International Journal of Engineering, Science and Mathematics
Vol. 7 Special Issue 4(3), April 2018,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijmra.us, Email: editorijmie@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

# The non-biting midge species, *Chironomus striatipennis* Kieffer (Diptera: Chironomidae) as a potent bio-indicator in water quality assessment

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**Abstract**

The non-biting midge species occupy several aquatic habitat including lakes, ponds, rivers and man-made habitats e.g. sewage and water treatment plants etc. Majority of the species exhibits tolerance to pollution. Several heavily polluted aquatic habitats encompassing industrial establishments in West Bengal were explored for pollution considering the presence of heavy metals as Pb, Cd, As and Cu. All the habitats were inhabited by *Chironomus striatipennis* Kieffer. The fourth instar larvae of the species collected from the sites were investigated for their morphological features and salivary gland polytene chromosomes. The studies indicated that deformities appeared in several phenotypic characters and polytene chromosome of the larvae. These abnormal features of *Chironomus* larvae were in response to the heavy metal pollution in their habitats. *C. Striatipennis* larvae were reared in the artificially created polluted laboratory environment. Investigation revealed that the appearance of deformities of various structure and the aberrations along polytene chromosome arms. Thus the response of *C. striatipennis* Kieffer larvae in polluted habitats appeared to be in consistency with the response of the larvae of the species in the laboratory. Therefore, the larvae of the species of Chironomus acted as potent bio-indicator to detect the presence of heavy metals in the environment.



***Keywords:***

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*Chironomus striatipennis*;
fourth instar larvae;
polytene chromosome;
heavy metal toxicity;
bio-indicator.

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## 1. Introduction (12pt)

The Chironomids bear resemblance to mosquitoes and like mosquitoes they pass their immature life stages in aquatic bodies [1]. These dipterous flies are quite harmless and holometabolous in nature. From their eggs the larvae develop in water as minute quite colourless worm like creatures those are called as first instar stage. With the progression through the advanced stages they appear to be coloured and the larvae developed from the *Chironomus* species mostly appear reddish in colour. There are four instar stages of the larval development before they enter into pupation. At the larval stages they develop tubes to dwell and the tubes are made up of sediments and mud under water [1]-[3]. This tube dwelling habit have made them adaptive amongst many predatory organisms in water as in bared

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condition they may easily be prominent because of their red colouration. These aquatic larvae are found to develop in almost all habitats irrespective of their pollution level. In this consideration they may be considered highly tolerant to toxicity and they combat with the toxicity in the habitats by some mechanism. The Chironomid larvae develop characteristic polytene chromosomes in many of their somatic cells including the cells of the salivary glands. These polytene giant chromosomes with their band and interbands may be considered as cytological manifestation of the genetic makeup of the flies [4]. In consideration to this we have striven to see whether a Chironomid larva responds to a change in their habitat and we have witnessed such response with respect to change in heavy metal concentration in the habitats. Hence, a role of the Chironomid larvae to act as bio-indicator in polluted water bodies could be ascertained. The present investigation is related to species *Chironomus stratipennis* Kieffer which is commonly available in the aquatic habitats of West Bengal and the role of this species to act as a bio-indicator could be revealed with the help of some laboratory investigations.

**2. Research Method (12pt)**

Various heavily polluted sites of West Bengal due to industrial establishments were selected for the collection of samples. The egg masses, larvae and the adult flies were collected from the selected zones for their study in the laboratory. Along with these the sediments and water samples from the habitats were also collected to measure their heavy metal contents. The heavy metals in the samples were detected by Atomic Absorption Spectrophotometer (AAS). The sites selected for collection of samples were Haldia, Kolaghat, Dhapa of Kolkata (Dumping Ground) and Dankuni. The industrial effluents as well as wastes from the urban localities would mix with the water bodies located in these areas. The larvae of the species collected were brought to the laboratory for study of their morphological features and polytene chromosome organization. The normal karyological features of *C. striatipennis* already studied in our laboratory were compared with the polytene chromosome features of the larvae collected from polluted zones. For the preparation of the polytene chromosomes from the salivary glands the penultimate fourth instar larvae were sacrificed to obtain their salivary glands. The gland tissues were fixed with 1:3 aceto-methanol for 5 minutes. The fixed tissue materials were stained with 2% aceto-orcein and then squashed to obtain polytene chromosome spreads over the glass slides.

