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Effects of Paraquat and Lead on Fish *Oreochromis hornorum*

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Herbicides are used for controlling weeds in ponds, reservoirs, and irrigation canals. Paraquat (PQ) cause toxic effects on fish Roach fry and Perch fry (Metelev et al. 1971), and produce morphologic alterations on salmon (*Onccochynchus kitsuch*) liver and gills (Evans,1984). One of the most important properties of PQ is its rapid and strong adsorption on the soil particles, so that it is unavailable in plant roots. Adsorption is so complete that the concentration in solution in the soil fall below the limit of chemical or biological detection. However, since PQ is highly soluble in water, toxic effects on aquatic organisms under field conditions may be expected if sufficient quantities of paraquat reach aquatic ecosystem (Calderbank, 1974). Biochemical investigations about the mechanisms of the PQ effects imply that cyclic reduction and oxidation of PQ molecules take place, producing lipid peroxides in membrane (Hett, 1974).

In the aquatic contamination, the lead (Pb) also produced ecologic unbalance. It is accumulated in gills and liver of fish (Stoepppler, 1989) and diminished reduced-glutathion in eritrocites (Hernberg, 1980).

This work reports results of our studies from the concurrent exposition to PQ and Pb in fish.

MATERIALS AND METHODS

Tilapia *Oreochromis hornorum* weighing 4 ± 0.9 g were collected from a fish farm in Zacatepec, Mor. (Mexico). Fish were kept in the aquarium laboratory, fed daily with ground nut cake; prior to sacrifice, feeding was stopped for one day. After the usual adaption, the animals were separated into groups of five each one for LC_{50} determination, or of 30 each one for sublethal

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toxicity and distributed in groups of 6 animals in 17 L aquaria jars. The water characteristics were: pH 7.2 - 7.5, alkalinity 95-110 ppm, hardness 118-122 ppm at 20°C. All bioassays were carried out under static conditions using $Pb(NO_3)_2$ manufactured by J.B.Baker and paraquat chloride by ICI of Mexico. Solutions ranged from 0.0 to 45 mg/L of PQ and 0.0 to 342 mg/L of Pb were used for acute toxicity studies. Fish were separated in groups of 6 in 25 L rectangular glass aquaria containing 17 L of the test solution. Three replicates of each group were conducted for each concentration and mortality data were pooled to obtain average (%).

Preliminary range-finding tests were done in order to establish the mortality range of 0 to 100%, suitable for calculating LC_{50}. The Probit analysis was used to this purpose. Fish were fed only during acclimation period. Mortality was recorded after 96 hr.

In order to evaluate sublethal toxicity, groups of fish were exposed for 96 hr to the following concentration of PQ 0.1, 10.5, and 21 mg/L (LC_0).

Other groups were intoxicated at a fixed Pb concentration (47 mg/L, LC_0) using these sublethal PQ concentrations. After this intoxication period gills and liver were isolated to lipid peroxides determination, evaluated as nMol of malondialdehide (MDA)/mg of protein. Carbonic anhydrase activity was also measured in gills. Control groups were set aside to comparison.

Liver and gills lipid peroxide levels were estimated by the Buege's method (1978). These organes were homogenized in ice with deionized water (10 % W/V). From this homogenized a volume of 50 µL was taken and the pH was adjusted to 7.4 with tris HCl. The mixture was kept in a water bath at 37 °C for 30 min. Then 2 mL 0.37 % thiobarbituric acid in trichloroacetic acid solution was added, and kept in a water bath at 95 °C for 15 min, and after it was cold in ice. The colored reaction product was absorbans 532 nm recorded.

Gills carbonic anhydrase activity was estimated with Maren's micromethod (1960). Gills were removed, kept in ice , homogenized in ice-cold deionized water . All reagents were kept at under 5 °C . CO_2 flow was carefully stabilized, 0.4 mL of phenol red was added to the reaction vessel, followed to 0.3 mL of gills homogenized in ice cold deionized water 50 % (W/V) , then 0.1 mL buffer 0.0026 M of $NaHCO_3$ was added rapidly. The time of the CO_2 hydration was measured when the indicator turned from red to yellow. The enzyme unit was defined as the activity necessary to halve the time

of the uncatalyzed reaction (Maren, 1960).

