

Effect of copper fungicide on earthworm, *Lampito mauritii*

ABSTRACT

Copper as copperoxychloride has wide spread use as a protectant fungicide/bactericide which prevents infection on plants. The recommended dose for rice field is 3 g/l where copper present is 50% i.e 1.5g copper/l. so the recommended dose is 1500mg/l i.e 1500 ppm of copper. The earthworms that play a major role in soil physical, chemical and biological fertility of soil were exposed to the copper fungicide. During in the suspension culture the epi-anecic earthworm, *Lampito mauritii* (Kinberg) could not survive beyond 30 ppm. So the earthworms were exposed to subtoxic level of copper i.e. 0, 10, 20 and 30 ppm of copper and the effect on growth, feeding, respiration, excretion and regeneration was found to be significantly deleterious. On exposure to sublethal dose of copperoxychloride the respiration increased but there was marked reduction in growth, feeding, excretion and regeneration. The positive contribution of the earthworm was hampered but it continued to utilize energy from the system and this was the major findings of this work.

KEYWORDS

Sublethal dose, copperoxychloride, epi-anecic earthworm, growth, feeding, respiration and excretion

1. INTRODUCTION

Soil is a habitat for many micro and macro-organisms. The microorganisms include archaea, bacteria, actinomycetes, fungi, algae, protozoa and macrofauna like springtail, mites, nematode, earthworm, ants, insects and larger organism like burrowing rodents. Among all soil biota, earthworms are the key biotic components which plays a vital role in maintaining chemical, physical and biological property of temperate and tropical soils. Earthworms (macrofauna) belong to the taxonomic phylum Annelida and family Lumbricidae.

Aristotle called earthworm “the Intestine of earth”. By ingesting soil debris, earthworms have been shown to enhance the bioavailability of soil nutrients such as carbon, nitrogen, phosphorous. While burrowing earthworm ingest large amount of soil and are therefore exposed to contaminants through their intestine as well as through their skin. As a result substances are concentrated from the soil into their body making them ideally suited for assessing the bioavailability of many harmful substances [1].

Earthworms hosts millions of decomposer (bio degrader) microbes in their gut and excrete them in soil along with nutrients nitrogen (N) and phosphorus (P) in their excreta called ‘vermicast’ [2]. Edward and Fletcher [3] showed that the number of bacteria and ‘actinomycetes’ contained in the ingested material increased up to 1,000 fold while passing through the gut. The numbers, biomass, and activity of microbial communities in the earthworm gut have also been shown to be different from that of uningested soil [4]. Singleton et al. [2] studied bacteria associated with the intestine and casts of earthworms and it included *Pseudomonas*, *Paenibacillus*, *Azoarcus*, *Burkholderia*, *Spiroplasm* and *Actinobacterim*.

To increase the yield, pesticides are used to decrease the yield loss by the pest. In addition to destroying the pests the pesticides have detrimental effect on the non-target organisms like earthworm which play important role in soil fertility. The epigeic and epianecic earthworms are exposed to the pesticides when it is spread. Earthworms have been selected as a suitable representative soil organism as they contribute to the overall productivity of agricultural soils through their feeding, casting and burrowing activities [5]. Generally, pesticides cause toxicity on earthworms but indirectly can cause the reduction of the population by decreasing the organic matter input and weed coverage [6], [7]. Growth, reproduction and avoidance behaviour are also affected by pesticides [8].

Copper oxychloride (50% WP), trade name Blue copper is protective wettable fungicide having double effect of systematic, contact and prevent action. Copper can be used on numerous crops against fungal and bacterial diseases. It can serve as a fatal enemy of major obstinate disease in growing period of the crop. Its active molecule penetrates with high performance. It can enter the in vivo of the plant rapidly, and expand in the ailing plant, sterilizing inside and outside. It is used as fungicide to control Scab, Anthracnose, Downy mildew, Early blight, and Late blight diseases of various crops, vegetables, Potato, Tomato, Tea, Coconuts, spices, and Tobacco, fruits like grapes, banana, nuts etc.

Copper as copper oxychloride is a protectant fungicide/bactericide which prevents infection on plants. Its mode of action is by interfering with the enzyme system of spores and mycelium, a process which is usually irreversible. It forms a chemical barrier against fungal attack and is a foliar fungicide with preventative action.

2. MATERIAL AND METHODS

2.1 Copper fungicide

Copper oxychloride is used as a fungicide and the recommended dose for rice field is 3 g/l where copper present is 50% i.e 1.5g copper/l. so the recommended dose is 1500mg/l i.e 1500 ppm of copper.

2.2 *Lampito mauritii* (Kinberg)



Figure 1. Image of earthworm *Lampito mauritii*

Lampito mauritii (Kinberg) one of the endemic species classified under epi-anecic species. It is widely distributed in India. It can sustain temperature range of 25-26°C with soil moisture 10-20 % and pH of 5.82 – 7.2 where its growth and reproduction is favoured [9]. The species live in vertical burrows about 1-2 metres into the soil profile and extend to the surface and burrows are channels for preferential flow of air and water and are often lined with organic matter especially protein rich mucus.

