Metal Accumulation and Biomarker Responses of Odanata Larvae, *Ischnura elegans* (Vander Linden, 1820) Exposed in a Lead-Zinc Mining Area in Turkey

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Abstract. This study was conducted in September 2014 to determine the effects of metal accumulation on the Odanata larvae which is a freshwater macro-invertebrate. Polluted area in the lower part of the mine founded on Umurbey Stream (Çanakkale, Turkey) and unpolluted area in the upper part of it are defined as the sampling stations. In this study, GSH (Glutathione), TBARS levels and Na^+, K^+ -ATPase activity were measured after the determination of metal accumulation (Cd, Cu, Fe, Pb, Zn) in the water and in the Odanata larvae, *Ischnura elegans* (Vander Linden, 1820). There was a decrease in Na^+, K^+ -ATPase activity; although the increase in GSH and TBARS levels in organisms sampled from polluted area. These results indicate that; metal accumulation caused to oxidative stress in Odanata larvae *I. elegans* and this organism reacted by running the compensate mechanisms for it.

Keywords: Metal accumulation, Biomarkers, Oxidative stress, Umurbey Stream, Odanata, *Ischnura elegans*

1 Introduction

Aquatic resources are gradually polluted by the natural and anthropogenic effects, day by day. Metals are one of the most important reasons of inorganic pollution in aquatic environments (Selvi, 2015). Metals which are discharged to aquatic ecosystem do not only dissolve in water, also accumulate in the food chain by taking aquatic organisms or sink to the bottom depending on the environmental conditions (Rainbow, 2002).

Benthic macro-invertebrates as Odanata larvae play a major role in ecosystems and take up the metals in different stages of the food chain (the food cycles,

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decomposition and production) -whether essential or not- from water, sediments and foods (Rainbow and Wang, 2001). By accumulating within the tissue of such organisms; metals may lead to irreversible and adverse changes in later stages in molecular level. Many chemical pollutants such as heavy metals cause the production of reactive oxygen species (ROS) which in turn result in oxidative stress (Stohs and Bagghi, 1995).

Umurbey Stream passes and located on the northeast of Çanakkale, is fertile for lead and zinc; it would be metal discharges into water from the rocks. So it causes deterioration of water quality and accumulation of metal in macro-invertebrates. In terms of assessing the ecological status of the river ecosystem of Umurbey Stream; there is no study that examines the effects of the pollution on the invertebrate physiology as well as the accumulation of the heavy metals available. In this study, the physiological effects of the metal pollution in Umurbey Stream, resulted from the Pb-Zn mine, established in the location, on macro invertebrates (*Ischnura elegans* Vander Linden, 1820) were analyzed by using biomarkers. For this purpose; the analyses of the physico–chemical parameters of the water, analyses of the heavy metals in water and invertebrate tissue and the biomarker analysis in the tissue were conducted.

2 Material and Methods

Umurbey is a stream which varies flow rate and water carrying capacity depending on the seasons, arises from the Koru Mountain that is located within the boundaries of Umurbey town (Çanakkale) and flows into the Dardanelles. However, there is a mine founded on Umurbey Stream (Çanakkale, Turkey) to obtain lead and zinc by utilizing this water resource.

Water samples and Odanata larvae were collected from the lower part (polluted area) and the upper part (unpolluted area) of the mine in September, 2014. The locations of the sampling sites are shown in Figure 1.

2.1 Physico-Chemical Analysis

The temperature (T), pH, electrical conductivity (EC), salinity (S) and dissolved oxygen (DO) of each water sample were measured at the sampling points by a pH meter and oxygen meter, respectively.

