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Z.D Kenzhin, A.B Kerimkulova, A.B Bigalyev
Department of Molecular Biology and Genetics, Faculty of Biology and Biotechnology,
al-Farabi Kazakh National University, Almaty, Kazakhstan

The principles of methods in determining level of bioindication of damaged ecosystems

Abstract

This paper describes a bioindicator methodic for the northern part of Caspian Sea - *Abramis sapa* P. (Bream white eye), individuals who were caught and fixed in 10% formalin at a time in the area near Atyrau city Balykshy settlement in August 2011. It is shown that the specie *Abramis sapa* P., can be used as an indicator species for the determination of environmental pollution by oil products and heavy metals. These, studies have shown that the specie *Abramis sapa* P., can be used as an indicator species for environmental pollution by oil products and heavy metals.

Keywords: bioindicator, *Abramis sapa* P., environmental pollution.

Introduction

In the short term development of oil and gas deposits on the continental shelf of the Caspian (Kashagan) may dramatically increase the environmental burden not only on the Kazakhstan part of Caspian Sea region, but throughout the waters of the central and northern part of the Caspian Sea. The most alarming cases with emergency release of crude oil, during which the NO_x content in some areas reaches 15 mg/m³ and above that is 176 times greater than the MCL (0.085 mg/m³) Research areas Caspian oil fields showed that the main source of pollution are oil contained in the waste of man-made fishing waters, and heavy metals coming from the man-made water from wells. Oil content of technogenic waters ranges from 52 to 71.5 mg/l, and the territory of the oil fields in the soil samples ranged from 5.4 to 1.2 mg/kg [1, 2].

One of the most common and dangerous for biota associated petroleum contaminants are heavy metals, from which the cadmium, lead, copper and zinc are attributed to a number of priorities. Heavy metals pollute at the same time as the water environment and soils of the coastal zone. Getting together with petroleum in the aquatic environment, and then into the soil,

heavy metals accumulate inhabitants of data media and cause several adverse effects.

In this paper we describe the quality aim was widespread in the North Caspian Sea and Ural River bream-lime nodules - *Abramis sapa* P., and analyze the ability of aquatic organisms to accumulate heavy metals and petroleum [3].

Materials and methods

In experiments using object environment - fish *Abramis sapa* P., was prepared sample in R. 1g of organs and tissues for the decomposition of organic material each sample was poured 2 ml of concentrated perchloric acid, 3 mL of concentrated nitric acid and placed in a dry-flame thermostat for 4-6 hours. Determination of heavy metals in biological materials was performed by atomic adsorption spectrophotometer AAS 1N. Calculations of the metal content was carried out by conventional methods [4].

To determine the benzo (a) pyrene sampled gills, kidneys, gonads and myshschy. Sample preparation was carried out by the usual method [3]. Hexane extraction method used substances; the measurement was carried out on chromatograph "LHM-80." For histological analysis of tissue preparations by conventional methods and stained with hematoxylin and eosin. Analysis was performed visually, with increasing

*Corresponding author: A.B. Bigalyev, e-mail:aitkhazha@gmail.com

x 200. Analyzed at different magnification on a light microscope DX 500.

Results and discussion

As specie - indicators we have chosen the type of fish *Abramis sapa* P.- Bream white eye (Figure 1). White eye is common in rivers flowing into the Black Sea to the north, as well as in the Volga. In the basins of the Caspian and Aral seas white eye is formed by several forms. We selected fish species *Abramis sapa* (Pallas, 1811) refers to the order of carp fish to the carp family, genus bream. The body of lime nodules - laterally compressed, more elongated in length than its sister bream - *Abramis brama*. Silver-gray coloration, the fins are grayish, with dark edges. Eyes are large (up to 30% of head length) with a silvery-white iris (hence the name). Mature white eye is a 4 or 5 years of age with a body length of 18-22 cm and weight 100-200 g, while males mature a year earlier than usual female [5].