They are light greyish to brownish body coloured but deeply pigmented at their head and tail region. Their body is of medium size. Clitellum covers 14th to 17th segment. Male pores on slightly raised areas of 18th segment; paired female pore on 14th segment. Length is about 95mm -155mm. body live weight usually 1 g. This species have the capability to produce 12-45 per year cocoons on an average by an adult [10].

Immature *Lampito mauritii* earthworms were utilized for conducting survivility experiment. Gut contents were cleaned by keeping them in distilled water for three hours [11]. Earthworms were exposed to 0 to 1500 ppm of copper oxychloride solution. After 240 hours, the earthworms exposed to beyond 30 ppm of copper solution could not survive. So the sub lethal dose of 10, 20 and 30 ppm of copper were used for the study and control sets were maintained at 0 ppm copper.

2.3 Growth

The biomass of earthworms reflects the physiological requirements of tissue. In the present study the change in the biomass of the earthworms i.e., change in weight of earthworms at different concentrations of copper solution i.e., 0 ppm 10 ppm, 20 ppm and 30 ppm has been studied.

500 g(300g soil and 200 g cowdung) 2mm sieved, air dried soil was taken in polythene packets. Moisture was maintained at 20 ± 2 g% by adding of distilled water to it called the control sets i.e. '0' ppm and respective copper solution in experimental sets i.e. 10, 20, 30 ppm of 10 replicates of each

concentration were taken. Approximately 1.5 g of earthworm was inoculated in each experimental packet after 5 days of moisture addition after microbial activation. Soil temperature was maintained at $25 \pm 2^\circ\text{C}$.

The change in the weight over initial weight was observed after 10, 20 and 30 days and percentage change in weight of earthworms over zero day culture was estimated.

2.4 Feeding (Stable Aggregate Formation)

Earthworm conserves nutrients by forming stable aggregates. As the formation of stable aggregates is directly proportional to feeding, feeding is estimated by estimating the stable aggregates of earthworms. Formation of stable aggregates is governed by percentage of clay, amount of organic matter in soil, bacterial mucilage, fungal mycelia and colloid contribution of macrofauna [12], [13].

In the present study the effect of 0, 10, 20, and 30 ppm concentration of fungicide on the stable aggregate formation has been observed. The earthworms were inoculated in zero day and estimation of stable aggregates was done at an interval of 10 days. After 10 days culture sets were sieved with water. The stable aggregates formed were collected, air dried and weighed. Carbon content of aggregate formed was estimated by Walkley and Black [14] titration method. Amount of carbon by energy conversion has been calculated and according to available standard values energy conversion for carbon is 41.44 kJ, g^{-1} , dry wt. [15]. Statistical analysis, ANOVA was performed to infer the results.

2.5 Respiratory rate

Respiratory rate was quantified by Alkali absorption method [16]. Carbon dioxide evolution was measured at $25 \pm 2^\circ\text{C}$ and expressed as $\text{mg of CO}_2 \cdot \text{gm}^{-1} \text{ live worm tissue, hr}^{-1}, \text{kg}^{-1} \text{ soil}$.

500gm (300 g soil and 200 g cow dung) 2mm sieved, air dried soil was taken in plastic jars with moisture content maintained at $20 \pm 2 \text{ g\%}$ by addition of distilled water in control sets i.e. '0' ppm and respective copper solution in experimental sets i.e. 5, 10 and 20 ppm. 10 replicates of each concentration were taken and 5g of earthworm was inoculated into them. The jars were kept undisturbed for 5 days for microbial activation. The respiratory metabolism was maintained at 0, 10, 20 and 30 days.

2.6 Excretion

Estimation of ammonia on exposure to the fungicide copper was done by Kaplan method [17]. *Lampito mauriti*, the anecic earthworms were collected from uncontaminated site and inoculated in culture packets which is prepared by mixing soil and cow dung in 3:2 ratio.

40 replicas were taken, 10 for each ppm of fungicide (0, 10, 20, 30). $20 \pm 2 \text{ g\%}$ Moisture level was maintained by giving distilled water to 0 ppm replica (control), 10 ppm fungicide to 10ppm and 20 and 30 ppm fungicide to 20 and 30 ppm replica respectively. Approximately 1.5 g earthworms were inoculated in each replica consisting of a mixture of 300 gm of sieved pasture soil and 200 gms of sieved cow dung.

After inoculating the earthworms, 1.6 gm of moist soil (dry wt. 1 gm) was taken from each replica of 0ppm, 10 ppm, 20 ppm, and 30 ppm. Amount of ammonia was estimated by measuring OD at 655 nm. This process was repeated in every 10 days interval. And 0 day, 10 days, 20 and 30 days reading were observed. The ammonia excretion was determined.

