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AL-FARABI KAZAKH NATIONAL UNIVERSITY

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GROWTH AND DEVELOPMENT OF THE GREEN TOAD (*BUFO VIRIDIS*) FROM THE WATER BODIES OF THE OIL PRODUCING REGIONS OF KAZAKHSTAN

Environmental pollution with oil and petroleum products leads to a decrease in animal biodiversity and human diseases. Due to the intense pollution of Kazakhstan's water bodies located on the territory of oil producing regions, the purpose of this study was to study the effect of different concentrations of oil on the growth and development of the green toad (Bufo viridis). This species of anuran amphibians is widespread in Kazakhstan, which is especially important given the aridity of the lands of the oil-producing regions. A chronic exposure to the concentrations of oil hydrocarbons found in the water of the reservoirs of the Aktobe, Atyrau and Mangistau regions on the tadpoles of the green toad (Bufo viridis) was carried out The results of the study revealed suppression of growth (size and weight) and a developmental delay in tadpoles from experimental groups. To determine the reasons for the slowdown in growth and development, the content of lipid peroxidation products (LHO and MDA) and the activity of antioxidant defense enzymes in the liver of tadpoles of the green toad (Bufo viridis) were studied. It has been shown that the production of LHO and MDA increases after exposure of the tadpoles to oil hydrocarbons, while the activity of antioxidant enzymes, on the contrary, decreases, which indicates enhanced oxidative stress in the liver of the treated tadpoles. Thus, exposure to oil hydrocarbons in concentrations found in the water bodies of the oil producing regions of Kazakhstan suppresses the growth and development of the green toad (Bufo viridis), one of the reasons of which is the increase of oxidative stress.

Key words: water soluble fraction of oil, Bufo viridis, growth, development, oxidative stress, lipid peroxidation, antioxidant enzymes.

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Қазақстанның мұнай өндейтін өңірлерінің суқоймаларында мекендейтін жасыл құрбақаның (*Bufo viridis*) өсуі және дамуы

Қоршаған ортаның мұнаймен және мұнай өнімдерімен ластануы жануарлардың алуантүрлілігінің азаюы мен адамдардың ауруына әкеледі. Мұнай өндіретін аймақтарда орналасқан Қазақстан суқоймаларының қарқынды ластануына байланысты біздің зерттеуіміздің мақсаты

түрлі мөлшердегі мұнайдың жасыл құрбақаның (Bufo viridis) өсуі мен дамуына әсері. Құйрықсыз амфибияның бұл түрі Қазақстан аумағында кеңінен тараған, бұл әсіресе мұнай өндіретін аймақтардың әсерінің құрғақтығын ескергенде өте маңызды. Ақтөбе, Атырау және Маңғыстау суқоймаларында кездескен концентрацияларының мұнай көмірсутектері арқылы жасыл құрбақа (Bufo viridis) итшабақтарына созылмалы әсер етті. Зерттеулер нәтижесі сынамалық топтағы итшабақтардың даму қарқынының төмендеуі (ұзындығы және салмағы) және өсуінің басылып қалғанын көрсетті. Дамуы және өсуінің баяулауының себебін түсіну мақсатында жасыл құрбақа (Bufo viridis) итшабақтарының бауырындағы липидтердің гидроперекисі және малон диальдегиді (ЛГП және МДА) мен антиоксиданттық қорғау ферменттердің белсенділігі зерттелді. Зерттеулер нәтижесінде ЛГП және МДА өнімдері мұнай көмірсутектерінің әсерінен өседі, ал антиоксиданттық ферменттердің белсенділігі керісінше төмендейді, бұл жағдай зерттелген итшабақтардың бауырындағы оксидативті күйзелістің күшейгенін көрсетеді. Осылайша Қазақстанның мұнай өндіретін аймақтарындағы суқоймаларынан табылған концентрацияларындағы мұнай көмірсутектері жасыл құрбақаның (Bufo viridis) өсуі мен дамуының тежелуіне әкелетін себебі оксидативті күйзелістің өрбуі болып табылады.

