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Full Length Research Paper

Evaluation on physical, chemical and biological properties of casts of geophagous earthworm, *Metaphire tschiliensis tschiliensis*

Suk Kuan Teng[1], Nor Azwady Abd. Aziz[1]*, Muskhazli Mustafa[1], Suraini Abd. Aziz[2], and Yi Wei Yan[1]

[1]Department of Biology, Faculty of Science, Universiti Putra Malaysia.
[2]Department of Bioprocess Technology, Faculty of Biotechnology, Universiti Putra Malaysia.

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Earthworms are soil dwellers that have profound effects on soil ecosystem. Their feeding and burrowing activities help to incorporate soil particles with organic matter and redistribute them back into the soil via casts. The casts produced enhance microbial activities in soil that promote nutrient cycling. The present study was carried out to examine the physical, chemical and biological properties of casts produced by soil dwelling earthworm, *Metaphire tschiliensis tschiliensis*. Prior to inoculation of earthworms, the soil was incubated for two weeks at room temperature with moisture content of 20%. Worm worked soil and earthworm casts produced were collected for analysis after one month. Our results demonstrated that earthworm casts contained higher organic matter (9.84 ± 0.60%) and humic acid (1.33 ± 0.25%) compared to bulk soil. Total N, Ca, S, Al and Zn contents were also found to be higher in earthworm casts. Besides that, earthworm casts exhibited higher colony forming units (CFU), indicating the presence of higher microbial population. The study suggested that cast produced by *M. tschiliensis tschiliensis* could improve the physical and chemical properties of the soil. It also contained higher microbial population compared to soil without earthworm.

Key words: Earthworm cast, *Metaphire tschiliensis tschiliensis*, colony forming unit, humic acid, organic matter, CEC.

INTRODUCTION

Throughout the decades, intensive agricultural activities in the tropics had lead to soil degradation and decrease in soil biodiversity (Jouquet et al., 2008; Lavelle et al., 1992). Protection and preservation of the soil habitat would be an important step in ensuring long term cultivation and productivity (Bhadauria and Saxena, 2010). Hence, organic farming and land restoration activities are generating more attention among farmers recently in sustaining the crop yield and prolonging the lifespan of their agricultural lands.

Earthworms are soil inhabitants that play a key role in maintaining desirable properties in soil (Jouquet et al., 2008; Römbke et al., 2005). They are important agents in many temperate and tropical ecosystems in regulating soil organic matter, nutrient cycling processes and even create new habitats for other organisms through their activities in soils (Bhadauria and Saxena, 2010; Brown, 1995; Decaëns et al., 1999). Soil burrowing earthworms actively consume soil and humified organic matter on the surface and upper soil layers and form burrows in the soil. They feed on soil rich in organic matter and formed the most abundant species in many forest and agriculture lands (Bottinelli et al., 2010; Marhan and Scheu, 2005).

Various shapes of casts are deposited by earthworms on the soil surface. Cast production is influenced by

*Corresponding author. E-mail: azwady@science.upm.edu.my. Tel:+60389466632. Fax: +60386567454.

earthworm species, temperature and moisture as well as the quality of organic source provided (Whalen et al., 2004). These surface casts would be dissociated upon weathering, human and livestock trampling where the nutrients and biogenic contents bound in the cast aggregates can be released into the soil (Jouquet et al., 2008). Earthworm casts resulted in modification of soil structural characteristics and soil matrix properties (Jouquet et al., 2008). However, the available information on these cast properties are limited to widely distributed species such as *Pontoscolex corethrurus* and *Lumbricus terrestris* with little attention has been paid on other endemic species in Asia region (Jouquet et al., 2008; Lavelle et al., 1992; Mariani et al., 2007).

In the northern part of Perlis province in Peninsular Malaysia, some of the agroecosystems were found to be dominated by a geophagous earthworm, *Metaphire tschiliensis tschiliensis* (Teng, 2012). It has an average density of 15.62 ± 2.11 ind/m^2 and mean biomass of 2.60 ± 1.00 g. These earthworms deposit large amount of casts on the soil surface. However, unfortunately to date there is no study concerning the effect of these casts on the surrounding environment. There was also no record on the physiochemical and biological properties of these casts and how these earthworms affect the soil matrix. Therefore, the present research was carried out to examine the physical, chemical properties and microbial (bacteria, fungi and actinomycetes) populations in surface casts produced by the earthworm, *M. tschiliensis tschiliensis*. The study also compared the properties of worm worked soil with soil without earthworm.

