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Buccal deformities in chironomid larvae (Diptera: Chironomidae) as an indicator Risk Assessment and Anthropogenic stresses of pollution in fresh water of the River Nile, Sohag Governorate, Egypt

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Abstract

Mouth parts deformities in chironomid larvae are considered as indicators of environmental stress in aquatic systems caused by water and sediment pollution such as heavy metals, pesticides as well as organic contamination. Fourth instar chironomid larvae were collected from two sites (SI and SII) located in the River Nile, Sohag Governorate, Egypt. The chironomid community of the two SI and SII was dominated by *Dicrotendipes nilophilus* (Diptera: Chironomidae). The percentage numbers of chironomid larvae with deformed mouthparts were 29% (SI) and 14% (SII). Although there were no significant differences in the sediment metal concentrations between the two sites, significant differences were recorded among the metal concentrations of iron (Fe), zinc (Zn) and copper (Cu). Moreover, the present results revealed that some physicochemical parameters played an important role in increasing the mouthparts abnormalities.

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Introduction

Results from many field studies strongly indicate a relationship between increased incidence and morphological deformities and toxic sediment stress (Hamilton and Saether, 1971; Köhn Frank, 1980; Warwick, 1980 a,b; Warwick, 1985; Janssens de Bisthvenand Van Spieybrok, 1994; Nazarova *et al.*, 2001; Nazarova *et al.*, 2013).

The larvae of most chironomid larvae species live on or in sediments; where they feed on organic matter (detritus) and the associated microfuna and flora. Because of their benthic feeding habits, these larvae are directly exposed to contaminants in sediments throughout their development (Nazarova *et al.*, 2013) and also they remain exposed to different toxicants. Chironomid larvae have been used extensively as biological monitors for the assessment of benthic pollution according to Nazarova *et al.* (2013).

Morphological deformities in chironomid larvae have been reported worldwide and it has been suggested that pollutants present at the site of collection of larvae are responsible for such deformities (Hamilton and Saether, 1971; Pettigrove, 1989; Warwick, 1990). Deformities can be defined as morphological features that depart from the normal configuration (Warwick 1998; Madden *et al.*, 1995; Nazarova *et al.* 2013). The chironomid larvae are considered as ideal organisms for bioassays because they spend most of their developmental age in sediment's surface where they remain exposed to different toxicants; also they are easy to culture and have short life cycle (Al-Shami *et al.*, 2012). Such abnormalities reflect sublethal effects and can be considered as early warning signals for environmental degradation by chemical contaminants (Warwick, 1990).

Mouthparts deformities of, especially in the mandible and mentum, are quite sensitive indicators of levels of a wide variety of anthropogenic pollutants found in bottom sediments (Warwick, 1985). For instance, Hamilton and Saether (1971) noted a higher level of deformed mouthparts in *Chironomus sp.* (Diptera: Chironomidae) larvae from sites receiving substantial agricultural and industrial runoff than from a less polluted site. Numerous field data demonstrated correlation between deformity frequencies and contamination of aquatic ecosystems (Janssens de Bisthoven *et al.,* 1997; Meregalli *et al.,* 2000, 2001; Wise *et al.,* 2001; Nazarova *et al.,* 2004).

Laboratory experiments have been shown that chironomid deformities can be induced by trace metals (Janssens de Bisthoven, 1995; Nazarova *et al.,* 1999), organochloro- pesticides (Warwick, 1985; Madden *et al.,* 1992), cholinesterase inhibitors (Nazarova, 2000). Such abnormalities reflect sub lethal effects and can be considered as early biological warning signals for environmental degradation by chemical contaminants (Warwick, 1990).

Moreover, larvae of the genus *Chironomus* (Diptera: Chironomidae) have been widely used in the studies of bioaccumulation of organic and inorganic contaminants by Krantzberg and Stokes (1989) and Lydy *et al.* (1992). The relationships between trace metal levels in water or sediment and concentrations in aquatic organisms are seldom encountered in field surveys (Arain *et al.,* 2008). Studies dealing with the accumulation of trace metals in the different aquatic insect communities are still few especially in Egypt.

