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Comparative analysis of benzo(a)pyrene accumulative ability in the organs of aquatic organisms living at the coastal zone of Northern and Central Caspian Sea areas of Kazakhstan

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Abstract

The aim of the study was the accumulative ability of the oil product benzo(a)pyrene in organs and tissues of fish species widespread in several Northern and Central Caspian Seas areas Abramis sapa P., Sander lucioperca L and Central Neogobius melanostomus P., Rutilus rutilus caspicus Y. This research focuses on these species because they are the main biomarkers of water in the study area and have considerable analytical value for the condition of its environment as it relates to benzo(a)pyrene. This work studies benzo(a)pyrene accumulation level in somatic and reproductive cells and organs of the presented fish species which are widely distributed at several biologically significant sites of the Caspian Sea, using the standard method of high performance gas-liquid chromatography (HPGLC). The presence of the studied chemical element in fish organs also demonstrated its accumulative effect in two different areas. The content of benzo(a)pyrene in the waters of the Northern Caspian Sea and the Ural River and benzo(a)pyrene's migration capability are also highlighted in this study. One of the main results of the work was the determination of positive correlation between the levels and frequency of accumulation of the presented chemical in tissue organs of the investigated species in two separate sites of Northern and Central areas of the Caspian Sea.

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Introduction

The Kazakhstan part of the Caspian Sea, particularly its Northern and Central areas, has been studied by many researchers (Bigaliev, 2009) for environmental monitoring of environmental objects for several years. They have also studied these parts which have more than 64% of the main hydrocarbon reserves or eight milliard ton of the extractive oil reserves. The main center of the petrochemical industry in Kazakhstan is the Caspian Region that includes the Atyrau and Mangistau areas, as well as adjacent parts of the Caspian Sea water area.

Polycyclic aromatic hydrocarbons (PAH) are a group of hydrophobic organic pollutants placed in the bottom sediments and biota of aquatic ecosystems including Caspian Region of Kazakhstan. Benzo(a)pyrene is a type of PAH classified as one of the top pollutants and is selected on the basis of its expected teratogenicity or acute toxicity on aquatic organisms (Alam and Tanaka, 2002). The ability of benzo(a)pyrene to make contact with organic substances, including body of most living facilities, and its further ability for bio concentration and migration in the food chain has important toxicological implications for animals, including aquatic organisms and fishes (Penry and Weston 1998). As benzo(a)pyrene always presents in PAH samples and being the main component, it is serving as the main criterion and special attention of the environmental risk assessment for the marine ecosystem monitoring (Haritash and Kaushik, 2009).
From the available data, it is safe to conclude that there is significant concentrations of benzo(a)pyrene in the North and Central Caspian Sea; this fact is confirmed by previous studies (Zhanburshin, 2005), where in similar areas of the monitoring study was significant migration activity of B(a)P in surface waters and sediments (Neff, 1984). As the previous results show (Gennadiev *et al.*,2000), contamination by benzo(a)pyrene as a specific xenobiotic formed during production, processing and transportation of oil is an extremely urgent environmental problem in many places and especially for the Caspian Sea, of Kazakhstan. Insufficient knowledge migration of Benzo(a)pyrene under the Caspian Sea and it's accumulating feature caused the main focus and aim of this study. The next aim was a poorly understood component of the migration and accumulation of the benzo(a)pyrene in the bodies of marine fish living in the described region. Fish as organisms capable of accumulation of benzo (a) pyrene in the conditions of the Caspian Sea, are mentioned for the first time. That is also able to use them as the main bio-indicators in the development of the principles of model organisms in assessing migratory activity of benzo(a)pyrene in a marine environment. For this reason were selected widely-distributed species of fish - Abramis sapa P, Sander lucioperca L, Neogobius melanostomus P, Rutilus rutilus caspicus Y, belonging to the families of Cyprinidae and Perciformes. They are endemic to the study area; they spawn, breed, live actively and migrate over substantial distances in appropriate ecological niches and habitats.

