EFFECTS OF THE CHELATING AGENT NITRILOTRIACETIC ACID (NTA) ON THE TOXICITY OF METALS (Cd, Cu, Zn and Pb) IN THE SEA URCHIN PARACENTROTUS LIVIDUS LMK

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NITRILOTRIACETIC ACID
TOXIC METALS
Cd, Cu, Zn, Pb
SEA URCHIN

Abstract – The toxicity of Cd, Cu, Zn and Pb alone and in presence of increasing doses of the chelating agent nitrilotriacetic acid (NTA) was tested in embryos of the sea urchin *Paracentrotus lividus* Lmk. NTA is able to completely reduce the toxicity of Cu, Zn and Pb, but not that of Cd. The effect of NTA on metal toxicity is discussed.

ACIDE NITRILOTRIACETIQUE
METAUX TOXIQUES
Cd, Cu, Zn, Pb
OURSIN

RÉSUMÉ – La toxicité du Cd, du Cu, du Zn et du Pb isolés ou en présence de doses croissantes d'agent chélateur, l'acide nitrilotriacétique (NTA) a été testée sur des embryons d'Oursin *Paracentrotus lividus*. L'acide nitrilotriacétique peut supprimer complètement la toxicité du cuivre, du zinc et du plomb, mais pas celle du cadmium. L'effet de cet acide sur la toxicité de ces métaux est discuté.

INTRODUCTION

The evaluation of the effects of metal complexation in the aquatic environment is a matter of interest since chelating agents have been considered for use as detergent builders in substitution for polyphosphates (Perry *et al.*, 1984).

Among the chelating agents, particular attention has been devoted to nitrilotriacetic acid (NTA). To date there is evidence that NTA is generally not toxic for marine animals (Eisler *et al.*, 1972; Bott *et al.*, 1980; Brunetti *et al.*, 1986 a,b) although it is able to influence the early development of marine invertebrates (Brunetti *et al.*, 1989).

In the present paper the results regarding the effects of NTA on the toxicity of cadmium, copper, zinc and lead in embryos of the sea urchin *Paracentrotus lividus* Lmk. are presented.

MATERIALS AND METHODS

Sea urchins were acclimatized for a week in laboratory with running sea water (salinity 35 %, temperature 18 °C) and fed with *Ulva* sp. Sperms and eggs were obtained by current KCl-method (Ruggieri, 1975). As it is known that this organism is characterized by a high degree of interindividual variability in the response to changes in the environmental conditions (Bougis, 1967), in each experiment eggs from a single female were fertilized with sperm from a single male and, after fertilization, they were washed to eliminate the excess of sperm and distributed in 100 ml aliquots of experimental water at a concentration of about 50 eggs per ml.

Three replications per experimental condition were performed. After 40 hours at 25 °C the length of the somatic rod of eighty 4-arm echinoplutei per replicate was measured according to the technique previously described (Brunetti *et al.*, 1989).

Subsamples were drawn at 24 and 40 hours after fertilization in order to determine the frequencies of the different developmental stages.

Metals were added to sea water as $CdCl_2.H_2O$, $ZnCl_2$, $PbCl_2$ (Merck), $CuCl_2.2H_2O$ (BDH-Chemicals Ltd) and the ratio metal : NTA was calculated considering the weight of the metal cation alone. All experiments were repeated three times.

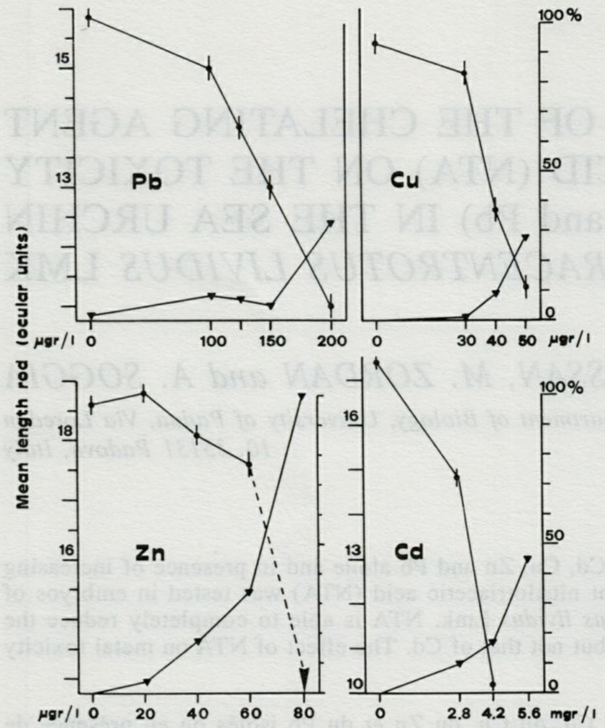

Fig. 1. – Mean length of rods of normal plutei (dots : sample size = 240) and frequencies of plutei with skeletal anomalies (triangles) at 40 hours from fertilization. Vertical bars represent the 95 % confidence interval of the mean. The arrow head indicates the impossibility of determining size as a 100 % of anomalies were present.

