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Biochemical and Hematological Effects of a Titanium Dioxide Industrial Effluent on Fish

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The effluent from a titanium dioxide industry, located at the south west coast of Finland, has polluted the water area outside the factory since 1961. The daily discharge amounted to 10 000 m^3, or more, a day in 1976.

According to HÄKKILÄ (1978), the effluent contained iron sulphate (4 %), sulphuric acid (3 %) and various amounts of heavy metals, such as zinc, lead, cadmium and copper. Additionally, the effluent is characterized by an extreme acidity (pH 1). When the effluent is discharged into the recipient, a rapid increase of the pH and a subsequent precipitation of the heavy metals present occurs.

Previous works, mainly field investigations, have shown that the effluent is deleterious to bottom dwelling organisms and to the fisheries of the area (SEPPÄNEN & SHEMEIKKA 1972, LEHTONEN 1976, VOIPIO & NIEMISTÖ 1975). The acute toxicity of the effluent to various water organisms, both vertebrates and invertebrates, has been tested (BAGGE & ILUS 1975, HÄKKILÄ 1978).

In a previous experiment (LEHTINEN 1980), the sublethal effects of the effluent were studied by means of the rotatory flow technique. In that study, it was found that a brown precipitate was formed on the gills of the fish, Perca fluviatilis and Alburnus alburnus, exposed for two to four weeks to low concentrations of the effluent. It was concluded that this precipitate was the main reason to the impaired capacity of the fish to manage in the rotatory flow tests. The mechanism behind the noted effects remained so far obscure.

The present work illustrates some physiological effects of the titanium dioxide industrial effluent, including the observed gill precipitate on the flounder (Platichthys flesus L.), a marine benthic fish species. As a bottom dwelling and relatively stationary organism, it is possible that this fish species will be very subjected to the outflocking, sinking effluent.

MATERIAL AND METHODS

Test organism

In total, 30 flounders of mean length 23 cm (range 20.5 - 25.5 cm) and mean body weight 133 g (range 94 - 179 g), were evenly distributed into six 60 L aquaria with a continuous flow (24 L/h) of

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filtered brackish water (salinity: 7 o/oo), which was taken from the Tvären, a bay of the Baltic outside the laboratory (80 km SW of Stockholm). The fish were caught by gill nets and then acclimated to laboratory conditions for 7 days. Both during the acclimation and exposure period, the fish were fed live common mussels, Mytilus edulis. Two flounders died during the exposure to the highest effluent concentration.

The effluent

The effluent was transported from the factory in Finland to the Brackish Water Toxicology Laboratory (NBL) in Sweden in two 250 L airtight polyethylene barrels. The pH (~1) did not change during 2 months' storage of the undiluted effluent at 13°C at the laboratory. The solid particles in the undiluted effluent were allowed to sedimentate in order to make continuous dosage through the narrow pump tubes possible.

Exposure

The experiment consisted of a control group, a low dose group (370 µl effluent/ L brackish water) and a high dose group (685 µl effluent/ L brackish water).

The effluent was distributed to the aquaria by a persistaltic pump (Ismatec mp 13) and accurated pump tubes (Elkay). The dosages were maintained during 14 days.

Temperature, pH and dissolved oxygen content and salinity were measured daily (Table 1). Water samples for heavy metal determinations were taken every 48 hours and frozen until analysis (Table 2).

TABLE 1

Chemical physical characteristics of the test water. The values are mean ± S.D. of 6-13 measurements.

	pH	Oxygen (ppm)	t (°C)	Salinity (o/oo)
Control	7.8 ± 0.1	10.6 ± 0.3	4.6 ± 0.1	7.0 ± 0.1
370 µl/L	7.2 ± 0.2	10.5 ± 0.4	"	"
685 µl/L	7.1 ± 0.3	10.4 ± 0.4	"	"

Analytical procedures

In order to get a picture of the amounts of heavy metals (Zn, Pb, Cd and Cu) in the solid phase, 50-250 ml of undiluted effluent were later neutralized with sodium hydroxide. The obtained precipitate was filtered, dried and analyzed for the metals mentioned above. The amounts of heavy metals in undiluted, unprecipitated effluent were also measured. Table 2 summarizes the results from these analyses.

