MORFOLOGICAL DEFORMITIES OF MOUTHPARTS IN GENUS *CHIRONOMUS* (DIPTERA: CHIRONOMIDAE) INDUCED BY HEAVY METALS

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MORFOLOŠKI DEFORMITETI USNOG APARATA RODA *CHIRONOMUS* (DIPTERA: CHIRONOMIDAE) IZAZVANI TEŠKIM METALIMA

Apstrakt

U slatkovodnim ekosistemima larve *Chironomidae* su izložene svim prisutnim toksičnim materijama u vodi i sedimentu i kao takve mogu se koristiti kao idealni biotest organizmi. Kod larvi hironomida često se razvijaju deformacije usnih delova, posebno mentuma, za koje se pretpostavlja da su uglavnom prouzrokovani neorganskim hemikalijama. Kod gajenih hironomida (*Chironomus*) na sedimentu iz šaranskog ribnjaka utvrđen je stepen i tip deformiteta u zavisnosti od koncentracije i tipa teških metala. Larve *Chironomus*-a (prikupljene iz Jelenačkog potoka) bile su izložene olovo (II) acetat-3 hidratu i bakar (II) sulfat-pentahidratu u koncentracijama 30, 120 i 200 µg/g suve mase sedimenta. Za procenu stepena deformiteta korišćen je indeks toksičnosti TSI po Lenatu. Kod larvi *Chironomus*-a, izloženih olovu primećen je porast učestalosti deformiteta sa povećanjem koncentracija i to najčešće deformitet medijalno-lateralnih zuba, koji su kraći, i nedostatak zuba. Larve *Chironomus*-a izložene bakru pokazuju pad stope deformiteta od najniže ka najvišoj koncentarciji, a najčešći tipovi deformiteta su kraći i podeljeni medijalni zubi i kraći medijalno-lateralni zubi.

Ključne reči: deformitet usnog aparata, teški metali, olovo, bakar, sediment, Chironomidae
Key words: mouthpart deformity, heavy metals, lead, copper, sediment, Chironomidae

INTRODUCTION

In developing countries, a large number of aquatic environments is exposed to degradation which also includes trace metal pollution. Benthic community represents one of the most sensitive community in aquatic ecosystems, which reacts on different type of processes in these ecosystems. Only by simultaneous monitoring of trace metals content in aquatic ecosystems and benthic communities we can fully understand into which extant aquatic environment is contaminated. Chironomid larvae are considered to be ideal biotest organisms, because they are exposed to toxic material in water and sediment. Chironomid larvae represent good candidates for ecotoxicological studies because of their relatively short life cycle and because they are mainly grazers (Beneberu and Mengistou, 2014). During feeding, these larvae can metabolize different type of compounds in water and sediment. These compounds can induce deformities in some body parts, firstly in mouthparts (Al-Shami et al., 2010). With the goal to determine the toxicity level of trace metals in *Chironomus* larvae, based on the deformities of mouthparts, as well as to establish the degree and the type of the deformity depending on the concentration and the type of trace metals, an experiment which results are shown in this essay was realized.

MATERIAL AND METHODS

Chironomid larvae were collected in the Jelenački brook. Material was sampled with Surber net. The sediment for the experiment was taken from the fish pond and dried in sterilizer for four hours at 100ºC. Further on, the sediment was mixed with lead (II) trihydrate and copper (II) sulfate pentahydrate in concentrations: 30, 120 and 200 µg/g of dry weight for both metals. The experiment was carried out using 3 concentrations with 3 replicates (3x3) for each metal. Water and sediment were placed for two days to achieve the toxic equilibration of suspended firm substances (OECD, 2004). At the beginning of the experiment, 25 chironomid larvae were put in each aquarium. Larvae were fed with finely ground extruded feed used for carp nutrition. After seven days, larvae were separated from the sediment. Microscopic mouthparts slides were made according to Namiotko et al. (2011).

Toxic Score Index (Lenat, 1993) was used in order to estimate the level of deformities in chironomid larvae. TSI index can be calculated as follows: [No. of Class I+ 2 (No. of Class II) + 3 (No. of Class III)] × 100/ Total No. of larvae. According to TSI index, deformities of mentum are divided into three classes: Class I – larvae with slight noticeable deformities, Class II – larvae with noticeable deformities: extra teeth, missing teeth, teeth with large gaps, Class III – larvae with significant deformations, including at least two deformities from the Class II.

