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Combined Toxicity of Four Toxicants (Cu, Cr, Oil, Oil Dispersant) to *Artemia salina*

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In sea waters multicontaminant pollution appears to be the rule rather than the exception. Mixtures of toxicants may produce unexpected effects, some are hazardous and some beneficial. Organisms are not probably safeguarded from the effects of combined toxicants by water quality standards based on simple toxicants.

For a realistic approach to pollution effects it is essential to estimate the combined toxicity of two or more chemicals. There is a need to understand the mechanisms and quantify the effects of multiple toxicity in order to provide responsible authorities with rational estimate of the effects of chemical mixtures. Thus the potential toxic effects of mixtures of toxicants has recently become a subject of growing scientific interest.

In this paper we have tried to estimate the joint toxicity of some pollutants commonly found in nearshore polluted waters: two metals, copper and chromium; an oil (Tunesian crude oil zarzaitine type); and an oil dispersant (Finasol OSR-2).

MATERIALS AND METHODS

Adult Artemia salina (25 days old; 3.5-4.5 mm long) were used as test animals. These were hatched from commercially available cysts under the same experimental conditions (22 °C, 38 o/oo S, light conditions: 12-h day, 12-h dark, fed with Germalyne[1]). The acute toxicity of the four toxicants (copper, chromium, oil, oil dispersant) to Artemia salina acting individually or jointly (all combinations of two, three of four chemicals) was estimated by determination of the 48-h LC50 (concentration of a toxicant which kills 50% of the test animals after 48 h of exposure) according to the Bliss (1938) method.

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

All experiments (static bioassays) were carried out in temperature conditioned rooms (22 °C). In all cases we used synthetic sea water (Synthetica type). The experimental animals were placed individually in 200-mL vials.

In order to prepare the solutions of Cu ($CuSO_45H_2O$) and Cr($Na_2C_2O_4$) necessary for the experiments we initially prepared concentrated solutions, with distilled water, and then diluted these with sea water. Oil (tunesian crude oil, zarzaitine type) - water dispersions (OWDs)[2] and oil dispersant (Finasol OSR-2) solutions were prepared by diluting the calculated amount of the toxicant in sea water.

After the addition of the toxicant the solution was shaken at approximately 2000 cycles per min for 30 min. Detailed characteristics of the tested OWDs and the resulting concentrations of hydrocarbons at various times could not be obtained. The range of tested toxicant concentrations is shown in the mortality curves (Figures 1 to 3).

Two series of experiments were performed: in the first we evaluated the toxicity of the two metals when acting alone. The toxicity of oil and Finasol was estimated in a previous paper (Verriopoulos and Moraitou-Apostolopoulou 1982). In the second we evaluated the toxicity of the various combinations of toxicant mixtures. Artemia was exposed to the following types of mixtures:

1) Cr + oil
2) Cu + Cr
3) Cr + Finasol
4) oil + Finasol
5) Cu + Finasol
6) Cu + oil
7) Cu + oil + Finasol
8) Cu + Cr + oil
9) Cr + oil + Finasol
10) Cu + Cr + Finasol
11) Cu + Cr + oil + Finasol

As the LC50 of each pollutant causes the same (59%) mortality, parts of the LC50 (e.g.10, 20%) are theoretically expected to cause the same mortality (e.g. 10% of the LC50 causes a 5% mortality). In the experiments of evaluation of the multiple toxicity we calculated each toxicant concentration to be added in the mixture as part of the relevant LC50. In each mixture for each toxicant we added the same percentage of its LC50. In this way all constituents are equitoxic contributing equally to the toxicity of the mixture. The concentrations of each pollutant in the test mixtures are shown in Table 1.

The differences between the toxicities of the various toxic mixtures were tested statistically by the t-test.

The toxicity of a mixture of two toxicants was determined by using the additive toxicity index (A.I.) developed by Marking and Dawson (1975). The necessary calculations for the index are:

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[2]OWDs have a hydrocarbon composition very closely resembling that of the parent oil (Anderson et al 1974).

