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Toxicity of Heavy Metals and Detergent Inducing Morphological Deformities and Survival Rate of Chironomous Circumdatus

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Abstract

In Chironomidae larvae, the potential effects of heavy metals and detergent can be seen as a result of morphological deformities in larval mentum, mandibles, oral cavity, and in different body regions. In this experiment, the objectives were to examine (I) The potential effects of heavy metals forming morphological deformities in Chironomidae larvae. (II) The survival rate of Chironomidae larvae in exposure to detergent and heavy metals. (III) To establish a dose-response relationship between toxicant and chironomid larvae. Consecutive developmental stages of larvae from egg to fourth instar were chronically exposed to the four sublethal concentrations of lead (0, 5, 50, 200 µg/g), copper (0, 1, 10, 100 µg/g), and detergent (0, 100, 200, 300 mg/g). Tetraphyll flakes were given as food. This experiment reveals the assessment of toxicity by the sublethal concentration of copper (Cu), Lead (Pb), and detergent induce low to high range of mouthpart and body deformities and survival rates in different instars of *Chironomous circumdatus* larvae during chronic exposure tests over several generations. According to the consecutive observations, it was found that the deformation frequency of oral region and body were high in detergent medium while lead and copper showed comparatively less deformation rate and high survival. In both lead and copper media, the survival rate shows a declining pattern and deformation frequency increased on increasing the dose.

Keywords: *Chironomous circumdatus*, Lead, Copper, Detergent, Deformation

1. Introduction

Water bodies in urban areas are getting extremely polluted in modern times. Streams are highly contaminated with heavy metals such as Lead (Pb), Copper (Cu), Zinc (Zn), and Cadmium (Cd). These metals are essential for the survival of aquatic biota, but when absorbed in excess they become toxic. The municipal waste including detergent major ingredients such as sodium laureth sulphate causes a high rate of mortality due to the alteration of hemoglobin production in chironomids. [2]

Chironomous (Order: Diptera; Family: Chironomidae) are bright red color (due to the presence of hemoglobin) larval worms that follow up 3 consecutive developmental stages (egg> 1st instar larva> 2nd instar larva> 3rd instar larva> 4th instar larva> adult. It belongs to the most abundant and widespread aquatic biota found near the sediment of ponds, lakes, and oceans, and performs an important role in the aquatic food web [3]. Chironomous larvae serve as a high-quality nutritional food for several species of fish due to their digestible protein and water content [3]. Chironomids larvae are extremely resistant; they

are able enough to survive for several hours in oxygen deprivation and are categorized as scavengers that feed only on organic matter [4]. Chironomids ingest a wide range of food, including macrophytes, algae, detritus and associated microorganisms, wood debris, and several invertebrates, collectively called omnivorous [4].

Population growth increases the level of pollution in the atmosphere which also affects aquatic and amphibians' life. Chironomous larvae are potential bioindicators of environmental degradation as their presence indicates low and polluted water quality [4]. Various human activities cause a great negative impact on the environment in which heavy metals are also considered a prime pollutant [5]. Heavy metals are non-biodegradable and have long-term acute and chronic toxic effects [6].

These heavy metals induce toxicity in sediments which serves as food for Chironomidae. Chironomid larva is one of the most selective and suitable organisms to test the aquatic toxicity caused by heavy metals and other pollutants such as contaminants of detergent in water [7]. Metals can form ions when released into the water bodies and these ions either binds with the limnic sediment or remain suspended [6]. In both conditions, they cause harm to the aquatic biota. Chironomous larvae are sensitive to many pollutants including heavy metals and detergents [8]. Chironomid larvae, when continuously exposed to pollutants, their late instars develop morphological deformities (generally mouthparts deformities especially in mentum and mandibles) [2]. In recent times, Chironomid morphological deformities analysis has become a valuable and established tool in the sediment assessment programme. [9]

In the experiment, performed in a laboratory, deformities in mouth have been induced by the contaminant sediment [9]. A significant correlation is found between the toxicity of sediment and the development of mouthpart deformities in chironomid larvae which can be demonstrate as sediment toxicity assessment and contamination for aquatic life [1]. These metals also induce the deformative tendency to the body. In the experimental analysis, a noticeable body deformation was assessed in chironomids. The tested larval bodies were dissociated when these metals were introduced which results in improper segment alignment, and improper body mass in chironomids. Copper (Cu) and Lead (Pb) are considered as most abundant and essential elements found in aquatic ecosystems [6]. However, the impact of these metals will become more toxic when their intake (through food or diffusion) is in excess [6]. Excess intake through feeding causes oral deformities which may vary according to the sublethal concentrations. These environmental contaminants may also interfere with hormonal activities mainly the activity of estrogen [9].

