Bull. Environ. Contam. Toxicol. (1993) 50:164–170

Environmental Contamination and Toxicology

Acute Toxicity of Sulfide and Lower pH in Cultured Rainbow Trout, Atlantic Salmon, and Coho Salmon

J. A. Ortiz,[1] A. Rueda,[1] G. Carbonell,[1] J. A. Camargo,[1] F. Nieto,[2] M. J. Reoyo,[1] and J. V. Tarazona[1]

[1]Group of Environmental Toxicology, INIA—Animal Health, E-28130 Valdeolmos, Madrid, Spain and [2]Marcultura S.A., P.O. Box 29, Cariño, La Coruña, Spain

Hydrogen sulfide (H_2S) and sulfide ion (HS^-), which together constitute dissolved sulfide, are in equilibrium with hydrogen ions in the water, and the distribution of dissolved sulfide between the two forms can be easily calculated using the ionization constant (APHA 1980). Sulfides are constituents of many industrial wastes such as those from tanneries, paper mills, chemical plants, and gas works (USEPA 1976). Hydrogen sulfide is found as an anaerobic degradation product of both organic sulfur compounds and inorganic sulfates, including those of sewage, sludge beds, algae and other naturally deposited organic material (USEPA 1976). The fact that hydrogen sulfide is oxidized in well-aerated water by natural biological systems to sulfates, or is biologically oxidized to elemental sulfur has caused investigators to minimize the toxic effects of H_2S on fish and other aquatic life (USEPA 1976).

This paper reports a field event in which rainbow trout (Oncorhynchus mykiss) Atlantic salmon (Salmo salar) and coho salmon (Oncorhynchus kisutch) were lethally poisoned by sulfide in a Spanish fish farm at Orol. Different mortality incidents have been observed since 1989, always during rainstorms, with mortalities up to 50-90%. Mortality of coho salmon was always lower and during some incidents they were not affected at all. The study also includes the experimental confirmation of the diagnosis.

MATERIALS AND METHODS

The water quality of the fish farm has been biannually checked since 1986, analyzing pH, temperature, dissolved oxygen, alkalinity, hardness, chlorides ,nitrites, orthophosphates, surfactants, organic matter, and suspended matter by standard procedures (APHA 1980).

Send reprint requests to Dr. J.V. Tarazona at the above address

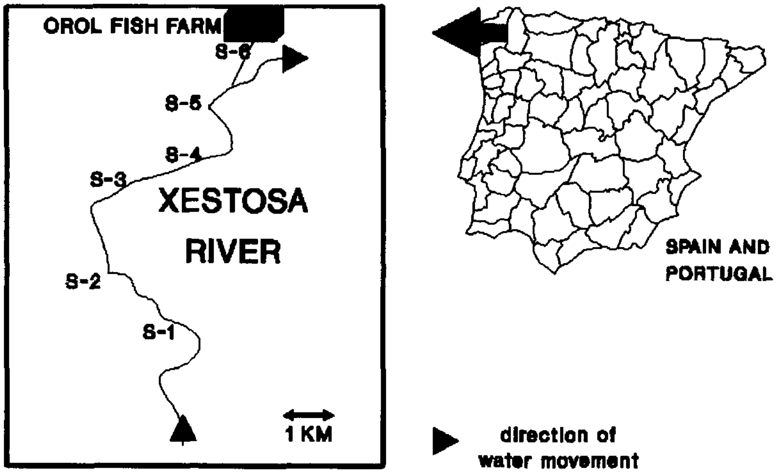

Figure 1. Map of sampling stations in the Xestosa River

During mortality episodes, heavy metals, cyanides and sulfides, in addition to the above mentioned parameters, were analyzed at the fish farm and in the Xestosa River upstream of the fish farm (sampling sites S3, S4, S5 and S6). Metal concentrations were measured in 3% HNO_3-acidified, unfiltered water samples by atomic absorption spectrophotometry (model 3030B Perkin Elmer) using a graphite furnace (HGA 300 Perkin Elmer). Cyanides were measured using a cyanide ion electrode (Orion, USA), and sulfides measured according to APHA (1980).

In addition to the physicochemical assessment, supplementary biological studies were performed on the river macroinvertebrate community, and on fish surviving at the farm. Samples of the benthic riffle macroinvertebrate community were collected at five sampling stations (S1 to S5), using a Hess cylindrical sampler (Hellawell 1986) which enclosed a sampling area of 0.1 sq m and had a mesh size of 250 μm. Sampling site S-6 was an artificial channel and, therefore, was not studied. All samples were preserved in 4% formalin until their study. The environmental impact on the macrobenthic community was quantified by the ecotoxicological index performed by Camargo (1990).