Materials and methods

Sample preparation and analysis

Several individuals of fish belonging to Abramis sapa P, Sander lucioperca L, Neogobius melanostomus P., Rutilus rutilus caspicus Y., were cached in five points of biological object sample in the investigated area near city Atyrau in the North Caspian region (47 ° 6'4.53" N, 51 ° 54'48.99" E), (46 ° 53'46.64" N, 51 ° 38'12.96" E) and city Aktau (47 ° 6'4.53" N, 51 ° 54'48.99" E), (43 ° 37'0.77" N, 51 ° 11'41.27" E), in the coastal zone of Central Caspian Sea. These were brought to the laboratory and studied by the method of morphometric analysis. The sampling was conducted between 10th of July and 20th of August 2011-2013 year.

Determining benzo(a)pyrene in organic samples

Evaluation of benzo(a)pyrene concentration was done with common methods of the benzo(a)pyrene allocation in the following form:

Individuals of fish were seized and their organs and

tissues—liver, gonads, kidney, gill and muscle tissue extracted. The liver is the most sensitive organ in any toxic effects and reactions and muscle tissue covers a large part of the living organism and has close contacts with a variety of external factors. Each organ was weighed based on the 1 g and subsequently prepared for future analysis. The individual fish in the control group were taken from identical species contained in the aquarium in clean tap water without adding any contaminants and impurities.

Test bodies of fish were placed in a paper filter pre-dried and finely ground. Then the prepared sample was flooded with the necessary quantity of demitilhloramide in an amount of 45 ml and subjected to an extraction process by extraction in a Soxhlet apparatus for 30 min (Berthou and Friocourt, 1981). The resulting liquid was subsequently placed in a rotary evaporator where it was exposed to partial evaporation until the residue in a small amount-0.7ml, more fluid sample- was transferred to 1.0ml flask and held at the metering contents of benzo(a)pyrene with gas-liquid chromatography. The instrument was calibrated and prepared using a mixed standard solution. The system was washed using distilled water for one minute before the start of the analysis of each sample.

Determining benzo(a)pyrene in water

Determination of the contamination of water sample component was done through chromatographic analysis of one of the calibration solutions for further chromatographic analysis of the prepared sample. For the reliability of measurements, a chromatographic analysis of the calibration solution and the prepared sample was performed at least two times in a row.

For the obtaining of results, two parallel measurements as well as two chromatograms were provided. For the result measurements, middle arithmetic meaning of benzo(a)pyrene contamination in analytical sample concentrate $\mathbf{C_{ch}}$,mkg/l (counting from two meaning of benzo(a)pyrene mass concentration in analytical sample concentrate) was taken.

Mass benzo(a)pyrene concentration in sample analysis (in original sample) **X**, mkg/l is calculated by the formula:

where:

$$X = \frac{Cch \times V2}{Cspe\ extract \times V1} \times 1000$$

$\mathbf{V_1}$- water sample value in ml, chosen for the concentration.

$\mathbf{V_2}$ – final sample value – ml.

$\mathbf{C_{SPE\ extract}}$– coefficient of solid phase benzo(a)pyrene extraction is equal to 0.95.

$\mathbf{C_{ch}}$ - middle meaning from parallel measurements of benzo(a)pyrene concentration in chromatographic analysis (from chromatograph), mkg/l.

Statistical analysis

The normality of variables was evaluated using T-test for independent samples by groups. The statistical differences between the damage observed in the contamination of benzo(a)pyrene in sea water and main fish organs and also between control and experimental test groups were carried out using the non-parametric Mann-Whitney (for independent samples). Correlations between different variables were determined by Spearman rank correlation test. The critical level for rejection of null hypothesis was considered to be a P value of 5%, two-tailed. All statistical counting were made using the program CSS Statistica 8.0 (Stat Soft Inc., 2007).

Results

The results, as presented in Table 1 and Table 2, indicate a very definite accumulative process of benzo(a)pyrene in groups Aktau, Atyrau and settlement Makhambet. The following results of research analysis in identification of the benzo(a)pyrene contamination and accumulation level lead us to suppose the permeation of this element in tissues of water organisms and participation in metabolism processes in 4 discovered

fish species.