Accordingly, this study is an attempt to determine the relations between trace metals in aquatic sediments and in the 4th instar larvae of *Dicrotendipes nilophilus* (Kieffer, 1925) collected from the River Nile in Sohag Governorat and to examine the effects of changes in the physicochemical parameters of the Nile water on the basis of the levels of mouthparts deformities during the period of investigation.

Materials and methods

Study area

Two sampling sites were chosen based on their location relative to agricultural and anthropogenic activities and representing two different habitats at Sohag Governorate, Egypt. The first site (SI) is an irrigation canal (ca 35 km length, 11 m width and of a maximum depth of 1.5m). It is located at about 21.3 km South of Sohag City (26° 20´ 05´ N and 31° 51´12´E). This site (SI) receives enormous amounts of agricultural waste water (Fig. 1). The second site (SII) is located on the East Bank of the River Nile, 11.7 km far from Sohag City (26° 33´ 26´ N and 31° 48´ 30´ E). This site (SII) was exposed to anthropogenic stress sources (street dusts, fishing and cultivation activities) as a result of the presence of many islands (Fig. 1).

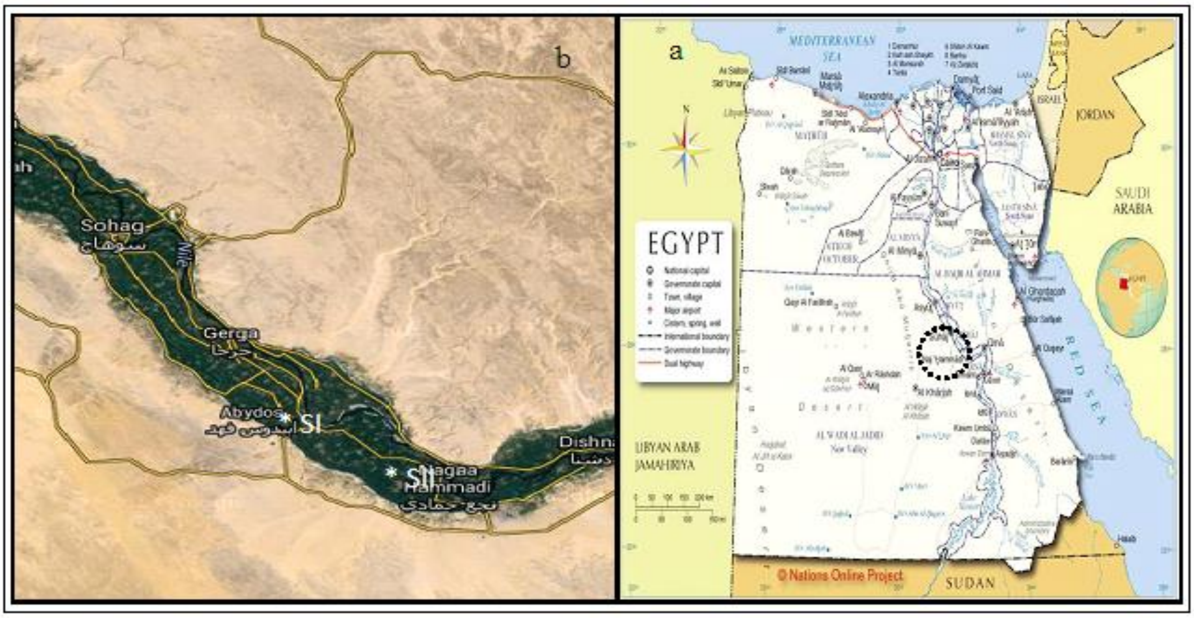

Fig. 1. a. Map of Egypt showing Sohag Governorate (dotted circle), **b.** an enlarged part of Sohag City showing the location of the two sampling sites (SI and SII).