The metal analyses were performed at the Institute of Analytical Chemistry, Åbo Academy, Turku, Finland.

TABLE 2

Heavy metal concentrations in the test water (µg/L) and in the undiluted effluent (µg/L), and amounts of heavy metals in the precipitate (µg/g dry precipitate).

	Zn	Pb	Cd	Cu	Method of analysis
Test water					
Control	3.4	0.1	0.1	0.6	Differential pulse anodic stripping voltametry (ASPLUND 1977)
Low dose	9.2	0.3	0.2	0.8	
High dose	6.3	0.1	0.1	1.9	
Undiluted effluent	33.60	0.22	0.12	1.50	" "
Precipitate	3137	53.0	5.8	202.0	Atomic absorption spectrophotometry (Perkin-Elmer with graphite oven (KARJALA 1980, to be published)

At the end of the exposure period, the fish was sacrificed with a blow on the head and blood was taken from caudal vessels with a heparinized syringe. The sampling procedure, as well as the analytical methods, used for determination of hematological and biochemical variables, have previously been described in detail (DAVE et al. 1975, JOHANSSON-SJÖBECK & LARSSON 1978, HAUX & LARSSON 1979).

Statistical treatment

Statistical analysis of the data was performed by the Pitman trend test (BRADLEY 1968). Significant correlations were established at $p \leq 0.05$.

RESULTS AND DISCUSSION

Water and electrolyte status

Two weeks of exposure to the effluent caused significant dose-dependent reductions of sodium and chloride concentrations, as well as the osmolality in the blood plasma of flounders (Figure 1). The blood plasma potassium concentration of the exposed fish showed no obvious alterations when compared to the control fish(Figure 1).

The effects of the effluent on the osmolality and the osmotically important ions indicate an impaired ability for osmoregulation. Probably, there is a leakage of sodium and chloride from the extracellular fluids of the exposed fish to the surrounding hypotonic brackish water (Na^+: 95 mM; Cl^-: 109 mM) and/or a decreased active uptake of these ions through the gills. Such disturbances may be