The other toxicant named detergent or surfactant also contaminates the water bodies and causes mortality and deformities in aquatic organisms. It is generated by human activities or sewage discharge into water-bodies containing toxic substances such as Sodium sulphates, nonylphenol (NP), and Bisphenol A (BPA) [10]. It was investigated that these toxicants cause genotoxicity in several organisms such as *Daphnia magna* and *Chironomous circumdatus* which increases the mortality rate [10]. In our experiment, the rate of mortality is assessed by introducing detergent particles in the larval culture.

This experiment aimed to examine *Chironomous circumdatus* sensitivity towards heavy metals and detergent by assessing their survival rate and deformation frequencies of the mentum, mandible, and body.

2. Materials and Methods

2.1. Sample collection

Collection of Chironomous circumdatus was performed in the freshwater pond, located in Isabella Thoburn college. Larvae of II, III, and IV instars were collected with the help of an aquatic hand net. A

cluster of benthic mud containing a bunch of larvae extracted from the pond. Number of larvae was around 620. The collected samples were then cultured in the laboratory to perform the further experiment.

2.2. Required material

Chironomous larvae (n = 620), aquatic hand net for collection, benthic mud for larval culture.
The material used to culture the larvae – A basal tray (87cm × 64cm × 6.5cm) to carry the aquariums, small aquariums (n = 30) to contain larvae (Measurement – Length = 11.2cm; Area = 50.24cm), mosquito net to cover the aquarium tray, wooden sticks to hold the net, a Petri dish, pond water, and Tetraphyll.

2.3. Experimental design and analysis

Larvae were reared in the plastic aquariums containing sediment (10g) mixed with fish flakes as food, benthic mud (2g), pond water (10ml), de-chlorinated tap water (130 ml), covered with a mosquito net to prevent the escaping of adult flies. Continuous ventilation was provided through the net. Aquariums contain water Hyacinth (*Eichhornia crassipes*) to maintain the natural habitat for larvae and also enable the breeding ease. Moulting of lower instars to higher occurred in 3-4 days, the II and III instars were moulted into the IV instars, and the IV instars were hatched out in 2-4 days as midge flies. Chironomids are the indicators of toxicity or domestic pollution in their habitat. The collected larvae have several low deformities in their oral structure and body. The hatched Chironomids laid egg masses in the aquarium water surface which was undergo embryogenesis and the eggs were hatched into the first instar larvae in 4 – 5 days. Further moulting processes were held on and the 1st instars were developed into the II instars. The metal toxicity exposure began when the larvae were about at their II instar stage.

2.4. Parameters of experiment

To assess the survival rate, oral deformities (mentum and mandibles) and body deformities during the sublethal and lethal exposure of heavy metal (Copper and lead) and detergent.

Figure A. Experimental set-up showing Chironomid larvae culture in lead, copper and detergent media

Figure b 1 Test *Chironomous circumdatus*

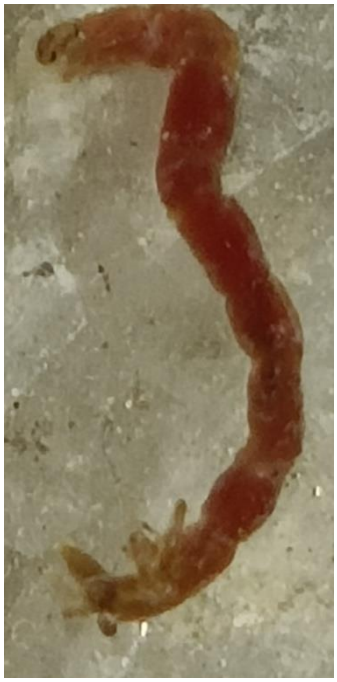

Figure. b 2. Magnified view of *Chironomous circumdatus* showing well segmented body and proper organs before test conditions

