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Comparative Cytogenetics Analysis of Chromosomal Aberrations Level in Two Marine Species of Fish, under the Influence of Benzo(a)pyrene Accumulation Ability

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Abstract: Fishes are sensitive biomarkers of genotoxic effects of environmental pollutants. This study aimed to evaluate the chromosomal aberrations frequency, under accumulative influence of petroleum product benz(a) pyrene, in fish species *Sander Lucioperca L*. and *Neogobius Melanostomus P*. Comparative value of levels and chromosomal aberrations frequency in somatic cells of liver and muscle tissue, at two fish species organs *Sander Lucioperca L*. and *Neogobius Melanostomus P.*, widely and overall living in more important sites of Northern and Central Parts of Caspian Sea, was recorded using common karyotyping method. The study revealed that one of the types of (PAH) s, petroleum, benzo(a)pyrene, can cause frequent chromosomal abnormalities in somatic cells, in the most sensitive organs of fish living in conditions, of the active development of oil industry.

Key words: Caspian Sea · Poly Aromatic Hydrocarbons (PAH) · Benz (A)Pyrene

INTRODUCTION

The Northern part of Caspian Sea and one of the largest rivers of the region that falls into it – Ural, with a large oil-producing Atyrau City situated in the delta where the river falls into the sea, suffer a considerable anthropogenic pressing due to the extending oil production and oil transportation. The Central part of Caspian Sea with a large port city of Aktau near it, as a zone of active production and development of gas deposits, also dramatically increases the ecological load all over the water area of the Central and Northern parts of Caspian Sea.

Anthropogenic activity connected with hydrocarbon resource development in studied region, brings variable number of the chemical elements of different nature, to environment. From huge number of elements that having chemical nature and their influence value degree on genetic apparatus, were chosen as several dominated types of chemical elements, in studying area. Mutagenic pollutant of environment as petroleum product benz(A)pyrene, invades into natural biochemical processes of organism and accumulates in organs and tissues, as a way of entrance to environment. This petroleum product belongs to group of mostly wide and commonly spread in studying area and marked by number of authors during for several years [1].

Accumulative influence of the above named chemical elements, shortly increasing chromosomal alteration both their numeric and structural changes and decrease mutagenic changes of the living organism Figure 1.

As a result, long-term monitoring studies and observations established a permanent presence, interaction and distribution of benz(a)pyrene in the region and described the accumulation of objects in the environment [2].

In view of wide distribution of these groups of chemicals petroleum products benz(a)pyrene, as compared with other types of elements in regions of interest and sufficient scrutiny. Accumulative effect and the activity of this group of elements are of interest on the part of chromosome analysis and its owned systemic impact on the structure and frequency of chromosome aberrations at the cellular level [3].

For evaluation the chromosomal apparatus damage level in somatic cells of living organisms objects, ability to assess changes in the state of chromosomes caused by exposure to adverse factors, namely one of the inorganic

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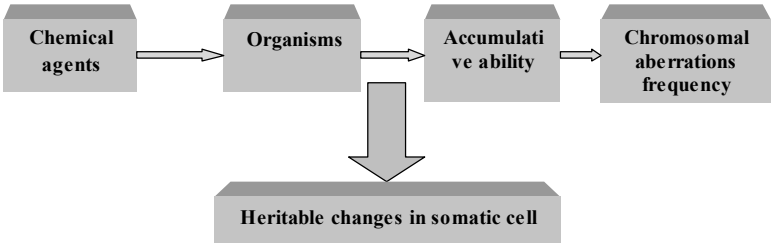

Fig. 1: The relationship of chemical pollution and the frequency of chromosomal changes in biological objects of the environment

chemical agents, petrochemicals benzo(a)pyrene, there have been chosen the widely-spread in the described region of investigation species of fish - *Sander lucioperca L*. and *Neogobius melanostomus P*.. The both species belong to one and the same family of perch-like fishes, they are widely spread and represent endemics in the investigated points, they spawn and propagate, live actively, migrate to considerable distances, occupy similar ecological niches and habitats.

