ACUTE WATER INUNDATION STRESS: THE RESPONSE OF AN EARTHWORM

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Abstract: Earthworms are soil invertebrates whose roles are essential and indispensable to sustainable agroecosystem services. In the soil environment, earthworms have to cope with a number of natural and anthropogenic stressors, including water inundation. A good understanding of earthworms' physical and biochemical responses to environmental stressors is therefore needed to conserve their population diversity and density. In this study, we assessed the responses of a tropical, endogeic earthworm to simulated acute water inundation. Earthworms were divided into batches, and each batch of earthworms was subjected to different levels of above-soil water inundation (0, 1.5, 5.0, 10.0, and 15.0 cm) fora period of 7 days, during which their survival and biochemical responses were observed. Results indicated that different inundation water depths produced differences in survival, dissolved oxygen, ammonia, and temperature responses. Earthworm survival decreased with rising water inundation, implying increased mortality with rising water inundation. The concentration of ammonia in soils increased with rising water inundation. Dissolved oxygen was highest in soil of no water inundation, and least in 10 and 15 cm water inundated soils. In addition, pH was highest in soil with no water inundation (6.8), and least in 10 cm water inundated soil (6.5). These results have implications for earthworm conservation and culture. Though earthworms are moisture-loving, excessive water flooding is inimical to their survival. A proper moisture balance is therefore needed to optimize the conservation and culture of earthworms.

Keywords: Earthworm's environment, dissolved oxygen, flood, irrigation

Introduction

The environment is the totality of all the factors and conditions that affects an organism in its habitat. The environment is not static, but always subjected to variations brought about by climatic changes, natural disasters, and human (anthropogenic) activities. The survival of an organism is therefore dependent, in part, to its ability to cope with, and adapt to changes in its environment. Species ability to cope with challenges in their environment relate directly to their behavioural adjustments (Langenhof and Komdeur, 2018) and biochemical responses (Dedeke *et al*., 2016), with some species being more successful than others.

Earthworms are among the most widespread of invertebrate animals whose role in the soil ecosystem has been well documented (Wood and James, 1993). They improve soil texture, aeration, and fertility (Owa *et al*., 2013). Earthworms are diverse in form, behaviour, and habitat. They could be aquatic or terrestrial, with different species found in marsh, swamps, grasslands, woodlands, scrublands, and forest. Earthworms enhance plant growth and crop productivity by their physical and chemical effects of burrowing and casting (Renu *et al*., 2006). They facilitate soil aeration, water infiltration, and turnover of large soil organic matter. Earthworms have also been identified as good soil bio-monitoring agents (Olayinka *et al*., 2011) and potential soil bioremediators (Owagboriaye *et al*., 2020; Dada *et al*., 2016).

Earthworms' survival and activity are principally determined by the presence of moisture. They burrow down the soil and remain in a state of inactivity during a period of extreme temperature and low soil water (Wood and James, 1993). In the soil environment, earthworms have to cope with a number of natural and anthropogenic stressors, including water inundation. Meanwhile, water inundation is always a serious challenge to many organisms as it can lead to hypoosmotic shock, acidic environmental condition, insufficient dissolved oxygen and so on. These conditions are potentially fatal to many organisms, especially in waterlogged environments, like marshes and swamps.

Water inundation in earthworm habitat is always brought about by flooding and human (anthropogenic) activities like irrigation farming. Though extensive works have been done on the effects and responses of earthworms to stressor factors such as metal pollution and ecotoxicity (Dada *et al*., 2016), it is also important to have a good understanding of earthworms' physical and biochemical responses to water inundation. This will positively impact efforts and attempts aimed at conserving earthworm population diversity and density for research, environmental and agricultural sustainability. This study therefore aimed to evaluate the responses of an earthworm species, *Libyodrilusviolaceus*, to water inundation stress. *Libyodrilusviolaceus* is an endogeic (dwelling and feeding below the soil surface) wetland earthworm species native to West Africa.

Material and Methods

Collection and identification of earthworm

This study was conducted in Ago-Iwoye, Ogun State, Nigeria, geographically located in the coordinates: 6° 57' 0" N, 3° 55' 0E. Earthworm samples were collected in the month of February along the banks of streams, marsh and farm settlements in Odogbolu Local Government Area of Ogun State, Nigeria. Earthworms were collected by digging with a shovel and hand sorting as described by Owa (1992), and transferred into plastic containers. The collected earthworms were further washed with clean sterile water (Owa *et al*., 2013) to make them free of soil particles. The species was identified as *Libyodrilusviolaceus*.

