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Effects of Sublethal Concentrations of Cadmium as Possible Indicators of Cadmium Pollution for Two Populations of *Acartia clausi* (Copepoda) Living at Two Differently Polluted Areas

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Toxicity of heavy metals to marine organisms is a subject of interest because due to technological development,the sea receives an increasing amount of heavy metals.Acute toxicity studies have played a major role in estimating the effects of heavy metals to marine organisms because survival is considered the best index of a pollutant stress,being in general,the least variable.However,long term exposure to sublethal concentration of a pollutant may markedly alter the normal functioning of organisms and thus destroy a population as effectively as lethal doses.Therefore,the study of the effects of sublethal doses is indispensable if we want to set realistic standards of water quality criteria.

The Saronic gulf(gulf of Athens) area of Greece,is a polluted area,especially in its N.E. part(Elefsis-Keratsini bay)where large quantities of industrial wastes and domestic sewage are disposed.High concentrations of trace elements in Saronic bay sediments have been reported (GRIMANIS et al.1976).

Recent work at the Zoological laboratory of the University of Athens studing the effects of heavy metals to planktonic organisms (MORAITOU-APOSTOLOPOULOU 1978,MORAITOU-APOSTOLOPOULOU and VERRIOPOULOS, 1979, MORAITOU-APOSTOLOPOULOU et al.,in press)has proved that there is a significant difference in the resistance to lethal doses of copper and cadmium and in the effects of sublethal concentrations of copper between two populations of the planktonic Copepod *Acartia clausi* living at two differently polluted areas of the Saronic gulf.

The population of *Acartia clausi* living at the heavily polluted area of Elefsis bay is more resistant to lethal and sublethal copper stress and to lethal doses of cadmium,than a population of *Acartia* living at an area situated about 25Km S.E. of Elefsis and considered as a relatively nonpolluted area(MORAITOU-APOSTOLOPOULOU 1974,1976,MORAITOU-APOSTOLOPOULOU and KIORTSIS 1976).

The purpose of this study was twofold:first to establish and characterize the sublethal effects of cadmium on different physiological processes(longevity,feeding,respiratory rates),and second to verify whether there exist differences in the effects of sublethal concentrations of cadmium between the two populations of *Acartia* adapted to differently polluted areas.

MATERIAL AND METHODS

Acartia clausi,a common planktonic Copepod in the Mediterranean Sea,is now considered as a key marine organism because of its extreme abunda-

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nce in polluted areas(CITARELLA 1973,GUGLIELMO 1973).In the heavily polluted area of Elefsis bay *Acartia* constitutes,during the cold period,the almost exclusive component of a quantitatively very rich zooplanktonic community(MORAITOU-APOSTOLOPOULOU 1974,1976).

As *Acartia clausi* plays a major role in the transfer of energy,and presumably,in the accumulation of pollutants,to higher trophic levels, it is important to evaluate the impact of pollutants on it.

Acartia was collected by horizontal hauls using a WP2 plankton net. The haulings were performed at two stations: a)in the heavily polluted Elefsis bay,characterized as "polluted area" and b) in a relatively clean area about 25Km S.E. of Elefsis characterized as "nonpolluted area"

The methods employed in this work have been described in detail by MORAITOU-APOSTOLOPOULOU and VERRIOPOULOS(1979)and will be given in outline only:The sea water was previously filtered in Millipore filter and autoclaved.All experiments were conducted at 18$\pm$0.5C.Test solutions were prepared by diluting a stock solution(20 mg Cd/L) of cadmium($CdCl_2.2H_2O$) in order to obtain the following concentrations of Cd ions: a) 0(control),b)0.1,c)0.2,d)0.3,e)0.4,f)0.6,g)0.9,h)1.0,i) 1.2 mgCd/L.

All quoted Cd concentrations are nominal values because the complexing capacity of sea water may markedly alter the concentrations.

These concentrations have been characterized as sublethal for *Acartia* because in previous experiments(MORAITOU-APOSTOLOPOULOU a et al.in press),the LC50 48h(concentration of a toxicant lethal to 50% of the test animals after 48hours of exposure) of cadmium for *Acartia* was determined as follows:

	LC50 48h(mgCd/L) area	
Temperature	polluted	nonpolluted
14°C	1.50$\pm$0.03	1.20$\pm$0.02
22°C	0.70$\pm$0.02	0.60$\pm$0.04

Three indices were used to assess the impact of sublethal concentrations:a) the mean longevity,b)the feeding activity as estimated by ingestion rates and c) the metabolic responses as estimated by respiratory rates.

