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Dissolution of Phosphorus from Rock Phosphate using Earthworms (*Eudrillus euginae*)

Debajyoti Chakrabarty[1*] • Sanjib Kumar Das[2]

[1] Department of Zoology, Krishnagar Government College, Krishnagar–741101, Nadia, West Bengal, India
[2] Waste Management Cell, West Bengal Pollution Control Board, Paribesh Bhawan, Bidhan Nagar, Block-LA, Salt Lake City, Sector-III, Kolkata-700098, West Bengal, India

Corresponding author: * debajyoti_chakrabarty@yahoo.co.in

ABSTRACT

Earthworms (*Eudrillus euginae*) were used to test their effects in the dissolution of phosphorus (P) from insoluble, naturally occurring, Purulia rock phosphate (PRP) through vermicomposting of two different substrates for 90 days. The same substrates (partially decomposed water hyacinth and cow dung with or without rock phosphate) were also allowed to decompose without earthworm (controls). The substrates were monitored for concentration of P, nitrogen and potash and changes in pH. The observations showed that the treatment combination with earthworm and rock phosphate (RP) was the best among four treatments employed in terms of P and N release. There were significant differences in concentration of available phosphorus among all the treatments and K concentration was not affected by the treatments. The findings suggest that the earthworms facilitated the dissolution of P from RP which affects mineralization of N in the substrates.

Keywords: earthworm, *Eudrillus euginae*, rock phosphate, vermiculture, water hyacinth

INTRODUCTION

Phosphorus (P) is a major element, which controls the productivity of aquatic ecosystems. Fertilization with chemically processed phosphate fertilizer is common in aquaculture for improvement in production (PPCL 1987), but these fertilizers render a pollution load on aquatic body after repeated use (Rand 1995). There has, thus been a vigorous search for alternative and equally effective, easily available and cheaper sources of P. Rock phosphate (RP) is an easily available, cheaper and eco-friendly source of P fertilizer, which can be equally effective as chemically processed P fertilizer (Chakrabarty 1994). RP is available easily elsewhere in India and vast deposits of such RPs and easily available elsewhere in the globe. However, RP is sparingly soluble in water.

Several methods, like acidulation and composting, have been tested to increase dissolution of rock phosphate, but the results are not encouraging. Earthworms are able to release phosphorus from insoluble sources (Springett and Syers 1979; Ismail 1996; Sinha *et al*. 2002; Chakrabarty *et al.* 2007; Gupta *et al.* 2007; Chakrabarty 2009; Chakrabarty *et al.* 2009; Hirota *et al.* 2010; Park *et al.* 2011). However, no effort has been made to use earthworms to increase its dissolution of RP. The earthworm behaves as bioreactor with many nutrient solubilizing bacteria in its gut (Akiyama *et al.* 1984; Omorinkoba *et al.* 1985; Biswas and Narayanasam 2006; Chakrabarty 2009; Prasanna *et al.* 2011). Several species of earthworms are being used in vermiculture and sometimes 2-3 different species of earthworms are simultaneously used for better result. However, *Eudrillus euginae* is mostly used by the vermiculturist for vermiculture. The objective of the study was to determine the effects earthworms (*E. euginae*) on the dissolution of phosphorus from insoluble RP.

MATERIALS AND METHODS

Rock phosphate and earthworms

The composition of naturally occurring Purulia rock phosphate (PRP) is: P_2O_5 – 21.6%, CaO – 39.4%, Fe - 9.2%, MgO – 5.8%, S – 9.5%, SiO_2 – 8.1%, C (organic) – 4.1%. The origin and nature of PRP is igneous and contains no heavy metals; it is an eco-friendly fertilizer commonly used in agriculture.

Earthworms ranging length and weight from 2.7–2.8 g (mean 2.75 ± 0.5 g) and 23–26 cm (mean 24.5 ± 0.8 cm), respectively were collected from the Akshay Krishi Vikash vermiculture farm, Karimpur, Nadia. A mixture of water hyacinth (*Eichhornia crassipes*) and fresh cow dung (equal proportions) was used as the substrate for the worms. The substrate materials were collected from nearby Krishnagar Govt. College Campus and properly mixed (1:1) and left to partially decompose for 15 days in an earthen vat (0.5 m diameter). The substrate was poured into plastic boxes (0.8 m × 0.5 m × 0.2 m).

Experimental design and management of the trial

The experiment had four treatments (T–1 to T–4): T–1: Substrate with no RP and no worms; T–2: Substrate without RP, with worms; T–3: Substrate with RP, without worms; T–4: Substrate with RP and worms In T3 and T4, RP was added at 20 g Kg^{-1} substrate whereas in T2 and T4 33 earthworms were added per box.

Three boxes (as replicates) in batches (for 0, 30, 60 and 90 days) were used for each treatment to allow for monthly destructive sampling. The bottom of the every box was lined with polythene sheet. The boxes with earthworms were screened with 3 mm wire screen net to protect the earthworm from predators and other animals. Then the earthworms were introduced at 33 worms box^{-1} and covered with the substrate to a height of about 10 cm (Dynes 2003). Water spraying was done with a watering can twice a day on sunny days and once on cloudy days to maintain moisture content at 60%.