Figure c. Larva cultured in an aquarium with spiked sediment

Figure d. Adult Chironomous midge emerging from the IV instar larvae

Figure e. Adult Chironomous midge

Figure f. IV instar larva of control media

2.5. Lead and copper exposure and assessment

Fig.1 shows the set-up or design performed for this experiment. Lead and copper exposure assessment was performed in the consecutive time-period ranges as 24h, 48h and 72h. The assessment of lead and copper toxicity effects on Chironomous were under observation for 3 days. The test concentrations used for lead were 5µg/g (3R), 50µg/g (3R), and 200µg/g (3R) and for copper were 1µg/g (3R), 10µg/g (3R), and 100µg/g (3R) respectively. Here the quantity of salt is mixed with 1 gram of sediment. The summation and distribution of different concentrations of lead in aquariums are 1 metal (lead) × 3 concentrations × 3 replica/ concentration + 1 control = 10 aquariums. For Copper, the summation is the same as 1 metal (copper) × 3 concentrations × 3 replica/ concentration + 1 control = 10 aquariums. The metal salt used for the lead was $Pb(NO_3)_2$ (Qualigens, 99%) and for copper was $CuSO_4$ (Qualigens, 98.5%). The larvae were cultured in aquariums containing sediment, benthic mud, pond water, and dechlorinated tap water. The introduced sediment was extracted and the metal accumulation was performed by mixing lead with the sediment. The metal-accumulated sediment was then re-introduced in the testing aquarium. Likewise, the process was performed for three consecutive concentrations of lead and copper. In each test aquarium, 20 larvae of II instars were introduced, and the remaining larvae were used as control. The temperature was 34 ± 2ºC and the photoperiod was 16L:8D, for both culture and assay. The assay was ended in three days and the concluded parameters showed the body deformation in several ranges, mentum deformation of larvae in which median tooth gap and dissociation were found, and mandible deformation.

2.6. Detergent exposure and assessment

The detergent powder has some severely toxic components to aquatic life such as sodium laureth sulphate, bisphenol A, nonylphenols, etc and it is an active contaminant for water bodies. So, it was selected as a toxic substance to assess the chironomids' survival rate in exposure to detergent.

Three test concentrations were selected as 100mg/g, 200mg/g, and 300mg/g to assess the mortality and survival rate of Chironomous larvae. In the culture medium, the sediment was accumulated with different concentrations of detergent (Domestic usable detergent) for 3 days and the exposure period were 24h, 48h, and 72h respectively. After introducing the detergent-mixed sediment, it was carefully mixed/stirred with the help of a spatula to distribute uniformly in the aquarium. The summation and distribution of different concentrations of detergent in aquariums are 1 salt (detergent) × 3 concentrations × 3 replica/ concentration + 1 control = 10 aquariums. The temperature was 34 ± 2ºC and the photoperiod was 16L:8D, for both culture and assay. The assay was ended in three days and concluded with the following observations as low survival rate, oral deformities, and body deformation in Chironomous larvae.

2.7. Statistical analysis

An accurate measurement and calculation were performed to analyse the survival rate, deformation frequencies of Chironomous larvae. The data of several parameters such as I. Survival rate, II.

Deformation frequencies of mentum, mandible and body in different experimental conditions were analysed using I. Mean ± Standard deviation and II. One way ANOVA. All calculations were performed with the help of Microsoft Excel 2019.

3. Observation Tables

Table 1

Survival rate and deformation frequencies of mentum, mandible and body in lead exposure with their respective standard deviations

S.No.	Test Condition (µg/g)	Survival Rate (%) [b]	Deformation frequency (%)		
			Mentum [c]	Mandibles [d]	Body [e]
1.	Control	71.5 ± 3.5	43.0 ± 1.5	12.0 ± 0.5	21.5 ± 2.0
2.	5µg/g	93.0 ± 0.5	53.0 ± 4.0	17.0 ± 1.5	28.0 ± 3.2
3.	50µg/g	81.5 ± 1.5	46.5 ± 3.5	20.0 ± 2.6	45.0 ± 2.6
4.	200µg/g	66.5 ± 2.0	58.0 ± 4.1	8.0 ± 0.5	17.0 ± 1.5

[a] Statistical analysis was performed by concluding mean and its respective standard deviation of R and the application of One-way ANOVA to conclude the variance; R = no. of replicates

[b] ANOVA results: $F_{3,8} = 3.44$, $P = 0.07$

[c] ANOVA results: $F_{3,8} = 0.44$, $P = 0.72$

[d] ANOVA results: $F_{3,8} = 1.28$, $P = 0.34$

[e] ANOVA results: $F_{3,8} = 3.05$, $P = 0.09$

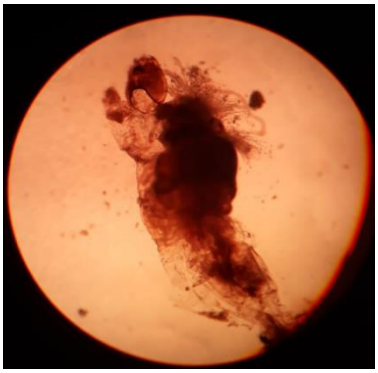

Figure A. Anterior end of larva showing mouthpart deformities (Microscopic view)

