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Effects of Zinc and Cadmium on the Burrowing Behavior, LC_{50}, and LT_{50} on *Donax trunculus* Linnaeus (Bivalvia-Donacidae)

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The bivalve *Donax* is a widespread clam that lives in exposed clean sandy beaches of the world (McLachlan et al; 1996). In spite of the fact that their habitats are very susceptible to pollution from industrial effluents, sewage, oil spillage at the sea or from major tank accidents (Brown and McLachlan, 1990; Bresler et al; 1999; Herut et al; 1999) few studies have dealt with the pollutants impact on *Donax* populations. Haifa Bay, in the northern part of Israel, is the most industrialized area in the country with nearby plants discharging their effluents into the bay directly or through the river.

Donax trunculus is found in higher densities in the sandy beaches of Haifa Bay (Neuberger-Cywiak et al, 1990). This filter feeder bivalve is able to coordinate its movements and maintain its position in the sediments and respond by behavioural and physiological changes to environment events that produce waves and changes in current direction, among others. Due to the streamlined shape of the shell, *Donax* is a rapid and efficient burrower, achieving complete burial by using relatively few digging cycles (Ansell and Trevallion, 1969; Trueman, 1971; Trueman, 1983a). Metal toxicity can produce sub lethal effects that reduce animal performance of crucial activity for survival in physically controlled habitats, like is the burrowing behaviour. This changing in behavioural pattern can prove indirectly lethality to the organism and the population

The purpose of this study was to determine the effect of two heavy metals, zinc and cadmium on the burrowing behaviour on *D. trunculus* and estimate the median lethal concentration LC_{50} (concentration estimated that produces a 50% mortality in a test population over a specific period of time) and median lethal time LT_{50} (time estimated that produces a 50% mortality in the test population at the studied concentration) (Rand and Petroccelli, 1985) on bivalves exposed to both ions separately. Acute lethal toxicity tests were carried out to estimate the range of concentrations over which the pollutants affected *D.trunculus*.

MATERIALS AND METHODS

Specimens of *D.trunculus* were collected at Hof Hargaman in Acre, Israel during the same year period (march-june), over two years, to ensure similar physiological

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conditions (Neuberger –Cywiak et al, 1990), and were kept in aquarium with sand and circulated sea water at the laboratory. The sand used came from the sampling area. The temperature was 20^0C $\pm 1^0$C; salinity was $40^0/_{00} \pm 1^0/_{00}$ and the photoperiod included 12 hr of daylight and 12 hr of darkness. These conditions were maintained during the experiment. The bottom of the aquarium was covered with a 5 cm layer of carbonate gravel and then the sand was added. The organisms were acclimatized for at least 10 days to these experimental conditions. If the mortality was higher than 5% in the first three consecutive days, the organisms were discarded.

Four sets of experiments, two for each metal, Zn and Cd were done at different times to determine the burrowing behaviour and the LC_{50} and LT_{50} on *D. trunculus*, conducting a static-renewal whole-sediment laboratory assay. Concentrations were selected based on a preliminary test in the laboratory. For Zn (as $ZnCl_2$) were 32 ppm, 10 ppm, 3.2 ppm, 1 ppm, 0.32 ppm, 0.1 ppm and a control. For Cd (as $CdCl_2$) were 10 ppm, 5.6 ppm, 3.2 ppm, 1 ppm, 0.32 ppm, 0.1 ppm and control (Reish and Oshida, 1986). Disposable 3 L plastic containers filled with 10 cm layers of sand and 1500 mL of filtered seawater (Millipore 0.22 µm) containing the experimental heavy metal concentrations were used. For each set of experiments, ten bivalves, whose shell lengths were between 20-30mm, were placed in each plastic container. Three containers were used at each concentration (30 organisms/concentration/set) and seven concentrations including the control were run each time (210 organisms/each metal/per set). Plastic containers were continuously aerated and sealed with a transparent cap to reduce water loss by evaporation. Water with the same ion concentration was changed every other day, in order to compensate the metal concentration for possible metal binding on the experimental vessel walls and sand, among others (Hennig and Greenwood, 1981). If the control presented mortality higher than 10% between each observation, the experiment was discarded. Burial assays were always started at the same time of day.

