Sorption of Ni(II) and Cr(III) ions by the Chironomus yoshimatsui larvae from wastewater

Keon Sang Ryoo*
*Department of Applied Chemistry, Andong National University, Andong 760-749, Korea
E-mail address: ksr@andong.ac.kr

ABSTRACT
In this study, Chironomus yoshimatsui larvae were applied to remove Ni(II) and Cr(III) ions from wastewater. The sorption studies were carried out using laboratory-reared C. y. larvae. It was found that C. y. larvae are very susceptible to Cr(III) as compared to Ni(II). The survival capacity of C. y. larvae was sharply reduced when exposed to even low Cr(III) concentration. Sorption isotherm and kinetics of C. y. larvae for Ni(II) were determined by means of controlled experiments in a batch system. It was observed that sorption efficiency of Ni(II) was largely concentration dependent and more effective at lower concentration. At each equilibrium, Ni(II) was removed up to roughly 44~80%. Sorption data were better fitted to the Langmuir isotherm model because of its correlation coefficient R² value greater than that of the Freundlich isotherm model. The sorption kinetics by C. y. larvae for Ni(II) was well described a pseudo-first-order rate expression. C. y. larvae have enormous potential for application in wastewater treatment technologies because they are widespread and abundant all around the world and can be easily kept in culture.

Keywords. Chironomus yoshimatsui larvae ; Ni(II); Sorption; Kinetics; Isotherm

1. INTRODUCTION
Water bodies including reservoirs, lakes, streams, rivers and oceans have been contaminated with a large quantity of toxic metals due to the rapid industrialization. The presence of toxic metals in water is actually harmful to the humans and aquatic life. Chromium and its compounds have been used for electroplating, leather tanning and metal finishing in various industry field [1]. Nickel has been widely used for the several industrial purposes like electroplating, production of batteries, manufacturing of sulfate and porcelain enameling [2]. However, if these toxic metals are into the human body at higher level, it has been known that they causes various diseases like dermatitis, nausea, chronic bronchitis, gastrointestinal distress and reduced lung function to humans. Therefore, lots of methods have been proposed for the removal of toxic metals from wastewater. These include adsorption, chemical precipitation, ion exchange, membrane filtration, coagulation, biological and electrochemical treatment [3-24].

C. y. larvae are widely distributed in Korea and have been used as a model species to assess the quality of freshwater and sediments [25]. They inhabit the sediments and feed on organic detritus from fish and plants in water. C. y. larvae are abundant in sediments and water contaminated by certain metals. It has been demonstrated that C. y. larvae are less susceptible to exposure to metals at metal-polluted sites [26]. They acclimate themselves to metals by either regulating body burdens or sequestering them in selected tissues. Until now, many researchers have focused on evaluation of C. y. larvae as a test organism for use in sediment toxicity bioassays [27-29]. However, as far as author know, no studies have dealt with C. y. larvae for sorption of toxic metals in wastewater.

The objective of this study is to investigate the feasibility of C. y. larvae for sorption of Ni(II) and Cr(III) ions in wastewater. C. y. larvae were chosen because they is one of the species able to survive in heavily metal-polluted streams in Korea. The experiments were carried out using aqueous solution contaminated with selected toxic metals like Ni(II) and Cr(III). The sorption performance of Ni(II) and Cr(III) was done with contact time and different initial concentrations. Moreover, isotherms and kinetics were also analyzed to reveal the sorption characteristics.

2. MATERIALS AND METHODS
2.1. Materials and reagents
In this study, C. y. larvae were used as the sorbent for sorption of Ni(II) and Cr(III) from aqueous solution. C. y. larvae were acquired from sediments in organic rich urban streams in Korea. Mass larval rearing of C. y. larvae was conducted in plastic containers (length 250, width 270, and height 170 mm) containing pre-aerated distilled water (depth 40~50 mm) and fine sand (depth 30~40 mm), which were placed inside a rearing cage (acrylic box with attached screen: length 420, width 550, and height 420 mm) for emerging adults. Tetramin® (< 0.2 mm) (TetraWerke, Melle, Germany) was provided as feed for the larvae. Adult C. y. were retained using a plastic cage covered with steel wire mesh and reproduced continuously. Detailed rearing method of C. y. was presented in Baek et al [30]. Anhydrous NiCl₂ and Cr(NO₃)₃ 9H₂O were purchased from Sigma-Aldrich, USA. The reagents are of analytical grade and were used without further purification.

