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EFFECTS OF SUBLETHAL CONCENTRATIONS OF ZINC, CHROMIUM AND COPPER ON THE MARINE COPEPODS *Tisbe holothuriae* AND *Acartia clausi*

by

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A B S T R A C T

The effects of sublethal concentrations of zinc, chromium and copper on the mortality, the fertility, the longevity, the food and oxygen consumption of the marine harpacticoid copepod *Tisbe holothuriae* (benthic) and *Acartia clausi* (planktonic) are discussed.

Our experiments led to the following. After prolonged exposure, the percentage of animals producing egg-sacs decreases significantly from generation to generation. The mortality of non-exposed animals, fed with contaminated food, increases proportionally with the exposure concentration. Longevity, fertility and food consumption demonstrate a progressive decrease with increasing concentrations, while respiration rates increase.

The influence of low sublethal concentrations, on the pollution "adapted" population is less pronounced than on the "non-adapted" one.

1. INTRODUCTION

The purpose of this paper is to present the effects of sublethal concentrations of zinc, chromium and copper on the mortality, the fertility, the longevity, the food and the oxygen consumption of the marine copepods *Tisbe holothuriae* and *Acartia clausi*.

Heavy metals are considered to be among the most harmful aquatic pollutants. Their toxicity to marine organisms has been found to vary not only between, but also within species. This has been attributed to various factors: temperature, salinity (Vernberg *et al.*, 1974; Verriopoulos, 1980), population density (Verriopoulos and Moraitou-Apostolopoulou, 1981), life stage (Calabrese *et al.*, 1973, 1977; Calabrese and Nelson, 1974; Verriopoulos and Moraitou-Apostolopoulou, 1982).

Sublethal doses affect physiological functions and behaviour without causing direct death. After exposure, a postponement or even inhibition of growth, of various marine animals, has been mentioned by Calabrese *et al.* (1973), Saliba and Ahsanullah (1973) and Benijts-Clauss and Benijts (1975).

Zinc toxicity is related to the inhibition of enzymic reactions of crustaceans. It affects respiration and osmoregulation (Chen and Slinn, 1980; Bittar *et al.*,1982; Haya *et al.*, 1983); reduces the regeneration rate (Weis, 1980); and decreases larval growth rate (Benijts-Clauss and Benijts, 1975), longevity, body length, reproduction (Winner, 1981), and the sex-ratio (Lalande and Pinel-Alloul, 1984).

Hexavalent chromium affects reproduction (Biesinger and Christensen, 1972; Oshida and Reish, 1975), feeding activity (Capuzzo and Sasner, 1977) and respiratory rates (Raymont and Shields, 1964).

Copper usually increases the respiration rates of marine organisms (Raymont and Shields, 1964; O'Hara, 1971); reduces egg production; decreases the feeding rate (Reeve et al., 1977; Moraitou-Apostolopoulou and Verriopoulos, 1979); produces changes in the blood (Christensen et al., 1972) and inhibits immunity (Roales and Perlmutter, 1977).

2. MATERIALS AND METHODS

The test animals were collected from two areas in the Saronikos Gulf: a "polluted area" in Elefsis bay and a "non-polluted area" near the Fleves islands.

All experiments were run in constant temperature rooms under the following experimental conditions: Salinity 38; food for Acartia clausi (Exuviella baltica, Skeletonema costatum, Nitzschia closterium and Chaetoceros danicus) for Tisbe holothuriae (Germaline and Ulva lactuca contaminated with different concentrations of Zn); Ulva collected from a non contaminated area was exposed for 48 hours to the following concentrations of zinc 10; 50; 100; 200; 500 and 1000 ppm; photoperiod for Tisbe holothuriae 12 hrs dark - 12 hrs light, for Acartia clausi 24 hrs dark; pollutants, concentrations, experimental temperatures and the lethal concentrations are shown in Table 1.

LC50 values for Acartia and Tisbe had been determined in previous studies, with the same experimental conditions.

Statistical analysis of the results was performed by the paired t-test.