Experimental design

A total of 30 high density plastic containers were obtained for the experiment. Humus soil was put into each container up to a depth of about 6 cm. Water was added to each container to make the soil friable (neither water-lodged nor dry). The containers were divided into five treatment groups, including

the control. Enough water was added to the soil each treatment group to simulate above-soil water inundation to appropriate levels (0, 1.5, 5.0, 10.0, and 15.0 cm). Ten earthworms were introduced into each treatment container. Each treatment was replicated six times. The experimental containers were placed under a tree at the bank of the River Ome, in Ago-Iwoye, Ogun State, Nigeria to provide a cool, simulated natural habitat for the earthworms. Each container was screened with tiny-mesh sized nettings to prevent interference from insects, rodents, birds and other animals. The earthworms were fed decaying leaves during the period of the experiment to avoid hunger stress (Spurgeon *et al*., 2003). The experiment lasted for seven days.
Mortality was determined as the difference between the initial number and final number of earthworm in each pot. Dissolved Oxygen (DO)was measure using a portable oxygen meter. A test multimeter was used to measure pH and temperature. The concentration of ammonia was determined by spectrophotometry.

Statistical analysis of data
The data obtained from the study were subjected to analysis of variance (ANOVA). All analyses were done using SPSS (version 17) software.

Results and Discussion

Survival of earthworms in varying levels of water inundation
High earthworm mortality was generally recorded in soils inundated with water after the 7-day experimental period (Fig. 1). Significantly lower ($p < 0.05$) earthworm mortality was recorded in the soil of no water inundation (control). Generally, the number of earthworm survivors decreased with increasing water inundation levels. This implies increasing mortality with rising water inundation. Hence, 10 and 15 cm water inundated soil recorded the highest mean mortality of 9±1.

