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Toxicity of Paraquat to Three Marine Organisms[1]

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Extensive use of pesticides has increased the incidence of environmental pollution. Among pesticides, herbicides are the most dangerous for seawater contamination since they are used on or near the soil, and in many instances, in the water for aquatic weed control.

We report here the effects of paraquat, a bipyridyl herbicide, on three marine animals. One species was chosen from each of three different groups of marine animals: molluscs, crustaceans and bony fish. In this research we determined the LT_{50} values of several concentrations of paraquat, its histopathological effect after an acute and a subacute exposure, the rate of the herbicide accumulation and the tissue distribution of this compound in the fish. An additional experiment was performed to estimate the rate of paraquat loss in test containers during a period of 24 h, that is to establish the real concentrations of the herbicide in the containers during the period of 24 h time of changing the seawater and redosing.

MATERIALS AND METHODS

Test animals. Test organisms used were a bony fish, Mugil cephalus, a small decapod crustacean, Pagurus sp., and a gasteropod mollusc, Murex brandaris. Fish were collected from the fish ponds of Messologhi Gulf and transported to the laboratory in seawater supplied with ice and under continuous aeration. The two other marine animals were collected from Evoikos Gulf and transported to the laboratory under the same conditions. All animals were acclimated for 15 days prior to exposure to the herbicide.

Test compound. The herbicide used was a formulation of paraquat known as Gramoxone (20% A.I.).

Toxicity tests.

a) Determination of the LT_{50}.

Static bioassays were conducted in 75 and 35 L all glass aquaria for the fish and the other two organisms, respectively; in the latter

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case the seawater had a height of 15 cm. Seawater was maintained at 20 to 22°C and the salinity at 30 to 31°/oo; aeration was continuous. For each concentration 10 juvenile fish averaging 17 cm and 20 individuals of the other two species were used. The seawater was changed and redosed every 24 h.

The herbicide dissolved in water, was used at 10, 5, 0.5 and 0.1 ppm (A.I.) concentrations.

Observations of mortality were made 1, 2, 4, 6, 24 h and every 24 h thereafter for a period of 3 mo. Data were plotted on a semilogarithmic paper and estimation of the LT_{50} values was made visually.

b) Rate of accumulation in the 3 species and tissue distribution in fish.

For this determination, 5 adult specimens of M. cephalus, weighing 600 to 650 g were used at 1 ppm concentration for a 15-day exposure time. We examined muscles, skin (washed with water), ovaries and digestive tract.

For the determination of the accumulation rate in the crustacean and molluscan species the animals were exposed for 3 days at 5, 2.5 and 1 ppm. Paraquat concentration in the media and the test animals was determined by the method of CALDERBANC & YUEN (1965) with minor modifications depending upon the amounts of the examined tissues. Additional research established the percentage recovery of paraquat in the examined tissues.

RESULTS

Figure 1 shows that 50% of the fish exposed at 10 ppm died within 1 h, while the LT_{50} for the crustacean and the gasteropod was 36 and 24 h, respectively; among the 3 species the crustacean was the least sensitive and the fish the most sensitive organism to the acute exposure to high concentration. On the contrary, the sensitivity to repetitive exposures to low concentrations was inversed; thus, at 1 ppm the fish survived longer than the crustacean. At 1 ppm the LT_{50} values were 16, 18 and 10 days for the fish, gasteropod and crustacean, respectively. No death occurred whichever the species at 0.1, 0.05 and 0.01 ppm throughout the experiment (3 mo for fish, 1 mo for other species). In the case of the crustacean and the gasteropod, the prolongation of the exposure time longer than 1 mo was difficult because of the high natural mortality occurring during the second month of their preservation in the laboratory.

Histology. Fish killed through mass concentration of the herbicide, within 24 h, presented signs of intense inflammation. Their skin was swollen, slimy and with hematomes, their gills damaged and covered with mucus, the liver and kidney severely affected by hemorrhage and necrosis, and the digestive tract distended with gas and presenting multiple hemorrhagic ulceration.