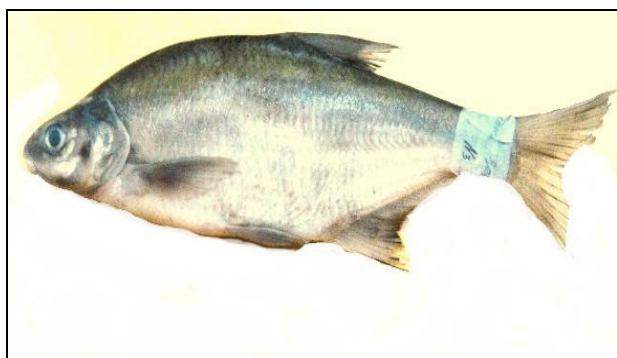


Figure 1 - *Abramis sapa* P.

Age was determined visually by the number of annual rings in scales and spine bone (figure 2) [6]. As the table shows the analyzed specimens population as females and males reach reproductive age, which in this species

begins in the fourth year of life. In a sample of the population were five females and eight males who were captured at one time in the near Atyrau Balykshy in August 2011. Captured specimens were fixed in 10% formalin and subsequent analytical procedures were performed in the Kazakh National University in Almaty.

To determine nutrition security in the study area calculated condition factor fish bream lime nodules by the method of Fulton and Clark [6].

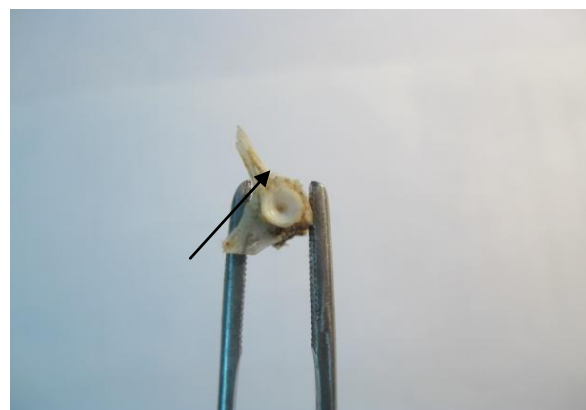
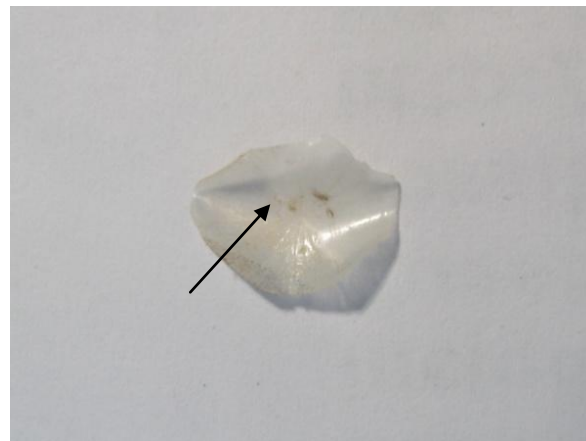


Figure 2 - Spine bone and fish scale *Abramis sapa* P. for age determination (→ three rings).

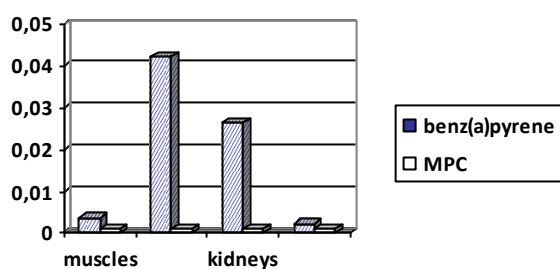
As it seen from Table 1, condition factor of males and females of the analyzed specimens statistically different from the table, which indicates that their excess weight. Indicator of nutritional status and provision ensuring are not the only factors in Fulton and Clark, but the difference between them. In our case, the last increase compared with the tabulated data - a result of amplification of power and accumulation of cavity fat, which we found at the opening of the fish.