RESULTS AND DISCUSSION

The mentum (labium) of *Chironomus* larvae consists of 15 dark pigmented teeth (Al-Shami et al., 2010): median which is tripartite (M), two larger medio-lateral (ML) and four smaller lateral teeth (L) each side (Figure 1).

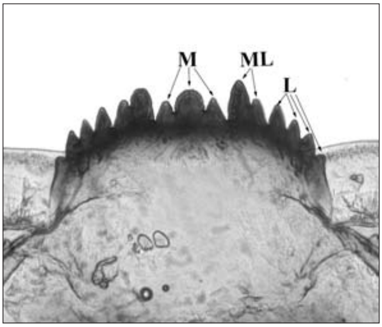

Figure 1. Mentum structure: M – median tooth; ML – medio-lateral teeth; L – lateral tooth

The appearance of the deformities is classified according to teeth position. Larvae in our experiment were exposed to copper and lead, and the following deformities were noticed: shorter median tooth and both medio-lateral teeth, missing third lateral tooth, missing median tooth, shorter medio-lateral teeth, median tooth split, missing first lateral tooth (Figure 2. A-F) Shorter teeth (Fig. 2, A, B) belong to the Class I, missing one tooth (Fig. 2 C) and split tooth belong to the Class II while Class III includes deformities with more teeth in mentum (Fig. 2, E, F). With the increase of lead (II) trihydrate (Fig.3) in the organisms exposed to lead, an increase of deformity frequency was noticed.

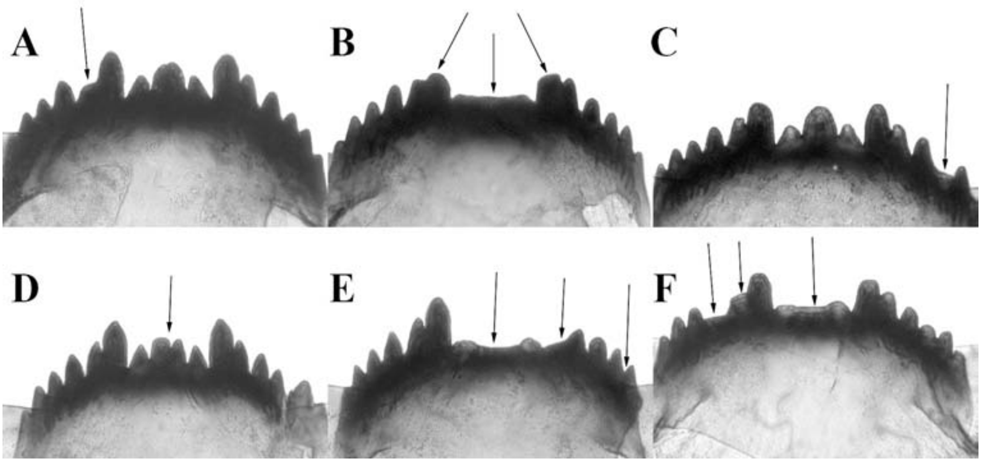

Figure 2. Deformity types: A: shorter tooth; B: shorter median and mediolateral teeth; C :missing teeth; D: split median tooth; E: shorter teeth and missing lateral tooth; F: more shorter teeth and missing teeth

Chironomus larvae exposed to copper showed decrease in rate of deformities from the lowest to the highest concentration (Fig. 3) Larvae exposed to the lowest, average and the highest Cu and Pb concentrations showed significantly higher rate of deformities than larvae reared in control aquariums (Fig. 3)