A histopathological study was performed on different organs of ten rainbow trout survivors. Gills, liver, spleen, kidney, intestine and caudal fin were fixed in a

10% formalin solution, dehydrated in ethanol, embedded in paraplast, sectioned at 4 μm and stained with haematoxylin and eosin.

The effects of sulfide and pH decrease, alone or in combination, were experimentally assessed by rainbow trout exposures to different sodium sulfide (Merck) and/or sulfuric acid (Merck) concentrations. All exposures were performed in flow-through systems and for 10 hr. Sulfide concentrations and pHs were monitoring by selective electrodes; final sulfide concentrations were analytically verified (APHA 1980).

RESULTS AND DISCUSSION

Table 1 depicts the water quality of the Xestosa River and the Orol fish farm prior to any mortality. Low alkalinity, pH and hardness values were observed. All parameters measured were suitable for salmonid farming (Alabaster and Lloyd 1980; USEPA 1976). The slightly high concentrations of anionic surfactants detected in the Xestosa River were not observed at the fish farm.

During the mortality events, several changes in water quality were observed (Table 2), i.e., a decrease in pH, which could lead to the decrease in alkalinity by the displacement of carbonates' equilibria, and an increase in water hardness by the increase in the capability to dissolve and remove Ca and Mg. Metal levels were within the acceptable range for salmonid fish (USEPA 1976, 1986; Mance 1987). At most sampling sites and during mortality episodes, cyanide was always below the level of detection, and only slightly harmful concentrations (USEPA 1976) were detected sporadically. Sulfide concentrations around 0.5 ppm were found in both the Xestosa River and at the Orol fish farm. More than 90 percent of this sulfide was in the toxic form of hydrogen sulfide. Sulfide concentrations were about 250-fold the recommended level, 2 μg/l of hydrogen sulfide, for the protection of aquatic life (USEPA 1976).

Table 3 describes damage to the benthic riffle macroinvertebrate community in sampling sites S-2 to S-5, which is reflected by the drastic increase in Carmargo's ecotoxicological index. The sensitivity of macroinvertebrate species to hydrogen sulfide has been previously reported (Oseid and Smith 1974; 1975)

Histopathological examination (Figs 2 to 5) revealed adherence between the secondary branchial lamellae, at the basal and apical levels, and seldom along the whole lamellae. Thrombus, edemas and karyorhexis were also observed in gills, while congestion and interstitial hemorrhages were observed in the kidney without

Table 1. Water quality in the Xestosa River and the Orol fish farm before mortality of fish occurred.

	XESTOSA RIVER			FISH FARM PONDS		
PARAMETERS.	MEAN	S.D.	RANGE	MEAN	S.D.	RANGE
ALKALINITY ($mgCO_3Ca/l$).....	6.08	±0.2	(6.6 -7)	6.8	±0.20	(6.6-7)
CHLORIDE (mg ClNa/l)......	26.82	±17.7	(8.33-43.69)	15.4	±8.26	(7.74-25.09)
CO_2 (mg CO_2/l)............	7.74			4.57	±1.22	(3.17-5.28)
HARDNESS (mg CO_3Ca/l)......	7.50	±4.67	(4.34 -8.33)	7.67	±3.93	(3.41-13.7)
ANIONIC SURFACTANTS (mg/l).	0.30	±0.31	(0.00 -0.63	0.04	±0.05	(0.08-0.131)
CATIONIC SURFACTANTS (mg/l).	N.D.			N.D.		
ORTOPHOSPHATES (mg P/l).....	N.D.			N.D.		
ORGANIC MATTER (mg O_2/l)...	2.58	±0.33	(2.30 -2.95)	2.58	±0.82	(2.22-4.4)
TOTAL SUSP. MATTER (mg/l).	21.91	±17.8	(11.2 -42.5)	19.43	±16.6	(7.6-45.5)
ORG.SUSP. MATTER (mg/l)...	16.76	±17.1	(4.6 -36.3)	16.86	±14.9	(5.4-41.3)
INORG.SUSP. MATTER (mg/l)..	5.13	±2.20	(2.6 -6.6)	2.56	±2.29	(0.0-6.2)
NITRATES (mg N/l)..........	1.42	±1.89	(0.07 -3.59)	1.37	±1.51	(0.07-3.54)
NITRITES (mg N/l)...........	N.D.			0.03	±0.06	(0.0-0.154)
DISSOLVED OXYGEN (mg/l)...	11.80	±0.2	(11.6 -12)	11.06	±1.41	(7.5-11.4)
pH.........................	6.26	±0.11	(6.2 -6.6)	6.26	±0.11	(6.2-6.6)
TEMPERATURE (ºC)...........	8.21	±0.21	(8.0 -8.43)	8.43	±0.32	(8.0-8.83)

Table 2. Water quality in the Xestosa River and the Orol fish farm when mortality of fish occurred.

