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Acute Toxicity to Freshwater Benthic Macroinvertebrates of Fluoride Ion (F-) in Soft Water

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Fluorine is the most electronegative of all elements. It does not occur naturally as a free element and only appears with a valence of -1. The most abundant fluorine-containing minerals are fluorite (CaF_2), fluorapatite ($Ca_5(PO_4)_3F$) and kryolite (Na_3AlF_6).

The fluoride concentration in sea water normally ranges from 1.2 to 1.4 mg/l (Dobbs 1974). Most fresh waters contain less than 0.2 mg F-/L, although total concentrations can be considerably higher if the fluoride is bound to small suspended particles (Dave 1984). However, fluoride concentration in surface waters is increasing as a result of industrial pollution (Martin and Salvadori 1983).

Toxic effects of fluorine compounds have been described in aquatic animals like *Daphnia magna* (Le Blanc 1980; Dave 1984), *Artemia salina* (Pankhurst et al. 1980), *Penaeus indicus* (McClurg 1984) and *Oncorhynchus mykiss* (Pimentel and Bulkley 1983; Smith et al 1985). However, very little is known about fluoride toxicity in benthic macroinvertebrate community of fresh waters.

In this paper is described the fluoride acute toxicity for five species of aquatic insect larvae which are ordinary benthic macroinvertebrates in rivers from The Iberian Peninsula.

MATERIALS AND METHODS

Last instars of trichoptera aquatic larvae were collected from fluoride unpolluted areas of Spanish rivers: *Chimarra marginata* Linnaeus, *Hydropsyche lobata* MacLachlan and *Hydropsyche bulbifera* MacLachlan from Río Aulencia (Madrid), *Hydropsyche exocellata* Dufour from Río Jarama (Madrid), and *Hydropsyche pellucidula* Curtis from Río Duratón (Segovia). In the laboratory, test organisms were randomly selected and placed into test aquaria.

Laboratory bioassays were conducted in glass aquaria each with a volume of 10 L dechlorinated tap water. Necessary water oxygenation and turbulence were produced by two air pumps per aquarium. Chamber environmental temperature and natural photoperiod were utilized. Test fluoride solutions were made from sodium fluoride (NaF pro analysi, Merck, FRG).

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Methods for these static acute toxicity bioassays were those recommended for standardized laboratory toxicity tests (US Environmental Protection Agency 1975; American Public Health Association 1980). Five fluoride tests were designated as A, B, C, D, and E bioassays. A control and 5 to 6 fluoride concentrations were used per bioassay. Two species were tested simultaneously in each bioassays with 10 larvae per species, excepting *H. pellucidula* with 12 larvae in the A test.

Test organisms were acclimatized to water quality conditions for 4-5 days prior to tests and were not fed during acclimatization nor during toxicity bioassays. During the acclimatization there were no dead animals and during toxicity tests dead animals were removed every day.

Hardness, alkalinity, chlorine, chloride, sodium, potassium, ammonia, nitrite, pH, water temperature, dissolved oxygen and conductivity, were analysed at the start and at the end of each toxicity bioassay using analytical methods described by American Public Health Association (1980) and Rodier (1981).

Fluoride concentration were monitored daily using an Orion-USA model 94-09 specific ion electrode and an Orion-USA model 90-02 calomel reference electrode. Water samples were analysed at pH 5.5 after adding total ionic strength adjustment buffer (TISAB-III) with cyclohexanediamine tetra acetic acid (CDTA) as complexing agent for total fluoride ion analysis. The specific ion electrode was calibrated using analytical method described by Orion Research (1983).

The 96-hr median lethal concentration (96-hr LC50), its 95% confidence limits (95% cl), and X^2 values, were calculated by the method of Litchfield and Wilcoxon (1949), using simultaneously the fluoride ion median concentrations of duplicate tests for each species. The death was defined as test larvae floating upside down and not moving.

