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Heavy metals effect on unviable larvae of *Dicrotendipes simpsoni* (Diptera: Chironomidae), a case study from Saguling Dam, Indonesia

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Abstract. Heavy metals pollution can create negative effects on living organisms, as a teratogenic effect, which result in reproduction disorder that caused unviable embryos, mortality, etc. These unviable embryos can occur in the chironomidae of Saguling Dam, this being assumed as a result of heavy metals pollution in the dam. This study was aimed to analyze the relationship between the occurrence of heavy metals in the water surface of Saguling Dam with the unviable head morphology of insect larvae of diptera chironomidae: *Dicrotendipes simpsoni*. The study was done descriptively. Data of water quality (heavy metals) and *D. simpsoni* larvae were analyzed with Principal Component Analysis (PCA) and then followed by simple correlation of Pearson Product Moment. This study found that Saguling Dam has been polluted by heavy metals resulting in some deformations in chironomidae larvae. The results of the study revealed that most of chironomidae suffered deformation, mainly on the head part. Antennal deformities were most likely to be characterized by high Cr concentration ($r = 0.7099$). Total deformities had a tendency to be characterized by Pb ($r = 0.7055$), and there is a high probability that pecten epipharyngis deformities was caused by Cu ($r = 0.6131$).
Key Words: pollution, heavy metals, teratogenic agents, deformities, Chironomidae.

Introduction. Saguling Dam is one of three lakes located in West Java which has a big influences on biodiversity as well as human life, especially in West Java. Yet, nowadays Saguling Dam is facing serious problems, such as high sedimentation process, declined water quality due to contamination of organic matter, heavy metals, pesticide, and so forth. These pollutants have negative effects on the living organisms in the lake, they cause unexpected fish mortality, eutrophication, and so on. The most significant negative impact experienced by fish farmers in the lake is mass mortality in different fish species that can lead to losses of thousand of tones. At the moment, it is assumed that the mortality was caused by the process of upwelling, which rise the toxic pollutants from the sediment up to the water column. However, more important are the pollutants that lead to the result of indirect mortality (not acute), as these pollutants may create some complicated problems. One of the pollutants is represented by heavy metals. In this case, although the heavy metals enter the water system in the concentration below the background level, if they enter the system continuously, they will cause serious problems. These heavy metals may become teratogenic, which may result in unviable embryos.

There are various heavy metals entering the Saguling Dam (Riani 2010a, 2010b). However, there are certain heavy metals with high concentrations which have been found in the lake, namely Hg, Cu, Zn, Cr, Cd, and Pb. The dynamics of these heavy metals in the water has been studied by many researchers, mainly in monitoring heavy metal pollution. The metals in the water may change and they merely depend on the environmental conditions and climate. For example, the concentration of heavy metals in rainy season is lower than the concentration in dry season. This is because in the rainy season, dilution process occurs. Meanwhile in dry season, the concentration of heavy metals becomes higher, as the metals are concentrated. However, as in the body of organisms, the heavy metals will be concentrated in certain life tissues, which one of them is gonad. This, in turn can bring to teratogenic effect that create a variety of disablement of resulted embryos (Riani & Cordova 2011).

There are numerous aquatic organisms living in the water. However, the organisms that are most frequently used and recommended for the monitoring of water quality are some benthic macro-invertebrates larvae, as diptera chironomidae (Lenat & Barbour 1994). Researches on chironomid deformation have been done many times in many countries: in Belgium - Meregalli et al (2000), Vermeulen et al (2000) and Bervoets et al (2004); in Denmark - Vermeulen et al (1998); in Canada - Liber et al (2007) and Baird et al (2007); Columbus, Georgia - Banning (2010); in Malaysia - Al-Shami et al (2011); in Argentina - Cortelezzi et al (2011); in France - Arambourou et al (2012); in Germany - Galluba et al (2012); in Italy - Di Veroli et al (2010).