Behavioural responses were observed at different periods of time and were characterized directly as buried, partially buried, unburied and dead (Fig 1 and 2). Clam's death was determined when there was no reaction to touch with a metallic rod on the foot and siphon or while the animal remained unburied and its valves opened (Bodoy, 1976). Observations from extended siphons and open/closed valves were simultaneously done. The results of mortality at 48hrs and 96hrs were used to calculate the LC_{50}. The values of mortality for each metal at different concentrations over the period studied were utilized to determine the LT_{50}. The LC_{50} and LT_{50} was obtained using a computer program (Stephan, 1977 and it was reviewed and updated by the U.S. Environmental Protection Agency, EPA, 1985). The binomial, moving average, probit and logit method were calculated. The best fit model for the data was presented.

RESULTS AND DISCUSSION

Locomotor impairment, i.e. a decrease in the population burial response of *D. trunculus* exposed to metals, can be observed in the Fig.1 and 2. During normal conditions (Control, Fig.1 and 2), the clam remained unburied for a few seconds. This behaviour was followed with the projection of its foot outside its valves while touching and testing the sand and the environment. Then, the digging behaviour started. This process is not always completely at once and the animal can remain partially buried or when the cycle is completed, totally buried. The burrowing response for *D. trunculus* at 10 ppm of Zn was different than that observed at the same concentration for Cd. During the first 6 hr of exposure to Zn at 10 ppm, less than 50% of the organisms were buried, and then they burrowed themselves avoiding the metal in the water and later re-emerged from the sand, possibly avoiding the Zn that had accumulated in the sediment. After re-emerging from the sediment, the clams died. At 10 ppm of Cd it was observed that the organisms could burrow themselves in the first 6 hr and they remained burrowed until they died. The rest were unburied. No changes in this pattern were observed (Fig. 1 and 2). Ansell and Sivadas (1973) and Ansell et al.; (1980) found that some individuals of *D. trunculus* return to the surface in temperature tolerance experiments under stressed conditions prior to death. In addition, Stenton-Dozey and Brown (1994a) reported that large animals of *D. serra* surface at a temperature below the 50% lethal limit and lie on the sediment gaping for some time before dying. Phelps (1989), working with *Mya arenaria*, found that copper spiking of sediment did not affect burrowing speed up to 13.2 µg Cu/g sediment, but at 51.4 µg Cu/g sediment, the clams had a significant delay in burrowing. Kaschl and Carballeira (1999), who worked with two species of *Venerupis*, suggested that pore water copper concentrations rather than sediment Cu concentrations were responsible for both burial delay and re-emergence. In our experimental results, the re-emergence could be explained as a survival strategy to avoid toxic sediment conditions. This could be a dangerous strategy in the natural environment, because the organism is then exposed to predation (birds, crabs, pigs) or susceptible to desiccation by becoming stranded by the waves. These behaviours may threaten the permanence of the population in some of its natural environments.

Mizrahi and Achituv (1989) found in *D. trunculus* that Cd is a high inhibitor of the respiratory enzymes, lactate dehydrogenase and cytochrome oxidase at 1 and 10 ppm. About 50% of the activity of cytochrome oxidase was inhibited by 10 ppm Zn, and it was hardly effective on the lactate dehydrogenase. Cd was found to inhibit cytochrome activity in the gastropod *Nassarius gibbolosa* whereas Zn caused less than 10% inhibition in the activity of the respiratory enzyme (Mizrahi and Achituv, 1994). In the same article was found a positive correlation between cytochrome oxidase activity and oxygen consumption. Earnshow et al, (1986) observed a decrease in the spermatozoa motility of *Mytilus edulis* exposed to heavy metals explaining as a consequence of respiratory inhibition by theses ions. Oxygen consumption on *D. trunculus* is affected more by Cd than Zn (Neuberger-Cywiak et al; in prep.). We suggest that at Cd high concentration, the respiratory