During the longevity experiments for every concentration and for each one of the two populations,20 matures females were put individually in glass coppels containing 50ml of solution. Coppels were covered with aluminium foil sheets and checked every 24h for survivals.

Experiments of oxygen consumption were performed by the polarographic method using a pHmeter.The principls of this method have been described by KANVISHER 1959).For each concentration tested and for each of the two sampling areas 20 animals were placed individually in 2mL syrings filled with 1.5mL oxygen saturated sea water.The *Acartia* was let to breath for 24h at 18C.In both types of experiments and for each one of the two populations 20 *Acartia* were put in sea water without added Cd(controls).

For the feeding experiments 10 females were put in 500mL Erlenmeyers.

Five jars were used for every concentration tested and for each one of the two areas. Concentration of the mixture of the four phytoplanktonic species added as food (Exuviella baltica, Nitzscia clostericum, Skeletonema costatum, Chaetoceros danicus) was approximately the same in all jars (fluctuated from 48.000 to 51.000 cells/mL). Phytoplanktonic cell counting was performed by haematocymetric cells type Malassez. The number of phytoplanktonic cells consumed (ingestion rate) was calculated 24h after the addition of food by the difference between the phytoplankton cells concentration after the addition of food and 24h later, corrected by a factor given from the control's jars mortality.

RESULTS AND DISCUSSION

Effects of chronic exposure to cadmium on survival

The results of sublethal concentrations of Cd on the longevity of the two populations of Acartia are presented in Fig.1

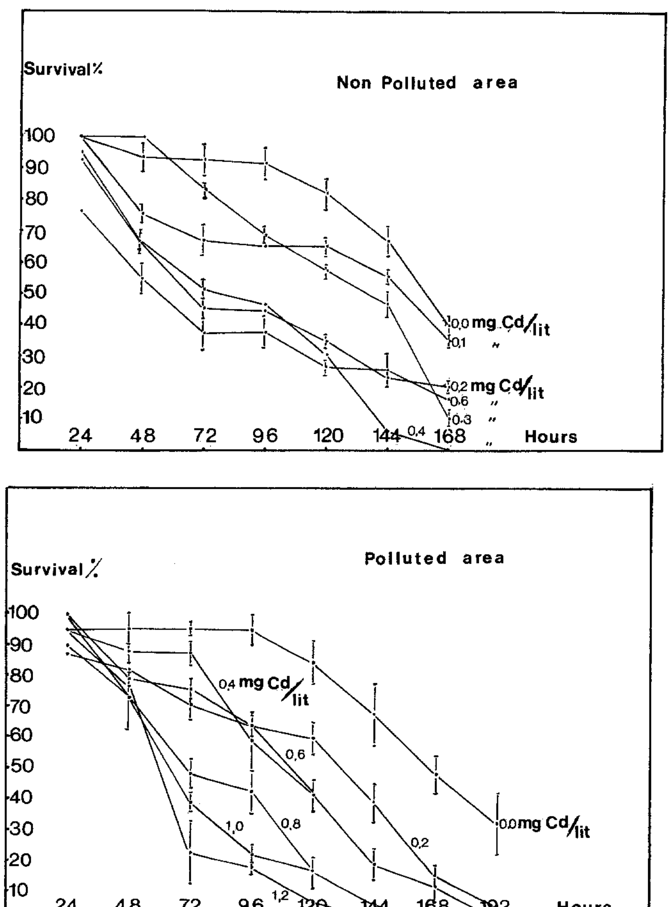

Fig.1 Survivorship curves of the two populations of Acartia exposed to sublethal concentrations of cadmium.

All tested concentrations reduce the longevity of Acartia with respect to controls.Generally,the mean longevity declines with increasing concentrations of cadmium(an exception was observed with the Acartia of the nonpolluted area:in the 0.3 mgCd/L concentration the animals seem to survive better than in the 0.3 concentration).

The results of t-test analysis for the differences in the mean longevity of Acartia between the tested concentrations are presented in Table 1.

Polluted area

Conc.of Cd (mg Cd/L)	0	0.2	0.4	0.6	0.8	1.0
0.2	VS					
0.4	VS	NS				
0.6	VS	VS	NS			
0.8	VS	VS	VS	VS		
1.0	VS	VS	S	NS	NS	
1.2	VS	VS	S	S	NS	NS

nonpolluted area

Conc.of Cd (mg Cd/L)	0	0.1	0.2	0.3	0.4
0.1	VS				
0.2	VS	VS			
0.3	S	NS	+		
0.4	VS	VS	NS	VS	
0.6	HS	VS	S	VS	NS

TABLE 1

Results of t-test analysis for the differences in the mean longevity of Acartia between the tested Cd concentrations.
(HS=significant at the level 99.9%,VS=significant at the level 99%, S=significant at the level 95% +=the mean longevity is higher at 0.3 than at 0.2mg).

