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International Journal of Current Research
Vol. 5, Issue, 01, pp.160-163, January, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

Effect of Congo Red on the Survival, Morphology and Behavior of the Earthworm, *Megascolex konkanensis*

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ARTICLE INFO

Article History:
Received 15th October, 2012
Received in revised form
20th November, 2012
Accepted 22th December, 2012
Published online 16th January, 2013

Key words:
Earthworm,
LC $_{50,}$
Avoidance behavior,
Toxicity.

ABSTRACT

The Toxicity of Congo red to earthworm, *Megascolex konkanensis* was studied. The toxicity was concentration dependent and the percent survival decreased with increasing concentration in water and soil. The concentration of congored which was sub lethal under normoxic condition became lethal under hypoxic condition. In soil, with increase in water content, a decreasing trend in LC_{50} value was observed. The severity of behavioral and morphological changes increased with increasing concentration of congored in water and soil.

INTRODUCTION

Protection of the soil habitat is the first step towards sustainable management of its biological properties that determine long-term quality and productivity. It is generally accepted that soil biota benefits soil productivity but very little is known about the organisms that live in the soil and the functioning of the soil ecosystem. Several studies have been undertaken to highlight the soil organisms' contribution to the sustainable function of all ecosystems. Soil macro fauna, such as earthworms, modify the soil and litter environment indirectly by the accumulation of their biogenic structures (casts, pellets, galleries, etc). The cycling of nutrients is a critical ecosystem function that is essential to life on earth. Studies in the recent years have shown increasing interest in the development of productive farming systems with a high efficiency of internal resource use and thus lower input requirement and cost. Earth worms are a major component of soil fauna communities in most natural ecosystems of the humid tropics and comprise a large proportion of macro fauna biomass. By their feeding, burrowing and casting activities, they also improve the soil quality, nutrient uptake, growth and yield of plants.

Earthworms in their natural habitat itself are at the risk of being exposed to foreign chemicals and increased levels of indigenous chemicals due to various human activities and technologic advancement. Earthworms like other organisms were reported to accumulate and live in soils containing large amount of persistent insecticides. Earthworms are sub terrestrial and aerobic. In its natural environment earthworms are prone to be subjected to progressive hypoxic condition as a result of removal of air contained between soil particles due to utilization of oxygen for various oxidative reactions or when the soil is flooded with rain and / or dye contaminated irrigated water. Therefore, the effect of Congo red under progressive hypoxic condition on the survival of the earthworms has also been assessed. Avoidance behavior of oligochaetes used as an index of stress caused by pollutants in various studies. Therefore, the borrowings are avoidance behavior has also been studied by exposing the earthworms *M.konkanensis* to soil mixed with congored.

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MATERIALS AND METHODS

Earthworms (*Megascolex konkanensis*) collected manually from uncontaminated soil of Mathampatti area, southwest of Coimbatore, at the depth of about 5 to 15cm were brought to the laboratory in polyethylene bags containing moist sand. Earthworms were kept in suitable vivaria containing moist organic debris. Leaf litters were provided as food daily and earthworms were free to feed ad libitum. They were acclimatized for period of not less than seven days and starved for one day before being used in various experiments. The effect of congored in water medium and the survival of the earthworms, *M.konkanensis* were assessed following the, static type of the bio-assay method. Acute toxicity studies were conducted to determine the 48 hr LC_{50} value of dyes following the procedures given in standard methods for the examination of water and waste water. Since the toxic potential are not known, exploratory tests were conducted to determine the approximate concentration range to be used 48 hr LC_{50} test. Based on the results of the exploratory test, series of 8 concentrations of congored (300,325,350,375,400,425,450 and 500 mgL^{-1}) were selected. While selecting concentrations, the concentrations which produced 0 and 100 percent mortality were fixed as the lowest and highest respectively. To fix the 48 hr LC_{50} value 6-8 animals were exposed to each concentration. Failure to respond to external stimuli was consider as criteria to determine the death. The effect of dyes under progressive hypoxic condition was studied by subjecting the earthworm to total submersion at different concentrations of congored in suitable containers over laid with liquid paraffin. The toxicity of congored to *M.konkanensis* in soil and their effect on its burrowing behavior were studied by introducing earthworms individually in each beaker containing soil with known concentration of congored to a height of 5cms. The soil

samples with different concentrations of congored were prepared by mixing appropriate amounts of congored. Earthworms were considered to be burrowed if ¾ of their body length was under soil. The effect of water content of the soil on the toxicity was studied by conducting similar experiment, however varying the water content of the soil from 20 to 35 percent at an interval of 5 percent. The maximum level of the water content of the soil was fixed as 35 percent, 5 percent above the water holding capacity of the soil. The water holding capacity of the soil was determined by Keen - Rackowski Box method. The LC_{50} and confidence limit values were determined by probit analysis.