2.2. Adsorption procedures
The Ni(II) and Cr(III) stock solutions with concentration of 100 mg L⁻¹ were prepared by using NiCl₂ and Cr(NO₃)₃ 9H₂O as a source of Ni(II) and Cr(III), respectively. Experimental solutions of the desired concentration were obtained by diluting the stock solutions successively. Batch sorption studies were carried out in a series of 500 mL of volumetric flasks filled with 100 mL Ni(II) solution and Cr(III) as well as the required amounts of C. y. larvae. The flasks were tightly closed with a silicone stopper. C. y. larvae were not fed during the sorption study. All flasks were aerated in order to maintain the dissolved oxygen levels. Sorption studies were conducted without agitation at room temperature. The samples were drawn from the flasks at regular intervals for determining the residual Ni(II) and Cr(III) concentrations. All experiments were
performed in triplicate and the average was taken for subsequent calculations. The concentrations of Ni(II) and Cr(III) in samples were determined using ICP-AES (Flame Modula S, Spectro, Germany).

Sorption isotherms were measured by varying the initial Ni(II) and Cr(III) concentrations and keeping C. y. larvae constant. The initial concentrations of Ni(II) and Cr(III) in aqueous solutions were in the range of 0.5 to 5.0 mg L\(^{-1}\) and the amount of C. y. larvae was 0.1 g. The sorption capacity of Ni(II) and Cr(III), \(q_e\) (mg g\(^{-1}\)), of C. y. larvae was evaluated by using the following expression:

\[
q_e = \frac{(c_0 - c_f)V}{m}
\]  

(1)

Where, \(c_0\) and \(c_f\) (mg L\(^{-1}\)) are the concentrations of metals at initial and equilibrium time \(t\), respectively. \(V\) (L) is the volume of the solution and \(m\) (g) is the weight of C. y. larvae. In this isotherm study, the Freundlich and Langmuir isotherm models were used for comparison with experimental data.

The sorption kinetic studies were performed with initial metal concentrations at different time periods varying between 1 day∼11 day. The amount of metals adsorbed at time \(t\), \(q_t\) (mg g\(^{-1}\)), was calculated using the following relationship:

\[
q_t = \frac{(c_0 - c_f)V}{m}
\]  

(2)

Where, \(c_0\) (mg L\(^{-1}\)) is the concentration of metals at a certain period of time \(t\). The pseudo-first-order and pseudo-second-order models were selected to analyze the kinetics of the sorption process.

3. RESULTS AND DISCUSSION

3.1. Effect of initial Ni(II) concentration and contact time

Generally, the sorption of sorbate on sorbent is dependent on both contact time and the initial sorbate concentration. The effect of contact time on the sorption of sorbate at different initial concentrations represents the sorption pattern. The contact time corresponding to the maximum sorption is considered as the equilibrium sorption time.

The sorption study was conducted in the concentration range from 0.5∼5.0 mg L\(^{-1}\) for each Ni(II) and Cr(III) while maintaining 0.1 g L\(^{-1}\) of C. y. larvae at room temperature. The sorption patterns corresponding to four different initial Ni(II) concentrations are presented in Fig. 1. It is observed that for all initial Ni(II) concentrations, there is an increase in the amount sorbed by increasing the contact time. Sorption reached equilibrium in 7 day at low concentrations of 0.5 and 1.0. mg L\(^{-1}\). On the other hand, at higher concentrations, equilibrium time was 11 day. After equilibrium, the remaining concentration of Ni(II) in solution remained nearly constant.

![Fig. 1. Sorption pattern of Ni(II) by C. y. larvae with contact time at room temperature.](image)

The percent of Ni(II) sorption and the distribution ratio, \(K_d\) (mg g\(^{-1}\)), can be correlated by the following equations:

\[
\text{Sorption (\%)} = \frac{100K_d}{R_d + \frac{V}{m}}
\]

(3)

\[
K_d = \frac{\text{amount of solute in adsorbent}}{\text{amount of solute in solution}} \times \frac{V}{m}
\]

(4)

