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Sensitivity of the Native *Chironomus* Species in Monitoring of Riverine Ecosystems in the Catchments of Lake Victoria Drainage Basin, Kenya

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Abstract

Globally, anthropogenic activities continuous to pose wide spread pollution into the aquatic ecosystems such as rivers. This study set out to assess the sensitivity of Chironomus species to justify their use in monitoring of riverine ecosystems in the Lake Victoria Basin, Kenya. Chironomid midges sampled from upstream and downstream of paper mill factory in the River Nzoia and sugar cane factory in the River Mbogo for toxicity tests in the laboratory. In the laboratory, midges were exposed to different dilutions of paper-mill factory effluents and sugar cane factory effluents. Results for all the tests were evaluated for variability among treatment effects and control using analysis of variance (ANOVA). There were significant effects ($p<0.05$) of effluents on sensitivity. The study, therefore, concluded that Chironomus species are sensitive to pollutants emanating from sugar cane processing and paper mill effluents, hence can be used as test organism in monitoring the health of riverine ecosystems in LVB. It was recommended that on-field toxicity tests for the Chironomus species be done, studies on expanded toxicity testing monitoring to spatially and temporally characterize toxic conditions not only on the catchments of L. Victoria but on the entire aquatic ecosystems in the country be carried out, expanded toxicity testing and chemical analysis be done on other organisms and lastly studies to be carried out in establishing whether Chironomus species at contaminated sites have a tolerance that is genetically passed on or acquired.

Key Words: Bioindicators, Chironomus Species, Effluents, Sensitivity and Ecotoxicology

Introduction

River pollution is common in the present world and has caused severe environmental consequence (Castillo et al., 2000). In Kenya, most important contamination sources are domestic and industrial wastewaters, urban and agricultural runoff (Osano et al., 2003; Raburu et al., 2009a). As a management strategy, many regulatory efforts have been geared towards chemical analysis to identify the level of various pollutants in streams and rivers (Getenga et al., 2004; Mwamburi, 2003) and establishing discharge standard that maintain established threshold levels (EMCA, 2006). Chemical methodologies, (Kohler et al., 2000) are essential for regulatory purposes but mostly lack the ability to assess toxicity, bioavailability and potential antagonistic/synergistic effects of pollutants on aquatic ecosystems (Vangheluwe et al., 1999). The limitations of chemical analysis are particularly apparent when the chemical nature of pollutants is unknown, in which case an extensive array of instrumentation needs to be used, often in a time consuming and costly manner (Liess et al., 1999).

Because of the above mentioned shortcomings with chemical analyses, recent advances in Kenya to assess and monitor pollution on rivers have focused on the use of biological criteria *(*Masese et al., 2009b; Raburu and Masese, 2010; Raburu et al., 2009a). However, a prerequisite for the development of useful biological indicator systems is that different species should be ranked in

order of their sensitivity to the stress parameter of interest (Wogram and Liess, 2001). In Kenya, there is no information about macroinvertebrate sensitivities. Such sensitivities can be obtained using different contaminants, e.g., effluents, inorganic salts and pesticides. Hence the aim of this study was to determine the sensitivity of *Chironomus* species to industrial effluents from pulp and paper mill and sugarcane processing.

Materials and Methods

Field Sampling

Chironomus species for the tests in the laboratory were sampled on two locations in the Rivers Nzoia and Mbogo, a tributary joining R. Nyando within the LVB, i.e. polluted site (C for R. Nzoia and D for R. Mbogo) and pristine site (R for R. Nzoia and S for R. Mbogo) (reference site). Effluent contamination in R. Nzoia comes from Webuye kraft paper factory whereas R. Mbogo receives effluent contaminants from Chemelil Sugar factory. Reference sites were located upstream in the forest from the two factories whereas the polluted sites were 2 km downstream from the point of the factories. The larval sampling occurred in sediment banks using a scope net.

Collection of Effluents and Preparation. Liquid effluent samples from the two factories were sampled at a point between the final treatment and the discharge outfall. Samples were transported to the laboratory and if not used, were kept at 0-4^{o}C to inhibit microbial degradation, chemical transformations, and loss of highly volatile toxic substances. However, samples once collected were used within the 36-h period (USEPA, 2002).

Laboratory acclimatization. Larvae were transported to the laboratory and acclimatized following established protocols (Figure 3.2) (Fonseca and Rocha, 2004; Santos et al., 2007). Dechlorinated water was used for toxicity tests. The midges were fed on chick mash and a 12:12, light: dark photoperiod regime was maintained. The water temperature of the aquaria was maintained at 25±1^{o}C. All the tests were done under static non-renewal acute toxicity test.

