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Original Article

Evaluation of kidney histopathological alterations in Crucian Carp, *Carassius carassius*, from a pesticide and PCB-contaminated freshwater ecosystem, using light microscopy and organ index mathematical model

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Abstract: This study aimed to evaluate the potential toxic effects of chronic sublethal pesticides and polychlorinated biphenyl (PCBs) exposure on feral crucian carp, *Carassius carassius,* using histopathological alterations as an endpoint. Besides, a mathematical model of organ index was used to evaluate the severity of tissue damages. Circulatory disturbances, inflammation, regressive and progressive changes of tubules, glomerulus and interstitial tissue of kidneys were the most frequent damages observed. Organ index calculation revealed moderate occurrence of damage in kidneys of fish compared to the reference site. Findings highlight the effectiveness of organ index as a measuring kidney's damage severity and health status of fish. The present work is the first study that determines the levels and effects of pesticides and PCBs in water and fish kidneys in Seferani Lake in Albania. The results suggest that the observed changes in kidney structure of *C. carassius*, could possibly indicate a prolonged chemical stress caused by pesticides and PCBs suggesting continuous monitoring of the lake to protect human consumer's health.

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Introduction

Anthropogenic activities are led to stress on aquatic environments by a mixture of pollutants, which may cause molecular, biochemical, cellular and consequently physiological damages on aquatic organisms such as fishes (López-Barea, 1995; Rand, 1995; Oheet al., 2004; Faggio et al., 2014a, b, 2018; Savorelli et al., 2017). A finest example is the feedback of wildlife communities under constant influence of pesticides and industrial chemicals, such as PCBs (Jeremy, 2000). An ample number of pesticides are in use nowadays, belonging to a wide diversity of classes, with an extensive range of physicochemical characteristics (Haygarth and Jarvis, 2002; Chromcova et al., 2015; Fiorinoet al., 2018; Plhalova et al., 2018; Stara et al., 2019). Organochlorine pesticides and PCBs are a group of toxic substances that can get into water bodies from different routes (Carter and Heather, 1995), and are defined by their ability to have chemical and biological stability and persistence (Jackson et al., 1994; Ross and Birnbaum, 2001; Pfeuffer and Rand, 2004). Despite the presence of these hazardous compounds in aquatic biota, their chronic biological effects on aquatic life in the lake are still poorly studied.

Fishes as inhabitants of aquatic ecosystems are frequently exposed to contaminated water, particularly in those areas where contaminants are stable on the environment and has a high potential of accumulation and biological effects (Bernet et al.,1999). Thereby, fishes are good bio-indicators of environmental health status because of their position in the trophic chain and their responsiveness to low stimuli of pollutants (Aliko et al., 2018; Burgos-Aceves et al., 2018, 2019; Gobi et al., 2018).

Biological reactions to contaminants measured in an organism are defined as biomarkers, including

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parameters such as enzymatic, hematological, immunological, genotoxic, endocrine, physiological, histological and morphological biomarkers (van der Oost et al., 2003). Histopathology of various fish tissues have been used as reliable biomarker in assessment of stressor's effects both in laboratory and field studies (Wester and Canton, 1991; Hinton et al., 1992; Dutta, 1996; Leonardi et al., 2009; Marchand et al., 2009). Histopathological changes are displayed as a medium response to chemical contaminants, and histology provides a rapid method to detect a number of lesions in various tissues and organs (Bucke et al., 1996; Miranda et al., 2008). Liver (Hinton and Lauren, 1990; Camargo and Martinez, 2007; Miranda et al., 2008), kidney (Bucher and Hofer, 1993; Hinton, 1993; Miranda et al., 2008), and gills (Fernandes and Mazon, 2003; Oliveira et al., 2005) are suitable organs for histopathological investigation to determine the effects of various stressors. Kidney is especially involved in the maintenance of water and body fluids homeostasis (Hinton et al., 1992) and serves as an excretory organ for the metabolites of a group of xenobiotics to which fish are possibly exposed (WHO, 1993). In fish, kidney collects and filtrate a large amount of postbranchial blood and so renal lesions might be expected to be good indicators of environmental pollution (Hinton and Lauren, 1990; Camargo and Martinez, 2007).

Seferani Lake, is one of three biggest among 85 karstic lakes found in Dumrea region, central-north Albania. During two last decades, this freshwater Lake, is facing with an intense anthropogenic activity. Local agriculture is mainly relied on cultivation of wheat, corn and tobacco. Shifting the land use towards agriculture brought about several problems related to the extensive use of pesticides and herbicides, often favored by the lack of control by the authorities. The majority of Dumrea karst lakes have been used for irrigation without any restriction or regards to the consequent detrimental effects on the biota, reducing it critically to values below biological minimum. Hence, the natural lacustrine ecosystems are declining severely leading to the disappearance of many aquatic species, including fishes (Qiriazi and Sala, 2000). In addition, further developments in the area contributed to reduce of water quality and increase pollution due to the discharge of liquid waste and chemicals directly into the lakes, and intensive use of herbicides and pesticides for agricultural activity (Selenica, 1998; unpublished data from Sula, 2018, 2019).

