

STRUCTURAL CHROMOSOME DAMAGES IN THE CARP (*CYPRINUS CARPIO*, L.) FROM SOME LOCALITIES OF THE RIVERS KOLUBARA, SAVA, DANUBE AND TAMIŠ

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STRUKTURNA OŠTEĆENJA HROMOZOMA ŠARANA (*CYPRINUS CARPIO*, L.) SA NEKIH LOKALITETA REKE KOLUBARE, SAVE, DUNAVA I TAMIŠA

Abstrakt

U rekama Kolubari, Savi, Dunavu i Tamišu, tokom višegodišnjih istraživanja, utvrđivano je prisustvo genotoksičnih i potencijalno genotoksičnih materija: nafte, derivata nafte, fenola, pesticida, teških metala, među kojima su Hg i Cd detektovani ne samo u sedimentu reka, već i u ribi i školjkama. U ribi su utvrđeni policiklični ugljovodonici, od kojih su mnogi kancerogeni i genotoksični; pesticidi, među kojima: heksahlorocikloheksan-HCH i njegov izomer lindan, kao i DDT i njegovi metaboliti. Kako je povišena učestalost hromozomskih oštećenja-prekida i gapova, pokazatelj genotoksičnog dejstva, citogenetičke metode je moguće primeniti na organizme iz prirode, radi utvrđivanja prisustva genotoksičnih agenasa u životnoj sredini. Tokom ranijih istraživanja, na osnovu rezultata dobijenih u laboratorijskim kontrolama, kao i na osnovu učestalosti promena kod riba iz čistih voda, pretpostavljena je oblast kritične zone-iznad becgground-a ili nivoa "spontanijih" promena, pri čemu su sve vrednosti iznad 3,5 %, tj. iznad oblasti zone, one koje ukazuju na nesumnjivo prisustvo i delovanje genotoksičnih agenasa. Cilj istraživanja je bio da se putem analize hromozoma šarana-*Cyprinus carpio*, L., odredi učestalost oštećenja tipa prekida i gapa, radi procene eventualnog genetičkog rizika na nekim lokalitetima reka Kolubare, Save, Dunava i Tamiša.

Citogenetički su analizirani metafazni hromozomi šarana-*Cyprinus carpio*, koji su dobijeni klasičnom metodom preparacije iz tkiva bubrega. Kod svake jedinke je pregledano 30-50 metafaznih figura hromozoma. Utvrđivan je broj hromozomskih prekida i gapova, a dobijeni rezultati su analizirani primenom statističkih metoda.

Najviše vrednosti su ustanovljene, redom, u reci Kolubari, Tamišu, Dunavu kod Višnjice i Savi kod ušća i bile su iznad nivoa "spontanih" promena i iznad predpostavljene kritične zone (3,0-3,5 %). Značajne statističke razlike ($p < 0.001$) se javljaju poređenjem lokaliteta sa najnižom učestalošću promena, kao što su Dunav kod Beške i Slankamena, sa lokalitetima: Kolubare, Tamiša, Dunava kod Višnjice i Save kod ušća. Rezultati pokazuju da postoji genetički rizik od stalnog ili povremenog zagađenja genotoksičnim materijama na lokalitetu Kolubare kod Obrenovca, Tamišu kod Pančeva, Dunavu kod Višnjice, kao i u Savi kod njenog ušća.

Ključne reči: genotoksični agensi, hromozomi riba, prekidi i gapovi, *Cyprinus carpio*

INTRODUCTION

The largest group of genotoxis agents comprises *industrial genotoxic agents* and within the group oil and oil derivates are of the greatest importance (IARC, 1989; Vanzella et al., 2007; Kolwzan, 2009; Oleira - Martins & Grisolia, 2009; Araújo et al., 2010). Genotoxic and carcinogenic properties were found in *phenols* (Michalowicz, 2004) and *pesticides*, as *hexachlorocyclohexane* – HCH and its γ – isomer, *lindane* (Kalanž et al., 2004), whereas genotoxicity of DDT and its metabolites is controversy: no effect detected in bacterial test systems, but found in mammal cell cultures (ATSDR, 2002). These, and similar chemicals and heavy metals, like Hg and Cd, have been found in Kolubara, Sava, Danube and Tamiš waters as in shell and fish bodies (Fingler & Drevenkar, 1988; Janković & Jovicic., 1994; Maljević, 2004; Ekostatus of the river Tamiš, 2010).

Increasing frequency of chromosome breaks and gaps is a useful indicator of genotoxic actions (Bröger, 1982). Cytogenetic methods are commonly used in laboratory conditions (Preston et al., 1981) and are possible to apply the same methods in organisms in nature.

This study was performed to detect chromosome breaks and gaps frequencies in the carp – *Cyprinus carpio* L., in specimens which were caught in three consecutive years on some localities of the rivers: Kolubara, Sava, Danube and Tamiš, with the aim to evaluate eventual genetic risk of genotoxic agents presence in the river water.

MATERIAL AND METHODS

Carp specimens were collected during three consecutive years, 1986, 1987 and 1988, on different localities of the rivers Kolubara, Sava, Danube and Tamiš, and cytogenetically analyzed. Metaphase chromosomes were obtained by preparation of kidney tissue according the classical methods (Fontana et al., 1970). Procedure involving 6 – 12 fishes from the same locality simultaneously. In the every individual fish, 30 – 50 mitoses were examined. Breaks and gaps were detected and scored, and results were statistically analyzed.

