferred that earthworm feeding activity increases the humic acid content in worm castings, impacting soil physicochemical parameters through increased nutrient availability. Thus, boosting soil fertility on a sustainable basis and encouraging the use of worm cast as a biofertilizer. However, differences in the increase of humic acid in worm castings compared to surrounding soil between different land-use sites vary with the dynamics of biological matter, the type of substrate used, and the functional categories of earthworms (epigees or endogeics) that prefer different food sources. Thus, the changes in humic acid levels induced by different types of land use are explained.

ACKNOWLEDGEMENT

The authors are thankful to Late Shri O.N. Bhargav, Managing Secretary, and Principal, Dr. Manoj Tripathi, Feroze Gandhi Degree College, Raebareli, for providing the necessary laboratory facilities to carry out this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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