In the case of Tisbe holothuriae, after egg hatching, the parental copepods were placed in other containers filled with freshly prepared solutions. As soon as the females of the F1 generation matured and their first egg-sac appeared, 10-20 of them were placed individually in glass containers filled with 50 ml of toxicant solution.

During the experiments, every 24 hrs: a) all containers were examined under the binocular microscope and dead copepods were removed in order to observe the mortality of the various generations at the different concentrations; b) test animals were supplied with food and c) the toxic solutions in the containers were changed.

For Acartia clausi, the ingestion rate was estimated by haematocytometer, 24 hrs after the addition of food. Oxygen consumption was measured by polarography.

In the longevity and the fecundity observations, for each concentration and for each of the two populations, 20 mature females were placed individually in glass containers filled with 50 ml of solution.

Table 1

Experimental conditions. (*) Animals from polluted area.
(**) Animals from non-polluted area.

Concentrations are: a) Nominal for Ulva
b) as measured at the beginning of the experiment for Tisbe and Acartia.

Species	Pollutants	Concentrations ppm	Temperature °C	LC50 (48h) ppm
*Tisbe holothuriae**	$ZnSO4.7H_2O$	0.000 0.007 0.010 0.070	18 ± 0.5	0.713 Zn
	Na_2CrO_4	0.000 0.500 1.000 2.000	14 ± 0.5	14.370 Cr
Ulva lactuca (contaminated food)	$ZnSO4.7H_2O$	0.0 10.0 50.0 100.0 200.0 500.0 1000.0	18 ± 0.5	
Acartia clausi * *Acartia clausi* **	$CuSO4.5H_2O$	0.000 0.001 0.005 0.010	14 ± 0.5	0.080 Cu 0.034 Cu

The feeding and the oxygen consumption experiments were run in 500 ml Erlenmeyer flasks containing 10 adult females of *Acartia clausi*. At zero time, the concentration of phytoplankton fluctuated from 48000 to 51000 cells ml^{-1}.

3. RESULTS

3.1 *Tisbe holothuriae*

Table 2 shows the average percentage mortality for each generation (F1, F2, F3 and F4) as well as the mortality of nauplii (N) and copepodites (C) caused by the different concentrations of Zn. The highest mortality was observed for the F1 generation at a concentration of 0.07 ppm, and for the F4 generation at a concentration of 0.01 ppm, the latter mortality being slightly lower.

Table 3 shows the percentage of animals producing egg-sacs (fertility), for each generation, at the different sublethal concentrations of Zn. A statistically significant (99%) decrease of the % of animals with egg-sacs is observed with each increase of the sublethal concentration up to 0.07 ppm Zn.

Table 2

Total mortality [%] for each generation of <u>Tisbe holothuriae</u> (F1...F4) at different concentrations of Zn and percentage mortalities (%) in relation to the total mortality for nauplii (N) and copepodites (C).

	0.07 ppm Zn	0.01 ppm Zn	0.007 ppm Zn	Control
F1	↗79.2 (N) [7.6] ↘20.8 (C)	↗85.7 (N) [1.2] ↘14.3 (C)	↗72.9 (N) [1.7] ↘27.1 (C)	↗51.6 (N) [0.8] ↘48.3 (C)
F2		↗81.8 (N) [1.0] ↘18.2 (C)	↗67.5 (N) [0.8] ↘32.5 (C)	↗40.1 (N) [0.7] ↘59.9 (C)
F3		↗88.8 (N) [1.3] ↘11.2 (C)	↗52.2 (N) [1.5] ↘47.8 (C)	↗41.5 (N) [0.6] ↘58.5 (C)
F4		↗85.4 (N) [6.5] ↘14.6 (C)	↗64.3 (N) [1.0] ↘35.7 (C)	↗50.0 (N) [0.5] ↘50.0 (C)

Table 3

Percentage of <u>Tisbe holothuriae</u> producing egg-sacs per day at the different sublethal concentrations of Zn, for each generation (F1, F2 and F3).