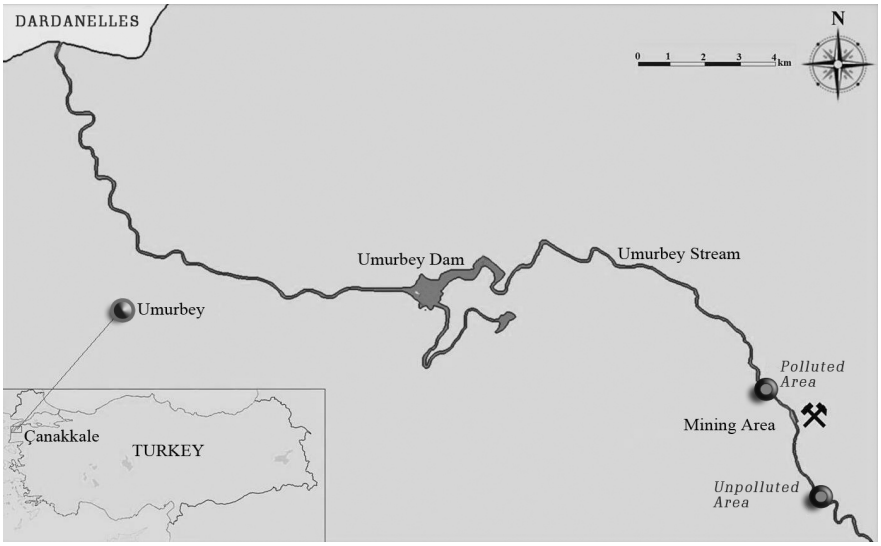

Fig. 1. Sampling Stations on Umurbey Stream

2.2 Metal Analysis in Water and Organisms

Water samples taken from the stations were filtered PTFE (0.45 mm pore size) for metal analysis and they were determined from ICP-OES Varian Liberty Sequential. However, the organisms were dried at the incubator set to 70°C for 24 hours after measuring the wet weight. Subsequently, dry weights of samples were weighted. Then, samples were burned over a hot-plate set to 70°C for an hour, following the addition of 5 ml HNO_3. After the samples were burned homogenously and cooled, they were filtered in a 0.45 μm syringe and diluted to 20 ml with distilled water (Smith et al., 2007). The metal analyses in organisms were determined with ICP-OES Varian Liberty Sequential.

2.3 Biomarker Analysis

Total glutathione (GSH) was determined according to Owens and Belcher (1965) method. Shortly, 40 μl homogenate, blank or standard, was added in triplicate to a microplate well containing 20μl of DTNB, 260 of assay buffer (K_2HPO_4, EDTA, pH7.5), and 20μl of glutathione reductase. Their action was started by the addition of 20μl of NADPH, with changes in absorbance at 412 nm (Thermo Multiscan FC, microplate reader) recorded over 10 min, and total GSH (μmol g^{-1} wet weight tissue) determined using the standard calibration curve (Smith et al., 2007).

Thiobarbituric Acid Reactive Substances (TBARS) assay was performed according to Camejo et al. (1998). Shortly, samples were defrosted and homogenized (Stuart SHM1, Homogenisator) in 5 volumes of buffer (Sucrose, EDTA, Hepes, adjusted to pH 7.4 with 20 mM Tris). Then 200 μL of homogenate was added to a

well of a 96-well microplate containing phosphate buffer. Following this, TCA and thiobarbituric acid were added, the plate was incubated at 60°C (1 hour) and then cooled on ice. Absorbances were recorded (in triplicate) at 530 nm in the microplate reader (Thermo Multiscan FC, microplate reader). All data from the assays were calculated per mg of cell protein. Protein was determined in 20 μL of each homogenate (in triplicate) according to Bradford, 1976. Samples were read at 595 nm (Optizen spectrophotometer) against to standards of bovine serum albumin (Bouskill et al., 2006).

