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ARAŞTIRMA MAKALESİ RESEARCH PAPER

The Evaluation in Different Temperature of Acute Toxic Effect of Cadmium on *Gammarus pulex* (Freshwater Amphipoda)

Osman SERDAR[1]* **Rahmi AYDIN[2]** **Metin ÇALTA[3]**

[1]* *Munzur Üniversitesi Su Ürünleri Fakültesi, 62000 TUNCELİ.* : https://orcid.org/0000-0003-1744-8883
[2] *Munzur Üniversitesi Tunceli MYO, Bitkisel ve Hayvansal Üretim Bölümü, 62000 TUNCELİ.* : https://orcid.org/0000-0002-3002-0892
[3] *Fırat Üniversitesi Su Ürünleri Fakültesi, 23000 ELAZIĞ.* : https://orcid.org/0000-0002-1652-8972

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Abstract: In this study, the LC_{50} values of the cadmium (Cd) toxicity on the *Gammarus pulex* were investigated. In this study, it was aimed to determine the change of LC_{50} values of Cd at *G. pulex* at 10, 14 and 18°C.The lethal concentration (LC_{50}) values for *G. pulex* which were exposed to various Cd concentrations for 96 hours were determined at different temperatures as 10, 14 and 18ºC. LC_{50} values obtained by probit analysis were found to be; 51.79 ± 1.2 µg L^{-1} for 10ºC, 47.67 ± 0.6 µg L^{-1} for 14ºC and 33.93 ± 0.6 µg L^{-1} for 18ºC. LC_{50} values were found to decrease due to the increase in temperature.

Keywords: Acute toxicity, cadmium, *Gammarus pulex*, temperature.

Kadmiyumun *Gammarus pulex* (Tatlısu Amfipodu) Üzerine Akut Toksik Etkilerinin Farklı Sıcaklıklarda Değerlendirilmesi

Öz: Bu çalışmada, *Gammarus pulex* üzerinde kadmiyum (Cd) toksisitesinin su sıcaklığına bağlı akut toksisite etkilerinden LC_{50} değerleri araştırıldı. Yapılan bu çalışma ile Cd'un *G. pulex*'te 10, 14 ve 18°C sıcaklıklardaki LC_{50} değerleri değişiminin tespit edilmesi amaçlanmıştır. Çeşitli Cd konsantrasyonlarına 96 saat boyunca maruz kalan *G. pulex* için LC_{50} değerleri, 10, 14 ve 18ºC olarak farklı sıcaklıklarda belirlendi. Probit analizinden elde edilen LC_{50} değerleri; 10ºC için, 51.79 ± 1.2 µg L^{-1}, 14ºC için 47.67 ± 0.6 µg L^{-1} ve 18ºC için 33.93 ± 0.6 µg L^{-1} olarak belirlenmiştir. LC_{50} değerleri, sıcaklık arttıkça daha düşük konsantrasyonlarda belirlenmiştir.

Anahtar sözcükler: Akut toksiste, *Gammarus pulex*, kadmiyum, sıcaklık.

INTRODUCTION

Heavy metals, which have increased concentration in water environment due to the effects of both natural resources and anthropogenic factors, can be carried by aquatic organisms and increased to upper trophic levels through the food chain (Hilmy et al., 1985; Nemsock & Hughes, 1988). While heavy metals cause mortality in high ambient concentrations, low ambient concentrations can cause various biochemical and physiological functions of aquatic organisms (Moraitou-Apostolopoulou and Verriopoulos, 1982; Heath, 1995).

Heavy metal pollution in aquatic ecosystems is one of the threats to ecosystem health and poses a great risk to aquatic organisms (Del Valls et al., 1998; Türkoğlu, 2008; Tokatlı, 2012).

Cadmium (Cd), which is one of the heavy metals with toxic effect in environmental pollutants, is very harmful for aquatic organisms even at low concentrations (Katalay and Parlak, 2002; Asri et al., 2007). In addition, Cd is the heavy metal element with the highest solubility in water. Therefore, the diffusion rate is high.

Cd, one of the heavy metals that have toxic effects in environmental pollutants, is very harmful to aquatic life even in low concentrations (Katalay & Parlak, 2002; Asri et al., 2007). The water solubility of Cd is one of the highest heavy metals. Therefore, the rate of diffusion is high. It is also not one of the elements necessary for human life. Due to its solubility properties, Cd^{+2} is taken into biological systems by plant and aquatic organisms and has the property of accumulation (Duffus, 1980).

