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Effect of Temperature on the Toxicity of Zinc, Copper and Lead to the Freshwater Amphipod *Gammarus pulex pulex* (L., 1758)

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Abstract: This study showed the effect of temperature on copper, zinc and lead toxicity to the freshwater amphipod *Gammarus pulex pulex*. 96-h LC_{50} values were estimated for copper, zinc and lead in this species using the static bioassay method. Increase in the concentration of copper, zinc and lead decreased the survival of the animals. The LC_{50} values of Cu, Zn and Pb for *Gammarus pulex pulex* ranged from 0.028 to 0.080, 5.2 to 12.1 and 11.2 to 23.2 mg/l, respectively. The results indicated that Cu was more toxic to the species, followed by Zn and Pb. The results were compared with those from other studies and discussed.

Key Words: Zinc, copper, lead, temperature, Gammarus pulex pulex

Tatlısu Amfipodlarından *Gammarus pulex pulex* (L., 1758)'lerde Çinko, Bakır ve Kurşun Toksisitesi Üzerine Sıcaklığın Etkisi

Özet: Bu çalışma, tatlısu amfipodlarından *Gammarus pulex pulex* (L., 1758)'lerde çinko, bakır ve kurşun toksisitesi üzerine sıcaklığın etkisini göstermektedir. Bu tür kullanılarak 96 saatlik öldürücü konsantrasyon değerleri (LC_{50}) statik biyolojik deneylerle çinko, bakır ve kurşun için tayin edilmiştir. *Gammarus pulex pulex* türleri için bakır, çinko ve kurşun LC_{50} değerleri sırasıyla 0,028-0,080, 5,2-12,1 ve 11,2 -23,2 mg/l arasında bulunmuştur. Bu türler için en toksik metal bakır olmuş ve bunu çinko daha sonra da kurşun izlemiştir. Bu sonuçlar diğer çalışmalarla karşılaştırılmış ve tartışılmıştır.

Anahtar Sözcükler : Çinko, bakır, kurşun, sıcaklık, *Gammarus pulex pulex*

Introduction

Hundreds of different pollutants can be found in water. The various pollutants and toxic chemicals can enter aquatic environments by several routes. These routes include direct precipitation, surface water, run-off, sewage discharges and industrial wastewater outfalls (1-6).

Heavy metals such as zinc, copper and lead can enter bodies of water in industrial wastewater and domestic wastes. Surface run-off and groundwater seepage carrying heavy metal residues can flow into rivers and lakes from such sites, causing contamination.

There are several characteristics of bodies of water that have a significant effect on the toxicity of a given heavy metal. Although environmental variables such as temperature can influence the solubility and toxicity of a toxicant, limited information is available on the effects of temperature on the toxicity of heavy metals (7-10).

Copper, zinc and lead were chosen in the present study because they represent a broad spectrum of potential pollutants in freshwaters and because recent reviews were available (1-4).

Many studies (5-11) show that invertebrates are generally more sensitive to heavy metals than either fish or algae. Therefore, many schemes for the protection of the freshwater ecosystem give equal weighting to the results of toxicity tests with macroinvertebrates.

Standard Methods for the Examination of Water and Wastewater (12) includes a coverage of the general terminology and procedures for performing bioassays. Tentative procedures for undertaking amphipod bioassays appeared for the first time in the 14th edition (1976) although only freshwater amphipods (gammarids) were recommended. Freshwater amphipod crustaceans, particularly those of the genus Gammarus, have been used as test animals in aquatic toxicology for many years (13-17).

However, no standardized procedure has been published. Amphipods are important components of freshwater food chains and toxicity tests with these animals can therefore be seen to have considerable environmental relevance. Short-term lethal exposures have shown freshwater amphipods to be extremely sensitive to a variety of toxicants. Gammarus pulex pulex has been used as a test animal in toxicological studies (14, 17).

Far more research is required to develop testing techniques in which secondary non-toxicant induced stress factors are removed. Research is needed to develop new methods and/or to standardize existing ones, the majority of which do not fulfil the requirements previously described.

This study was undertaken to establish the effect of temperature on copper, zinc and lead toxicity to the freshwater amphipod *Gammarus pulex pulex*.

Materials and Methods

The natural population of *Gammarus pulex pulex* is very high in Sinop freshwaters (personal observation). *Gammarus pulex pulex* was collected from an unpolluted area in Sinop. This area is called Keçideresi and is located on the Sinop-Ayancık road. Keçideresi is a spring water and its salinity is 0‰.

