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Combined Toxicity of Copper, Cadmium, Zinc, Lead, Nickel, and Chrome to the Copepod *Tisbe holothuriae*

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In recent years much work has been concerned with the determination of various contaminants in the environment and with the establisment of the toxicity of these compounds to marine animals. Heavy metals are of increasing concern as pollutants of marine and especially coastal environments. Mixtures of heavy metals may produce unexpected effects. The purpose of this study was to determine the acute toxicity of six heavy metals (Cu, Cd, Zn, Pb, Ni and Cr) to the marine copepod Tisbe holothuriae Humes and to see whether there is any interaction between these metals, when applied jointly.

MATERIALS AND METHODS

Ovigerous females of Tisbe holothuriae Humes, were used as test-aminals. Tisbe is a benthic copepod common in the littoral environment of the Mediterranean. The experimental animals were obtained from laboratory cultures; animals were cultured in synthetic sea-water (Ultra marine; water life, UK), under the same experimental conditions (22 ± 0.5 °C, 38 ‰ salinity, light conditions 12 h day - 12 h dark fed with germaline[1]).

The acute toxicity of the six toxicants (cadmium, copper, chromium, zinc, nickel and lead) to Tisbe acting individually or jointly (all combinations of two) was estimated by determination of the 48 h LC50 (concentration of toxicant which kills 50 % of the test animals after 48 h of exposure) according to the Bliss (1938) method. All experiments were run in constant temperature rooms (22 ± 0.5 °C). In all cases we used synthetic sea water. For each experimental concentration 20 specimens were placed individually in glass containers filled with 100 mL of the test solution. In order to prepare the solutions of Cd ($CdCl_2$ $2H_2O$), Cu ($CuSO_4$ $5H_2O$), Cr (Na_2CrO_4), Pb ($(CH_3COO)_2Pb$ $3H_2O$), Ni ($(CH_3COO)_2Ni$ $4H_2O$), Zn ($(CH_3COO)_2Zn$ $2H_2O$) necessary for the experiments we initially prepared concentrated solutions and then diluted these with synthe-

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[1] Germalyne (Select Germ, GR) is a dietetic human nutrient derived exclusively from wheat: it is a fine powder containing 48 % carbohydrates, 36 % proteins, 10 % lipids and 6 % minerals.

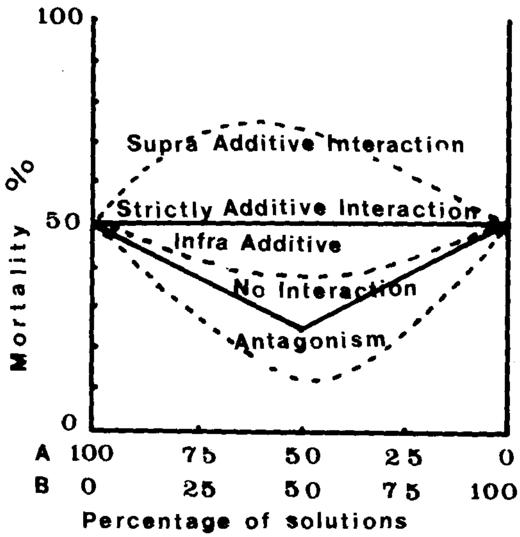


Figure 1. Possible types of responses which can occur between two hypothetical toxicants, A and B, which have similar actions. (Warren 1971).

tic sea water in order to obtain the desired concentration of metal ions. Two series of experiments were performed. In the first we evaluated the toxicity of the six metals when acting alone; for the determination of each 48 h LC50 we repeated the experiments three times. In the second series we evaluated the toxicity of the various combinations of toxicant mixtures; Tisbe was exposed for 48 hours to the following types of mixtures: Cd + Cu; Cd + Zn; Cd + Ni; Cd + Pb; Cd + Cr; Cu + Zn; Cu + Ni; Cu + Pb; Cu + Cr; Zn + Ni; Zn + Pb; Zn + Cr; Ni + Pb; Ni + Cr; Pb + Cr. In order to estimate the type of joint activity of each of the two metal mixture (supra - additive interaction, strictly additive interaction, infra - additive interaction, no interaction and antagonism) we used the Warren's (1971) test (Figure 1).
For each mixture (A and B polutants), we applied the following percentages of solutions:

A - :	100%	75%	50%	25%	0%
B - :	0%	25%	50%	75%	100%

where 100% corresponds to the 48 h LC50 of each polutant. Each combination of A and B correspond theoricaly to a 50% mortality. For the determination of the interactions we performed the experiments twice.