Table 1. Concentrations of each toxicant in the test mixtures in combined toxicity experiments

	10%	20%	30%	40%	50%	60%
Cu	0.056	0.052	0.168	0.224	0.280	0.336
Cr	1.291	2.582	3.873	5.164	6.455	7.745
Oil	29.78	59.56	89.34	111.12	148.90	178.69
Finasol	0.094	0.188	0.282	0.376	0.470	0.564
	70%	**80%**	**90%**	**100%**	**LC_{50}^{48-h}**	
Cu	0.392	0.480	0.504	0.560	mg/L	
Cr	9.037	10.328	11.519	12.910	mg/L	
Oil	208.46	238.24	268.02	297.80	mg/L	
Finasol	0.658	0.752	0.846	0.940	mg/L	

$$\frac{Am}{Ai} + \frac{Bm}{Bi} = S \qquad A.I. = \frac{1}{S} - 1.0 \text{ (when } S \leq 1.0) \text{ or } S(-1) + 1.0 \text{ (when } S \geq 1.0)$$

where A and B are chemicals, i and m are the toxicities (the LC50 values) of the individual toxicants and the mixture, respectively, and S is the sum of biological activity. Additive indices of -, 0, and + indicate less than additive, additive and more than additive toxicity respectively. The same formula was used with the three and four toxicant mixtures.

RESULTS AND DISCUSSION

When the two metals are acting individually to Artemia copper proved much more toxic (more than 20 times) than chromium. The calculated LC50 values for Artemia (Fig. 1) are as follows: Cu (0.485 ppm), Cr (12.838 ppm). The LC50 for oil is 297.8 ppm and for Finasol it is 0.90 ppm. Fig. 2 shows and compares the combined effects of mixtures of two and three pollutants, and Fig. 3 of four pollutants.

The results of the *t*-test are given in Table 2 for data of toxicity of various mixtures of toxicants. All differences are significant at the 95% level. Values marked by squares are significant at the 99% level (df=8).

The calculated LC50 values of the mixtures (% of the LC50 of the components), the concentrations (ppm) of the components, the linear regression equations (Bliss method) and the estimation of multiple toxicity according to Marking and Dawson (Sum of Biological activity and additive index) are given in Table 3.

When the pollutants are acting in mixtures of two, two types of joint toxicity are observed; a) strict additive: this is the case of the mixtures Cr + oil (A.I. = -0.016 practically 0), Cr + Finasol (A.I. = -0.109 practically 0), and b) less than additive: the less than additive effect was less intense in the mixture Cu + Cr (A.I. = -0.53) and clearly pronounced in the mixture oil + Finasol

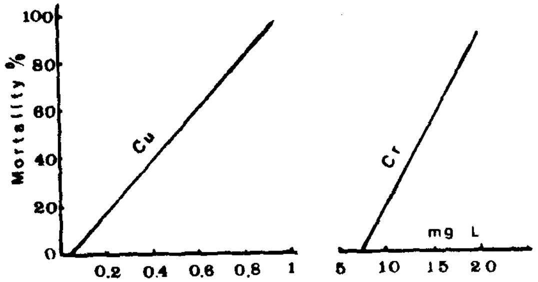
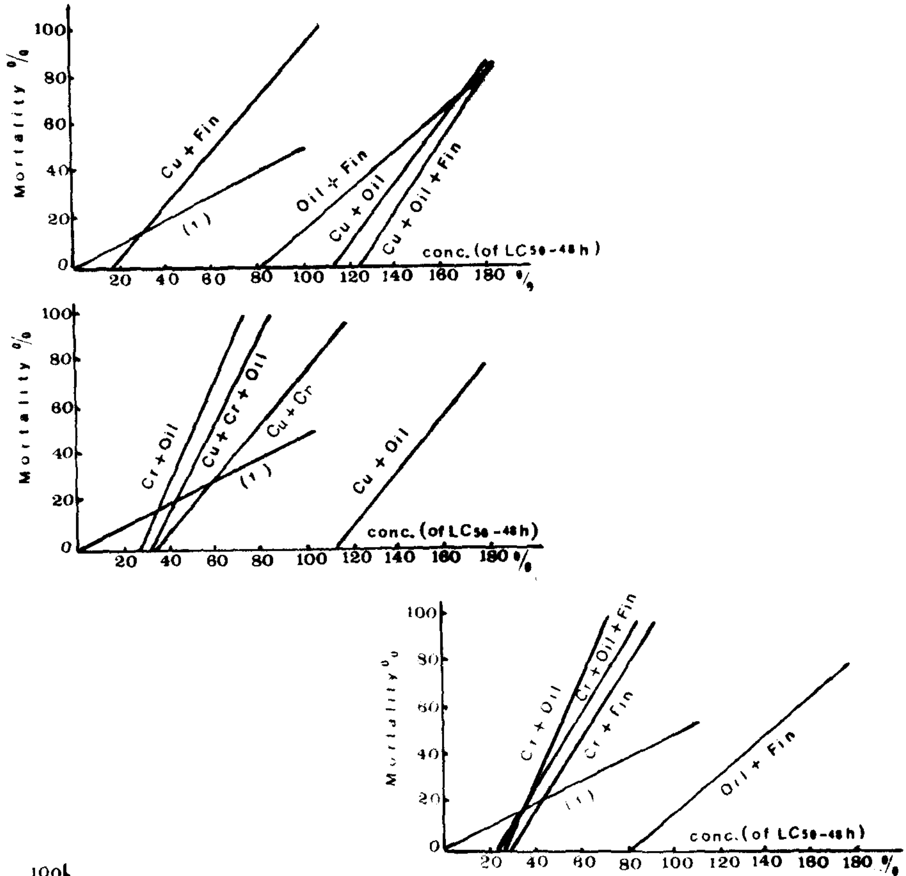


Fig. 1 Mortality curves of Artemia salina exposed to copper and chromium.