Where, \(V\) is the volume of the solution (mL) and \(m\) is the weight of C. y. larvae (g). The sorption percent of Ni(II) at equilibrium was found to be lower with increased initial concentration from 0.5 to 5.0 mg L\(^{-1}\). Depending on the initial concentrations, approximately 44∼80 % of Ni(II) sorption at equilibrium was achieved by C. y. larvae.
Fig. 2 represents amounts of Cr(III) sorbed by C. y. larvae with contact time for initial Cr(III) concentrations. As shown in Fig. 2, any sorption of Cr(III) by C. y. larvae was not observed through the contact time. Furthermore, additional contact time did not show the increase in sorption. The significant sorption difference for Ni(II) and Cr(III) can be explained by change in the survival capacity of C. y. larvae. A large number of C. y. larvae survived in all Ni(II) concentrations. Whereas, the survival of C. y. larvae declined sharply within the first day at all Cr(III) concentrations. The result indicates that C. y. larvae is capable of tolerating for Ni(II) compared with Cr(III) in solution.

3.2. Sorption isotherms

Sorption isotherms provide essential data source for understanding of relation with sorbent and sorbate when sorption equilibrium is arrived at a constant temperature. It is important for sorption system because it can be used to compare the properties with different sorbents quantitatively. In this study, the most commonly used Freundlich and Langmuir isotherm models were employed to investigate the sorption behavior of Ni(II) by C. y. larvae in aqueous solution. The Freundlich isotherm is an empirical equation employed to describe heterogeneous system. Whereas, the Langmuir isotherm takes an assumption that the sorption occurs at specific homogeneous sites within the sorbent.

The Freundlich isotherm equation is given as:

\[ q_e = k_f (c_e)^{1/n} \]  \hspace{1cm} (5)

Where, \( k_f \) (mg g\(^{-1}\)) and \( 1/n \) are the Freundlich constants related to sorption capacity and the sorbent affinity for the sorbate, respectively. A linear form of the Freundlich isotherm equation can be described as:

\[ \log q_e = \log k_f + \frac{1}{n} \log c_e \]  \hspace{1cm} (6)

A linear plot of log \( q_e \) versus log \( c_e \) yields a slope \( 1/n \) and an intercept of log \( k_f \).

The Langmuir isotherm equation is presented as:

\[ \frac{q_e}{q_s} = \frac{1}{Q} + \frac{c_e}{Q} \]  \hspace{1cm} (7)

Where, \( Q \) (mg g\(^{-1}\)) is the maximum amount of sorbate per unit mass of sorbent and \( b \) (L mg\(^{-1}\)) is the equilibrium sorption constant which is related to the affinity of the sorbate. The Langmuir constants are determined from the intercept of the linear plot of \( c_e/q_e \) against \( c_e \).

The choice between the Freundlich and Langmuir isotherms depends mainly on the nature of equilibrium data. The fittings of Freundlich and Langmuir isotherm of Ni(II) for C. y. larvae are presented in Figure 3 and 4. It was observed that the experimental data deviate more in the case of the Freundlich than Langmuir plot. This is verified by the correlation coefficient \( R^2 \) values of the linear plots, which revealed the extremely good applicability of the Langmuir isotherm model to the Ni(II) sorption by C. y. larvae.
Fig. 3. Freundlich sorption isotherm curve for Ni(II) at 298 K.

The corresponding isotherm parameters were evaluated from the linear plot and listed in Table 1. The values of $Q$ and $b$ of Langmuir isotherm constants were 10.59 and 0.14, respectively. Generally, the sorbates having higher $Q$ values have high affinity toward sorbents compared to others having lower $Q$ values. If the value of $b$ is smaller than 1, it indicates favorable sorption on sorbate to the surface of the sorbent. Whereas, if the value is larger than 1, the sorption is unfavorable.

Fig. 4. Langmuir sorption isotherm curve for Ni(II) at 298 K.

Table 1. Langmuir and Freundlich constants for sorption of Ni(II) at 298 K

| Sorbent       | $Q$ (mg g$^{-1}$) | $b$ (L mg$^{-1}$) | $R^2$ | $1/n$ | $k_F$ (mg g$^{-1}$) | $R^2$ |
|---------------|------------------|------------------|-------|-------|--------------------|-------|
| C. y. larvae  | 10.59            | 0.14             | 0.9846| 0.54  | 1.38               | 0.8682|
3.3. Sorption kinetics

In this study, two kinetic models were employed to evaluate the sorption processes of Ni(II) on C. y. larvae. The selected kinetic models are the pseudo-first-order and pseudo-second-order based on the assumption that the rate of adsorption is proportional to the number of free sites and the square of the number of unoccupied sites, respectively. The conformity between experimental data and the model-predicted values was expressed by the correlation coefficient $R^2$. The relatively higher value is more applicable model to the kinetics of Ni(II) sorption.