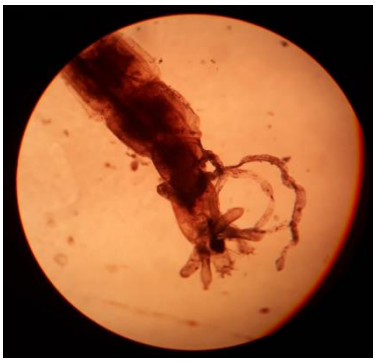

Figure B. Posterior end of larva showing deformation in ventral tubules (Microscopic view)

Table 2

Survival rate and deformation frequency of mentum, mandible, and body on exposure of copper

S.No.	Test Condition (µg/g)	Survival Rate (%) [b]	Deformation frequency (%)		
			Mentum[c]	Mandibles[d]	Body[e]
1.	Control	71.5 ± 3.5	43.0 ± 1.5	12.0 ± 0.5	21.5 ± 2.0
2.	1µg/g	95.0 ± 1.7	56.5 ± 3.0	16.5 ± 1.5	18.0 ± 1.1
3.	10µg/g	76.5 ± 2.5	36.5 ± 3.5	21.5 ± 1.5	28.0 ± 2.5
4.	100µg/g	53.0 ± 3.0	60.0 ± 3.6	15.0 ± 1.0	30.0 ± 3.0

[a]Statistical analysis was performed by concluding mean and its respective standard deviation of R and the application of One-way ANOVA to conclude the variance; R = no. of replicates.

[b] ANOVA results: $F_{3.8} = 4.54$, $P = 0.03$

[c] ANOVA results: $F_{3.8} = 1.57$, $P = 0.27$

[d]ANOVA results: $F_{3.8} = 1.38$, $P = 0.31$

[e]ANOVA results: $F_{3.8} = 0.69$, $P = 0.58$

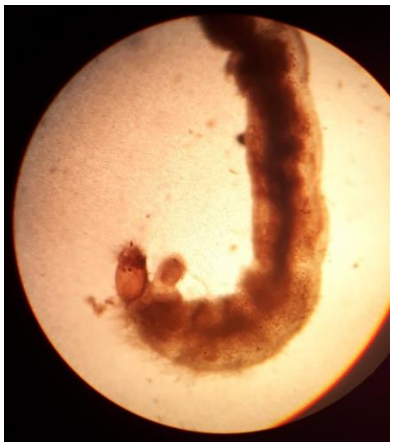

Figure C. Anterior end of larva showing mandible and anterior proleg deformation (Microscopic view)

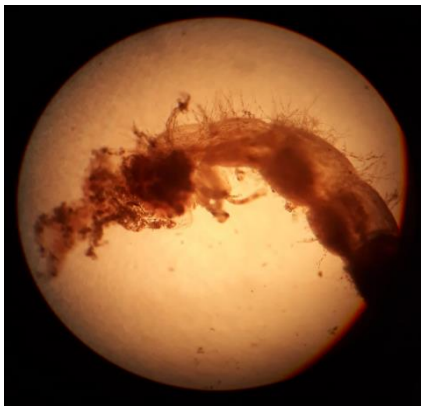

Figure D. Posterior end of larva showing high deformation in ventral tubules, anal tubules and posterior proleg (Microscopic view)

Table 3

Survival rate and deformation frequency of mentum, mandible and body on the exposure of detergent

S.No.	Test Condition (µg/g)	Survival Rate (%)[b]	Deformation frequency (%)		
			Mentum[c]	Mandibles[d]	Body[e]
1.	Control	71.5 ± 3.5	43.0 ± 1.5	12.0 ± 0.5	21.5 ± 2.0
2.	100mg/g	96.5 ± 0.5	38.0 ± 2.0	16.5 ± 0.5	21.5 ± 1.1
3.	200mg/g	73.0 ± 2.0	50.0 ± 3.6	26.5 ± 1.5	55.0 ± 1.0
4.	300mg/g	41.5 ± 2.5	73.0 ± 2.5	58.0 ± 2.0	83.0 ± 1.5