The larvae of *C. striatipennis* Kieffer reared in the laboratory were treated with heavy metals as Pb, As, Cu and Cd in the laboratory environment. For the purpose of such induction test, culture beds were prepared in the polythene tray of size of 12 x 10 inches with autoclaved clay and sand particles. Different culture trays were mixed with different doses of the salts of heavy metals under consideration. Each of the culture trays was then added with sterilized pond water to fulfil the requirement of inorganic salts. The culture trays were continuously aerated with the maintenance of required photoperiod with the help of 25 watt and 5 watt lamps. For the maintenance of the Chironomid culture, regular addition of nutrients, tetra bits and cellulose powder were supplied in the culture tray. The doses of the heavy metals considered for their addition with soil ranged from 1- 20mg/ kg of soil. The treated larvae were studied for their morphological features concerning the structure of the mentum, pecten epipharyngis and structure of the ventral tubules. At the same time the larvae were also studied for their polytene chromosome features. The method for preparation of their salivary gland polytene chromosomes was as stated before.

**3. Results and Analysis**

The sediment and water samples collected from the sampling sites were ascertained for their heavy metal content and result of the study on concentration of the heavy metals : As, Cd, Pb and Cu, may be shown in the following table (Table 1). The Chironomid larvae were found to survive in polluted water bodies without any difficulty, but they were found to exhibit some altered phenotypic features which could be correlated to the aberrant organization of the salivary gland polytene chromosomes. Normally each of the salivary gland cells showed the presence of four polytene chromosomes (Fig. 9) of which the fourth one was the smallest and being acrocentric in configuration represented the G arm. On the other hand the other three chromosomes appeared more or less metacentric but differing in their length. The chromosomes were characterized to be a combination of BF, CD and AE arms [5], [6] and these combinations were marked as chromosome I, II and III according to their decreasing order of length and the chromosomes were mapped by following standard mapping techniques [7]. On the contrary G arm was designated as chromosome IV (Fig. 9). Of many phenotypic features the structure of mentum (Figs. 2, 3), structure of teeth on pecten epipharyngis and ventral tubules were taken into consideration for study. In normal condition the mentum at the centre contained a trifid tooth with its medial shaft longer and sharply pointed (Fig. 3). The pecten epipharyngis was also found to be set with regularly arranged teeth having uniform height (Fig. 4). On the other hand the ventral tubules were paired structures curved distally and they were moderately long. The larvae collected from the polluted areas indicated deformities in the larval body structures (Fig. 1). The mentum which was found to have a symmetrical organization (Fig. 3) in normal larva showed an asymmetric organization (Fig. 5 - 7) in the larva developed under polluted condition in the habitats. The pecten epipharyngis having several teeth in regular arrangement (Fig. 4) in normal larva exhibited an irregular orientation of the teeth (Fig. 8) in the larvae obtained from polluted areas. The ventral tubules in the larvae present along the posterior abdominal segments underwent shortening in the affected larvae. The deformities observed in the larvae may be shown in the following table (Table 2). Along with these, the polytene chromosomes exhibited aberrant organization comprising heterozygous inversion along the chromosome arms, bleb formation at certain region of the arm and asynapsis (Figs. 10 - 13). Asynapsis again appeared to be of different forms, such as short terminal, intercalary loss (Fig. 13e) and long terminal type (Figs. 13a and b). Pompon configuration (Fig. 13d) i.e. only the chromatin mass devoid of any characteristic bands and interbands was also encountered [8]. Induction tests in the laboratory exhibited that not only the morphological deformities appeared in the larvae but also the aberrant forms of polytene chromosomes were generated in the treated larvae. In fact the treated larvae in the laboratory were more affected than the naturally occurring larvae. Chromosome wise analysis of the frequency of occurrence of aberrant forms with arsenic oxide [As2O3] indicated a picture as stated below (Table 2).