The product benz(a)pyrene, as one of the toxic and mutagenic factors, for living organisms, that are associated and followed with oil and gas production activity in the investigated zones of the Northern and Central Caspian Sea. As fact, this chemical agent, presents in natural environment and is classificated, as genotoxicant agent and has ability to interact with chromosome structure, inducting structure damages, that's brining to disorganization of the cell processes and disturbance of the biological system functions. So the aim of the present work was quantitative and comparative assessment of chromosomal apparatus damage in somatic cells and the frequency of chromosomal aberrations in the conditions of natural habitats of biological species *Sander lucioperca L*. and *Neogobius melanostomus P.,* under the influence, of one of the most widely spread types of petroleum products, polynuclear aromatic hydrocarbons.

MATERIALS AND METHODS

Sample Preparation and Analysis: Several specimens of fishes *Sander luciopercà L*. and Neogobius *melanostomus P*. were caught in 4 points of sampling biological objects in the investigated place adjacent to Atyrau City in the Northern Caspian Sea region (47° 6'4.53"N, 51°54'48.99"E), (46°53'46.64"N, 51°38'12.96"E) and Aktau City (47° 6'4.53"N, 51°54'48.99"E), (43°37'0.77"N, 51°11'41.27"E), on the territory of the Central Caspian Sea. Then they were delivered to the laboratory and studied with the usage of the method of morphometric analysis. The research was realized in summer period from July to August, 2011 – 2013.

Liver and muscle tissue out of the caught specimens of fish were taken. Because liver is more sensible organ for any toxic influence and reacting and a muscle tissue are occupying most part of living organism and having close contact with different external factors. There was impossible to extract the metaphase plate from gills tissue, because the fish in the tips of the filaments gills has a lymphopoiesis occurring process and most mitotic activity in lymphoid cells [4]. Each organ was weighed from 1 to 5 g. and then prepared for the further analysis. Control group were taken from identical species of fish contained in aquarium in pure running water without any admixtures.

Cytogenetic Analysis: Karyological analyses were collected from 400 fish species, which belonged to the following two species:

Sander luciopercà L – 200 individuals, karyotype 2n =48, NF=48;
Neogobius melanostomus P– 200 individuals, karyotype 2n = 46, NF = 46, according to Parish Chromosomal classification nomenclature.

Samples as somatic cell tissues was taken during summer because in this time of year, the fish have the highest mitotic index. Specimens caught fish were injected intramuscularly colchicine solution of 1 ml per 100 g body weight. After administration of fish placed in the water tank and after 4-5 hours the fish were killed by decapitation and the liver was dissected and muscle tissue. On the proposal of the International Commission for the nomenclature of different types for differential staining has taken certain notation: Q, G, C.

Technique of molecular cytogenetic diagnostics included: first DNA sample indicated tritium (3H). Then the sample was denatured by heating for 5 minutes

at 100 °C in 20 l of hybridization mix containing 2XSSC, 50% Dextran sulfate and 50% formamide - 500. Chromosomal DNA on a microscope slide and denatured in a solution of 0.07 M NaOH for 30 sec, followed by washing preparations in the 70 "(2 times) and 96° (2 times) ethanol for 5 min. Hybridization was performed for 18 hours at 37° C, followed by washing with 2 changes of drugs in 2XSSC, 50% formamide at 39 °C for 5 min; 2 shifts 2XSSC at room temperature, 2 shifts 70 "and 96 °C ethanol for 5 min. Preparations were dried in air followed by coating with an emulsion type M. Exposure to drugs under emulsion took an average of 6 days (from 2 to 12 days). Thereafter, chromosomal preparations were developed in the developer and stained amidovom 30% dye solution Wright 0.02M phosphate buffer at pH = 6.8 with a contrasting pattern Q-segmentation method chromosomes [5]. Analyzed by autoradiography and photographed on any optical microscope with a lens with an overall increase 100/1.32 h1125.