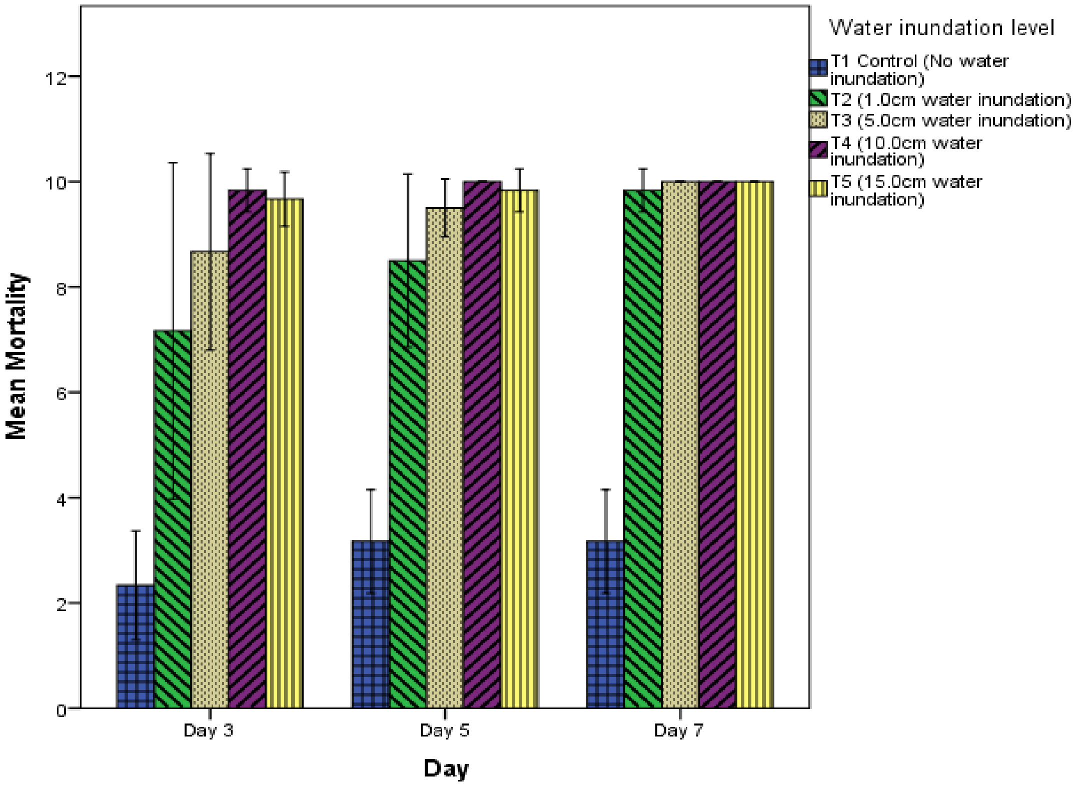

Fig. 1: Survival of earthworms in water inundated soils after 7 days

Table 1: Physicochemical chemical parameters of water inundated soils

Depth of water inundation in soil	Ammonia (mg/kg)	pH	Dissolved oxygen (mg/kg)	Temperature (^oC)
0 cm (Control, T1)	17±0.01	7.0±0.0	17.0±0.3	28.3±0.0
1.5 cm (T2)	24±0.15	6.8±0.1	4.0±0.1	27.3±0.1
5.0 cm (T3)	33±0.20	6.6±0.1	8.0±0.2	27.4±0.0
10.0 cm (T4)	31±0.01	6.5±0.2	2.0±0.1	27.6±0.0
15.0 cm (T5)	34±0.10	6.7±0.1	3.0±0.1	28.5±0.2

Physicochemical parameters of water inundated soils
The physicochemical parameters of soils inundated with different levels of water are presented in Table 1. The concentration of ammonia in the soils increased with increasing water inundation. An exception was the 10 cm water inundated soil which recorded lower ammonia concentration of 31 mg/kg, relative to the 5.0 cm and 15.0 cm water inundated soils which recorded 33±20 and 34±10 mg/kg, respectively.
The soil with no water inundation recorded the highest pH of 7.0, while the soil of 10 cm water inundation recorded the least (6.5). The soil of 15.0 cm water inundation recorded a pH of about 6.7. Dissolved oxygen was highest in the soil of no water inundation (17.0 mg/kg), and least in 10 cm and 15 cm water inundated soils. The temperature of the soil with no

water inundation (control soil) was 28.3°C. The highest temperature of 28.5°C was recorded in soil of 15.0 cm water inundation, and the least (27.3°C) was recorded in 1.5 cm water inundated soil.

Though earthworms are moisture-loving, inundating flood poses stress on them. Hypoosmotic stress is one of the mechanisms by which flood inundation kills earthworms. The observed increasing earthworm mortality with depth of inundating water must have resulted from the decreasing oxygen concentration (increasing hypoxia) with water depth. Like mammals, earthworms engage hemoglobin for transport of oxygen; but unlike mammals, the hemoglobin of earthworms are dissolved in the plasma, and not located inside the red blood cells. Therefore, earthworms are always quick to respond to osmotic stress brought about by inundating water flood.

Some of the surviving earthworms in the soils of higher water inundation were observably pale (gray) and weak. This was so because of the increased hypoosmos is of the body fluid and hypoxia brought about by flooding. Since flood water is always low in ions, there is a tendency for the earthworms to lose ions to the flood water. This may in turn prevent dysbalances in the metabolic equilibra around the mitochondria, leading to the inability of the electron transport chain to run normally. The resulting loss of the hemeprosthetic group with its Fe^{3+} can potentially make the inundated earthworm to become pale (gray) and weak.

The increasing ammonia concentrations recorded in the soils of higher water inundation in this study is also associated with hyposmosis and hypoxia-induced stress (Adlimoghaddam *et al*., 2016). The usual initial response to stress is the induction of stress hormones (adrenalin and cortisone). Those stress hormones, in their struggle to sustain energy generation, possibly also mobilized proteins which were deaminated to provide glucose for energy generation. But as a side effect, the resulting amino acids became converted into ammonia. Since ammonia potentially inhibits energy-generating ability of the mitochondria, the earthworms possibly had to release the ammonia generated from deamination to the environment.

Relative to the control which recorded a pH of 7, water inundated soil samples recorded a lower pH of up to 6.5. This is also likely due to the flood-induced hypoxia in the earthworms. The high hypoxia probably necessitated energy sourcing via fermentation (Toro and Pinto, 2015). Even though there was an increase in ammonia generation, the need to maintain homeostasis might have led to the general production of other catabolic acids, leading to the observed reduction of pH from 7.0 to 6.5. Nevertheless, it should be noted that some earthworms have several mechanisms of modifying and surviving in their water saturated environment, like paddy rice farms (Owa *et al*., 2003).

Conclusion

In this study, the responses of a tropical, endogeic earthworm to simulated acute water inundation were assessed. Different inundation water depths were found to produce differences in survival, dissolved oxygen, ammonia, and temperature responses. Earthworm survival decreased with rising water inundation, implying increased mortality with rising water inundation. These results have implications for earthworm conservation and culture. Though earthworms are moisture-loving, excessive water flooding is inimical to their survival. A proper moisture balance is therefore needed to optimize the conservation and culture of earthworms.

Conflict of Interest

The authors declare that there is no conflict of interest related to this work.

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