RESULTS AND DISCUSSION

Results of cytogenetic analysis of carp specimens are presented in Table 1.

Lower level of breaks and gaps has been registered in fish from the Danube near Beška and Slankamen (Table 1). Higher frequencies of breaks and gaps were detected in fish from Danube caught by Zemun and Grocka, and Sava by NT – B ($p < 0.05$). The highest values, above the 3.5 % changes, i.e. above the level of proposed critical zone (Fišter, 1992), were detected in river Kolubara near Obrenovac, Tamiš near Pančevo, Danube by Višnjica and Sava near the mouth, respectively. Statistically significant differences ($p < 0.001$) were found in comparison localities of Danube near Beška and Slankamen with Kolubara, Tamiš, Danube by Višnjica and mouth of the river Sava. Very high frequencies of chromosome breaks and gaps obtained in fish from the last announced localities indicate to genetic risk of permanent or periodical presences of genotoxic agents in river water.

Table 1. Frequency of breaks and gaps in carp – *Cyprinus carpio*

Locality	Year	Number of individuals	Mitoses examined	Breaks and gaps	Breaks and gaps %	\bar{x} for three years (%)
Lokalitet	Godina	Broj jedinki	Pregledanih mitoza	Prekida i gapova	Prekida i gapova %	\bar{x} za tri godine (%)
Kolubara – Obrenovac	1986	8	260	10	3,84	3,7845
	1987	7	210	8	3,80	
	1988	7	217	8	3,68	
		$\Sigma=22$	$\Sigma=687$	$\Sigma=26$		
Sava – "NT" B	1986	6	212	6	2,83	2,7536
	1987	6	236	6	2,54	
	1988	7	242	7	2,89	
		$\Sigma=19$	$\Sigma=690$	$\Sigma=19$		
Sava – ušće	1986	9	280	10	3,57	3,4246
	1987	9	331	11	3,32	
	1988	8	265	9	3,39	
		$\Sigma=26$	$\Sigma=876$	$\Sigma=30$		
Dunav – Beška	1986	10	346	7	2,02	2,0250
	1987	11	339	8	2,35	
	1988	11	352	6	1,70	
		$\Sigma=32$	$\Sigma=1037$	$\Sigma=21$		
Dunav – Slankamen	1986	12	384	8	2,08	1,9090
	1987	12	373	7	1,87	
	1988	11	343	6	1,74	
		$\Sigma=35$	$\Sigma=1100$	$\Sigma=21$		
Dunav – Zemun	1986	12	372	10	2,68	2,6022
	1987	10	340	9	2,64	
	1988	12	364	9	2,47	
		$\Sigma=34$	$\Sigma=1076$	$\Sigma=28$		
Dunav – Višnjica	1986	12	351	13	3,70	3,5989
	1987	12	396	14	3,53	
	1988	12	420	15	3,57	
		$\Sigma=36$	$\Sigma=1167$	$\Sigma=42$		
Dunav – Grocka	1986	11	363	12	3,30	2,9439
	1987	12	375	10	2,66	
	1988	10	315	9	2,85	
		$\Sigma=33$	$\Sigma=1053$	$\Sigma=31$		
Tamiš	1986	10	365	14	3,98	3,7442
	1987	11	354	13	3,67	
	1988	12	376	14	3,72	
		$\Sigma=33$	$\Sigma=1095$	$\Sigma=41$		

Sometimes, genotoxic contamination is difficult to prove immediately, especially in cases of some substances which are not genotoxic, but become genotoxic in metabolic detoxication processes – metabolic activation (Magee, 1982). Genotoxic agents could arise from biological degradations, or could be found after remediation of oil contaminated soil (Kolwzan, 2009). From the soil, chemicals could penetrate into

underground waters and by that way, to the rivers flow. Many of the oil products are known as carcinogens, mutagen and genotoxic (IACR, 1989; Vanzzella et al., 2007; Kolwzan, 2009; Oliveira-Martins & Grisolia, 2009; Araújo et al., 2010). Some chemicals, similar to DDT, could accumulate in animal tissues and long retained in the food-chains (ATSDR, 2002).

Examined carp specimens were collected from localities where oil products, phenols, pesticides and heavy metals, especially Hg and Cd have been continuously detected in the river water (Fingler & Drevenkar, 1988; Janković & Jovicic, 1994; Maljević, 2004; Ekostatus of the river Tamiš, 2010). In additions, findings in carp are in concordance with results which were obtained in other fish species: *Esox lucius*, *Carassius auratus gibelio*, *Tinca tinca*, *Alburnus alburnus*, *Stizostedion volgensis* and *Perca fluviatilis* (Fišter, 1992; Fišter et al., 1994; Fišter & Soldatović, 1996; Fišter et al., 1996).

CONCLUSION

High frequencies of chromosomal damages, breaks and gaps detected in the carp *Cyprinus carpio*, L. and significant statistical differences among localities examined, indicated to periodical or permanent presence of genotoxic chemical agents in the rivers Kolubara near Obrenovac, Tamiš near Pančevo, Danube near Višnjica and Sava near the mouth.

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