Statistical analysis, ANOVA was performed for inferring the data obtained [18].

3. RESULTS AND DISCUSSION

3.1 Growth

Figure 2 shows the growth i.e change in weight of earthworm of *Lampito mauritii* when exposed to sublethal dose of copper oxychloride.

During 30 days of observation it was noticed that weight of earthworm is gradually increased in control over 0 day. But growth was seen to be decreased in 10, 20 and 30 ppm exposure to copper. The weight decreased by 21.02%, 45.74%, 44.20% over initial weight on exposure to 10, 20 and 30 ppm copper respectively by 30 days. Two Way ANOVA showed significant impact of copper on growth of *Lampito mauritii* at 0.01 and 0.001 level of significance with respect to duration ($F_{cal} = 9.34$, $n_1=3$ $n_2= 9$) and dose ($F_{cal}=162.24$, $n_1=3$ $n_2= 9$), respectively.

The biomass is a good indicator of the physiological requirements of tissue and material cycling [19]. In present study significant reduction in biomass was found on exposure to sub-lethal dose of copper in copper solution. Rate of growth is gradually decreased. Similar results were reported by Khan *et al.*, [20]. They found a significant reduction in earthworm biomass after exposure to different concentration of copper chloride and concluded abnormal functioning of major physiological systems such as digestion and absorption. Helling *et al.*, [21] found that growth and reproduction of *Eisenia fetida* was significantly decreased in consecutive weeks after the exposure of copper fungicide. Bart *et al.*, [22] also reported reduction in growth of enchytraeids (potworms) in laboratory on exposure to copper oxychloride. Reduction in biomass was also found in the earthworm on exposure to pesticides by various studies [23]. [24]. [25], [26]. Owojori *et al.*, [27] found the reduction of growth acari (mites) of in the soils treated with chlorpyrifos, dimethoate, deltamethrin, copper. Zhang *et al.*, [28] found reduction in growth of *Acroboloides nanus*, *Pristionchus pacificus*, and *C. elegans* by mancozeb. Kreuzweiser *et al.* [29] reported that Imidacloprid reduced earthworm growth. According to Capowiez and Berard, [30] and Gomez-Eyles *et al.*, [31] reduction in body weight may reflect reduction in feeding by the earthworms. They also opined that worms excavate less when exposed to imidacloprid, which means that they feed less and have fewer intestinal contents. Loss in body weight may also be attributed to reduction of enzyme activities like cellulase activity which compromises the feeding efficiency of exposed organisms and it ultimately results in lower weight gain [32].

3.2 Feeding

Figure 3 indicates the stable aggregate formation of *Lampito mauritii* under the impact of copper. In control there is an increase of 30.42% in stable aggregate formation after 30 days was observed. But when earthworm were exposed to 10, 20 and 30 ppm of copper after 30 days of culture there was a decrease of about 4.06%, 13.18% and 17.24% in stable aggregate formation on exposure

to 10 , 20 and 30 ppm respectively over 0 ppm of copper. Two Way ANOVA showed that significant impact of copper in energy content of stable aggregate formation by the earthworm at .001 and .05 level of significance with respect to duration ($F_{\text{cal}} = 334.7$, $n_1=3$ $n_2= 9$) and dose ($F_{\text{cal}} = 168.6$, $n_1=3$ $n_2= 9$).

The feeding habit of earthworms results in physical aggregation of the soil. Earthworm activity creates structures, casts and galleries which modify circulation and accumulation of water [33]. In present study significant reduction in stable aggregate formation which is directly proportional to feeding on application of different doses of copper through fungicide is studied. Gunston et al. [34] also reported negative impact of pesticides on earthworms feeding and cast production.

The degree of stability of casts depends on food and behaviour of the worms [35]. Arthur, [36] noticed that bacterial population in the earthworm gut was about 1000 times more than the surrounding soil. The production of polysaccharides like levan and dextran by many bacteria bind quartz and clay particles. Soil particles are cemented by calcium humate produced by interaction of ingested organic matter and cacite excreted by calciferous glands of earthworm hypothesized by Meyer [37]. A significant reduction in earthworm cast of ground surface on application of fungicide like carbendazim, benomyl etc was observed by Keogh and Whitehead [38]. Reduction in surface casting, abundance of earthworms, biomass and litter accumulation when benomyl and related fungicides were sprayed [39]. Reduction in cast production was also reported in *L.terrestris* and *Aporrectodea caliginosa* on exposure to Imidacloprid and Chlorpyrifos, respectively [40], [41]. Larink and Sommer [42] also found that feeding behaviour was reduced by imidacloprid and cyfluthrin.

Burrows and Edward [43] correlate the reduction in feeding as a natural strategy for survival i.e. reducing food intake to avoid the toxins. Such strategy was reported to be commonly used in earthworms to avoid poisoning with heavy metals [43] but also organic chemicals such as pesticides [44], [45].