Until now, few studies on the effects of pollution of Seferani Lake on its biota have been conducted, viz. its microbiological load (Mali and Shumka, 2011), and histopathological changes of fish liver tissue (Sula and Aliko, 2017). The present study aimed to investigate the potential toxic effects of organochlorine pesticides and PCBs found in Seferani Lake, on feral freshwater crucian carp using kidney histopathology as an endpoint. A reference site, Dushku Bulcarit Lake, was used to allow comparison between a polluted and organochlorine and PCBs-free lake. To evaluate quantitatively tissue damages, an organ index mathematical model was applied. In addition, an identification of cellular alterations resulting from natural stressors other than contaminants, is also discussed.

Materials and methods

Fish collection: In total, thirty live adult male fish (six individuals at each station) with total body length of 18.7±2.4 cm, and weight of 183.9±24.5 g, were collected from the Seferani Lake during April 27 and May 11, 2019. Since the water data analysis showed no variation between stations, all individuals were pooled regarding the histopathological analysis. Before the experiments, fish were anesthetized with a 0.75% aminobenzoic acid ethyl ester (MS-222) solution and pH adjusted to 7.7 with $NaHCO_2$. The body cavity was opened and the kidneys were immediately removed and fixed into 10% formaldehyde solution. A total of five reference crucian carp fish were caught in the same period from Dushku Bulcarit Lake, Gramshi district, north east of study area as control. Dushku Bulcarit Lake was selected as a reference site due to its pristine natural conditions, its proximity to Seferani Lake, and absence of any anthropogenic influences.

Water analysis: Water samples were taken at 5 stations

Table 1. Histopathological assessment tool for fish kidney. An importance factor (w) ranging from 1 to 3 is assigned to every alteration: it is composed of the respective organ (org), the reaction pattern (rp), and the alteration (alt)*. # Extracted from (Bernet et al., 1999).

Reaction pattern	Functional unit of tissue	Alteration	Importance factor	Score value	Index
Circulatory disturbances		Hemorrhage/ hyperemia/aneurysm	$w_{KC1}=1$	a_{KC1}	I_{KC}
		Intercellular edema	$w_{KC2}=1$	a_{KC2}	
Regressive changes	Tubule	Architectural and structural alterations	$w_{KR1}=1$	a_{KR1}	I_{KR}
		Plasma alterations	$w_{KR2}= 1$	a_{KR2}	
		Deposits	$w_{KR3}= 1$	a_{KR3}	
		Nuclear alterations	$w_{KR4}= 2$	a_{KR4}	
		Atrophy	$w_{KR5}= 2$	a_{KR5}	
		Necrosis	$w_{KR6}= 3$	a_{KR6}	
	Glomerulus	Architectural and structural alterations	$w_{KR7}= 1$	a_{KR7}	
		Plasma alterations	$w_{KR8}= 1$	a_{KR8}	
		Deposits	$w_{KR9}= 1$	a_{KR9}	
		Nuclear alterations	$w_{KR10}= 2$	a_{KR10}	
		Atrophy	$w_{KR11}= 2$	a_{KR11}	
		Necrosis	$w_{KR12}= 3$	a_{KR12}	
	Interstitial tissue	Architectural and structural alterations	$w_{KR13}= 1$	a_{KR13}	
		Plasma alterations	$w_{KR14}= 1$	a_{KR14}	
		Deposits	$w_{KR15}= 1$	a_{KR15}	
		Nuclear alterations	$w_{KR16}= 2$	a_{KR16}	
		Atrophy	$w_{KR17}= 2$	a_{KR17}	
		Necrosis	$w_{KR18}= 3$	a_{KR18}	
Progressive changes	Tubule	Hypertrophy	$w_{KP1}=1$	a_{KP1}	I_{KP}
		Hyperplasia	$w_{KP2}= 2$	a_{KP2}	
	Glomerulus	Hypertrophy	$w_{KP3}=1$	a_{KP3}	
		Hyperplasia	$w_{KP4}= 2$	a_{KP4}	
		Thickening of Bowman's capsular membrane			
	Interstitial tissue	Hypertrophy	$w_{KP5}=1$	a_{KP5}	
		Hyperplasia	$w_{KP6}= 2$	a_{KP6}	
Inflammation		Exudates	$w_{KI1}=1$	a_{KI1}	I_{KI}
		Activation of RES	$w_{KI2}=1$	a_{KI2}	
		Infiltration	$w_{KI3}= 2$	a_{KI3}	
Tumor		Benign tumor	$w_{KT1}= 2$	a_{KT1}	I_{KT}
		Malignant tumor	$w_{KT2}= 3$	a_{KT2}	
					$I_{K.}$

