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CHANGES IN HAEMATOLOGICAL CHARACTERISTICS OF THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALB.) REARED IN THE MIXTURE OF NATURAL AND OIL-SHALE MINE DRAINAGE WATER

Abstract. Rainbow trouts (*Oncorhynchus mykiss* Walb.), reared in the mixture of natural and oil-shale mine drainage water for half a year, were analyzed haematologically and morphologically. The frequencies of micronucleated and dissolving erythrocytes were measured. Haematological characteristics were strongly unbalanced, showing blood dehydration, hyperglycemia and low levels of neutrophils. The frequencies of micronucleated erythrocytes were 30 times elevated as compared to the control ones. The frequencies of dissolving erythrocytes (indicating haemolysis) were in a correlation with gonadosomatic indices showing a possible chronical toxic effect on fish.

Introduction

During a conflagration in the oil-shale mine "Estonia" at the turn of 1988/1989, large quantities of phenols were carried by flowing waters into the northern part of Lake Peipus as well as into the Narva River and the Gulf of Finland. In order to estimate the quality of drainage waters from the oil-shale mine after the incident, several investigations were carried out, including physico-chemical (Тенно, Кооритс, 1991), microbiological (Хейнару et al., 1991) and algological (Кукк et al., 1991) ones. The present study considers the haematological, morphological and genotoxic data on the rainbow trouts (*Oncorhynchus mykiss* Walb.) reared in the mixture of mining water from the mine "Estonia" and natural water during half a year (May—November). Three-year-old rainbow trouts were reared in a separate corf in the river of Pungerja, 2 km downstream from the outfall of the water from the mine.

Material and methods

5 female and 5 male rainbow trouts (*Oncorhynchus mykiss* Walb.) were analysed on the spot (at Sõrumäe) on Nov. 29, 1990, in order to avoid damage from transportation. At that time the individuals were physically in a good condition, biting normally, although water temperature was essentially lower ($t = 2.8$ °C, oxygen approximately 10 mg/l, pH = 7.8).

Morphological measurements were made using Pravdin's (Правдин, 1966) instructions for Salmonids. The Smitt and standard lengths, total weight, weight without internal organs were measured. Condition indices were calculated according to Fulton. Hepatosomatic, heart, spleen and gonadosomatic indices were calculated as percentages of weight without internal organs. Maturity stages of gonads were determined.

Blood samples for haematological analyses were taken at 15 °C within 5 min after capture from the heart, using a heparinized syringe. Blood smears were prepared immediately, air dried, fixed in ethanol for 15 min,

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and stained with Giemsa for 20 min. Blood samples (1 ml) were centrifuged within 30 min after collection during 10 min at 3000 rpm, the plasma was stored at −20 °C until assayed. Leucocrit (Lt) and hematocrit (Ht) values (McLeay, Gordon, 1980) were measured within 5 min after centrifugation. In order to measure haemoglobin (Hb) (Миансарян, Полосян, 1982), red blood cells were lysed with 0.04% NH_4OH and measured photometrically with a haemoglobinometer (Гф-3). Red blood cell count (RBCC) was taken by means of a conductometric haemocytometer (ГЦПК-3). Glucose was determined using standard KIT (БИОЛАР). Chloride concentrations were determined with KIT (LACHEMA, Cl photo); a flame photometer (ПАЖ-1) was employed for determining sodium concentrations. The total serum protein was determined using the Biuret method. Numbers of lymphocytes (Lym) and neutrophiles (Neu) were counted from blood smears under a light microscope (magnification 900×) and expressed in percentages from all leucocytes. Micronucleated (MN) and dissolving (DE) erythrocytes were counted by a single observer from coded blood smears. The frequencies of MN (Hose et al., 1988) and DE were expressed in presentages from all erythrocytes (10,000 erythrocytes counted from each fish). All the dissolving erythrocytes counted had cell membranes; shaded cells were excluded from count.

To determine morphological and haematological deviations, the characteristics were compared with those of the rainbow trouts reared in several Estonian fish hatcheries. The values of glucose, total protein, haemoglobin, hematocrit and numbers of erythrocytes, lymphocytes and neutrophiles were compared with normative values for healthy rainbow trouts (Осетров, 1978). As the frequencies of micronucleated and dissolving erythrocytes were observed for the first time in Estonia, and as different fish species reared in the same conditions may have different MN frequency levels (Hose et al., 1988), 12 rainbow trouts from the Põlva Fish Hatchery were analysed, using the above described method, in order to obtain control data.