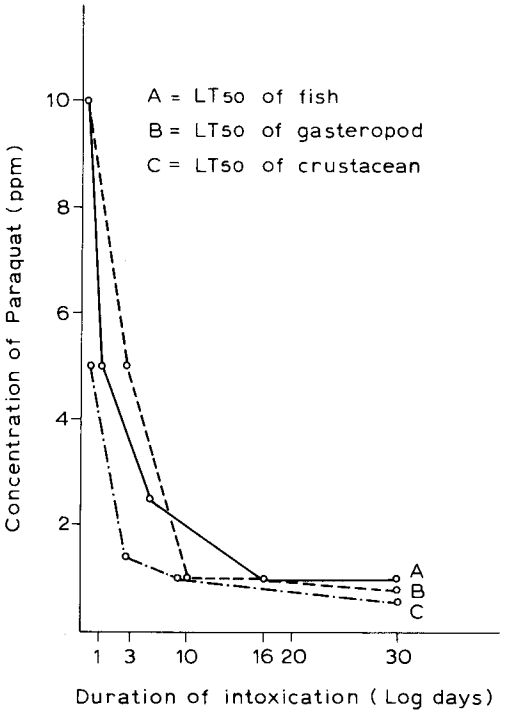

Fig. 1. LT_{50} values of M. cephalus, M. brandaris and Pagurus sp.

The histopathological findings in the case of the fish exposed at 1 ppm of paraquat during 15 days were less severe but more differentiated. Thus, were observed a pronounced hydropic degeneration, blood and bile stasis in the liver, hyaline degeneration and tubular necrosis in the kidney and mucosal erosion in the digestive tract. However, the gills of these fishes were the organs most affected: thickening of the gill filament, destruction of epithelial wall of the secondary lamelae and distention of the tip of the gill filament with simultaneous fibrosis of the base of the distended areas (Fig. 2, A); the latter lesions of the gill were also observed in the gills of the examined crustacean (Fig. 2, B) and even in these of the gasteropod.

Accumulation of paraquat. As indicated in table 1, paraquat in the fish body was found at higher concentrations in the digestive tube and the skin. Lower amounts were found in the muscles.

Crustaceans accumulated more paraquat than the other two marine organisms (Table 2); this effect must be related to increased sensitivity of this species to repetitive exposures to paraquat.