RESULTS

The toxic effect of Congo red

The toxic effect of congo red was concentration dependent and percentage survival of earthworm decreased with increasing concentration of congored in water and in soil (Table 1 and 3). The 48 hr median lethal concentration of congored was 372.25 mgL^{-1} in water (Table 4). When earthworms were subjected to progressive to hypoxic condition in congored solutions, higher percentage of mortality was observed in addition to decrease in the duration of survival. Further, apart from a decrease in the LC_{50} value from 372.25 to 323.89 mgL^{-1}, the concentration of congored (300 mgL^{-1}) which was sublethal under normoxic condition became lethal when the oxygen tension decreased (Table 2). The LC_{50} in soil at 15 percent water content was 974.45 $mgKg^{-1}$. When the water content of the soil was increased from 15 percent to 35 percent at an increment of 5 percent, a decreasing trend in LC_{50} values were observed. The observed LC_{50} at 20, 25, 30 and 35 percent were 899.70, 840.05, 795.15 and 750.15 $mgKg^{-1}$ respectively (Table 4). Further, the lower lethal concentration at the respective water content has also decreased. The lower lethal concentration at 15 percent water content was 900 $mgKg^{-1}$ and at 20, 25, 30 and 35 percent were 840, 810, 785 and 730 $mgKg^{-1}$ respectively (Table 3). In the control soil, irrespective of the water content, earthworm quickly burrowed and remained burrowed throughout 48 hr period of observation. There were no mortalities.

Table 1. Percentage survival of *Megascolex konkanensis* exposed to different concentration of Congo red solution under normoxic condition (n=8)

Concentration mgL^{-1}	Duration in hours	
	24	48
450	100	12.5
425	100	25.0
400	100	37.5
375	100	50.0
350	100	62.5
325	100	75.0
300	100	100
200	100	100

Table 2. Percentage Survival of *Megascolex konkanensis* exposed to different concentration of Congo red solution under hypoxic condition

Concentration mgL^{-1}	Duration in Hours							
	6	12	18	24	30	36	42	48
800	-							
700	-							
600	100	-						
500	100	100	-					
400	100	100	100	100	75.0	-	50.0	12.5
375	100	100	100	100	100	100	50.0	25.0
350	100	100	100	100	100	100	100	37.5
325	100	100	100	100	100	100	100	50.0
300	100	100	100	100	100	100	100	62.5
275	100	100	100	100	100	100	100	75.0
250	100	100	100	100	100	100	100	87.5
225	100	100	100	100	100	100	100	100

Table 3. Percentage survival of *Megascolex konkanensis* exposed to different concentration of Congo red in soil during 48hrs exposure period at different percent water content

Concentration mg kg^{-1}	35% n = 8	30% n = 8	25% n = 8	20% n=6	15% n=6
Control	100	100	100	100	100
720	100				
730	83				
740	67				
750	50				
760	33				
770	17				
780	0	100			
785		83			
790		67			
795		50			
800		33	100		
805		17			
810		0	87.5		
820			75	100	
830			62.5		
840			50	87.5	
850			37.5		
860			25.0	7.5	
870			12.5		
880			0	62.5	
875					
900				50	87.5
920				37.5	
925					75.0
940				25.0	
950					62.5
960					
975				12.5	50.0
980					
1000				0	37.5

Table 4. LC_{50} Values of Congo red in water and soil to the earthworm Megascolex konkanensis at 48 hrs exposure period

Media	Acute toxicity range (95% Confidence Limit)		Median LC_{50} $mgkg^{-1}$	Fold difference in toxicity
	Upper $mgkg^{-1}$	Lower $mgkg^{-1}$		
# Water-Normoxia	400.17	346.28	372.25	
# Water-Hypoxia	353.87	296.45	323.89	0.87
Soil-15%	999.30	999.30	974.45	2.62*
Soil-20%	919.64	919.64	899.70	1.083
Soil-25%	849.83	849.83	840.05	1.071
Soil-30%	800.36	800.36	795.15	1.056
Soil-35%	760.57	760.57	750.15	1.059

