Risk assessment and risk decrease of contamination of waters with blood in slaughter industry. Part 1: Risk assessment

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Abstract: The purpose of this work is to establish the influence of different concentrations of animal blood on some basic water indexes - quantity of dissolved oxygen, pH, transparency, chemical oxygen demand and change of general nitrogen content. The paper presents the experimental results and gives assessment of the degree of criticality of contamination of waters.

Key words: waters, animal blood, contamination.

Introduction

Characteristic contaminants of waters are inorganic salts, acids and bases, radioactive substances, dissolved organic substances and other substances, usually emitted from production. A specific contaminant is blood from slaughter industry. It is rich in basic biogenic substances – proteins, fats and carbohydrates. When these substances enter the water reservoirs, putrefactive processes occur under the influence of microflora. The medium becomes anaerobic and the quantity of dissolved oxygen decreases. There is a change also in the pH-value and the transparency of water; ammonia and hydrogen sulphide are formed and they affect the flora and the fauna.

This research aims to establish the criticality of contamination of waters with animal blood. The basic tasks of the research, related to its aim, are:

- to establish the influence of blood on some basic quality specifications of water quantity of dissolved oxygen, pH, transparency, chemical oxygen demand and general nitrogen content, by which the general protein is calculated;
 - to determine the differential risk of contamination on bio-objects.

Materials and Method

In this research we used fresh stabilized slaughter blood from large ruminants. It was added to drink water, which specifications were determined in advance.

We studied the change of:

• the quantity of dissolved oxygen:

Animal blood was added to drink water. The quantity of blood was in the range from 0 to 60 g/l water with step 2 g. Concentration of dissolved oxygen was measured every time when the blood concentration was increased. We used oxymeter OXI 91-WTW to determine the oxygen concentration. The medium was continuously stirred by means of laboratory homogenizer. The measuring range of oxygen was from 0 to 199 % saturation or from 0 to 50 mg/l. When measuring in percentage, the error is less than 1 %, and when measuring in mg/l – less than 0.1 mg/l.

The admissible value of dissolved oxygen is 4 mg/l for water reservoirs of second category, used for fish breeding, water sports, cultural needs, drinking pool, etc. [Anonym., 2000]

• pH of water:

We used the potentiometric method of measuring.

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The admissible pH value is 6.0-8.5 for water reservoirs of second category (Anonym.,

• transparency of water when adding blood:

To determine the transparency of water, we used the method of reading of the standard Snellen chart. We used the device of the same name.

• chemical oxygen demand:

We applied the biochromatic method of measuring. In sulphur-acid medium, the potassium biochromate oxidizes the reducing agents, which are contained in water. The excessive potassium biochromate is titrated with a solution of ammonium ferrosulphate with known concentration with a ferroine indicator. We use Ag₂SO₄ to catalyze oxidation.

The admissible value of COD is 70 mg/l (Anonym., 2000).

• concentration of general nitrogen:

We used the Keldal's method. The method involves wet incineration of nitrogencontaining products with concentrated sulphuric acid at high temperature in the presence of a catalyst to obtain carbon dioxide, water and ammonia, which together with sulphuric acid give ammonium sulphate. Under the influence of sodium hydroxide the ammonium sulphate forms ammonia, which is trapped by a specified volume of excessive sulphuric acid with known concentration and titration of the excessive acid with a hydroxide.

All experiments were repeated three times, for each experiment we used different sample of drink water from the Ruse region.

The Risk is evaluated through:

- admissible values of water specifications;
- mortality Pmort of bioindicators. The bioindicators for each experiment were 60 small carps with weight 10-14 grams.

To establish the common influence of two controllable factors on the dissolved oxygen, we made planned experiments, during which in addition to the quantity of added blood M, we changed the temperature T of the medium on three levels -10, 20, 30°C and the alkaline reaction pH respectively to 6, 7 and 8.

