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Kleine Mitteilung

Joint effects of four pollutants (copper, chromium, oil, oil dispersant) on the respiration of *Artemia*

By G. Verriopoulos, E. Milliou and M. Moraitou-Apostolopoulou

With 1 figure and 1 table in the text

Abstract

The effects of four toxicants (Cu in the form of $CuSO_4 \cdot {}^5H_2O$; Cr in the form of Na_2CrO_4; an oil: Tunesian crude oil zarzaitine type and an oil dispersant: Finasol OSR2) and their combinations (all mixtures of two, three and four toxicants) on the respiration of *Artemia* was studied. The toxicant concentration tested was in all cases the LC50 48 h (concentration of a toxicant which kills 50 % of the test organism after 48 hours of exposure). All toxicant solutions caused an important and statistically significant increase on the respiration rate of the exposed animals. Furthermore the exposure of *Artemia* to all types of mixtures of toxicants caused higher respiratory intensities than the exposure to the individual toxicants although all toxic solutions tested are equitoxic as far as the survival of *Artemia* is concerned (the LC50 48 h values). A linear relationship between the respiratory intensities of *Artemia* resulting from exposure to the toxic mixtures, and the additive indices of these mixtures (calculated in a previous paper from the effects of these mixtures on the survival of *Artemia*) was noticed. From the present data it becomes obvious that the interaction of pollutants, when they are acting jointly depends also from the effect considered.

Introduction

The nearshore marine environment is often polluted by a large number of toxic substances. Water quality standards which set permissible levels based on the impact of single toxicants are not realistic. The study of joint effects of pollutants on the aquatic organisms gives a more realistic approach to pollution effects in field conditions.

In a recent paper we have studied the impact of four pollutants, two metals (Cu, Cr) an oil (tunesian crude oil zarzaitine type) and an oil dispersant (Finasol OSR 2) on *Artemia*. The test animals were exposed to: a) the individual toxicants and b) to all combinations of two, three and four toxicant mixtures. The toxic effect measured for the estimation of the joint toxicity of the four pollutants was the survival of the test animals.

In order to investigate the impact of the four pollutants on the physiology of marine organisms, we have studied their joint action on the respiration of

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Artemia, a parameter which is considered as the more characteristic indices of the physiological activity of an organism. Furthermore with these experiments we could find out if the type of reaction of the four toxicants acting jointly on the survival of an organism is the same with that of the same pollutants acting on the physiological mechanism.

Material and methods

Adults of *Artemia* hatched from commercially available cysts (New Technology *Artemia* Revolution) were used as test animals. The sensibility of the respiratory metabolism to the stress exerted by pollutants is regulated by genetical factors and is also influenced by physiological conditions (age, sex, reproductive cycle etc.), by the life history of the test animals (rearing conditions etc.) and by the environmental variables. In order to overcome these disturbing factors, in our experiments both control and experimental animals came from the same batch, hatched simultaneously and were reared under the same conditions. All test animals were females, had the same age (four weeks) and length (3.8 $\pm$ 4 mm). All experiments were performed in constant temperature rooms at 22 °C in synthetic sea water (synthetica type). The solutions of the two metals: Cu in the form of $CuSO_4 \cdot {}^5H_2O$ and Cr in the form of Na_2CrO_4, were prepared by diluting a stock solution to the proper concentration of metal ions. Oil (tunesian crude oil zarzaitine type)-water dispersions (OWDs) and oil dispersant (Finasol OSR 2) solutions were prepared by diluting directly the calculated amount of the toxicant in sea water. After the addition of the toxicant the solution was shaken at approximately 2000 turns per minute for 30 minutes. Detailed characteristics of the tested OWDs and the resulting concentrations of hydrocarbons at various times could not be obtained.

Two series of experiments were performed: in the first we have measured the impact of the four pollutants, when acting individually on the respiration intensity of *Artemia*; in the second we have evaluated the effects of the various combinations of toxicant mixtures on the respiration. *Artemia* was exposed to the following 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

The respiratory reaction of *Artemia* was measured after exposure to the LC50 48 h of each one of the four toxicants and the 11 mixtures of toxicants.

