Journal of Sustainability Science and Management
Volume 9 *Number* 1, June 2014: 128-133
ISSN: 1823-8556

IMPACT OF STRESS ON EXCRETION IN EARTHWORM (*PERIONYX EXCAVATUS*)

BABUTHANGADURAI, A.[1*], JAWAHAR ALI[1], P., CHITRARASU[1]., SHAIK ALAWDEEN[1] AND JOHN, B. A.[2]

[1]*Unit of Aquaculture & Aquatic Toxicology, Department of Zoology, The New College, Chennai – 600 014, India.* [2]*Institute of Oceanography and Maritime Studies (INOCEM), International Islamic University Malaysia, Kuantan, 25200 Pahang.*

**Corresponding author: babudurai10@yahoo.com, akbarjohn50@gmail.com*

Abstract: In the present study, the nature of excretory products and the impact of stress on the rate of excretion of the megascolecid earthworm, *Perionyx excavatus* was studied. Excretion in *P. excavatus* was analyzed with reference to three aqueous media: tap water, distilled water and 1% Sodium chloride. Clitellate worms (N=5.) were taken for the study and nitrogenous products (ammonia and urea) eliminated in the three media were analyzed for twenty four hours, sampling was carried out at an interval of two hours. In order to assess immediate response in the first one hour, samples were also taken at the end of 30 minutes, 45 minutes and 60 minutes, during the first one hour of observation. After 60 minutes, the worms excreted 0.023 mg ammonia and 1.451 mg urea in the tap water; 0.014 mg ammonia and 1.397 urea in distilled water; and 0.030 and 1.076 mg ammonia and urea in 1% NaCl. After 60 minutes, under heat stress, the worms excreted 0.011 mg ammonia and 0.578 Urea in the tap water; 0.006 mg ammonia and 0.682 mg urea in the distilled water; 0.009 mg ammonia and 0.520 urea in the 1% NaCl solution. Animals exposed to H_2SO_4 stress, excreted 0.034 mg ammonia and 1.023 mg urea in the tap water; 0.040 mg ammonia and 0.645 mg ammonia and 0.620 mg urea in the 1 % NaCl solution. The worm survived 24 hours in the tap water excreting ammonia urea in the distilled water; 0.03 mg (0.188 mg) and urea (0.926 mg) and the latter at higher amount indicating that *P. excavatus* is primarily ureotelic and also capable of excreting ammonia. The worm did not survive beyond eight hours in the distilled water and 1% NaCl. The present study clearly demonstrates that the earthworm *P. excavatus* is primarily ureotelic and revert to ammonotelism under stress situation.

KEYWORDS: Excretion, earthworm, stress, nitrogenous waste.

Introduction

Over the past four decades, animal manures have been extensively used as a source of soil fertility in order to improve the crop yield. Many works has been attempted to use various earthworm species to improve the soil fertility by enhancing the ammonia and nitrate concentration in the soil (Callahan, 1988; Engelstad, 1991; Gilot, 1997; Binet *et al.,* 1998). Earthworms have been described primarily ammonotelic and ureotelic under stress situation. Cohen and Lewis (1949) found that the non-protein nitrogen excreted by *Lumbricus terrestris* was mainly in the form of ammonia together with traces of both urea and uric acid and that the quantity of urea increased significantly during starvation. Bishop and Campbell (1965) also observed similar effect of starvation on nitrogen excretion in *L. terrestris* and reported an increase in the production of enzymes involved in ornithine cycle with a corresponding decline in the quantity of ammonia excreted till it reached ureotelism. However, Haggag and El Duweinin (1959) reported that starvation had no remarkable effect on nitrogen excretion. Kale (1985) observed that nitrogen excretion was mainly ammonotelic in three species of tropical earthworms. The epigeic earthworm, *Octochaetona serrata* was found to be mainly ureotelic and excretion of ammonia was less than 2% under optimal condition (Shabeera, 1989). Excretion of nitrogen was reported to change under different condition of

the environment such as change in temperature, availability of water, food and stress conditions. The nature of excretory products also varies from species to species (Edwards and Lofty, 1971).