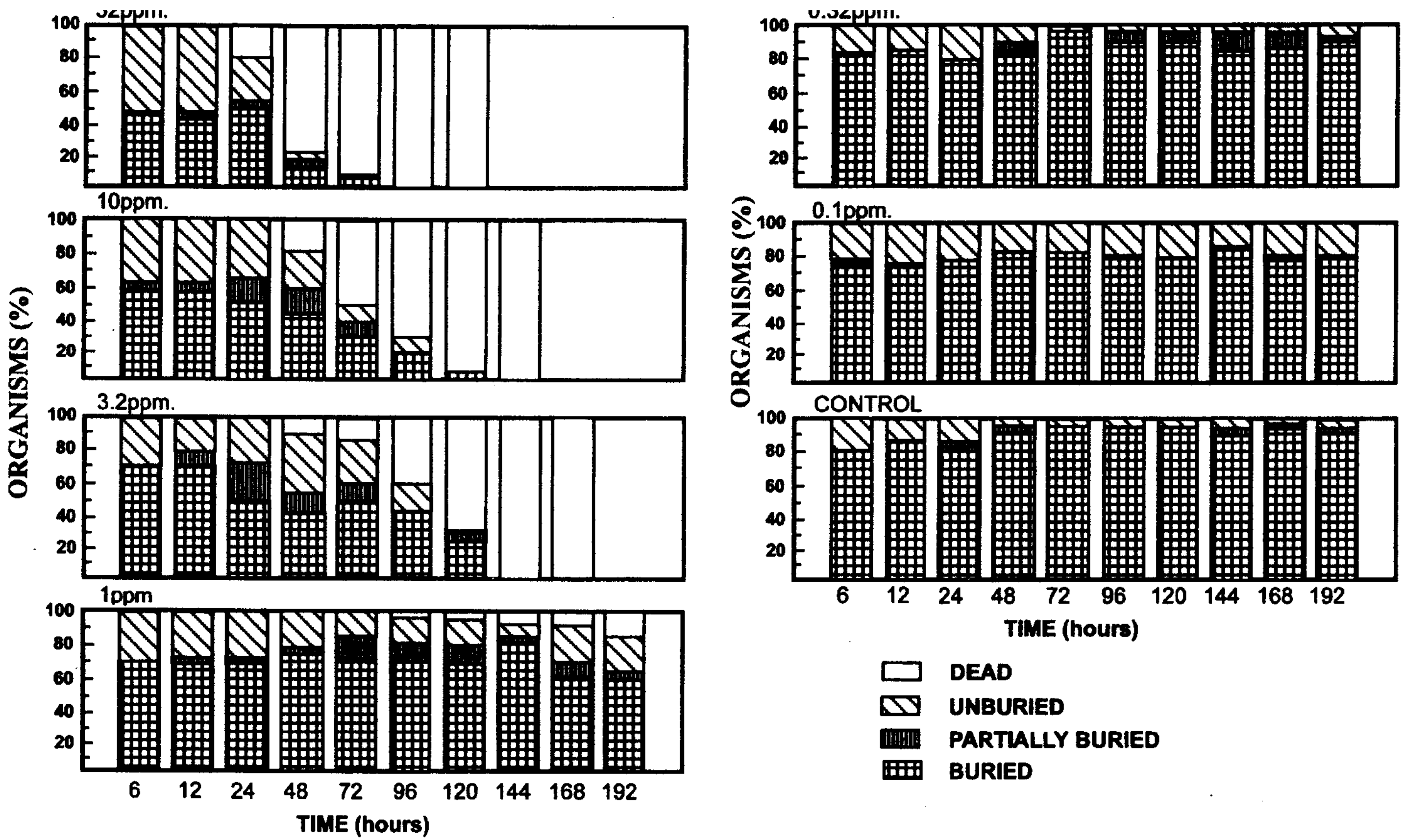

Figure 1. Effect of zinc on the burrowing behaviour of *D.trunculus*.