One of the biggest problems facing the world today is increasing global warming. In particular, the increase in global heat caused by the excessive use of fossil fuels affects the whole world. Greenhouse gases, pesticides, heavy metals, increase as a result of human activities and play a negative role in aquatic environments and aquatic organisms (Mol & Doğruyol, 2012).

LC_{50}, which is one of the acute toxicity tests, is expressed as the amount of the substance that kills 50% of the organisms in a given environment for a certain period time. These studies are usually carried out in 24, 48, 72 and 96 hour periods (Çetinkaya, 2005).

Water pollution is known to have a direct effect on living organisms. For this reason, bioindicator species are used to determine the decrease in the environmental quality of water pollution (Kazancı et al., 1997). They are the aquatic organisms most affected by water pollution. The Gammarus, known to water indicators, are known to be sentive to many toxic substances. Therefore,it is use in toxicological studies is increasing Gammarus, known as clean water indicators, are knownto be sensitiveto many toxic substances. Therefore, it is use in toxicological studies is increasing (Demirsoy, 1998).

The aim of this study was to determine the acute toxic effect of Cd heavy metal which is widely used in today's industry, on *G. pulex* at different temperatures.

MATERIAL and METHODS

Chemicals: $CdCl_2$ used in the experiments was purchased from Sigma-Aldrich Chemical.

Test organism: The Gammaridae family is one of the most important invertebrate groups found in clean water resources (Cold and Forbes, 2004). This family plays a major role in the nutrients of freshwater ecosystems and is an important nutrient for fish species in the world (Rosenfeldt et al., 2015). Species identification of the organisms used in the research was made by Prof. Dr. Serap SALER, a faculty member of the Fisheries Faculty of Fırat University. *G. pulex* organisms they were collected with dip nets from side arms of Munzur River in Tunceli, Turkey. Before the experimental study, organisms were adapted in the stock aquarium for least 30 days in a climate-controlled indoor environment at 18 ± 0.5 °C and a 12:12 light: dark cycle. Throughout adapt time, individuals were fed on refuted willow leaves. The organisms were checked per 24 h.

Acute toxicity (LC_{50}): For all experiments carried out in this study, *G. pulex* samples that have reached reproductive maturity from adapted organisms were selected as healthy ones. Care was taken to ensure that the selected samples were male individuals who had reached the maturity of the sex and completed the shell change (W:0.08±0.001g, L:1.03±02mm). Ten organisms individuals were used for all concentration groups and replications, including the control group. In this study was used static non-renewal tests (Weber, 1991). Ten organisms were exposed in 1 L glass vessels to concentrations of different Cd concentrations of 96 h. LC_{50} values at temperatures of 10, 14 and 18 ºC were determined for *G. pulex* specimens after interval determination tests. The organisms were not fed during the experiments. The organisms were checked daily and dead individuals were counted and removed. LC_{50} experiments were repeated three times for each temperature.

Experimental design: The LC_{50} value was determined experimentally for each of 10, 14 and 18 ° C. Interval determination was performed for each temperature. Cd concentrations of µg per liter were prepared for temperatures (Table 1).

Table 1. Concentration ranges used to determine LC_{50}.

Temperature	Control	Concentrations (µg L^{-1})						
10°C	0.0	40.0	42.5	45.0	47.5	50.0	52.5	55.0
14°C	0.0	37.5	40.0	42.5	45.0	47.5	50.0	52.5
18°C	0.0	30.0	32.5	35.0	37.5	40.0	42.5	45.0

Statistical analysis: The obtained data were calculated by probit analysis in SPSS packet (ver. 24.0) program ($p<0.05$) and LC_{50} values were determined for 96 hours (Weber, 1991).

RESULTS and DISCUSSION

Gammarus species are used to develop models that can be used for risk assessment of varying chemical concentrations (Ashauer et al., 2007; Beketov & Liess, 2008; Demirci, 2018).

When the effect of temperature on the acute toxicity of Cd is examined, the LC_{50} values of Cd at 10, 14 and 18°C are shown in Table 1.