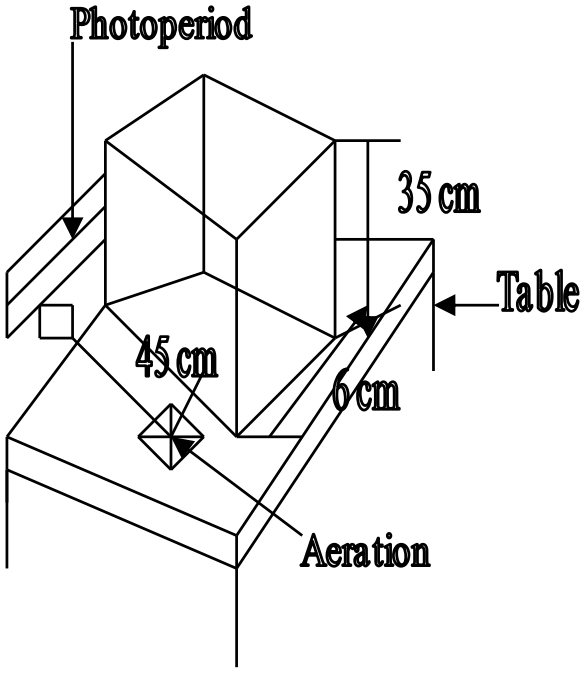


Figure 1. General view of the aquarium that was used for maintaining *Chironomus* species in the laboratory (Source: Author, 2015)

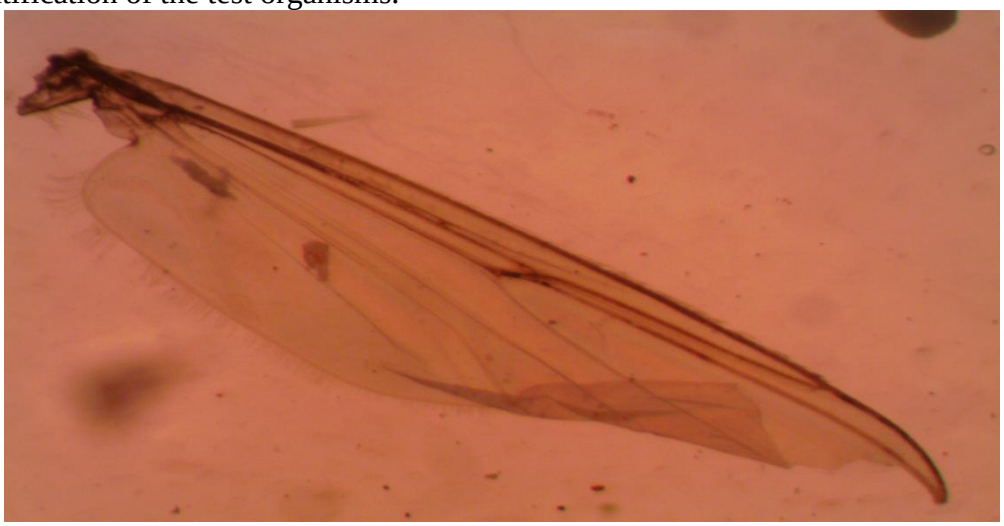
Identification of the chironomids. In order to ascertain that the organisms sampled belonged to the *Chironomus* species, the midges were identified to the lowest level possible using identification keys (Epler, 1995). Larval specimens stored in 70% ethanol were observed under a dissecting microscope to ascertain their physical features before the head capsule was removed for further investigations (Plate 3.1 below).

X5 magnification

Plate 1. Fourth instar Chironomid larvae used in this study (Photo by Kobingi Nyakeya, 7th May, 2014)

The head capsule of each individual specimen was carefully removed while observed under a dissecting microscope (GallenKamp) using a needle and a sharp thin metal plate. The head capsule was then put in a 10% potassium hydroxide (KOH) for 6-24 hours to remove the soft obscuring tissues. It was then mounted on the slide using Euparal mountant ventrally. The mentum teeth were examined and counted as well as the antennae segments. This is because such features alone can differentiate one group from another.

The wings of the reared Chironomid adults were also removed while observed under the dissecting microscope and mounted on the slides using Euparal mountant and observed under the compound microscope (plate 3.2) with different ranges of magnification in order to be used in the identification of the test organisms.



X40 magnification

Plate 2. A wing of an adult *Chironomus* species (Photo by Kobingi Nyakeya, 7th May, 2014)

Bioassays/ Bio Assessment

Experimental design. Five industrial effluent sample concentrations plus control for each test with four replicates was used for the test of the Chironomus midges. Ten live midges were exposed to each industrial effluent sample concentration (Jensen, 1972). Then serial dilutions of the effluent for each test was done with a factor of 0.5 i.e. 100%, 50%, 25%, 12.5% and lastly 6.25% (USEPA, 2002). Ten midges in all the experiments were added to 0.5 l beakers, containing the appropriate serial effluent dilutions, with approximately 1 cm layer of sterilized sand and 1.0 ml of food suspension. A temperature of 25±1^{o}C and a 12:12, light: dark regime was maintained. The experiment ran for 24 hours.

