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Individual and combined effects of copper and lead on the marine shrimp, *Palaemon adspersus* Rathke, 1837 (Decapoda: Palaemonidae)

Bakır ve kurşunun deniz karidesi *Palaemon adspersus* Rathke, 1837 (Decapoda: Palaemonidae)'lara ayrı ayrı ve birlikte etkisi

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Abstract
The acute toxicity by single or combinations of copper and lead to the marine shrimp *Palaemon adspersus* Rathke, 1837 (Decapoda: Natantia) was evalulated by static bioassays, calculating the LT_{50} (lethal time for 50%) and the LC_{50} (lethal concentration for 50%). Each bioassay lasted up to 30 days and survival time has decreased with increasing concentrations of copper and lead in the environment. Copper was 4.25 times more toxic to *Palaemon* than lead. When tested in combinations of paired metals, the expected mortalities were not similar to those observed mortalities which suggested that paired metals acted interactively. Toxicities of mixtures of copper and lead were assessed also using the toxic-unit concept. The results of this study were compared with the other studies and discussed.
Key Words: Copper, lead, lethal time, lethal concentration, toxic-unit, bioassay, *Palaemon adspersus*.

Introduction

The presence of certain heavy metals in a natural environment may alter the toxicity of other metals to marine organisms. Hence, it is important that the presence of all metals be taken into consideration when establishing water quality criteria (Ahsanullah *et al.*, 1981, 1988).

Water quality criteria for some heavy metals such as copper and lead, consistent with the protection of aquatic life, are derived from bioassay data on representatives of the major components of marine food chains, including macroinvertebrates.

Lead, as a pollutant, has assumed to have a 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. Copper is found in natural waters as a trace metal usually at concentrations <5 μ^{-1} but can also be present at much higher concentrations as a result of industrial processes (Bryan, 1976a,b; Moore and Ramamoorthy, 1984). European standards and Guidelines (MAFF, 1995), Food and Agriculture Organization (FAO, 1977) and Turkish Standards (Anonymous, 1990) recommended maximum copper concentrations (20 mg/kg wet weight, but higher levels in shellfish are permitted if copper is of natural occurrence), although the criteria given would not protect the more sensitive macroinvertebrate species.

The toxicological data base on any chemical should include information on the modifying of different water quality. Similarly, the toxicity of the chemical when in combination with the mixture of other common chemicals should be known. Relatively little is known about the combined effects of mixtures which may be dangerous (Negilski *et al.*, 1981).

However, simpler models exist for evaluating environmental toxicity resulting from chemical mixtures. The most basic model is the Toxic Unit Model which involves determinig the toxic strength of an individual compound, expressed as a toxic-unit (Marking, 1985; Rand and Petrocelli, 1985; Landis and Yu, 1995).

The decapods are the best-known crustaceans and include the commercially important shrimps (Fish and Fish, 1996; Demirsoy, 1998). *Palaemon adspersus* occurs along the coast of the North Sea, Baltic Sea, England, Ireland and along the coast of Spain, Portugal and Morocco, in the Mediterranean Sea and the Black Sea (Guerao and Ribera, 1995). It tolerates a wide range of temperatures and salinities and it is distributed in shallow water, often in estuarine conditions. It is important for small-scale commercial fisheries in many areas. They eat a wide variety of food, both plant and animal, and are preyed on extensively by fish and birds (Fish and Fish, 1996). In Sinop coast it is especially associated with *Cymodocea nodosa* beds and rockpools (Personal obsevations).

The aim of this study is to access the acute letality of copper and lead individually and paired combinations to marine decapod *Palaemon adspersus* Rathke, 1837 using various models proposed by Finney (1971); Negilski *et al.* (1981); Ahsanullah *et al.* (1988); Marking (1985); Landis and Yu (1995).

Materials and Methods

Palaemon adspersus were collected from littoral rockpools at Akliman, Sinop in April. After being collected the animals were transported in aerated sea water to the laboratory, where they were placed in plexiglass experimental tanks (30x30x20 cm) at 17°C for a week before the start of experiments.