RESULTS AND DISCUSSION

Figure 2 shows the results of the first series of experiments concerning the acute toxicity of Tisbe for the six heavy metals acting separately and the calculation of 48 h LC50 according to the Bliss (1938) method.

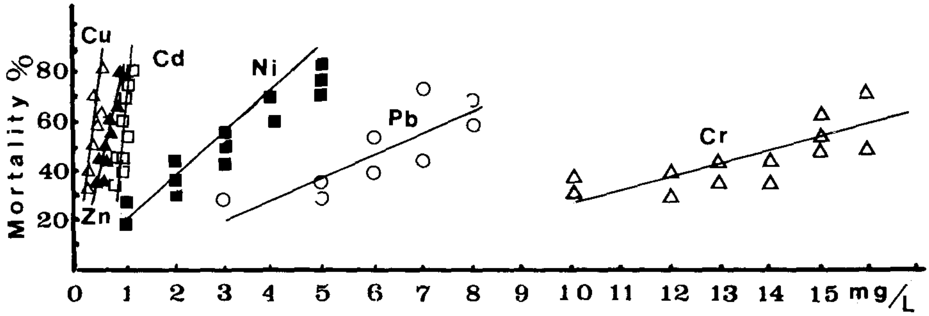
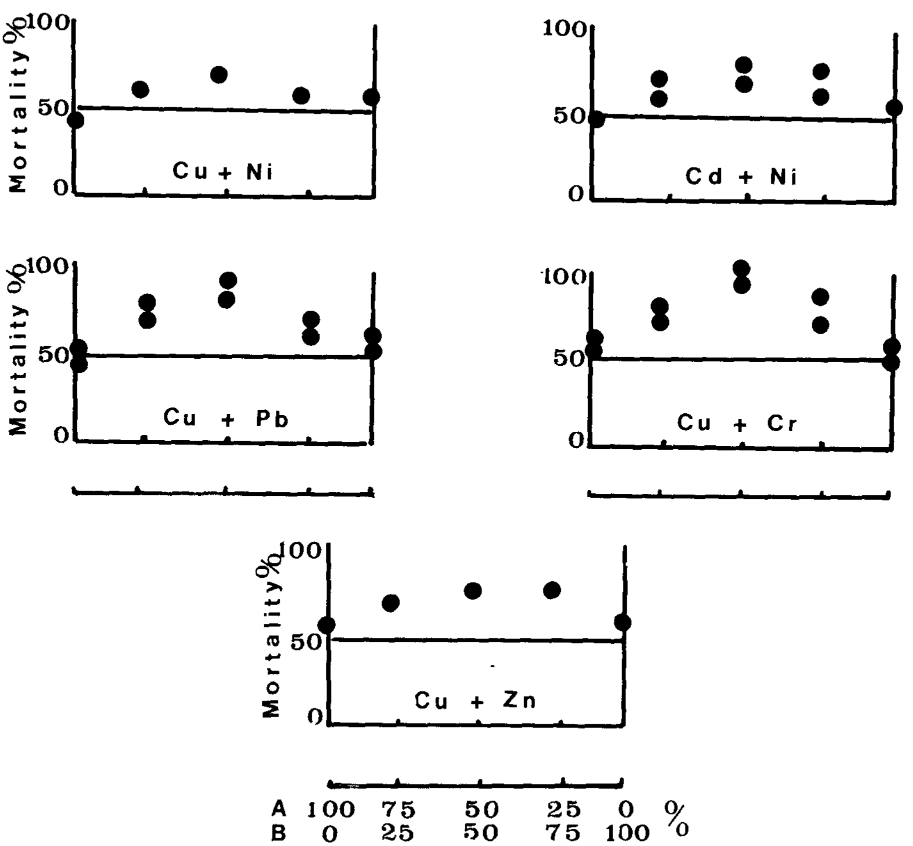


Figure 2. Determination of 48 h LC50 of the different heavy metals according to the Bliss method (1938).
Cu = 0.37 mg/L ; Zn = 0.62 mg/L ; Cd = 0.91 mg/L
Ni = 2.58 mg/L ; Pb = 6.34 mg/L ; Cr =14.13 mg/L

The order of toxicity of heavy metals when acting alone is the following: Cu > Zn > Cd > Ni > Pb > Cr. Figure 3 illustrates the combined effects of the metals (in mixtures of two) on the mortality of *Tisbe* after exposure for 48 h, according to the Warren (1971) method.