Түйін сөздер: мұнайдың суда еритін фракциясы, Bufo viridis, өсуі, дамуы, оксидативті күйзеліс, липидтердің тотықпен қышқылдандыруы, антиоксидантты ферменттер.

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**Рост и развитие зеленой жабы (*Bufo viridis*)
из водоемов нефтедобывающих регионов Казахстана**

Загрязнение окружающей среды нефтью и нефтепродуктами приводит к снижению биоразнообразия животных и заболеваниям человека. В связи с интенсивным загрязнением водоемов Казахстана, находящихся на территории нефтедобывающих регионов, целью настоящего исследования явилось изучение влияния различных концентраций нефти на рост и развитие зеленой жабы (Bufo viridis). Данный вид бесхвостых амфибий широко распространен на территории Казахстана, что особенно важно, учитывая аридность земель нефтедобывающих регионов. Было проведено хроническое воздействие углеводородами нефти на головастиков зеленой жабы (Bufo viridis) в концентрациях, обнаруженных в водоемах Актюбинской, Атырауской и Мангистауской областей. Результаты исследования выявили подавление роста (размер и вес) и снижение темпов развития головастиков в опытных группах. Для выяснения причин замедления роста и развития было изучено содержание продуктов перекисного окисления липидов (ГПЛ и МДА) и активность ферментов антиоксидантной системы защиты в печени головастиков зеленой жабы (Bufo viridis). Было показано, что продукция ГПЛ и МДА повышается при воздействии углеводородами нефти, в то время как активность антиоксидантных ферментов, напротив, снижается, что указывает на усиленный оксидативный стресс в печени исследованных головастиков. Таким образом, воздействие углеводородов нефти в концентрациях, обнаруженных в водоемах нефтедобывающих регионов Казахстана, вызывает подавление роста и развитие зеленой жабы (Bufo viridis), одной из причин которого является усиление оксидативного стресса.

Ключевые слова: водорастворимая фракция нефти, Bufo viridis, рост, развитие, оксидативный стресс, перекисное окисление липидов, антиоксидантные ферменты.

Introduction

Pollution of the environment by oil and oil products is one of the main problems in the world. In Kazakhstan, where oil production is one of the main branches of the economy, vast territories are actively involved in the development of oil and gas fields. Herewith, the bulk of oil and gas producing enterprises are concentrated in the west of the country: in the Aktobe, Atyrau and Mangistau regions. The result of the increased anthropogenic load is the deterioration of the ecological situation in the oil producing regions (Askarova 2014: 456).

The number of animal populations and biodiversity is negatively affected by intensive pollution of the environment with oil and oil

products (Kolesnikov 2011: 19). The most effective method for assessing the response of living organisms to environmental pollution is biological monitoring of terrestrial and aquatic ecosystems, including bioindication and biotesting. Amphibians are particularly sensitive organisms-bioindicators due to the biphasic lifestyle and developmental features, the early stages of which pass in the water, and the later ones are associated with the transition of young individuals to land. (Gutleb 1999: 1-14; NAVFAC 2004: 26). In Kazakhstan, one of the most widespread species of amphibians is the green toad (*Bufo viridis*), since it inhabits a variety of biotopes from the forest-steppe to the desert. This feature is of particular importance for bioindication of the oil-producing regions of the country, since most of the territories where oil production is located belong to the arid zones.

Normal passage of early individual development of amphibians determines the formation of systems and various functions of the organism, defining survival and other qualities of larvae (Simon 2011: 141-145). Many researchers note the relationship between the frequency of teratogenic phenomena and the degree of anthropogenic impact on the environment (Saka 2004: 1065-1073; Melvin 2012: 178-183; Melvin 2013: 22-27; Falfushynska 2015: 172–173; Cheng 2017: 3096-3102). Due to their low level of tolerance the amphibian larvae are extremely susceptible to low concentrations of chemical compounds. The impact of these substances is expressed not only in the appearance of developmental defects, but also in the modification of a number of cytological, morphophysiological and biochemical parameters (Salin 2012: 864; Salvaterra 2013: 191). The response to the toxic effects of xenobiotics is based on oxidative stress (Falfushinska 2008: 1100). In conditions of increased oxidative stress or enhanced formation of active forms of oxygen, the functioning of the enzymes of the antioxidant system and, as a consequence, the formation and accumulation of oxidative damages may occur, which accompanies a number of pathophysiological phenomena (Pigeolet 1990: 285). The use of change in enzyme activity of the antioxidant system as biomarkers helps to confirm the effects of anthropogenic action at the biochemical level (Venturino 2005: 338). For example, it was shown that morphological disturbances accompanied by changes in the work of the antioxidant system are observed in tadpoles exposed to petroleum products (Amaeze 2014: 4256; Wu 2017: 103).