Bioindication of criticality was made by variation of the degree of water contamination with blood and the period of stay – exposure of bioindicators in the contaminated water. In order to assess the influence of contamination, we calculated the mortality rate of bioindicators. We used planned experiment B2 (Tomov, 2003; Tomov & Vladimirov, 2005). The remaining indicators were controlled – general nitrogen, COD, pH and water temperature. Contamination with blood and exposure were changed on three levels.

Result and Discussion

When water is contaminated with animal blood, dissolved oxygen decreases lineally (Figure 1). With initial blood content 6.8-7.6mg/l in clean drink water, dissolved oxygen decreases when blood concentration increases. When the blood concentration is 60 g/l the dissolved oxygen is 0.63-0,86mg/l. When the blood concentration is above 28 g/l the dissolved oxygen drops below the admissible value of 4 mg/l.

To approximate the experimental results, we used the SPSS software. With this software we derived the following regression model of dissolved oxygen C, mg/l:

$$C=7.35-0.1174M$$
 (1)

where M is blood concentration in water, g/l.

During all experiments made with different initial concentration of dissolved oxygen, straight lines were formed, as the one on figure 1, parallel to one another. The coefficient in front of the concentration M (Eq. 1) indicates the quantity of molecular oxygen, which is absorbed by 11 g blood. It remains constant and the free member is changed.

When blood concentration is increasing, water transparency S is decreasing more than 10 times (Figure 2a). It affects the penetration of light and has negative influence on biological processes and photosynthesis.

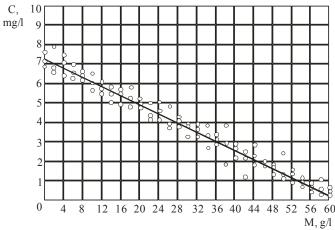


Figure 1. Variation of dissolved oxygen C depending on blood concentration M

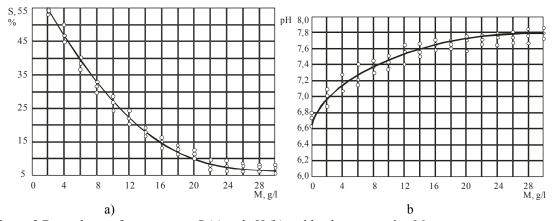


Figure 2.Dependence of transparency S (a) and pH (b) on blood concentration M

Figure 2b shows pH variation. From initial value 6.6-6.8 it rises to 7.8, but nevertheless it remains within the admissible limits for water reservoirs of second category. When pH rises above 7.0 the ammonium cations in water are transformed into ammonia, which is very toxic to fish.

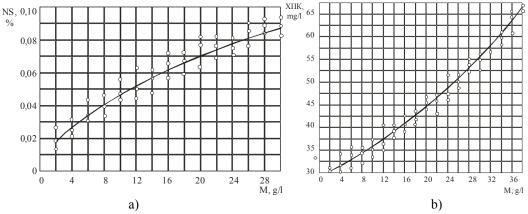


Figure 3. Dependence of general nitrogen NS (a) and the chemical oxygen demand (b) on blood concentration M

Figure 3a shows the tendency of change of general nitrogen. It is evident that general nitrogen in water changes almost proportionally with the increase of the quantity of added blood and increases from 0 to 0.088.