	XESTOSA RIVER			FISH FARM PONDS		
PARAMETERS.	MEAN	S.D.	RANGE	MEAN	S.D.	RANGE
ALKALINITY ($mgCO_3Ca/l$)...	0.36	±0.34	(0.0-1)	2.25	±1.79	(0.0-3.90)
CHLORIDE (mg ClNa/l).....	19.36	±0.96	(17.99-20.12)	19.97	±4.14	(16.63-26.95)
CO_2 (mg CO_2/l)..........	2.81	±1.01	(1.5-4.70)	7.72	±7.74	(2.10-21)
HARDNESS (mg CO_3Ca/l)....	11.52	±1.79	(10.34-15.51)	18.59	±6.81	(11.13-29.42)
ORGANIC MATTER (mg O_2/l).	2.64	±0.90	(2.00 -4.52)	3.53	±1.27	(2.01-5.55)
NITRITES (mg N/l).......	N.D.			N.D.		
DISSOLVED OXYGEN (mg/l)..	13.17	±1.93	(10.3-14.50)	10.13	±1.62	(8.41-14.50)
pH......................	5.66	±1.95	(5.13-6.37)	5.63	±0.11	(5.51-5.72)
TEMPERATURE (ºC)........	5.67	±1.95	(4.50-10)	9.16	±2.75	(6.50-12)
CYANIDE (mg/l)..........	0.05	±0.06	(0.00-0.1)	0.07	±0.05	(0.00-0.10)
SULFIDES (mg/l).........	0.55	±0.11	(0.44-0.66)	0.57	±0.02	(0.56-0.59)
COPPER (ppb)............				3.85	±4.03	(1-6.7)
IRON (ppb).............				65.50	±21.9	(50-81)
CADMIUM(ppb)............				0.02	±0.00	(0.02-0.02)
LEAD (ppb)..............				0.80	±0.28	(0.6-1)
MANGANESE (ppb).........				50.00	±50.0	(50 50)

Table 3.- Results of the benthic riffle macroinvertebrate community study.

Taxa	S-1	S-2	S-3	S-4	S-5
Polycelis sp.	+	-	-	-	-
Proponemure sp.	+	-	-	-	-
Rhyacophila sp.	+	-	-	-	-
Eusimulium sp.	+	-	-	-	-
Ecdyonurus sp.	+	-	-	-	-
Baetis sp.	+	-	-	-	-
Heptagenia sp.	+	-	-	-	-
Limnephilus sp.	+	-	-	-	-
Ancylus fluviatilis	+	-	-	-	-
Holobdella stagnalis	+	-	-	-	+
Perla sp.	+	-	-	-	-
Ephemerella sp.	+	-	-	-	-
Camargo's Ecotox. Index:	0	100	100	100	94