The excess of the MPC in the analyzed organs, groups Atyrau, according to a high degree in the kidneys and gonads: 56.8 - 41.7 MPC, - in specie Sander lucioperca L., 57,9-52,3 MPC- specie Abramis sapa P., which can cause some concern and fears about their manytime growth, because it is known that the sex glands involved in the reproductive process of aquatic fish, evidence raises concerns about the fact that in addition to the carcinogenic, benzo(a)pyrene has also mutagenic, embryotoxic and haematological effects. In the gills, muscle and liver level of maximum permissible concentration is expressed in numerical sequence, muscle 3.6 MPC, in the gills 4.4 times the MPC, in the liver of 2.9 MPC, specie Sander lucioperca L., from 3.1 to 5.2 times and the muscle and gills 2.3 MPC liver species Abramis sapa P., that can judge the lesser role in participating in the adsorption process liver benzo(a)pyrene.

Table 1. Benz(a)pyrene contamination ability in organs of fish groups in points Atyrau and Aktau. Significance in relation to non-exposed controls at P<0.05 (Mann-Whitney test).

Sander lucioperca L	n	Atyrau M±SD	Neogobius Melanostomus P	n	Aktau M±SD
Gills	10	0.044±0.029	Gills	10	0.046 ±0.021
Muscle tissue	10	0.036±0.019	Muscle tissue	10	0.042±0.023
Liver	10	0.029 ±0.015	Liver	10	0.032±0.019
Gonads	10	0.0417 ± 0.0297	Gonads	10	0.0327 ±0.0236
Kidneys	10	0.0568±0.0384	Kidneys	10	0.0473±0.0315
Abramis sapa P	n	Atyrau M±SD	Rutilus rutilus caspicus Y	n	Aktau M±SD
Gills	10	0.022±0.010	Gills	10	0.025±0.014
Muscle tissue	10	0.031±0.020	Muscle tissue	10	0.037±0.028
Liver	10	0.023±0.012	Liver	10	0.018 ±0.07
Gonads	10	0.0523±0.0257	Gonads	10	0.0551±0.0377
Kidneys	10	0.0579±0.0388	Kidneys	10	0.0427±0.0284
Sander lucioperca L, (control)	n	M±SD	Cyprinus carpio carpio L(control)	n	M±SD
Gills	10	0.0002±0.0008	Gills	10	0.0001±0.0001
Muscle tissue	10	** not found	Muscle tissue	10	0.00010±0.0003
Liver	10	0.0004±0.0002	Liver	10	0.00015±0.0007
Gonads	10	0.0009±0.0005	Gonads	10	0.00012±0.0008
Kidneys	10	** not found	Kidneys	10	0.0005±0.0003
Abramis sapa P (control)	n	M±SD	Neogobius kessleri G(control)	n	M±SD
Gills	10	0.0003±0.0001	Gills	10	0.0006±0.0002
Muscle tissue	10	0.0002±0.0001	Muscle tissue	10	0.0003±0.0001
Liver	10	0.0005±0.0002	Liver	10	0.00015±0.0007
Gonads	10	0.0002±0.0001	Gonads	10	0.0004 ±0.0001
Kidneys	10	0.0004±0.0001	Kidneys	10	** not found

Atyrau, as a form of Sander Lucioperca L., fluctuated slight 0.2 MPC in the gills, whereas in other organs, muscles, gonads, liver and kidneys, the content of B (a) P has not been revealed. Type Abramis Sapa P., indicating the excess of MPC at 0.2 to 0.3 times in the muscle and gills 0.4 to 0.5 times the kidneys and the liver.

The excess of the MPC, the group also noted Mahambet at a very similar level and is characterized by the excess of the permissible value in the kidneys and gonads from 59.3 to 40.5 MPC from 4.3 to 5 MPC muscles and gills and 2.3 MPC in the liver, type Sander lucioperca L., from the established norm. The excess of the MPC authorities Abramis sapa P., was in the muscle and gills from 4.7 to 6.1 MPC, the kidneys and gonads from 38.7 to 29.2 MPC, the MPC 2 in the liver.

