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Determination of Some Biochemical Parameters Changes in *Gammarus pulex* Exposed to Cadmium at Different Temperature and Different Concentration

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A B S T R A C T

In this study, the oxidative stress effects of cadmium (Cd) toxicity depending on water temperature were investigated on *Gammarus pulex*. The test organism individuals were exposed to sublethal concentrations for 96 hours at certain rates (C1; 1/10, C2; 1/20 and C3; 1/40) of LC50 values of Cd for each temperature (10, 14, 18 °C). Malondialdehyde (MDA) level, glutathione peroxidase (GPx), and catalase (CAT) enzyme activities were investigated at the temperatures determined in *G. pulex* exposed to Cd. With the increasing temperature, the MDA level and CAT enzyme activity increased while GPx enzyme activities decreased. The results of this study revealed that the biochemical response caused by Cd on *G. pulex* had statistically significant differences (p<0.05) with temperature. In this study, the use of MDA levels with CAT and GPx-related enzymes, Cd exposure, toxicity, and temperature change as biomarkers for risk assessment may be useful.

Keywords: *Gammarus pulex*, cadmium, malondialdehyde, glutathione peroxidase, catalase

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Farklı Sıcaklık ve Farklı Konsantrasyonlarda Kadmiyuma Maruz Bırakılan *Gammarus pulex*'te Bazı Biyokimyasal Parametrelerin Değişikliklerinin Belirlenmesi

Öz: Bu çalışmada *Gammarus pulex* üzerinde kadmiyum (Cd) toksisitesinin su sıcaklığına bağlı olarak oksidatif stres etkileri araştırılmıştır. Test organizması bireyleri, her sıcaklık (10, 14, 18 °C) için belirli oranlarda (C1; 1/10, C2; 1/20 ve C3; 1/40) LC50 değerlerinin 96 saat boyunca subletal konsantrasyonlara maruz bırakıldı. Cd'ye maruz kalan *G. pulex*'te belirlenen sıcaklıklarda malondialdehid (MDA) seviyesi, glutatyon peroksidaz (GPx) ve katalaz (CAT) enzim aktiviteleri araştırıldı. Sıcaklık arttıkça MDA seviyesi ve CAT enzim aktivitesi artarken GPx enzim aktiviteleri azaldı. Bu çalışmanın sonuçlarına göre, *G. pulex* üzerinde Cd'nin neden olduğu biyokimyasal yanıtın sıcaklık ile istatistiksel olarak anlamlı farklılıklara (p<0,05) sahip olduğu belirlenmiştir. Bu çalışmada, risk değerlendirmesi için biyobelirteç olarak MDA seviyelerinin CAT ve GPx ile ilişkili enzimler, Cd maruziyeti, toksisite ve sıcaklık değişimi ile kullanılması yararlı olabilir.

Anahtar kelimeler: *Gammarus pulex*, kadmiyum, malondialdehit, glutatyon peroksidaz, katalaz

Introduction

Aquatic environments are widely accepted today as a simple and inexpensive disposal option that is considered an ideal discharge area for most waste. This has led to increased ecological poisoning due to the bioaccumulation of toxic chemicals and some long-standing pollutants, including developed countries (Taylan and Özkoç 2007). Increasing industrial, urban, and agricultural developments increase the environmental problems of today and these environmental problems have a negative synergy effect with living things along with global warming. Heavy metals, which make up the majority of industrial pollutants, can enter into the structures of aquatic organisms through wastewater (Bat et al. 2000). One of the most important sources of ecosystem health is heavy metal pollution and it poses stress, threat, and a great risk to organisms in aquatic ecosystems (Del Valls et al. 1998). When a metal enters the biological system of any organism, it can damage the vital functions of that organism (Hu 2000; Kayhan 2006). Increasing concentrations of

heavy metals in aquatic environments are taken up by aquatic organisms and transported to upper trophic levels via food chains. Examination of heavy metal accumulation in living organisms living in the water environment is important in determining the species susceptible to heavy metals as well as in determining the structural and functional disorders that occur in the organism (Kayhan 2006).

Cadmium (Cd), one of the heavy metals with toxic effects in environmental pollutants, is very harmful to aquatic organisms even at low concentrations (Katalay and Parlak 2002; Asri et al. 2007). Cd is the heaviest metal element with the highest water solubility. For this reason, the broadcast speed is high. It is also not one of the necessary elements of human life. Because of its solubility property, it is released into biological systems by plant and aquatic organisms in the form of Cd^{+2} and has accumulation properties (Duffus 1980).

