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Sublethal effects of kraft pulp mill waste water on the perch, Perca fluviatilis, studied by rotary-flow and histological techniques

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One-year-old perch were semistatically exposed for 14 days in 1, 2 and 4 % (v/v) concentrations of total, unfiltered, bleached, kraft pulp mill waste water. The fish were tested with the rotatory-flow technique before and after exposure. In the two highest concentrations the perch displayed significantly impaired ability to compensate in the rotatory-flow test compared to the control perch. However, no difference was observed between the two highest effective test concentrations.

Histological analysis of liver showed hepatocytes with pycnotic nuclei, particularly in connection with the central veins. Increased cytoplasmic granularity with lysosome-sized particles was also discernible. Apically swollen secondary lamellae occasionally occurred in the gills. In addition, parasitic cysts were found between the secondary lamellae in all exposed fish but not in the controls.

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1. Introduction

The acute toxicity of untreated sulphate (kraft) pulp mill waste waters, as tested by standard 96h salmonid LC50 bioassay, generally lies between 5 and 50 % dilution of the original effluent (for a recent review see Oikari et al. 1979). Despite the considerable dilutive effect of the high-volume discharges, toxicological effects on the aquatic life, e.g. on salmonid river populations, have been stressed during recent years by several, mainly Canadian, workers (Walden & Howard 1977). A recent study (Holmbom & Lehtinen 1980) indicates that a similar impact may be prevalent in Finnish watercourses. However, test results from fresh water and seawater animals cannot be unequivocally applied to the Baltic Sea, because its fish fauna have diversely adapted themselves to exceptionally low but nevertheless, permanent water salinity. More information is therefore required concerning the physiological effects of waste waters from the pulp mill industry on the local fish fauna.

Apart from the acute toxic effects, great significance has been attributed to the determination of sublethal and long-term effects. However, many histological and haematological methods, for example, do not permit direct quantification of the apparent adverse effects of waste water on an animal's fitness.

This report deals with some histologically demonstrable sublethal effects on the liver and gills of the perch, *Perca fluviatilis* (L.), after subjection to low concentrations of total effluents from a typical Nordic kraft mill on the southwestern coast of Finland. The general condition of the fish was simultaneously tested using the rotatory-flow technique which has been described in detail by Lindahl & Schwanbom (1971) and Lindahl (1978). This method is considered to be able to detect sublethal poisoning or other physiological impairment of fish by recording their ability to compensate for torque in a rotating current.

2. Material and methods

2.1. The effluent

The experiments were carried out from 1st to 18th June, 1979, in a temporary laboratory in the vicinity of the pulp mill. Samples of the total unfiltered effluent from the aeration lagoon were collected during two days using a continuous flow proportional sampler and kept in polyethylene containers until used in the experiments. This procedure was repeated during two weeks so that fresh test solution was available every two days. The test solutions in the test aquaria were renewed to 90 % every 48 h.

During the experimental period the mill produced both pine and birch bleached cellulose.

For the chemical and physical characteristics of the test solutions see Tables 1 and 2. The analyses were performed as reported previously (Holmbom & Lehtinen 1980).

2.2. Experimental design

One-year-old perch (mean total length and *SD* 5.2 ± 0.5 cm, were used. After capture by bownet in a fresh water basin 80 km NE of the mill, the fish were acclimatized to laboratory conditions in a 400 l container with a continuous flow of brackish water (2 l/min, salinity 0—3 $^0/_{00}$) for seven days (Homes & Donaldson 1969). The perch were fed daily *ad libitum* with live speciments of *Neomysis integer* (Leach) and *Aedes* larvae. At the end of the acclimatization period the fish were placed into four (control = 0, 1,2 and 4 % v/v) 45 l silicone-sealed glass aquaria which were submerged in a trough with running brackish water for constant temperature maintenance. The effluent concentrations used were chosen on the basis of our previous results on acute toxicity tests performed at the same mill (Holmbom & Lehtinen 1980). Each aquarium was divided into compartments by perforated acrylic plates to enable recognition of individual test fish. One control and one 2 % concentration perch died during the 14 day exposure. A photoperiod of 12:12 hrs was maintained.