**Table 1. Heavy metal concentration at different collection sites**

| **Collection sites** | **Heavy metal concentration** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **As(mg/L)** | | **Cd(mg/L)** | | **Cu(mg/L)** | | **Pb(mg/L)** | |
| | **Wat.** | **Sed.** | **Wat.** | **Sed.** | **Wat.** | **Sed.** | **Wat.** | **Sed.** |
| **Dhapa(Kolkata)** | <.005 | 2.04 | <.005 | 1.37 | .03 | 103.25 | 0.008 | 157.36 |
| **Kolaghat (TPS )** | <.005 | 1.56 | <.005 | <1.0 | <.01 | 26.37 | 0.008 | 109.93 |
| **Haldia** | <.005 | 3.19 | <.005 | <1.0 | <.01 | 32.7 | 0.008 | 226.43 |
| **Dankuni** | BDL | BDL | BDL | 0.0018 | BDL | 0.1155 | BDL | BDL |

**Wat.=Water, Sed. = Sediment, TPS = Thermal Power Station, BDL = Below Detection Limit**

**Table 2. Characterization of the morphological deformities in the larvae of C. striatipennis collected from polluted water bodies**

| Sl. No. | Structure in the larval body | Nature of deformities |
|---|---|---|
| 1 | Mentum | i) Asymmetry of the lateral shaft of the medial tooth<br>ii) Stubby medial tooth & reduced spines in the tooth |
| 2 | Pecten epipharyngis | i) Irregular teeth arrangement<br>ii) Sharpening of the teeth |
| 3 | Ventral tubule | Shortening of the tubule length |
| | | |

**4.**

**Table 3. Frequency of occurrence of polymorphic polytene chromosomes after As treatment**

| Aberrant chromosomes | 1st | Freq. | 2nd | Freq. | 3 rd | Freq. | 4th | Freq. |
|---|---|---|---|---|---|---|---|---|
| **Dose(mg/kg)** | | | | | | | | |
| 2 mg | + | 0.025-0.05 | + | 0.02 5 | - | > .025 | ++ | 0.85- 1.0 |
| 4 mg | - | 00 | - | 00 | - | 00 | + | 1.0 |
| 6 mg | + | 0.5 | + | 0.5 | + | 0.5-0.625 | + | 0.75 |
| 8mg | + | 0.5 | + | 0.5 | + | 0.625-1.0 | +/- | - |

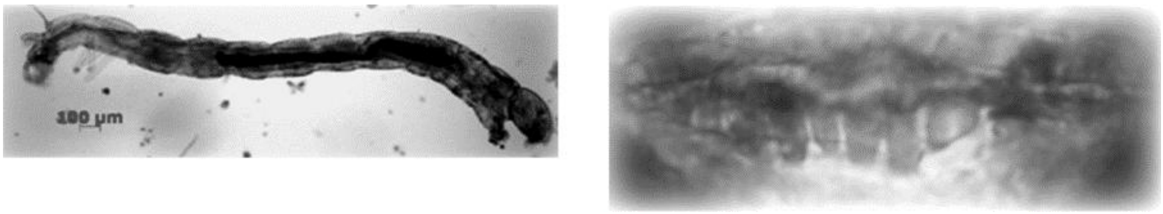
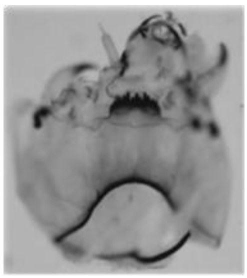
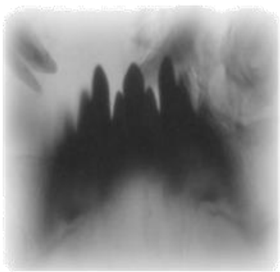


Figure 1. Fourth instar larva of *C. striatipennis* Kieffer

(2) (3)
Figure 2 and 3. (2) Head capsule showing position of mentum in 4th instar larva (3) Mentum normal structure showing medial tooth