The utilization of epigeic earthworm in waste management practices is well documented in scientific literature on wastes from industries (Subramanian *et al.,* 2010; Pramanik and Chung, 2011; Gomez-Brandon *et al.,* 2011). However, the optimizing the level of necessary nutrition such as N, P and K in soil using earthworms are still scanty (Deepika and Surindra, 2011). Hence, the present study was aimed to analyze and assess the nature of excretory products of the earthworm *P. excavatus* and to study the effect of heat stress on the excretion. The impacts of acid stress on the excretion were also studied.

Materials and Methods

Five adult worms of equal weight were taken for the experiment. Samples were washed and blotted to remove moisture on the surface of the body. The weight of the worm was taken using electronic monopan balance (Model: CA _34). The worms were taken in a group of five for assessing excretory products, ammonia and urea separately in triplicates for each of the excretory product since earthworms are reported to survive in water medium and it is easy for sampling research experimental setup. Since much of the nitrogen is reported to be excreted through nepheridia phores the worms were kept in water for sampling nitrogenous product from the water medium. Tap water with salinity <5ppm was used as control medium while the distilled water and 1% sodium chloride solution were selected as experimental media.

150ml of each of the experimental media were taken in 500 cc beaker. The worms were let into each of the media in groups of five and the excretory products were analyzed following the standard procedures (Kaplan, 1965). The study was conducted in room temperature for 24 hours. In order to study the changes in the excretory response in the experimental media, sampling was also done during the first hour observation at the end 30 minutes, 45 minutes and 60 minutes.

To study the response to stress condition, the excretion was monitored at increased temperature (40˚C) inside a hot air oven, and exposing the worms to acidified media adding diluted H_2SO_4 (10^{-5}). Sampling was done at the end of 30 minutes, 45 minutes and 60 minutes of exposure to stress. Samples were analyzed for ammonia and urea.

Results and Discussion

After 60 minutes of experiment, the worms excreted 0.023 mg ammonia, 1.451 mg urea in the tap water, 0.014 mg ammonia and 1.397 urea in distilled water, and 0.030 mg ammonia and 1.076 mg urea in 1% NaCl (Figure 1a & 1b). After 60 minutes, under heat stress, the worms excreted 0.011 mg ammonia and 0.578 urea in the tap water; 0.006 mg ammonia and 0.682 mg urea in the distilled water; 0.009 mg ammonia and 0.520 urea in the 1% NaCl solution (Figure 2a & 2b). Animals exposed to H_2SO_4 stress, excreted 0.034 mg ammonia and 1.023 mg urea in the tap water; 0.040 mg ammonia and 0.645 mg urea in the distilled water; 0.03 mg ammonia and 0.620 mg urea in the 1 % NaCl solution (Figure 3a & 3b). The worm did not survive beyond eight hours in the distilled water and 1% NaCl.

The present study demonstrates that the earthworms *P. excavatus* excretes ammonia at 0.188mg/g w.wt and urea at 0.926mg/g w.wt/ day indicating that the earthworms *P. excavatus* is primarily ureotelic in comparison to the quantity of ammonia and urea excreted at an interval of two hours during the sampling. It is crystal clear that urea is excreted several times at higher quantity than ammonia. The impact of stress situation on excretion in the earthworms has been studied in relation to starvation and temperature variation. Starvation shifts nitrogen excretion to urea (Cohen and Lewis, 1949). Increases in urea as much as five times than normal level after 20 days of starvation