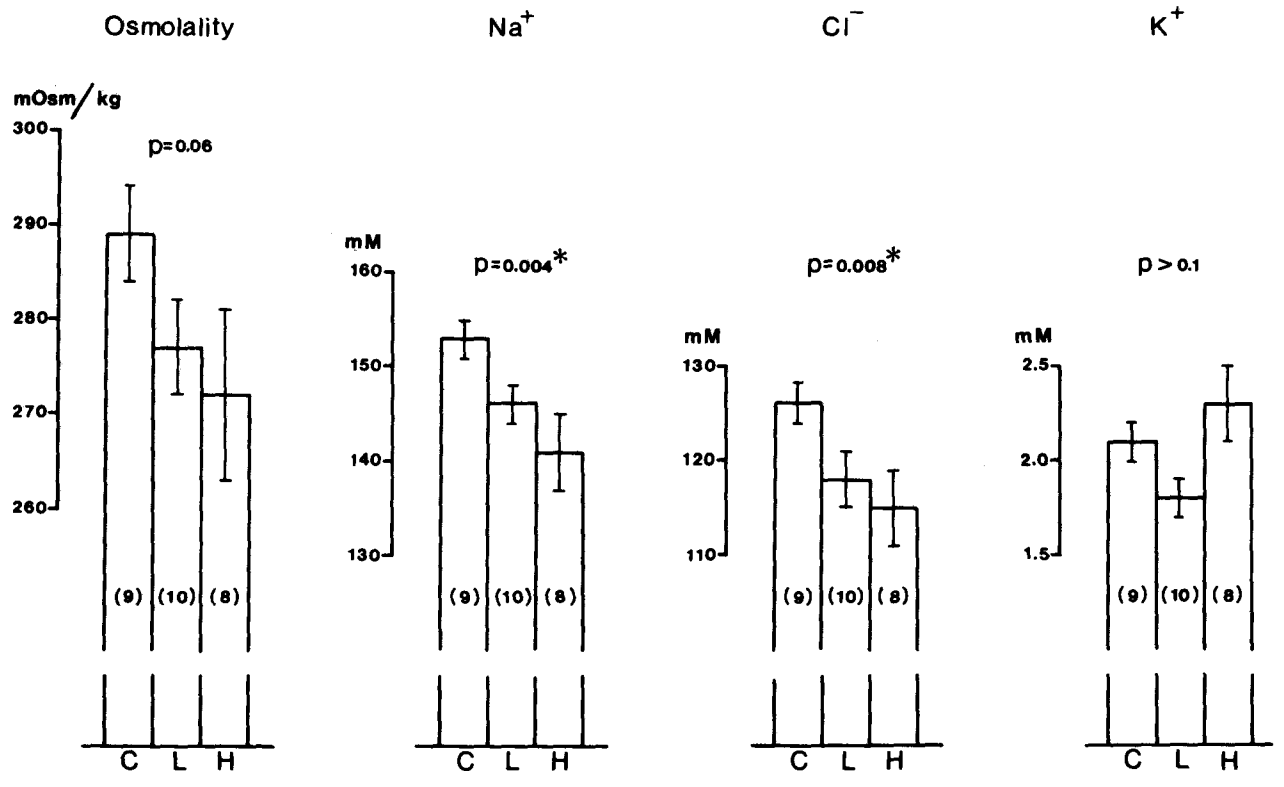

Figure 1. Effects of titanium dioxide industrial effluent on osmolality, sodium, chloride and potassium in blood plasma of flounders (P.flesus). Each column respresents the mean value and vertical bars indicates the standard error of the mean. Number of fish in parenteses. C = control group, L = low dose group, H = high dose group.
* indicates statistically significant alterations ($p < 0.05$).

a result of the observed brown precipitation of effluent products on the gill lamella from the exposed fish. The nature of this gill precipitation is not fully elucidated (HÄKKILÄ 1978). Preliminary results (LEHTINEN & KLINGSTEDT 1980, to be published) showed, however, that this precipitate is mainly constituted of an iron-titanium complex. Furthermore, it can be seen from Table 2 that a co-precipitation of other heavy metals occurs in connection with this iron-titanium complex. Despite slow oxidative processes, due to the low water temperature, similar amount of precipitate seemed to be formed on the gills as that found in a previous experiment with considerably higher test temperature (LEHTINEN 1980). This implies that besides strictly mechanical effects, a biochemical action of heavy metals present in the precipitate is possible. In addition, although in a minor degree, effects of dissolved heavy metals (zinc, cadmium, copper, lead, etc) on the gill function cannot be excluded.

Several earlier investigations have shown that fish acutely or sub-acutely exposed to heavy metals suffer from various pathological alterations in gill tissues (BAKER 1969, SKIDMORE 1970, GARDNER & YEVICH 1970, GARDNER & LaROCHE 1973, MATTHIESSEN & BRAFIELD 1973, HUGHES 1976).

Carbohydrate metabolism

The exposure of flounders to the effluent produced significantly enhanced levels of blood glucose and blood lactate (Figure 2). This dose-dependent hyperglycemic and hyperlactemic response indicates a typical stress response, which certainly includes an increased breakdown of liver and muscle glycogen. Such a response may be caused by a general state of stress, due to the exposure to the mixed effluent, but it can also be a result of a hypoxic state caused by strictly mechanical and/or heavy metal action on the gill function.

Hypoxia is known to cause elevations in blood glucose and blood lactate in fish (BLACK et al. 1961, HEATH & PRITCHARD 1965). It has also been shown that exposure of fish to heavy metals, like chromium (STRIK et al. 1975), zinc (WATSON & McKEOWN 1976), cobalt (KHANNA & GILL 1975) and cadmium (LARSSON 1975) cause significant elevations in blood glucose. In addition, fish exposed to pesticides (SILBERGELD 1974), industrial effluents (McLEAY & BROWN 1975, OIKARI & SOIVIO 1977) and polluted river (Rhine) water (POELS & STRIK 1975) often show a hyperglycemia.