Stock solutions of MERC grade chemicals, copper (II) sulphate ($CuSO_4.5H_2O$) and lead (II) nitrate $Pb (NO_3)_2$ were prepared in sea water and diluted as required. The concentrations of 0.01, 0.05, 0.1, 0.5, 1, 2, 5, 10, 20, 30, 50 and 100 mg l^{-1} of copper and lead were tested and the thirteenth beaker was used as a control (clean sea water). Four replicated series of experiments were carried out simultaneously for copper and lead. The test solutions were then slowly added to each experimental beaker containing the test shrimp. All control and test solutions were aerated by Rambo EP-8500 air pumps. Preliminary tests were carried out to establish suitable concentration ranges.

All experiments with decapods were conducted under static test conditions in 1 litre beakers containing 800 ml of solution. Active and apparently healthy individuals were selected from the stock tanks. Two shrimps were added to each beaker. *Palaemon adspersus* could survive in this manner for 40 days or more if there were no contaminant in the water (personal observations). The mean body length with rostrum of *Palaemon adspersus* was 4.78 (SE=0.124) and 5.62 (SE=0.255) mm and the mean weight was 0.93 (SE=0.091) and 1.61 (SE=0.195) g for male and female, respectively.

Animals were checked daily for mortality. Death criteria was the absence of movement when the test organisms were prodded. Dead individuals were removed after each observation, but not replaced. Moreover, all test organisms were periodically examined under a stereomicroscope in order to detect possible morphological modifications.

The average concentrations were used in subsequent data analysis. Experiments were conducted at 17 °C ±2, 15±0,5 ‰ salinity, 92 ±2 % dissolved oxygen and 7±0,2 pH for each of the two metals and for paired mixtures.

LT_{50} is the time elapsed from the beginning of the course of the experiment to the death of half the individuals tested.

From comparison of 4 and 10 days concentration-mortality curves for each metal tested simply, experiments with paired mixtures were designed according to the method described Finney (1971) and Negilski *et al.* (1981) to test the independent dissimilar action model. The expected mortalities for the mixture were calculated according to the formula:

$P=1-(1-P_1).(1-P_2)$

where

P= Percentage of individuals predicted to respond to the mixture of metals;

P_1, P_2= Percentage of individuals responding to each metal, based on data from the individual concentration - response curves and measured metal concentration, from mixture test solutions.

Student's *t* values were calculated between expected and observed mortalities according to Zar (1984), to test for significant differences.

The toxicity of the mixture (TU) is determined by summing the strength of the individual compounds using the following model (Landis and Yu, 1995):

$$TU=\frac{P_S}{P_{T50}}+\frac{Q_S}{Q_{T50}}$$

where S represents the actual concentration of the metal in solution and T_{50} represents the lethal threshold concentration (a concentration above which some effect will be produced and below which it will not). If the value is greater than 1.0, less than 50 % of exposed population will survive; if it is less than 1.0, greater than 50 % will survive. A toxic unit of 1.0 is equal to incipient LC_{50} (Marking, 1985).

In all mixtures, if mortalities were observed in the controls, then results were corrected according to Abbot's formula (SMEWW, 1976) (Standard Methods for the Examination of Water and Wastewater). However, the mortalities have never been greater than 10 %.

Abbott's (SMEWW, 1976):

$$P=\frac{P*-C}{1-C}$$

where P and P*= the corrected and observed proportions responding to the experimental stimulus and C= the proportion responding to the control test.

Results for any decapod dying of moulting before 24 h of the experimental period were not included in the data analyses.

Results and Discussion

The primary criterion of a toxicity test is the survival after exposure to contaminated and uncontaminated (control or clean) waters (ASTM, 1990; U.S. EPA and U.S. COE, 1991). 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% (ASTM, 1990). Mortality of *Palaemon adspersus* increased with increasing copper and lead concentrations in the environment.

96 hours LC_{50} values for copper and lead for *Palaemon adspersus* are shown in Table 1. No mortalities were observed at the end of the 96 hours exposure to concentrations of 5 mg Cu l^{-1} and 10 mg Pb l^{-1} or less in water. The results of LC_{50} analyses show that copper was more toxic to *Palaemon adspersus* than lead.

Table 1. The 96-hour LC_{50} values (mg l^{-1}) with 95% fiducial limits (FL) for marine decapod *Palaemon adspersus* exposed to copper and lead.

