

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*.

Lampito mauritii is one of the anecic earthworm found widely in Odisha and especially in Western Odisha, where this work has been conducted. The main contribution of the anecic earthworms is fragmentation of litter and its transportation to the lower layers of soil. It also produces mucus and helps in macrostructure formation. It ingests and disperses the of soil biota and controls population of some soil biota including microbes and mineralization process is facilitated [5].

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 [6]. Generally, pesticides cause toxicity on earthworms but indirectly can cause the reduction of the population by decreasing the organic matter input and weed coverage [7], [8]. Growth, reproduction and avoidance behaviour are also affected by pesticides [9].

Copper oxychloride (50% WP), trade name Blue copper is protective wetttable 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.

There are several reports that the pesticides used for reduction of loss of yield due to the pests also result in reduction of non target organisms like earthworms which play important role in soil fertility [10], [11], [12], [13]. Most of the studies show the lethal effect the various pesticides. In this study an attempt has been made to find the effect of a widely used fungicide copper oxchloride on the anecic earthworm, *Lampito mauritii* at subtoxic level. Although at subtoxic level the organism is not killed, but its metabolic parameters like growth respiration, excretion etc. are hampered and these changes may also be considered as the bioindicators of the toxic substances entering into the ecosystems.

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 [14]. 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 [15].

Immature *Lampito mauritii* earthworms were utilized for conducting survivility experiment. Gut contents were cleaned by keeping them in distilled water for three hours [16]. 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 [17], [18].

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 [19] 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. [20].

2.5 Respiratory rate

Respiratory rate was quantified by Alkali absorption method [21]. Carbon dioxide evolution was measured at $25 \pm 2^\circ\text{C}$ and expressed as mg of CO_2 , gm^{-1} live worm tissue, hr^{-1} , kg^{-1} 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 ± 2 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 [22]. *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 ± 2 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, two way ANOVA was performed for inferring the data obtained [23].

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 (Table 1).

The biomass is a good indicator of the physiological requirements of tissue and material cycling [24]. 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.*, [25]. 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.*, [26] found that growth and reproduction of *Eisenia fetida* was significantly decreased in consecutive weeks after the exposure of copper fungicide. Bart *et al.*, [27] 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 [28]. [29]. [30], [31]. Owojori et al., [32] found the reduction of growth acari (mites) of in the soils treated with chlorpyrifos, dimethoate, deltamethrin, copper. Zhang et al., [33] found reduction in growth of *Acroboloides nanus*, *Pristionchus pacificus*, and *C. elegans* by mancozeb. Kreuzweiser et al. [34] reported that Imidacloprid reduced earthworm growth. Dichlorovos application leads to reduction in earthworm biomass [35]. According to Capowiez and Berard, [36] and Gomez-Eyles et al., [37] 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 [38].

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 ($F_{cal} = 334.7$, $n_1=3$ $n_2= 9$) and dose ($F_{cal} = 168.6$, $n_1=3$ $n_2= 9$) (Table 2).

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 [39]. 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. [40] also reported negative impact of pesticides on earthworms feeding and cast production. **Weight loss and reduction in feeding on exposure to toxicants in soil has been reported by various workers [41], [42], [43].**

The degree of stability of casts depends on food and behaviour of the worms [44]. Arthur, [45] 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 [46]. A significant reduction in earthworm cast of ground surface on application of fungicide like carbendazim, benomyl etc was observed by Keogh and Whitehead [47]. Reduction in surface casting, abundance of earthworms, biomass and litter accumulation when benomyl and related fungicides were sprayed [48]. Reduction in cast production was also reported in *L.terrestris* and *Aporrectodea caliginosa* on exposure to Imidacloprid and Chlorpyrifos, respectively [49], [50]. Larink and Sommer [51] also found that feeding behaviour was reduced by imidacloprid and cyfluthrin.

Burrows and Edward [52] 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 [51] but also organic chemicals such as pesticides [53], [54].

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 ($F_{cal} = 296.01$, $n_1=3$ $n_2= 9$) and dose ($F_{cal} = 135.34$, $n_1=3$ $n_2= 9$) (Table 3).

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 [55]. Earthworms on exposure to carbaryl and endosulfan showed an increased value of CO₂ evolution. Temperature stress result in increase in the oxygen consumption reported by Senapati and Dash [56]. Bolton [57] 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 expose to different concentration 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 [10] where exposure of eartworms, *Perionyx excavates* to cartap hydrochloride resulted in 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 [58], [59]. The increase in respiratory metabolism might be attributed to to higher muscular activities [60], [61] and energy utilization to overcome oxidative impairment [62]. According to Maboeta et al. [63] energy budget model describes the underlying physiological processes as catabolisation 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 ($F_{cal} = 275.24$, $n_1=3$ $n_2= 9$) (Table 4).

Mucous protein and nitrogenous metabolic by-product like ammonia, urea and free amino acids are the main nitrogenous compounds excreted by earthworms [64]. 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*, [65]. Reduction in ammonia excretion on exposure to copper fungicide has been reported by Patnaik and Senapati, [66].

