

**Toxicity of Zinc and Lead to the Polychaete
(*Hediste diversicolor* Müller 1776)**

**Çinko ve Kurşun'un Poliket (*Hediste diversicolor*
Müller 1776)'a Toksisitesi**

**Levent Bat, Ayşe Gündoğdu, Mehmet Akbulut,
Mehmet Çulha, Hasan Hüseyin Satılmış**

University of Ondokuz Mayıs, Sinop Fisheries Faculty, Department of Basic
Sciences, 57000 Sinop, Turkey

Abstract

The acute toxicity of zinc and lead in water with clean sediment to the polychaete *Hediste diversicolor* was evaluated by static 10-day and 28-day bioassays, calculating the LC₅₀ (lethality concentration for 50%). Mortality has increased with increasing concentrations of zinc and lead. The results indicated that Zn was more toxic to the species than Pb. The results also showed that small worms are more sensitive to Zn and Pb than bigger worms.

Key Words: Zinc, lead, bioassay, *Hediste diversicolor*.

Introduction

Toxicity of heavy metals in marine and estuarine organisms is a subject of increasing interest. Only relatively few species have been extensively used for toxicity testing (Cairns and Mount, 1990) and there is no single biological response or test species that can meet all environmental and legislative requirements for effective toxicity testing (Widdows, 1993; Ingersoll, 1995; Rand *et al.*, 1995). Nevertheless, benthic invertebrates have great potential for sediment toxicity tests (Reynoldson and Day, 1993), because they are intimately associated with sediments either

through their burrowing activity or by ingestion of sediment particles (Luoma, 1983; Reynoldson, 1987; Bryan and Langston, 1992; Reynoldson and Day, 1993; Bat, 1998).

Polychaetes are frequently employed in sediment toxicity tests (Luoma and Ho, 1993; Ingersoll, 1995). Species used to date include: *Cirriformia spirabrancha* (Milanovich *et al.*, 1976), *Neanthes arenaceodentata* (Pesch and Morgan, 1978; Pesch, 1979; Pesch and Hoffman, 1983; Dillon *et al.*, 1993), *Glycinde picta* (Swartz *et al.*, 1979), *Crenodrilus serratus* (Reish, 1980), *Arenicola cristata* (Schoor and Newman, 1976; Rubinstein, 1979; Rubinstein *et al.*, 1980; Walsh *et al.*, 1986), *Arenicola marina* (Bat, 1998; Bat and Raffaelli, 1998), *Nereis virens*, *Glycera dibranchiata* and *Nephtys caeca* (Olla *et al.*, 1988), *Dynophilus gyrocoliatu*s (Åkesson, 1980; Long *et al.*, 1990), *Ophryotrocha labronica*, *O. diadema* (Åkesson, 1980) and *Streblospio benedicti* (Cheng *et al.*, 1995).

Polychaetes are an important component of the biota in marine and estuarine environments. As a group they possess a variety of feeding habits, the majority being either filter or detritus feeders. They are important food for snails, larger crustaceans, fishes and birds.

However, the polychaete *Hediste diversicolor* were recommended as a test species for sediment bioassays (Pocklington *et al.*, 1992). Thus this species was chosen for the present investigation. It burrows into intertidal estuarine sediments and is usually distributed from near the mouth of a river into regions which although still tidal are exposed to fresh water for some of the time. It lives in a more or less permanent, often U-shaped, burrow in mud and muddy-sand, and is tolerant of salinities down to 1‰. It is omnivorous, feeding on a variety of plant and animal material on the surface of the sediment and also on suspended particles present in the respiratory current down into the burrow. The food is trapped on a mucous funnel secreted and periodically eaten by the worm (Fish and Fish, 1996; Demirsoy, 1998).

Under certain conditions some polychaetes are able to absorb metals from ingested sediment as well as from solution in the surrounding water (Bryan and Hummerstone, 1971; Bat, 1998).

Polychaete bioassays can be conducted without sediment, however, it is well known that some toxicants, particularly heavy metals, interact with the sediment. By conducting a bioassay without sediment, the interaction of the polychaete, the metal and the sediment is ignored. The present study on the toxicity of zinc and lead in water with sediment was made to identify to tolerance limit of the hazardous substances to the polychaete *Hediste diversicolor*.

Materials and methods

The polychaete selected for static bioassay is *Hediste diversicolor* which are divided into two groups about 55-70 mm and 20-30 mm in lengths and about 250-500 mg and 125-250 mg in weights, respectively. Total 500 individuals of *Hediste diversicolor* were collected. They are commonly found in the nearshore damp soil of the coastal waters. Specimens were collected from the nearshore regions of Sarikum, Sinop, Turkey and were acclimatized to the laboratory conditions for 5 d. They were starved for 24 h before the bioassay. No response to prodding was considered as death. All experiments were conducted at 20°C±2.

