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Acute Lethal Toxicity and Accumulation of Copper in *Gammarus pulex* (L.) (Amphipoda)

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Abstract: *Gammarus pulex* (L.) collected from clean sites around Kabaklı pond (Diyarbakır) was acclimatized at 15°C for 10 days. The individuals were placed in 1lt. vessels and exposed to Cu^{++} concentrations of 0.05, 0.08, 0.10, 0.15, 0.20, 0.30 and 0.50 ppm to determine both 24, 48, 72 and 96 h LC50 values and Cu^{++} accumulation over 96h. LC_{50} values at 24, 48, 72 and 96 h were calculated to be 0.2, 0.17, 0.12 and 0.1 ppm, respectively. Acute copper accumulation in gammarids exposed to various copper concentrations over 96 h was determined using an atomic absorption spectrophotometer. An equation of logY=1.523+0.078logx, r=0.976 was obtained from the relationship between the copper concentration of the surrounding water (X) and the body copper accumulation (Y) in *G. pulex.* This relatonship was found to be statistically significant (P<0.05). There was an increase in copper accumulation rate in proportion to water copper concentrations.

Key Words: Copper toxicity testing, accumulation, bioindicator, *Gammarus pulex*.

Bakır'ın *Gammarus pulex* (L.) (Amphipoda)'deki Akut Letal Toksisitesi ve Birikimi

Özet: Kabaklı Göleti (Diyarbakır) civarındaki kirletilmemiş küçük bir su kaynağından alınarak laboratuara getirilen *Gammarus pulex* (L.) örnekleri, 15°C sıcaklıkta 10 günlük bir adaptasyon süresine tutulmuştur. Adaptasyon gösteren bireyler, 0.00 (kontrol), 0.05, 0.08, 0.10, 0.15, 0.20, 0.30 ve 0.50 ppm Cu^{++} konsantrasyonlarında su bulunan kaplar içine alınarak 24, 48, 72 ve 96 saatlik sürelerdeki LC_{50} değerleri hesaplanmıştır. LC_{50} değerleri 24 saat için 0.2 ppm; 48 saat için ise 0.17 ppm; 72 saat için 0.12 ppm ve 96 saat için 0.10 ppm olarak bulunmuştur. Akut letal konsantrasyonlarda 96 saat tutulan örneklerdeki akut Cu^{++} birikimi atomik absorbsiyon spektrofotometresinde ölçülmüştür. Ortamın Cu^{++} konsantrasyonu (X) ile *G. pulex* vücudunda biriken Cu^{++} konsantrasyonu (Y) arasındaki ilişkiden logY=1.523+0.078logX, r=0.976. denklemi elde edilmiştir. Bu ilişki istatistiksel olarak da anlamlı bulunmuştur (P<0.05). *G. pulex*'deki Cu^{++} birikimi artan konsantrasyona bağlı olarak artış göstermiştir.

Anahtar Sözcükler: Bakır toksisite testi, akümülasyon, biyoindikatör, *Gammarus pulex.*

Introduction

Heavy metals are continually released into the aquatic environment from natural sources such as volcanic activity or weathering of rocks. Moreover, industrial processes and some agricultural uses (e.g. $CuSO_4$ is used to control aquatic vegetation) have greatly increased the mobilization of many metals in freshwater (1). Therefore, in recent years, concern has increased over heavy metal pollution.

Heavy metals include both essential elements (Mn^{++}, Zn^{++}, Cu^{++} etc.) and metals with no known biological function such as Cd^{++}, Hg^{++}, Ag^{++} and Sn^{++}. These metals are potentially harmful to most organisms at some level of exposure and absorption. Some aquatic species can regulate the body levels of essential metals such as Cu^{++} and Zn^{++} at constant levels, but the regulation is broken down at higher metal exposures and metal accumulation occurs. Metal regulation is mainly achieved by the rate of metal excretion being increased to match the rate of the metal uptake. However, the body concentrations of non–essential metals such as cadmium and mercury are not regulated, and it is accumulated in proportion to dissolved cadmium concentrations (2, 3, 4, 5, 6). It is also known that the final body concentrations of a metal is dependent on the accumulation strategy of the species for that metal (4, 7).