In order to clarify deviation trends, all characteristics were compared using Correlation Matrix calculation, and sexual differencies were determined using Two-Sample Analysis.

Results

No indication was found of the existence of chronic diseases in the habit or in the morphology of the internal organs of the rainbow trouts reared in the mixture of mining and natural water. Morphologically, these rainbow trouts had smaller head indices (mean 18.5±0.1%, average of Estonian fish hatcheries 19.8±0.1%), while their condition indices were higher (mean 1.51±0.01, average of Estonian fish hatcheries 1.29±0.02). It was typical for the female individuals ($N=5$) that eggs in the gonads were nearly mature; their livers were significantly ($P<0.01$) heavier (3.17±0.16%) than those of the males (1.87±0.08%). Sexual differences were not expressed by heart (♀ 0.19±0.03%; ♂ 0.21±0.01%; $P>0.1$) or spleen (♀ 0.1±0.05%; ♂ 0.15±0.04%; $P>0.1$) indices.

The haematological analysis of the mixture-reared rainbow trouts showed higher levels of haemoglobin, hematocrit, total protein and glucose, and lower levels of Cl^- and Na^+ (Fig. 1). The White Cell Formula was strongly distorted, showing great numbers of lymphocytes and small numbers of neutrophiles. The only sexual difference among haematological data was in the total protein level (♀ 10.7±1.8 g%; ♂ 7.6±0.7 g%; $P<0.01$).