3.3 Respiration

Figure 4 indicates the rate of respiratory of *Lampito mauritii* under the impact of copper. It was found that rate of respiration was seen to be increased with duration and treatment. i.e after 10 days of exposure the rate of respiration was found to be 3.93, 7.04, 7.04, and 8.11 mg of CO₂ evolved/g of live tissue, hr⁻¹,kg⁻¹ at 0, 10, 20, 30 ppm of copper, respectively. At 20 days 15.98%, 28.99%, 51.49% increase in respiration was observed in 10, 20, 30 ppm exposure of copper respectively over 0 ppm. After a month there is a increase of about 13.40%, 45.49%, 65.78% was observed under 10, 20 and 30 ppm of exposure of copper. ANOVA showed significant impact of copper on respiratory metabolism of earthworm at 0.001 level of significance with respect to duration ($F_{\text{cal}} = 296.01$, $n_1=3$ $n_2= 9$) and dose ($F_{\text{cal}} = 135.34$, $n_1=3$ $n_2= 9$).

Energy loss of an organism is represented by respiratory metabolism. Most of the organism's energy is utilized in respiration. Soil respiration is being used for estimation of biological activity [46]. Earthworms on exposure to cabaryl and endosulfan showed an increased value of CO₂ evolution. Temperature stress result in increase in the oxygen consumption reported by Senapati and Dash [47]. Bolton [48] measured the respiration of earthworm species *Dendrobaena rubida* and *Lumbricus castaneus* at 10°C and reported values between 75 and 100 µl CO₂ h⁻¹g⁻¹. In the present study

enhancement in the respiratory metabolism when exposed to different concentrations of copper fungicide has been observed which results in the loss of more energy utilized by the earthworms. Similar results were also observed by Panda and Patnaik [49] where exposure of earthworms, *Perionyx excavates* to carboxin hydrochloride resulted in an increase in respiration. It is suggested that exposure to toxicants like heavy metal or other pesticides impedes food consumption, while at the same time energy was required to eliminate the toxicant [50], [51]. The increase in respiratory metabolism might be attributed to higher muscular activities and energy utilization to overcome oxidative impairment [52]. According to Maboeta et al. [53] energy budget model describes the underlying physiological processes as catabolism of tissue under stress condition, with rates of weight loss accompanied by the increase in maintenance rates.

3.4 Excretion

Figure 5 represents the impact of copper on ammonia excretion of *Lampito mauritii* (Kinberg). In control ammonia excretion was gradually increased where as in 10, 20 and 30 ppm of exposure of copper, excretion was seen to be decreased upto 13.33%, 26.66%, 46.66% respectively. After a month excretion was decreased drastically i.e 53.60%, 88.14% and 97.42% in 10, 20 and 30 ppm exposure of copper respectively. ANOVA test shows significant impact of copper on ammonia excretion at 0.001 level of significance in laboratory culture with respect to duration ($F_{cal} = 174.7$, $n_1=3$ $n_2= 9$) and dose ($F_{cal} = 275.24$, $n_1=3$ $n_2= 9$).

Mucous protein and nitrogenous metabolic by-product like ammonia, urea and free amino acids are the main nitrogenous compounds excreted by earthworms [54]. In the present study the alteration in ammonia excretion of earthworm was found on exposure to sub-lethal dose of copper fungicide. The rate of excretion of earthworm *Lampito mauritii* increases day wise and decreases with increase concentration of copper.

On exposure to sub lethal dose of copper the organism remain in the ecosystem but it fails to contribute its positive role towards the system as a result of toxicity of copper. Decrease in excretion by earthworm has been reported on application of sublethal dose of malathion by Senapati *et al*, [55]. Reduction in ammonia excretion on exposure to copper fungicide has been reported by Patnaik and Senapati, [56].

The maximum channelization of the energy towards the respiratory metabolism may be one of the major reasons for decrease in the excretion of earthworm under stress condition.

4. CONCLUSION

Copper which one of the most frequently used fungicide by the farmers do have detrimental effect on the non target organisms like earthworms. At sub lethal dose although the earthworms are not eliminated from the system, but their positive contributions like growth, aggregate formation, nitrogen contribution towards soil in form of ammonia are drastically decreased. At the same time most of the energy is channelized towards maintenance of the body on exposure to copper in the present study. On exposure to the sub lethal dose it remains in the system utilising the resources but does not contribute positively towards the system. So there should be study of effect of sublethal dose of pesticides and

alternate bio pesticides should be chosen for use in the croplands for reduction of yield loss due to pest. The sublethal effects like effect on the parameters like growth, feeding, respiration and excretion can also be used as indicators for presence of toxic substances.