Sensitivity. To test for sensitivity the proportion data was derived using the following formula:

Sensitivity = No. of true positives/ (No. of true positives) + (No. of false negatives)

Data Analysis

Results for the effluent toxicity tests were presented as means (±Standard Error of Mean, SEM) derived from sensitivity. Data for the tests was first transformed by the Probit transformation to stabilize the variance and satisfy the normality requirement before analysis. Two way ANOVA for the tests of the *Chironomus* species midges was run with the station and concentration as the main factors with the station x concentration interaction effect and where there was no interaction then 1-way ANOVA was run with the main individual factors only (i.e. effluent, station and concentration). *Post hoc* Tukey test was then performed to identify means of concentrations of the different test parameters for the *Chironomus* midges differing from one another. One-Way ANOVA was used to determine significant difference between the control and the effluent concentrations. All analysis was done in Sigma Plot and the significant differences were inferred at α=0.05 level.

Results

Species Identified in the Study

Four species were identified and classified in this study. They included *Chironomus decorus, Chironomus riparius, Chironomus stigmaterus* and *Goeldichironomus c.f. natans*.

Sensitivity

Station S of R. Mbogo which acted as the reference point in this river, recorded the highest percent sensitivity level (64.0±9%) of the *Chironomus* species exposed to sugar cane effluent, followed by station R (reference point) of R. Nzoia (54.0±8%) as illustrated in Figure 4.1 below.

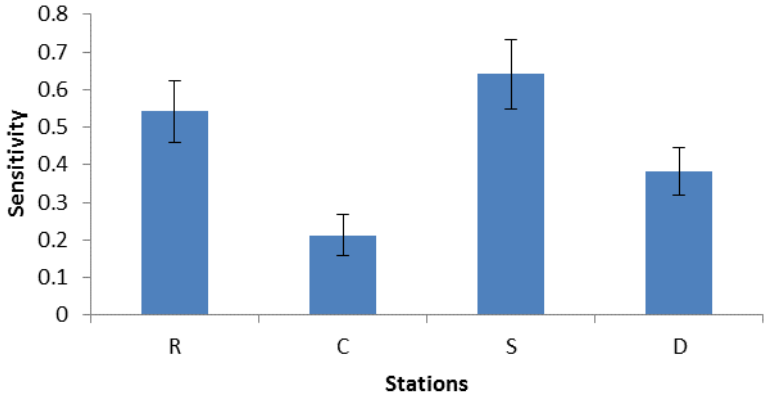


Figure 2. Mean (± SEM) percentages of the sensitivity of the *Chironomus* species from the four stations of rivers Nzoia and Mbogo

The least percent sensitivity (20.0±5%) was noted in station C of R. Nzoia in which the midges were exposed to paper and pulp effluent. Station D of R. Mbogo on the other hand recorded (38.0±6%) percent sensitivity.

The sensitivity of the *Chironomus* species in both the effluents increased steadily with an increase in the level of effluent concentration. However, the sensitivity of the midges exposed to sugar cane effluents showed a small decrease at 25% concentration before indicating an upward trend again. In both the effluents, sensitivity of the midges is seen to be constant once in 50% and 100% (Figure 4.2).

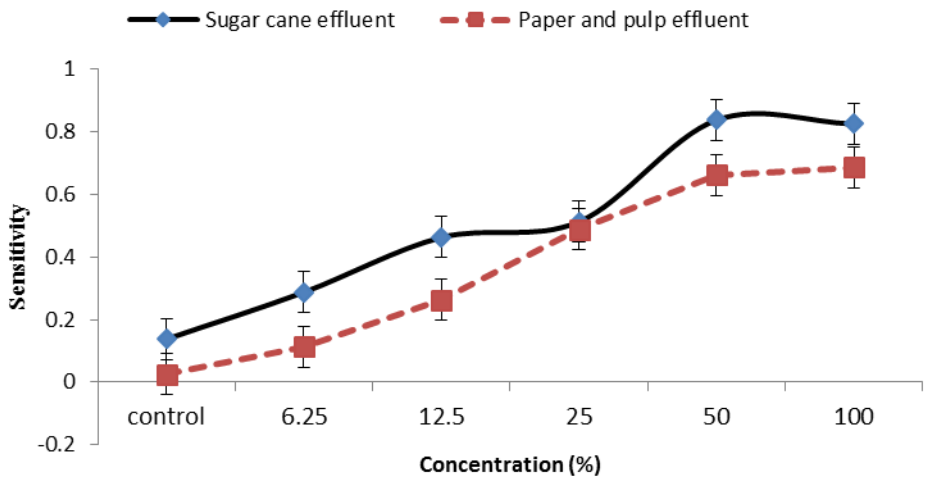


Figure 3. Mean (± SEM) percentages of the sensitivity of the *Chironomus* species under different levels of effluent concentrations