RESULTS

Preliminary experiments and selection of the metal doses

The effects of the metals along on echinopluteus size (expressed as mean length of the somatic rod), and the percentages of skeletal anomalies with increasing metal concentrations are shown in Figure 1. These data, as well as the frequencies of the prism stage (Table 1), indicate that the metals induce a slowing down of development. The data also indicate that the developmental stages included between 0 and 24 h seem to be relatively insensitive to the metal toxicity.

The concentration of metal which induces a size reduction of two units with respect to the control (Pb = 150 µg/l; Cu = 40 µg/l; Cd = 2.8 mg/l) was selected for subsequent experiments with metal-NTA mixtures. In the case of Zn this reduction in size was not reached but at 80 µg/l the frequency of skeletal anomalies rose to 100 %. This dose was selected for the following experiments with NTA.

Effects of NTA on metal toxicity

In all cases the chelating agent strongly reduces the frequency of skeletal anomalies produced by the metal and, with the exception of Cd, it increases the echinopluteus sizes (Figs 2). It also brings the developmental rate back to control levels (Fig. 3).

DISCUSSION

The first evidence regarding the ability of NTA to reduce the toxic effects of heavy metals is due to Bizzi *et al.* (1955) who found a 100 % survival in mice injected with $CuCl_2$ plus NTA, while the same dose of metal alone was lethal.

Later this property of NTA, as well as of other chelating agents (i.e. EDTA), was evidenced also in the natural freshwater environment. NTA was found to reduce the toxicity of Cu and Zn (Sprague, 1968), Cd and Pb (Muramoto, 1980), Al

Table I. – Frequencies of prism stage (sample size = 200) at 24 and 40 hours from fertilization. The persistence of high frequencies of this stage indicates a slowing down of the developmental rate.

Zn			Cu			Pb			Cd		
Conc. (µgr/l)	Hours 24	40	Conc. (µgr/l)	Hours 24	40	Conc. (µgr/l)	Hours 24	40	Conc. (mg/l)	Hours 24	40
0	0	0	0	6	0	0	–	0.5	0	13	0
20	7	0	30	20	2	100	–	3.5	2.8	16	0
40	12	0	40	24	3	125	–	6.5	4.2	25	0
60	34	4	50	50	30	150	–	5.5	5.6	51	0
80	61	10	–	–	–	200	–	18	–	–	–

– = not determined

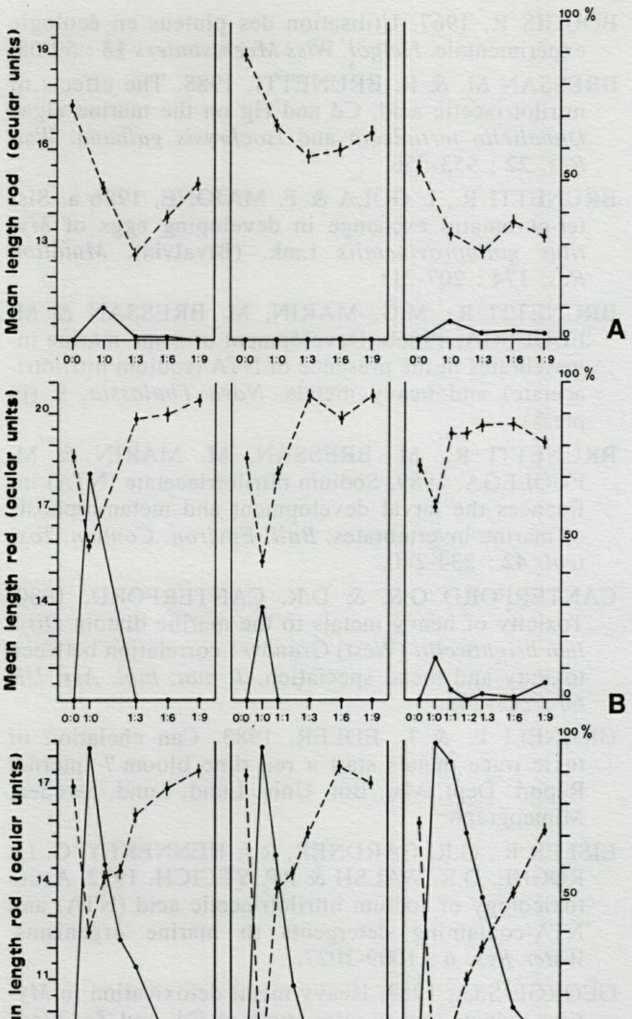

Fig. 2. – Experiments with various metal : NTA ratios (three experiments per metal). A = cadmium (2.8 µg/l), B = copper (40 µg/l); C = zinc (80 µg/l); D = lead (150 µg/l). Mean length of the somatic rod (dashed line) and percentages of plutei with skeletal anomalies (continous line). Vertical bars represent the 95 % comparison intervals by T-method (see Sokal and Rohlf, 1981). The arrow head in C indicates the impossibility of determining the size as 100 % of anomalies were present.