		Egg-sacs					
	ppm Zn	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
F1	(0.070)	3.6	2.1	0.4			
F1	(0.010)	25.3	19.5	18.8	11.7	4.5	1.3
F1	(0.007)	35.2	35.0	31.2	25.6	14.8	3.7
F1	Control	41.9	36.6	28.5	26.8	14.3	8.9
F2	(0.010)	19.6	5.6	3.4	2.1	0.7	
F2	(0.007)	15.4	10.9	6.4	4.4	2.1	1.4
F2	Control	21.5	20.6	19.4	15.3	8.7	6.3
F3	(0.010)	6.6	4.7	2.8	1.1		
F3	(0.007)	24.2	16.9	12.1	5.6		
F3	Control	21.9	21.9	20.7	16.5	12.2	10.9

After 3 days exposure to a Zn concentration of 0.07 ppm only 0.38% of the Fl generation produced egg-sacs. With a Zn concentration of 0.01 ppm a statistically significant (99%) decrease of the % of the population producing egg-sacs is observed, from generation to generation. On the other hand there is a non-statistically significant difference in fertility between the control and the 0.007 ppm concentration of Zn.

Tisbe holothuriae fed with zinc contaminated Ulva lactuca presented a mortality which increased proportionally to the degree of contamination of Ulva lactuca as well as with time (Table 4).

Table 5, shows the fertility (egg-sacs/female), the time between successive sacs (in days), the abortion of egg-sacs, the hatching (offspring/female) for the F2 generation and the maturation time (in days) for the different concentrations of Cr.

Table 4

Mortality (%) in 48 hrs of Tisbe holothuriae fed with contaminated Ulva lactuca for different exposure periods.

ppm of Zn	days of exposure	mortality %
10	2	0
	5	5
	7	47
50	2	17
	5	60
	7	100
100	2	52
	5	60
	7	100
200,500,1000	2	90
	5	100

Table 5

Tisbe holthuriae population dynamics (fertility, time between egg-sacs, abortion, hatching and maturation time) after exposure to different concentrations of Cr.

Cr ppm	Fertility egg-sacs/ female	time between egg-sacs (days)	Abortion %	Hatching offspring/ female	Maturation (days)
Control	5.8	4.0	36.9	110.2	12
0.5	3.5	4.5	41.0	52.8	20
1.0	2.5	4.0	89.0	44.1	-
2.0	1.4	3.4	97.0	6.6	-

In comparison to the control animals all chromium concentrations reduce the longevity of F2 females. Very pronounced differences in the longevity of the F3 generation have been observed between the tested concentrations. Not one F3 Tisbe holothuriae survived longer than 4 days at a concentration of 2 ppm of Cr and the development of eggs did not pass the first naupliar stage. Table 6 presents the survival of F2 and F3 generations at the tested chromium concentrations.

Table 6

Survival (%) of F2 and F3 generations of Tisbe holothuriae exposed to different Cr concentrations.

Survival %					
ppm Cr	Day 5	Day 10	Day 15	Day 20	Generation
Control	95	90	80	70	F2
0.50	93	87	75	68	F2
1.00	82	60	20	0	F2
2.00	87	37	0		F2
0.50	96	62	25	12	F3
1.00	25	12	0		F3
2.00	0				F3

3.2 Acartia clausi

There is a statistically significant (95%) decrease in survival with increasing Cu concentration. The sensitivity of Acartia clausi living in the non-polluted area is more pronounced (statistically significant) than that from the polluted area (Table 7).

Table 8 shows the fecundity (number of eggs/copepod on the 4th day), the ingestion rate (cells per copepod in 24hrs) and the oxygen consumption (µl O_2 in 20 hrs) of the female Acartia clausi, from the polluted and non-polluted areas, exposed to the different sublethal concentrations of copper.

The fecundity of the "polluted" Acartia clausi was higher than that of the "non-polluted" one. There is no egg production in 0.01 ppm Cu for the "non-polluted" animals. There is a progressive decrease in the egg production with increasing concentrations of Cu, for the "non-polluted" animals and there is a statistically significant difference for the "polluted" animals.

A reduction in food consumption was observed for the "non-polluted" Acartia clausi, between the controls and 0.050 ppm of Cu. For the "polluted" copepods, there is no difference between controls and 0.001 ppm and a sharp decrease was observed at 0.005 ppm Cu. In the control, both populations show a similar ingestion rate.