Thus, the purpose of this work was to study the growth and development of the green toad (*Bufo viridis*) from various water bodies of the oil producing regions of Kazakhstan.

Materials and methods

Collection of material was carried out during expeditions to the Temir river (Aktobe region), Uil river (Atyrau region) and the coast of the Caspian Sea (Mangistau region). Here, the eggs of the green toad (*Bufo viridis*) were collected, the water parameters (temperature, pH, dissolved oxygen content) were measured and water samples were taken from the collection sites for further chemical analysis. The eggs were delivered to the laboratory and placed separately in accordance with the reservoir from which it was taken in 50 L aquariums with pure aerated dechlorinated water with a temperature maintained within 21-25°C. The eggs were incubated for 5 days under the conditions described until the hatching of tadpoles. Next, after tadpoles reached Gosner stage 25 they were moved to 18 L aquaria (15 tadpoles per each), filled with 15 liters of pure aerated declorinated water. The tadpoles were divided into 4 groups, each consisting of 45 tadpoles in total: 1) control (pure water); 2) Temir river (Aktobe region); 3) Uil river (Atyrau region); 4) the coast of the Caspian Sea (Mangistau region). Tadpoles were fed daily with boiled lettuces and aquarium fish food *ad libitum*, and excess food and feces were removed every day. To determine the content of oil hydrocarbons in water from the studied water bodies, the water samples were analyzed using the method of gas chromatography-mass spectrometry (GC/MS) in accordance with GOST 31953-2012 (GOST 31953-2012: 1-18). Water-soluble fraction of crude oil (WSFO) was added into the aquaria with *B. viridis* tadpoles at the concentrations corresponding to the results of GC/MS for each natural reservoir (Temir river, Uil river, Caspian sea). Every 2 days following 80% water change the corresponding concentration of the WSFO was added into aquaria. The preparation of the water-soluble oil fraction was carried out according to Anderson J.W. et al. (Anderson 1974: 75-88) taking into account the recommendations of Singer et al. (Singer 2000: 1007-1016).

The weighing and morphometric measurements of *B. viridis* tadpoles were carried out on the first day of the experiment and every 2 weeks thereafter until the tadpoles reached metamorphic climax, when all 4 limbs appear and metamorphosis begins (Gosner stage 42). The tadpoles that reached the Gosner stage 42 were euthanized in a buffered solution of MS-222 (3-aminobenzoic acid ethyl

ester, Sigma-Aldrich), weighed, photographed using a stereoscopic microscope Motic DM 143 (China) to measure the morphometric parameters, and the liver was sampled for further biochemical research.

In the liver of tadpoles, the content of lipid hydroperoxides was determined by a method based on measuring the light absorption of conjugated diene structures of lipid hydroperoxides in the 232-234 nm region (Gavrilov 1983: 23-25). The content of malonic dialdehyde was determined by the method with thiobarbituric acid (Pradnya 2006: 262-265). The activity of superoxide dismutase (SOD) was determined by measuring the red-formazan precipitation reaction during the reduction of nitroblue tetrazolium and superoxide radicals generated by xanthine oxidase (Guengerich 1994: 1259-1313). The catalase activity was determined by the method of H. Luck (Guengerich 1994: 1259-1313).

Results and discussion

The results of the chemical analysis of water samples from the reservoirs in which the eggs were collected by GC/MS are shown in table 1.