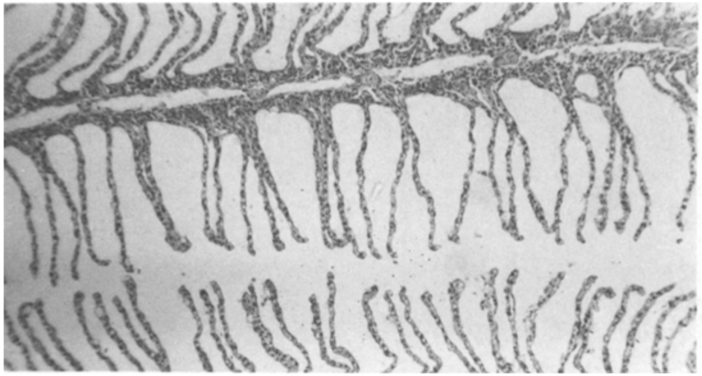

Figure 2. Gills. Adherence between lamella HE 10x

Figure 3. Gills. Thrombus in branchial lamella HE 25x

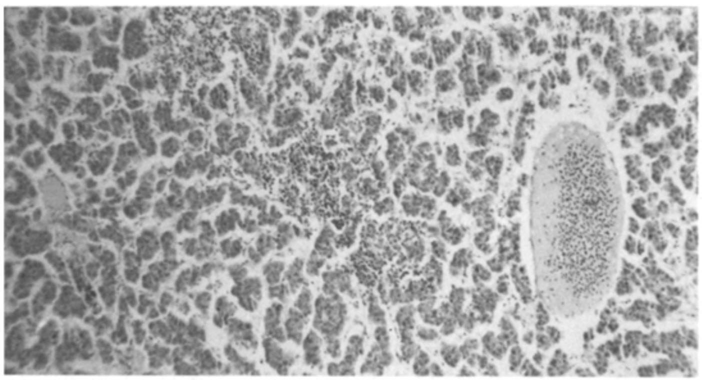

Figure 4. Liver. Congestions HE 25x

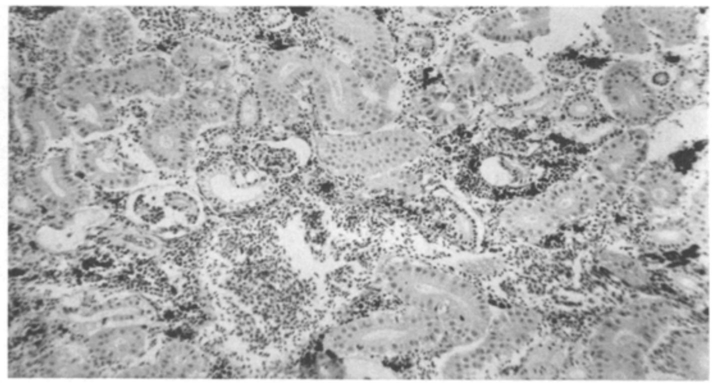

Figure 5. Kidney. Haemorrhages HE 10x

significant lesions in other organs. Coccaceae bacteria were observed in both the gills and kidney.

Although the lethal toxicity of sulfides for different fish species ranges between 0.047 and 1 mg/l (Adelman and Smith 1970; USEPA 1976; Thurston et al. 1979) and different methods for sulfide removal from industrial wastes have been developed (Buisman and Lettinga 1990), the high oxidation rate of hydrogen sulfide has motivated investigators to minimize the toxic effects of H_2S on fish and other aquatic life (USEPA 1976). Until now, we had observed only a few sulfide poisonings in fish, and always in cyprinid species related to very low dissolved oxygen levels. However, sulfide concentrations in the Xestosa River and the fish farm were high enough to be considered harmful.

Table 4 summarizes the results on rainbow trout mortality observed after the experimental exposure to various sulfide and pH levels. The combination of sulfides and low pH led to 100% mortality after 8 hours of exposure, even for sulfide concentrations lower than those reported for the fish farm, while single exposures to sulfides or pH gave mortalities below 50%. Percentages of dissolved sulfide present as hydrogen sulfide were about 75% and 95% at pHs of 6.5 and 5.5, respectively. The increase in the unionized form does not justify the observed results; therefore, synergistic effects between sulfides and pH must be suggested.

Table 4. Rainbow trout mortalities after 8 hours of flow-through exposure to sulfide and/or pH.

Sulfide (mg/l)	pH	mortality (%)
0	6.5	0
0.45	6.5	20
0	5.5	40
0	5.2	70
0.4	5.5	100
0.5	5.5	100

The Xestosa River does not receive industrial or urban sewage which can explain the observed sulfide concentrations; therefore, a soil composition study was requested by Marcultura S.A.. The study revealed the presence of sulfide-rich metamorphic geological materials between S1 and S-2 (F. Macias-Vázquez, personal communication). These materials had been air-exposed as a result of road construction, and when it rained the sulfides were dissolved and released into the river. The study also suggested that the oxidation of sulfide to sulfate would be responsible for the decrease in the pH.

As a result, sulfide poisoning of salmonid fish in a well-aerated river without exposure to industrial or major urban waste dumping could be explained.

Acknowledgments. This work was funded in part by INIA Research Project grant number 8569.

REFERENCES.

American Public Health Association (1980) Standard methods for the examination of water and wastewater, 15th edition. APHA-AWWA-WPCF, Washington DC

Adelman IR, Smith LL (1970) Effect of hydrogen sulfide on northern pike eggs and sac fry. Trans Amer Fish Soc 99:501-508

Alabaster JS, Lloyd R (1980) Water quality criteria for freshwater fish. Butterworths, London

Buisman CJN, Lettinga G (1990) Sulphide removal from anaerobic waste treatment effluent of a papermill. Wat Res 24:313-319

Camargo J (1990) Performance of a new ecotoxicological index to assess environmental impacts on freshwater communities. Bull Environ Contam Toxicol 44:529-534

Hellawell JM (1986) Biological indicators of freshwater pollution and environmental management. Elsevier Applied Science Publishers, London

Mance G (1987) Pollution threat of heavy metals in aquatic environments. Elsevier, London

Oseid DM, Smith LL Jr (1974) Chronic toxicity of hydrogen sulfide to Gammarus pseudolimnaeus. Trans Am Fish Soc 103:819-822

Oseid DM, Smith LL Jr (1975) Long-term effects of hydrogen sulfide on Hexagenia limbata. Environ Entomol 4:15-18

Thurston RV, Russo RC, Fetterolf CM Jr, Edsall TA, Barber YM Jr (1979) A review of the EPA Red Book: Quality criteria for water. Water Quality Section, American Fisheries Society, Bethesda, MD

US Environmental Protection Agency (1976) Quality criteria for water. US Environmental Protection Agency, Washington DC

US Environmental Protection Agency (1986) Quality criteria for water. US Environmental Protection Agency, Washington DC

Received July 30, 1991; accepted June 30, 1992.