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 there 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. Further research work should include effective biopesticides for protection of crops against pests which will not be detrimental for the soil fauna.

Table 1. Two way ANOVA for growth of *Lampito mauritii* earthworm under the impact of copper with respect to treatment and duration.

Source of Variation	Sum of squares	df	MS	F-cal	P-value	F tab	Significance
Between duration	1.38	3	0.46	9.34	0.01	7.0	Significant
Between Treatment	23.95	3	7.98	162.24	0.001	13.9	Significant
Interaction	15.22	9	1.79	34.37	0.05	1.95	Significant
Within	7.08	144	0.05				
Total	47.63	159					

Table 2: Two Way ANOVA for energy content of stable aggregate formed by earthworm *Lampito mauritii* under the impact of copper with respect to treatment and duration

Source of Variation	Sum of Square	df	MS	F-cal	P-value	F tab	Significance
Between Duration	0.295985	2	0.147993	334.7241	0.001	27.0	Significant
Between treatment	0.2237	3	0.074567	168.6523	0.001	23.7	Significant
Interaction	0.062995	6	0.010499	23.74646	0.5	2.18	Significant
Within	0.04775	108	0.000442				
Total	0.630431	119					

Table 3: Two way ANOVA for respiration of *Lampito mauritii* earthworm under the impact of copper in laboratory culture.

Source of Variation	Sum Square	df	MS	F cal	P-value	F tab	Significance
Between duration	875.59	2	437.80	296.01	0.001	27.0	Significant
Between treatment	406.02	3	135.34	91.51	0.001	23.7	Significant
Interaction	58.91	6	9.82	6.64	0.05	2.18	Significant
Within	159.73	108	1.48				
Total	1500.26	119					

Table 4. Two way ANOVA for Ammonia excretion of *Lampito mauritii* earthworm under the impact of copper with respect to treatment and duration.

Source of Variation	Sum of square	df	MS	F tab	P-value	F cal	significance
Between duration	0.10	2	0.05	174.70	0.001	27.0	Significant
Between treatment	0.24	3	0.08	275.24	0.001	23.7	significant
Interaction	0.11	6	0.02	65.35	0.05	2.18	significant
Within	0.03	108	0.01				
Total	0.48	119					

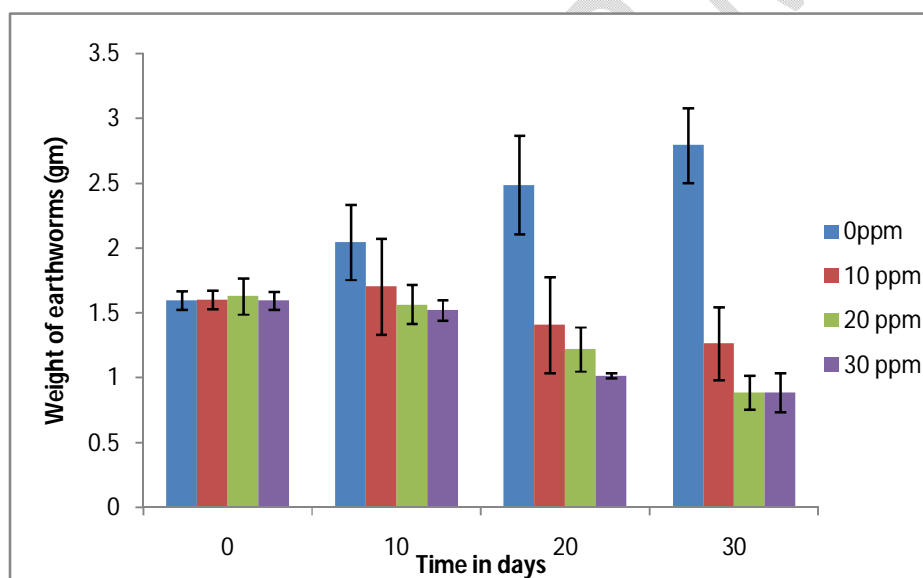


Figure 2: Change in weight of *Lampito mauritii* (Kinberg) on exposure to copper.