Table 1 - Indicator of nutritional status and age of fish

The average fatness by Fulton		The average fatness by Clark		For fatness the table		The average age of individuals in a population	
♀	♂	♀	♂	♀	♂	♀	♂
2,36	2,06	1,70	1,69	1,72	1,52	4	4,1

Perhaps the reason for this is the increased organic pollution, where the dominant role belongs to the petroleum habitat; to test the extended position determined the content of a petroleum derivative, namely, benzo(a)pyrene in tissues and organs of bream. The choice of benzo(a)pyrene is due to the fact that it is an indicator of oil pollution and carcinogenic, that is a factor of environmental stress.

The content of carcinogen determined in muscle, gonads, kidneys and gills by gas-liquid chromatography (Figure 3). The action of xenobiotics on fish is manifested primarily in the pathological changes in liver and gills [7].

**Figure 3** - Content of benzo (a) pyrene in indicator species organs, mg/kg.

When comparing the concentration of benzo(a)pyrene in the bodies of *Abramis sapa* P., MAC was calculated from the multiplicity of the excess. For comparison, we used the limit values for meat and fish, which is equal to 0.001 mg/kg. Of all the analyzed organs and tissues of the greatest cumulative properties of benzo (a) pyrene found gonads (multiple MPC excess of 42 times). The second place on the same index is the kidney, then muscle and gills. All organs accumulated benzo(a)pyrene above the norm. In order to detect the influence of a carcinogen at the tissue level, histological analysis was performed gills and liver.

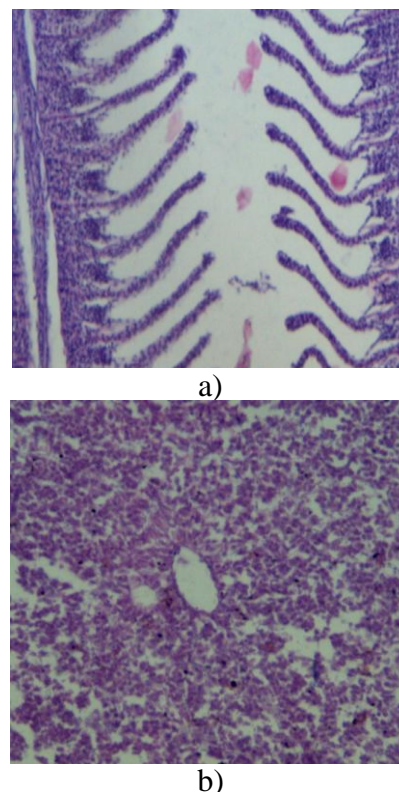
The liver is known to provide the biotransformation and toxic (for PAH) toxic substances and is therefore the first target of the action of organic pollutants. The gills are also damaged, as the body directly in contact with water containing xenobiotics.

Thus, under light microscopy of internal organs *Abramis sapa* P., in the liver and gills

were marked pathological changes, the nature and severity of which indicate chronic toxic effects on fish (Figure 4).

Microscopic examinations of internal organs *Abramis sapa* P., were observed changes in liver and gills of fish. In the liver, circulatory disturbances observed bed, per vascular edema and swelling of the stroma (Figure 4). In the liver parenchyma observed phenomena micro vesicular statuses and necrosis.

In the field of view marked hepatocytes with strongly eosinophilic cytoplasm and nucleus piknotichnym, while there is moderate infiltration, predominantly lymphocytic cell number, activation of resident macrophages. Such changes are characteristic of fish liver in chronic effects of heavy metals, the development of fatty liver in fish indicate internal damage to cell membranes as a result of uncontrolled under the influence of organic (mainly chlorinated and aromatic) compounds.

**Figure 4** - Histostructure of gills and liver of (a) (b) *Abramis sapa* P., coloring by hematoxylin-eosin. Microscope magnification x200.

The structures of the gills are marked less pronounced changes (Figure 3). In many petals gill epithelium retained the typical structure. Outside, the lamellae are covered with double-layered secondary gill epithelium; respiratory cells formed a flattened shape. However, microscopic examination revealed pathological changes in the form of elimination of lamellae, epithelial tissue from the basal plate, necrosis of respiratory cells, and infiltration of leukocytes. Such changes in the microstructure is characterized by the gills when exposed to a number of pollutants, especially polycyclic aromatic hydrocarbons and heavy metals [7].