Because of rapidly occurring stress effects caused by handling (Oikari & Soivio 1975, Soivio & Oikari 1976), the fish were not netted or removed from the water. Instead, a 10 cm long tube open at one end and connected to a shaft handle at the other was submerged in the aquarium. The test fish was then allowed to swim into the tube and the open end was closed with a plug. The fish was thereupon lifted out and placed into the rotatory-flow apparatus, thus preventing removal of the fish from the aquatic environment at any step.

The four or five fish in each group were tested with the rotatory-flow technique, both before and after exposure. The tube diameter used was 21 mm with a water current velocity of 3 cm/s. A rotational acceleration of 120 rev/min was applied. Every specimen was tested five time in succession with two-minute intervals without prior acclimatization to the rotatory-flow tube (Lehtinen 1980). The fish were killed after 12 hrs of recovery after the final test and pieces from the outermost right gill arch and from the caudal part of the liver were fixed in Bouin's solution. The pieces were paraffin sectioned in 5 μm slices and stained with alcian blue-PAS-Mayer's hematoxylin (Pearse 1968). The control group (n = 3) was made up by sampling three additional perch kept in brackish water with the same chemical and physical characteristics. No differences were revealed between the histological appearance of these and rotatory-flow treated controls.

3. Results

In comparison to the results obtained prior to exposure, the mean compensation capacity of the fish significantly decreased (analysis of variance $P < 0.05$) in the two highest concentrations whereas the mean compensation capacity of the fish in the lowest concentration, as well as that of the controls, remained the same (Table 3). Some individuals in the lowest test concentration and in the control group even displayed an increased capacity to compensate for increased torque of the water current. An interesting point was that the clear reduction in compensation capacity was almost equal in fish exposed to the two highest doses (Table 3).

Histological analysis of the liver revealed structural changes in all three test groups, but these were clearly less frequent with the lowest

Table 1. Chemical and physical characteristics of the test water samples (diluted bleached kraft pulp mill waste water) during the 14 d exposures (mean ±*SD* of 4 samples/aquarium).

	Control	Concentration of waste water v/v % 1	2	4
Temperature °C	12.6 ± 1.4	12.6 ± 1.4	12.6 ± 1.4	12.6 ± 1.4
pH	7.8 ±	7.8 ± 0.2	7.7 ± 0.2	7.6 ± 0.2
Oxygen mg/l	10.2 ± 0.4	10.1 ± 0.4	9.8 ± 0.5	9.3 ± 0.8
Conductance mS/m	930 ± 94	894 ± 60	874 ± 56	870 ± 56
$KMnO_4$ mg/l	27 ± 5	51 ± 9	76 ± 10	125 ± 18
Suspended solids mg/l	13 ± 19	22 ± 22	31 ± 23	26 ± 18
Colour Pt mg/l	37	105	238	261
Turbidity (Control = 1)	1.0	2.1	3.2	4.0

Table 2. Concentrations ($\mu g/l$) of some identified toxic or potentially toxic compounds originating from the waste water in the test aquaria (mean of 4 subsamples/aquarium). Detection limit 0.5 $\mu g/l$.

	Control	Concentration of waste water v/v % 1	2	4
Fatty acids				
Palmitic	38	26	40	48
Stearic	20	12	30	24
Oleic	11	31	58	48
Linoleic	—	3	3	42
Pinoleic	—	19	8	8
Resin acids				
Pimaric	—	5	6	16
Palustric	—	—	5	3
Isopimaric	—	4	5	11
Abietic	—	—	17	30
Dehydroabietic	4	10	20	40
Neoabietic	—	6	4	4

concentration. Shrunken dark nuclei were often observed sparsely distributed in the tissue, particularly in the vicinity of central veins. The nucleolar surface of the hepatocytes was more diffuse than in the control fish. No statistically significant differences were revealed between the maximum diameters of 20—30 round or oval nuclei from each of the groups. In addition, the cytoplasm of the hepatocytes exhibited increased numbers of granulae similar in size to lysosomes, which implies symptoms of toxicity.