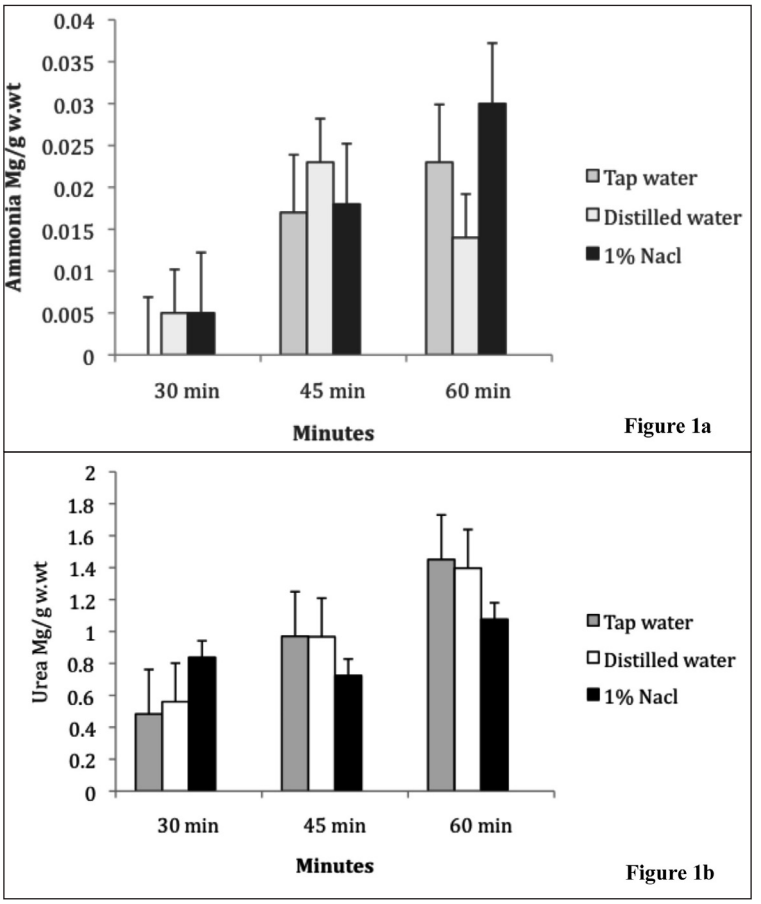

Figure 1: Indicates the Amount of Ammonia (Figure 1a) and Urea (Figure 1b) Excretion by *P. escavatus* in Control (Tap Water) and Experimental Media (dH_2O and 1%NaCl) at Room Temperature 32°C.

in *Lampito mauritii* has been observed. In the earthworm *Octochaetona serrata*, remarkable shift in the rate of excretion of ammonia to urea was found (Shabeera, 1989). Similarly stress of heat stimulated excretion of urea was found in higher concentration. In the present study, of exposure the earthworms, *P. excavatus* to the distilled water and 1% sodium chloride media offset the excretion of both ammonia and urea decreasing the output compared to excretion in the tap water. The worm died in the latter two media, obviously due to osmotic and ionic stress. Elimination of ammonia during the first one hour period of observation decreased output of ammonia by 39.1% after initial increase in the distilled water but showed steady increase in 1% sodium chloride reaching 30.4 % after one hour of exposure. Conversely, urea excretion initially increased but decreased steadily in both the distilled water and 1% sodium chloride, probably indicating that the earthworm *P. excavatus* revert to ammonotelism in response to stress, resulting from exposure to acidity and heat stress further corroborate the inference that urea output decreased under acute stress. However output of ammonia increased in all three media under acidic irritation stress but decreased under heat stress. It could be inferred that the need to conserve water under increased temperature reduces the output of the excretory products under heat.