Two-Way ANOVA (Table 4.1) showed that sensitivity of the *Chironomus* species that were sampled from the two rivers and exposed to both sugar cane; and paper and pulp effluents under different dilutions in the laboratory differed significantly between effluents (F=52.54, p=0.001); stations (F=111.474, p=0.001); concentrations (F=158.453, p=0.001) and among stations combined with concentrations (F=6.768, p=0.001). Analysis of the effluents combined with the concentrations did not have any effect on the sensitivity of the exposed midges.

Table 1. Summary of Two-Way ANOVA showing variation for sensitivity, with effluent, station, concentration, effluent x concentration and station x concentration *=p<0.05

Source of Variation	DF	SS	MS	F	P
Sensitivity					
Effluent	1	0.45375	0.45375	52.54	<0.001*
Station	3	2.648	0.883	111.474	<0.001*
Concentration	5	6.272	1.254	158.453	<0.001*
Effluent x Concentration	5	0.08	0.016	1.85	0.115
Station x Concentration	15	0.804	0.0536	6.768	<0.001*
Residual	66	0.57	0.00792		
Total	95	10.293	0.108		

*Denotes significant difference

Relationship among the concentrations. Post hoc Tukey test for sensitivity showed a significant difference between the Chironomid midges in the control and all other effluent concentrations (F=21, p=0.001).

Table 2. *Post hoc* Tukey test among means of different concentrations for sensitivity, specificity, LC50, deformity and emergence (p<0.05)

Concentration (%)	Sensitivity
0	0.08±0.025a
6.25	0.2±0.048b
12.5	0.4±0.048c
25	0.5±0.048d
50	0.75±0.05e
100	0.76±0.028e

A,b,c,d,e shows an effect

Discussion

The results of this study revealed that chironomid midges sampled at upstream stations of R. Nzoia and R. Mbogo were more sensitive to sugarcane and paper pulp effluents in the laboratory as compared with those sampled from exposed sites downstream. This is most probably due to the fact that downstream stations receiving effluents from the factories may have contributed to adaptation changes in the parental chironomids that may have passed the environmental traits (non-genetic) to their off springs (Fernandez et al., 2011; Bonduriansky et al., 2011). It is also

argued that adaptation of organisms in an environment is likely to play an important role in allowing populations to persist through periods of rapid environmental change (Fernandez et al., 2011).

Dose response relationship of the chironomids under different levels of dilutions indicated that sensitivity increased with an increase in the effluent concentration. However, this did not matter once it reached a level of 50% meaning any further increase of the effluent concentration would not have a lethal effect. This could be explained by the fact that the chironomids in the sampling points had adapted to the changing environment in terms of seasons whereby during the rainy season effluents were diluted hence its strength reduced unlike the dry spell when its lethality was enhanced. Such an occurrence therefore, is repeated in a predictable way making the *Chironomus* species thrive through adaptation.

The *Post hoc* test that indicated a significant difference between the control of the measured parameters and the different treatments is an indication that paper pulp and sugarcane effluents had adverse effects on the *Chironomus* species. It may also be explained by the fact that *Chironomus* species are sensitive to both effluents hence can be relied upon in testing for pollution in any aquatic ecosystem.

The significant differences noted on the sensitivity of *Chironomus* species and the different levels of paper pulp and sugar cane effluents were as a result of the increased levels of concentrations causing more adverse effects on the organisms under test.

Conclusions

From the results of this study, sensitivity of the *Chironomus* species exposed to the two effluents increased with an increase in the effluent concentration. Sensitivity also increased downstream in regard to the sampling stations of the Chironomid midges under study. It is also, concluded that *Chironomus* species are sensitive to pollutants emanating from sugar cane processing and paper mill effluents, hence can be used as test organism in monitoring the health of riverine ecosystems in LVB.Based on the findings of the study, the following were the recommendations:

1. There is need to carry out chemical analysis of sugar cane and paper pulp effluents in order to identify the responsible toxicants for the adverse effects observed on the *Chironomus* species.
2. There is need to carry out on-field toxicity tests for the *Chironomus* species.
3. Expanded toxicity testing and chemical analysis to be done on other organisms.
4. Studies should be conducted on expanded toxicity testing monitoring to spatially and temporally characterize toxic conditions not only on the catchments of L. Victoria but on the entire aquatic ecosystems in the country.
5. Studies to be carried out in establishing whether *Chironomus* species at contaminated sites have a tolerance that is genetically passed on or acquired

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