Sampling

During an annual cycle (from January, 2007 till December, 2007), samples were collected monthly from the two sampling sites (SI and SII). Chironomid larvae were collected from the top (5-10 cm) of the sediment along the banks of each sampling sites using 5 µm mesh hand net. Upon collection, the 4thinstar chironomid larvae were isolated using a white screen mesh and were preserved in 4% formalin solution until they were taken to the laboratory where they were conserved in 70% ethanol and then processed for inspection and identification using binocular 80X magnification according to Epler (2001). Chironomid larvae belonging to the genus *Dicrotendipes* were used in this study while other genera of *Chironomus* were not considered because of the relatively low numbers or due to the absence of deformities.

The 4th instar larvae of *Dicrotendipes nilophilus* (Kieffer, 1925) were selected and placed in hot 7% KOH solution for 30 minutes to clear pigments and to improve visual inspections. Larval head capsules were excised and rinsed thoroughly in distilled water, dehydrated in graded series of ethyl alcohol (70% ~ 100% ethyl alcohol) and then mounted on glass slides and photographed and scanned by scanning electron microscopy. Deformities were scored according to definitions supposed by Janssens de Bisthoven *et al.* (1992) with some modifications as follows:-For the mandibles, the scoring system was:

A- Lacking of the apical teeth
B- Lacking of the apical and dorsal teeth
C- Lacking of the apical and broken of the middle teeth
D- Lacking most of the teeth.

-For mentum, the scoring system was:

1- Broken of middle teeth
2- Gap of mentum

3- Broken of one of the outer teeth
4- Broken of more than one of the outer teeth

The number, percentage as well as the types of deformed mouth parts were assessed from one hundred slides previously prepared from each site (SI and SII).

Stainless steel trowels were used to collect the bottom sediments seasonally. Considerable care was taken in order to gather the toxic layer of the riverbed which is then placed in 0.5 litre-capacity bags. These bags were refrigerated at 4 °C till the analyses of the all gathered layers.

Metal analyses

The total concentration of the four trace metals (Fe, Zn, Cu and Cd) in water, sediment samples and bodies of the4th instar larvae of *Dicrotendipes nilophilus* were measured seasonally using Atomic Absorption Spectrophotometer (Perkin-Elmer® 2380 and Buck Scientific® 210 VGP) and applying the methods described by Timmermans *et al.* (1992) and Omer (2003).

Physicochemical analyses

Physicochemical parameters for sampling water quality were measured monthly in the field and in the laboratory of Geochemistry at the Faculty of Science, Sohag University, Egypt. The water temperature, pH, electric conductivity, chloride (Cl^-), sulphate (SO_4^{2-}), calcium (Ca^2), sodium (Na^+), and potassium(K^+) concentrations were measured using the methods described by Chen (1995) and Omer (1996).

Statistical analysis

All statistical analyses were set at $P < 0.05$. SAS package (SAS Institute, 2002) was adopted for performing the statistical analysis.

Results

Physicochemical parameters

The mean values of the selected physicochemical parameters at the two sampling sites (SI and SII) were presented in Table (1). No significant differences were recorded in the mean water temperature and pH between the two sampling sites. However, conductivity and most of the studied chemical parameters (Ca^2, K^+, Na^+, Cl^-, SO_4^{2-}) were significantly higher ($P < 0.05$) in the first site (SI) than in the second one (SII).

Table 1. Mean values of physicochemical parameters at sites SI and SII on River Nile from January 2007 to December 2007.

Parameter	Site I	Site II
Water temperature (ºC)	$23.1 \pm 5.2^{a*}$	23.5 ± 3.2^{a}
Conductivity (µs/cm)	775.51 ± 16.8^{a}	337.3 ± 13.6^{b}
pH	8.04 ± 0.08^{a}	8.2 ± 0.14^{a}
Potassium	51.55 ± 1.80^{a}	38.65 ± 1.72^{b}
Sodium	111.05 ± 2.22^{a}	34.82 ± 2.47^{b}
Calcium	38.66 ± 1.86^{a}	11.40 ± 0.47^{b}
Chloride	35.29 ± 1.84^{a}	18.3 ± 2.11^{b}
Sulphate (ppm)	44.29 ± 5.64^{a}	23.55 ± 3.48^{b}

* Means in rows followed by the same letters are insignificantly different ($P > 0.05$).