*Compared with Normoxia LC_{50} Value
Expressed in mgL^{-1}

In soils containing higher concentration of congored (1250 to 12,000 $mgKg^{-1}$) the burrowing activity increased with further decreased in the concentration of Congo red down to 1000 $mgKg^{-1}$ and with increase in the duration of exposure in the respective concentration. Once full burrowing activity commenced (42 hrs in 1000 $mgKg^{-1}$) practical burrowing activity decreased. The number of earthworm which was fully burrowed increase from 1000 $mgKg^{-1}$ downwards. Earthworm was fully burrowed at 48 hrs in 925 $mgKg^{-1}$ and with further decreased in the concentration, 100 percent burrowing was observed at lesser duration of exposure (Table 5). When the water content of the soil was increased progressively, the initiation of partial and full burrowing activities was observed at progressively lower concentration of Congo red (Table 5). Death of the earthworm in the soil was proceeded by formation of segmental bulging throughout the body (800 $mgKg^{-1}$), body construction (4000 – 7000 $mgKg^{-1}$), precilitellar swelling (6000 $mgKg^{-1}$), oozing of coelomic fluid (2000-8000 $mgKg^{-1}$), shortening of the body (1250-2000 $mgKg^{-1}$), fast wriggling movement (7000 to 8000 $mgKg^{-1}$), violent curling and coiling (1250 to 6000 $mgKg^{-1}$) and curling and coiling (1000 – 1100 $mgKg^{-1}$). In the concentration below 1000 $mgKg^{-1}$ no visible symptoms of stress preceding death was observed. From 1000

to 1100 mgKg^{-1}, behavioral changes alone were observed (Table 6). The concentrations above 1250 mgKg^{-1} at which morphological changes in the earthworm were observed, there was no burrowing activity 100 percent burrowing was observed only at concentration where there was no stress symptoms (Table 5).

Table 5. Effect of CR on the burrowing behavior of *Megascolex konkanensis* in Soil during 48 hrs exposure period at different percent water content

Concentration mgkg^{-1}	35% n=6			30% n=6			25% n=6			20% n=6			15% n=6		
	P	B	U	P	B	U	P	B	U	P	B	U	P	B	U
Control		100			100			100			100			100	
720		100													
730		100													
740		100													
750		100													
760	50	50													
770	100	50													
780					100										
785					100										
790				33	67										
795				50	50										
800				100				100							
810								100							
820								100			100				
830							17	83							
840							50	50			100				
850							100								
860											100				
870										17	83				
880															
900										50	50			100	
925														100	
950													17	83	
975													50	50	
1000													100		

P-Partially burrowed; B-Burrowed; U-Unburrowed

Table 6. Behavioral and morphological manifestations of *Megascolex konkanensis* to different concentration (mgkg^{-1}) of Congo red in soil at various water content

	Water content				
Manifestation	**15%**	**20%**	**25%**	**30%**	**35%**
Fast wriggling movement	7000-8000				760
Coiling and Curling	1000-1100	900-960			
Violent coiling and /or curling	1250-6000	980			
Slow movement	950-975	860-880	830-840	795-810	
Construction formation at the anterior end	4000-7000			810	770-780
Constriction at intervals throughout the boby	5000				
Segmental bulging	8000				
Blacking of the boby	8000				
Shortening of the body	1250-1500				700-750
Elongation of the body		900-980	840-880	785	
Preclitellar swelling	6000				760-770
Oozing out of coelonic fluid	2000-8000				
No visible change	<1000	<860	<830	<780	<700

The magnitude of the toxic effects of Congo red increased with increasing water content of the soil. Some of the behavioral changes observed at higher concentration of Congo red at 15 percent water content were initiated at progressively lower concentration of congo red when soil was increased. With increasing water content of the soil, behavioral changes were observed to 760 mgKg^{-1} and morphological changes down to 700 mgKg^{-1}. Body construction observed from 4000 to 7000 mgKg^{-1} at 15 percent water content was observed at 770 and 780 mgKg^{-1} at 35 percent, water content, preclitellar swelling observed at 6000 mgKg^{-1} at 15 percent water content was observed from 760 -770 mgKg^{-1} at 35 percent water content (Table 6). With increasing water content of the soil morphological changes were observed even on earthworms which showed partial to full burrowing activity. In water, the effect of congo red was similar to that observed in soil. Stress responses preceding death was not observed below 300 mgKg^{-1}. Behavioural changes alone were observed up to 350 mgKg^{-1}. The behavioural changes observed were fast movement and lifting the body (300-375 mgKg^{-1}), curling and coiling (325-400mgKg^{-1}) violent erratic jerky movements (425-450mgKg^{-1}) and hyperexcitability 9500mgKg^{-1}). Above 500 mgKg^{-1} the earthworms were almost labile after initial hyperexcitability. The morphological changes observed were body swelling (9375-400mgKg^{-1}), anal swelling (425-500mgKg^{-1}) body constructions (425-450mgKg^{-1}) segmental bulging (600-650mgKg^{-1}) and peeling of epidermis (450-600mgKg^{-1}). The severity of behavioral and morphological changes increased with increasing concentration of Congo red in water and soil (Table 7).