Aquatic organisms have been widely used to assess environmental pollution because of their ecological and economic importance and their morphological, physiological and ecological diversity in aquatic habitats (8). The use of these organisms in toxicity tests is also essential in the protection of aquatic ecosystems. Many studies have been carried out on the effects of environmental pollutants on various aquatic organisms, and on biological assesment of water quality using certain indicator organisms (9). Most toxicity tests have been concerned with measures of acute lethality; the results are expressed as a concentration or dose of toxicant at which a specified percentage (e.g. LC_{50}) of the test organisms are killed over a standard period of time (e.g. 24, 48, 72 or 96h) (10, 11, 12, 13). The uptake and accumulation of known pollutants by internal organs such as vertebrate kidneys, or by the whole organisms in the case of crustaceans, can also provide useful data on the type and nature of toxicants (14, 15, 16, 17, 18).

Many studies have been also carried out on the lethal toxicity and accumulation of heavy metals in various crustacean species (2, 3, 19, 20, 21, 22, 23, 24, 25). Most toxicity studies are concerned with non–essential metals such as cadmium (26, 27, 28, 29, 30), but little is known about the lethal effects of essential metals such as Cu^{++} on gammarids and about the pathways or mechanisms by which Cu^{++} is accumulated (31, 32).

Gammarus pulex (L.) is an extremely widespread and abundant crustacean which may occur at high densities and forms an important component of the diet of a number of fish. As it is particularly sensitive to several toxicants, it is used as standard toxicity test organism in the U.S.A. and has been recommended for use in Europe (26, 33). In the present study, we investigate the acute lethal toxicity (LC_{50} values for 24, 48, 72 and 96h) and accumulation of copper in *G. pulex* exposed to a range of copper concentrations to evaluate the use of this species as an indicator organism for monitoring pollution.

Materials and Methods

Test animals

G. pulex samples were collected from clean sites around Kabaklı pond (Diyarbakır) and acclimatized in laboratory conditions at 15°C in aerated tanks for 10 days prior to experimentation. We selected specimens of similar size for all species. Appropriate numbers of the animals were then placed in 1 lt. glass vessels containing various concentrations of natural spring water and well aerated. Spring water with known composition (Table 1) was used in all experiments for both accumulation and toxicity tests as water quality parameters (particularly pH, alkalinity and hardness) are important factors likely to influence the uptake and toxicity of metal salts. During acclimation and testing, the animals fed on leaves brought from their original habitat.

Metal exposure and sublethal toxicity tests

Stock copper solution ($CuSO_45H_2O$) was freshly prepared, and added to each test vessel containing spring water to achievement the required range of copper concentrations of 0.00 (control), 0.05, 0.08, 0.1, 0.15, 0.20, 0.30 and 0.50 ppm for each species. The metal concentrations used were calculated on the basis of mg of copper in the salt. Initial range–finding experiments were performed to derive the concentrations suitable for LC_{50} determinations. Following metal exposure, the animals were checked daily for mortality. Death was defined as a total lack of movement. In all experiments the spring water and metallic ion were renewed every two days. All tests were performed at least six times. LC_{50} values of 24, 48, 72 and 96h were determined using Tox29.bas Probit Analysis Program, prepared by Charles E. Stephan, (6201 Congdon bluid, Duluth MN 55804).

Determination of copper accumulation

Cu^{++} concentrations selected for the accumulation tests for *G. pulex* were in the range of Cu^{++} concentrations used to determine the LC_{50} values for 24, 48, 72 and 96h. After exposure, the animals were removed and washed in several changes of distilled water. Animals used in the accumulation tests were those surviving 4 days of exposure. *G. pulex* specimens corresponding to each experimental overload were pooled. For metal analysis, specimens were oven–dried to constant weight at 70°C, powdered, and digested in 4ml of concentrated HNO_3 and $HClO_4$ (3:1) overnight at 80°C on a hot plate (6). All specimens were weighed both before and after drying. On average, dry weights were 27% of the wet weights for *G. pulex*. The digested samples were then brought to a constant volume (10 ml) with distilled water. The copper concentrations of

Table 1. Water quality parameters (mg/l).

pH	:	8.0
Disolved O_2	:	6.5
Cations		
Ca	:	23.2
Mg	:	5.4
Na	:	3.7
K	:	0.3
Anions		
HCO_3	:	107.0
SO_4	:	8.2
Cl	:	5.4
NO_3	:	1.3

these digests were determined by A.A.S (UNICAM–929 Spectrometer). All metal concentrations are expressed in terms of wet weight (mg/kg=ppm).

Results and Discussion

Copper accumulation in *G. pulex* exposed to various copper concentrations for 96 hours is shown in Figure 1.