Metal analyses

Zinc (Zn), copper (Cu), iron (Fe) and cadmium (Cd) analyses in waters, sediments as well as in the 4th instar *Dicrotendipes nilophilus* larvae were shown in fig.2, 3and 4, respectively. The measured concentrations of trace metals in sediments, waters and larvae in both sampling sites (SI and SII) increased in the following order: Cd <Cu< Zn< Fe. Significant changes in the concentrations of all trace metals were recorded for sediments, waters and larvae during the different seasons.

The concentrations of trace metals in water column were much lower than those in sediments in both sites and the highest amounts of all metals were recorded in larvae. Statistical analysis showed that no significant differences ($P > 0.05$) in the concentrations of sediment trace metals between the two sampling sites. However, significant difference ($P < 0.05$) was recorded only in the concentration of water iron between the two sampling sites. However, the accumulation of Fe, Zn and Cu was significantly higher in the larvae collected from the first site than those from the second site ($P < 0.05$). Moreover, no significant differences ($P > 0.05$) were recorded in the amount of Cd accumulated in the larval body between the two sampling sites. On the other hand, Zn, Cu and Fe concentrations in *Dicrotendipes nilophilus* (Kieffer, 1925) larval bodies were strongly correlated with those of sediments of both sampling sites ($r > 0.8$). However, this correlation was weak as compared with those in the waters ($r < 0.4$).

Table 2. The types, numbers and percentages of deformed menta and mandibles of 4th instar larvae of *Dicrotendipes nilophilus* collected from sites SI and SII. Letters from A to D refer to mandible deformity type while numbers from 1 to 4 refer to menta deformity type (see context for more details).

			Site I	Site II
Deformities	Mandibular	A	5	7
		B	2	4
		C	1	5
		D	1	4
	Total percentage (%)		9	8
	Mentum	1	5	3
		2	3	0
		3	0	3
		4	0	0
	Total percentage (%)		20	6

Morphological deformities in the 4th instar larvae

The two non-deformed mandibles of the 4th instar chironomid larvae consisted, each, of one basal tooth, two middle teeth, apical and dorso-lateral teeth (Fig. 5). The mentum possessed six outer teeth per side and a single middle tooth (Fig.6).

Scanning electron micrographs as well as light photographs revealed the different types of deformities recorded in the mandibles of the 4th instar larvae of *Dicrotendipes nilophilus* (A, B, C and D) and menta (1, 2,3 & 4) as presented in figures5& 6, respectively. The percentage of larvae with deformed mouth parts in SI ranged from 9% having deformed mandibles to 20% with deformed menta (table 2). However, in SII, the percentage of larvae with deformed mandibles was higher (8%) than with deformed menta (6%) as illustrated in table (2).

In the first sampling site (SI), mandible deformity of type A was the most common one (Fig. 5c), while type 1 mentum deformity was the most common one (Fig. 6b). However, in the second sampling site (SII) types A&B of deformed mandibles (Fig. 5c, d) and types 1 & 3 of deformed mentum (Fig. 6 b, c) were the most common ones.

Table (2) showed that the total percentage of larvae possessing deformities of mandibles and menta in the first site was about twofold (29%) as compared to the second site (14%). Although some larvae displayed either a deformed mentum or mandible, some of them exhibited both deformities.