UNDER PEER REVIEW

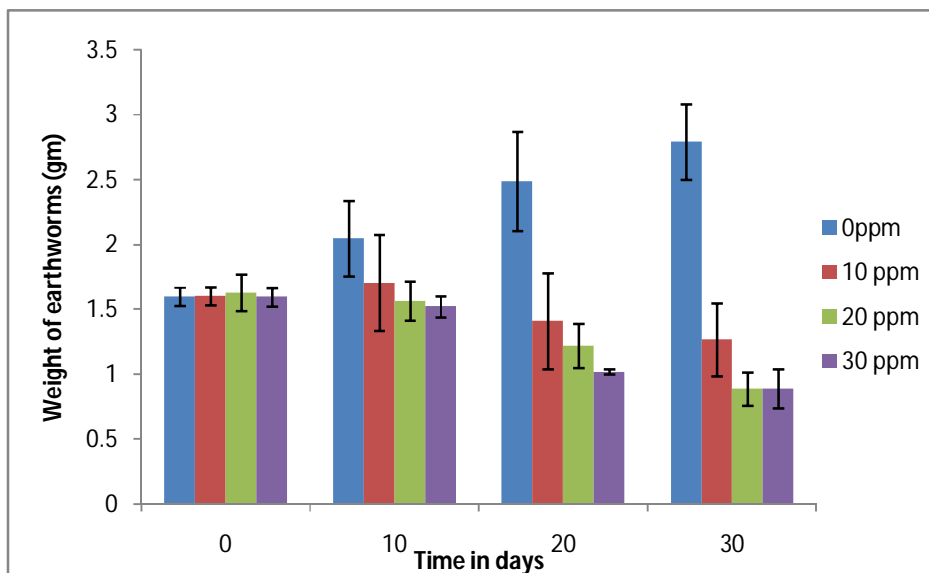


Figure 2: Change in weight of *Lampito mauritii* (Kinberg) on exposure to copper.

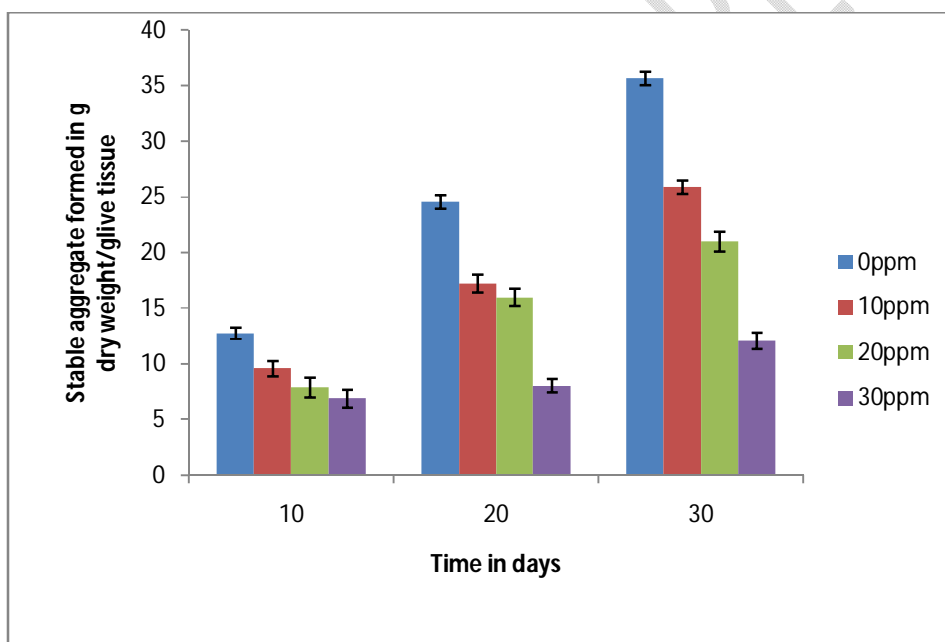


Figure 3. Change in weight of stable aggregate formed by *Lampito mauritii* (Kinberg) on exposure to copperoxychloride.

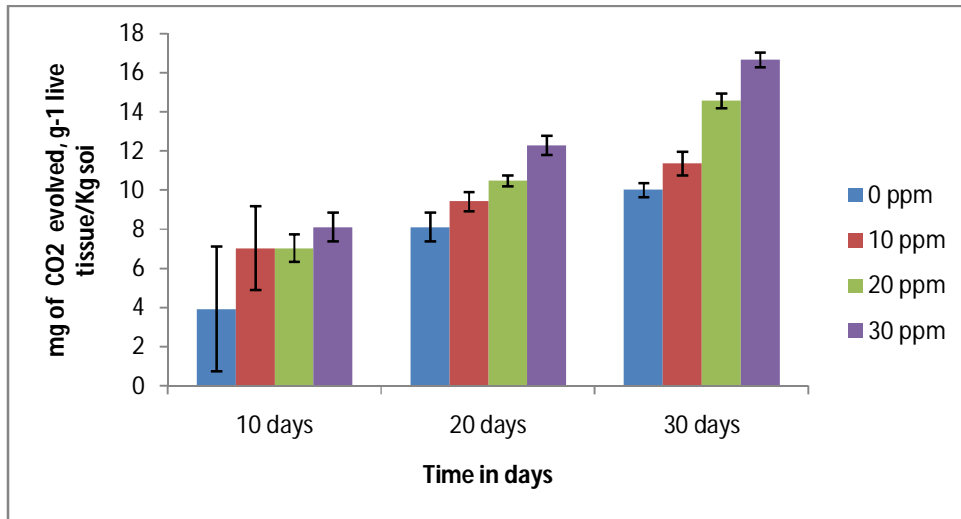


Figure 4. Respiratory rate of *Lampito mauritii* under the impact of copper in laboratory culture.