However, in Indonesia, chironomid deformation research does not exist yet. Therefore, this study was aimed to analyze the relationship between the occurrence of heavy metals in the water surface of Saguling Dam (West Java) with unviable head morphology respond of insect larvae of diptera chironomidae: *Dicrotendipes simpsoni*.

Material and Method. The study was conducted in Saguling Dam, one of three cascade dams on Citarum River, West Java, Indonesia. This is the first dam on Citarum River, where Cirata and Jatiluhur dams are in downstream of Saguling. Saguling Dam has received amount of pollutans most from the upstream area of the Citarum River where along its waterside is used for multipurposes practices, such as agricultural, domestic and industrial activities. Due to this reason, the water of river entering Saguling Dam is tend to be the high contaminated water. This research was an advanced study of the samples which were taken formerly. The larvae samples were taken during the period of May to July 2002, and the identification of chironomidae larvae was carried out from August to November 2003. A total of three sites in Saguling Dam area were visited (Figure 1). Those observation sites were site 1 that was located in Batujajar region, closed to Citarum River outlet, site 2 was in Ciminyak region, the most dense floating cage farming region, and site 3 was located in the dam building area, an outlet of lake and a dangerous area (with water depth more than 50 m).

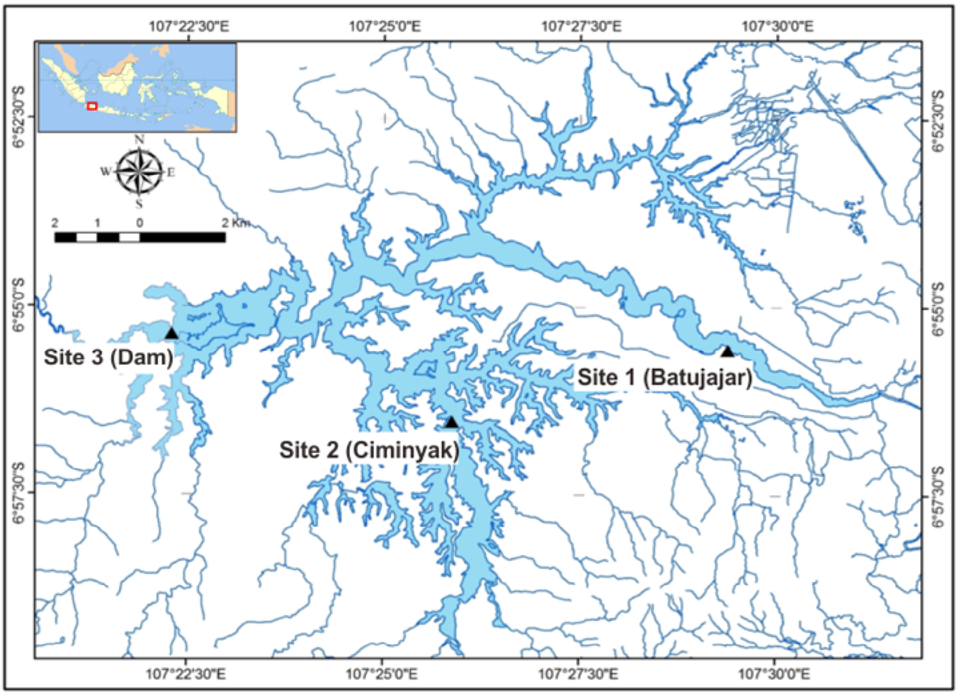


Figure 1. Map of Saguling Dam showing different observation sites.

The larvae sampling was conducted once a month in three months (May to July 2002) in each site. The chironomidae larvae were collected by submerging the artificial drifted media, which was an artificial substrate placed on an iron frame (30 x 30 cm). The frame was equipped by a plastic net with mesh size of 2 mm. This artificial substrate was submerged in between floating cages at a water depth of 2 m for one month. After that, the larvae were collected from the plastic net and preserved with 4% formalin. The collected chironomidae larvae were then identified (and at the same time were sorted) by using a stereo microscope at 15-45x magnification. The species (*D. simpsoni*) was identified according to Hawking & Smith (1997) and Epler (2001).