[a] Statistical analysis was performed by concluding the mean and its respective standard deviation of R and the application of One-way ANOVA to conclude the variance; R = no. of replicates

[b] ANOVA results $F_{3,8} = 10.45$, $P = 0.00$

[c] ANOVA results $F_{3,8} = 4.45$, $P = 0.04$

[d] ANOVA results $F_{3,8} = 28.72$, $P = 0.00$

[e] ANOVA results $F_{3,8} = 47.24$, $P = 1.96$

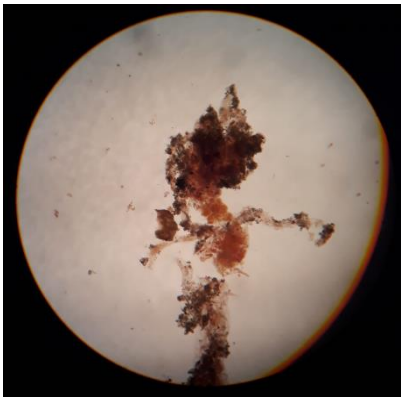

Figure E. Posterior end of larva showing complete deformation of ventral tubules, posterior proleg, anal tubules (Microscopic view)

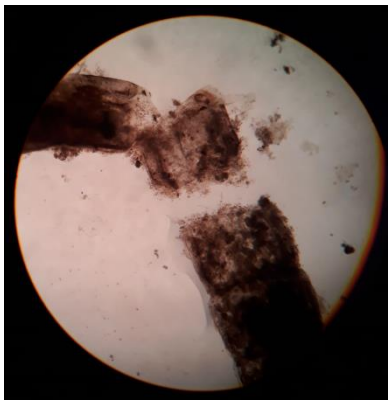

Figure E. Anterior segmented portion of larva showing dissociation in abdominal region (Microscopic view)

4.2 Copper exposure and toxicity

The observations were performed for the test time of 72h (3days). The observations on exposing copper on Chironomids larvae were concluded according to the following parameters – I. Survival rate was relatively varied according to the test concentrations of lead (ranged between 95% - 53%), II. Mentum deformation (ranged between 60% - 36%), III. Mandible deformation (ranged between 21% - 12%), IV. Body deformation (ranged between 30% - 18%). The amount of organic matter (sediment, food) was the same in all test concentrations and control.

Survival rate – In the given Table 2, the survival rate of Chironomids larvae was lowest (53.0 ± 3.0%) at the dose of 100µg/g and highest (95.0 ± 1.7) at the dose of 1µg/g. Even on the several observations performed on control larvae, it was found that the survival rate of control was comparatively low (71.5 ± 3.5%) with 1µg/g copper test condition (One-way ANOVA, P = 0.03)

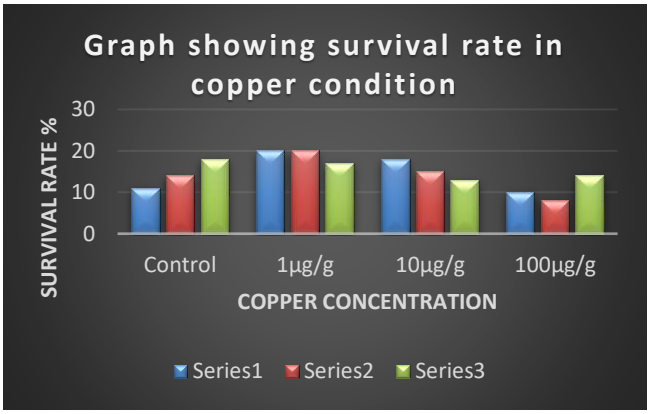

Figure B. Survival rate of Chironomids larvae on exposure of copper

Mentum deformation observation – According to the observations concluded in the given Table 2, the noticed mentum deformation frequency was highest (60.0 ± 3.6%) at the dose of 100µg/g and lowest (36.5 ± 3.5%) at the dose of 10µg/g. The frequency of mentum deformation at the dose of 1µg/g was 56.5 ± 3.0% which was comparatively high with the control condition (43.0 ± 1.5%). Variation in mentum deformation rate was observed in replicates (One-way ANOVA, P = 0.27)