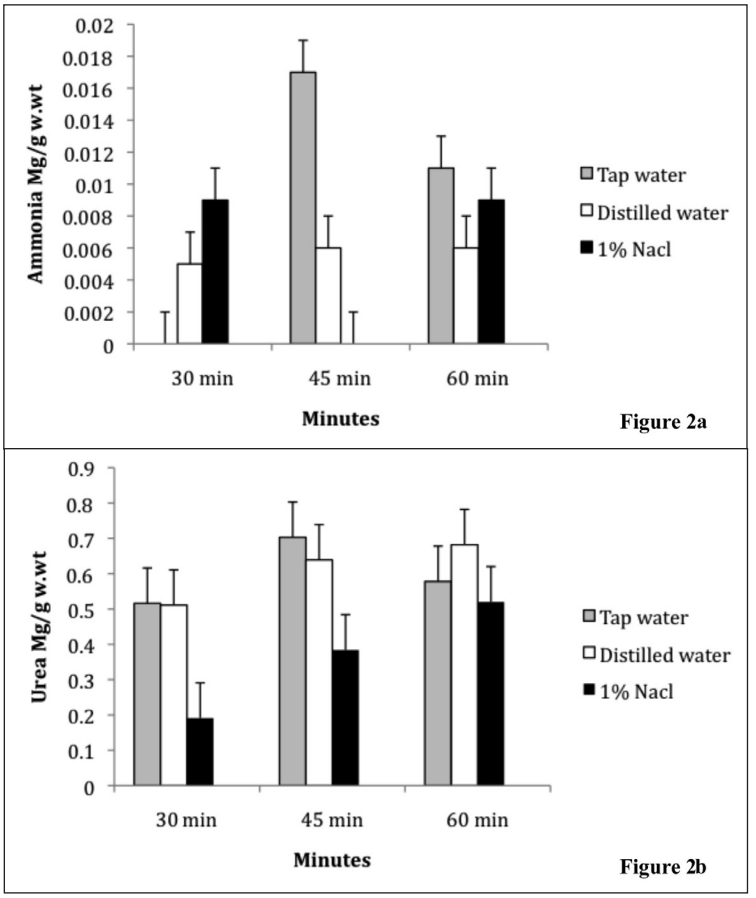

Figure 2: Indicates the Amount of Ammonia (Figure 2a) and Urea (Figure 2b) Excretion by *P.escavatus* in Control (Tap water) and Experimental Media at Heat Stress Condition (Temperature 40°C).

Acknowledgement

Thanks are due to the Management, Principal and HOD (Zoology), The New College, Chennai.

References

Binet, F., Fayolle, L., & Pussard, M. (1998). Significance of Earthworms in Stimulating Soil Microbial Activity. *Biology and Fertility of Soils,* 27: 79-84.

Bishop, S. H., & Campbell, J. W., (1965). Arginase and Urea Biosynthesis in the Earthworm, *Lumbricus terrestris*, *Comp. Biochem. Physiol.*, 15: 51-71.

Callahan, C. A. (1988). Earthworms an Ecotoxicological Assessment Tools. In: Edwards CA Neuhauser EF (eds.). *Earthworms in Waste and Environmental Management*. SBP Academic publication the Hague the Netherlands. 295-301.

Cohen, S., & Lewis, H. B. (1949). The Nitrogenous Metabolism of the Earthworm, (*Lumbricus terrestris*), *J. Biol. Chem*., 180: 79-91.

Deepika, S., Surindra, S. (2011). Vermicomposting of Herbal Pharmaceutical Industry Waste: Earthworm Growth, Plant-Available Nutrient and Microbial Quality of End Materials. *Bioresource Technology*, 112: 179-185.