Larvae preservation was done by mounting. For unviable types determination purpose, 15 individual larvae of chironomidae from instars IV were taken from each artificial substrate (Meregalli et al 2000). For the purpose of larvae identification, clearing was first done, so that their internal tissues become minimum (Vermeulen et al 2000), subsequently, dehydration process, and then blood smear was created on object glass, with ventral side faced up. The specimen was then identified for its unviable types using fluorescence microscope at 100-1000x magnification. The observed deformation included total deformities, mentum, antennal, mandible and pecten ephipharyngis deformities.

In addition to deformities, heavy metal concentrations were also measured. The collected data were then analyzed by Principal Component Analysis (PCA) using MVSP software, version 3.1 (Kovach 1999). The correlation among the accumulated heavy metal variables (Hg, Cu, Zn, Cr, Cd and Pb) and deformities observed on different parts of *D. simpsonii* larvae were analyzed by using the simple correlation of Pearson Product Moment ($p = 0.05$).

Results and Discussion. The observation results of heavy metals (Hg, Cu, Zn, Cr, Cd, and Pb) in Saguling Dam were in the range of 0.001–0.003 mg L^{-1}; 0.004–0.010 mg L^{-1}; 0.023–0.027 mg L^{-1}; 0.005–0.011 mg L^{-1}; 0.006–0.013 mg L^{-1}; and 0.012–0.017 mg L^{-1}, respectively (Table 1). Concentrations of several heavy metals in the water in each observed site were above the background level of class C appointed by Government Regulation of Republic of Indonesia Number 20 Year 1990. The high concentration of heavy metals in the lake will enter the body of aquatic organisms that live in the lake, including chironomidae, and then it will accumulate in their body tissues, so that it may create negative effect on the organisms.

Table 1

Average concentration of heavy metals in Saguling Dam, West Java*

No	*Heavy metals*	*Observed sites*			*Average range*	*Background level of class C (Indonesian Law No. 20)*
		Batujajar	*Ciminyak*	*Dam*		
1	Hg (mg L^{-1})	0.001	0.003	0.001	0.001–0.003	< 0.001
2	Cu (mg L^{-1})	0.010	0.004	0.004	0.004–0.010	< 0.02
3	Zn (mg L^{-1})	0.027	0.027	0.023	0.023–0.027	< 0.02
4	Cr (mg L^{-1})	0.011	0.003	0.005	0.005–0.011	< 0.05
5	Cd (mg L^{-1})	0.007	0.013	0.006	0.006–0.013	< 0.01
6	Pb (mg L^{-1})	0.014	0.012	0.017	0.012–0.017	< 0.03

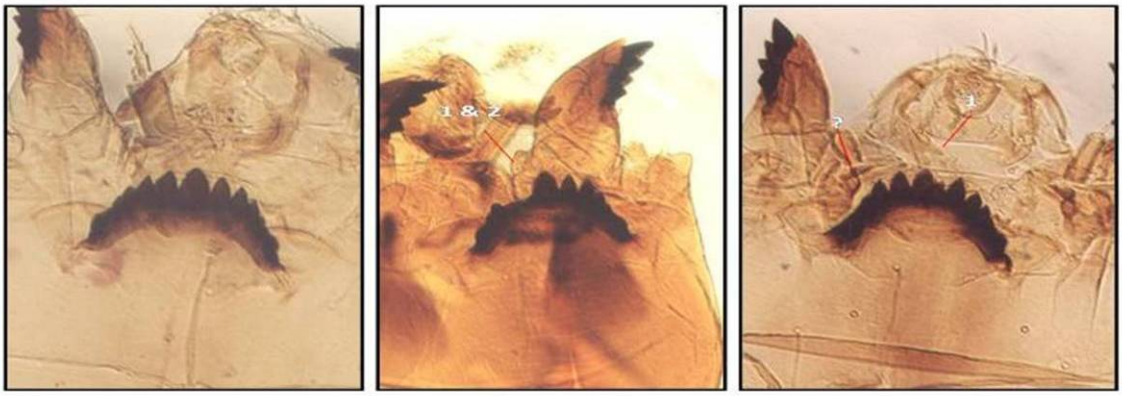
*Source: report results of water quality monitoring of Saguling Lake, and Government Regulation of Republic of Indonesia Number 20 Year 1990 regarding aquatic pollution control for background level class C.