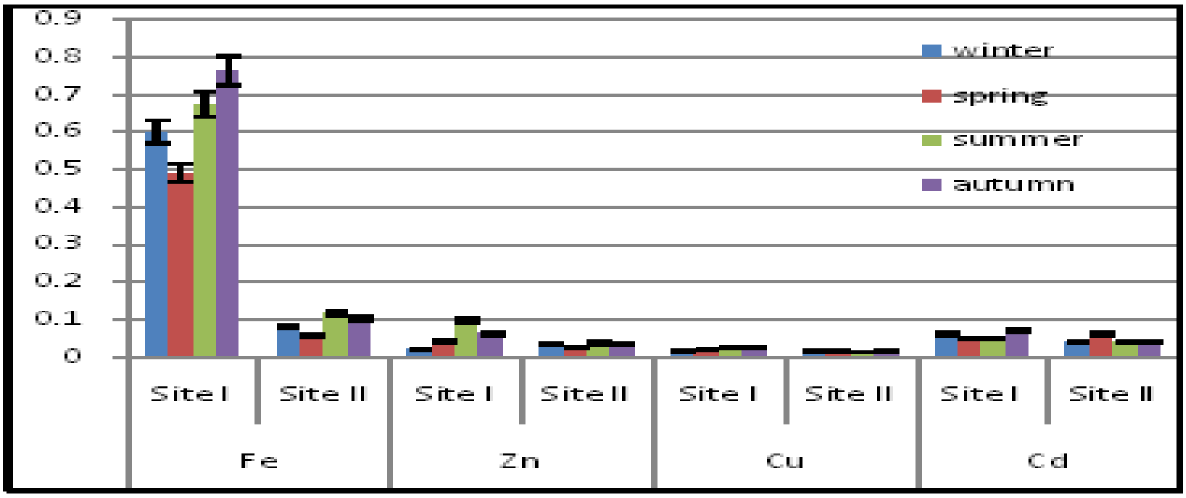

Fig. 2. Seasonal variations in the concentrations of trace metals (Fe, Zn, Cu & Cd) in the waters of sites I and II.

Discussion

Local variations in the input of trace metals into the studied two sampling sites (SI and SII) reflected not only changes in their concentrations in sediment and water column (Suschka *et al.,* 1994; Ciszewski, 1998; Labus, 1999), but also in their bioavailability to organism as reflected in their accumulated metal concentration. The present results indicated that metals concentration in sediment was much higher than in water.

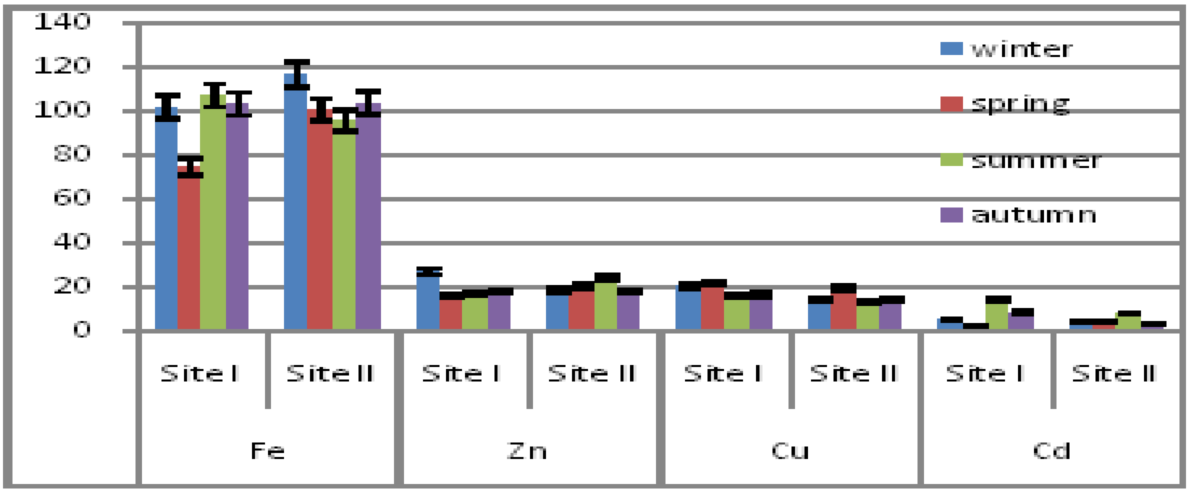

Fig. 3. Seasonal variations in the concentrations of trace metals (Fe, Zn, Cu & Cd) in the sediments of sites I and II.