In general, the total index overshoot was from 0.2 to 0.6 MPC of the threshold values that indicate a relatively low concentration in all organs. Identical

control group Mahambet kinds, exceeding indicators MPC ranged from 0.3 to 0.2 in the gill tissue and the liver, type Sander Lucioperca L., while in other organs the concentration of benzo(a)pyrene was observed. View Abramis Sapa P., is also characterized by minor figures exceeded MPC by 0.7 times to 0.6 times the gills and liver. In muscle tissue, gonads and kidneys the presence of benzo(a)pyrene was found.

Analysis of the cumulative performance of benzo(a)pyrene in the body group of Aktau, as mentioned earlier, showed similar intensity of content in the examined organs of fish species: Neogobius Melanostomus P and Rutilus rutilus caspicus Y., with views of groups and Makhambet Atyrau. In species of this group is identical accumulation observed in the kidney and the gonads in other organs has a uniform distribution of values. The value of the threshold is exceeded MPC representation of a group of Aktau, characterized virtually the same threshold is exceeded with the 1st and 2nd groups, so the species Neogobius Melanostomus P., the total number of exceedances in the kidneys and gonads ranged from 47.3 to 32.7 from the set MPC rules. The muscles and gills from 4.2 to 4.6 times, in the liver of 2.2 MPC. View Rutilus rutilus caspicus Y., marked by exceeding the maximum permissible concentrations in muscle and gill tissues from 3.7 to 4.5 times the magnitude of the MPC, the kidneys and the reproductive glands from 42.7 to 55.1 times in the liver - 2 8 times.

Table 2. B(a)P contamination ability in organs of fish groups in point Makhambet. Significance in relation to non-exposed controls at P<0.05 (Mann-Whitney test).

Sander lucioperca L	*n*	Makhambet *M±SD*	Abramis sapa P	*n*	Makhambet *M±SD*
Gills	10	0.050±0.021	Gills	10	0.087±0.034
Muscle tissue	10	0.043±0.015	Muscle tissue	10	0.036±0.028
Liver		0.023±0.0011	Liver	10	0.017±0.011
Gonads	10	0.0292± 0.0288	Gonads	10	0.025±0.0150
Kidneys	10	0.0387±0.0390	Kidneys	10	0.0423±0.0146
Sander lucioperca L (control)	*n*	*M±SD*	Abramis sapa P (control)	*n*	*M±SD*
Gills	10	0.0003± 0.0001	Gills	10	0.0006±0.0002
Muscle tissue	10	** not found	Muscle tissue	10	** not found
Liver	10	0.0008±0.0003	Liver	10	0.0005±0.0003
Gonads	10	** not found	Gonads	10	0.0002±0.0001
Kidneys	10	0.001±0.0001	Kidneys	10	** not found

The control group of species Neogobius kessleri G and Cyprinus carpio carpio L., is exceeded in the reproductive glands and gill tissues from 0.1 to 0.12 times and 0.4 to 0.5 MPC, liver and kidneys of both species did not show the presence of benzo(a)pyrene, whereas a slight excess MPC - 0.3 times, was found in muscle tissue type Cyprinus carpio carpio L., the content of benzo(a)pyrene, in muscle tissue Neogobius kessleri G was noted.

From the available data, laboratory and health study analysis of sea water, revealed a high percentage of the concentration of benzo(a)pyrene, indicating the excess of the permissible threshold concentration in the aqueous medium, areas of Northern and Central areas of the Caspian Sea. The pH of the water in investigated part manifested itself in the range of from 5.4 to 9.0 per unit and the lowest quality indicator, while the control samples, were in 7.0 to 7.2. It is known that the estimated average acidity of pH value, is equal to 7.0, Table 3. This fact is confirmed by the study of the control samples of

water that existed in the present analysis, where contamination of benzo(a)pyrene does not exceed the permissible concentrations and limit values.