The observation results of abnormalities showed that there were deformation on the observed organs, namely mentum, mandible, antenna and pecten ephipharyingis (Figures 2-5 and Table 2).

Table 2
Frequencies (%) of mouth and antennal deformities type in *D. simpsoni* in Saguling Dam

Sites	*Total deformities*	*Mandible deformities*	*Mentum deformities*	*Antennal deformities*	*Pecten epipharyngis deformities*
Batujajar	95.56	20	22.22	77.78	35.56
Ciminyak	68.89	31.11	11.11	13.33	28.89
Dam	82.223	22.22	4.44	66.67	35.56

Table 2 revealed that the highest total deformation frequencies are present in the chironomidae larvae collected from Batu jajar site, and then followed by Dam and Ciminyak sites. However, percentage of mandible teeth deformities in Ciminyak site was found relatively higher than in the other sites.



a b c

Figure 2. Normal mentum (a) and abnormal mentum (b and c) (teeth loss from mentum (b) and new additional teeth and asymmetric (c)).

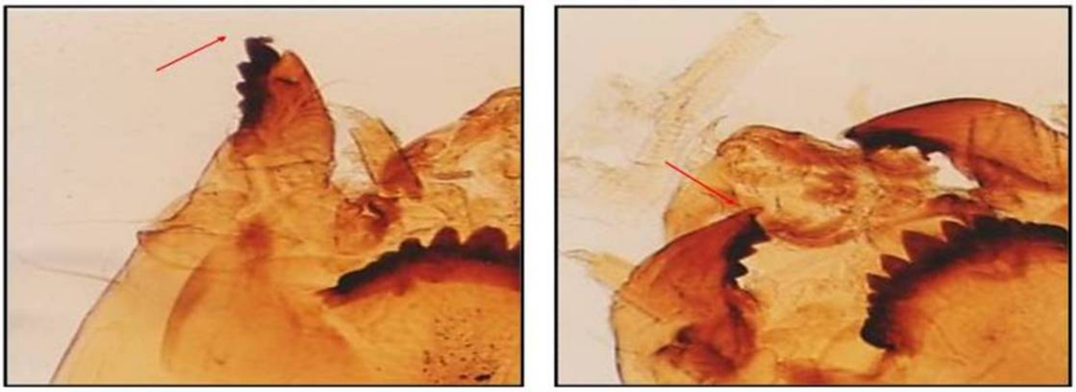


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Figure 3. Normal mandible (a) and abnormal mandible (one teeth loss from mandible) (b).