Species	Cu LC_{50} (95% FL)	Pb LC_{50} (95% FL)
Palaemon adspersus	16 (12-21)	68 (55-74)

Although *Palaemon elegans* were collected from the same habitat, their 96 hours LC_{50} values (2.18-2.91; mean 2.52 and 5.50-7.90; mean 5.88 mg l^{-1} for Cu and Pb, respectively), (Bat *et al.*, 1999a) were different from *Palaemon adspersus* in this study (Table 1). This indicates that similar or different results can be measured with marine invertebrates living in the same niche.

The mean LT_{50} values and relative standard errors (SE) for *Palaemon adspersus* are shown in Figure 1. Lead, which is a non-essential metal, is significantly less toxic to *Palaemon adspersus* than copper, which is an essential metal. These results agree with toxicity studies on other invertebrates (Ahsanullah *et al.*, 1981 and 1988; Bat *et al.*, 1999a,1999b). This may be due to part of geography since the sites which have a high background concentrations of metals such as copper (Bryan, 1976a,b) and invertebrates may have developed either a physiological or genetic adaptation or a combination of both to some metals (De Nicola *et al.*, 1987, 1989, 1992a,b).

The computed value of t for both Cu and Pb is less than the tabulated value for P=0.1, so it is concluded that the differences between observed mortality percentage and that expected by independent dissimilar action is unsignificant.

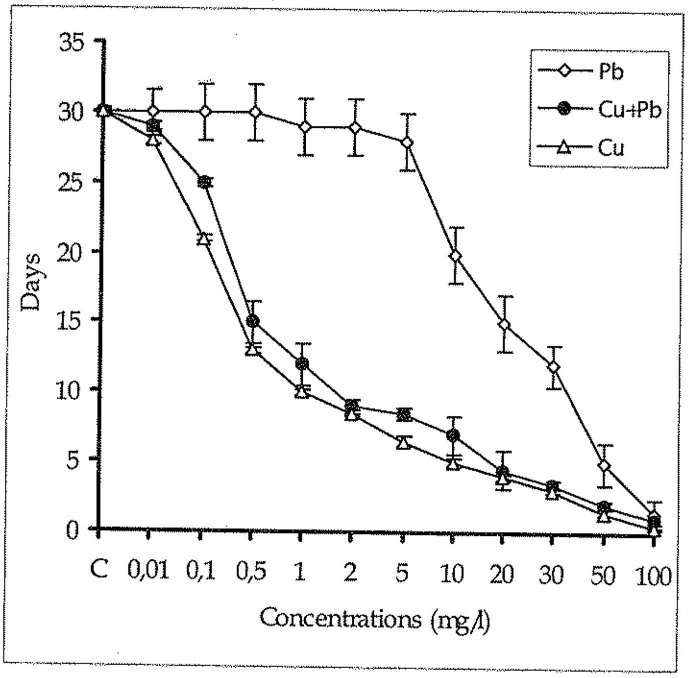

Figure 1. The mean LT_{50} values and related standard errors (SE) for *Palaemon adspersus* at different concentrations (mg / l) of Cu, Pb and paired mixtures of Cu and Pb.

The mortalities expected from the independent model were different from those observed mortalities at all concentrations (Table 2). In this study, the toxic-units were also calculated for 4 and 10 days. Table 2 indicated that the Cu and Pb in paired mixtures acted in an interactive manner. Moreover, the toxic-units were less than 1.0 at lower concentrations of Cu and Pb, indicating that more than 50% of exposed population of *Palaemon* would survive, while they were greater than 1.0 at higher concentrations of paired mixtures,and so more than 50% of population would die.

Table 2. Concentrations of Cu and Pb with expected percentage mortalities, expected and observed mortalities and toxic units for each mixture concentrations for 4 and 10 days.