Benz (A) Pyrene Extraction: In the course of estimation of concentration of benz(a)pyrene there was realized the commonly accepted method of extraction of the petrochemical benz(a)pyrene in the following way. The studied organs of fish (liver and muscle tissue) weighed from 2 to 7 g were placed into a paper filter being preliminarily thoroughly minced and dried. Then into the prepared sample there was poured a necessary quantity of demitilchloramide (45 ml) and was subjected to extraction process by the method of Soxhlet extraction during 30 min [6]. The gained liquid was subsequently placed into the rotary evaporator apparatus, where it was subjected to partial evaporation until there is a small remnant in the quantity of 0.7 ml, then the liquid sample was placed into 1.0 ml flask and there was realized the measurement as to the concentration of benz(a)pyrene with the usage of gas-liquid chromatographer. The instrument was calibrated and profiled using the mixed calibration standard solution. The system was rinsed for one minute with the deionized water before the analysis of each sample.

Statistical Analysis: Statistical differences between chromosomal abnormalities in particular organs of *Sander luciopercà L. è Neogobius melanostomus P.,* with respect to mean concentrations of studied element were evaluated by ANOVA on log-transformed data to obtain a normal distribution of features [7]. Normality of analyzed features was checked by Shapiro–Wilk's test and the homogeneity of variances by Bartlett's test. Statistical confidence was set at $p < 0.05$. Fish's ability to take up the elements from its environment and translocation rate of the elements within the fish were, evaluated by the Index Bioaccumulation or Translocation Factor (TF), expressed by the following ratios: benz(a)pyrene concentration in gills and benz(a)pyrene concentration in muscle tissue. All statistical calculations were carried out using the CSS StatisticaStatsoft [8].

RESULTS AND DISCUSSIONS

It is has been accepted that the toxic effects of the polynuclear aromatic hydrocarbons (PAH) are provoked by their metabolites, basing on the example of one of the famous dangerous chemical secondary components of PAH, benz(a)pyrene, that belongs to the 1st rate of danger. Benz(a)pyrene is always found in PAH samples and represents an essential component; it is produced in the course of oil production and burning of the liquid hydrocarbon, solid and gaseous fuel. Being relatively chemically stable benz(a)pyrene can migrate for a long time from objects to objects. It has been proved, that benz(a)pyrene, along with other types of PAH, the most rapidly causes a disturbance of the chromosome set and consequently alters the structure of DNA, leading to its frequent injury. Constitutes one of the major mutagens for the body. Many authors including have managed to identify among hundreds of polynuclear aromatic hydrocarbons (PAH) of different structure that are produced at the conditions of oil production and found in the objects of the environment, benz(a)pyrene is more foreground for monitoring of the investigated area [9].

Our results on detection of petrochemical benz(a)pyrene accumulation and a chromosomal aberrations level, gives the grounds for assumption that PAH and their secondary product benz(a)pyrene penetrates into the tissues of studied marine organisms and takes part in the processes of metabolism and chromosomal damage in the two studied species of fish. Figure 2 shown the comparative degree of accumulation of the petrochemical product benz(a)pyrene and its influence – reacting on chromosomal aberrations process in various organs of the studied objects.

As in Table 1, accumulation of benz(a)pyrene in Atyrau and Aktau populations showed mainly in muscle tissue of the fish, which 3 - 4.8 and 2.7- 4.9 times exceeds the maximum permissible concentration (MPC) in compare with the control group where the concentration exceeds the MPC 1.5 – 1.8 and 1.6 – 1.8 times. Then the accumulation of the product is realized in liver tissue