An indirect mechanism for the free radical production caused by Cd is suggested. Cd increases the amount of non-bound forms of these metals by taking the place of zinc (Zn), calcium (Ca), copper (Cu), and iron (Fe) in metalloenzymes. It binds to thiol groups of free radical scavengers such as glutathione (GSH) and inhibits antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). Although it is Fenton metal, it is thought that it causes the production of superoxide and nitric oxide, and many other free radicals and thus leads to peroxidation, DNA damage, and protein oxidation of cell membrane structures (Brzóska and Moniuszko-Jakoniuk 2001; Waisberg et al. 2003; Bertin and Averbeck 2006).

One of the methods of determining pollution in the aquatic environment is to determine the levels of the organisms living in the environment that are affected by this pollution. The evaluation of biological markers as an early warning of adverse changes and damage has been shown as a suitable tool for *Gammarus pulex* (Dat et al. 2000; Achary et al. 2008; Yildirim et al. 2018; Serdar 2019). Reactive oxygen species (ROS) suppress the antioxidant system, inducing oxidative stress, lipid peroxidation, and oxidation of proteins during metabolism (Almroth et al. 2008; Tatar et al. 2018; Cimen et al. 2020). There is an important balance between the production of ROS and the removal of antioxidant defense systems by organisms. ROS is cleared by antioxidant enzymes and non-enzymatic antioxidants (Hermes-Lima 2004; Halliwell and Gutteridge 2007; Serdar et al. 2018). Lipid peroxidation is chain reaction in which oxidants break down membrane phospholipids with polyunsaturated fatty acids. Lipid

peroxidation causes damage to bio-membranes, which can lead to significant consequences for living organisms (Hermes-Lima 2004; Jemec et al. 2012; Serdar et al. 2018).

This study was designed to determine the effect of pollution in the water environment on the organism in the water together with the global temperature with the development of the industrial sector. Therefore, it is aimed to experimentally determine the effect of sublethal Cd concentrations on *G. pulex*, a water organism, at different temperature levels.

Materials and Methods
Test Organisms
The test organism *G. pulex* used in the study was collected from the source part of the tributaries of the Munzur River of Tunceli province, with hand nets from the areas that are virgin in terms of domestic and industrial pollution. Before the experimental study, organisms were adapted at 10, 14, and 18 ± 0.5 °C, respectively, at the test temperature. Ambient lighting 12:12 bright: dark it is set to be climate controlled in its cycle. In this process, the test organisms were fed with rotten willow leaves. Organisms were checked daily.

Acute Toxicity (LC$_{50}$)
Serdar et al. (2019a) determined their Cd LC50 values in *G. pulex* at 10, 14, and 18 ºC. LC50 values for 10, 14, and 18 ºC temperatures are 51.79 ± 1.2 µg L^{-1} Cd, 47.67 ± 0.6 µg L^{-1} Cd, and 33.93 ± 0.6 µg L^{-1} Cd, respectively. LC50 values used in this study Serdar et al. (2019a)'s taken from the work they have done.

Experimental Design
It was recorded that the water temperature in the natural environment where the test organism was collected varied between 12 and 14 ºC throughout the year. In the study, experimental temperatures were chosen as 10, 14, and 18 ºC to prevent the test organism from adapting to laboratory conditions.

In this study, the test organisms were adapted for at least 25 days for each determined temperature. To minimize systematic errors, healthy, similar-sized (w: 0.0474 ± 0.0053 g and L: 10.35 ± 0.055 mm) and male organisms were selected.

Exposure of *G. pulex* to Sublethal Cd Concentrations
Sublethal concentrations in this study were chosen from Cd concentrations of 1/40, 1/20, and 1/10 ratios of LC50 values for each temperature. For this purpose, four different experimental groups, one of which is the control group, were created. These experimental groups created as follows are applied for each temperature experiment;

1. Group C0, control group, Cd-free
2. Group C1, 1/40 LC50 values of Cd
3. Group C2, 1/20 LC50 values of Cd
4. Group C3, 1/10 LC50 values of Cd

In the experimental design, glass aquariums and containing 0.5 L Cd concentrations were used. 15 test organisms were added to the aquariums for each group. Test organisms were exposed to three different Cd concentrations for 96 hours for each temperature group. All experimental procedures were performed in three replicates. Test organisms were not fed in all experimental procedures, including Cd toxicity tests.