The pseudo-first-order model is given as:

$$\log \left( \frac{q_e}{(q_e - q_t)} \right) = \frac{k_1}{2.303} t$$  \hspace{1cm} (8)

Eq. (8) can be rearranged to obtain the following linear relationship:

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t$$  \hspace{1cm} (9)

Where, $k_1$ (min$^{-1}$) is the equilibrium rate constant of the pseudo-first-order kinetics. The values of $q_e$ and $k_1$ are determined from the slope and intercept of the straight line.

The pseudo-second-order kinetic model is presented as:

$$\frac{1}{q_e - q_t} = \frac{1}{q_e} + k_2 t$$  \hspace{1cm} (10)

Eq. (10) can be linealized:

$$\frac{1}{q_e} = k_2q_e^2 + \frac{1}{q_e} t$$  \hspace{1cm} (11)

Where, $k_2$ (g mg$^{-1}$ min$^{-1}$) is the equilibrium rate constant of the pseudo-second-order kinetics. The values of $q_e$ and $k_2$ are determined from the slope and intercept of the straight line.

Fig. 5 and 6 show the pseudo-first-order and pseudo-second-order kinetic model fitting to the experimental data for C. y. larvae at different initial concentrations. The experimental amounts of sorption equilibrium ($q_{e,exp}$), the rate constants of pseudo-first-order and pseudo-second-order kinetic ($k_1$ and $k_2$), the calculated amount of sorption equilibrium ($q_{e,cal}$) and the correlation coefficients ($R^2$) are shown in Table 2.

Fig. 5. Plots of pseudo-first-order kinetics of Ni(II) sorption on C. y. larvae at 298 K.
Fig. 6. Plots of pseudo-second-order kinetics of Ni(II) sorption on C. y. larvae at 298 K.

From Table 2, it was observed that the calculated amounts of sorption equilibrium \( q_e,\text{cal} \) from both pseudo-first-order and pseudo-second-order kinetic models deviated slightly from the experimental amounts of sorption equilibrium \( q_e,\text{exp} \). The \( q_e \) values obtained experimentally and from the pseudo-first-order kinetic model for each initial concentration were found to be 3.80, 7.80, 17.9, 22.0 and 1.75, 3.68, 8.34, 9.60 mg g\(^{-1}\), respectively. Meanwhile, the pseudo-first-order kinetic model, as shown clearly in Table 2, appeared to be the better fitting model because it has higher \( R^2 \) than that of the pseudo-second-order kinetic model for larvae. The correlation coefficients \( (R^2) \) for the pseudo-first-order kinetic model are higher than 0.90 in all cases. This result suggests the Ni(II) sorption on C. y. larvae can be approximated more appropriately by the pseudo-first-order kinetic model. The rate constant \( k_1 \) of pseudo-first-order kinetic model was almost the same when increasing the initial Ni(II) concentration from 0.5 to 1.0, 2.5 and 5.0 mg L\(^{-1}\).

Table 2. Pseudo-first and pseudo-second order kinetic model parameters for different initial Ni(II) concentrations at 298 K

| Sorbent                  | Initial concentration (mg L\(^{-1}\)) | \( q_e,\text{exp} \) (mg g\(^{-1}\)) | \( q_e,\text{cal} \) (mg g\(^{-1}\)) | \( k_1 \) (day\(^{-1}\)) | \( F^2 \) | \( q_e,\text{cal} \) (mg g\(^{-1}\)) | \( k_2 \) (g mg\(^{-1}\) day\(^{-1}\)) | \( F^2 \) |
|--------------------------|--------------------------------------|-------------------------------------|-------------------------------------|--------------------------|--------|-------------------------------------|-------------------------------------|--------|
| Chironomid midge larvae  | 0.5                                  | 3.80                                | 1.75                                | 0.19                     | 0.997  | 1.90                                | 0.22                                | 0.9915 |
|                          | 1.0                                  | 7.80                                | 3.68                                | 0.19                     | 0.9963 | 3.78                                | 0.11                                | 0.9997 |
|                          | 2.5                                  | 17.9                                | 8.34                                | 0.12                     | 0.9491 | 8.12                                | 0.025                               | 0.8522 |
|                          | 5.0                                  | 22.0                                | 9.60                                | 0.17                     | 0.9775 | 10.66                               | 0.033                               | 0.9661 |