Na^+, K^+-ATPase activity was determined in raw tissue homogenates Silva et al. (1977). Shortly, samples were defrosted and homogenized (Stuart SHM1, Homogenisator) in 5 volumes of buffer (Sucrose, EDTA, Hepes, adjusted to pH 7.4 with 20 mM Tris). Homogenates was added to a K^+ containing medium, while a second homogenate aliquot (0,4 mL) was added to a second K^+ free medium. After incubation (37°C for 10 min), there actions were stopped (with using 1 mL TCA), free phosphate was measured by adding 1 mL freshly prepared color reagent ($FeSO_4.6H_2O$, ammonium heptamolybdate), and absorbances were measured at 660 nm (Optizen, spectrophotometer) against phosphate standards (Bouskill et al., 2006).

2.4 Statistical Analysis

The data obtained from biomarker and metal analyses of Odanata were subjected to t-test by using the Minitab-User Guide program. In these statistical comparisons, a value of $p<0.05$ (95% Confidence Interval) was considered significant (Anonymous, 1996).

3 Results and Discussion

In this study, metal concentrations, GSH, TBARS levels and Na^+, K^+ -ATPase activities were examined in Odanata larvae sampled from Umurbey Stream. Water quality parameters, their units are summarized in Table 1. Furthermore, the results of metal levels and biomarkers responses in organisms are given in Fig 2 and Fig 3, respectively.

Metal accumulation in Odanata larvae sampled from the polluted area was measured higher levels, depending on the metal concentration in water. Odonata larvae need water with plenty oxygen and constant flow as they spend their growth period in water (Mandaville, 1999).

Depending on the mining activities; levels of all metals measured in Odanat larvae sampled from polluted area are determined higher than unpolluted area; and the differences were identified statistically significant ($p<0.05$). The metal which is uptaken by macro-invertebrates with different ways (water, sediment and foods); immediately excreted from their bodies or detoxified, if is not suitable for their metabolic activity. Besides, if the percentage of uptaking metals is higher than the percentage of excreting and detoxifying, non-essential metals accumulate in their bodies and also the toxicity occurs (Rainbow and Luoma, 2011).

Table 1. The results of physico-chemical parameters and metal concentrations of surface water sampled from Umurbey Stream

Data	Unit	Unpolluted Area	Polluted Area
T	ºC	13.44±0.02	14.57±0.14
S	ppt	0.24±0.02	0.31±0.02
pH	pH	7.52±0.01	7.24±0.03
EC	μS cm^{-1}	229.53±2.26	247.68±4.46
DO	mg L^{-1}	8.82±0.04	7.04±0.11
Cd	mg L^{-1}	n.d.	0.02±0.01
Cu	mg L^{-1}	0.04±0.01	0.11±0.01
Fe	mg L^{-1}	1.21±0.08	2.13±0.04
Pb	mg L^{-1}	0.39±0.04	0.84±0.07
Zn	mg L^{-1}	0.62±0.05	1.61±0.09

In this study, in parallel to the increase of the heavy metal levels in the water and the invertebrate species in Umurbey Stream; it was observed that the GSH, which forms up a significant part of the antioxidant defense system, increased due to the rise of the production of ROS. Also the differences between the stations were found to be significant ($p<0.05$). In similar studies, conducted on macro invertebrates, it was also reported that the enzyme activates were affected by the pollutants and such pollutants caused oxidative stress and led to changes in anti-oxidant enzyme activities (Parkes et al., 1993; Sivori et al., 1997; Kaya, 2014).

In the study, depending on the pollution, it was measured in TBARS levels of organisms. Moreover was determined to be significant differences between the stations. The increases on TBARS levels of the Odanat larvae, collected in polluted area from Umurbey Stream, can be interpreted as an onset of lipid – peroxydation. The oxidative stress increases the free oxygen radicals or disrupts the antioxidant defense mechanisms. The antioxidants, affected by the oxidative stress, either by being stimulated or being inhibited, may lose their ability to prevent the formation of ROS or to prevent cell damages (Kavitha and Rao, 2009). The metal water pollution being excessive in this region of river ecosystem, which is under the threat of pollution and the excessive metal accumulation in live organisms are the reasons of the formation of lipid – peroxydation. In many studies (Di Giulio et al., 1993; Livingstone et al., 2003; Barata et al., 2005; Kaya, 2014), it has been reported that enhanced lipid peroxidation in aquatic organisms exposed to high concentrations pollutants.