The effects on the gill epithelium were not as apparent or as frequent as those on the liver. However, apical swelling was discernible in the secondary lamellae of only a few fish in the two highest concentrations. In addition, a few fish exhibited sporadic increased mucus production on or between the lamellae. An interesting observation was also that parasitic cysts, identified as *Oodinium sp.*, were found between the secondary lamellae in all exposed fish, but not in the controls. Nevertheless, most lamellae and filaments appeared normal or only slightly histologically changed, even in the most heavily parasitized individuals.

Table 3. Capacity of perch, *Perca fluviatilis* (L.), to resist increasing torque of water current before and after 14 d exposure in three concentrations of bleached kraft pulp mill waste paper. Each fish was tested five times in succession with two-minute intervals in a 3 cm/s water current and a rotational acceleration of 120 rev/min. The average value of each fish was used for calculation of the mean of each group. Numbers refer to group means $\pm SD$. (*) indicates statistically significant differences between pre-exposure and post-exposure capacity of the test fish in the different groups (analysis of variance $P < 0.05$). N = number of fish tested.

Conc. % (v/v)	Before exposure	After exposure	N
0	22.8 ± 2.5	23.8±3.3	3
1	23.6 ± 2.0	23.9±2.5	4
2	24.1 ± 2.1	19.7±3.2*	4
4	23.5±1.3	18.8±6.2*	5

4. Discussion

It is generally recognized that resin acids and phenols, as well as their chlorinated derivatives, are major contributors to fish toxicity from pulp mill effluents (Leach & Thakore 1973, Rogers 1973, Landner & Sörensen 1975, Landner et al. 1977, Oikari et al. 1979, Holmbom & Lehtinen 1980). Table 2 shows that the present test conditions were very similar to those described by the above authors. Total resin acid concentration in the 4 % waste water samples was about 0.1 mg/l, which is within 10—20 % of 96 h LC50 values in salmonid bioassays (Holmbom & Lehtinen 1980). Unfortunately, chlorinated compounds, especially the most important chlorophenols were not analysed in this work. According to Walden & Howard (1977) the 0.1 mg/l concentration level, in relation to acute toxicity, is well within the concentrations which can be expected to cause diverse sublethal physiological effects. To our knowledge, structural changes in the liver of fish exposed to total effluents of a bleached kraft pulp mill have not previously been reported. Due to the relatively small number of fish studied, our results must primarily be considered as indicative rather than absolute. As such they indicate clear and uniform effects from concentrations as low as 2—4 % of a total aerated effluent from a Finnish kraft mill (Table 3). It is interesting to note, however, that an investigation which used the effects on detoxification enzymes in trout liver as criteria revealed almost identical results with regard to the lowest effective dilution (Castren & Oikari 1979).

The observed effects, such as the diminished capacity of the fish to compensate for the increasing torque of the water current in the highest concentrations, could be due to many different factors, including purely mechanical influences. The results of rotatory-flow tests with ethanol (see e.g. Lindahl 1978) clearly point to intoxication of the central nervous system, whereas the results of Lehtinen (1980) and Larsson et al. (1980) show that a secondary mechanical effect on the gills might also be the reason for physiological impairment of the test fish.

Macleod et al. (1966) found that fibres in pulp mill effluents impaired both the oxygen uptake and swimming ability of minnows, *Pimephales promelas*. Smith et al. (1966) reported that cellulose fibres induced a stress response in fish which manifested itself as increased metabolism, decreased hematocrit value and in increase in the number of mucous cells in the gills. Since the effluent used in this work was not filtered, and therefore contained about twice as much suspended solids as the control water, the rotatory-flow results might have been affected by the fibres present in the test water. As the histological effects on the gills were not very pronounced, factors other than the expected impairment of the diffusion of respiratory gases (Hughes 1976, Soivio unpubl.), such as resin acids, phenols and their chlorinated derivatives, apparently contributed to the decreased compensation capacity noted in the fish during the rotatory-flow test. This idea is supported by the more frequent structural effects on the liver tissue of the perch.

The rotatory-flow technique must therefore be used to complement other techniques which permit more specific detection of toxic effects on fish.

The discovery of parasites in all test fish, but not in the controls, implies increased susceptibility of perch to parasites during exposure and, perhaps therefore, in water affected by pulp mill effluents. Increased susceptibility may be preceded by minor structural changes, invisible with conventional paraffin techniques, in secondary lamellae of the gill. Further research into this point is essential.