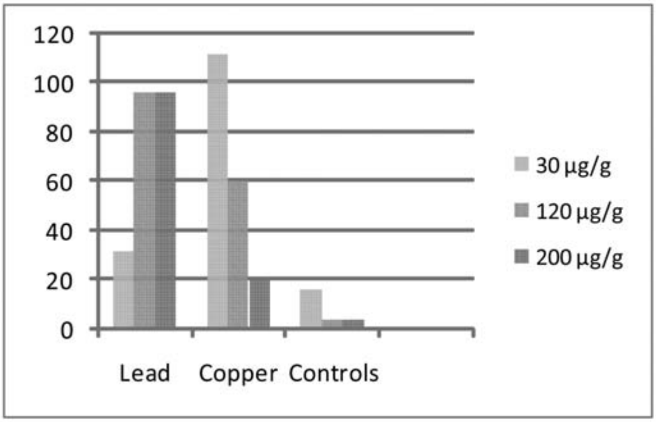

Figure 3. TSI index for the larvae exposed to different Pb and Cu concentrations

In larvae reared in sediment with the increasing lead concentrations, higher frequency of deformities can be noticed. This can be explained by the fact that lead in the sediment acts directly on organisms, causing appearance of deformities, or acts indirectly through bioacumulation in organisms, entering in food chains.

Deviation of the results in our experiment was a consequence of high mortality of individuals exposed to concentrations of 120 µg/g and 200 µg/g of $CuSO_4$x $5H_2O$ (Fig. 3).

Martinez et al. (2003) used the same concentrations of copper like in our experiment, and significant difference of rate of deformities was noticed between larvae exposed to various copper concentrations and those from the control. However, the linear relationship between metal concentrations and rate of deformities hasn’t been established.

In our experiment, the lowest copper concentration induced the highest deformity percentages like shorter median and shorter lateral tooth (Fig. 4) As copper concentrations increased, the number and type of deformities decreased which can be explained by high mortality of larvae on higher concentrations

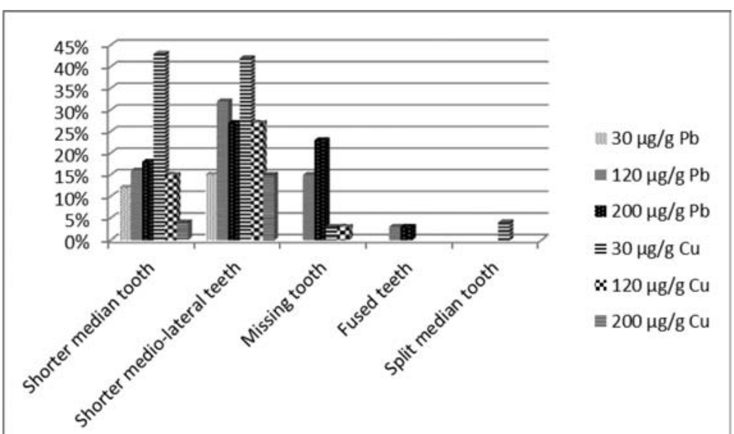

Figure 4. Percentage of deformities type in relation to different Cu and Pb concentrations

Larvae exposed to average concentration of lead had the highest percentage of deformities with shorter medio-lateral teeth and missing teeth as dominant (Fig. 4). Larvae exposed to the highest lead concentration had the lowest pecentage and deformity types which can be explained by high mortality of larvae, as well as by the presence of different pollutants in natural sediment, which can influence the deformity types.

Comparing with the results published by Martinez et al (2003), the copper concentrations in our experiment were the same. Deformity types at the lowest concentration were missing teeth, fused teeth and split median tooth, while in the experiment conducted by our team the most common deformities were shorter median tooth, missing teeth, split median tooth and absence of fused teeth. Comparing to their results at average concentration, the most common deformities were missing and fused teeth, while in our experiment prevailed deformities on medio-lateral teeth. Martinez et al. (2003) showed, when using the highest concentration of copper, the most common deformities were: missing teeth, split median teeth and fused teeth, while in our experiment, we could not define the most common deformity type at the highest copper concentration because of high mortality.

CONCLUSION

Larvae of *Chironomus* exposed to lead showed increase in frequency of mouthpart deformities with increase of lead concentration, while larvae exposed to copper showed decrease in frequency of mouthpart deformities from the lowest to the highest lead concentration. The possible reason could be due to high mortality of individuals on higher concentrations. The most common deformity of *Chironomus* larvae exposed to lead were shorter medio-lateral teeth and missing teeth, while in *Chironomus* larve exposed to copper, the most common deformities were observed on medial tooth and medio-lateral teeth.

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