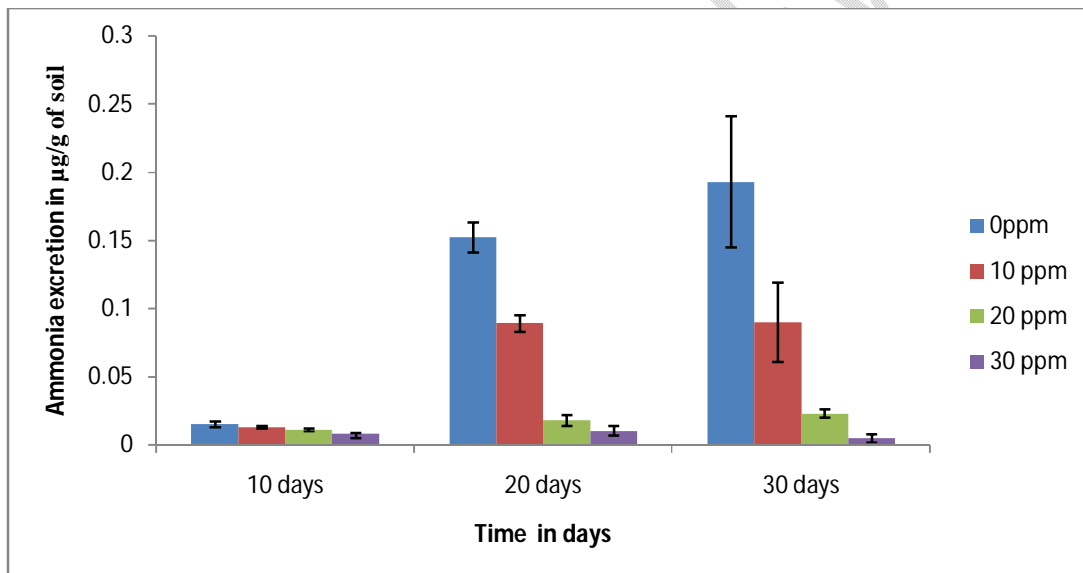


Figure 5 Ammonia excretion of *Lampito mauritii* (Kinberg) under the impact of copper

5. REFERENCES

[1] Morgan JE, Morgan AJ. Earthworms as biological monitors of cadmium, copper, lead and zinc in metalliferous soils. *Environmental Pollution*. 1988;54(2): 123–38.

[2] Singleton DR, Hendrix BF, Coleman DC, Whitmann WB. Identification of uncultured bacteria tightly associated with the intestine of the earthworms *Lumricus rubellus*. *Soil Bio. Biochem.* 2003;35: 1547–1555.

- [3] Edwards CA, Fletcher KE. Interaction between earthworms and microorganisms in organic matter breakdown. *Agric. Ecosyst. Environ.*, 1988;20: 235–249.
- [4] Schonholzer F, Hahn D, Zeyer J. (1999). Origins and fate of fungi and bacteria in the gut of *Lumbricus terrestris* studied by imageanalysis. *FEMS Microbiol. Ecol.* 1999;28: 235–248.
- [5] Culy MD, Berry EC. Toxicity of soil applied granular insecticides to earthworm populations in cornfields. *Down to Earth.* 1995; 50: 20- 25.
- [6] Booth LH, O'Halloran KA. Comparison of biomarker responses in the earthworm *Aporrectodea caliginosa* to the organophosphorus insecticides diazinon and chlorpyrifos. *Environmental toxicology and chemistry.* 2001;20 (11): 2494-502.
- [7] Abdul Rida AM, Bouche MB. 1997. Earthworm toxicology: from acute to chronic tests. *Soil Biology and Biochemistry.* 1997; 29: 699-703.
- [8] Kula H, Larink O. Development and standardization of test methods for the prediction of sublethal effects of chemicals on earthworms. *Soil Biology and Biochemistry.* 1997;29(3-4): 635-639.
- [9] Kale RD. Earthworms and soil. *Proceedings of National Academy Science India*, 67(B): 1997;13-24.
- [10] Dash MC, Senapati BK. 1980. Cocoon morphology, hatching and emergence pattern in tropical earthworms. *Pedobiologia.* 1980;20 : 316-324.
- [11] O.E.C.D. (Organization for economic Co-operation and Development) Earthworm, Acute toxicity text. *OECD Guidelines for testing of chemicals* 1984;(207):194.
- [12] Swaby RJ. The influence of earthworms on soil aggregation. *Journal of Soil Sci.* 1950;1: 195-197.
- [13] Lavelle P. The soil system in humid tropics. *Bio. Int.* 1984;9: 2-17.
- [14] Walkley A, Black IA. (1934). An examination of Degtareef method for determination of organic carbon in soil: Effect of variations in digestion condition and of inorganic soil constituents. *Soil Sci.* 1934;63: 251-263.
- [15] Remmert H. *Ecology A Text Book : Springer – Verlag, Berlin* pp. 289, 1980.
- [16] Witkamp M. Rates of CO₂ evolution from forest floor. *Ecology.* 1996;7 : 492-494.