Table 1 – Results of quantitative determination of oil concentrations in water samples

№	Sample	Concentration of total oil content in water samples, mg/L
1	Tengiz river (Aktobe region)	0,52
2	Uil river (Atyrau region)	0,38
3	Caspian sea coast (Mangistau region)	1,07

As can be seen from the table 1, the greatest content of oil hydrocarbons (HC) was found in water samples taken from the coastal sections of the Caspian Sea. It should be noted that in all three reservoirs, an excess of maximum permitted concentration (MPC) of petroleum products in water used for fishery purposes (0.05 mg / l) (Obobschennyi perechen' predel'no dopustimyh koncentracii 1990: 11) was detected. The concentration of petroleum products exceeded the MPC by 10-fold In the Tengiz river, 7-fold in the Uil river, and 21-fold in the Caspian Sea.

To assess the effect of oil hydrocarbons on the growth and development of *B. viridis*, the following parameters were studied: the change in full body length, weight, and rate of development (development time from stage 25 to stage 42) of tadpoles during a chronic experiment.

The results of measurements of the full body length and weight of *B. viridis* tadpoles are shown in figure 1 and 2. Analysis of the change in full body length during 3 months of chronic exposure to WSFO showed that the presence of oil hydrocarbons in water suppresses the growth of *B. viridis* tadpoles. Herewith, the tadpoles that had the smallest body length at the end of the experiment were those exposed to WSFO in the concentration found in the coastal waters of the Caspian Sea (1.07 mg/L): the tadpoles were 1.4-fold smaller ($p < 0.01$) than the those of control group. Similarly, the weight of the body of tadpoles exposed to different concentrations of oil HC (0.38 mg/L, 0.52 mg/L, and 1.07 mg/L) was 1.3-fold, 1.8-fold and 2.1-fold lower, respectively ($p < 0.01$).

The developmental rate was assessed according to the time required for *B. viridis* tadpoles to undergo developmental stages from Gosner 25 (free swimming and feeding stage), to stage 42 (metamorphic climax, onset of metamorphosis) (figure 3). As shown in figure 3, tadpoles of experimental groups developed more slowly than those in the control group. The tadpoles of the control groups passed the stages 25-42 for 82±3 days, while tadpoles exposed to 0.38 mg/L, 0.52 mg/L and 1.07 mg/ L, required 97±5, 105±4, 116±6 days, respectively.

The decrease in the rate of development, accompanied by the suppression of the growth of amphibian tadpoles, has been noted by many researchers (Eriyamremu 2008: 284-290; Brunelli 2009: 135-142; Gürkan 2015: 153-163). This reaction is caused by intoxication with heavy metals, pesticides, petroleum hydrocarbons and other xenobiotics. Thus, disruption of growth and development of amphibian tadpoles is an important indicator of environmental pollution. However, it is necessary to identify the cause of such violations.

To identify the causes of suppression of growth and development, the content of primary and secondary LPO products, as well as the state of the antioxidant defense system in the liver of *B. viridis* tadpoles were studied (figures 4 and 5). As can be seen from fig. 4, in the liver of *B. viridis* tadpoles exposed to oil hydrocarbons, enhanced production of lipid hydroperoxides (LHO) and malonic dialdehyde (MDA) occurs. The content of LHO in the liver of tadpoles

exposed to WSFO at concentrations of 0.38 mg/L, 0.52 mg/L, and 1.07 mg/L was 17%, 44% and 80% higher, respectively ($p<0.01$). The level of MDA in the liver of tadpoles exposed to the same corncentrations was also increased by 23%, 36% and 68%, respectively ($p<0.01$).