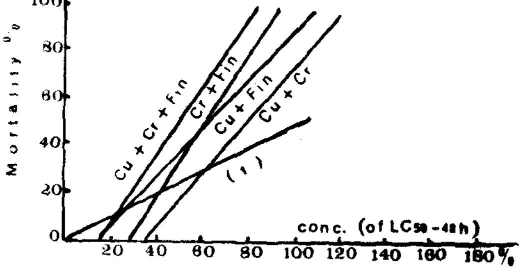


Fig. 2 Mortality curves of two and three pollutant mixtures in comparison with the curve of these pollutants when acting alone (curve 1)

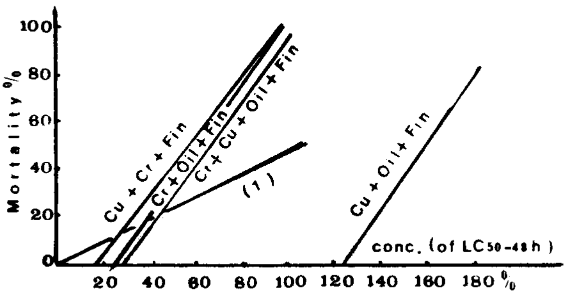


Fig. 3 Mortality curve of a mixture of four toxicants in comparison with those of the three toxicants and that of the pollutants acting alone.

Table 2. -test analysis of the percentage mortalities of _Artemia salina_ exposed to various mixtures of the four toxicants (d.f. = 8). All values are statistically significant at the 95% level. Values marked by squares are significant at the 99% level.

	Cr + oil	Cr + Fin.	Cr + Fin.	Cu + Fin.	Cu + Cr	Cu + + oil	Cu + Cr + Fin.	Cu+Cr+ oil+ Fin.
Cr + oil	-	3.4614	2.308	2.300	5.017 ■	3.481	0.734	4.0845
Cr + Fin.		-	4.320	0.093	4.962 ■	1.5965	5.7615 ■	4.0629
Cr+oil+Fin.			-	1.957	5.517 ■	2.2586	3.472	4.750 ■
Cu + Fin.					3.126	0.7689	4.637 ■	2.797
Cu + Cr						3.9866	7.805 ■	5.132 ■
Cu+Cr+oil						-	2.9336	2.7367
Cu+Cr+Fin.							-	7.117 ■

(A.I. = -1.87) and Cu + oil (A.I. = -2.03)

When three of the pollutants are acting jointly in all cases a less than additive reaction was observed. This type of joint activity was very clearly pronounced in the case of the mixture Cu + oil + Finasol (A.I. = -3.75).

The mixture of the four pollutants exhibited an important (A.I. = -1.63) less than additive reaction.

The toxicities for _Artemia_ reported in the present paper are similar to those mentioned for other small marine crustaceans (Moraitou-Apostolopoulou 1978; Moraitou-Apostolopoulou and Verriopoulos 1982a,b). The order of individual toxicities of the four toxicants

Table 3. LC50 values of the various mixtures of the four toxicants (% of the LC of the components), concentrations (ppm) of the components, and estimations of multiple toxicity (S = sum of Biological Activity, A.I. = additive index, A = strict additive effect, LA = less than additive effect).