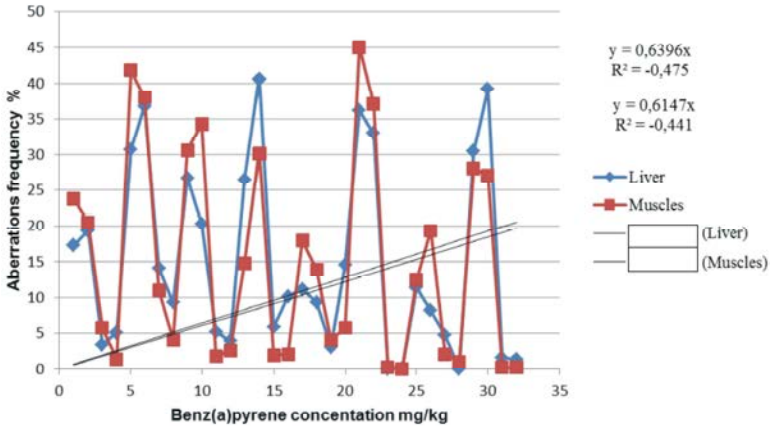

Fig. 2: Correlation effect of chromosomal abnormalities in somatic cell of studied individuals and benz(a)pyrene accumulative concentration

Table 1: Comparative concentration of benz(a)pyrene in organs of *Sander lucioperca L. and Neogobius melanostomus P*

Benz(a)pyrene concentration M±m	Multiplicity Exces, MPC	Control	Multiplicity Excess, MPC	Benz(a)pyrene concentration	Multiplicity Excess, MPC	Control	Multiplicity Excess, MPC
S.Lucioperca Liver0.033^a ±0.016^a	3.3^a	0.0004^a±0.0001^a	0.2	N.Melanostomus Liver 0.035^a ±0.011^a	3.5	0.0002^a±0.0001^a	0.2
S.Lucioperca Liver 0.040^a ±0.013^a	4.0^a	0.0002^a±0.0001^a	0.2	N.Melanostomus Liver 0.042^a±0.14^a	4.2	0.0002^a±0.0001^a	0.2
S.Lucioperca Liver 0.046^a ±0.015^a	4.6^a	Not found	0.1	N.Melanostomus Liver 0.048^a±0.016^a	4.8	0.0003^a±0.0001^a	0.3
S.Lucioperca Liver 0.057^a ±0.028^a	5.7^a	0.0003^a±0.0001^a	0.3	N.Melanostomus Liver 0.055^a±0.027^a	5.5	0.0003^a±0.0001^a	0.3
S.Lucioperca Liver 0.059^a ±0.023^a	5.9^a	0.0004^a±0.0001^a	0.1	N.Melanostomus Liver 0.053^a±0.021^a	5.3	0.0006^a±0.0002^a	0.6
S.Lucioperca Muscles 0.035^a ±0.014^a	3.5^a	0.0002^a±0.0001^a	0.2	N.Melanostomus Muscles 0.038^a±0.015^a	3.8	0.0002^a±0.0001^a	0.2
S.Lucioperca Muscles 0.027^a ±0.017^a	2.7^a	Not found	Not found	N.Melanostomus Muscles 0.029^a±0.011^a	2.9	Not found	Not found
S.Lucioperca Muscles 0.066^a ±0.026^a	6.6^a	0.0003^a±0.0001^a	0.3	N.Melanostomus Muscles 0.070^a±0.028^a	7.0	0.0003^a±0.0001^a	0.3
S.Lucioperca Muscles 0.079^a ±0.031^a	7.9^a	Not found	Not found	N.Melanostomus Muscles 0.081^a±0.032^a	8.1	Not found	Not found
S.Lucioperca Muscles 0.083^a ±0.041^a	8.3^a	0.0005^a±0.0002^a	0.5	N.Melanostomus Muscles 0.084^a±0.033^a	8.4	0.0005^a±0.0002^a	0.5
S.Lucioperca Muscles 0.074^a ±0.029^a	7.4^a	0.0002^a±0.0001^a	0.2	N.Melanostomus Muscles 0.075^a±0.037^a	7.5	Not found	Not found

MPC-Maximum permissible concentration^{a}mg/kg

approximately in same equal proportion 3.1 – 5.0 and 2.5 – 4.7 times exceeds the MPC, as in muscle tissue, in compare with the control group 1.3 – 1.6 and 1.5 -1.9 times.