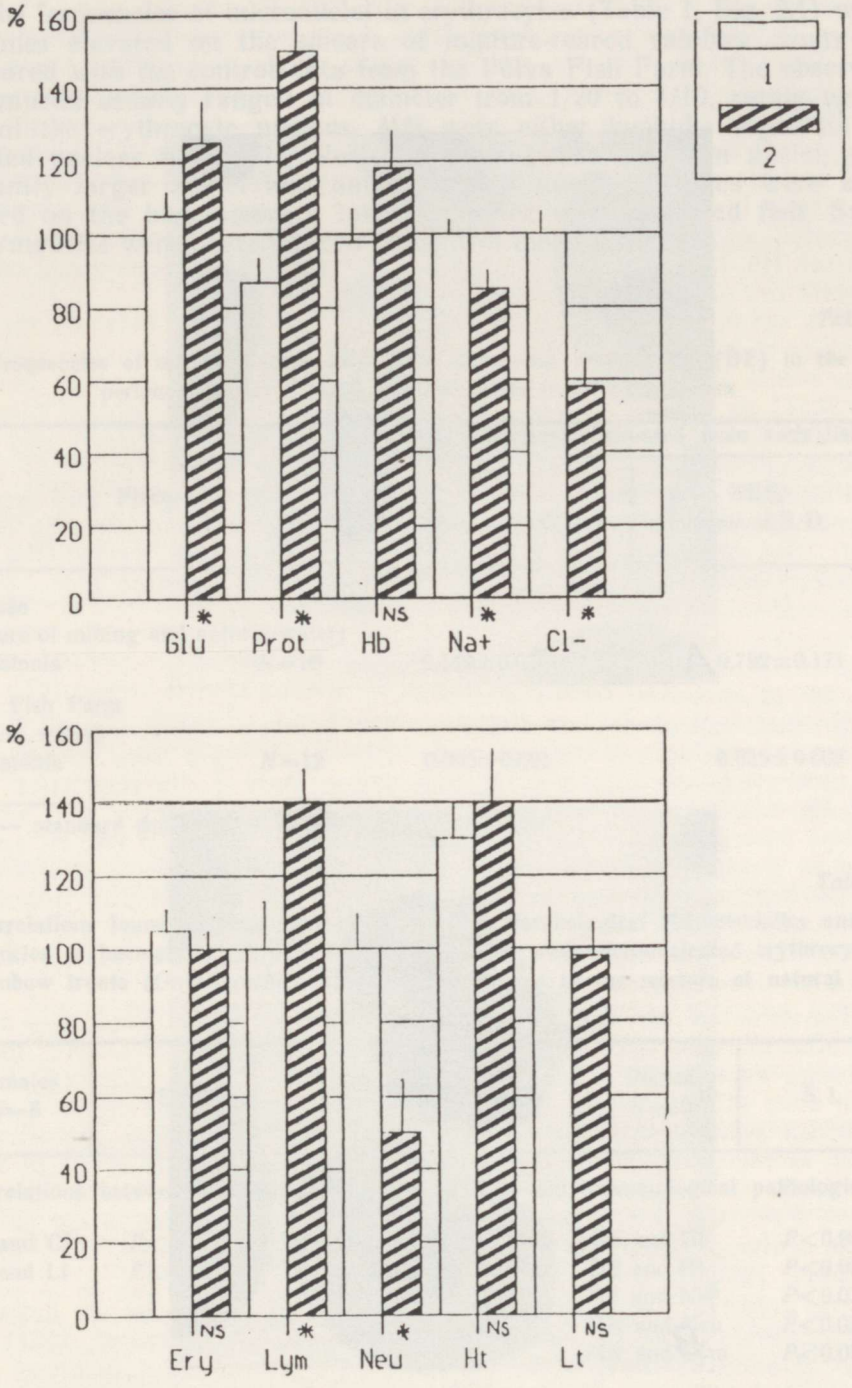
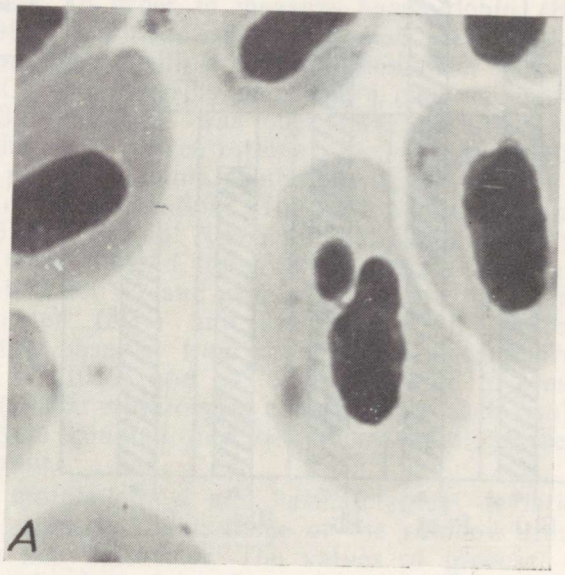


Fig. 1. Haematological characteristics of rainbow trouts reared in the mixture of mining and natural water as compared to those normal for healthy rainbow trouts.
1 — rainbow trouts reared in artesian well water as control, *2* — rainbow trouts reared in the mixture of natural and mining waters. 100% for Glu, Prot, Hb, Ht, Ery, Lym and Neu are taken from Осетров, 1978, and 100% for Lt, Na^+, Cl^- are taken from Тувикене, 1989. Values are mean ± standard deviation. * — Significantly different from control value ($P<0.05$); NS — not significantly different from control value.



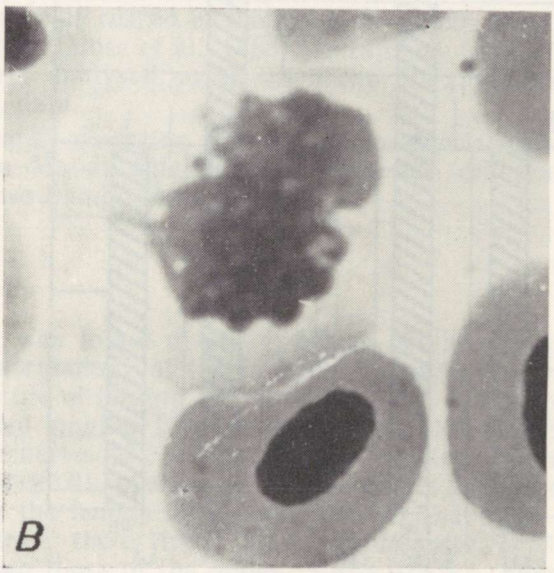


Fig. 2. Pathologies found in the smears of the perpheral blood of rainbow trouts reared in the mixture of mining and natural waters — micronucleated erythrocyte (*A*) and dissolving erythrocyte (*B*).