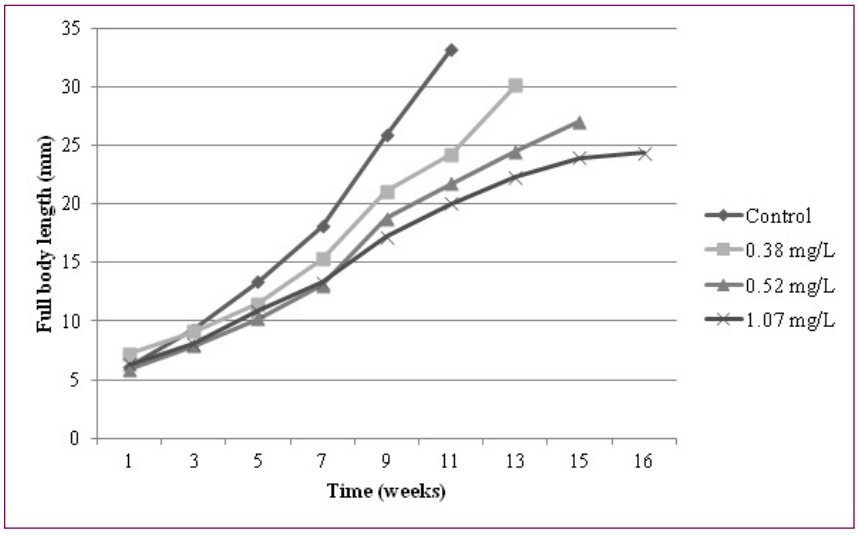

Figure 1 – Change in full body length of *B. viridis* tadpoles during chronic exposure to different concentrations of the WSFO. All data presented are statistically significantly differ from control ($p<0.01$)

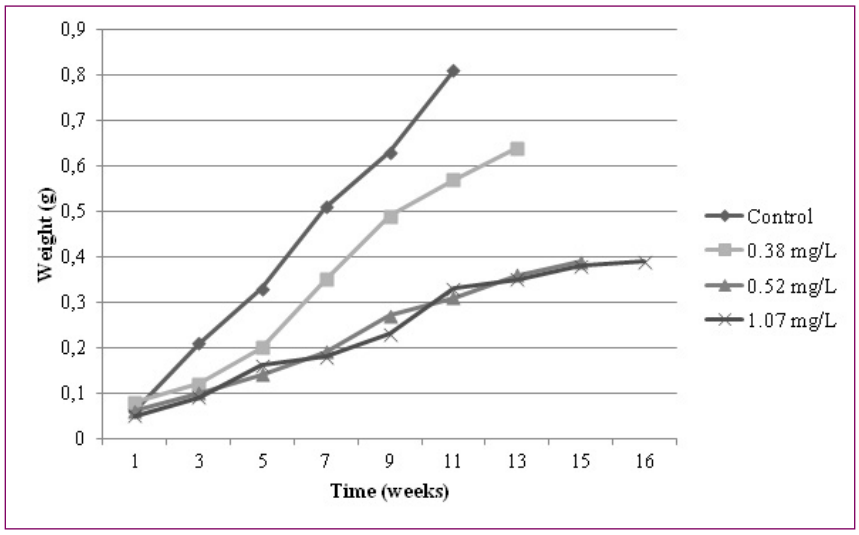

Figure 2 – Change in body weight of *B. viridis* tadpoles during chronic exposure to different concentrations of the WSFO. All data presented are statistically significantly differ from control ($p<0.01$)

The level of LPO processes is normally regulated by endogenous antioxidants and the activity of antioxidant enzymes. In case of insufficient protection, the process of peroxidation becomes uncontrolled, and leads to the accumulation of reactive oxygen species and free radicals and the development of pathological processes in cells, primarily the destruction of internal membranes (Wu 2017: 102).

The state of the antioxidant system of tadpoles of *B. viridis* after 3 months of exposure to different concentrations of WSFO was assessed by the

activity of key antioxidant enzymes, superoxide dismutase and catalase in the liver (figure 5). Superoxide dismutase performs inactivation of oxygen radicals, which can arise during biological reactions of electron transport or under the action of metals with variable valence, toxic substances, and radiation. Catalase is a heme enzyme that catalyzes the hydrogen peroxide decomposition reaction, with the formation of water and molecular oxygen (Eriyamremu 2008: 285).