Concerning respiration, all tested concentrations produce increases in oxygen consumption. Furthermore, the "polluted" population showed higher respiratory rates than the "non-polluted" one.

Table 7

Survival (%) per day of female Acartia clausi from polluted and non-polluted areas exposed to different concentrations of Cu.

	Survival %										
ppm Cu	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	(days)
Control	92	80	80	75	70	70	65	60	55	50	polluted
0.0010	80	80	75	70	60	55	45	35	25	15	polluted
0.0025	75	75	75	70	65	60	45	30	15	10	polluted
0.0100	75	70	65	45	40	40	30	15	10	0	polluted
Control	90	80	80	80	65	55	45	40	35	35	non-poll
0.0010	90	72	60	50	30	25	15	0			non-poll
0.0050	85	75	65	35	25	20	10	0			non-poll
0.0100	85	60	15	0							non-poll

Table 8

Effects of Cu on the fecundity, the ingestion rate and the oxygen consumption of the female Acartia clausi from polluted and non-polluted area.

	Ingestion rate (cells ingested per 24h)		Fecundity (eggs per copepod on day 4)		Oxygen consumption (µl O_2 consumed in 20h)	
ppm Cu	Poll	non-Poll	Poll	non-Poll	Poll	non-Poll
Control	25600	25550	5.25	3.12	0.010	0.006
0.0010	24950	14440	6.00	1.00	0.018	0.009
0.0025			7.06			
0.0050	12290	3065		0.28	0.022	0.019
0.0100			5.69	0.00		

4. DISCUSSION

The exposure of four subsequent generations of Tisbe holothuriae to three concentrations of Zn (0.07, 0.01 and 0.007 ppm) shows that the toxicity of Zn increases with concentration and prolonged exposure.

The results show that exposure to 0.07 ppm Zn (1/10 of the 48h LC50) does not lead to high population mortality for the first generation but that the copepods cannot produce a second generation; this is due mainly to the small number of egg-sacs (up to 3) and the small % of animals producing egg-sacs. The % of animals producing egg-sacs decreases significantly from generation to generation.

The observed results are due, firstly to the progressive decrease of the % of animals producing egg-sacs, as the concentration of zinc increases from 0.007 to 0.07 ppm Zn; secondly, to the increased mortality of the nauplii as compared to the copepodites and thirdly, to the capacity of the organisms to support certain sublethal concentrations of Zn. Alaysed-Danet et al. (1979) attributed the decrease of the developmental rate of Artemia salina exposed to zinc, to the disturbance of the amylase and trypsine systems. According to Bittar et al. (1982), zinc stops the catalytic action of the cyclic AMPkinase on the sodium flux of the muscle of Balanus nubilis.

For the 0.07 and 0.01 ppm concentrations of Zn, a disequilibrium between the "adaptation-detoxification" rate and the growth rate is observed; this is why population size decreases with time, from generation to generation.

When non-exposed Tisbe holothuriae were fed with zinc contaminated Ulva, the medium was probably contaminated with Zn from food. In aquatic plants, losses of heavy metals into the surrounding water occurs over the general body surface by diffusion or in association with secretions. However, the ingestion of contaminated food must be the main cause of Tisbe holothuriae mortality. We may assume that, during exposure, Ulva lactuca had absorbed significant quantity of Zn (Bryan, 1974). In most cases the uptake of the heavy metals by aquatic plants seems to be a passive process. The concentration of metals in marine plants tends to reflect those of the water because marine plants are not able to regulate heavy metals.

Mortality of Tisbe holothuriae rises with increasing contamination of Ulva lactuca with Zn. Experimental work on the plaice Pleuronectes platessa (Pentreath, 1973; 1976) and on the euphasiid Meganyctiphanes norvegica (Small et al. 1973; Benayoun et al., 1974) has shown that, even at the earliest stages, food is the main source of most metallic contaminants and their radionucleides including, iron, cobalt, zinc, manganese, methyl mercury, cadmium and silver.