Taxonomic characteristics of *Gammarus pulex pulex* (L., 1758)

Body smooth. Antennae 2 with a swollen compressed flagellum, bearing a flag-like brush of setae at the inner surface, calceoli present, pereiopods 3 and 4 with long curled setae, pereiopods 5 to 7 almost without setae at the anterior margin of the segments. Epimeral plates moderately pointed, armed with spines only uropod 3 densely setose, the inner ramus being about 3/4 to 4/5 as long as the outer ramus. Urosome flat without dorsal elevations.

The first antenna of male samples was half as long as the body of the animal. The segments in the flagellum and accessory were 28 and 5 in number, respectively. The second antenna of the animals was shorter than the first. The number of segments in the flagellum of the second antenna was 18.

Experimental procedure

Experiments were conducted at a constant temperature of 15, 20 and 25ºC (±1ºC). The acute toxicity of copper as copper (II) sulphate, zinc as zinc chloride and lead as lead (II) nitrate was determined using static tests. Stock solutions of MERCK grade $CuSO_4$, $ZnCl_2$ and $Pb(NO_3)_2$ were prepared in deionized water. Stocks were acidified by adding a few drops of concentrated analar nitric acid in order to reduce the precipitation/adsorption of the metal ions (14,18,19). *Gammarus pulex pulex* has been observed to actively avoid a pH below 6 (personal observation). Therefore, the pH of the culture medium chosen was above 6.

Preliminary tests were carried out to establish suitable concentration ranges. The nominal zinc, copper and lead concentrations were in the range 0.0005 to 50 mg l^{-1}. All experiments were conducted using 6 test concentrations plus a control series under static test conditions in 1-litre beakers containing 800 ml of solution.

Animals (9-11 mm long) were fully acclimated to the appropriate temperature for a week before testing. Mean dry weight was 5.12 mg (19.25 mg wet wt). Experiments were conducted during the appropriate season (e.g., 15ºC experiments in autumn and 25ºC experiments in summer). Active and apparently healthy individuals were selected from the stock tanks. Each series consisted of 3 replicates with 10 animals.

Gammarids have been described as predaceous animals that feed on other invertebrates (16). Therefore, during acclimatization Chironomid larvae and/or Ostracod have been used as a food source to avoid cannibalism. However, *Standard Methods for the Examination of Water and Wastewater* (12) recommended that statistic bioassays in which the animals are not fed should be restricted to 96 hours. Therefore, in this study each toxicity test lasted 96 hours and observations for mortality were made twice daily.

Many species require substrates of sediment for sheltering or hiding places (16). Preliminary tests also showed that 50% of the control animals died without sediment. Therefore, sediments were taken from the same area that the animals were collected from. The sediment was washed through a 1 mm mesh sieve into a tank to remove any associated macrofauna and to ensure a standard particle size for the sediment in all experiments. Sediments were stirred and rinsed 3 times with distilled water, then allowed to stand for 24 hours in tap water. The overlying water was then poured off and the sediment was provided as substrate in all the test containers and no food was provided throughout the course of the experiment.

All containers were aerated without disturbing the sediment surface in order to maintain the dissolved oxygen levels above 60% of the air saturation value, and covered by black material to exclude direct light except from directly above. Moreover, amphipods including Gammarids as a group have been described as reacting negatively to light (16).

Dissolved oxygen and pH were measured in all experiments and the design of the experiments ensured that all replicates and treatments were exposed to the same factors. The average pH was 7.5±0.3 and oxygen saturations were 80% in all containers. These were acceptable conditions for toxicity tests (12,15,16,20).

Samples for total sediment organic carbon analysis were dried at 60ºC in an oven for 48 h. A 5 gram sample was then treated with hydrochloric acid vapour overnight in a desiccating jar to convert any calcium carbonate to chlorides. Weighed, dried samples were then placed in a muffle furnace at 600ºC for 4 hours and the loss on ignition was taken as the organic carbon content of the sediment (21).

A number of metals may bind to organic matter, leading to reduced toxicity, so the toxicity of heavy metals may be modified by the levels of organic matter present in the body of water. This is unlikely to be the case in this study because the average total organic content of the sediment was less than 1.00 % (SE 0.09).

Results and Discussion

The primary criterion of a toxicity test is the survival after exposure to contaminated and uncontaminated (control) waters (15,16,20). None of the control animals died, demonstrating that the holding facilities and handling techniques were acceptable for conducting such tests, as required in the standard EPA/COE protocol where mean survival should be ≥90% (15). The mortality of *Gammarus pulex pulex* increased with increasing copper, zinc and lead concentrations and temperature regimes. However, the toxicity of copper, zinc and lead to *Gammarus pulex pulex* was decreased by decreasing temperature. Similar effects of temperature were shown by Bryant et al. (7-9) on the toxicity of chromium, arsenic, nickel and zinc to a variety of marine and estuary invertebrates.