4. CONCLUSION

In this study, we have provided an useful information for the sorption of Ni(II) and Cr(III) from wastewater using C. y. larvae. As far as author knows, any sorption studies have not dealt with C. y. larvae as a sorbent material until now. It was found that the population of C. y. larvae larvae declined sharply when exposed to Cr(III). However, C. y. larvae were less affected by exposure to the Pb(II). The sorption efficiency of C. y. larvae for Ni(II) was highly concentration dependent, and the maximum sorption efficiency of Ni(II) at equilibrium was approximately 44–80 % in the studied concentration ranges. Isotherm studies showed that the Langmuir isotherm model were more suitable for describing the sorption system of Ni(II) and C. y. larvae. The sorption kinetic studies demonstrated that the process for sorption of Ni(II) followed the pseudo-first-order kinetic model, which provided the best fit for the experimental data. It reveals that C. y. larvae can be a good candidate as a sorbent for removal of Ni(II) in wastewater.

ACKNOWLEDGEMENTS

This research was supported by the Basic Science Research program(NRF-2017R1D1A1B03034241) through the National Research Foundation of Korea(NRF) funded by the Ministry of Education, Science and Technology.
REFERENCES

[1] Nietro-Marquez, A., Pinedo-Flores, A., Picasso, G., Atanes, E., and Kou, R. S. 2017. Selective adsorption of Pb\textsuperscript{2+}, Cr\textsuperscript{3+} and Cd\textsuperscript{2+} mixtures on activated carbons prepared from waste tires. J. Environ. Chem. Engineer. 5: 1060-1067.

[2] Gupta, V. K., Nayak, A., Agarwal, S., Chaudhary, M., and Tyagi, I. 2014. Removal of Ni(II) ions from water using scrap tire. J. Molecular Liquids 190: 215-222.

[3] Zhou, Y., and Haynes, R. J. 2011. Removal of Pb(II), Cr(III) and Cr(VI) from aqueous solutions using alun-derived water treatment sludge. Water Air & Soil Pollut. 215: 631-643.

[4] Bradil, H. B. 2004. Adsorption of heavy metal ions on soils and soil constituents. J. Col. & Interface Sci. 277: 1-18.

[5] Garg, U., Kaur, M. P., Sud, D., and Garg, V. K. 2008. Removal of cadmium(II) from aqueous solutions by adsorption on agricultural waste biomass. J. Hazard. Mater. 154: 1149-1157.

[6] Lasheen, M. R., Ammar, N., and Ibrahim, H. S. 2012. Adsorption/desorption of Cd(II), Cu(II) and Pb(II) using chemically modified orange peel: Equilibrium and kinetic studies. Solid State Sciences 14: 201-210.

[7] Martins, A. E., Pereira, M. S., Jorgette, A. O., Martins, M. A. U., Silva, R. L. V., Saeki, M. J., and Castro, G. R. 2013. The reactive surface of Castor leaf [Ricinus Communis L] powder as a green adsorbent for the removal of heavy metals from natural river water. Applied Surface Science 276: 24-30.

[8] Chen, J. H., Xing, H. T., Guo, L. X., Li, G. P., Weng, W., and Hu, S. R. 2013. Preparation, characterization and adsorption properties of a novel 3-aminoptyrotriethoxysilane functionalized sodium alginate porous membrane adsorbent for Cr(III) ions. J. Hazard. Mater. 248-249: 285-294.

[9] Ciopec, M., davidescu, C. M., Negrea, A., Grozav, L., Lupa, L., Negrea, P., and Popa, A. 2012. Adsorption studies of Cr(III) ions from aqueous solutions by DEHPA impregnated onto Amberlite XAD7-Factorial design analysis. Chem. Eng. Res. Des. 90: 1660-1670.

[10] Volesky, B. 1994. Advances in biosorption of metals: selection of biomass types, FERMS Microbiol. Rev. 14: 291-302.

[11] Say R., Yilmaz, N., and Denizli, A. 2004. Removal of chromium(VI) from aqueous solution by activated carbons: kinetic and equilibrium studies. J. Hazard. Mater. B 123: 223-231.

[12] Goyal, N., Jain, S. C., and Banerjee, U. C. 2003. Comparative studies on the microbial adsorption of heavy metals. Adv. Environ. Res. 7: 311-319.

