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COMBINED TOXICITY OF FOUR TOXICANTS (Cu, Cr, Oil, Dispersant) TO *Artemia salina*

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In sea waters multicontaminant pollution appears to be the rule rather than the exception. For a realistic approach of pollution effects it is essential to estimate the combined toxicity when two or more pollutantants are acting in combination to marine animals.

In this study 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 OSR2) to *Artemia salina*.

The acute toxicity of the four toxicants acting individually+ or jointly(all combinations of two,three or four chemicals) was estimated by determination of LC_{50} 48h (concentration of a toxicant which kills 50% of the test animals after 48 hours of exposure) according to the Bliss method.

In the experiments of evaluation of multiple toxicity we have calculated each toxicant concentration to be added in the mixture as part of the relevant LC_{50} (eg 10%,20% of the LC_{50}). In this way all components in the solution are equitoxic contributing equally in the toxicity of the mixture. The toxicity of two toxicants mixture was determined using the additive toxicity index developed by Marking and Dawson. The necessary calculations for the index are: $\frac{Am}{Ai} + \frac{Bm}{Bi} = S$ where A and B are chemicals, i and m are the toxicities (the LC_{50} values) of the individual toxicant and the mixture respectively and S is the sum of Biological Activity. To establish linearity and to assign a reference point of zero for simple additive toxicity ,Marking and Dawson calculated the index as follows: Additive index (A.I.) = $S-1.0$ for $S \leqslant 1.0$ (greater than additive toxicity) or $S(-1)+1.0$ for $S \geqslant 1.0$ $\frac{S}{S}$ (less than additive toxicity). Additive indices of $-$,0,+ indicate less than additive,additive and more than additive toxicity respectively. The same formula was used with the three and four toxicants mixture.

When the two metals are acting individually to *Artemia* ,copper proved much more toxic(more than 20 times) than chromium. The calculated LC_{50} values(ppm) are: Cu:0,485,Cr:12,838. The LC_{50} values for oil is 297,8 ppm and for Finasol 0,90 ppm. When the two pollutants are acting in mixture 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.=-0,105 practically 0),Cu+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 (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 problem of toxic effects of pollutants acting jointly seems a very complicated one. The interaction of pollutants depends not only from the components of the mixture but also from 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 sequences of events eg the binding of toxicants to the target tissue. Much remain to be done concerning such interactions. The various mechanisms involved in pollutants interactions remain little known;extrapolation of laboratory data to field situation is difficult due to the sheer complexity of the interacting factors.

+ The acute toxicity(LC_{50} 48h)of oil and Finasol to *Artemia* was estimated in a previous paper

L-VI7

Cadmium toxicity to marine biota

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ABSTRACT: Cadmium influence on unicellular algae in short term and chronic toxicity conditions as well as mussels contamination by nutrition were studied.

Cadmium has no known useful biological function; it is ranked among the most hazardous trace elements in the environment. As free ion this metal ranges in the Romanian Black Sea area from 0.05 to 1.6 $\mu g\ l^{-1}$ (COUSTEAU, 1978; PECHEANU, pers. commun.). Observations on short term effect of Cd were described previously (MIHNEA and cowork., 1980). Little information is available regarding both the acute and chronic effect of Cd on aquatic biota, particularly in a food chain transmission experiment.

In this paper we present data to demonstrate the degree of cadmium toxicity in unicellular algae (*Chaetoceros simplex* v.*calcitrans* Pauls., *Cyclotella caspia* Grun., *Skeletonema costatum* Grev., *Chlamydomonas* sp. and *Platymonas impellucida* McLaughlan & Parke), its intimate action on the metabolism as well as the accumulation to filter feeding consumer *Mytilus galloprovincialis* Lam.

MS medium was based essentialy on sea water sampled 10 Nm from the coast, filtred and sterilized, with a salinity of 17 g‰ and pH 7.5. Five concentrations of Cd-Cl_2Cd ranging from 0.04-2 $\mu g\ l^{-1}$ were used and duplicate controls without Cd additions were included. Acute contamination effect was observed in 72 hours and that of chronic one in 14 days.

A 5000 lx light intensity in a L/D=8/16 hours cycle and 19-21°C temperature were maintained. For chronic assay bach cultures were performed; each three day period a volume of suspension was sampled and fresh medium with Cd supplied.

Mytilus galloprovincialis was scaled in length classes, fed for 30 days with contaminated algae at a 0.5 μg Cd l^{-1} level, and kept at 15-17 g‰ salinity, 7.8 pH and 19-21°C temperature.

Short term effect. It was noted:

(1) Cd causes destruction during the first 24 hours proportional with its concentration in *Chlamydomonas* and *Platymonas*. The adaptative capacity occured after 48 hours when algae could resume division and show a certain stimulation. (2) Stimulating concentrations were very specific (in $\mu g\ l^{-1}$): all of them for *Chlamydomonas*, 0.04-0.5 for *Platymonas*, 0.04-0.1 for *Chaetoceros*, 0.04-1 for *Cyclotella*, 1 but only for 24 hours in *Skeletonema*. (3) A rise in the organic compounds synthesis in *Chaetoceros* and *Cyclotella* when Cd level ranged between 0.04-0.1. (4) An exacerbation of the mineral metabolism in *Chaetoceros*, *Cyclotella* and *Skeletonema* when Cd ranged between 0.1 and 0.5. (5) An absorbtion of Cu over control values in *Chaetoceros* (63.76-76.52%) and *Cyclotella* (27.53%). (6) No Cd accumulation.

Chronic effect (was assayed exclusively on *Chlamydomonas* for 0.1 and 0.5 $\mu g\ l^{-1}$ Cd concentrations). The observations are: (1) division rate was stimulated three days and then dropped to 50% under the control sample (0.5 μg Cd); (2) neoxanthin, violaxanthin, lutein, carotens, and chlorophyll a/b ratio reduced; (3) the total aminoacids synthesis was stimulated, exceeding the control value by 71.33% (0.1 μg Cd l^{-1}) and 46.51% (0.5 μg Cd l^{-1}); a special increase for alanine, phenilalanine and cystine was noted; (4) Cd accumulation ranged from 6.27 to 15.42 $\mu g\ g^{-1}$ dry matter and took place after 168 hours of contamination; (5) a strong divalent ion absorbtion was observed, exceeding the control value by 1,300% for Zn and 2,800% for Cu; (6) Cd accumulation in mussles was bigger than control value by 19.4-36.26%, the highest level (1.88 $\mu g\ g^{-1}$ fresh weight) was reached by the individuals of the 4.5-7 cm length class.

Concerning short term effect of Cd, our results are a confirmation of different responses observed by other authors (BERLAND and cowork., 1976).

The division rate stimulation was obtained at a level of 25-100 $\mu g\ l^{-1}$ in *Skeletonema* isolated from the Mediterranean Sea (BERLAND & cowork., 1977).

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