It can be clearly seen that the body copper concentration of the amphipod *G. pulex* increases in proportion to water copper concentration. An equation of *log Y=1.523 + 0.078 log X, r=0.976* was obtained from the relationship between copper concentration of the surrounding water (X) and the body coper accumulation (Y) in *G. pulex*. This relationship was found to be statistically significant ($P<0.05$). In crustaceans, there are two different accumulation strategies for essential metals such as Cu and Zn: one is that even at a wide range of metal concentrations, body copper concentrations stay at constant levels (meaning that body metal regulation occurs);

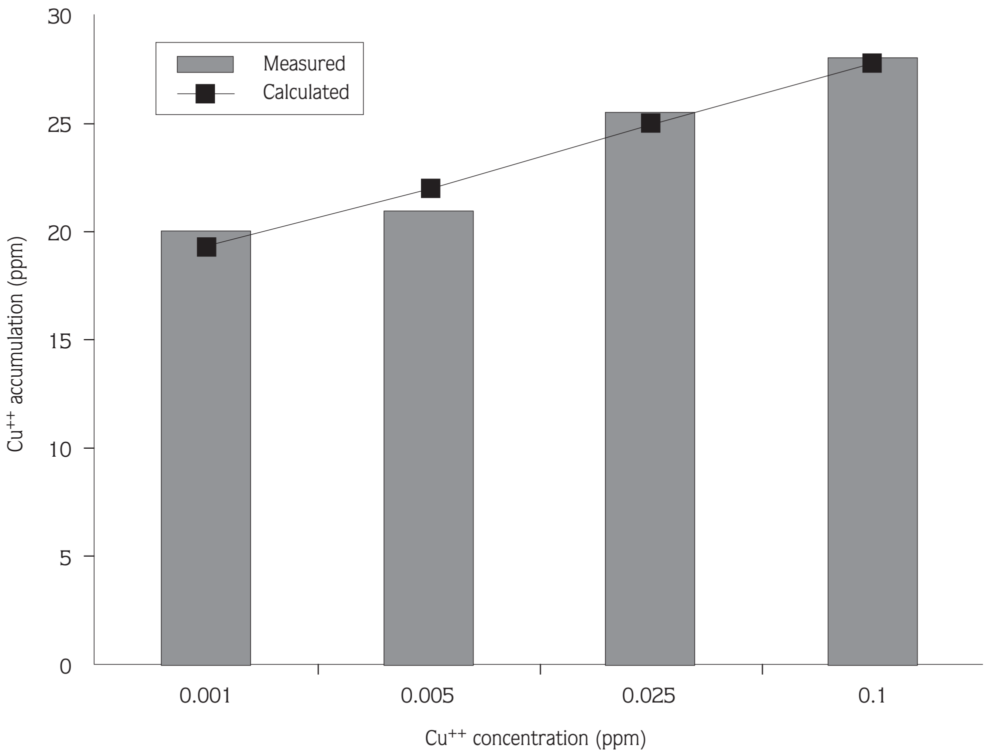

Figure 1. Copper accumulation in *G. pulex exposed* to various concentrations of Cu^{++} over 96 h. Each data point represents the mean of three experiments. The calculated values were estimated from the regression equation given in the text.

the other is that the body copper concentration increases with increasing metal concentrations (meaning that body metal regulation does not occur) (8). These two different strategies help the organisms in the detoxification of heavy metals.

It is also known that the metal binding protein metallothionein plays a role in detoxification of copper (8, 15). Studies show that in crustaceans copper and zinc regulation is a characteristic of species belong to the order Decapoda, but not observed in the other Crustacea orders (8, 34). From this point of view, body copper accumulation in *G. pulex* (Amphipoda) is not regulated, leading to an exponential increase in body copper concentrations. Our previous study on the same species with nonessential metal cadmium also showed there to be an inrease in the cadmium accumulation rate in proportion to water Cd^{++} concentrations and that body cadmium concentrations were not found to be regulated in accordance with other studies carried out on crustaceans (3, 6, 14, 35). In crustacean species, it is also thought that there is a relationship between metal permeability, regulation and accumulation. If the metal uptake rate in organisms permeable to metals is higher than the excretion rate, then metal accmulation occurs. Metal accumulation in crustaceans seems to occur mainly in hepatopancreas and exoskeleton (36, 37). Organisms permeable to metals are also extremely susceptible to metal ions. The sensitivity of different species to heavy metals is not only determined by their rates of absorption.