Dead animals were found on the sediment surface and were usually dark in colour. No mortalities were observed at the end of the exposure to concentrations of 0.0005 mg Cu l^{-1} , 1 mg Zn l^{-1}, 8 mg Pb l^{-1} or less in water. All the animals were dead after 3 hours exposure to a concentration of 1 mg l^{-1} Cu in water. Twenty percent of the animals were dead after 4 days exposure to concentrations of 25 mg l^{-1} Pb in water. The results of LC_{50} analyses show that copper was more toxic to *Gammarus pulex pulex* than either zinc or lead, the LC_{50}s for survival being 0.028, 5.21 and 11.2 mg l^{-1} Cu, Zn and Pb at 25°C, respectively. Whereas LC_{50} values were 0.080, 12.1 and 23.2 mg l^{-1} Cu, Zn and Pb at 15°C, respectively.

LC_{50} values for copper, zinc and lead for *Gammarus pulex pulex* are shown in Table 1 . An examination of the 96 h LC_{50} values for *Gammarus pulex pulex* indicates a rank order of metal toxicity of Cu>Zn>Pb. Lead was only toxic at very high concentrations at all temperatures. Lead as a pollutant has assumed particular importance due to its relative toxicity and increased environmental contamination via car exhaust and highway run-off. Effects of lead in the aquatic environment with the effect of fluctuating temperature, however, have not been studied and relevant literature is scarce.

Some of the LC_{50} values for zinc, copper and lead recorded for different amphipods in other studies are similar to those recorded in the present study, whereas some LC_{50} values for some metals are not. The toxicity of heavy metals including copper, zinc and lead to amphipods is summarized in Table 2. Acutely lethal concentrations fall in the range 0.02 to 1.3 mg Cu l^{-1}, 0.58

Table 1. The 96-hour LC_{50} values with 95% fiducial limits (FL) for *Gammarus pulex pulex* (L., 1758) exposed to copper, zinc and lead at 15, 20 and 25ºC (mg l^{-1}).

Temperatures	Cu LC_{50} (95% FL)	Zn LC_{50} (95% FL)	Pb LC_{50} (95% FL)
15ºC	0.080 (0.076-0.087)	12.1 (10.2-15.1)	23.2 (20.3-25.8)
20ºC	0.041 (0.035-0.048)	9.3 (8.5-9.7)	16.1 (13.6-18.4)
25ºC	0.028 (0.024-0.034)	5.2 (4.8-6.3)	11.2 (9.7-12.8)

Table 2. Acute toxicity of heavy metals to amphipods.

Species	Habitat^a	Metal	Method^b	Time	End point^c	Temp. (°C)	Sal. (‰)	Results	Ref.
Allorchestes compressa	SW	Cd, Zn	WAT, ST	96-120h	S	16.8-20.5	34.5	120h Cd LC$_{50}$= 0.2-4 ppm; 96h Zn LC$_{50}$= 0.58 ppm; this amphipod was more sensitive than crab, shrimp, mollusc and worm.	18
Allorchestes compressa	SW	Se	WAT, CF	96h	S	18	34.8-35.3	LC$_{50}$= 4.77 and 6.17 ppm from two different areas; juveniles were more sensitive than adults.	30
Allorchestes compressa	SW	Cu	WAT, ST	96H	S	20	32±1	LC$_{50}$ values for juveniles and adults were 0.11 and 0.50 ppm, respectively.	31
Allorchestes compressa	SW	Zn, Cd, Cu	WAT, CF	96h	S	20.3±0.8	34.1±0.7	Cu was 1.6 times more toxic than Cd and 4 times more toxic than Zn; the toxicity of a combination of two and three metals is different from that of individual metals	32
Allorchestes compressa	SW	Cd, Cr, Cu, Zn	WAT, CF	4wk	S,G	19±1	31±1	Cu was the most toxic metal; the sublethal effects of the four metals appear to be in similar proportion to their lethal effects.	33
Chelura terebrans	SW	Cd	WAT, ST ST	96h 7 day	S	19.5	35	96h LC50 = 0.63 ppm; 7day LC$_{50}$ = 0.2 ppm.	34
Austrochiltonia subtenuis	FW	Cd	WAT, ST ST	96h	S	15±1		96h LC$_{50}$ = 0.04 ppm.	35
Crangonyx pseudogracilis	FW	Cd, Cu, Cr Pb, Hg, Mo, Ni, Sn, Zn	WAT, ST	48h 720 96h	S	13		48h LC$_{50}$ values were 34.6, 2.4, 2.2, 43.8, 0.47, 3618, 252, 72 and 121 ppm; 96h LC$_{50}$ s were 1.7, 1.3, 0.42, 27.6, 0.001, 2623, 66 (72h), 50 and 19.8 ppm in order listed.	14
Corophium insidiosum	IN	CD	WAT, ST	96h 7 days	S	19.5	35	96h LC$_{50}$ =1.27 ppm; 7day LC$_{50}$ = 0.51 ppm.	34
Corophium insidiosum	IN	As,Cd,Cr, Cu,Pb,Hg, Zn	WAT, ST	96h-20 days	S, A	19±1		96h LC$_{50}$ s were 1.1, 0.68, 11, 0.6, >5, 0.02 and 1.9 ppm in order listed.	36
Eohaustorius estuarius	IN	Cd	WAT,	4 days SED	S		30	4-day LC$_{50}$s were 41.9, 36.1 and 14.5 ppm (in water) for animals held in the laboratory for 11, 17 and 121 days, respectively.	37
Elasmopus bampo	C	Cd	WAT, ST	96h 7 days	S	19.5	35	96h LC$_{50}$ = 0.57 ppm and 7day LC$_{50}$ = 0.2 ppm.	34
Elasmopus bampo	C	As,Cd,Cr, Cu,Pb,Hg, Zn	WAT, ST	96h-20 days	S, A	19±1		96h LC$_{50}$ s were 2.75, 0.9, 3.4, 0.25, >10, 0.02, and 12.5 ppm in order listed.	36