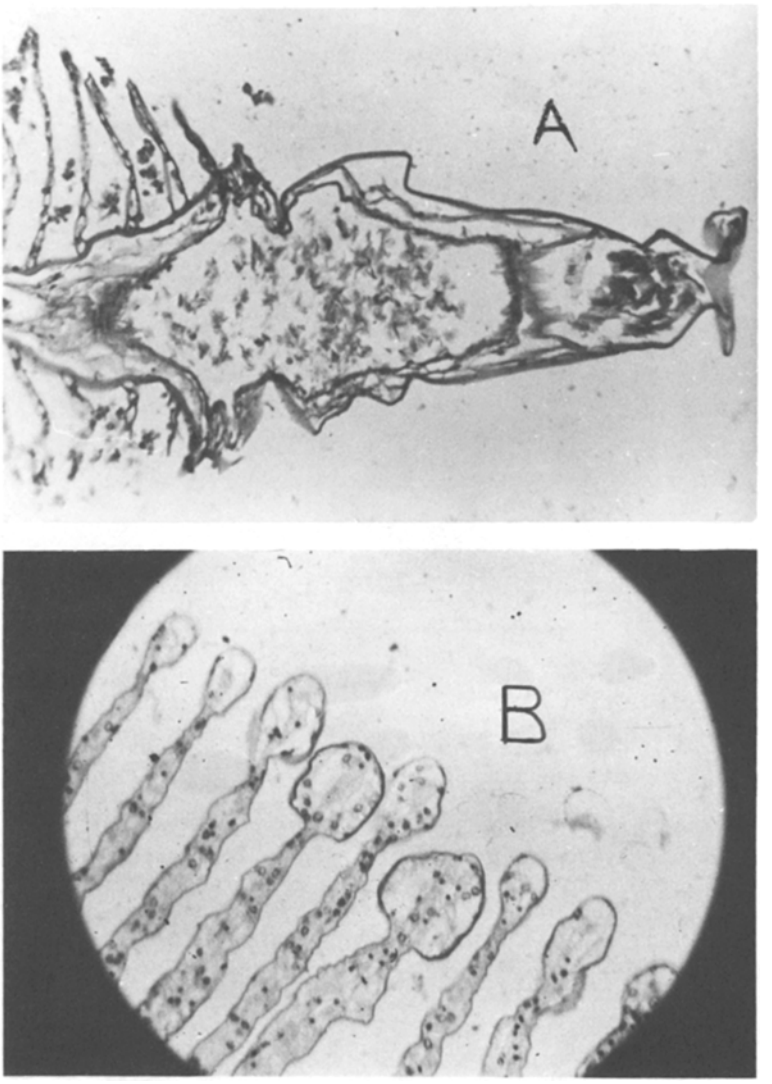

Fig. 2. Characteristic gill filament lesion of M. cephalus (A) and Pagurus sp. (B). In addition to the changes of the epithelium of secondary lamelae the tip of gill filament of both species are distended, and in the case of M. cephalus, fibrotic tissue is formed in the base of distended areas.

Paraquat loss rate in test containers during 24 h. According to the data presented in Table 3, the quantities of paraquat detected in the aquaria, 10 min after the dosing were almost one third of the initial. Thereafter and up to 24 h (time of changing the seawater and redosing), the quantities increased slightly

TABLE 1. Paraquat concentration in various tissues of M. cephalus after 15 days exposure at 1 ppm.

Organs	Paraquat concentration* in µg/g
Muscles	0.19 ± 0.07
Ovaries	0.23 ± 0.14
Skin	4.7 ± 1.8
Digestive tract	6.1 ± 1.8

*Individual tissues were analyzed. Data presented are averages of 5 individuals ± standard deviation.

TABLE 2. Paraquat concentrations in the whole body of M. brandaris and Pagurus sp. after a 3 days exposure in various concentrations.

Concentrations ppm	Detected quantities of paraquat (µg/g)*	
	M. brandaris	Pagurus sp.
10.0	2.8 ± 0.8	-
5.0	2.2 ± 1.0	15 ± 4
2.5	-	9.2 ± 2.5
1.0	1.5 ± 0.5	3.2 ± 0.9

*Each sample is constituted by 20 specimen.

TABLE 3. Quantities of paraquat detected in seawater at different times after dosing.

Initial concentrations of paraquat (ppm)	Detected quantities* (in ppm)				
	10 min	1 h	4 h	6 h	24 h
10.0	-	2.44±0.05	2.55±0.06	2.66±0.06	2.53±0.04
5.0	1.32±0.07	1.38±0.04	1.38±0.05	1.48±0.04	1.63±0.12
1.0	0.33±0.03	0.43±0.03	0.50±0.04	-	0.52±0.04

*Data presented are average of 3 samples for seawater.

for the high doses and significantly for the low one.

DISCUSSION

The estimation of the LT_{50} values was based on the initial quantities and not on the real ones inducing mortality, since the

aim of our experiment was the investigation of an eventual harmful effect on the marine organisms associated with the discharge of a given quantity of a herbicide.

The toxicity of paraquat on M. cephalus seems to be higher than that reported from freshwater fish (ALABASTER 1969, ALABASTER & ABRAM 1965), while for Pagurus sp. our findings are similar to that reported by PORTMAN (1970) for Crangon crangon.

However, as the concentrations inducing mortality used in this experiment are hardly met in the environment and there was no mortality among animals exposed to lower concentrations during the 3 mo period of observation, the toxicity of paraquat must be regarded as a moderate one.

The main lesion induced by paraquat in mammals is the lung fibrosis (CLARK et al. 1966, SMITH et al. 1973, SMITH & HEATH 1974). A similar lesion was found in the gills of M. cephalus, Pagurus sp. and M. brandaris. It is likely that the respiratory system, whichever its type is, is the system the most affected by paraquat and the induced lesion can be considered as a characteristic effect of this toxin.

It has been reported that paraquat is not hazardous for fish because it is rapidly absorbed by aquatic plants and mud (ANONYMOUS 1975). However, the data of the present experiment indicate that, although paraquat concentrates in fish muscle at low quantities, it has an accumulative capacity very high in crustaceans where it concentrates in considerable amounts. Since crustaceans constitute a large part of the zooplankton and an important link of the trophic chain, such an effect could be of great ecological significance.

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