Figure 2 shows mean survival rates of *G. pulex* samples exposed to various copper concentrations for 24–96 hours. The LC_{50} values for 24, 48, 72 and 96 hours were calculated as 0.2, 0.17, 0.12 and 0.1, respectively, indicating that this organism is sensitive to copper ions.

Indeed, metal toxicty studies show that *G. pulex* is a sensitive organism to heavy metals (19). The magnitude of change in the LC_{50} values from day 1 to day 4 was rather small (0.2–0.1 ppm). These types of results have been observed with peracarid crustaceans (e.g. Isopoda and Amphipoda) (8). Taylor et al. (22) reported that LC_{50} values for 48–240 hours in *G. pulex* were calculated to be 0.047–0.033 ppm. These values are lower than those in the present study. Differences may be caused by different experimental conditions such as water quality. The effects of water hardness, salinity, temperature and pH on the toxicity of metals are well known (23, 38, 39). We have therefore used spring water with constant Ca (25 mg/l, regarded as soft water) in all experiments to circumvent these effects.

The tests employing single species may provide information about the environmental risks of a toxicant (22). There is also a need to employ appropriate species in toxicity tests, particularly those that are potentially vulnerable organisms in order to predict the impact of toxicants on a particular environment (40). The use of aquatic organisms to monitor trace metal bioavailability is well known (41, 42). An ideal bioindicator should be a net accumator of the trace metal in question which does not the body metal concentrations to relatively constant levels over a range of metal bioavailability, with a correlation between the metal content of the organism and the average metal concentration in surrounding water. *G. pulex* possesses these criteria and is a sensitive indicator of copper both at acute toxicity and at accumulation levels, indicating the possible use of this species in monitoring pullution. For a more realistic approach, studies concerned with the effects of other essential and non–essential metals alone or in combination should be carried out on this species.

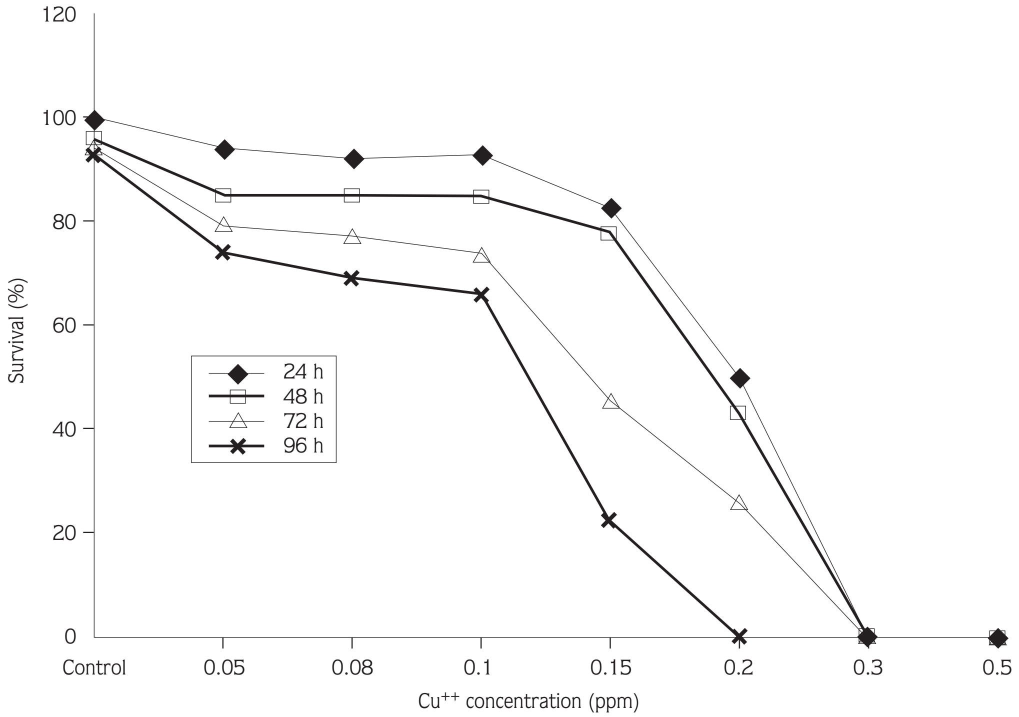

Figure 2. Mean survival rates of *G. pulex* exposed to Cu^{++}. Each data point represents the mean of six experiments.

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