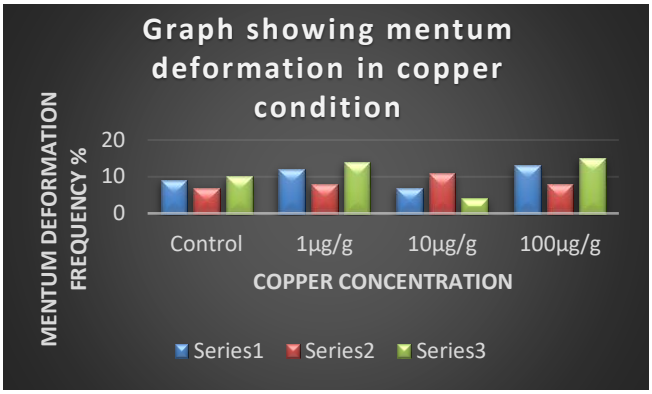

Figure C. Mentum deformation frequency of Chironomids larvae on the exposure of copper

Mandible deformation observation – In reference to the following observation concluded in Table 2, the rate of mandible deformation was comparatively low with the frequency of mentum and body deformation. At the test dose of 10µg/g, the mandible deformation frequency was highest (21.5 ± 1.5%) whether, on the exposure of a test dose of 100µg/g, the deformation frequency was lowest (15.0 ± 1.0%) but comparatively high with the control condition (12.0 ± 0.5%). At the dose of 1µg/g, the mentum deformation rate was 16.5 ± 1.5% (One-way ANOVA, P = 0.31)

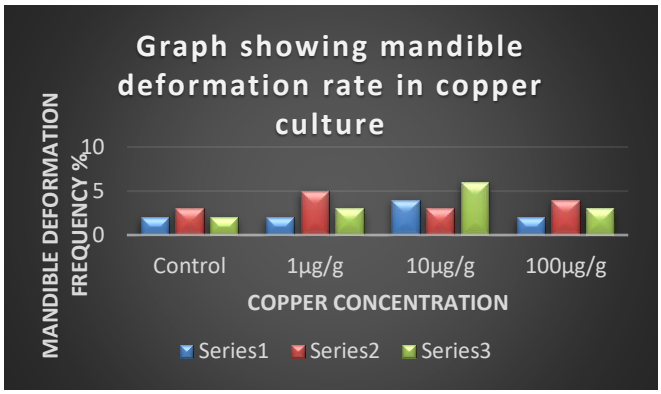

Figure D. Mandible deformation frequency of Chironomids larvae on the exposure of copper

Body deformation frequency – The body deformation frequency was followed in accordance with the dose-response relationship as the deformation rate was increased with the increasing dose of copper. The body deformation frequency was highest (30.0 ± 3.0%) at the dose of 100µg/g and lowest (18.0 ± 1.1%) at the dose of 1µg/g. At the dose of 10µg/g, the deformation frequency was 28.0 ± 2.5% which was comparatively high with the control condition 21.5 ± 2.0%. A slight variation was observed in replicates (One-way ANOVA, P = 0.58)

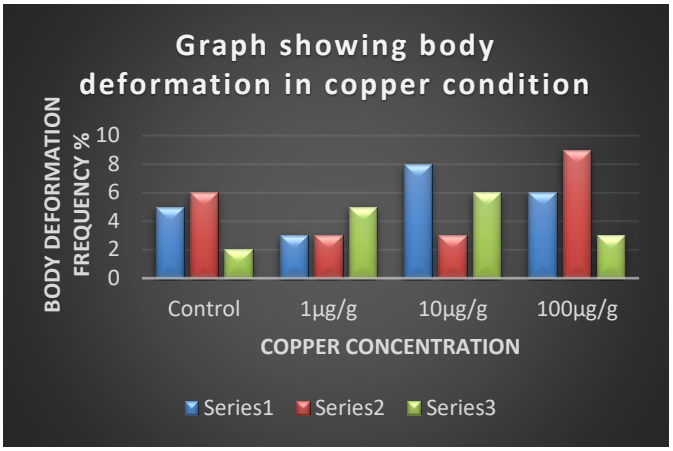

Figure E. Body deformation frequency on the exposure of copper

4.3. Detergent exposure and toxicity

Following parameters were effectively concluded according to our observations such as - I. Survival rate was relatively varied according to the test concentrations of detergent (ranging between 95% - 53%), II. Mentum deformation (ranged between 60% - 36%), III. Mandible deformation (ranged between 21% - 12%), IV. Body deformation (ranged between 30% - 18%). The amount of organic matter (sediment, food) was the same in all test concentrations and control.