These results were consistent with previous studies reported by An and Kampbell (2003) and Anazawa *et al.* (2004). Additionally, body burdens of trace metals were much higher than concentrations in sediment from which the larvae were collected. These results agree with those of Kiffiney and Clements (1993) and Cain *et al.* (1995), these authors reported that whole-animal metal concentrations in river-dwelling insects were correlated with concentrations in sediments. Although water metal concentrations were very less than those in *Dicrotendipes nilophilus* bodies and sediments; the concentration gradient among metal levels in organism, sediment and water was the same as Cd < Cu < Zn < Fe. Thus, the *Dicrotendipes nilophilus* larvae were interacting with both sources of the metals. Moreover, the strong correlations between the concentrations of trace metals in the larval body of *Dicrotendipes nilophilus* and those in sediment suggested that the organism metals concentrations were greatly influenced by the sediment metals levels. Also, the significant relationships between metals levels in sediments (Zn, Cu and Fe) and *Dicrotendipes nilophilus* larvae showed their ability to reflect ambient metal availabilities and proved them to be good biomonitor of trace metals in aquatic system.

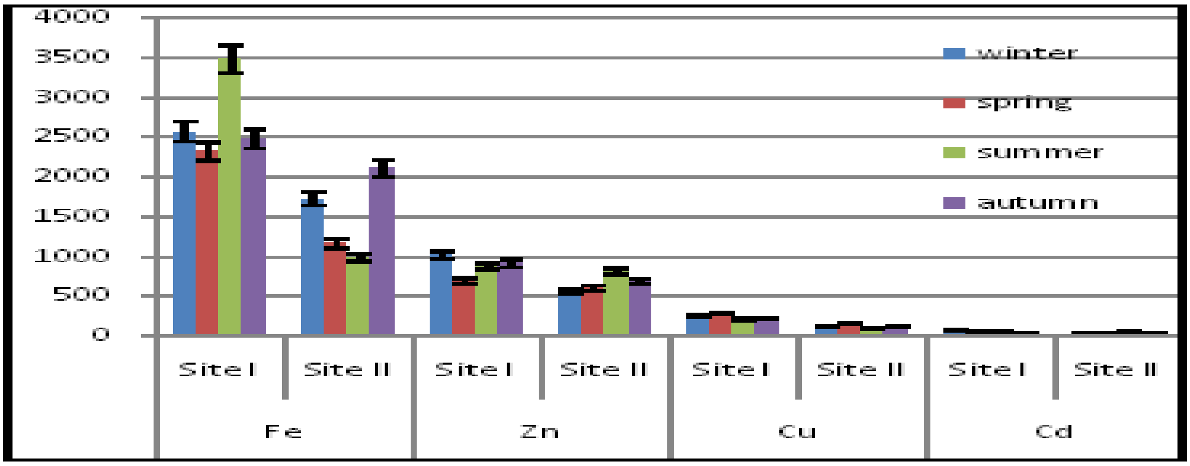

Fig. 4. Seasonal variations in the concentrations of trace metals (Fe, Zn, Cu& Cd) in the bodies of 4th instar larvae of *Dicrotendipes nilophilus* (Kieffer, 1925) larvae at sites I and II.

The present result revealed that *Dicrotendipes nilophilus* larvae collected from SI accumulated higher concentrations of trace metals than those collected from SII. Although there were no differences in the sediment metals concentrations between the two sampling sites, significant differences were recorded between body burdens of Cu, Zn, and Fe for the larvae collected from the two sampling sites. The possible explanation for this difference was the higher water salinity of the first sampling site (SI) than in the second one (SII). In this study, salinity was not measured directly, but specific conductance may be used as substitute, because it is proportional to the concentration of major ions that contribute to more than 99% of total salinity (Rodhe, 1949). Weinstein (2003) and Bidwell and Gorrie (2006) observed that, whenever water salinity increased beyond certain tolerance levels, the larvae of *Chironomus maddeni* (Martin and Cranston, 1995) (Diptera: Chironomidae) accumulated significantly more Cu and Zn from the sediment. In addition, there is some indication that chironomid larvae may experience an increase in membrane permeability as they are acclimated to increasing salinity which can lead to enhanced uptake of metals (Barjaktarovic and Benddl –Young, 2001).