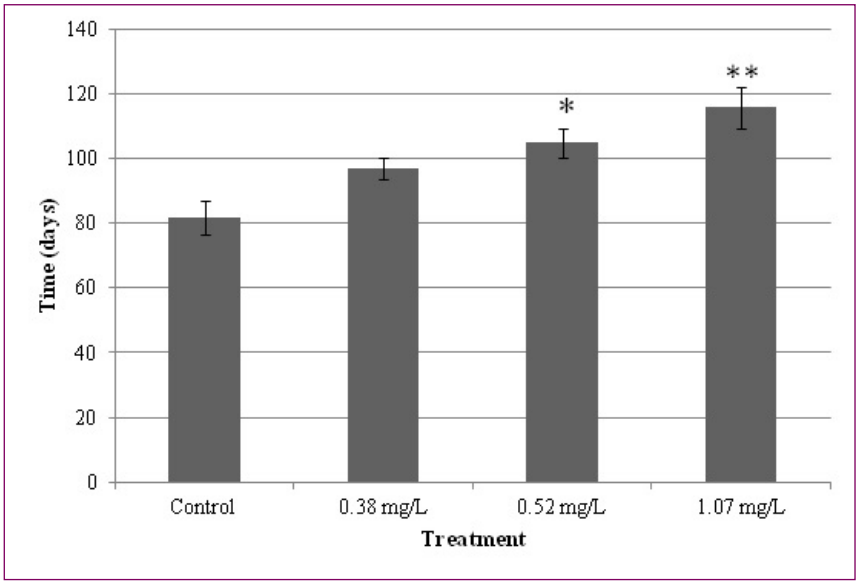

Figure 3 – The time of development of *B. viridis* tadpoles from Gosner stage 25 to stage 42 during chronic exposure to various concentrations of the WSFO. *P≤0,05; **P≤0,01

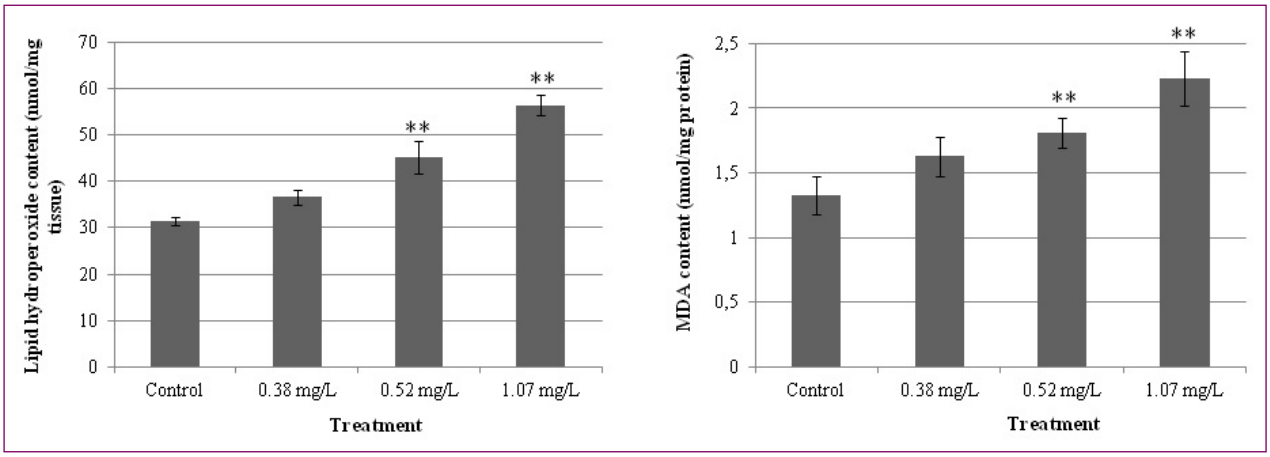

Figure 4 – The content of LHO and MDA in the liver of *B. viridis* tadpoles after chronic exposure to various concentrations of the WSFO. **P≤0,01

The results of biochemical analysis of SOD activity showed a decrease in the activity of this enzyme in the liver of *B. viridis* tadpoles exposed to the three concentrations of WSFO (0.38 mg/L, 0.52 mg/L and 1.07 mg/L) by 17%($p<0.05$), 29% ($p<0.01$) and 43% ($p<0.01$), respectively. The activity of CAT was also reduced by 23% ($p<0.05$), 33%($p<0.01$) and 49% ($p<0.01$) after chronic exposure to 0.38 mg/L, 0.52 mg/L and 1.07 mg/L of WSFO, respectively.