Hematological variables

The effluent caused increased values for hematocrit, hemoglobin content and number of erythroblasts (Figure 3). These elevated values are, however, not statistically significant, when the whole fish groups, i.e. both sexes, are considered. When the sexes are treated separately, the females show a significant increase in hematocrit and hemoglobin content (Table 3), while these hematological variables were unaffected in the males.

The alterations in the red blood cell picture were accompanied by a reduction of the spleen-somatic index, SSI (Figure 3). Thus, a

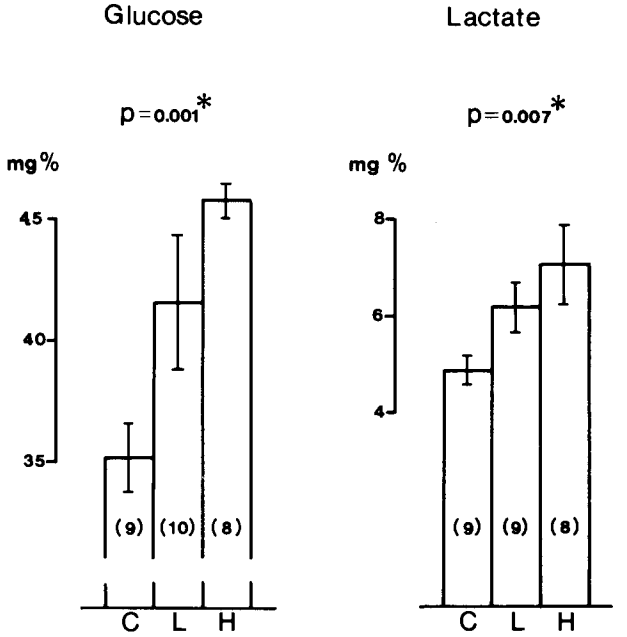

Figure 2. Effects of titanium dioxide industrial effluent on blood glucose and blood lactate in flounders (P.flesus). Symbols as in Fig.1.

probable explanation of the hematological changes is that the exposed fish compensate for an impaired oxygen uptake (due to a disturbed gill function) by a release of erythrocytes from the spleen. However, also severe stress can cause a similar polycythemic effect. Such a state of stress is associated with an increased secretion of catecholamines (NAKANO & TOMLINSON 1967, NILSSON et al. 1976), which have been shown to cause a polycythemia by contracting and partly emptying the spleen on the supply of red blood cells (LARSSON 1973).

The fish exposed to the effluent showed a slightly decreased number of lymphocytes (Figure 3). Such a lymphopenia is a common finding in severe stress situations in mammals (SELYE 1950) and other vertebrates BENNET & JOHNSON 1973, BENNET & SEVILLE 1975). In teleosts, it has been shown that stress (e.g. industrial effluents) causes a decreased number of circulating lymphocytes (McLEAY 1975). These alterations are probably mediated by an increased release of corticosteroids (McLEAY 1973, JOHANSSON-SJÖBECK et al. 1978). It is suspected that a stress-induced lymphopenia is a main contributing factor in the decreased disease resistance of fish in stress situations (WEDEMEYER 1970, SNIESZKO 1974).

Hematological effects of titanium dioxide effluent on female flounders (P.flesus). Values are mean ± S.E. Number of fish in parenthesis.