Survival rate – A great variation was observed when Chironomids larvae were exposed to different test concentrations of detergent (100mg/g, 200mg/g, 300mg/g). In the given Table 3, when the larvae were exposed to a dose of 100 mg/g, the survival rate was found to be the highest (96.5 ± 0.5%) and it was lowest (41.5 ± 2.5%) at the dose of 300mg/g. The survival rate was moderately comparable (73.0 ± 2.0%) with the control condition (71.5 ± 3.5%) at the dose of 200mg/g with a slight inactivation state. Variation was seen in replicated (One-way ANOVA, P = 0.00)

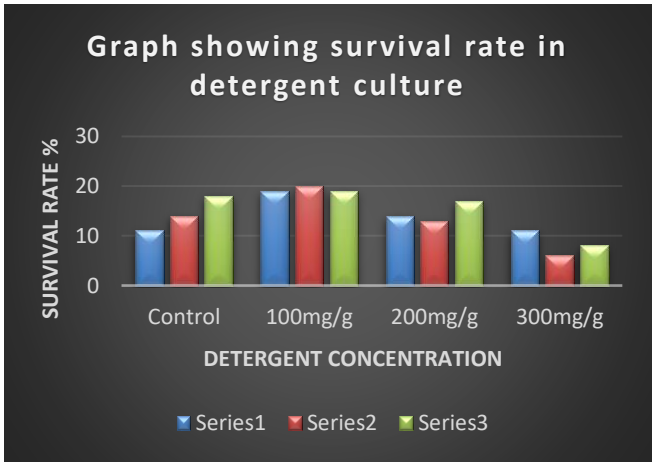

Figure F. Survival rate of Chironomids larvae on the exposure of detergent

Mentum deformation observation – Mentum deformation was found to be varying greatly in different test conditions. In the given Table 3, The frequency of mentum deformation was highest (73.0 ± 2.5%) at the dose of 300mg/g and lowest (38.0 ± 2.0%) at the dose of 100mg/g. At the dose of 200mg/g, the deformation frequency was 38.0 ± 2.0%. The control condition was found to be varied (43.0 ± 1.5%) with

three different test conditions. A dose-response relationship was followed parallelly as the frequency of mentum deformation increases with the increasing dose (One-way ANOVA, P = 0.04).

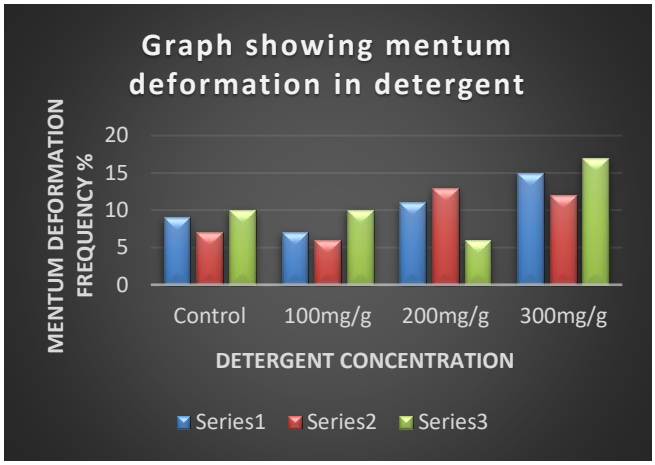

Figure G. Mentum deformation frequency on the exposure of detergent

Mandible deformation observation – According to the observations concluded in Table 3, the rate of mandible deformation was highest (58.0 ± 2.0%) at the dose of 300mg/g and lowest (16.5 ± 0.5%) at the dose of 100mg/g. At the dose of 200mg/g, the frequency of mandible deformation was 26.5 ± 1.5%. The least mandible deformation frequency 12.0 ± 0.5% was noticed in the control condition (One-way ANOVA, P = 0.00). The tendency of mandible deformation was observed as high in the toxicity of detergent.