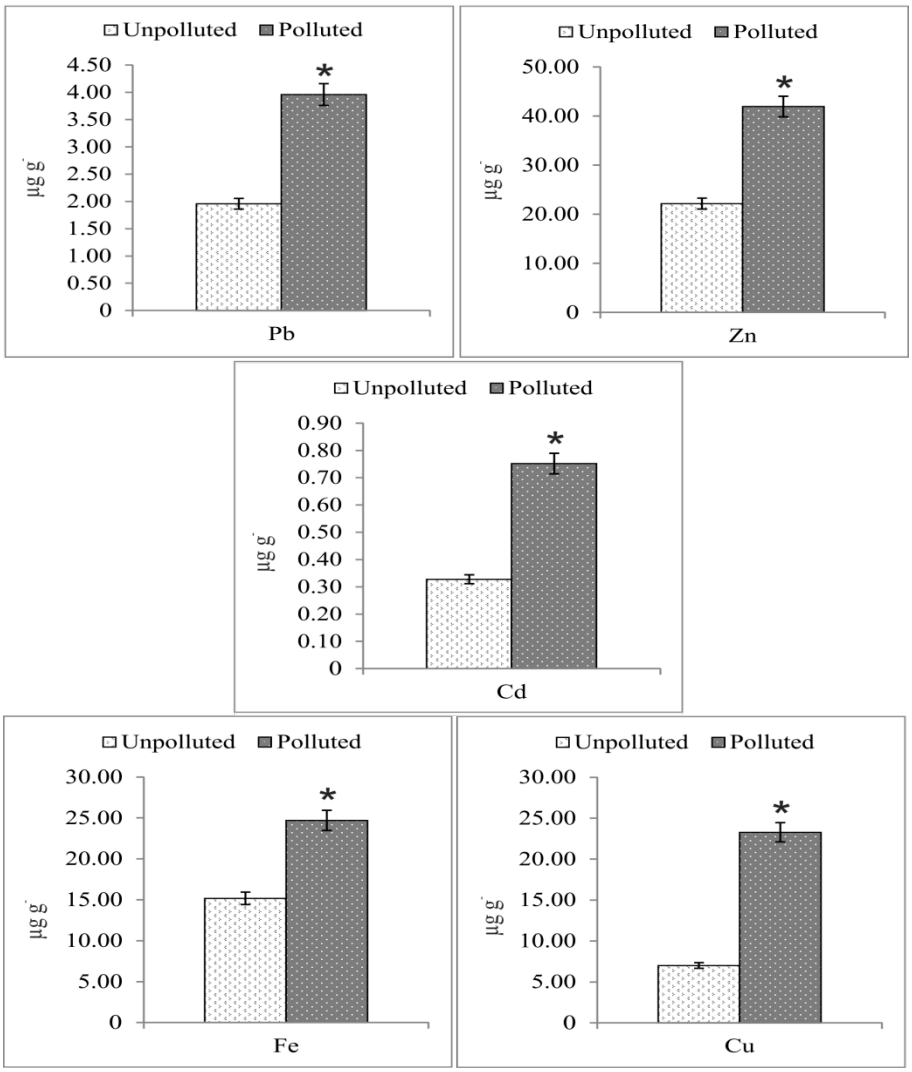

Fig. 2. Heavy metal bioaccumulation parameters of *Ishnura elegans* collected from Umurbey Stream in September 2014 (Value along a column with (*) was significantly different from other values in column; * $p<0.05$).