Clean sediment was collected from the same area as the worms and washed through a 500 µm mesh sieve into a tank to remove any associated macrofauna and then washed again at least 6 times with tabwater before use in subsequent experiments. This procedure ensured a standard particle size (<500µm) for all experiments (Bat, 1997 and 1998; Bat and Raffaelli, 1998). Clean sediment was added to the beakers to create a 4 cm deep layer. The acute toxicity of zinc as zinc chloride and lead as lead (II) nitrate was determined using static tests. Stock solutions of MERCK grade ZnCl₂ and Pb(NO₃)₂ were prepared in deionized water. The concentrations were selected on the basis of preliminary screening tests conducted to ascertain the lethal and sublethal concentrations for each metal. Experiments were conducted in 1 l beakers which were closed with a glass plate to prevent evaporation. For each test solution 4 animals were used. Each test concentration was run in duplicate and experiments repeated 3 times till comparable results were obtained. Appropriate controls were maintained. All beakers were aerated in order to maintain the dissolved oxygen levels above 60% of the air saturation value (ASTM,1990; U.S. EPA/COE Manual,1991). All beakers were covered by black material to exclude direct light except from directly above. All glassware was acid-washed in 10% HCl for 24h

and then thoroughly rinsed in distilled water prior to use (Bat and Raffaelli, 1998; Bat *et al.*, 1998).

Damaged worms were discarded. The worms were not given any food during the course of the experiment. Test solutions were not changed. Each tank was checked daily and any dead organisms recorded but not replaced. After 10 and 28 days, the content of each beaker was sieved and the number of surviving worms recorded. The LC₅₀ was calculated by probit analysis (Finney, 1971).

Sediment samples for the determination of organic matter were dried at 105°C for 24 h. Five grams of dried sample were then treated with hydrochloric acid vapour overnight in a desiccating jar to convert any calcium carbonates to chlorides. Samples were then placed in a muffle furnace at 600°C for four hours. The loss on ignition was taken as the organic matter in sediment (Buchanan, 1984).

The average total organic matter of the sediment at the start of the experiment was 6.5 % (SD=1.30). The mean temperature over the experimental period in all bioassays was 20°C±2, dissolved oxygen saturation was 70%±6 and pH was 7.5±0.25.

Results and discussion

The primary criterion of toxicity tests is the survival after 10-day and 28-day of exposure to test and control sediments (Swartz *et al.*, 1985). None of the control worms died, demonstrating that the holding facilities, water, control sediment and handling techniques were acceptable for conducting sediment bioassays, as required in the standard U.S. EPA/COE protocol where mean survival should be ≥90%. Survival decreased with increasing zinc and lead concentrations in water with clean sediment for 10-day and 28-day (Table 1).

The polychaete *Hediste diversicolor* had a greater resistance to the contaminated water with clean sediment. This was based on survival. It can be said that that *Hediste diversicolor* is a very robust organism, capable of tolerating quite high levels of zinc and lead. Moreover, if heavy metals were removed from the environment, *Hediste diversicolor* would be able to survive and behave normally (personal observations).

Table 1. The 10-day and 28-day LC₅₀ values with 95% fiducial limits (FL) for the polychaete *Hediste diversicolor* exposed to zinc and lead in water with clean sediment.

	Animals	
	55-70 mm in lengths and 250-500 mg in weights	20-30 mm in lengths and 125-250 mg in weights
Zn (mg l ⁻¹)		
10-day LC ₅₀ (95% FL)	35 (31-39)	25 (21-29)
28-day LC ₅₀ (95% FL)	18 (15-23)	9 (6-13)
Pb (mg l ⁻¹)		
10-day LC ₅₀ (95% FL)	65 (60-71)	48 (45-54)
28-day LC ₅₀ (95% FL)	28 (24-34)	19 (15-12)

At the end of the experiment, all sediments were sieved through a 500 µm mesh and survivors only were weighted without permitting depuration. Sediment was probably present in the gut, affecting the weight but all treatments were the same in this respect. Individual weight increased with increasing Zn and Pb concentrations, but this wasn't significant ($P > 0.05$). It is indicating that all survivors fed on the organic carbon content of the sediment (approx. 6.5%) and all deaths result from toxic effects and not from cannibalism. The results of this study also showed that the smaller individuals are more susceptible at zinc and lead concentrations so that only heavier individuals remain alive at higher concentrations.