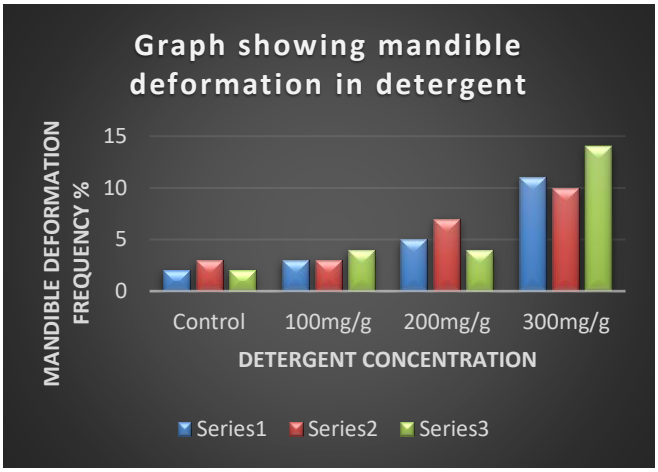

Figure H. Mandible deformation frequency on the exposure of detergent

Body deformation observation – The most affected and deformed part was the larval body by the active ingredients of detergent. A major dissociation was observed in their body at the tissue level. Segmental regions were deprived, and ventral tubules were deformed along with the whole-body tissues. In the given Table 3, the highest frequency of body deformation (83.0 ± 1.5%) was observed at the dose of 300mg/g, and the lowest (21.5 ± 1.1%) was found at the dose of 100mg/g which was comparatively similar with control condition having a slight variation in replicates (21.5 ± 2.0%). At the dose of 200mg/g, the observed body deformation frequency was (55.0 ± 1.0%). The dose-response relationship was shown in all test conditions as the deformation rate increased on increasing dose (One-way ANOVA, P = 1.96)

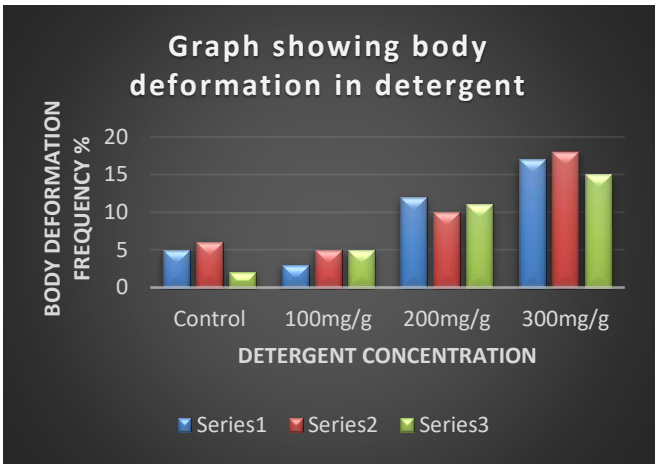

Figure I. Body deformation frequency on the exposure of detergent

5. Discussion

Aquatic life including aquatic insects or any developmental aquatic stage of insects are threatened by many of chemicals which results in the deformations of their body parts or can even be fatal by retarding their survival rate. From these such threatening chemicals, lead, copper and detergent are extremely injurious to these little Chironomids larvae. These metals have adverse effects on freshwater invertebrates [11]. Earlier studies found that copper causes oral deformities in aquatic invertebrates and it also delays adult emergence [12]. It was found that copper was more sensitive to the larval stages while least sensitive to the egg stage [12, 2]. In our experiment, the outcome shows similarity with the result of Kosalwat and Knight (2002) as the highest test dose caused high mentum deformation. The other metal lead is mainly responsible for skeletal deformities [13]. Lead has caused the highest body deformation rate on the dose of 50µg/g which can be called the sublethal concentration or sub-lethal dose for the body deformation rate of Chironomid larvae. In the exposure of detergent, a linear dose-response relationship was shown as the survival rate decreased on increasing concentration and the deformation frequency of mentum, mandible, and body had increased on increasing dose of detergent. The lowest survival rate and highest deformation frequency were observed at the highest dose given (300mg/g). Out of these three-test salt, the highest mortality rate was obtained by detergent salt which is 59%. The rate of mouthpart and body deformation was highest in the exposure of detergent in comparison with lead and copper. All the test larvae were found to be most sensitive in 72 hours i.e., on 3rd day after exposure.

In the first 24 hours after exposure, 90% of the larvae exhibit low activities and low locomotion which can be noted as a weak period. On the next day, after 48 hours, their physiological response was the same but the anatomy deformation was increased slightly. On the 3rd day, after 72 hours of exposure80% of them became completely inactivated and died (data is mentioned in the above table 1,2, and 3) and the remaining 20% were weak and very little activated. Their physiological responses were retarded and their anatomy was majorly deformed including mouthparts and the whole body. The larvae were considered to be dead if they are not reacting to the external natural or artificial stimuli [14]. In our experiment, sediment was chosen to be contaminated for the metal exposure because larvae are supposed to love inhabiting the sediment and benthic mud and also, they extract some of the nutrition from sediment. Sediment was considered as a substrate; it also exhibits an adsorption mechanism for toxicants and impacts harmful effects on the epidermis of larvae [15].

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