The survivorship curves of the two areas prove that in all common concentrations,the longevity of the pollution adapted population was less affected.These differences checked with the t-test analysis proved statistically significant for the concentrations of 0,0.2,and 0.4 mg Cd/L,but non significant for the 0.6 concentration.

Metabolic responses of Acartia to cadmium

The respiratory rates of the experimental and control animals are indicated in Table 2 and represented graphically in Fig.2

Conc.of Cd (mg/L)	polluted	nonpolluted
0	0.032±0.016	0.025±0.017
0.2	0.037±0.005	0.025±0.008
0.4	----	0.026±0.014
0.6	0.038±0.012	0.025±0.026
0.8	0.043±0.012	0.044±0.015
1.0	0.059±0.913	

TABLE 2

Oxygen consumption(μL O_2/cop/24h) of the two populations of Acartia after exposure to cadmium.

The reaction of the two populations to cadmium differs markedly:in the pollution adapted population a continuous increase of oxygen consumption was observed with increasing concentrations of cadmium at all doses tested.The rythm of increase of respiratory rates is slow between 0.2 and 0.8 mgCd/L and an increase of 35% was observed between 0 and 0.8 mgCd/L. An abrupt increase was noticed at the solution of 1mgCd/L and the oxygen consumption at this concentration was 86 % higher than that of the control animals.

The oxygen consumption of the animals of the nonpolluted area seemed not affected between 0.2 and 0.6 mgCd/L concentrations.On the contrary at the 0.8 concentration an important increase in respiratory rates was observed:the oxygen consumption at this concentration was

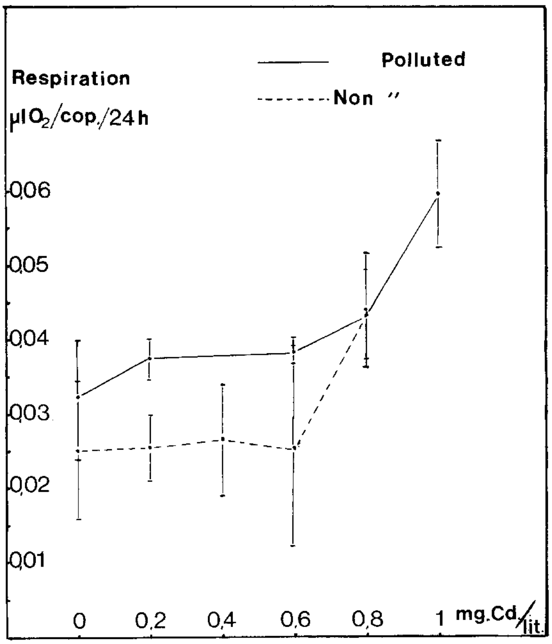

Fig.2 Metabolic responses of the two populations of Acartia after exposure to sublethal concentrations of cadmium.

76% higher than that of the control animals.At the lower concentrations of cadmium(0.2 to 0.6 mg/L) the Acartia of the nonpolluted area seem not affected because they maintain constant respiratory rates. The concentration of 0.8 seem to overpass the compensation mechanisms of this population of Acartia resulting at an abrupt increase in respiratory rates.The population of Acartia living at the polluted environment reacting with a regular progressive increase of the oxygen consumption with increasing cadmium concentrations presents a slight increase even at the concentration of 0.8 and only at the 1mg concentration an intense respiratory reaction is observed.

Furthermore the respiratory rates of the pollution-adapted population of Acartia at all concentrations tested were higher than those of the population living at the nonpolluted area.The same difference was observed even for the control animals.These differences tested with the t-test proved not statistically significant.

In a previous paper(MORAITOU-APOSTOLOPOULOU AND VERRIOPOULOS,1979) we noticed a continuous increase of respiratory rates of both populations of Acartia exposed to sublethal concentrations of copper.

The observed increases in respiratory rates must be mainly related to modifications of activity of the organisms caused by the pollutant concentrations.

Similarly other authors(RAYMONT AND SHEILD 1962,O'HARA 1972)have also observed increase in respiratory rates of different organisms in the presence of heavy metals.