- [17] Kaplan A. The determination of urea, ammonia and urease. In: Methods of Biochemical Analysis. Ed Glick D. John Wiley and Sons New York, 311-321, 1969.
- [18] Gupta SP. Statistical methods (14th eds). Sultan Chand and Sons. New Delhi, 1980.
- [19] Thompson AR. Effects of nine insecticides on numbers and biomass of earthworm in pasture. Bull. of Env. Cont. and Toxicology. 1970;5: 577-586.
- [20] Khan I, Ayesha S, Sharique A. Biomass and behavioral responses of earthworm, *Lumbricus terrestris* to copper chloride. Iranian Journal of Toxicology, 2007;1(2): 2-2.
- [21] Helling B, Reinecke SA, Reinecke AJ. 2000. Effects of the fungicide copper oxychloride on the growth and reproduction of *Eisenia fetida* (oligochaeta). Ecotoxicology and Environmental Safety. 2000;46(1): 108-116.
- [22] Bart S, Laurent C, Péry ARR, Mougin C, Pelosi C. Differences in sensitivity between earthworms and enchytraeids exposed to two commercial fungicides. Ecotoxicol. Environ. Saf. 2017;140: 177–184.
- [23] Farrukh S, Ali AS. Effects of Dichlorovos Organophosphate on Growth, Reproduction, and Avoidance Behavior of Earthworm *Eisenia foetida*. Iranian Journal of Toxicology, 2011;5(14): 495-501.
- [24] Ahmed ST. The impact of four pesticides on the earthworm *Lumbricus terrestris* (Annelida; Oligochaeta). Int. J. Cur. Res. Rev. 2013;5(21): 1-5.
- [25] Travlos IS, Gkotsi T, Roussis I, Kontopoulou CK, Ioanna Kakaboukl K, Bilalis DJ. Effects of the herbicides benfluralin, metribuzin and propyzamide on the survival and weight of earthworms (*Octodrilus complanatus*). Plant Soil Environ. 2017;63(3): 117–124.
- [26] Treder K, Jastrzebska M, Kostrzevska MK, Makowski P. Do long-term continuous cropping and pesticides affect earthworm communities? Agronomy, 2020;10: 586
- [27] Owojori OJ, Waszak K, Roembke J. Avoidance and reproduction tests with the predatory mite *Hypoaspis aculeifer*: effects of different chemical substances: avoidance and reproduction tests with *Hypoaspis aculeifer*. Environ. Toxicol. Chem. (2014);33:230–237.
- [28] Zhang J, Liang W, Wu X, Jiang S, Li Q. Toxic effects of acetochlor on mortality, reproduction and growth of *Caenorhabditis elegans* and *Pristionchus pacificus*. Bull. Environ. Contam. Toxicol. 2013;90 :364–368.