	Control	Low dose	High dose	P value
Hematocrit (%)	14.9 ± 0.6 (6)	20.2 ± 2.3 (6)	21.0 ± 0.9 (5)	< 0.02
Hemoglobin (g/100 ml)	4.1 ± 0.2 (6)	5.2 ± 0.6 (6)	5.5 ± 0.3 (5)	< 0.04

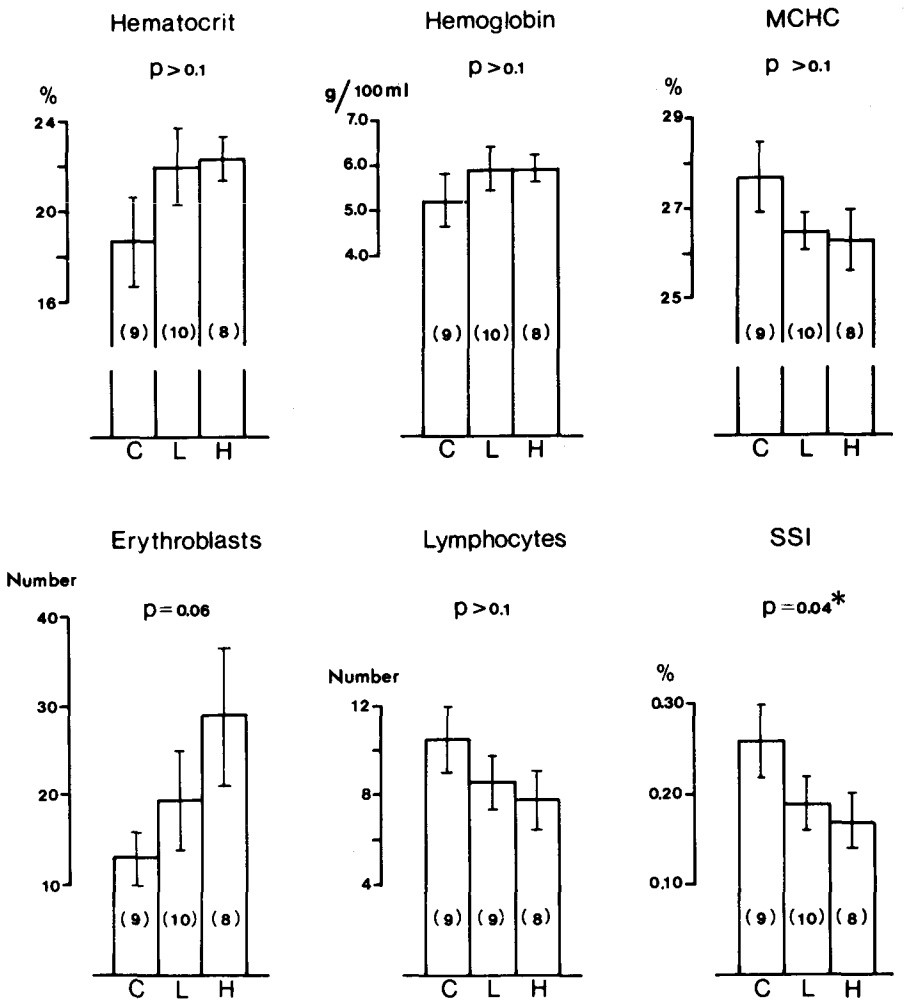

Figure 3. Effects of titanium dioxide industrial effluent on hematological variables in flounders (P.flesus). Erythroblast and lymphocyte counts are expressed as total number per 1000 erythrocytes. Symbols as in Fig.1.

CONCLUSIONS

The present work shows that clinic-chemical methods seem to be very suitable in controlled laboratory experiments in order to detect sublethal disturbances of industrial effluents on fish. The results indicate that the diluted titanium dioxide industrial effluent causes marked effects on the ion balance, the carbohydrate metabolism and the hematology in fish. A direct effect of heavy metals in solution is not probable as the concentrations of dissolved metals are relatively low. A more plausible explanation is that the observed disturbances are results of strictly mechanical action of the precipitate on the gill tissue and/or a disturbed gill function caused by biochemical action of heavy metals present in the precipitate. In addition, some of the noted responses (hyperglycemia, hyperlactemia and polycythemia) may also be attributed to a general stress syndrome due to the exposure. Altogether the registered disturbances certainly reduce the condition of the fish. This may be a contributing reason for the previous observation that the present effluent causes an impaired capacity of fish to manage in the rotatory flow test (LEHTINEN 1980).

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