Comparisons to previous studies on the effect of some heavy metals must be made with caution because of the variability of exposure periods, bioassay conditions, test duration, as well as different species employed (Table 2). However, some valid comparisons can be made. However, zinc has also been found to be more toxic than lead to the polychaete *Capitella capitata* (Reish *et al.*, 1976). Similarly, Bryan and Hummerstone (1973a) found that cadmium was not very toxic to *Nereis diversicolor*. Interestingly, copper was found to be less toxic than cadmium or zinc in a complementary sediment bioassay organism *Corophium volutator* (Bat and Raffaelli, 1998). Bryan (1974) also reported that tolerant individuals of *Nereis diversicolor* were about four times more resistant than non-tolerant individuals and this may go some way to explain why no clear patterns have been found between different populations in toxicity bioassays.

Polychaetes are abundant, soft-bodied and cover a wide size range. They have a short life-cycle, can be transported easily and laboratory cultures of certain species can be maintained. Moreover use of the polychaetes in monitoring marine environmental quality is of special value because of their direct contact with the water column and sediments of their environment.

Table 2. Polychaete toxicology studies involving water and sediment exposures in laboratory and/or field bioassays.

Species	Habitat ^a	Metal	Method ^b	Time	End Point ^c	Tem. (°C)	Sal. (‰)	Results	Reference
<i>Nereis diversicolor</i>	IN	Cu	WAT, SED	7day 37day	S, U	13		Tolerance to the toxic effects of Cu is very different in two populations of the same species.	Bryan and Hummerstone, 1971
<i>Nereis diversicolor</i>	IN	Zn, Cd	WAT, SED	96h 816h	S, U	13	0.35- 17.5	Zn is regulated by the worm, whereas Cd is not; in laboratory, increasing concentrations in solution the rate of absorption of Cd increases more rapidly than that of Zn; in the field, concentrations of Zn in the worms vary less than those of Cd and populations from high-Zn sediments are better at regulating Zn than normal populations, these worms more resistant to Zn than normal worms.	Bryan and Hummerstone, 1973a

Table 2, cont'd

Species	Habitat ^a	Metal	Method ^b	Time	End point ^c	Tem. (°C)	Sal. (%)	Results	Reference
<i>Nereis diversicolor</i>	IN	Mn	WAT, SED	1wk 2wk	S, U	13	1.6- 20	With decreasing salinity, the concentration factor increases; most of Mn was absorbed from solution.	Bryan and Hummerstone, 1973b
<i>Nereis diversicolor</i>	IN	Cu, Zn	WAT, SED	96h	S, U			96h Cu LC ₅₀ = 2.3 and 0.54 ppm tolerant and non-tolerant animals, respectively; metal levels 22 and 1140 ppm Cu normal and contaminated areas; 163 and 194 ppm Zn normal and contaminated areas, respectively.	Bryan, 1976
<i>Nereis diversicolor</i>	IN	Zn	WAT, SED, CF	days - two months	A	20±2		Worms can accumulate ⁶⁵ Zn from sediments; the presence of worms in the sediment causes the release of ⁶⁵ Zn to overlying water.	Renfro, 1973

Species	Habitat ^a	Metal	Method ^b	Time	End point ^c	Temp. (°C)	Sal. (‰)	Results	Reference
<i>Nereis diversicolor</i>	IN	Fe	SED, C	10-88 days	U, A	15±1		Bioavailability of ⁵⁵ Fe depended on its concentration in sediment and not on sediment type; accumulation of ⁵⁵ Fe appeared to be complete after 25 to 35 days.	Jennings and Fowler, 1980
<i>Nereis virens</i>	IN	Cu, Zn, Cd, Pb	SED, ST	30 days	A		10±0.5	Cu and Zn levels in worms exposed to the sediments showed no significant changes from initial values.	Ray <i>et al.</i> , 1981
<i>Nereis virens</i>	IN	Cd	SED	7, 14, 21, 28 (days)	B, U		15, 20-25	After 28 d, uptake was highest in this species (20 ppm) compared to <i>dibranchiata</i> .	

^a IN= infaunal, C= cultured animals; ^b WAT= water, SED= sediment; ^c U= burrowing, A= accumulation

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Hediste diversicolor kullanılarak temiz sedimentli sudaki çinko ve kurşunun akut toksisitesi 10 ve 28 günlük statik biyolojik deneylerle ölçülmüş öldürücü konsantrasyon (LC_{50}) hesaplanmıştır. Çinko ve kurşun konsantrasyonlarının artmasıyla ölüm oranı da artmıştır. Bu tür için çinko ve kurşuna daha duyarlı olduklarını göstermiştir.

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