All tested Cr concentrations affect the population dynamics of Tisbe. The longevity of both F2 and F3 generations decreased after exposure to Cr and the survival time became progressively shorter with increasing chromium concentrations.

The nauplii of the F3 generation were much more sensitive than the F2 ovigerous females: at 2 ppm their survival time was about 1/15 of that of the control animals. Due to the increased sensitivity of embryonic stages, even at low pollutant concentrations which do not significantly affect adult animals, a population may be destroyed.

Another effect of Cr, on the population density of a species, is the inhibition of sexual maturation or the prolongation of the developmental period. Only very few Tisbe of the F3 generation, which survived at the 0.5 ppm concentration, arrived at maturity and coupled. Chromium also affects sexual maturation or prolongs the developmental period thereby influencing population density. The first ovigerous sac of these females appeared on the 20th day and in the control animals on the 12th day.

Longevity, fertility and food consumption shows a progressive decrease with increasing copper concentrations, while respiration rates increase.

The influence of the low copper concentrations, on the pollution "adapted" population of Acartia clausi, is less pronounced. According to Bryan and Hummerstone (1971), Brown (1976) and Bradshaw (1970), there is a genetic adaptation. This adaptation seems to develop separately for each metal, depending on its presence in the specific environment.

5. REFERENCES

Alayse-Danet, A.M., J.L. Charlou, M. Jezequel and J.F. Samain, 1979. Model of rapid detection of sublethal effects of pollutants modification of amylase and trypsin levels of Artemia salina contaminated by copper or zinc. Mar.Biol., 51:41-46

Benayoun, G., S.W. Fowler and B. Oregioni, 1974. Flux of cadmium through euphasiids. Mar.Biol., 27:205-212

Benitjts-Clauss, C. and F. Benijts, 1975. The effect of low zinc concentrations on larval development of the mud crab Rhithropanopeus harrisii Gould. In: Sublethal effects of toxic chemicals on aquatic animals, edited by J.H. Koeman and J.T.W. Strik, Amsterdam, Elsevier, pp.43-52

Biesinger, K.E. and G.M. Christensen, 1972. Effects of various metals on survival, growth reproduction and metabolism of Daphnia magna. J.Fish.Res.Bd.Can., 29:1691-1700

Bittar, E.E., G. Chambers and E.H. Fischer, 1982. The influence of injected cyclic AMP protein kinase catalytic subunit on the sodium flux in barnacle Balanus nubilis muscle fibres. J.Phys., 339:39-52

Bradshaw, A., 1970. Pollution and plant evolution. New Scientist, 17:497-506

Brown, B, 1976. Observations on the tolerance of the isopod Asellus meridianus. Rac. to copper and lead. Water Res., 10:555-559

Bryan, G.W. and L.G. Hummerstone, 1971. Adaptation of polychaete Nereis diversicolor to estuarine sediments containing high concentrations of heavy metals. I. General observations and adaptation to copper. J.Mar.Biol.Ass.U.K., 51:845-863

Bryan, G.W., 1974. Adaptation of an estuarine polychaete to sediments containing high concentrations of heavy metals. I. General observations and adaptation to copper. J.Mar.Biol.Ass.U.K., 51:845-863

Calabrese, A., R.S. Collier, D.A. Nelson and S.R. MacIness, 1973. The toxicity of heavy metals to embryos of American oyster Crassostera virginata. Mar.Biol., 18:162-166

Calabrese, A. and D.A. Nelson, 1974. Inhibition of embryonic development of the red clam Mercenaria mercenaria by heavy metals. Bull.Envir.Contam.Toxicol., 2:92-97

Calabrese, A., S.R. MacInnes, D.A. Nelson and S.E. Miller, 1977. Survival and growth of bivalve larvae under heavy metals stress. Mar.Biol., 41:179-184

Capuzzo, J.M. and J.J. Sasner, 1977. The effects of chromium on filtration rate and metabolic activity of Mytilus edulis L. and Mya arenaria. In: Physiological responses of marine biota to pollutants, edited by F.S. Vernberg, Academic Press, pp.225-237

Chen, H.C. and J.D. Slinn, 1980. Osmoregulation of prawn Palaemon elegans exposed to some heavy metals. J.Fish.Soc.Taiwan, 7:1-13