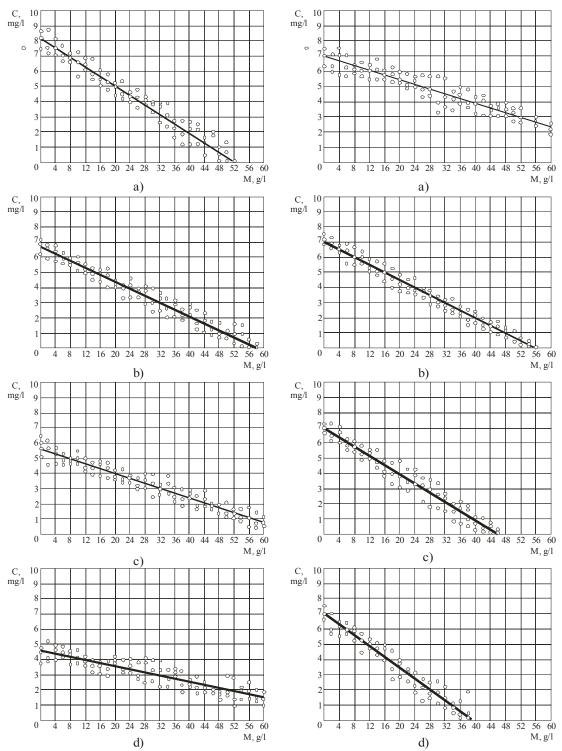


Figure 4. Dependence of dissolved oxygen C on blood concentration M at water temperature 10°C (a), 20°C (b), 30°C (c) μ 40°C (d)

Figure 5. Dependence of dissolved oxygen C on blood concentration M at pH= 6.3 (a), pH=6.8 (b), pH=7 (c) μ pH=7.7 (d)

The chemical oxygen demand increases from 31 mg/l at blood concentration 2 g/l to over 68 mg/l at blood concentration 38 g/l.

By means of the above software we obtained models of variation of the other studied water specifications as a result of contamination with animal blood:

$$S=66.2635-5.4380M+0.1653M^2-0.0017M^3; (2)$$

$$pH=6.,6626+0,1214M-0.,0045M^2+5.,8.10^{-5}M^3;$$
(3)

$$NS=0.0110M^{0.6129};$$

$$COD=26.2095+1,1507M-0.0190M^2+0.004M^3.$$
(4)

Water temperature has strong influence on the dissolved oxygen. At temperature $10\,^{\circ}\text{C}$ (Figure 4a) the dissolved oxygen falls below the admissible value, which happens at blood concentration 25-26 g/l, at 20 $^{\circ}\text{C}$ - 22-23 g/l (Figure 4b), 30 $^{\circ}\text{C}$ - 20-21 g/l (Figure 4c) and 40 $^{\circ}\text{C}$ -8-9 g/l (Figure 4d). Therefore when temperature increases the admissible value of dissolved oxygen is exceeded at lower values of blood concentration.

Based on the research, namely measuring of variation of the concentration of dissolved oxygen in water when adding blood at different temperatures, we established that the quantity of absorbed oxygen from 1 gram blood at one and the same temperature does not depend on the initial oxygen concentrations. However this quantity is different at different temperatures. Relationship between concentration of dissolved oxygen in water and the quantity of contaminating blood at different temperatures is given on fig. 4. It is evident that when temperature increases the slope of the curves becomes smaller. It means that in case of equal initial oxygen concentrations, the quantity of blood to bind the oxygen will be less at lower temperature than at higher temperature.

Relationship between angle and temperature is inversely proportional. When temperature increases, the angle, respectively the coefficient in front of M decreases, which means that the ability of blood to bind the dissolved oxygen decreases too. The explanation is that the process of binding the haemoglobin to oxygen is reversible. At a specified temperature a dynamic equilibrium is established between oxygen, haemoglobin and oxyhaemoglobin. When temperature increases the stability of oxyhaemoglobin decreases and the equilibrium goes to decomposition to haemoglobin and oxygen. When temperature decreases the equilibrium goes to formation of oxyhaemoglobin.

Research was made to establish the influence of variation of the intitial pH of water samples and the ability of blood to bind the dissolved oxygen. We determined the quantity of dissolved oxygen at different pH and one and the same initial quantity of dissolved oxygen. Results are given on Figure 5, where you can see the strongly expressed influence of active reaction. When alkalinity increases, drop of the quantity of dissolved oxygen below the admissible value occurs at lower blood concentrations in water.

We carried out planned bi-factor experiments.