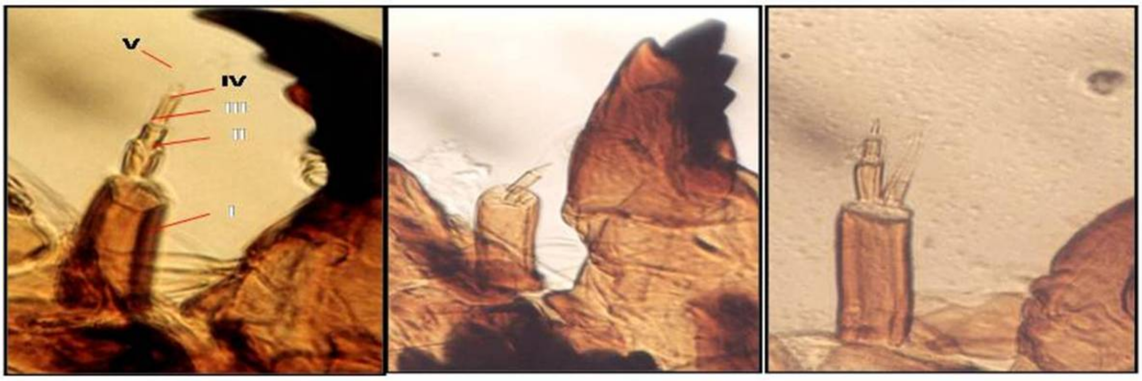

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Figure 4. Normal antenna (a) and abnormal antenna (segment loss on antenna) (b and c).

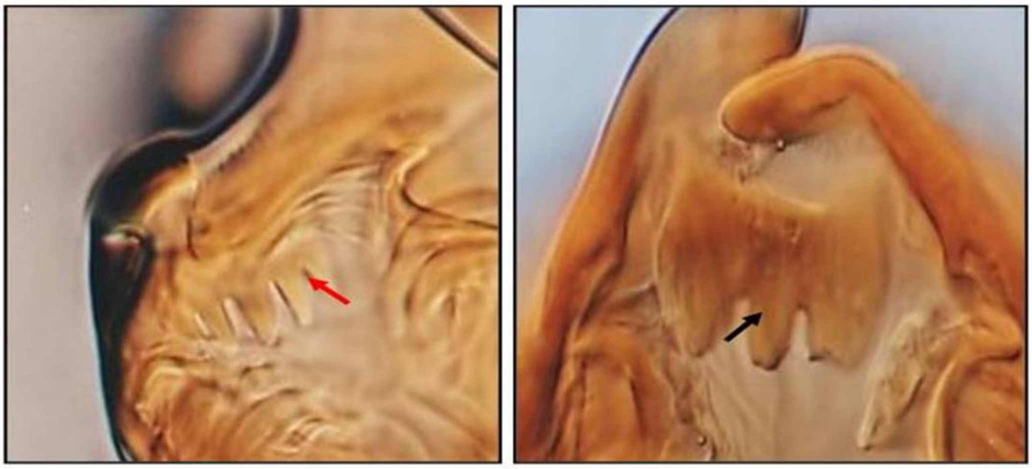

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Figure 5. Normal pecten epipharyngis (a) and abnormal pecten epipharyngis (fused teethfrom pecten epipharyngis and increased pecten teeth width) (b).

The relatively high percent of deformities in chironomidae larvae in Batujajar site was most likely due to the high toxic pollutant matters, mainly heavy metals that came from Citarum River that enter to the inlet of Saguling Dam (Table 1). The source of heavy metals was likely from industrial activities that disposed their waste water into Citarum River. Additionally, according to the field observation, there was an indication that there were many small scale industries concentrating on tanned dead animal skin around Saguling Dam. In the tanning process Cr was used. The resulted tanned waste, containing Cr, was subsequently disposed into Citarum River. The river water will then enter the Saguling Dam. Therefore, deformities in chironomidae larvae was assumed to be caused by heavy metals, mainly Cr, which was coming from the tanned dead animal skin done by local community, especially who lives nearby the Saguling Dam. According to Mutukumaravel et al (2007), heavy metals on sub-lethal concentration may endanger the aquatic organisms. Vinodhini & Narayanan (2008) explained that Cr is a toxic heavy metal for fresh water organisms, such as carp (*Cyprinus carpio*). In carps body, Cr will be accumulated in body organs, such as gill, liver, kidney, and flesh. This, in turn, will result in unviable as it is teratogenic.