MATERIALS AND METHODS

Site description and earthworms sampling

Sampling of earthworms was conducted at Kampung Guar Jentik (06°37.482 N, 100°13.490E) in May 2009. The sites were surrounded by limestone hills and experience a distinct dry season from January to April. The soil is characterized as clay soil with pH 6.08 on average. Earthworms, *M. tschiliensis tschiliensis* were sampled using chemical expellant method (Römbke et al., 2005) and rinsed with distilled water before carried to the laboratory.

Soil preparation

Soil collected from sampling site and decomposed cattle manure were used as the culture medium in the ratio of 9:1. Prior to the experiment, the soil and cattle manure were air-dried, grounded, sieved and re-moistened to 20% (v/w). Prepared medium (10 kg each) was incubated for 2 weeks in round containers (diameter=20.5 cm) with small holes at the base for discarding excess water. Throughout the period, the soil was incubated in the dark and soil moisture was maintained by spraying distilled water on the soil surface.

Earthworm culturing

Earthworms collected were maintained in their natural soil for about 2 weeks and fed with decomposed cattle manure. Ten adult earthworms (average weight of 5.56 g) were rinsed with distilled water before introduced into the soils (5 replicates). The control was soils without earthworms' inoculation. All containers were kept in shady area and covered with dark-colored net to create a conducive condition throughout the culture period. Distilled water was sprayed on soil surfaces and orchid net on daily basis to maintain moisture content of 20%.

Soil and cast analysis

After a month, earthworm casts (EC) produced on the soil surface was collected. Earthworms were removed from the containers to obtain worm worked soil (WWS). Earthworm casts that were found within the burrows were carefully removed before the collection of WWS. WWS collected were homogenized for analysis. The control was soil without earthworms' inoculation, namely bulk soil (BS). The casts and soil samples were undergone serial dilution and cultured on nutrient agar (NA), potato dextrose agar (PDA) and actinomycetes agar (AA) plates to determine microbial populations by colony forming unit (CFU) (Cappuccino and Sherman, 2005).

Casts and soil samples were air dried, grounded with pestle and mortar, sieved (2mm) and stored in air tight plastic bags. Sieved soil and cast samples were subjected to different physical and chemical analysis. pH was measured in soil/water (1: 2.5) suspension and total organic matter was determined via loss of ignition (LOI) (Jones, 2001). For cation exchange capacity (CEC) determination, the samples were treated with ammonium cations at pH 7. Kjeldahl digestion was carried out on casts and soil samples to determine total nitrogen (N) (Van Ranst et al., 1999). In the digestion, 0.5g of samples was boiled with concentrated sulphuric acid with salicylic acid. Kjeldahl tablet and sodium thiosulphate was added as catalysts. Besides that, aqua regia digestion were performed to determine total phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and other micronutrients (Van Ranst et al., 1999). Homogenized sample (1.00g) was boiled with the combination of hydrochloric acid and nitric acid before preserved in 2M nitric acid. All the digested samples were analyzed with Induction Coupled Plasma (ICP). The standard method described by Ahmed et al. (2005) was adopted for humic acid analyses with some modifications made (Lee et al., 2009) during the purification process.

Statistical analyses

Data obtained was analyzed using SPSS (Statistical Package for Social Sciences), 17.0. The data was undergo normality test and tested for homogeneity of variance using Levene's test. Microbial populations, nutrient contents and humic acid content in WWS, ES and bulk soil (BS) were analyzed by One-way analysis of variance (ANOVA) with post hoc Duncan test. A significant level of $p<0.05$ was considered throughout the analysis.

RESULTS

Physical and chemical properties of WWS, EC and BS

EC recorded the highest percentage of total organic matter content (9.84%; $p < 0.05$) compared to WWS and BS. EC was found to have almost two folds of organic matter more than WWS. Interestingly, percentage of organic matter in WWS was lower compared to BS.

Table 1. Organic matter, pH, CEC and humic acid contents in worm worked soil (WWS), earthworm casts (EC) and bulk soil (BS) (n=5).

	Worm worked soil (WWS)	Earthworm casts (EC)	Bulk soil (BS)
Organic matter (%)	5.69 ± 0.03[a]	9.84 ± 0.60[c]	7.29 ± 0.13[b]
pH	6.43 ± 0.10[a]	6.70 ± 0.09[a]	6.65 ± 0.07[a]
CEC (cmol kg^{-1})	0.66 ± 0.13[a]	0.65 ± 0.07[a]	0.57 ± 0.03[a]
Humic acid (%)	0.43 ± 0.08 [a]	1.33 ± 0.25 [c]	0.70 ± 0.06 [b]

Similar letter within the same row indicates no significant difference at p=0.05.