The frequencies of micronuclei in erythrocytes (Table 1, Fig. 2*A*) were 30 times elevated on the smears of mixture-reared rainbow trouts as compared with the control data from the Põlva Fish Farm. The observed micronuclei usually ranged in diameter from 1/20 to 1/10, rarely up to 1/3, of the erythrocyte nucleus. MN were either knoblike segments or isolated nuclear fragments. Nuclear pleomorphism, holes in nuclei, significantly larger nuclei and amitoses with nuclear bridges were also noticed on the blood smears taken from the mixture-reared fish. Such abnormalities were never noticed on control blood smears.

Table 1

Frequencies of micronucleated (MN) and dissolving erythrocytes (DE) in the peripheral blood of rainbow trouts reared in different waters

Place		10,000 erythrocytes counted from each fish	
		MN% Mean ±S. D.	DE% Mean ±S. D.
Sõrumäe (mixture of mining and natural water) NE Estonia	$N=10$	0.149±0.030	0.792±0.171
Põlva Fish Farm (natural water) SE Estonia	$N=12$	0.005±0.001	0.035±0.002

S. D. — standard deviation, N — number of individuals.

Table 2

Correlations found between haematological and morphological characteristics and frequencies of haematological pathologies (dissolving and micronucleated erythrocytes) of rainbow trouts (*Oncorhynchus mykiss* Walb.) reared in the mixture of natural and drainage water

Females $N=5$	S. l.	Males $N=5$	S. l.	Pooled $N=10$	S. l.
Correlations between haematological characteristics and haematological pathologies					
MN and Cl^-	$P<0.05$	DE and Hb	$P<0.05$	DE and Hb	$P<0.05$
MN and Lt	$P<0.01$	DE and Ht	$P<0.01$	DE and Ht	$P<0.05$
		DE and Lt	$P<0.05$	DE and Na^+	$P<0.07$
				MN and Neu	$P<0.05$
				MN and Lym	$P<0.05$
Correlations between morphological characteristics and haematological pathologies					
DE and HI	$P<0.01$	DE and HI	$P<0.07$	DE and GI	$P<0.05$
				MN and HI	$P<0.07$

MN — frequencies of micronucleated erythrocytes; DE — frequencies of dissolving erythrocytes; Hb — haemoglobin concentrations; Ht — hematocrit values; Lt — leucocrit values; Neu — neutrophile counts; HI — hepatosomatic indices; GI — gonadosomatic indices; N — number of individuals and S. l. — significance level.

Dissolving erythrocyte (Table 1, Fig. 2*B*) frequencies were 23 times higher in the blood smears taken from mixture-reared fish in comparison with those taken from the rainbow trouts of the Põlva Fish Farm. The difference in the frequencies of dissolving erythrocytes was rather great, ranging from 0% up to 1.8%.

The correlations found between erythrocyte pathologies (micronucleated and dissolving erythrocytes), morphological and haematological data are shown in Table 2. No plausible correlation was found between micronucleated and dissolving erythrocyte frequencies. Plausible correlations between Hb, Ht and dissolving erythrocyte frequencies (pooled and males) suggest the association of these parameters.

The sexual difference is observable in correlations between the frequencies of micronucleated erythrocytes. Males had no plausible correlations, while females had plausible correlations between MN and Lt ($P<0.01$) as well as between MN and Cl^- ($P<0.05$). In pooled calculations MN had plausible correlations with neutrophile ($P<0.05$) and lymphocyte ($P<0.05$) counts.

Plausible correlations between erythrocyte pathologies and hepatosomatic indices suggest the importance of the detoxifying capacity of the liver. The correlation between dissolving erythrocyte frequencies and gonadosomatic indices may prove the chronic toxic effect on fish.

Discussion

Haematological characteristics of the rainbow trouts reared in the mixture of mining and natural water differed strongly from normal data for rainbow trouts reared in natural water. Only 4 characteristics (Hb, Ery, Ht, Lt) out of 10 were normal or deviated only slightly (not significantly) from normal. Although being normal, the values of Hb, Ht and Lt were in a plausible correlation with dissolving erythrocyte frequencies. The lack of any correlation between erythrocyte counts and dissolving erythrocyte frequencies proves that perished erythrocytes are replaced by normal ones from deposits (spleen), indicating that haemopoietic organs could not have been strongly damaged.