The type of deformities observed in the present study were similar with the results described by other authors (Köhn and Frank, 1980; Warwick and Tisdale, 1988; Janssens de Bisthouen *et al.*, 1998; Wise *et al.*, 2001; Nazarova *et al.*, 2004). Warwick (1988) assumed that a frequency of deformities in a chironomid community exceeding 8% of total larvae number can be taken as an indicator of some unfavourable impact.

Accordingly, in the present study the significant difference in the accumulated metal concentrations (Fe, Zn, and Cu) between larval bodies of *Dicrotendipes nilophilus* in SI than those in SII, suggest that higher bioaccumulation might be responsible for the higher percentage of deformed larvae obtained in SI (29%) than in SII (14%). Mouthparts abnormalities were strongly influenced by a variety of environmental stress, not only by trace metals but also by high deposition rates of fine mineral particles. The latter factor could be the cause of conspicuously higher deformities of mentum found in the larvae collected from SI (20%) than those collected from SII (6%).

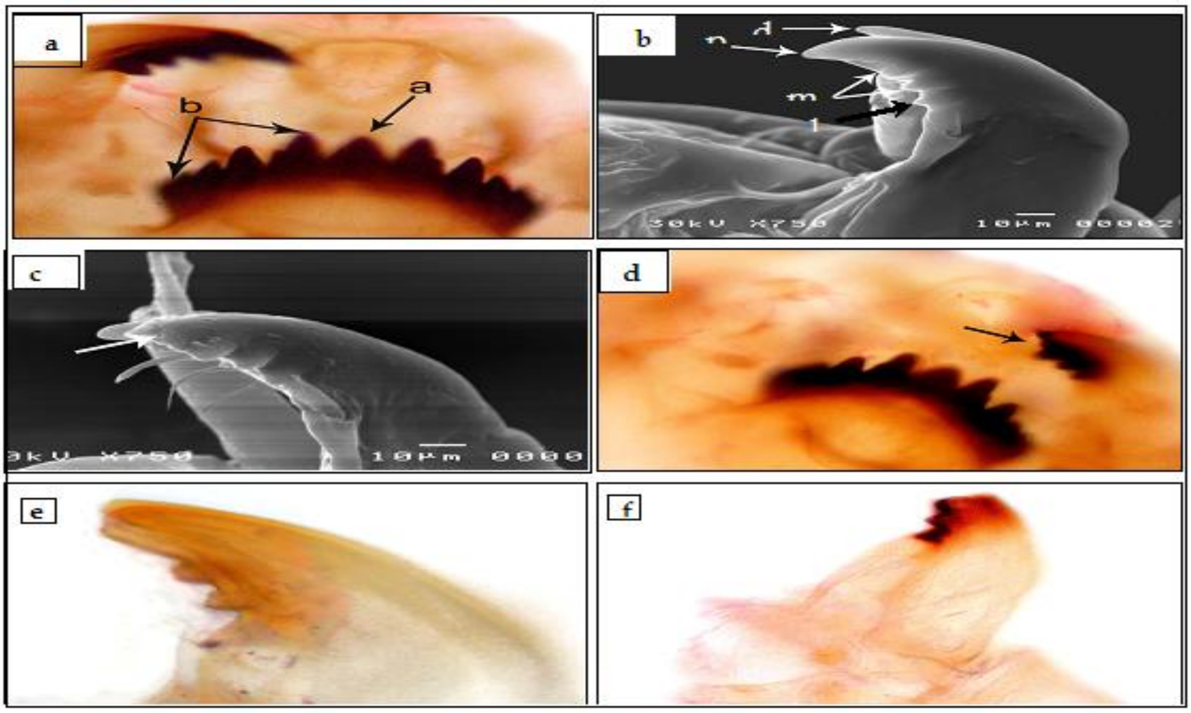

Fig. 5. Mandible of the 4th instar chironomid larva, *Dicrotendipes nilophilus* (Kieffer, 1925): a- photograph showing normal mandible and mentum (a= middle tooth, b= outer teeth), b- scanning electron micrograph showing left normal mandible (d= dorso-lateral tooth, p= apical tooth, m= middle teeth, l=basal tooth), c- scanning electron micrograph showing type A deformity (arrow= lacking of the apical tooth), d- photograph showing type B deformity (arrow= lacking of apical and dorsal teeth), e- photograph showing type C deformity, f- photograph showing type D deformity.