Table 2. Total nutrient contents of worm worked soil (WWS), earthworm casts (EC) and bulk soil (n=5).

Elements (mg/kg)	WWS	EC	BS
N	2.34±0.07[a]	3.78±0.11[c]	3.39±0.12[b]
P	1.11±0.06[a]	1.42±0.12[a]	1.24±0.13[a]
K	2.42±0.23[a]	2.54±0.18[a]	2.48±0.29[a]
Ca	3.67±0.22[a]	5.00±0.31[b]	3.92±0.35[a]
Mg	1.14±0.05[a]	1.42±0.07[a]	1.16±0.13[a]
S	0.41±0.03[a]	0.73±0.04[b]	0.43±0.03[a]
Al	16.30±1.70[b]	17.05±1.83[b]	12.86±0.93[a]
B	0.06±0.01[a]	0.06±0.00[a]	0.05±0.01[a]
Mn	0.34±0.02[a]	0.39±0.03[a]	0.33±0.05[a]
Zn	0.09±0.00[a]	0.11±0.01[b]	0.08±0.01[a]
Cu	0.02±0.00[a]	0.03±0.00[a]	0.02±0.00[a]
Fe	16.70±1.03[a]	15.25±0.55[a]	15.12±1.62[a]

Similar letter within the same row indicates no significant difference at p=0.05 between treatments.

However, pH and CEC were not significantly different ($p > 0.05$) between the three categories of soil.

In addition, humic acid content between the categories varied significantly. EC contained highest amount of humic acid (1.33%). We also found that BS has higher humic acid content (0.70%) compared to WWS (0.43%). The results are summarized in Table 1.

Plant nutrient contents

Present findings suggested improved nutrient content in EC as compared to WWS and BS (Table 2). This was shown by higher content of macronutrients (N, Ca and S) in EC than in both WWS and BS. Total N was found to be the highest in EC (3.78 ± 0.11 mg/kg), followed by BS (3.39 ± 0.12 mg/kg) and WWS (2.34 ± 0.07 mg/kg). Additionally, total Ca and S contents were approximately 20 and 40% higher in EC compared to WWS and BS. However, differences on Ca and S contents in WWS and BS were not significant. When comparing other micronutrients, total Zn content was found to be the highest in EC ($p < 0.05$).

Microbial populations

Microbial populations in EC, WWS and BS are summarized in Figure 1. EC recorded higher bacteria and fungi populations compared to WWS and BS. However, WWS and BS did not show significant difference in both bacterial and fungal populations. On the other hand, our results showed different trend for actinomycetes population between the three soil categories. We found that actinomycetes populations in WWS was remarkably lower ($p < 0.05$) as compared to both EC and BS.

DISCUSSION

The present study showed that organic matter and humic acid content in EC were significantly higher compared to WWS and BS. Prior to earthworms' inoculation, same amount of cattle manure was added to all treatments. They were thoroughly mixed and homogenized to ensure even distribution of organic matter. High accumulation of organic matter in EC suggested that *M. tschiliensis tschiliensis* exhibits selective feeding, with preference on