In conclusion we note that a combination of physiological and morphological methods provides useful and sensitive tools with which the sublethal effects of pulp mill effluent can be investigated.

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References

Castren, M. & Oikari, A. 1979: Use of detoxification reactions (glucoronidation) in evaluation of the water toxicity of fishes. — 2nd Symposium of Fish Physiology, June 7—8, Gothenburg. Abstract/Poster.

Holmbom, B-H. & Lehtinen, K-J. 1980: Acute toxicity to fish of kraft pulp mill waste waters. — Paperi ja Puu — Papper och Trä 11: 673—684.

Homes, W. N. & Donaldson, E. M. 1969: The body compartments and the distribution of electrolytes. — In: Hoar, W. S. & Randall, D. J. (Eds.) Fish Physiology I. Academic Press N. Y., London.

Hughes, G. M. 1976: Polluted fish respiratory physiology. — In: Lockwood, A. P. M. (Ed). Effects of Pollutants on Aquatic Organisms: 163—183. Cambridge Univ. Press, Cambridge.

Landner, L. & Sörensen, L. 1975: Toxiska och andra effekter av blekeriavloppsvatten. — En kritisk litteratursammanställning (review; in Swedish). SSVL, Project 7. Report no 12: 1—61.

Landner, L., Lindeström, L. & Linden, O. 1977: Effekter av skogindustriella avloppsutsläpp i recipienterna. Sammanställning av nuvarande kunskap (review; in Swedish). NM 80. Report no 2 B:8.

Larsson, Å., Lehtinen, K-J. & Haux, C. 1980: Some physiological effects of titanium dioxide industrial effluents on fish. — Bull. Env. Cont. Tox. 25: 427—435.

Leach, J. M. & Thakore, A. N. 1973: Identification of the constituents of kraft pulping effluent that are toxic to juvenile coho salmon (Onchorynhus kisutch). — J. Fish Res. Board Canada 30: 479—484.

Lehtinen, K-J. 1980: Effects on fish exposed to effluent from a titanium dioxide industry and tested with rotatory-flow technique. — Ambio 9: 31—33.

Lindahl, P-E. 1978: Rotatory-flow technique for detection of sublethal poisoning and other impairment of fitness in fish. — SNV (National Swedish Environmental Protection Board) PM 1058: 1—72.

Lindahl, P-E. & Schwanbom, E. 1971: A method for detection and quantitative estimation of sublethal poisoning in living fish. — Oikos 22: 210—214.

Macleod, J. C. & Smith, L. L. 1966: Effect of pulpwood fiber on oxygen consumption and swimming endurance of the fathead minnow. — Trans. Am. Fish. Soc. 95: 71—84.

Oikari, A. & Soivio, A. 1975: Influence of sampling methods and anaesthetization of various haematological parameters of several teleosts. — Aquaculture 6: 171—180.

Oikari, A., Soivio, A., Vuorinen, M., Vuorinen, P-J. & Nyholm, K. 1978: Metsäteollisuuden jätevesistä ja jätevesikomponenteistä sekä niiden vaikutuksista kaloihin (review; in Finnish). Mimeograph, Pupro-Helsinki. Can be obtained from the registrar's office of the Fisheries and Game Div., Min. of Agriculture Helsinki.

Pearse, A. 1968: Histochemistry, theoretical and applied. — Williams & Williams, Baltimore.

Rogers, I. H. 1973: Isolation and chemical identification of toxic components of craft mill wastes. — Pulp Paper Mag. Canada 74: 111—116.

Smith, L. L., Kramer, R. H. & Oseid, D. M. 1966: Long-term effects of conifer groundwood paper fibre on walleyes. — Trans. Am. Fish. Soc. 95: 60—70.

Soivio, A. & Oikari, A. 1975: Haematological effects of stress on a teleost, Esox lucius (L.). — J. Fish Biol. 8: 397—411.

Walden, C. C. & Howard, T. E. 1977: Toxicity of pulp and paper mill effluents. A review of regulations and research. — Tappi 60: 122—125.

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