In the same organs of bream, which revealed the accumulation of polycyclic aromatic hydrocarbons benzo(a)pyrene, analyzed the content of eight heavy metals (Table 3).

Data for heavy metals in excess of the limit values for the multiplicity of food presented to histogram (Figure 5-7). As it can be seen from the histogram of Pb and Cd content higher than the MPC in 12 and 2 times respectively, and the excess copper is observed only in the liver. These elements are highly toxic to living organisms and are 1-hazard class (Figure 5).

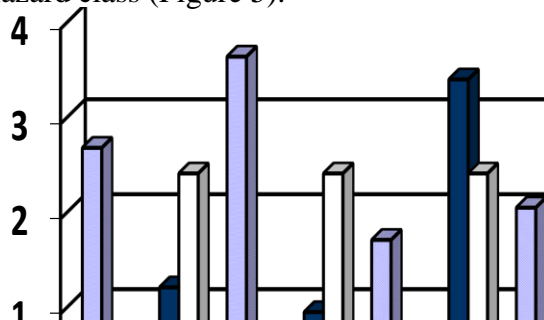


Figure 5 - Content of heavy metals (Pb, Cd, Cu) in organs of Abramis sapa P., mg/kg.

The nickel content in all organs of the form of the indicator exceeds the MCL, but his maximum accumulation is observed in the gills, which accumulates and other heavy metals - Co (cobalt) (Figure 6).

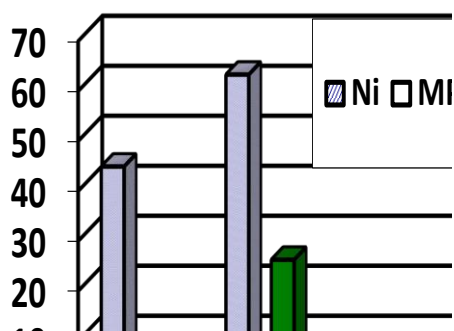


Figure 6 - Content of heavy metals (Ni, Co) in organs of Abramis sapa P., mg/kg.

Of zinc (Zn) and manganese (Mn) in all studied organs of the form of the indicator is much higher than the MAC, and of iron a clear excess is observed only in the gills (Figure 7).

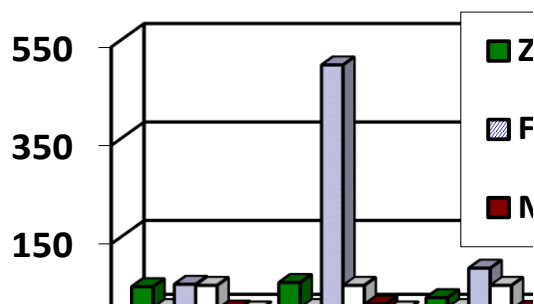


Figure 7 - Content of heavy metals (Zn, Fe, Mn) in organs of Abramis sapa P., mg/kg.

These, studies have shown that the species Abramis sapa P., can be used as an indicator species for environmental pollution by oil products and heavy metals. Was found high levels of accumulation of petroleum-derived carcinogen - benzo (a) pyrene in the gonads, kidneys and small - in the gills and muscles. Histological analysis showed pathological changes in the gills of eliminations in the lamellae of epithelial tissue, and liver revealed fatty degeneration.

Oil discharges are accompanied by heavy metal pollution, since it was discovered accumulation of eight heavy metals in the form of indicator. Seven of the eight metals differentially accumulate in the gonads, muscle, liver and gills, and only the contents of eight metals - copper does not exceed the MCL in all studied organs except the liver. The highest amounts of metals found in the gills, then the muscles. A significant excess of the limit values for manganese (Mn) and zinc (Zn) found in the gonads. Thus, we can talk about the target organs for heavy metals in the form of the indicator, Abramis sapa P.

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