Preparation of Homogenates

The *G. pulex* specimens for the preparation of homogenates were passed through pure water. A pool was created from 15 individuals to be able to form homogenates from the samples. After the water was removed with the drying paper, organisms were weighed and homogenized by diluting 1/10 in 1.15% potassium chloride (KCl). The obtained homogenates were centrifuged at +4 ºC for 15 minutes at 3200 rpm in a glass tube, after which the supernatants were separated.

Biochemical Analyses

Changes in the MDA levels were measured spectrophotometrically according to Placer et al. (1966) the modified method. The CAT activity was determined according to Aebi (1984). The determination of the GPx activity was made according to the method described by Beutler (1975). Protein quantities were determined according to Lowry et al. (1951) to calculate specific enzyme activities and MDA levels.

Biochemical analyzes have shown changes in oxidant/antioxidant parameters were evaluated by Two Way-ANOVA variance analysis.

Results

The MDA Level

The changes in MDA level of control and experimental groups in *G. pulex* which exposed to sublethal Cd concentrations were given in Table 1.

When the MDA level at 10 ºC was examined, C3 group was higher than the control and the difference between them was statistically significant ($p<0.05$). The MDA levels at 14 ºC were found to be higher in C1, C2, and C3 groups compared to the control, but the difference between them was statistically insignificant ($p>0.05$). The MDA levels at 18 ºC were found to be higher in C1, C2, and C3 groups compared to the control and the difference between them was statistically ($p<0.05$).

Belong to temperature the changes in MDA levels of control and experimental groups in *G. pulex* which exposed to sublethal Cd concentrations were given in Table 1.

When the MDA levels at 10, 14, and 18 ºC temperatures of control group samples were examined, the MDA level of the group 14 ºC was higher than at 10 and 18 ºC and the difference between them was statistically significant ($p<0.05$).

When the MDA levels at 10, 14, and 18 ºC temperatures of the C1 group samples were examined, the MDA level of group 10 ºC was lower than the other groups and between differences were found to be significant ($p <0.05$).

When the MDA levels at 10, 14, and 18 ºC temperatures of C2 group samples were examined, MDA level at 18 ºC was found to be statistically higher than 10 ºC temperature ($p <0.05$). The MDA level at 14 ºC was found to be similar to the temperature values of 10 and 18 ºC ($p>0.05$).

When the MDA levels of C3 group samples were examined at 10, 14, and 18 ºC temperature, the MDA level determined at all temperatures was not been shown any statistically significant difference ($p>0.05$).

Table 1. Changes in MDA levels at different Cd concentrations depending on temperature

Temperature (ºC)	Control	Sublethal Cd Concentration Groups		
	C0	C1	C2	C3
10	1.03 ± 0.4^{bcY}	0.75 ± 0.3^{cY}	1.41 ± 0.4^{abY}	1.85 ± 0.7^{aX}
14	1.93 ± 0.6^{aX}	1.86 ± 1.0^{aX}	1.81 ± 1.7^{aXY}	2.13 ± 1.7^{aX}
18	0.90 ± 0.3^{cY}	2.02 ± 1.0^{bX}	2.71 ± 0.2^{aX}	3.15 ± 1.0^{aX}

a, b, c: The difference between the values of different letters in the same row was statistically significant ($p<0.05$).
X, Y: The difference between the values carrying different letters in the same column was statistically significant ($p<0.05$).

The GPx Activity

The changes in the GPx activity of the control and experimental groups in *G. pulex* which exposed to sublethal Cd concentrations were given in Table 2.

The GPx activity at 10 ºC was higher in the C1, C2, and C3 groups than Control, and the difference between them was statistically significant (p<0.05). The GPx activity at 14 ºC was higher in the Control and C2 groups than C3 and C1 groups and the difference between them were statistically significant (p<0.05). The GPx activity at 18 ºC was higher in the C1, C2, and C3 groups than in the control group, and the difference between them was statistically significant (p<0.05).

Belong to temperature the changes in GPx activity in the C0, C1, C2, and C3 groups of *G. pulex* which were exposed to sublethal Cd concentrations were given in Table 2.

When the GPx activity of the control group was examined at 10, 14, and 18 ºC temperatures, the GPx activity was the highest at 10 ºC and the lowest at 18 ºC, and the difference between groups was statistically significant (p<0.05).

When the GPx activity of the C1 group was examined at 10, 14, and 18 ºC. The GPx activity was the highest in the C1 group. Between them, the difference was statistically significant (p <0.05).

When the GPx activity of the C2 group, at temperatures of 10, 14, and 18 ºC was examined in the GPx activity was the highest at 10 ºC and the lowest at 18 ºC and the difference between groups was statistically significant (p<0.05).