Mixture (mg l^{-1})		No. of animals	4 days					10 days				
			% mortality expected for each metal alone		% mortality expected by independent dissimilar action for mixture	Observed mortality (%) for mixture	combine toxic units	% mortality expected for each metal alone		% mortality expected by independent dissimilar action for mixture	observed mortality (%) for mixture	combine toxic units
Cu	Pb	N	Cu	Pb				Cu	Pb			
Cont.	Cont.	8	--	--	--	--	--	--	--	--	--	--
0.01	0.01	8	--	--	--	--	0.003	--	--	--	--	0.06
0.05	0.05	8	--	--	--	--	0.015	--	--	--	--	0.03
0.1	0.1	8	--	--	--	--	0.03	--	--	--	--	0.30
0.5	0.5	8	--	--	--	--	0.15	--	--	--	--	0.60
1	1	8	--	--	--	--	0.30	--	--	--	12.5	1.20
2	2	8	--	--	--	12.5	0.60	12.5	--	12.5	25	2.40
5	5	8	--	--	--	25	1.50	25	--	25	25	6.00
10	10	8	37.5	--	37.5	37.5	3.00	37.5	12.5	45.3	37.5	12.0
20	20	8	50	25	62.5	50	6.00	50	25	62.5	62.5	24.0
30	30	8	62.5	37.5	76.56	62.5	9.00	87.5	50	93.75	75	36.0
50	50	8	75	50	87.5	87.5	15.0	100	62.5	100	87.5	60.0
100	100	8	87.5	62.5	95.31	100	30.0	100	100	100	100	120.0
					t=0.432					t=0.449		
					P=0.681					P=0.667		

Although aquatic organisms may be exposed in the field to many different combinations of pollutants and different environmental conditions, most acute toxicity tests have considered only the individual effects of these pollutants. There is little information about the combined effects of mixtures which may be dangerous. Therefore, the acute toxicity of copper and lead to the decapod *Palaemon adspersus* was also assessed in paired combinations. In some studies the effects of mixtures of some heavy metals have been reported as synergistic (Negilski *et al.*,1981; Bat *et al.*,1998) or strictly additive (Thorp and Lake,1974) or antagonistic (Oakden *et al.*,1984; Bat,1995).

For example, *Callianassa australiensis* was exposed to zinc, cadmium and copper in pairs for 14 days static toxicity test and the results indicated that the metals in all paired mixtures acted interactively (Negilski *et al.*,1981). When the mixture of the three metals (zinc, cadmium and copper) was considered for *Allorchestes compressa*, there was no difference between observed and expected values according to the simple similar action model (Ahsanullah *et al.*, 1988).

In the present study, the toxicities of mixtures of copper and lead were also assessed according to the toxic-unit concept. The results of the present study showed that the toxicity of combination of these metals was different from that of individual metals. These results compared with those of Negilski *et al.*(1981) who reported that the expected mortalities of *Callianassa australiensis* in all paired and triad mixtures for zinc, cadmium and copper were overestimated. Similarly, toxic unit calculations overestimated the toxicity of combinations of two and three metals (zinc, cadmium and copper) to *Callianassa australiensis* except for the Zn-Cd combination (Ahsanullah *et al.*, 1988). However, no clear trends have emerged that permit a simple generalization (Negilski *et al.*, 1981). Indeed, further research is needed before any conclusion is drawn.

There is, therefore, a need for further research on the toxicity of heavy metals to invertebrates. This should concentrate on the effects of sublethal chronic exposures. Toxicity test should also be carried out on species from a range of taxa (Bat *et al.*, 2000).

Özet

Deniz karideslerinden *Palaemon adspersus* Rathke, 1837 (Decapoda: Natantia) kullanılarak bakır ve kurşunun akut toksisiteleri LT_{50} ve LC_{50} statik biyolojik deneylerle hesaplanarak ölçülmüştür. Bu organizmaların hayatta kalma süreleri bakır ve kurşun konsantrasyonlarının artmasıyla azalmıştır. Bakır, kurşuna göre 4.25 kez daha toksik bulunmuştur. Metallerin birlikte etkileri denendiğinde, beklenen ölümler gözlenen ölümlere benzerlik göstermemiştir, bu da metal çiftlerinin birbirlerini etkilediği sonucuna varılmıştır. Bakır ve kurşunun karışımlarının toksisitesi toksik-birim kavramı kullanılarak da değerlendirilmiştir. Bu sonuçlar diğer çalışmalarla karşılaştırılmış ve tartışılmıştır.

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