Mortality rates: In this study, the death rates of Cd concentrations were determined for 96 hours at 10°C (Figure 1). The average mortality rates in three replicated experiments were determined as 0., 13.3, 33.3, 43.3, 53.3, 63.3, 76.7 and 86.7, respectively, depending on the concentrations (0.0 (control), 45.0, 47.5, 50.0, 52.5, 55.0, 57.5 and 60.0 µg L^{-1} Cd).

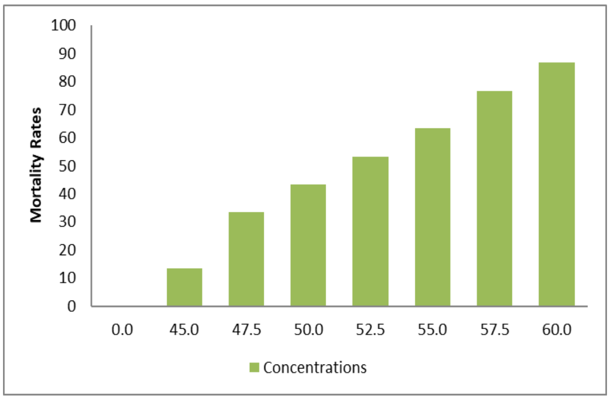

Figure 1. Mortality rates of *G. pulex* exposed to Cd at 10˚C for 96 h.

In this study, the death rates of Cd concentrations were determined for 96 hours at 14°C (Figure 2). The average mortality rates in three replicated experiments were determined as 0., 10.0, 23.3, 36.7, 53.3, 56.7, 73.3 and 90.0, respectively, depending on the concentrations (0.0 (control), 40.0, 42.5, 45.0, 47.5, 50.0, 52.5 and 55.0 µg L^{-1} Cd).

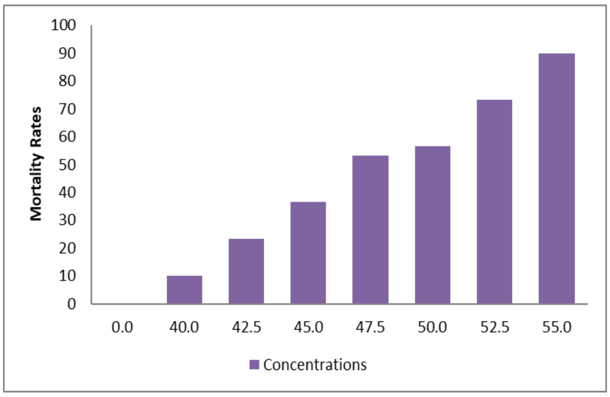

Figure 2. Mortality rates of *G. pulex* exposed to Cd at 14˚C for 96 h.

In this study, the death rates of Cd concentrations were determined for 96 hours at 18°C (Figure 3). The average mortality rates in three replicated experiments were determined as 0., 13.3, 33.3, 46.7, 56.7, 70.0, 76.7 and 86.7, respectively, depending on the concentrations (0.0 (control), 40.0, 42.5, 45.0, 47.5, 50.0, 52.5 and 55.0 µg L^{-1} Cd).

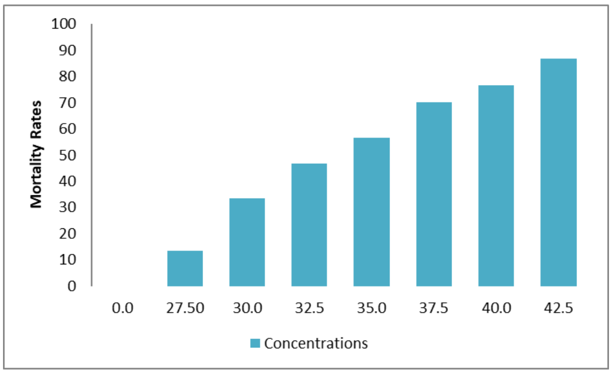

Figure 3. Mortality rates of *G. pulex* exposed to Cd at 18˚C for 96 h.

Acute toxicity values: The LC_{50} value of the Cd at 10°C was found to be 51.79 ± 1.2 µg L^{-1}, the lower band value was 49.03 ± 1.3 µg $L^{-1}$and the upper band value was 54.43 ± 1.3 µg L^{-1} (Figure 1).