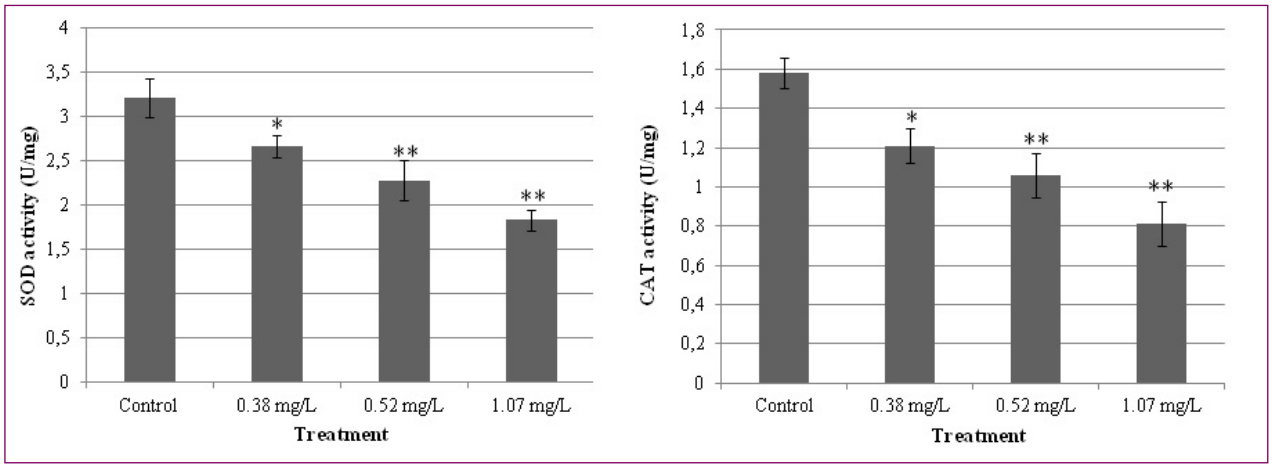

Figure 5 – Активность СОД и КАТ в печени головастиков *B. viridis* после 3 месяцев воздействия различных концентраций ВРФН *P≤0,05; **P≤0,01

One of the reasons for the suppression of the antioxidant defense system (decrease in the activity of SOD and catalase) in *B. viridis* tadpoles may be the accumulation of reactive oxygen species and toxic peroxide products (lipid peroxides, aldehydes, ketones and other products) as a result of the toxic effect of oil HC. In addition, a high level of LPO products and a decreased activity of antioxidant enzymes may indicate the damage and death of liver cells (Costa 2008: 160; Burraco 2016: 471). It should be noted that in the study of the size, weight and growth rate, as well as the biochemical parameters of the liver of the *B. viridis* tadpoles, the dose-dependent character of the disruptions of the investigated endpoints was observed. This may indicate a correlation between the changes in the biochemical functions of the cells and the external morphological manifestations observed in our study.

Conclusion

Thus, it was shown that the chronic impact of crude oil hydrocarbons in the concentrations found in the water bodies of the oil producing regions of Kazakhstan adversely affects the developmental rate, and size and weight of *B. viridis* tadpoles. The increased oxidative stress can be a cause of suppression of growth and development, as evidenced by increased accumulation of lipid peroxidation products and reduced activity of antioxidant defense enzymes in the livers of the tadpoles studied.

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МАЗМҰНЫ–СОДЕРЖАНИЕ

Шолу мақалалары – Обзорные статьи

1-бөлім Қоршаған ортаны қорғау және қоршаған ортаға антропогендік факторлардың әсері

Раздел 1 Воздействие на окружающую среду антропогенных факторов и защита окружающей среды

2-бөлім Қоршаған орта ластаушыларының биотаға және тұрғындар денсаулығына әсерін бағалау

Раздел 2 Оценка действия загрязнителей окружащей среды на биоту и здоровье населения

3-бөлім Биологиялық алуантүрлілікті сақтаудың өзекті мәселелері

Раздел 3 Актуальные проблемы сохранения биологического разнообразия