The source of high concentration of Pb in Saguling Dam was likely from terrestrial daily transportation, either in the vicinity or within the Saguling Dam. Just like Cr, Pb in the aquatic ecosystem may also be dangerous to aquatic organisms. It is because Pb is a heavy metal with high toxicity on living organism (Dartmouth Toxic Metals Research Program 2001). The high concentration of this metal in the gill of fish is positively correlated with the organ damage level, even can cause animal death (Riani 2010a,b,c; Tresnati & Djawad 2012), and the metal within the aquatic organisms body will cause ion absorption disability (Riani & Cordova 2011; Zeitoun & Mehana 2014). Besides, Pb is teratogenic, so that it may result in aquatic animal embryos deformation (Jezierska et al 2000).

The analysis results of head deformation types, including antennal, mentum teeth, mandibles, and pecten epipharyngis comb deformities (Figures 2-5), showed deformation types in *D. simpsoni* were segment losses on antenna, from one to two segment losses. Mentum deformities in the animals, included teeth loss from mentum and new additional teeth (it will not happen in normal condition). Mandible teeth and pecten epipharyngis deformities, included increased pectin teeth width; according to Meregalli et al (2000) that was one of the indicators of sub-lethal toxicity responses, as the cause of toxic pollutant (e.g. heavy metals) exposures. This was supported by studies results of de Bisthoven et al (1992, 1998) and Dickman et al (1992) that showed the positive correlation between deformation frequency and accumulation level of pollutants, such as heavy metals and poly-aromatic hydrocarbon (PAH), in the body of deformed chironomidae.

The result of multivariate analysis (using PCA) and simple correlation Pearson Product Moment (that correlated heavy metal concentration, mg L^{-1}, and percentage of organ deformities showed that antennal deformities percentage was likely characterized by high concentration of Cr (significant correlation value = 0.7099 and p = 0.032, with 95% confidence interval). The antennal deformities were probably due to the occurrence of unviable embryos and this was aggravated by direct contact of heavy metals, occurred in the water, in the contact with the antenna. This was supported by Warwick (1985) statement that antennal deformities were caused by direct contact between antenna and toxic pollutant, including the dissolved pollutant in the water.

Total deformation was likely to be characterized by high concentration of Pb with correlation value of 0.7055 (p = 0.034). This was parallel to Hudson & Ciborowski (1996) statement that Pb may cause head structure change.

Pecten epipharyngis deformities were inclined to be caused by Cu with correlation value of 0.6131 (but not significant at p level = 0.05). This was contrary to the study result of Kosalwat & Knight (1987) who showed that there was a significant linear correlation between pecten epipharyngis deformities percentage and Cu concentration on 95% confidence interval. The insignificant correlation was probably due to the different animal that was tested (chironomidae larvae), so that they have the ability to respond differently to toxic agent. According to Razak (1980) fishes were very sensitive to Cu because they do not have effective defenses in Cu absorption process, and it is toxic to algae and mollusks. While according to Connell & Miller (1995) that Cu can be accumulated by marine organisms with concentration factor of 5000 times in mollusk and 100 times in fish.

In this study, it was identified that Cd causes relatively little effect on mentum deformities. It was different from the study results of de Bisthoven et al (1998) Cd with concentration of 9 µg and 27 µg caused mentum deformities in *Chironomus*. This was probably due to the Cd concentration in the water was still tolerable by *D. simpsoni* larvae, as the result, it does not cause deformation. Meanwhile, in the study done by Bisthoven et al (1998), Cd concentration was not tolerable by the organisms which resulting relative high mentum deformities. In addition, it was also probably because the type of tested organisms were different, so that, there were different responses to Cd.

Conclusions. Insect larvae deformation in diptera chironomidae *D. simpsoni* was an insensitive signal in indicating stress that was caused by the complexe influence of several pollutants from Saguling Dam. There was a significant correlation between heavy metals contamination (represented by Cr and Pb) and frequency as well as total antennal deformities, with correlation values of 0.7055 and 0. 710, respectively, in 95% confidence interval (p = 0.05). Moreover insect larvae of diptera chironomidae, a macro-benthic invertebrate organism, can be used as an important aquatic bio-indicator in Indonesia.

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