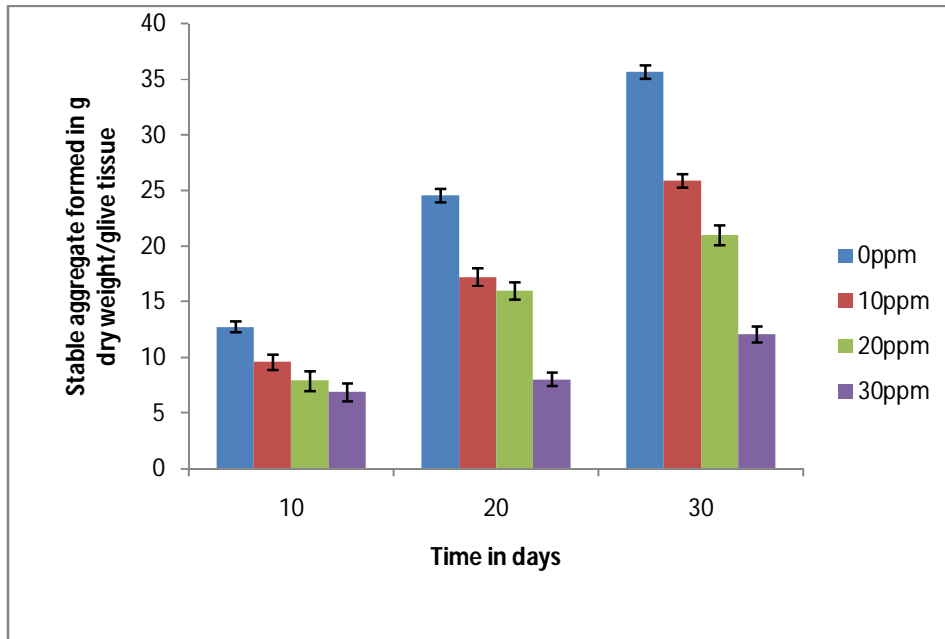


Figure 3. Change in weight of stable aggregate formed by *Lampito mauritii* (Kinberg) on exposure to copperoxychloride.

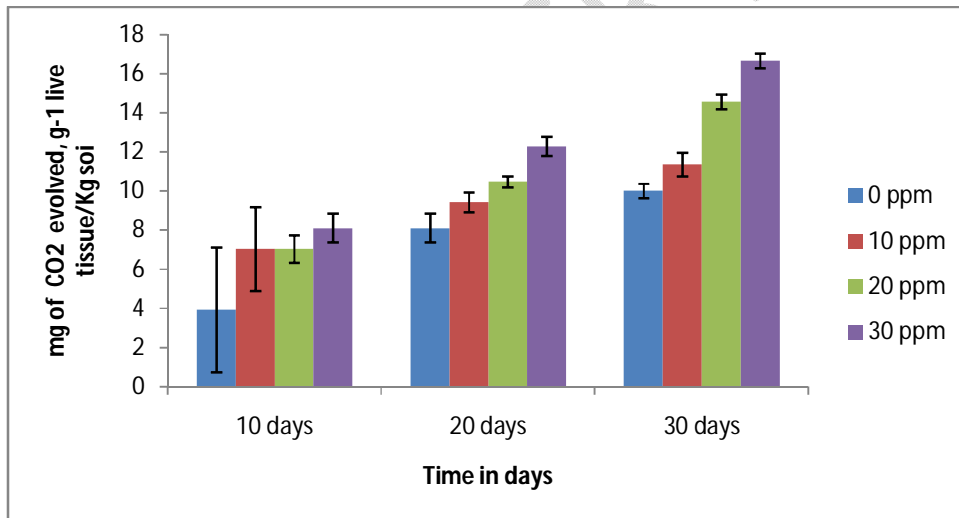


Figure 4. Respiratory rate of *Lampito mauritii* under the impact of copper in laboratory culture.

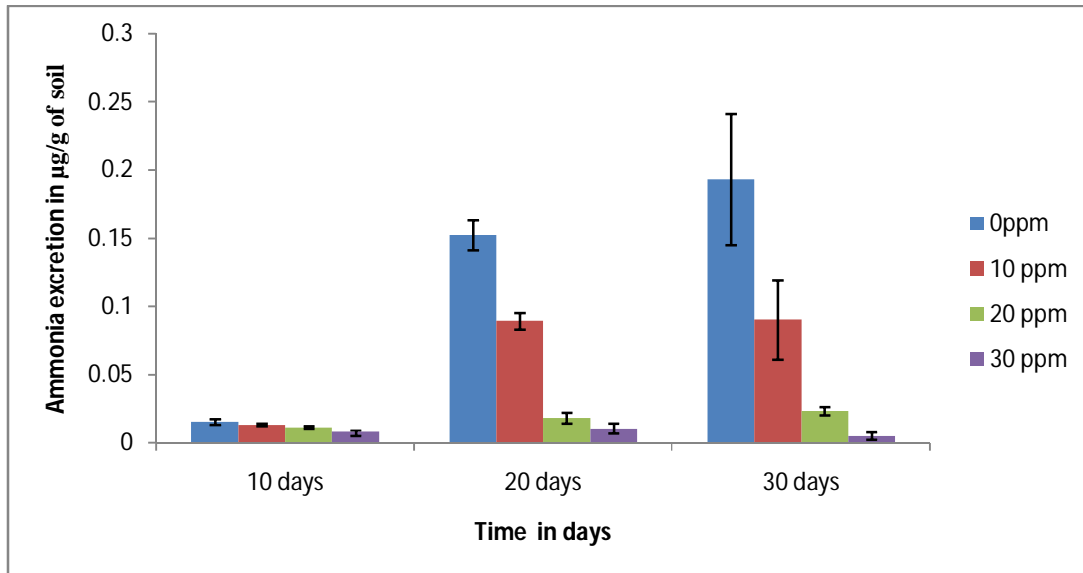


Figure 5 Ammonia excretion of *Lampito mauritii* (Kinberg) under the impact of copper

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