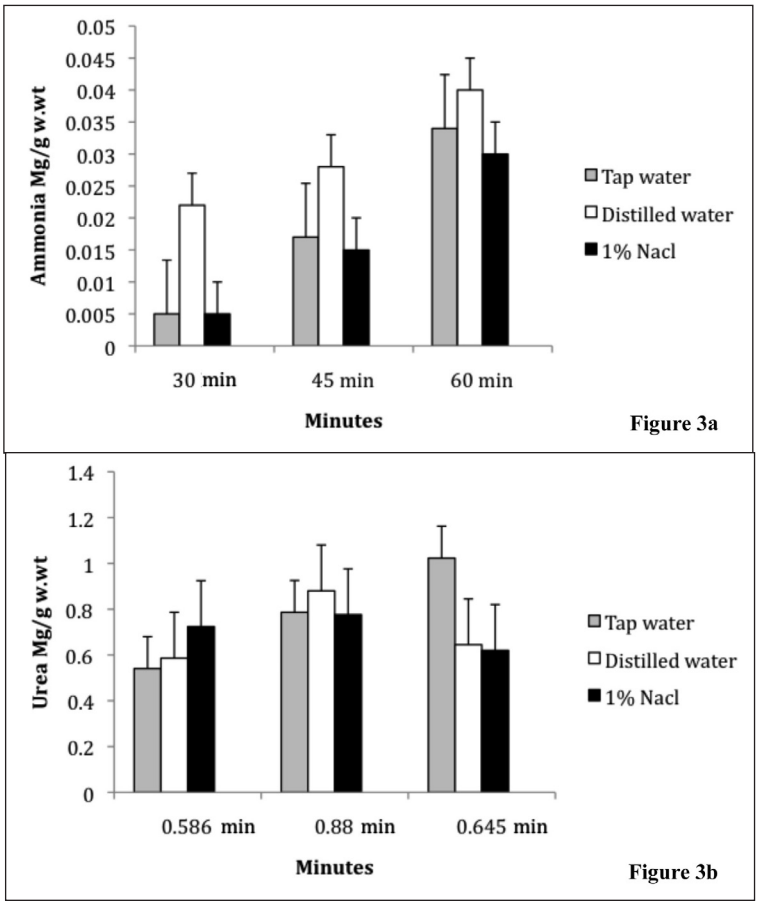

Figure 3: Indicates the Amount of Ammonia (Figure 3a) and Urea (Figure 3b) Excretion by *P. escavatus* in Control (Tap water) and Experimental Media Under Acid Stress (H_2SO_4).

Edwards, C. A., & Lofty, J. R. (1972). *Biology of Earthworms*. London: Chapman and Hall. 147-150.

Engelstad, F. (1991). Impact of Earthworms on Decomposition of Garden Refuse. *Biology and Fertility of Soils*. 12: 137-140.

Gilot, C. (1997). Effects of a Tropical Geophagous Earthworms *Millsonia anomala* (Megascolecidae) on Soil Characteristics and Production of a Yarn Crop in Ivory Coast. *Soil Biology and Biochemistry*, 29: 353-359.

Gomez-Brandon, M., Lazcano, C., Lores, M., & Dominguez, J. (2011). Short-term Stabilization of Grape Marc through Earthworms. *J. Haz. Mat.,* 187: 291-295.

Haggag G., & El.-Duweini, A. K., (1959). Main Nitrogenous Constituents of the Excreta and Tissue of Earthworms, *Proc. Egyptian Acad. Sci*., 13: 1-5.

Kale, R. D., (1985). Effect of Starvation on Ammonia and Urea Excretion in Three Species of Tropical Earthworms (Personal Communication).

Kaplan, A. (1965). The Determination of Urea Ammonia and Urease. *Methods in Biochemical Analysis,*17: 311-321. New York: John Wiley and Sons.

Pramanik, P., Ghosh, G. K., Ghosal, P. K., & Banik, P. (2007). Changes in Organic – C, N, P and K and Enzymatic Activities in Vermicompost of Biodegradable Organic Wastes under Liming and Microbial Inoculants Bioresour. *Technol.,* 98: 2485-2495.

Shabeera Begum, H. (1989). Study of Nitrogen Excretion in the Earthworm, *Octochaetona serrata* and Impact of Certain Stress Factors, M.Phil Thesis, University of Madras. 17.

Subramanian, S., Sivarajan, S. & Saravanapriya, M. (2010). Chemical Changes during Vermicomposting of Sago Industry Wastes. *J. Haz. Mat.,* 179: 318-322.