To verify whether the toxicity of sodium fluoride was due to fluoride ion fundamentally, sodium and conductivity toxicity controls were conducted parallel to fluoride toxicity bioassays, using sodium chloride (NaCl pro analysis, Merck, FRG). For that, 10-15 larvae of each test species were exposed in soft water (13-18 mg $CaCO_3$/L) for 96 hr to high sodium concentrations (255-313 mg Na^+/L) and conductivities (650-740 µmhos/cm) which were higher than those values measured in fluoride bioassays. Sodium concentration and conductivity were measured using an Orion-USA model 97-11 specific ion electrode and a Yellow Springs-USA model 33 conductivity meter, respectively. The possible mortality was checked daily.

RESULTS AND DISCUSSION

There were no dead animals in sodium and conductivity controls after 96-hr exposure to sodium chloride. Mean values and their standard deviations of water quality parameters analysed during fluoride toxicity bioassays are shown in Table 1. Chlorine was not detected.

All mean values of those parameters are within water quality criteria for aquatic organisms (US Environmental Protection Agency 1986). The variation of obtained values between different bioassays and between aquaria for a same test (Table 1) was probably due to slight shifts in the tap-water quality and the influence of test larvae on physico-chemical characteristics of water during each fluoride toxicity test.

Fluoride mean concentrations and mortality percentages of fluoride five static acute toxicity bioassays are presented in Table 2. Standard deviations were lower than 10% of their respective mean values. There was no mortality in control aquaria. The mortality percentage increased with regard to the sodium fluoride concentration. Mean values of sodium concentration and conductivity ranged from 6.65 to 244 mg Na^{+}/L and from 55 to 665 µmhos/cm respectively, depending on the NaF concentration into each aquarium.

The 96-hr median lethal concentrations, their 95% confidence limits and X^2 values obtained for each test species are shown in Table 3. All X^2 values are lower than X^2 values (P=0.05), indicating that data are not significantly heterogeneous.

The present study has demonstrated that the toxicity of sodium fluoride is due to fluoride ion (F^-) principally, since in sodium and conductivity toxicity controls there was no mortality of test species.

From a simple comparison of median lethal concentrations for five species, we can infer that *H. bulbifera* and *H. exocellata* are the most sensitive species to fluoride, since their 96-hr LC50s are smallest and their 95% confidence limits do not significantly overlap with the 95% confidence limits of the other species tested.

Compared with other aquatic invertebrates, trichoptera test larvae appear to be more sensitive to fluoride. McClurg (1984) obtained a 96-hr LC50 of 1,118 mg F^-/L for the estuarine prawn *Penaeus indicus,* and Le Blanc (1980), in tests with NaF in hard water, found 24 and 48-hr EC50s and a "no discernible effect concentration" of 680, 340 and 110 mg NaF/L, respectively, in *Daphnia magna.*

This may be due to the formation of innocuous complexes with one or more ions of seawater (Oliveira et al. 1978), and the precipitation of insoluble calcium fluoride from hard water.

Thus, Smith et al. (1985) have deduced that the acute toxicity of fluoride ion to *Gasterosteus aculeatus, Pimephales promelas,* and juvenile *Oncorhynchus mykiss* varied with fish species and initial water hardness due to the precipitation of CaF. The smallest 96-hr LC50 obtained directly by them was of 200 mg F^-/L to 23-62 mg $CaCO_3$/L of initial hardness in rainbow trout.

On the other hand, Pimentel and Bulkley (1983) found a 96-hr LC50 of 51 mg F^-/L to 17 mg $CaCO_3$/L of hardness in *Oncorhynchus mykiss,* and Prochnow (1978) observed that 25 mg NaF/L did generate no acute toxictity in *Cyprinus carpio.*

All this could indicate that some freshwater benthic macroinvertebrates like *H. bulbifera* and *H. exocellata* can be more sensitive than freshwater fish to fluoride ion. Water quality criteria, based on the more sensitive species, should provide adequate protection to fluoride pollution.

Table 1. Mean values and standard deviations of water quality parameters analysed in static fluoride acute toxicity bioassays (A, B, C, D and E).