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Short Communication

Table 1 Growth performance, nutrient utilization productivity indices and prediction equation of earthworm using different substrate in 90 days (t).

Parameters	T–1	T–2	T–3	T–4
Initial total weight of worms (g)	No earthworm	91.60	No earthworm	91.60
Final total weight of worms (g)	No earthworm	305.20 **	No earthworm	399.80 *
Total weight gain of worms (g)	No earthworm	213.60 **	No earthworm	308.20 *
Relative growth rate (%)	No earthworm	233.18 **	No earthworm	336.46 *
Specific growth rate	No earthworm	0.74 **	No earthworm	0.91 *

Note. Level shown as * = $P < 0.05$, ** = $P > 0.05$
T–1 and T–3 did not have worms
T–2 = Cow dung, water hyacinth and earthworm, T–4 = Cow dung, water hyacinth, Rock Phosphate and earthworm

Sampling

The collection of worms was done by spreading neem leaves soaked in water (for about three days) on top of the substrates. This served as attractant and the earthworms came up at the top layer of the substrate, from where they were collected using a forceps (Sogbesan and Ugwumba 2006).

Samples vermicompost (T–2 and T–4) and compost (T–1 and T–3) were analyzed for available N, P and K concentration following the methods as described below,

Concentration of available Nitrogen was determined by micro-Kjeldahl method (Black *et al.* 1965). Concentration of available Phosphorus was measured (colorimetrically using Olsen's extractant) applying the chlorostannous phosphomolybdic acid method (Jackson 1967). Concentration of available Potassium: was determined with the help of flame photometer using neutral ammonium acetate as extractant (Jackson 1967).

Data analysis

All data recorded were subjected to two-way ANOVA at $P < 0.05$ using statistical software package, SPSS 17.0 version. The means were separated using Duncan's multiple range test (Duncan 1955).

RESULTS

There was an increase in concentration of nutrients (N, P and K) in all treatments with vermicomposting period up to 60 days after which no further increase occurred (**Fig. 1A-C**). Highest amounts of available N (**Fig. 1A**), P (**Fig. 1B**) and K (**Fig. 1C**) were found in T–4 followed by T–3 then T–2 and T–1. Only treatment T4 had significantly higher N than the other treatments; there were no significant treatment effects in terms of K concentration. A significant difference ($P < 0.05$) was observed among treatments in terms of available P concentration. Treatment T–4 showed highest (1.59 mg Kg^{-1}) amount followed by T–3 (0.84 mg Kg^{-1}), T–2 (0.56 mg Kg^{-1}) and T–1 (0.20 mg Kg^{-1}). The value of pH became slightly lower in T–2 and T–4, but remained almost same in T–1 and T–3 (**Fig. 2**). There was a significance difference in earthworm biomass production between T–2 and T–4. The highest biomass of 30.82 g $week^{-1}$ was recorded in T–4 while the lowest (21.36 g $week^{-1}$) was in T–2 (**Table 1**). There was a gradual and continuous increase in the monthly growth of the cultured earthworms from the two tested substrates throughout the experimental period. Highest weekly weight of 399.80 g earthworm kg^{-1} of substrate was recorded in T–4, followed by 305 g earthworm kg^{-1} substrate in T–2 (**Table 1**).