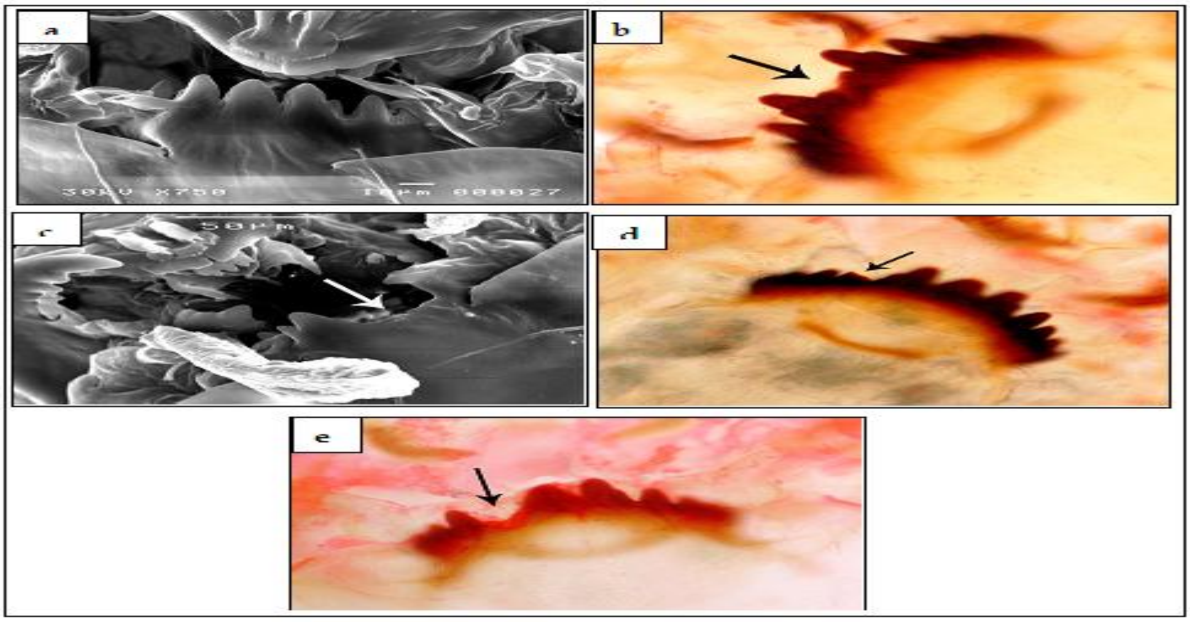

Fig. 6. Mentum of the 4th instar chironomid larva, *Dicrotendipes nilophilus* (Kieffer, 1925): a- scanning electron micrograph showing normal mentum, b- photograph showing type 1of mentum deformity (arrow= broken tooth of the middle teeth), c- scanning electron micrograph showing type 2 of mentum deformity (arrow = gap of mentum), d- photograph showing type 3 of mentum deformity (arrow = broken one tooth of the outer teeth), e- photograph showing type 4 of mentum deformity (arrow = broken of more than one tooth of the outer teeth).

These results were supported by previous results obtained by Nazarova *et al.* (2004) in which higher mentum abrasions were recorded whenever the concentrations of different minerals were higher.

Conclusion

Based on these results; the first site (SI) was facing the most serious impact of water pollution resulting from agricultural activities. Therefore, the authors suggest that, from attitude of practical issue, conditions prevailing in site (SI) may exert stress on the community of *Dicrotendipes nilophilus*.

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