to 19.8 mg Zn l^{-1} and 5-27.6 mg Pb l^{-1} for 96h. These differences may be attributed to different collecting sites and periods, different species, different experimental temperatures, different size beakers and different laboratory conditions. Moreover, some invertebrates may develop either a physiological or genetic adaptation or a combination of both to copper (22). For example, Bryan (23,24) reported that the amphipod Corophium volutator was one of the most copper-tolerant organisms, both in laboratory experiments and in contaminated field conditions, either because it regulates copper against changes in the environment or because Corophium is basically

Table 2. cont'd

Species	Habitat[a]	Metal	Method[b]	Time	End point[c]	Temp. (°C)	Sal. (‰)	Results	Ref.
Hyallella azteca	FW	Pb	WAT, ST	12-120h	S			Free Pb concentration reflects Pb's biochemical activity better than total Pb; the highest mortality rates are associated with the highest free Pb concentrations.	38
Gammarus pulex	FW	Cu	WAT, ST	96h	S	15		LC$_{50}$ value was 0.02 ppm.	39
Gammarus pulex	FW	Cu	WAT, ST	24-96h	S, A	15		LC$_{50}$ values at 24,48,72 and 96h were 0.2,0.17,0.12 and 0.1 ppm.	17
Gammarus pseudolimnaeus	FW	Pb	WAT, CF	96h- 28 days	S, A	15		Pb was toxic and caused more than 50% mortality at 136 ppb and above after 96h; 28-day LC$_{50}$ = 28.4 ppb and 96h LC$_{50}$ = 124 ppb.	40
Grandidierella japonica	IN	Cd	WAT, ST	96h 7 days	S	19.5	35	96h LC$_{50}$0 = 1.17 ppm and 7day LC$_{50}$ = 0.5 ppm.	34
Rhepoxynius abronius	IN	Cd	WAT, ST	96h	S	19.5	35	96h LC$_{50}$ = 0.24 ppm.	34

[a] IN= infaunal, SW= seawater, FW= freshwater, C= cultured animals
[b] WAT= water, SED= sediment, ST= static system, CF= continuous-flow system
[c] S= survival, G= growth, A= accumulation

impermeable to copper (24), or because copper can be excreted directly in an insoluble form (25). This procedure may be ineffective in the case of the freshwater amphipod *Gammarus pulex pulex*. More research is needed to clarify the effect that temperature has on the toxicity of copper, zinc and lead.

Copper is found in natural waters as a trace metal usually at concentrations of $<5\ \mu^{-1}$ but can also be present at much higher concentrations as a result of industrial processes (1,4). European standards and guidelines (26,27), FAO (28) and Turkish Standards (29) recommended maximum copper concentrations, although the criteria given would not protect the more sensitive macroinvertebrate species. There is, therefore, a need for further research on the toxicity of heavy metals to freshwater invertebrates. This should concentrate on the effects of sublethal chronic exposures. Toxicity tests should also be carried out on species from a range of taxa.

The principal conclusion that emerges from this study and other studies (7-10) is that it is essential to consider the effects of temperature when assessing the toxic effects of heavy metals on the survival of aquatic organisms.

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