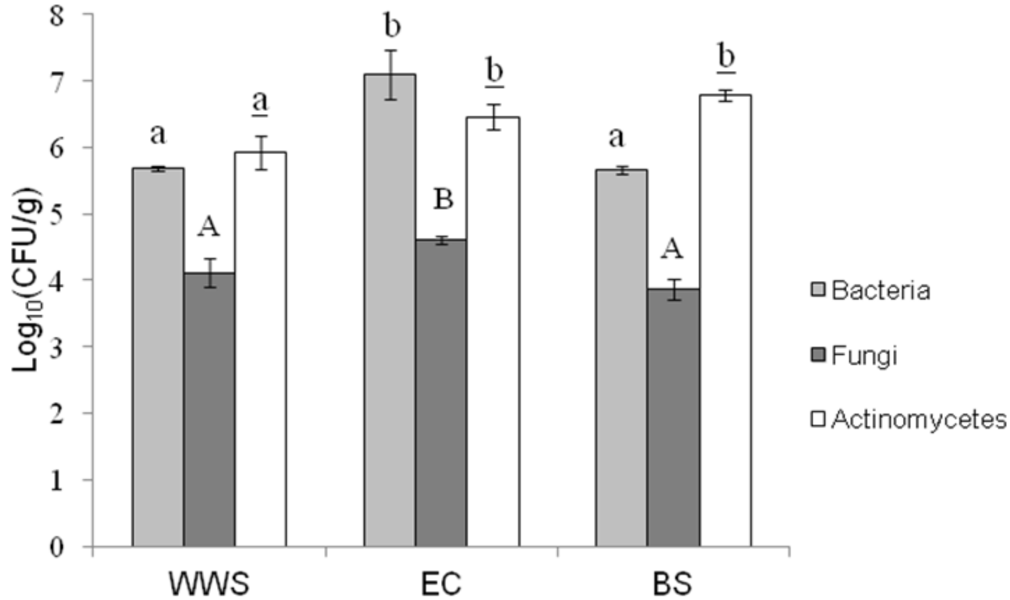

Figure 1. Bacteria, fungi and actinomycete populations in earthworm casts and soils (n=5, error bars represent standard error). **NB:** Different lower case letters show significant difference between treatments ($p<0.05$); Different capital letters show significant difference between treatments ($p<0.05$); Different underlined lower case letters show significant difference between treatments ($p<0.05$).

soil rich in organic matter. Formation of new aggregates in the form of cast helps to prevent rapid loss of nutrients by enclosing them in a more stabilized structure, as shown by higher humic acid content in EC. The presence of binding agents from the mucus of earthworms' gut might helped in binding organic and mineral particles together upon gut passage. Thus, it protects the cast from rapid microbial degradation (Bossuyt et al., 2005).

Besides that, higher organic matter was observed in BS (7.29%) as compared to WWS (5.69%), suggesting the utilization of organic matter in WWS by the earthworms. Soil burrowing earthworms such as *M. tschiliensis tschiliensis* tend to mix and incorporate organic materials with mineral soil as they form burrows and deposit casts on soil surface (Edwards and Bohlen, 1996). Due to their low assimilation rate, these excess materials would be accumulated in the casts. In soils without earthworm, the nature of organic material tends to accumulate in the soil aggregates complicating the decomposition process (Lavelle, 1988). Activities of earthworm facilitate the breaking of these aggregates for decomposition and nutrient cycling thus increases the amount of available nutrients in their casts (Chaudhuri et al., 2009).

According to Konare et al. (2010), the use of loss on ignition (LOI) on organic matter determination on clay soil might resulted in inaccuracy due to the dehydroxylation and decomposition of inorganic constituents. This will result in increase weight loss that causes inaccurate estimation of organic matter in the soil samples. However, the soil and cast samples analyzed in the present study were of the similar texture thus would have a deletable effect on the error.

There was no significant variation observed in pH and CEC among the soil categories suggesting earthworm inoculation did not significantly change the pH of their surrounding soil. The presence of pH-homeostasis condition in earthworm gut would help to maintain pH of digestive tract near to neutral (pH of 6.70 ± 0.09) upon excretion via casts. In our study, changes in pH of earthworm casts was not obvious as the bulk soil used was calcareous in nature (pH = 6.65 ± 0.07). Thus, the finding demonstrated that presence of earthworms would not create distinctive pH changes in soil that might lead to alteration of physiochemical conditions in a specific area. Soil pH and CEC are interrelated. Increase in soil pH would result in higher CEC in soil (Brady, 1984). In the study, soil pH was almost the same in three categories (WWS, EC and BS) and thus it did not create significant change in CEC.

Organic matter that passes through the earthworm gut is broken down into finer particles and becomes more readily available for microbial decomposition (Edwards and Bohlen, 1996; Scullion and Malik, 2000). This was shown by higher bacterial and fungal populations in EC compared to WWS and BS. Casts excreted by earthworms provide an optimal condition for microbial activity, hence resulted in improved organic matter and microbial populations in EC compared to WWS.

EC contained approximately 50% higher organic matter and recorded almost 30% higher in bacterial populations than WWS. Soil microorganisms are responsible for nutrient changes mechanism as they are active decomposers. Earthworm activities in soil govern the growth of microbial populations as they are sensitive towards various changes in the soil (Brown, 1995). With the formation of new microhabitats via earthworm casts, it would create a conducive condition for the growth of microorganisms. This is primarily due to the structure of casts that is able to retain higher moisture and organic matter for microbial utilization.