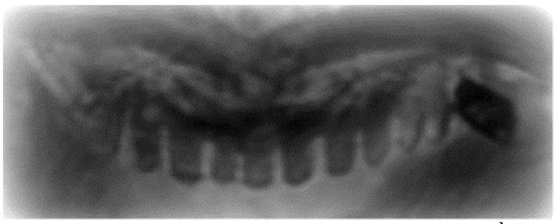


Figure 4. Structure of pecten epipharyngis in $4^{th}$ instar larva

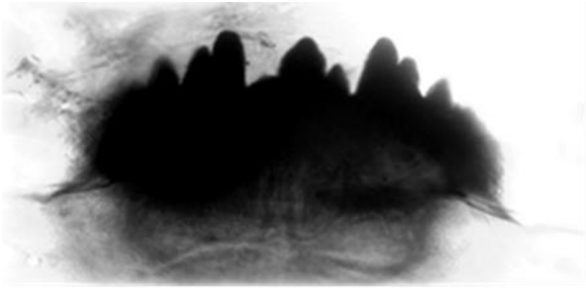


Figure 5. Defective mentum with medial tooth showing asymmetrical lateral shafts

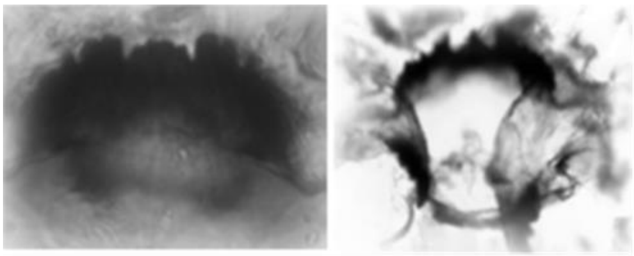


Figure 6 and 7. (6) Mentum with stubby tooth structure and medial tooth without spiny shafts (7) Highly reduced teeth in the mentum

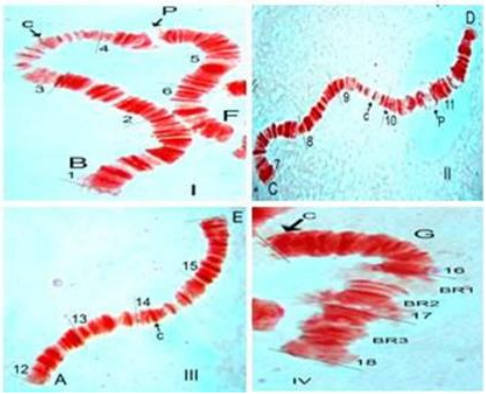



Figure 9. Four polytene chromosomes in the salivary gland cell of *C. striatipennis* Kieffer. Arrows with C and P indicate centromeric positions and puffs respectively in the chromosomes. The 4th chromosome in acrocentric called G arm and the other three chromosomes are marked as I, II and II according to their decreasing order of length. Combination of arms has been shown in the chromosomes

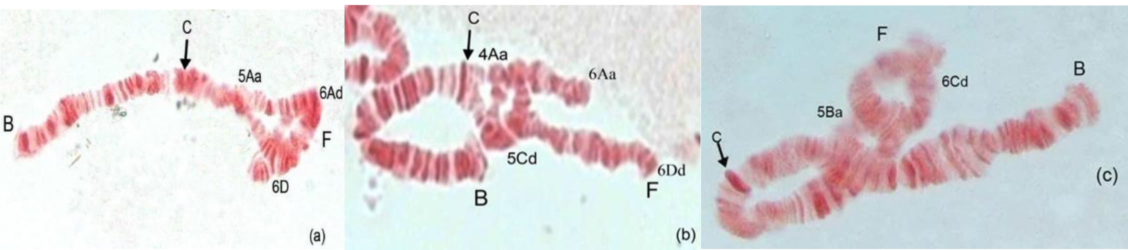



Figure 10. (a) Chromosome I with heterozygous inversion along arm F (b) Heterozygous inversion and loss with cross bridge formation in arm F (c) Looped segment with deficiency at the intercalary position along F arm