Respiration rates were determined potentiometrically with a Radiometer PHM 73 Analyser, using 20 ml syringes as respiration chambers. The analyser chamber was connected to a Haake constant Temperature Circulator maintained at 22 °C throughout. *Artemia* were placed in syringes filled with test water and clean water (controls). The syringes were kept in constant temperature rooms and tested 5 hours later. Controls without *Artemia* exhibited negligible changes in oxygen concentration. Respiration rates in μl 02 were calculated directly from the change in percentage saturation before and after the experimental period.

The differences between the various respiration rates measured were tested statistically by the t-test.

Results and discussion

Table 1 gives the measured respiration rates of *Artemia* in: a) clean synthetic sea water (controls) and b) after exposure to the LC50 48 h of the four pollutants and their 11 mixtures. In the same Table are shown the additive indices (A.I) according to Marking & Dawson (1975) of the 11 toxic mixtures taken from Verriopoulos et al. (in press). These indices demonstrate the mode of interaction of pollutants when acting jointly (more than additive, strict additive, less than additive) when the effect measured is the survival of the test animals. Additive indices of 0 indicate a strict additive toxicity, negative indices (−) indicate a less than additive toxicity. The exposure of *Artemia* to all types of toxic solutions resulted in a statistically significant (99 % level) increase of the respiration rate in comparison with the respiration of the non exposed animals. Furthermore all toxic mixtures have caused higher respiration intensities from those caused after exposure to the solutions of the four toxicants acting individually. This was unexpected because all toxic solutions tested (single toxicants, mixtures of two, three and four toxicants) are equitoxic as far as their impact on the survival of *Artemia* is considered (all concentrations are the LC50 48 h values of the tested solutions) and were expected to cause the same physiological (respiratory) disturbance.

Table 1. Respiration rates of *Artemia* exposed to four toxicants (Cu, Cr, oil, Oil Dispersant) and their mixtures.

Toxicant (LC50 48 h)	Respiration rate (μl/ind/hour)	Additive Index (A.I)
clean sea water (control)	0.001675	
Cu	0.002913	
Cr	0.002964	
Oil	0.002851	
oil Dispersant	0.002903	−1.87 (L.A.)
Cu + Cr	0.002902	−0.53 (L.A.)
Cu + oil	0.003289	−2.03 (L.A.)
Cu + Dispersant	0.003388	−0.109 = 0 (S.A.)
Cr + oil	0.003450	−0.016 = 0 (S.A.)
Cr + Dispersant	0.003304	−0.105 = 0 (S.A.)
oil + Dispersant	0.003288	−1.87 (L.A.)
Cu + Cr + oil	0.003135	−0.706 (L.A.)
Cu + Cr + Dispersant	0.003357	−0.49 (L.A.)
Cr + oil + Dispersant	0.003176	−0.66 (L.A.)
Cu + oil + Dispersant	0.003135	−3.75 (L.A.)
Cu + Cr + oil + Dispersant	0.003150	−1.63 (L.A.)

S.A. = Strict additive.
L.A. = Less than additive.

The statistical analysis of the various respiration intensities measured proved that the following mixtures caused a significant (90 % level) increase of respiration rate from that measured after exposure to one of their components acting individually:

Cu + Cr + Dispersant; Cr + Dispersant; Cr + oil	from Cr
Cu + oil; Cu + Cr + Dispersant; Cu + Dispersant	from Cu
Cr + oil; Cu + oil; oil + Dispersant	from oil
Cu + Dispersant; Cr + Dispersant Cu + Cr + Dispersant	from Dispersant
oil + Dispersant	from Dispersant

There is a linear relationship between the respiration intensities caused by these mixtures and their (negative) additive indices calculated in a previous paper (VERRIOPOULOS et al., in press) (Fig. 1). The deviation from the respiratory intensities caused by the components of the mixture acting individually (Cu = 0.002913; Cr = 0.002964; oil = 0.002851; dispersant = 0.002903 μl/individual/hour) becomes less intense as the negative value of the additive indices increases. This means that as antagonistic reaction of the components of a mixture increases (increase of the negative additive indices) their effects approaches that of the individual components of the mixture. Respiration represents an important physiological index of an organism because respiratory rates reflect the metabolism and the overall functional well being of an animal.