The maximal concentration of two toxicants, Cd^{2+} and NH_4^+, was above the allowed one year value for fish (Alabaster, Lloyd, 1980) in the drainage water from the oil-shale mine "Estonia" (Тенно, Кооритс, 1991). Even after mine drainage water blended with natural water, as a result of which contamination with toxicants should have been significantly lower in the place where the fish were reared, the toxic effect of these chemicals (Cd^{2+} and NH_4^+) can appear, as supported by other toxicants (Zn^{2+}, Pb^{2+}, Cu^{2+}) present in the water, and by the fluctuating salinity of the drainage water (Brown et al., 1969; Alabaster, Lloyd, 1980; Тенно, Кооритс, 1991).

The dissolving of erythrocytes itself may be caused by an increased contamination of water with hemolytics like phenol (Linhardt, 1951) and NH_4^+ (Danecker, 1964) or by the elevated levels of NH_4^+ in blood after a sublethal influence of a number of various toxicants including Cd^{2+}, phenols and oil (Лукьяненко, 1983).

Decreased levels of Na^+ and Cl^- in peripheral blood, indicating blood dehydratation, appear in the case of chloride cell misfunctions (Матей, 1991), but also in the case of chronical stress (Wedemeyer, McLeay, 1981). High levels of glucose in peripheral blood (hyperglucemia) are caused by disorders in hydrocarbon metabolism appearing in the conditions of physical or chemical stress conditions (Wedemeyer, McLeay, 1981). High numbers of lymphocytes associated with low numbers of neutrophiles

($P<0.01$ in our study) has been observed in Cd^{2+} polluted perch (Larsson et al., 1986).

Elevated frequencies of micronucleated erythrocytes compared to control data show a clear genotoxic effect of the environment (Hose et al., 1988). Whether this situation is common in the whole industrial area of NE Estonia should be established in further investigations.

It would be necessary to carry out more detailed analysis in order to determine whether the factors causing changes in haematological parameters can affect the reproductivity of fish.

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MUUTUSED PÕLEVKIVIKAEVANDUSE KUIVENDUSVETE JA LOODUSLIKE VETE SEGUS KASVATATUD VIKERFORELLIDE (*ONCORHYNCHUS MYKISS* WALB.) VERES

Pool aastat põlevkivi kaevandamisvete ja looduslike vete segus kasvatatud vikerforelle (*Oncorhynchus mykiss* Walb.) on analüüsitud morfoloogiliselt ja hematoloogiliselt. Analüüsitud kalade veri oli tugevate kõrvalekalletega normist. Seda näitavad vere dehüdratatsioon, hüperglükeemia ja madal neutrofiilide arv. Mikrotuumadega erütrotsüütide hulk oli kontrolliga võrreldes 30 korda tõusnud. Lagunevate erütrotsüütide esinemissageduse seos gonadosomaatilise indeksiga võib näidata toksilise mõju pikaajalisust kaladele.

Ану ПАЛЬМ, Арво ТУВИКЕНЕ, Теет КРАУЗЕ

ИЗМЕНЕНИЯ ГЕМАТОЛОГИЧЕСКИХ ДАННЫХ У РАДУЖНОЙ ФОРЕЛИ (*ONCORHYNCHUS MYKISS* WALB.), ВЫРАЩЕННОЙ В СМЕСИ ПРИРОДНОЙ И ШАХТНОЙ ВОДЫ

Выращенная в смеси природной и шахтной воды радужная форель (*Oncorhynchus mykiss* Walb.) изучалась морфологическими и гематологическими методами. Установлено, что гематологические показатели сильно отклонились от нормы, выражая гидролиз крови, гипергликемию и низкое содержание нейтрофилов. Эритроцитов с микроядрами в периферической крови было в 30 раз больше, чем в крови контрольного варианта. Корреляция между частотой разрушающихся эритроцитов и гонадосоматическими индексами может свидетельствовать о продолжительности токсического влияния на рыбу.