When the GPx activity of the C3 group at temperatures of 10, 14, and 18 ºC was examined GPx activity was lowest at 18 ºC. Between them, the difference was statistically significant (p <0.05).

Table 2. The GPx activity changes in Cd concentrations depending on temperature

Temperature (ºC)	Control	Sublethal Cd Concentration Groups		
	C_0	C_1	C_2	C_3
10	24.54 ± 0.4 [aX]	7.29 ± 0.2 [dY]	19.67 ± 0.7 [cX]	20.88 ± 1.6 [bX]
14	17.03 ± 0.2 [bY]	20.33 ± 0.9 [aX]	17.48 ± 0.9 [bY]	19.67 ± 0.7 [aX]
18	3.54 ± 0.8 [cZ]	7.15 ± 0.8 [bY]	11.64 ± 2.8 [aZ]	13.18 ± 0.3 [aY]

a, b, c, d: The difference between the values of different letters in the same row was statistically significant (p<0.05).
X, Y, Z: The difference between the values carrying different letters in the same column was statistically significant (p<0.05).

The CAT Enzyme Activity

The changes in the CAT activity of the control and experimental groups in the *G. pulex* organisms exposed to sublethal Cd concentrations were given in Table 3.

When the CAT activity at 10 ºC was examined, C2, and C3 groups it was found to be statistically higher than the control group (p<0.05). When the CAT activity at 14 ºC was examined, the C1 group was found statistically higher than the other groups (p <0.05). When the CAT activity at 18 ºC was examined, C2 and C3 groups were higher than the control group and the difference between them was found statistically significant (p<0.05).

Belong to temperature the changes in CAT activity of control and experimental groups in *G. pulex* which exposed to sublethal Cd concentrations were given in Table 3.

When the CAT activities of the control group were examined at 10, 14, and 18ºC temperatures, the CAT activity was higher at 14 ºC and the lowest at 18 ºC temperature, and the difference between groups was statistically significant (p<0.05).

When the CAT activities of the C1 groupwere examined at 10, 14, and 18 ºC temperatures, the CAT activity was higher at 14 ºC and the lowest at 18 ºC temperature, and the difference between groups was statistically significant (p<0.05).

When the CAT activities of the C2 group were examined at 10, 14, and 18 ºC temperatures, the CAT activity was the highest at 10 ºC and the lowest at 18 ºC, and the difference between groups was statistically significant (p<0.05).

When the CAT activities of the C3 group were examined at 10, 14, and 18 ºC temperatures, the CAT activity was the highest at 10 ºC and the lowest at 18 ºC, and the difference between groups was statistically significant (p<0.05).

Table 3. The CAT activity changes in Cd concentrations depending on temperature

Temperature (ºC)	Control	Sublethal Cd Concentration Groups		
	C_0	C_1	C_2	C_3
10	64.40 ± 40.4 [bY]	76.91 ± 60.7 [bY]	159.66 ± 66.6 [aX]	179.94 ± 78.8 [aX]
14	82.16 ± 33.7 [bX]	157.02 ± 13.1 [aX]	103.42 ± 39.0 [bY]	108.32 ± 32.7 [bX]
18	4.86 ± 1.8 [cZ]	9.20 ± 2.5 [cZ]	28.29 ± 3.6 [bZ]	45.32 ± 17.6 [aY]

a, b, c: The difference between the values of different letters in the same row was statistically significant (p<0.05).
X, Y, Z: The difference between the values carrying different letters in the same column was statistically significant (p<0.05).

Discussion

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 LC50 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 LC50 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 on the final test results. As a result, they reported that temperature and salinity were effective in organisms. Qiu and 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 organisms' low temperature and low salinity stress affect larval growth after 7 and 40 days and that these environmental changes can directly affect.

In the aquatic environment, pollution can cause toxic effects such as lipid peroxidation by increasing ROS production resulting from the imbalance between ROS concentration and antioxidant defense system (Regoli et al. 2004). Key antioxidant enzymes and non-enzymatic antioxidants are influenced by various single pollutants known to increase ROS levels (Valko et al. 2006; Ryter et al. 2007). Oxidative stress, detoxification, and neurotoxicity biomarkers were used in Gammarids (Yildirim et al. 2019).