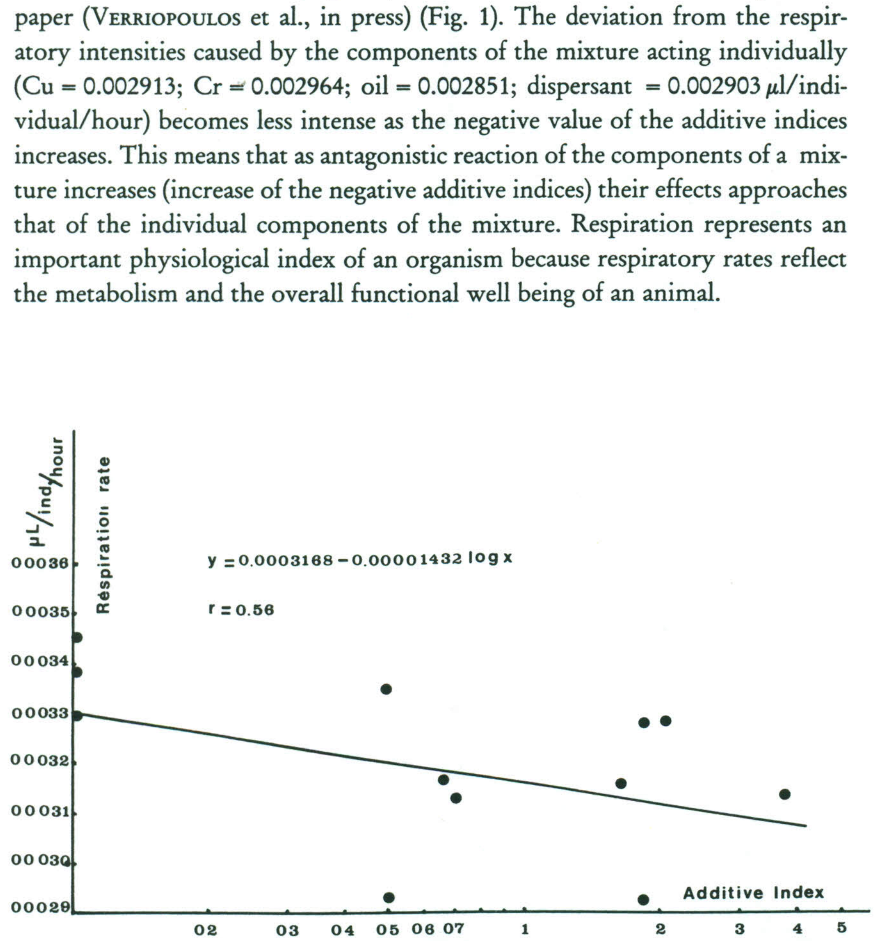

Fig. 1. Relationship between the respiratory intensities of *Artemia* exposed to the various toxicants mixtures and the additive indices of these mixtures.

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It has been well documented in the literature that stresses caused by changes in environmental parameters as well as by exposure to pollutants cause respiratory responses of animals. Sublethal impairment of the capacity of an animal to perform and adapt, as occurs when important respiratory changes are caused, can reduce the changes for survival and the potential for growth and reproduction.

The respiratory responses to pollutants present great variability (RAYMONT & SHIELD, 1964; ANDERSON et al., 1974; STRUHSAKER et al., 1974; KAPOOR & GRIFFITHS, 1976; VERRIOPOULOS et al., in press). Generally consistent respiratory changes have been referred at high pollutant concentrations (REEVE et al., 1977; VERRIOPOULOS et al., in press).

The tests of respiration have become valuable for the estimation of the impact of pollutants because respiration represents a critical physiological process. Furthermore respiration is an effect relatively easy to monitore and especially tests are rapid (about 5 hours VS 24 to 48 hours of the survival tests).

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 tested (BRAEK et al., 1976). Our results indicate that the interaction of pollutans when acting in mixture may also differ according to the effect considered; e.g. mixtures of pollutants may act additively producing acute effects, but interact in an unpredictable way when acting on the physiological mechanism. This is probably because the uptake, site of action and toxic mechanisms differ in the two cases.

The present results have demonstrated that for a more realistic estimation of the impact of pollutants on an organism, when acting in mixture more than one type of toxicity tests must be performed.

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The address of the author:

Zoological Laboratory, University of Athens, Athens 15771, Greece.