The LC_{50} value of the Cd at 14°C was found to be 47.67 ± 0.7 µg L^{-1}, the lower band value was 45.27 ± 0.7 µg L^{-1} and the upper band value was 50.10 ± 0.7 µg L^{-1} (Figure 4).

The LC_{50} value of the Cd at 18°C was found to be 33.93 ± 0.7 µg L^{-1}, the lower band value was 31.39 ± 0.8 µg L^{-1} and the upper band value was 37.00 ± 0.8 µg L^{-1} (Table 2).

Table 2. LC_{50} values of Cd at temperatures of 10, 14 and 18°C belonging to *G. pulex* individuals

Temperature	LC_{50} Value (µg L^{-1})	Lower Band (µg L^{-1})	Upper Band (µg L^{-1})
10°C	51.79 ± 1.2	49.03 ± 1.3	54.43 ± 1.3
14°C	47.67 ± 0.7	45.27 ± 0.67	50.10± 0.7
18°C	33.93 ± 0.7	31.39 ± 0.8	37.00 ± 0.8

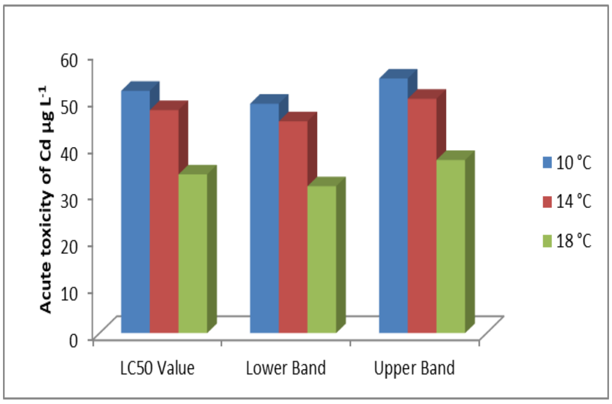

Figure 4. Temperature-dependent LC_{50} values of cadmium.

Toxicology examines the damage and destructive effects of physical or chemical agents on living organisms. In this context, aquatic toxicology tests aim to determine at what concentration any substance harms organisms on aquatic organisms (Karataş, 2005).

Bat et al., (2000) determined the LC_{50} values of zinc (Zn), copper (Cu) and lead (Pb) toxicity in freshwater amphipods at *G. pulex* at three different temperatures (15, 20 and 25 ° C). They reported that LC_{50} values were decreased with temperature. Zauke, (1982) investigated the relationship between Cd's acute toxicity to seasonal variation and environmental variables in *Gammarus tigrinus* natural populations and reported that there is a relationship between Cd concentration and water temperature. Piazza et al. (2016), conducted a study to evaluate the nature of the toxicity test in the study, in particular, the temperature and salinity changes in the presence of a toxic substance, and the environmental impact of information on the role of these parameters. Changes in temperature and salinity were observed separately, regardless of whether reference toxic substances were present, to obtain initial information the final test results. As a result, they reported that temperature and salinity were effective on organisms. Qiu & Qian, (1999) were indicated that *Amphitrite amphitrite* at the larval stage is significantly affected by temperature, as well as markedly by both survival and development. Nasrolahi et al. 2013, showed that model organism low temperature and low salinity stress affects larval growth after 7 and 40 days and that these environmental changes can directly affect. In this study, the changes in the toxic effect of the calculated Cd were found to interact with the temperature increase (Figure 1). In this study, similar results were found in the previous literature.

CONCLUSIONS

Many factors that cause chemical pollution arising from various industrial activities, which accumulated in living organisms, can be transported in ecosystems from in the lowest of the food chain to in the top ring chain of the food chain (Demirci, 2013).

Physiological factors, such as temperature can be an important actor in ecotoxicological analyses when exposed to the stressors of organisms (Piazza et al., 2016).

In this study, it was determined that Cd heavy metal has a toxic effect to *G. pulex* aquatic indicator living individuals even at very low concentrations. The effect of Cd on organisms increased with the increase in temperature. With this study, it is estimated that global warming, which is one of the environmental problems of today, will affect all aquatic and hence land life.

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***Corresponding author's:**
Osman SERDAR
Munzur Üniversitesi Su Ürünleri Fakültesi, 62000 Tunceli, Turkey.
E-mail : oserdar@munzur.edu.tr
ORCID : https://orcid.org/0000-0003-1744-8883
GSM : +90(505) 695 93 34