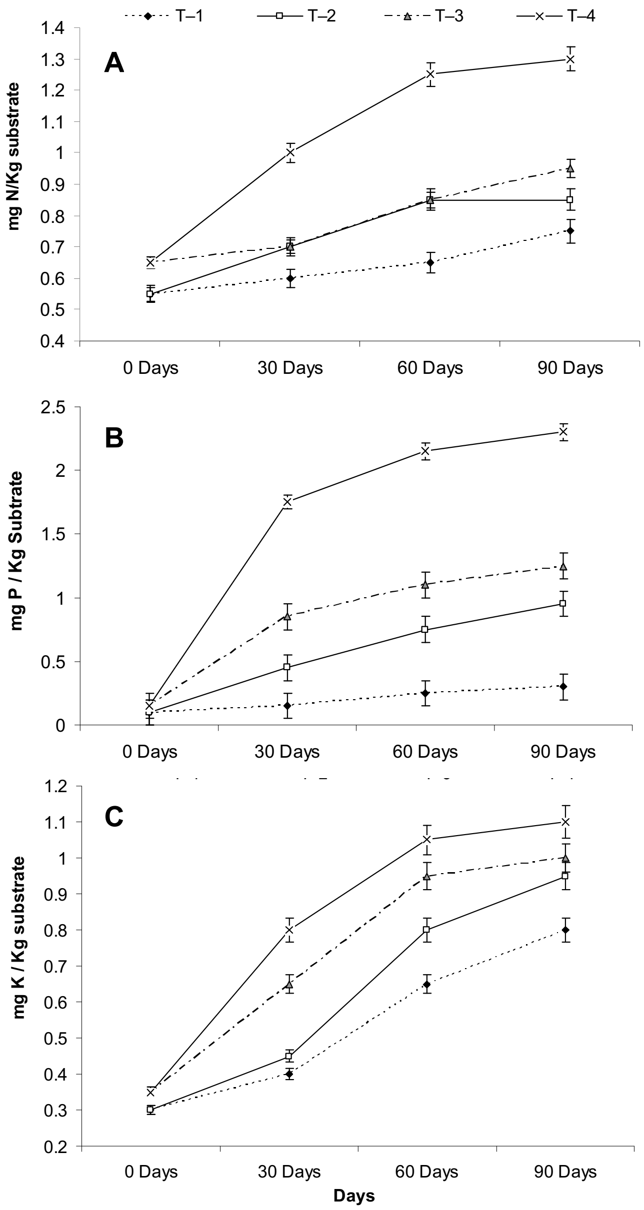

Fig. 1 Changes in the concentration of N (**A**), P (**B**) and K (**C**) in various treatments with the passage of time.

DISCUSSION

The trends of N as result appear to suggest that increasing the P content of cow dung and hyacinth will enhance the effect of earthworms on composting of the materials. This could be because the N: P ratio in the substrate was too high, making P limiting (PPCL 1987). Addition of P therefore appears to encourage earthwork activity in breaking down the hyacinth and the cow dung releasing more N and P. Increased P levels in treatments with earthworms suggest that gut microorganisms, in earthworms, and other secretions could be involved in the solubilization of P. The RP contain huge amount of phosphorus in bound form and its release through weathering, partial dissolution or composting has been tested with limited success (Chakrabarty 1994). However, vermicomposting of RP has not been tried earlier for dissolution of P from bound P. It has been reported by various groups (Atlavinyte and Daclulyte 1969; Ismail 1997; Lee 1985; Kale 1998; Ping and Boland 2004; Song *et al.* 2009) that worms secret enzymes, including proteases, lipases, amylases, cellulsases and chitinases, which bring about rapid biochemical conversion of cellulosic and proteinous materials in a variety of organic wastes that helps to enhance the speed of decomposition activities of bacteria and other microbes, convert the insoluble nutrients into soluble (Sinha *et al.* 2002) and to increase the nutrient release from

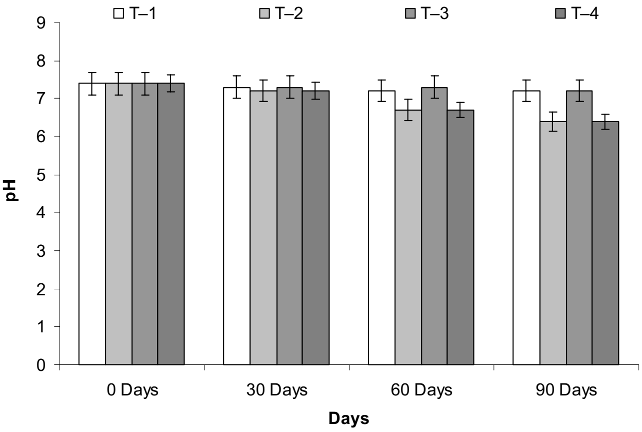

Fig. 2 Changes of pH in the rearing substrate during different days of treatment.

insoluble sources like RP as well as finally increase the manorial quality of vermicompost. The similar K contents in all treatments could be because most of the K in manure and hyacinth could be in inorganic form and the addition of earthworms or rock phosphate may not have an effect on this element.

Vermicomposting of RP proved to be a potential one in releasing nutrients from RP in this experiment. The lowering of pH in the rearing substrata was as expected in the vermicomposting process due to microbial action. This slight lowering of pH may have been effective in the release P from the insoluble RP. Chakrabarty (1994) also found a similar nature of P release from Mussorie RP with slight lowering of pH.

The significantly higher average biomass production and relative growth rate in T–4 earthworm compared to T–2 substrate shows favorable developmental condition of earthworms in the former. The best biomass production recorded in T–4 because of P, iron and other nutrient present in RP. The increase in P from RP may have resulted in a better balance of nutrients in the substrate for the worms. The iron supplement provided the necessary ingredient for production of haemoglobin in earthworm serum. Chakrabarty (2009) also found that the earthworms absorbed the iron part of the iron-bound phosphate from PRP in a similar experiment, which in turn emancipated the bound form of PRP-P. Another explanation for better growth of earthworms is the possible production of higher number of bacteria and biochemical by products in T–4 by the mixture combination of cow dung and RP (Aston 1984). These bacteria served the purpose of decomposition of organic matter as well as served as the protein source for the developing worms. Suzuki and Kurihara (1981) demonstrated that aquatic oligochaete, *Aelosoma hemprichi* could feed exclusively on bacteria, showing remarkable rapid growth on cow dung. Williams *et al.* (2004) reported that the faecal material produced by earthworms provides a ready supply of labile organic substrates to the bacteria within soil and compost, thus promoting microbial activity in release of N, P and K nutrients.

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