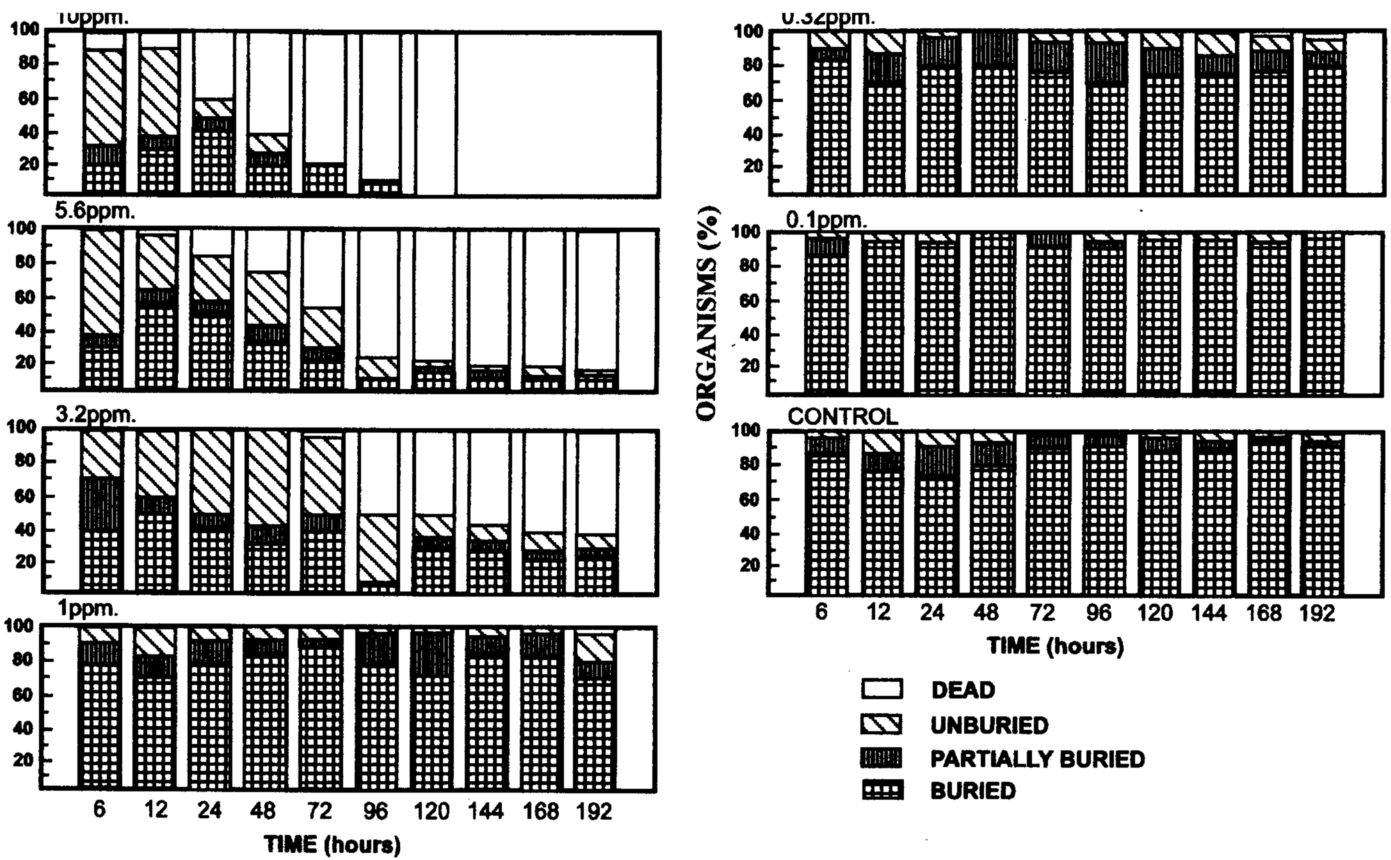

Figure 2. Effect of cadmium on the burrowing behaviour of *D.trunculus*.

enzyme -among others- is affected and the respiratory chain collapse so there is no energy to burrow and re-emerge from the sand, therefore the animals die. If the organisms are buried they die in the sand and if they are on the surface, they remain unburied until they die. This behaviour is found at high levels of metal concentrations in the surrounding water, and is not found normally on the coastal areas, but may be present at an industrial sporadic discharge. A constant exposure to pollutants at low concentration can produce different effects, like an acute toxicity of the contaminant. A sub lethal dose does not kill the organisms in a short period of time, but can affect its ability to respond to the environment and therefore shortening its life. Also a prolonged exposure to a sub lethal dose can promote genetic changes in the population (Walker et al, 2001).

It was observed at lower concentrations of Zn and Cd that 20% of the Zn treated bivalves (1 ppm and 0.32 ppm) remained between partially buried or unburied and began to die after 196 hrs. At 0.1ppm of Zn almost all the organisms were buried and no mortality was observed as the control. Meanwhile, after 96 hr exposure to Cd, mortality was observed at 1 ppm, but at 0.32 ppm no mortality was detected. Approximately 20% of *D. trunculus* remained unburied after being exposed to 0.1 ppm Cd for 216 hr. During the first stages of the experiment, both metals incited the closure of the valves and the withdrawal of the siphons. This is a typical response among bivalves suddenly exposed to a chemical pollutant, as well as to a drastic change in salinity or temperature (Stenton-Dozey and Brown, 1994b; Trueman, 1983b).