Discussion

Intensification of oil and gas activity in the study region poses a growing threat to the lives of the majority of living organisms in the Kazakhstan area of the Caspian Sea, since the presence and distribution of the high number of oil and PAH, including benzo(a)pyrene, could in the future lead to the loss of the hydraulic system and biodegradation of biodiversity in the region. Our study demonstrated that one of the dangerous chemical agents benzo(a)pyrene in both areas of the Northern and Central parts of the Caspian Sea can widely and intensively accumulate in the main organs of the studied fish. Contamination of benzo(a)pyrene in the main organs of fish and water area, correlation part between fish organs and sea water samples, experimental and control groups can give us informative knowledge about the main accumulative behavior of benzo(a)pyrene agent in the studied region and give its estimation in monitoring system. The high bioaccumulation of benzo(a)pyrene in water organisms suggests the need for further research aimed at assessing the actual contribution of industrial activities, especially oil production, to the level of contamination in these areas.

Table 3. B(a)P contamination (mkg) in seawater samples. Significance in relation to non-exposed controls at P<0.05. (Mann-Whitney test).

Point Makhambet	Water pH	Benz(a)pyrene contamination	Point Atyrau	Water pH	Benz(a)pyrene contamination	Point Aktau	Water pH	Benz(a)pyrene contamination
Point 1	pH – 7.1	0.048±0.021	Point 1	pH – 6.4	0.0440±0.0205	Point 1	pH – 7.2	0.0492±0.0293
Point2	pH – 8.2	0.027±0.0134	Point2	pH – 8.5	0.075±0.048	Point2	pH – 5.4	0.0279±0.0116
Point3	pH – 7.2	0.034± 0.012	Point3	pH – 7.0	0.056±0.020	Point3	pH – 9.0	0.034±0.015
Point4	pH – 8.0	0.0533± 0.017	Point4	pH - 8.8	0.090±0.078	Point4	pH - 7.2	0.053±0.023
Point 5	pH – 7.1	0.0487±0.011	Point 5	pH – 6.4	0.077±0.046	Point 5	pH – 7.0	0.049±0.018

Our study demonstrated that groups of fish species are exposed by the influence of a benzo(a)pyrene product as demonstrated by the Gas-Liquid chromatography method. This method completely showed high accumulation activity in organs of the reproductive and excretory systems in both groups of fishes. Further assessing of benzo(a)pyrene migration activity in water and living organisms can give a good recommendation as to using fish organisms in monitoring of hydro systems.

An analysis of the accumulation of benzo(a)pyrene in surface sea waters near the Northern and Central Caspian Sea, as well as the fresh waters of the Ural river, confirms the presence of the chemical element in the selected samples. According to the getting results correlation relationship between benzo(a)pyrene in the tissue test organisms and the aquatic environment showed a positive effect of total exposure at all points of the study as a result of penetration and absorption into the body of the objects.

In our study was found, that with the regard to the migration activity of benzo(a)pyrene, the cause of most interest, there is a significant accumulation effect in excretory organs and reproductive systems. So both groups showed similar concentration and the excess of MPC, as mentioned earlier several times, from the established norm and similar high content in water. It may speak of more active absorbent processes along with the other examined organs. Accumulation in the gonads directly can lead to destabilization of the reproductive system, reduce the number of offspring, as well as the possible mutagenic and teratogenic effects on future generations. Results of presented analysis revealed particulary hight concentration of studying element in gill epithelium

similary correlated with muscle tissue, but gills of all fishes are involved in the process of filtering the water environment and especially in the respiratory system and hematopoiesis, with the broader activity and close contact with water, the gill epithelium cells may also suffer from the toxic stress caused by prolonged exposure to these numerous xenobiotic components, including, benzo(a)pyrene. The liver is one of the depots of the bodies and harbors most toxic substances involved in the process of neutralization by oxidation and decomposition. By binding to the benzo(a)pyrene, the agency may also have the ability to see to its own disintegration and removal from the body, which may explain its low presence. A small concentration of benzo(a)pyrene in muscle tissue is identical to the rest of the bodies.

As it is known, muscle tissue occupies the entire volume of the body, essentially being the main mechanism involved in the movement along with the skeletal system. Minor concentrations of benzo(a)pyrene in the muscle of fish and groups Atyrau and Aktau may indicate the uniform distribution of the material over the entire area of the body, unlike the reproductive glands and kidney, with lower volume and mass weight. All of the above calls for further more research into the issue of migration of the substance in various organs as this type of chemical elements cause for concern in view of its high toxicity and intensifies the effect of the state of the fish fauna species diversity represented in the region.

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