Frequency of chromosomal aberrations as a result of benz(a)pyrene contamination in studied biological species had 10.7 - 23.8 times in Atyrau population, 1.01 – 5.07 (Control) in Aktau population had 20.4 - 41.8 times, in control group number was 0.2 - 4.06.

Noting the frequency of chromosomal aberrations in the form of such violations as deletions, duplications and polyploidy. And it is worth noting the increase in the amount of chromosomal abnormalities such as deletions and duplications in the species *Sander Lucioperca L.* Table 2 and observed frequency of polyploidy in the species *Neogobius melanostomus P.* Table 3 associated usually with fish species differences and characteristics and with habitat two types of study, but it should be noted, as mentioned previously [9] virtually identical to petroleum product accumulation benzo(a)pyrene in two different test subjects.

Most PAHs do not dissolve easily in water. In an aqueous environment they adsorbed on particulates and solubilized in any organic or oily part present in water, sediment and soil, thus, move through soil to contaminate underground water. The low molecular weights PAHs, such as three–ring PAHs, are more soluble in water and are volatile than high molecular weight compounds. In the works of Mastral and Callen [10], in which storm water samples were examined, it was found that PAHs are more predominant in particulate form than in the dissolved form. Furthermore, due to their low vapor pressure, PAHs adsorbed to soil and remain with it for a long time. Also, their hydrophobic and lipophilic natures facilitate their bioaccumulations and persistence in the environment, posing a long term threat to the ecosystem.

Similar studies conducted [10] in experiments in vivo by injecting diluted concentrated samples of petroleum products of benz(a)pyrene in body of species *Sander lucioperca L.* and *Neogobius melanostomus P.*, found very frequent process similar of chromosomal changes in

Table 2: Data analysis of chromosomal aberrations frequency at *Sander luciopercà L.* Atyrau site

Benz(a)pyrene concentration M±m	Number of cell studied	Duplication	Deletion	Polyploidy	Number of aberrations	Frequency of aberrations (%)
S.Lucioperca						
Liver 0.033a ±0.016a Muscles	256	15	11	13	17.2	23.8
0.035[a] ±0.014[a]	245	19	17	15	19.3	20.5
Control						
Liver 0.0004[a] ±0.0001[a]	241	4	Not found	2	3.39	5.7
Muscles 0.002a±0.001a	236	7	Not found	5	5.1	1.4
S.Lucioperca						
Liver 0.040[a] ±0.013[a] Muscles	302	18	27	10	23.1	14.2
0.027[a] ±0.017[a]	297	15	30	7	16.4	10.7
Control						
Liver 0.0002[a] ±0.0001[a]	290	1	2	Not found	Not found	Not found
Muscles Not found	305	3	2	Not found	Not found	Not found
S.Lucioperca						
Liver 0.046[a] ±0.015[a]	291	22	10	12	26.7	30.6
Muscles 0.066[a] ±0.026[a]	285	16	14	19	20.3	34.3
Control						
Liver Not found	295	Not found	6	1	5.2	1.79
Muscles0.0003[a] ±0.0001[a]	280	Not found	4	1	3.9	2.64
S.Lucioperca						
Liver 0.057[a] ±0.028[a]	400	34	22	11	11.21	17.9
Muscles 0.079[a] ±0.031[a]	389	26	25	7	9.4	13.9
Control						
Liver 0.0003[a] ±0.0001[a]	397	5	Not found	5	3.08	4.1
Muscles Not found	355	1	3	3	14.6	5.7
S.Lucioperca						
Liver 0.059[a] ±0.023[a]	400	31	20	9	11.4	12.5
Muscles 0.083[a] ±0.041[a]	270	27	20	5	8.2	19.3
Control						
Liver 0.0004[a] ±0.0001[a]	410	1	3	4	4.7	2.04
Muscles 0.0005[a] ±0.0002[a]	296	1	1	Not found	Not found	1.01

MPC-Maximum permissible concentration [a]mg/kg

Table 3: Data analysis of chromosomal aberrations frequency at *Neogobius melanostomus P.*, Aktau site