When the organism opens its valves and extends its siphons to begin the process of burrowing, or when it filters the surrounding water to obtain food and oxygen, the clam is in contact with the metal. From our results, we conclude that Zn -an essential ion- it is not so deleterious at the same concentration as Cd. This metal is a non-essential ion and is a higher stressor that forces the valves to close at the first moment and the siphons are withdrawn longer than normal. The organism must open valves to continue with physiological activities and must be in contact with the ion. At 12 hr exposure to high concentrations of Cd, the valves remained open, and the foot and siphons were extended. The siphons were very thin and long, closure of the valves were very slow, and in many cases there was no synchronization between contractions of the foot and siphons, and this caused the cutting of the foot or siphons by the valves. Ansell et al.(1999) have determined that natural populations of *D. vittatus* showed damaged siphons, caused by non-lethal predation by juvenile flatfishes. Although the clams were removed from the sand, they rapidly resumed normal activity as confirmed by re-burrowing. This process took place by healing, and regenerating siphons appeared almost normal at 10 days. In our results, no recovery was observed and clams died.

The LC_{50} and LT_{50} determinations are shown in Table 1 and 2. The deviation values obtained for 30 organisms at each experimental metal group were among the same order of magnitude, in the confidence limits established. The results pointed out in this tables were product of a 60 organisms group per metal.

Table 1. LC_{50} values for *D. trunculus* exposed to Zn and Cd at 48 hr and 96 hr.

Metal:Zn			Metal:Cd		
Time	**LC_{50}**	**95% confidence limits**	**Time**	**LC_{50}**	**95% confidence limits**
(h)	(ppm)	(ppm)	(h)	(ppm)	(ppm)
48	22.3	10.0-32.0	48	7.6	5.6-10.0
96	6.7	3.2-10.0	96	3.8	1.0-5.6

Table 2. LT_{50} values for *D. trunculus* exposed to Zn and Cd at different concentrations.

Metal:Zn				Metal:Cd			
Conc.	**LT_{50}**	**95% confidence limits**	**slope**	**Conc.**	**LT_{50}**	**95% confidence limits**	**Slope**
(ppm)	**(hr)**	**(hr)**		**(ppm)**	**(hr)**	**(hr)**	
32	31.18	26.32-36.91	3.6	10	33.36	29.69-37.13	8.1
10	66.57	56.66-77.19	4.37	5.6	48.75	48.75-93.99	7.32

The LC_{50} obtained based in the binomial and moving average method were very similar. The binomial method was selected because the data distribution fit to the model, specifically in this study where no partial death exists. Low mortality was found at low concentrations and high mortality was found at elevated concentrations being the LC_{50} an arithmetic media between the maximal concentration in which 0% mortality was found and the minimum concentration that produces 100% mortality. In this study, the others methods present warnings associated with the correspond method, i.e. in the moving average method, the number of spans used were less than 5 in all three cases, being this a limitation of this method. The LT_{50} was obtained using the logit method, that was suitable for all the four concentrations being a parametric model produced by a sigmoidal function.

At 24 hr, 50% mortality was no detected for both metals at the concentrations studied. Compared to Cd, higher Zn concentrations are needed to obtain 50% of mortality at 48 hr and 96 hr. This means that Cd produced more alterations in *D. trunculus* than Zn. Brown (1982) has studied the short-term effect on adult *Bullia digitalis* of some metals and found that the LC_{50} at 96 hr for Cd was 0.9 $mg.L^{-1}$ and Zn was 3 $mg.L^{-1}$. Similarly, as seen in Table 2, we also conclude that Cd has a stronger toxicity because at the same concentration (10 ppm) the time to obtain the 50% mortality was almost half that of Zn.

Behaviour responses have been used previously as a tool in studies of environmental stress. The burrowing behaviour in bivalves and gastropods was used as a tool to understand the effects of some pollutants on the biota (Avolizi and Nuwayhid 1974; Stirling 1975; De Mahieu et al. 1980; Chapman et al. 1985; Axiak and George 1987; Phelps 1989; Cheung and Wong 1999; Kaschl and Carcalleira 1999). Similarly, Cheung and Wong (1999) found that copper, induces subtle behavioural changes on gastropoda, *Babylonia lutosa*, and could cause ecological death by disrupting the normal function and life history of the exposed organism. Our results indicate that there is an effect on the burrowing behaviour of *D. trunculus* produced by the effect of the metals. We conclude according to the results of the mentioned works that the monitoring of the burrowing behaviour of *D. trunculus* in the environment is a very important indicator to consider in marine pollution research.

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