Table 7. Behavioral and morphological manifestations of *Megascolex konkanensis* exposed to different concentrations (mgL^{-1}) of Congo red, Neolan grey and Cr (III) in water

Manifestation	Congo red
Segmental bulging	600-650
Flaccid	500-600
Thinning	500-600
Peeling of epithelium	450-600
Body Construction	425-450
Anal Swelling	
Oozing out Coelomic fluid	
Body Swelling	375-400
Hyperexcitability	500
Violent coiling and curling	400-450
Violent erratic jerky movement	425-450
Coiling and curling	325-400
Lifting the body	300-375
Vigorous movement	300-375
No visible change	<300

DISCUSSION

Earthworms are common soil organisms and play an important role in improving structure and fertility of soil ecosystems (Bartlett *et al*., 2010). They modify soil organic matter both chemically and physically, mix leaf litter with the soil, facilitate the formation and stabilization of soil aggregates and improve soil porosity (Lavelle and Spain, 2001). It has been indicated that earthworms may represent up to 60–80% of the total animal biomass in soil (Ouellet *et al*., 2008; Jouquet *et al*., 2010). Unlike many other soil organisms that are protected by thick cuticle on the exterior of their bodies, earthworms are particularly susceptible to soil chemicals (Lanno *et al*., 2004; Nahmani *et al*., 2007). The results of the studies show that Congo red was toxic to the earthworm, *M.konkanensis*. The magnitude of their toxicity was high in water and low in soil. Differences in their toxicity may be attributed to difference in their toxic potential as determined by their chemistry and their effective internal concentration. The internal concentration may in turn be determine by their bio available concentration in the external medium (water or soil) therefore exertion of toxic effect at low concentrations in water may be due to their high bio availability resulting in an increased rate on entry in to the integument and susceptibility of the earthworm due to the stress imposed by water. Soil may modify their bio availability by absorbing them. The time for which they remain in soil solution it is sufficient for them to find their way in to the earthworm through the moist skin or through the gut wall during the passage of contaminated soil on consumption. At external concentration where burrowing activity is absent, the duration of survival implicates

increased rate of entry through the integument due to increased bio availability. The amount of contaminants that enters the earthworms body might have increased with increasing water content of the soil, hence the observed reduction in the LC_{50} values. Although the water content was increased 5 percent above the water holding capacity of the soil, the toxic effects were observed still at comparatively higher concentration ranges than that observed in water. This may probably due to comparatively low bioavailable concentration due to absorption, difference in the area of earthworm body available for transport and heterogeneous nature of the soil solution involving interactions among the component of the soil solution for transport across the integument Congo red produced behavioral changes and morphological symptoms. The symptoms of toxicity were dose dependent. The signs and symptoms of toxicity range from violent movement, shifting of body, curling and coiling, hyperexitability and swelling, body constructions, segmental swelling and peeling of epidermis in soil, and swelling was observed. Many of the observed symptoms were also recorded in the earthworm, *Pheretima posthuma,* exposed to different pesticides (Hans *et al.*, 1990).

Most of the symptoms observed in the present study were also recorded in Lambito mauritii exposed to zinc (Shyamala,1991) and Polypheretima elongate (Ramaswami and Subbu Ram, 1992) Mechanism of action of Congo red in causing death to earthworm is not known. Death also occurred at lower concentration range in water and soil without the occurrence of morphological symptoms. Therefore, death may be the result of action of them on some physiological mechanisms, and morphological changes may considered as the secondary effect which appeared preceding death. The decrease in the LC_{50} values under hypoxic condition indicates the synergistic action of oxygen tension with congo red. Earthworms were found to burrow in soil containing even lethal concentration of congo red. Full burrowing activity was observed only when there were no morphological symptoms. Failure to burrow may therefore be considered as due to the effect of congo red on the muscular system, probably degenerating changes which may render the animal inefficient to burrow rather than an exhibition of avoidance behavior. Earthworms are suitable bioindicators of soil contamination, and can be used to provide safety thresholds for contaminants.

This study provides important information on the ecological relevance of the types of toxicity data for use in ecological risk. Assessments or derivation of soil quality standards. Exposure to chemicals in higher concentration would affect their survival and productivity of the soil.

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