RESULTS AND DISCUSSION.

LC_{50} of Pb and PQ were 202 mg/L and 31.5 mg/L respectively for the teleost fish *Oreochromis hornorum*. No mortality occurred in control animals. The toxicity signs of fish treated with PQ was a tendency to swim erratically near to the water surface and Pb treated fish shown a loss equilibrium.

The PQ elicited lipid peroxides in gills and liver at all used concentrations . Table 1 shows that lipid peroxidation was increased with PQ concentration. These results are in agreement with those found by Hett (1974), who proved the formation of PQ radicals, fatty acid synthesis inhibition and lipid peroxidation in cellular membranes.

The groups exposed only to Pb (47 mg/L), also produced an important lipid peroxidation both on gills and liver (Table 2). Chvapil (1972) suggested that metals which form redox systems may catalyze lipid peroxidation and cause membrane damage . However, at the lowest PQ concentration (0.1 mg/L) plus Pb (Table 2), lipid peroxidation diminished approximately to the control values (without both PQ and Pb,table 1), and with higher PQ concentrations (10.5 and 21.0 mg/L) plus Pb, it was increased significantly (Table 2)

The fact that fish exposed simultaneously to Pb (47 mg/L) and PQ (0.1 mg/L) resulted in a disminution of lipid peroxidation suggests an antagonism between PQ and Pb under these conditions. The reason for this result it is not quite clear and, in order to explain this finding, further investigation will be necessary.

Effects of PQ alone on lipid peroxidation was more severe in liver that in gills. At the highest herbicide concentration it was 3.6 times the control value in liver, whereas in gills this increment was only 57 % (Table 1). The fish exposure at combined Pb plus PQ, at highest concentration of herbicide, lipid peroxidation was significantly higher (40%) in liver than in gills (Table 2). This result suggests that liver reduced PQ producing free radicals more efficiently than the gills; whereas the Pb could be in redox form which may catalyze lipid peroxidation potentiating the membrane damage caused by PQ. This is in accordance with Hett (1974) and Chvapil (1972).

Exposure to 21 mg/L of PQ resulted in an increase of the gill carbonic anhydrase activity (Table 1), but this effect was not observed when Pb was present (Table 2) .

Table 1. Effects of Paraquat on lipid peroxidation and Carbonic Anhydrase in liver and gills of the fish *Oreochromis hornorum.*

Paraquat mg/L	Lipid peroxidation nmol MDA/mg protein GILL	LIVER	Carbonic Anhydrase GILL (EU)
0.0	0.70±0.04	0.41±0.04	1066±50
0.1	0.79±0.11	0.56±0.06	998±73
10.5	0.85±0.10	1.16±0.19*	1146±35
21.0	1.10±0.13*	1.49±0.28*	1279±67*

Values are mean ± SEM of 18 animals. EU= enzyme unit.
* denote significancy respect to control (P< 0.05).

Table 2. Pb and Paraquat effects on lipid peroxidation and Carbonic Anhydrase activity in gills and liver of fish *Oreochromis hornorum.*

Paraquat mg/L	Lipid peroxidation nmol MDA/mg protein GILL	LIVER	Carbonic Anhydrase GILL. (EU)
0.0 + Pb	1.35±0.16	1.33±0.23	1038±36
0.1 + Pb	0.72±0.08	0.43±0.04	1099±12
10.5 + Pb	1.15±0.11*	0.76±0.05*	1116±25
21.0 + Pb	1.19±0.07*	1,67±0.27*	1063±28

Values are mean ± SEM of 18 animals. EU=enzyme unit.
* denote significancy respect to control (P< 0.05).

Further experiments are necessary to examine a possible PQ and Pb interaction related to AC activity.

Finally, the main conclusion of this study is the potential value of the lipid peroxidation as early indicator of cellular toxicity of herbicide Paraquat.

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