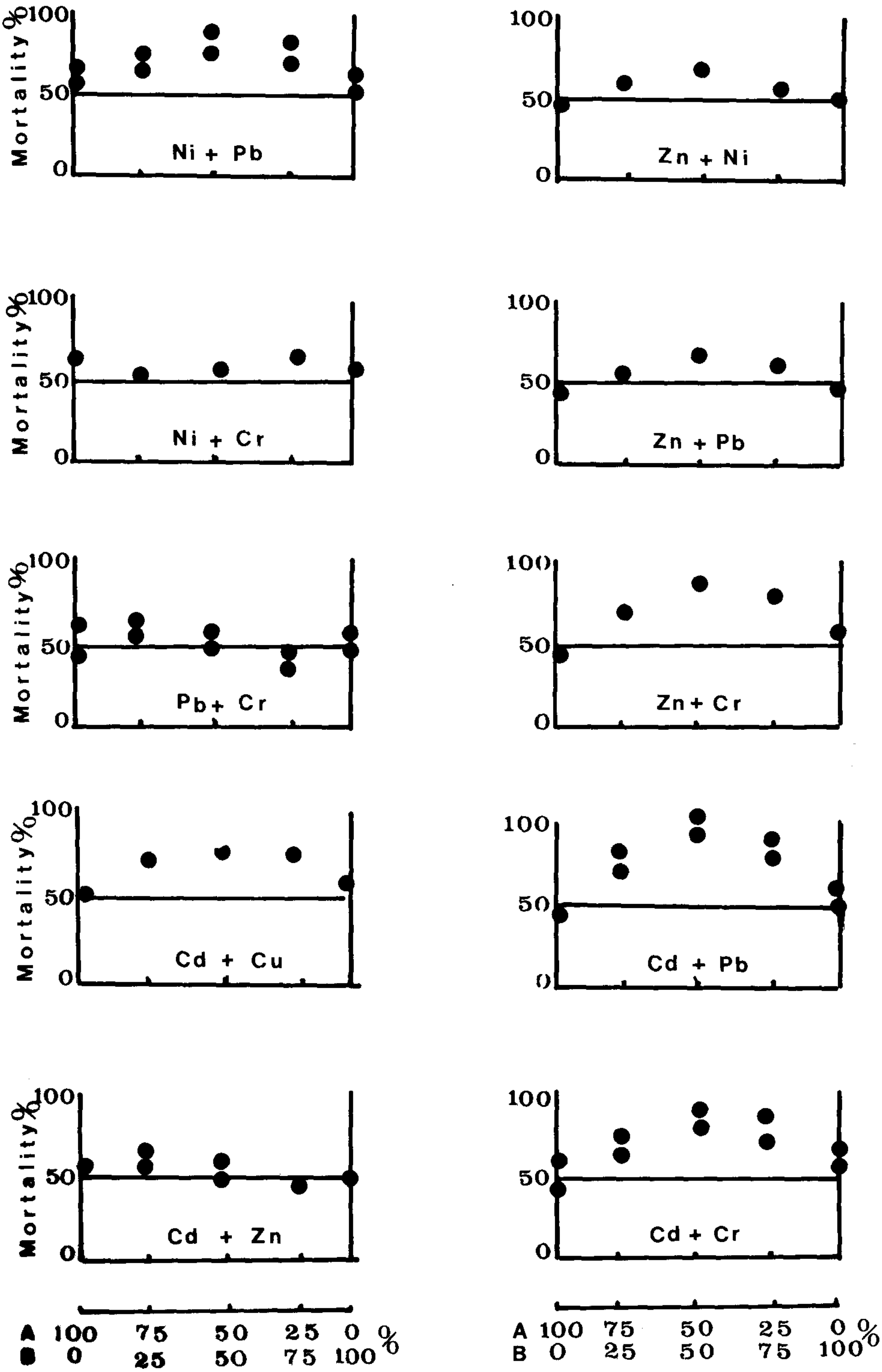


Figure 3. Combined effects of mixtures of two metals (A + B) on the mortality of Tisbe.