Lipid peroxidation, considered as a valuable indicator of oxidative damage of cellular components known as the first step in cellular membrane damage, is caused by pesticides, metals, and other xenobiotics (Gamble et al. 1995; Regoli et al. 1998). Duman and Kar (2015) reported that the MDA content of Cd accumulation in organisms increased depending on the exposure concentration and duration. Similarly, in this study, the MDA levels were also increased in sublethal Cd concentration groups when compared to the control group ($p < 0.05$). Chandran et al. (2005) investigated how to change the MDA levels of Cd and Zn in *Achatina fulica*. They reported that the MDA levels increased with increasing Cd concentration compared to the study data. In this study, the MDA levels increased with the concentration of Cd, and the data of the study showed parallelism.

Vellinger et al. (2013) found that the MDA levels in *G. pulex*, where Cd and arsenic (As) were single and co-administered, were higher than the control group. They also reported a significant increase (67.2%) in the MDA levels with increasing Cd concentration in the individuals. In this study, it was found that the MDA levels increased due to both temperature and concentration increase. Sroda and Cossu-Leguille (2011) investigated the effect of seasonal changes on antioxidant markers in *Gammarus roeselli*. In parallel with the increase in temperature, the model reported the increase of MDA level in living organisms. In this study, MDA levels increased with temperature.

The GPx is a component of a complex antioxidant defense system and its response is possibly accompanied by responses of other antioxidant enzymes and scavenger molecules; however, its induction may provide an indication of defense against oxidative stress (Tsangaris et al. 2007). Inhibition of the GPx activity may reflect the failure of the antioxidant system in contact with polluting (Ballesteros et al. 2009) or may be related to the direct effect of superoxide radicals or pollutes on enzyme synthesis (Bainy et al. 1993). In this study, GPx activity in *G. pulex* exposed to Cd decreased at 10 ºC compared to the control group. As well as GPx activity decreased with the temperature of exposure to Cd in the *G. pulex* organism ($p < 0.05$). In this study, GPx activity decreased with increasing temperature of exposure to Cd in *G. pulex* organism ($p < 0.05$). Depending on the temperature, the reduction of observed GPx activities may be associated with decreased glutathione levels. This decrease in GPx activity in the study is in line with the studies performed (Serdar et al. 2019b; Kutlu and Susuz 2004). However, in another study, CAT activity increased with Cd exposure and this increase suppressed the increase in GPx (Zhang et al. 2011). The reason for the increase in GPx activity at 14 and 18 ºC temperatures in this study can be explained by the decrease in CAT activity.

The CAT is a very common enzyme found in virtually all living organisms that use oxygen. It acts in water and oxygen formation by catalyzing the decomposition of hydrogen peroxide (Chelikani et al. 2004). The elevation of these antioxidant enzymes would be critical in minimizing cellular injury

(Rajeshkumar et al. 2013). On the other hand, CAT activity may increase or decrease in contaminated environments depending on the substance (Sobjak et al. 2017). In previous studies, it had been expressed that ROS species can inhibit CAT activity (Kono and Fridovich 1982; Escobar et al. 1996; Duman and Kar 2015). In this study, while CAT activity decreased due to increasing temperature but increased due to increased concentration (Table 3) ($p<0.05$). Antioxidative stress activity can change depending on sexuality, physiological phase, and species (Felten et al. 2008; Zhang et al. 2011). However, it was found that Cd exposure concentration and exposure duration can also alter antioxidative stress activity (Duman and Kar 2015). They further revealed that short-term exposure to organic chemical pollutants leads to the induction of antioxidant enzymes in aquatic organisms. However, CAT activity was negatively affected by redox-cycling-inducing chemicals (Pandey et al 2008; Rajeshkumar et al. 2013). In this study, the CAT enzyme activity is inhibited by the organisms under stress with temperature and Cd exposure. Similar to the present study, decrease in CAT activity have been reported in aquatic organisms exposed to various pollutants (Thomas and Murthy 1976; Hasspieler et al. 1994; Sayeed et al. 2003; Zhang et al. 2004; Crestani et al. 2007; Yildirim et al. 2018). According to the results found in the literature related to the activity of this enzyme, potential antioxidant changes by species, habitat, etc. can be explained (Glusczak et al. 2007).

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

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

In this study, it was determined the oxidative response of exposure to at different temperature sublethal Cd concentrations of *G. pulex*, which is used as a clean water indicator in ecotoxicological evaluations. In this study, it can be concluded that stress conditions provided by Cd exposure at sublethal concentrations and different temperatures evoked specific responses in *G. pulex*. Therefore, the above results indicate that Cd and temperature an environmental pollutants as oxidative stress.

Acknowledgments

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