- [29] Kreuzweiser DP, Good KP, Chartrand DT, Scarr TA, Thompson DG. Are leaves that fall from imidacloprid-treated maple trees to control asianlonghorned beetles toxic to non-target decomposer organisms? *J. Environ. Qual.* 2008;37:639–646.
- [30] Capowiez Y, Berard A. Assessment of the effects of imidacloprid on the behavior of two earthworm species (*Aporrectodea nocturna* and *Allolobophora icterica*) using 2D terraria. *Ecotoxicol. Environ. Saf.* 2006;64, 198–206.
- [31] Gomez-Eyles JL, Svendsen C, Lister L, Martin H, Hodson ME, Spurgeon DJ. Measuring and modeling mixture toxicity of imidacloprid and thiacloprid on *Caenorhabditis elegans* and *Eisenia fetida*. *Ecotoxicol. Environ. Saf.* 2009;72: 71–79.
- [32] Luo Y, Zang Y, Zhong Y, Kong Z. Toxicological study of two novel pesticides on earthworm *Eisenia fetida*. *Chemosphere* 1999;39: 2347–2356.
- [33] Tisdall JM, Oades, JM. Organic matter and water-stable aggregates in soils. *European Journal of Soil Science.*, 1982;33(2): 141–163.
- [34] Gunstone T, Cornelisse T, Klein K., Dubey A, Donley N. Pesticides and Soil Invertebrates: A Hazard Assessment. *Frontiers in Environmental Science*, 2021;9 Article 643847.
- [35] Guild, WJMCL. Earthworms and soil structure. **In** *Soil Zoology*. **Ed** Kevan DK, Mc Butterworths E, London,1955; 83–98.
- [36] Arthur DR. Form and function in the interpretation of feeding in Lumbricid worms. *Viewpoints Bio*, 1965;4: 204-251.
- [37] Meyer L. Experimenteller Beitr%oge zu makrobiologischen Wirkungen auf Humus and Boden Bildung, *Arch. Pflanzenerahrung Dungung Bodenkunde*, 1943;29:119-140.
- [38] Keogh RG, Whitehead PH. Observations on some effects of pasture spraying with benomyl and carbendazim on earthworm activity and litter removal from pasture. *New Zealand Journal of Experimental Agriculture*. 1975;3:(1)103-104.
- [39] Wright MA. Effect of benomyl and some other systematic fungicide on earthworms. *Ann. Appl.Biol.* 1977;87: 520-524.
- [40] Dittbrenner N, Triebkorn R, Moser I, Capowiez Y. Physiological and behavioural effects of imidacloprid on two ecologically relevant earthworm species (*Lumbricus terrestris* and *Aporrectodea caliginosa*). *Ecotoxicology*. 2010;19:1567–1573.

- [41] Capowiez Y, Dittbrenner N, Rault M, Hedde M, Triebkorn R, Mazzia C. Earthworm cast production as a new behavioural biomarker for toxicity testing. *Environ. Pollut.* 2010;158:388–393.
- [42] Larink O, Sommers R. Influence of coated seeds on soil organisms tested with bait lamina June 2002 *European Journal of Soil Biology.* 2002;38(3-4):287-290
- [43] Burrows LA, Edwards CA. The use of integrated soil microcosms to predict effects of pesticides on soil ecosystems. *European Journal of Soil Biology.* 2002;38 (3-4): 245–249.
- [44] Nunes MET, Espindola ELG. Sensitivity of *Eisenia andrei* (Annelida, Oligochaeta) to a commercial formulation of abamectin in avoidance tests with artificial substrate and natural soil under tropical conditions. *Ecotoxicology.* 2012;21(4): 1063–1071.
- [45] Wang JH, Zhu LS, Liu W, Wang J, Xie H. Biochemical responses of earthworm (*Eisenia foetida*) to the pesticides chlorpyrifos and fenvalerate. *Toxicology Mechanisms and Methods.* 2012; 22(3): 236–241.
- [46] Lundergarh H. Carbon dioxide evolution of soil and crop growth. *Soil Sci.* 1927;23: 417-453.
- [47] Senapati BK, Dash MC. Energetics of earthworms population in tropical pastures from India. *Proc. Indian Acad. Sciences (Anim. Sci.).* 1983;92: 315-322.
- [48] Bolton PJ. The use of an infrared gas analyser for studies on the respiratory metabolism of Lumbricidae. *In* *Methods of study in soil ecology.* Ed Phillipson, J. UNESCO, Paris, 1970.;pp. 269-273.
- [49] Panda M, Patnaik A. A study on ecotoxicity of cartap hydrochloride on "*Perionyx excavatus*". *Eco. Env. & Cons.* 2021;27 (1): 274-281.
- [50] Calow P. Physiological costs of combating chemical toxicants: ecotoxicological implications. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology.* 1991;100 (1–2): 3–6.
- [51] Johnstone ASA, Hodsonb ME, Thorbekc P, Alvarezd T, Sibly RM. An energy budget agent-based model of earthworm populations and its application to study the effects of pesticides. *Ecological Modelling.* 2014;280: 5–17.
- [52] Marigoudar SR, Ahmed RN, David M. Cypermethrin induced respiratory and behavioural responses of the freshwater teleost, *Labeo rohita* (Hamilton). *Veterinarski arhiv.* 2009;79(6): 583-590.

[53] Maboeta MS, Reinecke SA, Reinecke AJ. The relationship between lysosomal biomarker and organismal responses in an acute toxicity test with *Eisenia fetida* (Oligochaeta) exposed to the fungicide copper oxychloride. *Environmental Research*. 2004;96: 95–101.

[54] Laverack MS. *The physiology of earthworms*. New York, USA: *Pergamon Press*, Ltd. 1963.

[55] Senapati BK, Biswal J, Pani SC, Sahu SK. Ecotoxicological effects of malathion on earthworms. *Soil Biol. Biochem.* (U.K.).1992;24(12): 1719-1722.

[56] Patnaik A, Senapati BK. (). Impact of copper fungicide on the survivability, growth and regeneration of an anecic earthworm. *Ecol. Env. & Cons.* 1996;2: 109-113.

UNDER PEER REVIEW