(Allen *et al.*, 1980) and Cr (III) (Muramoto, 1981) in fishes; and that of Cd, Zn, Pb, Cu in phytoplankton (Hongve *et al.*, 1980; Allen *et al.*, 1980).

Only few studies have been devoted to the marine environment it was reported that NTA stimulates the population growth of some microalgae (Yentsch *et al.*, 1974) and this effect has been explained as the consequence of a lowering in the toxicity of copper ions present in the environment (Anderson & Morel, 1978). This effect on microalgae was also observed by other authors (Granéli & Edler, 1983).

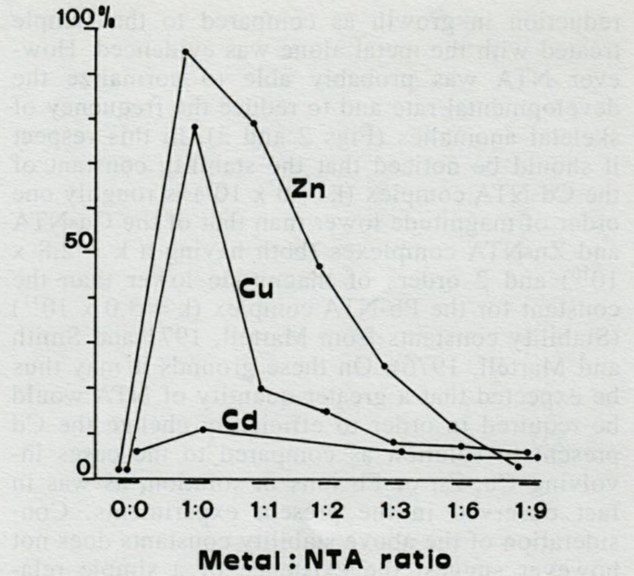

Fig. 3. – Developmental rate as frequency of prism stage at 24 hours from fertilization (data on Pb not taken).

Canterford & Canterford (1980) showed that EDTA is also able to reduce the toxicity of Cu, Zn, Cd and Pb in a marine diatom.

The detoxifying power of chelating agents on heavy metals is generally explained as a consequence of the reduced availability of the free metal ions (Spencer, 1957; Morris & Russell, 1973; Allen *et al.*, 1980; Canterford & Canterford, 1980).

In fact the degree of toxicity of the metal appears to be correlated with the concentration of the chelating agent, however data collected by Eisler *et al.* (1972), in an acute toxicity test on fishes, seem to indicate that the metal dose is also important, the molar ratio metal : NTA required to produce a detoxifying effect being a function of the metal concentration. The mechanism of detoxification is not however clear.

Our data show that NTA is able to completely reduce the toxicity of copper, zinc and lead but not that of cadmium. A lack of detoxification of cadmium was already noticed by us in experiments using invertebrates (Brunetti *et al.*, 1986b) and microalgae (Bressan & Brunetti, 1988). In the latter case only metal : NTA molar ratios up to 1 : 3 were tested and this amount of NTA was considered to be probably insufficient to produce detoxification. In the present experiments metal : NTA ratios up to 1 : 9 were tested but no reduction in cadmium toxicity was noticed. On the contrary at the lower concentration of NTA (1 : 3) a clear reduction in growth as compared to the sample treated with the metal alone was evidenced. However NTA was probably able to normalize the developmental rate and to reduce the frequency of skeletal anomalies (Figs 2 and 3). In this respect it should be noticed that the stability constant of the Cd-NTA complex (k=1.6 x 10^9) is roughly one order of magnitude lower than that of the Cu-NTA and Zn-NTA complexes (both having a k = 2.8 x 10^{10}) and 2 orders of magnitude lower than the constant for the Pb-NTA complex (k = 3.0 x 10^{11}) (Stability constants from Martell, 1971 and Smith and Martell, 1976). On these grounds it may thus be expected that a greater quantity of NTA would be required in order to efficiently chelate the Cd present in solution as compared to the cases involving Cu, Zn or Pb ions in solution, as was in fact observed in the present experiments. Consideration of the above stability constants does not however suggest the existence of a simple relationship between the large differences in detoxifying capacity observed for NTA in association with Cu, Zn and Pb and the stability constants of the respective metal : NTA complexes.

In addition George (1983) described a pathway of Cd metabolism in *Mytilus* which involves a very long biological half-live. This suggests that a possible detoxifying effect of NTA on Cd might be masked by a high metabolic cost involved in discharging this metal from the organism, resulting in the observed reduction in size of the experimental animals.

In conclusion the present data demonstrate that NTA displays a marked ability to decrease the toxicity of the metal ions Zn, Pb anc Cu even with respect to highly sensitive marine animals, and that since the concentrations of NTA required are sufficiently low to not produce toxic effects "per se", the possibility of using this substance as an anti-pollutant, firstly suggested by Sprague (1968), should be further investigated.

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