Table 1. Types of interaction of combinations of two metals (Su.- A: supra additive; St.- A: strictly additive; In.- A: infra additive).

Metal combination	Type of interaction	
Cd + Cu	Su.- A	(potentiation).
Cd + Zn	St.- A	(synergism).
Cd + Ni	Su.- A	(potentiation).
Cd + Pb	Su.- A	(powerful potentiation).
Cd + Cr	Su.- A	(potentiation).
Cu + Zn	Su.- A	(potentiation).
Cu + Ni	Su.- A	(synergism).
Cu + Pb	Su.- A	(potentiation).
Cu + Cr	Su.- A	(powerful potentiation).
Zn + Ni	Su.- A; St.- A	(synergism).
Zn + Pb	Su.- A	(synergism).
Zn + Cr	Su.- A	(potentiation).
Ni + Pb	Su.- A	(potentiation).
Ni + Cr	Su.- A	(synergism).
Pb + Cr	St.- A; In.- A	(synergism).

In all experiments, clear cases of synergism were observed (tab. 1). The order of toxicity of the various combinations of toxicant mixtures is the following: Pb+Cr = Cd+Zn < Ni+Cr < Zn+Ni < Pb+Zn = Ni+Cu = Zn+Cu = Cd+Cr < Cd+Ni < Cd+Cu < Pb+Ni < Zn+Cr < Pb+Cu < Cr+Cu = Pb+Cd The estimation of the toxicity is based on the toxicity of a solution containing 50 % of the LC50 of each toxicant. All six tested metals proved toxic to *T. holothuriea*. The 48 h LC50 of copper was found to be the most toxic; about 37 times higher than that of chromium. Our findings are in accordance with the data on acute toxicity of the six metals refered to by various researchers (Brown and Absanullah 1971, Calabrese et al. 1973, D'Agostino and Finney 1974). Some deviations in the different 48 h LC50 (for Cu, Cd & Cr) for *Tisbe holothuriae*, observed between this study and previous ones, (0.08 mg/L Cu; 8.14 mg/L Cr; 0.97 mg/L Cd; Moraitou-Apostolopoulou and Verriopoulos 1982, 0.42 mg/L Cu; 0.87 mg/L Cd; Verriopoulos et al.1987) must be due to different experimental conditions (temperature, water quality - synthetic or natural -,food e.t.c).

The problem of toxic effects of pollutants acting jointly appers very complicated. The interaction of pollutants depends not only on the components of the mixture but also on the organism affected. The combination Cu + Cr, for example presents a strictly additive action with the copepod *Tisbe holothuriae* (Moraitou-Apostolopoulou and Verriopoulos 1982; and the present results) and less-than-additive effects with *Artemia salina* (Verriopoulos et al.1987). According to Break et al.(1976) Cu + Zn gave clear cases of synergism with the dinoflagellate *Amhidirium carteri* and the diatom *Thalassiosira pseudonana* while the same metals acted as antagonists towards the diatom *Phaeodactylum tricornutum*. The existence of mixtures of metals that act jointly producing more pronounced effects than the individual components is an important and neglected aspect of pollution research. Doudoroff and Katz

(1953) and D'Agostino and Finney (1974) also found that copper and cadmium act synergistically.

Usually in combined toxicity studies, with two pollutants, the interaction is additive (Lloyd 1961; Herbert and Shurben 1964; Herbert and Vandyke 1964; Sprague and Ramsey 1965; Moraitou-Apostolopoulou and Verriopoulos 1982). Anderson and Weber (1975) investigated the multiple toxicity in fish with the following results: Cu + Ni present strictly additive action or concentration addition; Cu + Zn present synergetic action. According to Von Westernhagen et al. (1979) the mixtures of Cu and Pb present additive action in the herring's eggs and larvae. Although there appears to be apparent usefulness in adopting the described rationale for predicting the lethal effects of mixtures, there is a need to investigate whether the action of sublethal concentrations of constituants in a mixture can be extrapolated from their lethal effects as mixtures. Much remains to be done concerning toxicant interactions. The various mechanisms involved in pollutant interactions remain relatively unknow; extrapolation of laboratory data to a field situation is difficult due to the sheer complexity of the interacting factors.

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