Christensen, G.M., J.M. McKim, W.A. Brungs and E.P. Hunnt, 1972. Changes in the blood of the brown bullhed Ictalurus rubulosis following long and short term exposure to copper. Toxicol.Appl.Pharmacol., 23:417-422

Haya, K., B.A. Waiwood and D.W. Johnston, 1983. Adenylate energy charge and ATPase activity of lobster Homarus americanus during sublethal exposure to zinc. Aquat.Toxicol., 3:115-116

Lalande, M. and B. Pinel-Alloul, 1984. Toxicity of heavy metals to planktonic crustaceans from Quebec Canada lakes. Sci.Tech.Eau, 7:253-259

Moraitou-Apostolopoulou, M. and G. Verriopoulos, 1979. Some effects of sublethal concentrations of copper on a marine copepod. Mar.Pollut. Bull., 10:88-92

O'Hara, J., 1971. Alteration in oxyzen consumption by bluegills exposed to sublethal treatment with copper. Water Res., 5:321-327

Oshida, F. and D.S. Reish, 1975. Effects of chromium on reproduction in polychaets. So. Calif. Coastal Water Res. Proj. 1500. Imperial Hawy Segundo Calif.Ann.Rep., 55-65

Pentreath, R.J., 1973. The accumulation and retention of Zn(65) and Mn(54) by the plaice Pleuronectes platessa. J.Exp.Mar.Biol.Ecol., 12:1-18

Pentreath, R.J., 1976. Some further studies on the accumulation and retention of Zn(65) and Mn(54) by the plaice Pleuronectes platessa. J.Exp.Mar.Biol.Ecol., 21:179-189

Raymont, J.E. and J. Shields, 1964. Toxicity of copper and chromium in the marine environment. In: Advances in water pollution research, edited by E.A. Pearson, Academic Press, pp.275-290

Reeve, M.R., J.C. Gamble and M.A. Walter, 1977. Experimental observations on the effects of copper on copepods and other zooplankton. Controlled ecosystems pollution experiment. Bull.Mar.Sci., 27:92-104

Roales, R.R. and A. Perlmutter, 1977. The effects of sublethal doses of methylmercury and copper, applied singly and jointly on the immune response of the blue gourami Trichogaster trichopterus to viral and bacterial antigens. Arch.Environ.Contam.Toxicol., pp.325-331

Saliba, L.J. and M. Ahsanullah, 1973. Acclimation and tolerance of Artemia salina and Ophryotrocha labronica to copper sulphate. Mar.Biol., 23:297-302

Small, L.F., S.W. Fowler and S. Kečkeš, 1973. Flux of zinc through a macroplanktonic crustacean. In: Radioactive contamination of the marine environment. International Atomic Energy Agency, Vienna, pp.437-452

Vernberg, W., P.S. DeCoursey and J. O'Hara, 1974. Multiple environment factors effects on physiology and behaviour of the fidler crab, Uca pugilator. In: Pollution and physiology of marine organisms, edited by J. Vernberg and W. Vernberg, New York, Academic Press, pp.381-425

Verriopoulos, G., 1980. La toxicité du Cr sur le copepode harpacticoide Tisbe holothuriae en rélation avec la temperature. Journ.Etud. Pollut.CIESM., 5(1980):797-802

Verriopoulos, G. and M. Moraitou-Apostolopoulou, 1981. Impact of chromium to the population dynamics of Tisbe holothuriae. Arch.Hydrobiol., 93:59-67

Verriopoulos, G. and M. Moraitou-Apostolopoulou, 1982. Differentiation of the sensitivity to copper and cadmium in different life stages of a copepod. Mar.Pollut.Bull., 13(4):123-125

Weis, J.S., 1980. Effects of zinc on respiration in the fiddler crab Uca pugilator and its interactions with methyl mercury and cadmium. Mar.Environ.Res., 3:249-256

Winner, R.W., 1981. A comparison of body length size and longevity as indices of chronic copper and zinc stresses in Daphnia magna. Env.Poll.Ser.A.Ecol.Biol., 26:33-37