Incorporation of different nutrients in the earthworm casts depends mainly on the feeding and burrowing activities of earthworms (Lee and Foster, 1991). The present study found that EC contained higher nutrient contents compared to WWS and BS. This is most probably due to the intimate mixing of organic matter through the earthworm gut that would further enhance mineralization and humification process (Blanchart et al., 1999; Lavelle, 1988). Previous study had documented higher mineral N, P and S in earthworm casts compared to bulk soil and related the increase with earthworm feeding behavior (Haynes et al., 2003). Earthworms that feed on organically rich materials would produce casts with higher nutrient contents thus, contain higher microbial community (Haynes et al., 2003). Modifications of soil aggregates by earthworms and the formation of new structures (casts) lead to nutrient dynamics that increase their availability to soil microbes and plants (Jouquet et al., 2011).

Additionally, EC contained highest total N (0.35%), followed by BS (0.31%) and WWS (0.21%). The presence of earthworm is able to increase N mineralization due to active mineralization of carbon (decaying plant root and leaf litter) upon gut passage (Edwards and Bohlen, 1996). In the present study, cast of *M. tschiliensis tschiliensis* recorded higher concentrations of N, Ca and S as well as Zn. The result was supported by Kharin and Kurakov (2009) who found that cast of endogeic earthworm has high nitrification rate and led to an increase in nitrate concentrations. This could be contributed by higher bacterial and fungal activities in EC that further enhanced mineralization process it resulted in higher N compared to BS. In a study conducted on *M. anomala* population in grass savanna, the earthworms were estimated to produce 5 to 25 kg N ha^{-1} N annually and 63 to 71% of it was found being incorporated into fresh casts in the form of ammonium (Lavelle and Martin, 1992).

Total Ca and S contents were approximately 40% higher in EC compared to WWS and BS. Earthworm casts are usually found to have greater exchangeable K, Ca, and Mg than bulk soil (Edwards and Bohlen, 1996; Mariani et al., 2007). Improved Ca content in EC was probably due to the presence of active calciferous gland in earthworms that actively secrete mucus rich in calcium carbonate into esophagus (Drake et al., 2006; Schrader and Zhang, 1997). This led to elimination of excess Ca ions via casting activity and greatly increases Ca availability in soil. Improved Ca content in casts, together with clay and organic matter would in turns help to maintain the aggregate structure of casts and make them more stable compared to surrounding soil (Barois et al., 1993; Jouquet et al., 2008). Thus, they are more resistant when exposed to external and internal stresses (Edwards and Bohlen, 1996). These cast aggregates contain higher water holding capacity and water infiltration that contribute to soil fertility.

The present study also recorded higher bacteria and fungi populations in EC compared to WWS and BS. The results agree with previous findings that suggested fresh casts are always characterized with higher microbial biomass than aged casts (Blair et al., 1995; Perreault et al., 2007). According to a study done on casts of *A. caliginosa* fed with different litter (wheat straw or lucerna hay), it showed that microbial biomass in earthworm casts was higher in treatments with litter inputs than unamended treatments (Haynes and Fraser, 1998). It appeared that microbial activity was considerably influenced by organic matter availability in soil. EC are hot spots that promote high microbial biomass as they contain higher moisture content and available organic carbon than surrounding soil (Edwards and Bohlen, 1996). Meanwhile, actinomycetes populations in WWS was remarkably lower ($p < 0.05$) compared to both EC and BS. This was probably due to low organic matter content in WWS (5.69 ± 0.03%) that made it incapable of sustaining high actinomycetes populations.

Our study suggested the symbiotic relationship between earthworms and soil microbes. The joint activity of both organisms promoted the accumulation of organic matter and nutrients in the cast. The nutrients would be available to the plants upon cast dissociation. Thus, the casts are known as “patches of fertility” that improve soil properties and contained higher plant available nutrients for better plant growth (Choosai et al., 2010).

Conclusion

Positive results obtained from present study suggested that the presence of geophagous earthworms, *M. tschiliensis tschiliensis* and its casts that improve soil properties. In tropical regions, regular rain fall would prevent the formation of cast aggregates on soil surface and help to release nutrients back to the soil for plant absorption. This would help in maintaining soil fertility in tropical regions that are usually associated with soil compaction and nutrient loss issues. However, knowledge on the interaction of this earthworm with the soil system is still lacking. Therefore, more research should be conducted in order to gain comprehensive understanding and possible contributions of this earthworm to the agro-ecosystem in the tropics.

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