	A	B	C	D	E
Water temperature (ºC)	15.15 (0.36)	14.10 (0.31)	13.25 (0.16)	17.30 (0.93)	15.95 (0.26)
Dissolved oxygen (mg/L)	10.15 (0.08)	10.16 (0.10)	10.25 (0.09)	9.95 (0.22)	10.03 (0.06)
pH	7.84 (0.07)	7.49 (0.11)	7.46 (0.12)	7.43 (0.14)	7.41 (0.15)
Hardness (mg CaCO3/L)	17.60 (3.61)	18.77 (2.84)	16.28 (1.59)	12.04 (1.98)	13.18 (1.79)
Alkalinity (mg CaCO3/L)	33.24 (2.83)	24.07 (0.93)	24.67 (0.90)	22.94 (1.29)	22.18 (1.67)
Chloride (mg/L)	3.54 (2.08)	7.27 (2.01)	15.64 (3.46)	8.91 (1.52)	4.50 (0.66)
Potassium (mg/L)	0.09 (0.02)	0.09 (0.01)	0.08 (0.01)	0.08 (0.02)	0.07 (0.01)
Ammonia (mg N/L)	0.04 (0.04)	0.05 (0.05)	0.05 (0.05)	0.04 (0.04)	0.03 (0.03)
Nitrite (mg N/L)	0.02 (0.02)	0.04 (0.02)	0.03 (0.01)	0.02 (0.01)	0.02 (0.01)

Table 2. Results of A, B, C, D and E toxicity bioassays after 96-hr exposure to fluoride ion.

	Control	A1	A2	A3	A4	A5
Fluoride mean concentration (mg/L)	0.03	2.51	7.64	21.34	64.98	194.13
Mortality of *H. pellucidula* (%)	0.0	0.0	0.0	16.6	75.0	100.0
Mortality of *H. bulbifera* (%)	0.0	0.0	10.0	30.0	90.0	100.0

	Control	B1	B2	B3	B4	B5	B6
Fluoride mean concentration (mg/L)	0.05	12.18	19.12	30.16	48.52	77.12	121.8
Mortality of *H. pellucidula* (%)	0.0	0.0	20.0	40.0	70.0	80.0	100.0
Mortality of *H. lobata* (%)	0.0	0.0	10.0	30.0	40.0	60.0	100.0

	Control	C1	C2	C3	C4	C5	C6
Fluoride mean concentration (mg/L)	0.06	11.78	18.90	29.78	48.56	77.06	122.6
Mortality of *H. lobata* (%)	0.0	0.0	10.0	30.0	60.0	70.0	100.0
Mortality of *H. bulbifera* (%)	0.0	10.0	40.0	60.0	70.0	90.0	100.0

	Control	D1	D2	D3	D4	D5	D6
Fluoride mean concentration (mg/L)	0.06	12.40	19.44	30.08	49.90	77.18	124.9
Mortality of *H. exocellata* (%)	0.0	20.0	40.0	50.0	80.0	100.0	100.0
Mortality of *Ch. marginata* (%)	0.0	0.0	10.0	30.0	60.0	70.0	90.0

	Control	E1	E2	E3	E4	E5	E6
Fluoride mean concentration (mg/L)	0.07	12.46	20.52	30.80	50.42	78.38	121.8
Mortality of *H. exocellata* (%)	0.0	20.0	30.0	50.0	70.0	90.0	100.0
Mortality of *Ch. marginata* (%)	0.0	0.0	20.0	30.0	50.0	80.0	100.0

Table 3. 96-hr LC50s, their 95% confidence limits and X^2 values.

	96-hr LC50 (mg F-/L)	95% cl (mg F-/L)	X^2
Hydropsyche bulbifera	26.30	18.8-36.7	1.87
Hydropsyche exocellata	26.50	20.4-34.4	3.63
Hydropsyche lobata	48.20	37.9-61.2	4.80
Hydropsyche pellucidula	38.50	29.9-49.5	2.79
Chimarra marginata	44.90	35.2-57.3	4.08

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