Solutions	LC_{50}^{48h}		Y = b + mX	S	A.I.
	%LC_{50}^{48h}	of each pollutant (ppm)			
Cu	100	0.48545	Y = 0.03465 + 0.902 X		
Cu	100	12.83825	Y = 7.20495 + 0.112665 X		
Oil	100	297.8	Y = 289.625 + 0.1655 X		
Fin.	100	0.90	Y = 0.5329 + 0.00814 X		
Cr + oil	50.8108	6.52 Cr + 151.31 oil	Y = 26.4865 + 0.48649X	1.016(0)	-0.016 A
Cu + Cr	76.7857	0.37 Cu + 9.86 Cr	Y = 33.7586 + 0.6552 X	1.53	-0.53 LA
Cr + Fin.	60.5172	7.77 Cr + 0.54 Fin.	Y = 27.7586 + 0.6552 X	1.105(0)	-0.105 A
Cu + Fin.	60.8351	0.29 Cu + 0.55 Fin.	Y = 14.2758 + 0.9312 X	1.109(0)	-0.109 A
Oil + Fin.	142.79	1.29 Fin. + 427.8 oil	Y = 80 + 1.256 X	2.87	-1.87 LA
Cu + oil	152.5116	0.73 Cu + 454.17 oil	Y = 110.9972 + 0.8303 X	3.03	-2.03 LA
Cu+oil+Fin.	159.0538	0.76 Cu + 1.43 Fin. + 473.65 oil	Y = 124.2209 + 0.6966 X	4.75	-3.75 LA
Cu+Cr+oil	57.50	0.27 Cu + 7.38 Cr + 171.23 oil	Y = 32.5 + 0.5 X	1.706	-0.706 LA
Cr+oil+Fin.	54.375	6.98 Cr + 0.49 Fin.+170.86 oil	Y = 24.6875 + 0.59375 X	1.66	-0.66 LA
Cu+Cr+Fin.	49.90	0.24Cu+6.14Cr+0.45Fin.	Y = 15.79 + 0.6822 X	1.49	-0.49 LA
Cu+Cr+oil+Fin.	65.75	0.32Cu+8.44Cr+0.59Fin.+1958 oil	Y = 28.25 + 0.75 X	2.63	-1.63 LA

to Artemia adults (Cu>Finasol>Cr>oil) is also the same to that noticed for other marine animals (Brown and Absanullah 1971; Calabrese et al 1973; Verriopoulos and Moraitou-Apostolopoulou 1982).

It must be remembered that pollutant concentrations reported here are those added to the test solutions initially. Effective concentrations of the test toxicants may be significantly reduced by organisms' uptake, absorption to container walls and volatilization. The strict additivity of the mixtures Cr+oil, Cr+Finasol, Cu+Finasol shows that these pollutants when in mixtures of two are acting independently to Artemia. The less than additive toxicity of the mixtures Cu+Cr, oil+Finasol and Cu+oil indicates antagonism expressed when these chemicals are acting jointly. This antagonism could be related to competition for a common uptake site.

Our results indicate a tendency of independent toxic action of chromium in mixtures of two pollutants. In all solutions containing chromium a relatively strict additive joint action was noticed ranged from -0.016 to -0.53). This hypothesis was reinforced by the results of three pollutant joint action: the solutions containing copper, oil and Finasol demonstrated a strong less than additive reaction (A.I. = -3.75); in the three mixtures containing chromium the additive indices ranged from -0.49 to -0.706.

Oil on the contrary showed a tendency for a strong antagonistic joint action when in mixture. Thus the additive index of the mixture oil+Finasol was -1.87 and that of Cu+oil -2.03. Studies on the toxicity of methylmercury and copper to the blue gourami Trichogaster trichopterus (Roales and Perlmutter 1974) indicated that the two toxicants may interact antagonistically. Previous work (Moraitou-Apostolopoulou and Verriopoulos 1982) demonstrated an obvious synergism of the effects of three metals (Cu, Cd, Cr) to Tisbe holothuriae when acting in mixtures of two. The mixture of the three metals presented higher toxicity than that of the individual metals acting separately, but lower than that of two metals mixtures. It seems that the mode of action of pollutants in a mixture depends also on the organism tested. According to Braek et al (1976), Cu and Zn gave clear cases of synergism to the dinoflagellate Amphidinium carteri and the diatom Thalassiosira pseudonana, while the same metals acted antagonistically towards the diatom Phaeodactylum tricornutum. Mixtures of metals may be additive in producing acute but interact in unpredictable ways in producing chronic effects. This is probably because the uptake site of action and toxic mechanisms differ for acute and chronic toxicity. From the mortality curves of pollutant mixtures used in our experiments it seems that in all ranges of tested concentrations the type of joint action is the same.

The problem of toxic effects of pollutants acting jointly seems a very complicated one. The interaction of pollutants depends not only on the components of the mixture but also on the organism affected. Various forms of interaction either chemical or physiological may occur when pollutants are acting jointly. Chemical interactions involve the mutual influences between pollutants that result in new compounds, chelate complexes, etc. Physiological interactions can occur in altering the sequence of events, e.g., the binding of toxicants to the target tissue. Much remains to be done concerning such interactions. The various mechanisms involved in pollutant interactions remain little known; extrapolation of laboratory data to field situation is difficult due to the sheer complexity of the interacting factors.

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