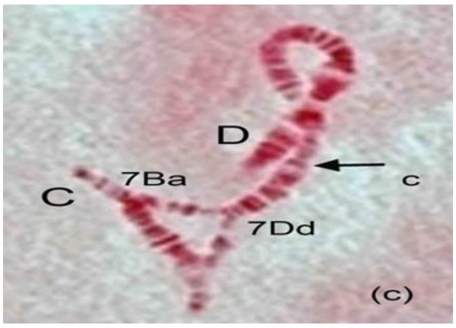



Figure 11.(c) Formation of a terminal hat in arm C of chromosome II

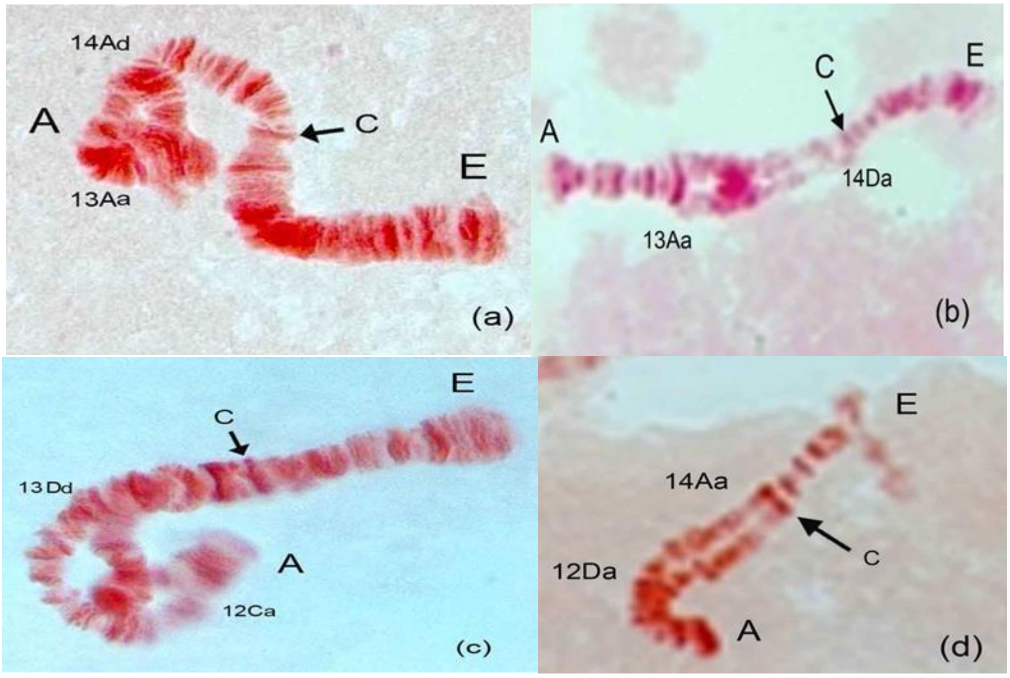



Figure 12. (a) A terminal loop in arm A of Chromosome III (b) Arm A of Chromosome III is affected showing asynapsis with cross-bridge formation (c) Heterozygous inversion in arm A of Chromosome III (d) Asynapsis along arm A of Chromosome III

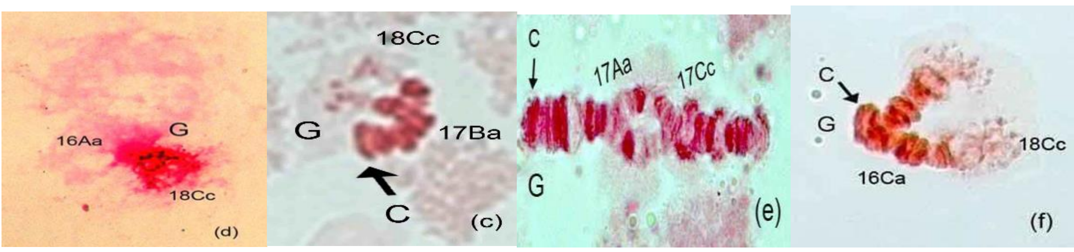



Figure 13. (c) Terminal asynapsis in arm G (d) Pompon configuration (e) Intercalary loss in arm G (f) Terminal asynapsis in arm G