On the other hand REEVE et al.(1977) working with the the influence of sublethal concentrations of copper on various planktonic organisms noticed that the observed respiratory reactions present a high variability and concluded that they cannot be considered as sensitive indicators of sublethal stress.

There are limited data concerning the influence of cadmium in the respiratory rates of marine organisms.VERNBERG et al.(1977) noticed differences in the metabolic responses of Palaemonetes pugio after exposure to cadmium:under static experimental conditions the respiratory rates of animals exposed were significantly lower than those unexposed.On the contrary an increase(statistically non significant)was noted in the experimental shrimp maintained in flow-through system. Therefore the authors conclude that respiratory rates are not a predictable and reliable indicator of cadmium pollution for the grass shrimp.NELSON et al.(1976) report that juveniles Bay Scallops (Argopecten irradians)exposed to cadmium exhibited significantly higher respiratory rates than those of the control animals.

Effects of cadmium on the feeding activity of Acartia

The ingestion rates(cells/copepod/24h) of the two populations at all cadmium concentrations are presented in Fig.3.

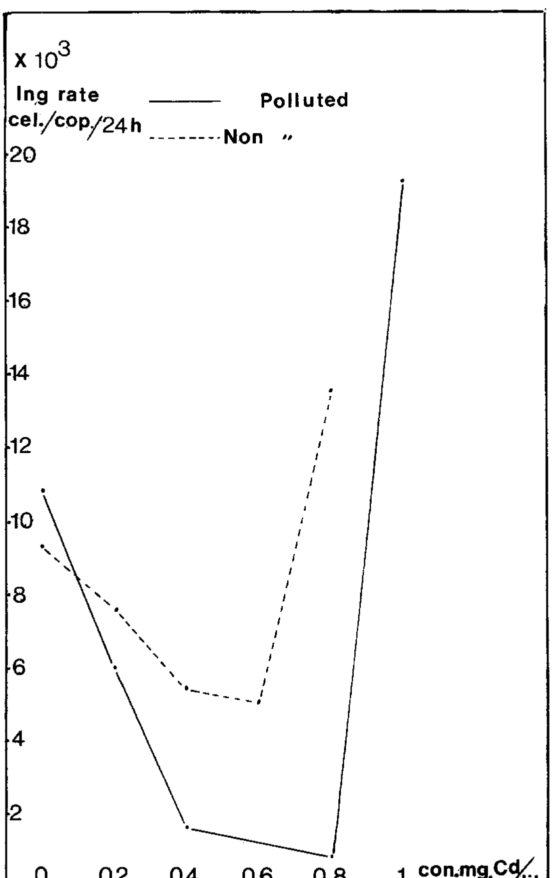

Fig.3.Feeding activity of the two populations of Acartia after exposure to cadmium.

The pollution adapted population of Acartia shows a continuous decrease of ingestion rates from 0 to 0.8 mgCd/L(at the 0.8 concentration a reduction of 91% in comparison with the control animals ingestion was observed).At the concentration of 1mgCd/L an abrupt increase of ingestion rate was noticed and the ingestion rate at this concentration was 76% higher than that of the control animals.

Acartia of the nonpolluted area maintained rather constant ingestion rates between 0 and 0.6 mgCd/L concentrations.A sudden increase in ingestion rates was noticed at 0.8 mgCd/L concentration and at this concentration the ingestion rate was 43 % higher than that of the control animals.

These abrupt increases in ingestion rate must be correlated with the analogous increases in respiratory rates observed at the same concentrations.

From the data of respiratory and ingestion rates one may conclude that the concentrations of 0.8 and 1mgCd/L are critical for the Acartia of the nonpolluted and polluted area reciprocally.It is probable that the stress exerted to Acartia at these concentrations,causes a high motility resulting at the abrupt increase of respiratory and ingestion rates. It is worthy to notice that these concentrations must be very near to lethal concentrations at 18C.

The experimental data of this paper prove that the mean longevity of both populations of Acartia can be considered as a reliable indicator of sublethal cadmium stress. Furthermore the population living at the polluted environment demonstrated an adaptation to the pollution conditions being more resistant than the population living at the nonpolluted area.

For the pollution adapted Acartia the metabolisms(expressed aw respiratory rate) and the feeding activity(expressed as ingestion rate)could be considered as an indicator of cadmium pollution because they demonstrate a continuous and significant change at the range of sublethal cadmium concentrations(0 to 0.8 mg/L) tested.

Concerning the Acartia of the nonpolluted area only at higher concentrations(0.8mg/L)these indices are markedly affected(more than those of the pollution adapted population at the same concentrations).

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