Blood concentration M was a controlled factor in both experiments. It was changed on three levels 10, 25 and 40g/l. For the first experiment the water temperature was changed from 10 °C to 30 °C at step 10 °C, and for the second experiment the alkaline reaction was from 6 to 8 at step 1. Experiments were carried out according to plan B2. After processing the results with the software for multifactor regression analysis we obtained the following models for dissolved oxygen content C:

$$C=3.10072-2.40239 \text{ M}-0.23320T+0.47500MT+0.09316 M}^2+0.09316 T^2;$$
(6)

$$C=3.40082-2.56352 \text{ M}-0.72118\text{PH}+0.08687\text{M}^2+0.33679 \text{ pH}^2.$$
 (7)

In order to determine borders of variation of controlling factors when biotesting the criticality, we studied the influence of the time of exposure Tv. Experiments were made with constant controllable factors – general nitrogen NS=0.07-0.09%, COD =45-66mg/l, pH=7 and T=20°C. The time of exposure Tv of the selected bioindicators was changed from 0.5 to 4h. Water was contaminated on three levels: M=28 g/l (C=3.4 mg/l); M=36 g/l (C=2.6 mg/l); M=44 g/l (C=1.7 mg/l). Results in variation of mortality rate Pmort of bioindicators are given on Figure 6.

On the basis of these results we planned bi-factor experiments with variation of contamination on the same levels. Exposure was changed on three levels -0.5; 2 and 3h. General nitrogen, COD, pH and T were maintained within the above limits. On Figure 7 you can see the graphic representation of obtained results for average value of Pmort from three experiments.

The data from this experiment were processed with the software for multi-factor regression analysis and the following model of criticality of contamination with blood was obtained:

 $Pmort (M,Tv) = 0.828695 - 0.083582M + 0.530554Tv - 0.011458MTv + + 0.001854M^{2} + 0.000517Tv^{2}.$ (8)

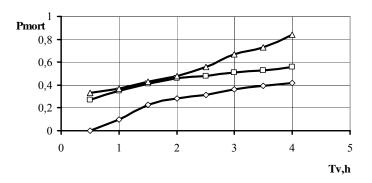


Figure 6. Change of mortality rate Pmort of bioindicators depending on the time Tv of stay in water with blood concentration M=28 g/l (\diamondsuit), M=36 g/l (\square), M=44 g/l (\triangle)

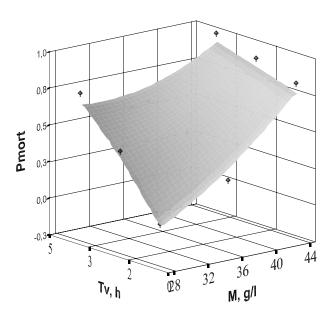


Figure 7. Dependence of the mortality rate Pmort of the bioindicators on the water contamination M and exposure time Tv

Conclusion

As a result of the carried out research, the following conclusions can be made:

• A linear dependence was established (formula 1) between the quantity of dissolved oxygen and the contamination with blood. With the increase of contamination from 2 to 60g/l the dissolved oxygen decreases from 6,8-7,6mg/l to 0,4-0,8mg/l. The coefficient in front of M-contamination with blood remains constant, and the free member is changed for the different water samples;

- Transparency has strong dependence on contamination and within the researched range, it was reduced more than 10 times:
 - The active reaction is within the limits:
- General nitrogen increases 5 times-from 0.015 to 0.088, nevertheless it is within the admissible limits;
- The chemical oxygen demand also increases with the increase of contamination with blood, but remains within the admissible limits;
- Regression models of studied indicators of water quality were obtained-dependences 1-7; In order to establish the degree of criticality of contamination we determined (formula 8) the dependence of the mortality rate of bioindicators-small carps 10-14g on the concentration of animal blood in water and the exposure time. It can be used to forecast dangerous effects and for the first time new information is given for the common influence of two basic factors.

The experimental research was made with samples of drink water. In future it is necessary to use for the experiments water from specific water reservoirs of second category, which we make the obtained results closer to the real ones.

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