Benz(a)pyrene concentration M±m	Number of cell studied	Duplication	Deletion	Polyploidy	Number of aberrations	Frequency of aberrations (%)
N.Melanostomus						
Liver 0.035[a] ±0.011[a]	350	9	5	27	30.71	41.8
Muscles 0.038[a]±0.015[a]	290	7	8	34	36.8	38.03
Control						
Liver 0.0002a ±0.0001[a]	365	3	2	6	14.1	11.09
Muscles 0.0002[a] ±0.0001[a]	295	1	2	2	9.35	4.06
N.Melanostomus						
Liver0.042[a] ± 0.14[a]	350	8	14	28	26.5	14.81
Muscles 0.029[a]±0.011[a]	345	5	19	31	40.65	30.12
Control						
Liver0.0002[a] ±0.0001[a]	361	2	3	4	5.9	1.93
Muscles Not found	380	2	7	9	10.2	2.07
N.Melanostomus						
Liver 0.048[a]±0.016[a]	294	9	1	22	25.07	31.08
Muscles0.070[a]±0.028[a]	374	7	-	25	29.3	20.4
Control						
Liver0.0003[a] ±0.0001[a]	290	1	2	3	Not found	Not found
Muscles0.0003[a] ±0.0001a	379	1	1	7	Not found	Not found
N.Melanostomus						
Liver0.055[a]±0.027[a]	294	7	10	34	36.19	45.02
Muscles0.081[a]±0.032[a]	280	4	16	30	33.01	37.15

Table 3: Continued

Benz(a)pyrene concentration M±m	Number of cell studied	Duplication	Deletion	Polyploidy	Number of aberrations	Frequency of aberrations (%)
Control						
Liver 0.0003a±0.0001a	310	Not found	3	7	Not found	0.27
Muscles Not found	287	Not found	6	Not found	Not found	Not found
N.Melanostomus						
Liver 0.053[a]±0.021[a]	300	7	4	27	30.4	28.05
Muscles0.084[a]±0.033[a]	300	11	6	39	39.2	27.1
Control						
Liver 0.0006[a] ±0.0002[a]	315	3	Not found	1	1.6	0.17
Muscles0.0005[a] ±0.0002[a]	311	Not found	Not found	5	1.3	0.2

MPC-Maximum permissible concentration [a]mg/kg.

two experimental identical fish species detectable as an increase in the duplication processes of species *Sander Lucioperca L*. and polyploidy in the species *Neogobius melanostomus P.,* similar to the process of chromosomal rearrangements have taking place in identical biological species, at zones of Northern and Central parts of Caspian Sea.

CONCLUSION

The analysis of the concentration benz(a)pyrene has shown that the content of the studied petroleum product benz(a)pyrene in the considered fish species, exceeds MPC more in several times in both investigated biological species.

The considerable content of benz(a)pyrene in bodies of the muscle tissue and liver, at *Sander luciopercà L.,* in comparison with *Neogobius melanostomus P.,* has no high accumulative ability and prevalence in muscle and liver tissues at *Neogobius melanostomus P*., in comparison with *Sander luciopercà L.*

Cytogenetic analysis has established a mutagenic effect of benz(a)pyrene concentration, which manifested in a significant increase in the chromosome aberration frequency in the muscle and liver tissue of two fish species as compared to control animals. The level of chromosome aberrations was found to benz(a)pyrene compound dependent. However, a surprisingly limited effect of repeated administrations were observed, strongly indicating that even single exposure events by these substances may lead to health damaging effects.

It's administrated by gavage in two types of tissue benz(a)pyrene compounds accumulation display virtually equal mutagenic potency to living organisms.

The significant, positive correlations found between the content of benz(a)pyrene in the studied environment and the chromosomal aberration levels under of these element in the organs of fishes that can indicate the potential use of species *Sander luciopercà L.* and *Neogobius melanostomus P.,* in bio monitoring process of environmental changes with benz(a)pyrene product contamination, on chromosomal aberrations level in animals species at Northern and Central Areas of Caspian Sea.

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