The Chironomid flies are considered to be good bio-indicator of environmental pollution and the Chironomid larvae have been the EPA and OECD approved test organism for water quality assessment. The present investigation has revealed their potentiality to assess the water and sediment quality in consideration to heavy metal concentration. Many investigators throughout the globe observed a relation of deformities of the polytene chromosomes in the Chironomid larvae and pollution in aquatic bodies with heavy metals [9]-[18]. A number of investigators also reported the appearance of morphological deformities in the larvae of Chironomid in polluted water [19]-[21]. In this consideration our findings regarding phenotypic deformities of the larvae of *C. striatipennis* in polluted water bodies and polytene chromosome aberrations appeared to be consistent with the previous reports on the effects of heavy metals on the larval development of Chironomids. The habitats in the present study were also highly polluted with heavy metals. A comparison of the heavy metal contents in the habitats under consideration and the reference limit of the respective heavy metal may be presented with the help of a graphical representation (Fig. 14).The guideline values for the heavy metals namely As, Cd, Cu and Pb are known to be 0.01, 0.003, 2 and 0.01 mg/L respectively.

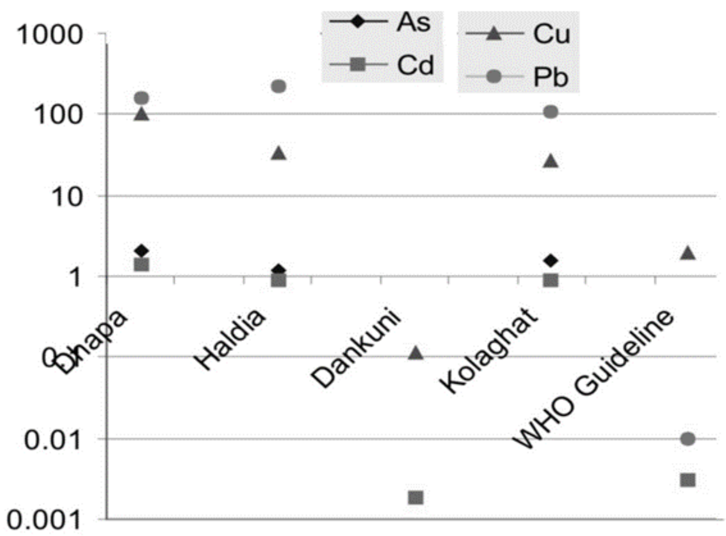



Figure 14. Logarithmic graph showing relative conc. of heavy metals; As, Cd, Cu and Pb in different habitats with reference to WHO guidelines

It is well evinced from the above comparative display of the heavy metal contents of the habitats in this investigation and WHO guidelines for trace metals that the habitats were highly polluted. Still the species of Chironomus, *C. striatipennis* thrived well with perpetuation of their generations. It is still shrouded in mystery that how the minute organisms manage to withstand the intense pressure of remaining in the unsolicited environment with so many heavy metals present in such higher concentration. The morphological deformities were some of the detrimental effects under the influence of the heavy metal pollution. Further, it may be conjectured that the phenotypic expressions of the deformed structures must be the result due an impact on the genetic makeup of the flies in polluted water bodies. Such a prediction is supported by the occurrence of chromosomal aberrations as manifested through their polytene chromosome organization. Incidence of chromosomal aberrations because of the influence of heavy metal toxicity was also reported by many investigators before [14]. Therefore, *Chironomus stratipennis* Kieffer which is commonly available in the aquatic habitats of West Bengal acted as a potent bio-indicator [22] to detect the presence of heavy metals in the environment.

**Conclusion**

Thus an inference may be drawn on the basis of these findings that the larvae of the species, *C. striatipennis* Kieffer are playing pivotal role as potent bio-indicator to detect the presence of heavy metals in the polluted aquatic habitats thereby assessing the quality of water of those aquatic bodies.

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