[13] Kowalczyk, M., Hubicki, Z., and Kolodynska, D. 2013. Removal of heavy metal ions in the presence of the biodegradable complexing agent of EDDS from waters. Chem. Eng. J. 221: 6-10.

[14] Kumar, K. Y., Muralidhara, H. B., Nayaka, Y. A., Balasubramanyam, J., and Hanumanthappa, H. 2013. Hierarchically assembled mesoporous ZNO nanorods for the removal of lead and cadmium by using differential pulse anodic stripping voltammetric method. Power Technol. 239: 208-216.

[15] Demir, A., and Arisoy, M. 2007. Biological and chemical removal of Cr(VI) from waste water: Coat and benefit analysis. ScienceDirect 147: 275-280.

[16] Alguacil, F. J., Alonso, M., Lopez, F., and Delgado, A. L. 2008. Uphill permeation of Cr(VI) using Hostarex A 327 as ionophore by membrane-solvent extraction processing. Chemosphere 72: 684-689.

[17] Nasef, M. M. and Yahaya, A. H. 2009. Adsorption of some heavy metal ions from aqueous solutions on Nafion 117 membrane. Desalination 249: 677-681.

[18] Shaiden, N. H., Eldemerdash, U., and Awad, S. 2002. Removal of Ni(II) ions from aqueous solutions using fixed-bed ion exchange column technique. J. Twain Inst. Chem. Eng. 43: 40-45.

[19] Bai, L., Hua, H. P., Fu, W., wan, J., Cheng, X. L., Ge, L. Z., Xiong, L., and Chen, Q. Y. 2011. Synthesis of a novel silica-supported dithiocarbamate adsorbent and its properties for the removal of heavy metal ions. J. Hazard. Mater. 195: 261-275.

[20] Ruta, F. L., Ribeiro, V. C., Soares, L. M., Costa, C. C., and Nascentes, C. 2012. Efficient removal of Cd\textsuperscript{2+} from aqueous solutions using by-product of biodiesel production. 237-238: 170-179.

[21] Kirkelund, G. M., Damoe, A. J., and Ottosen, L. M. 2013. Electrodeionytic removal of Cd from biomass combustion fly ash suspensions. J. Hazard. Mater. 250-251: 212-219.

[22] Tang, X. Li, Z., and Chen, Y. 2009. Adsorption behavior of Zn(II) on calcinated Chinese loess. J. Hazard. Mater. 161: 824-834.

[23] Kim, K. H., Keller, A. A., and Yang, J. K. 2013. Removal of heavy metals from aqueous solution using a novel composite of recycled materials. Colloids and Surfaces A: Physicochemical and Eng. Aspects 425: 6-14.

[24] Fan, H. J., and Anderson, P. R. 2005. Copper and cadmium removal by Mn oxide-coated granular activated carbon.
Sep. Purif. Technol. 45: 61-67.

[25] Mo, H. H., Son J., Ryoo, K. S., Bae, Y. J., and Cho, K. J. 2018. Burrowing behavior of Chironomus yoshimatsui larvae as an indicator of freshwater quality. Ecological Indicators 85: 377-382.

[26] Choi, J., Roche, H., and Caquet, T. 2000. Effects of physical (hypoxia, hyperoxia) and chemical (potassium dichromate, fenitrothion) stress on antioxidant enzyme activities in chironomus riparius Mg. (diptera, chironimidae) larvae: potential biomarkers. Environ. Toxicol. Chem. 19: 495-500.

[27] Sildanchandra, W., and Crane, M. 2000. Influence of sexual dimorphism in chironomus riparius meigen on toxic effects of cadmium. Environ. Toxicol. Chem. 19: 2309-2313.

[28] Beaty Jr, T. V., and Hendricks, A. C. 2001. The relationship of chironomus riparius larval Se body burden and body concentration to larval dry mass and effects on sensitivity to selenium. Environ. Toxicol. Chem. 20: 1630-1640.

[29] Fargašová, A. 2001. Winter third- to fourth-instar larvae of Chironomus plumosus as bioassay Tools for assessment of acute toxicity of metals and their binary combinations. Ecotoxicol. Environ. Safety 48: 1-5.

[30] Baek, M. J., Yoon, T. J., and Bae, Y. J. 2012. Development of glyptotendipes tokunagai (diptera: chironomidae) under different temperature conditions. Environ. Entomol. 41 (4): 950-958.