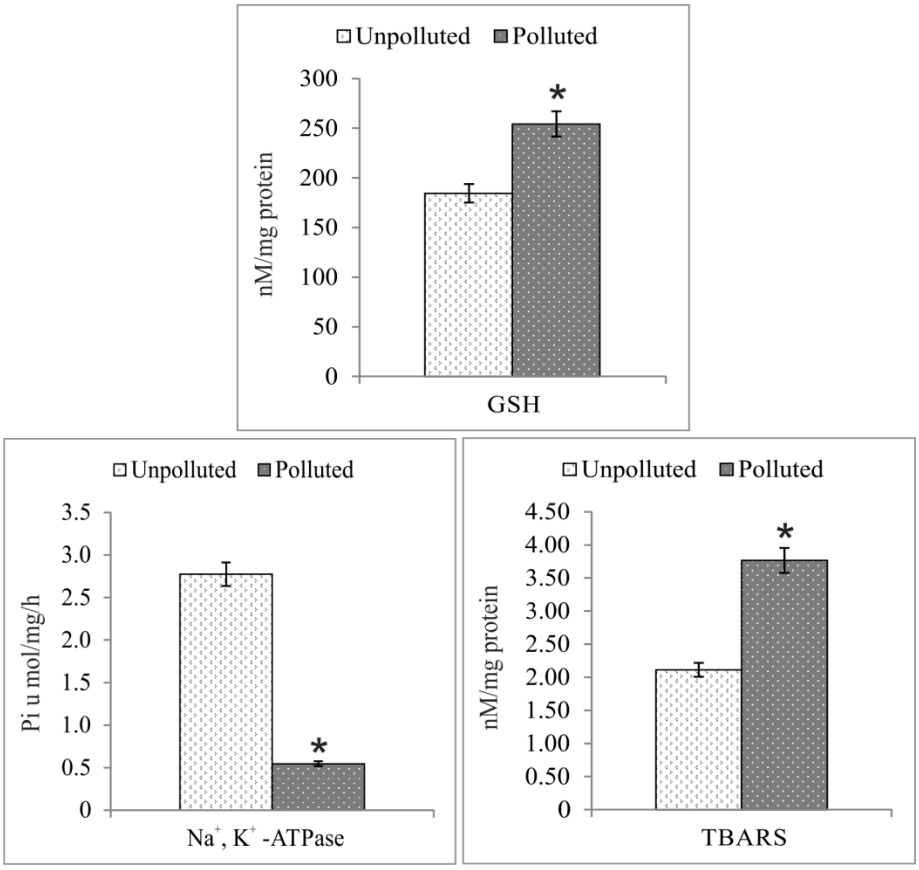

Fig. 3. GSH, Na^+, K^+-ATPase activity and TBARS levels of *Ishnura elegans* collected from Umurbey Stream in September 2014 (Value along a column with (*) was significantly different from other values in column; * $p<0.05$).

Na^+, K^+-ATPase enzyme activity in Odanata larvae *I. elegans* collected from the polluted area was found to be considerably inhibited. It could be interpreted as the running compensation mechanisms to return to control levels of the enzyme (Stagg and Shuttleworth, 1982). The major functions in excretion and osmoregulation of Na^+, K^+-ATPase in aquatic macro-invertebrates have been reported by several researchers (Peacock, 1981; Nicolson, 1993).

Many organisms, by their antioxidant defense systems, detoxification mechanisms and their ability to preserve the oxidant – antioxidant balance, have developed a basic cellular defense system. Since the enzyme activities are the first response to the environmental stress conditions; they are considered as the fastest markers (Depledge and Fossi, 1994). Livingstone (2001) has been reported that there was a significant correlation between free radical processes and antioxidant defense system in the toxicity of organisms.

These results indicated that combination of chemical and biochemical responses can be used to assess and specify the pollution in high stressed river ecosystems. Umurbey Stream has a great importance for fisheries and agricultural activities. Lots of vegetables and fruits are irrigated from this water supply. Therefore, to keep the pollution levels of stream under control is important for sustainability of the ecosystem. Besides, it is suggested monitoring studies depending on the seasons and other parts of the streams; by considering that pollution effects may vary with the parameters of water (pH, salinity, temperature) in subsequent research.

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