at 1.5 m depth. One liter of water was analyzed for each sample for physico-chemical characteristics of lakes water and presence of organochlorine pesticides and PCBs. The physico-chemical characteristics of the water were determined according to APHA (1998). The water quality parameters of Seferani Lake are shown in Table 1, where pH = 7.8, temperature = 19.73±1.34°C, DO = 3.91±1.38 mg/l, ammonium (NH^{4+}) = 1.86±0.12 mg/L, sulphates = (SO_4^{2-}) 1.65±0.41 mg/L, nitrites (NO^{2-}) = 0.4±0.02 mg/L, total phosphate = 2.95±0.3 mg/L, and N-NO_3 = 3.26±1.42 mg/L. Meanwhile, the reference site, Dushku Bulcarit Lake, showed a normal situation with: pH 8.0, temperature 19.89±2.1°C, DO 9.1±0.2 mg/l, ammonium (NH^{4+}) 0.07±0.02 mg/L, nitrites (NO^{2-}) 0.012±0.01 mg/L, and sulphates (SO_4^{2}), total phosphate and N-NO_3 in non-available amounts.

Analysis of pesticide and PCBs was done using gas chromatography HP 6890 series II with electron capture detector (GC/ECD). RTX-5 capillary column and detector ECD was used for the separation and detection of organochlorine and PCBs compounds. Quality and quantity analysis of pesticides and PCBs was based on EPA 8081B standard.

Table 2. Pesticides and PCBs measured concentrations in Seferani and D. Bulcarit Lakes

Parameter	Seferani Lake	D. Bulcarit Lake
Pesticides		
alfa-HCH	5.57±0.3 ng/L	n/a
beta-HCH	14.6±1.02 ng/L	n/a
Dieldrin	660.87±2.1 ng/L	n/a
4.4'-DDD	755.31±2.34 ng/L	n/a
EndosulfanSulfat	546.92±2.34 ng/L	n/a
PCB-s		
PCB 28	48.75±1.03 ng/L	n/a
PCB 52	145.14±3.1 ng/L	n/a
PCB 101	19.62±1.23 ng/L	n/a
PCB 118	17.47±2.01 ng/L	n/a
PCB 153	623.33±4.1 ng/L	n/a
PCB 209	203.66±2.61 ng/L	n/a

Histopathogical analysis: Histopathology was conducted based on Sula and Aliko (2017). After fixation, samples of the kidney were dehydrated in an ethanol series, cleared in xylene and embedded in paraffin wax and sectioned at 5 µm. After dewaxing with xylene and hydration in ethanol series of decreasing concentration, sections were stained with hematoxylin and eosin (Luna, 1968) and examined under a light microscope. Cytological results were expressed as a prevalence of fishes with histopathological lesions, the kidney lesion index was determined according to Bernet et al. (1999). This index is based on the sum of the score rankings (a) and importance factors (w) for each considered lesion in the tissue (Table 1). The score ranking was based on the percentage of lesions ranging from 0 to 6 depending on the degree and extent of alteration: (0) unchanged; (2) mild; (4) moderate; and (6) severe. Intermediate values were also considered. The importance factor (w) was determined for each lesion depending on the effects considered as minimal (1), moderate (2) and irreversible (3).

Mathematical calculation of lesion indices: Because the lesions within one organ are studied, two indices are applicable (Bernet et al., 1999).

Reaction index of an organ $(I_{org\ rp}) = \sum (a_{org\ rp\ alt} \times w_{org\ rp\ alt}) / alt$

Where: *org* = organ (constant); *rp* = reaction pattern; *alt* = alteration; *a* = score value; *w* = importance factor. The quality of the lesions in an organ is expressed by the reaction index.

Organ Index (I_{org}): $\sum \sum (a_{org\ rp\ alt} \times w_{org\ rp\ alt}) / rp\ alt$

Where: *org* = organ (constant) (for abbreviations see reaction index formula). This index represents the degree of damage to an organ.

Statistical analysis: Statistical analyses were refined by analysis of variance (ANOVA) using SPSS software, version 15. One-way analysis of variance (ANOVA) was used to test the level of significance at 0.05 level of probability for the pesticide and PCBs residue levels and kidney tissue alterations. A Pearson correlation analysis (PCA) was used to determine the relationship among the PCBs and pesticides and organ index coefficient. All data were expressed as mean ± SED. The significance was assigned for $P<0.05$.

Results

Water analysis: Water quality data regarding pesticide and PCBs amounts measured in Seferani Lake, are showed in Table 2. No pesticide residue was determined in the water samples of Dushku Bulcarit Lake. Among the pesticides, dieldrin (660.87±2.1 ng/L), 4.4”-DDD (755.31±2.34 ng/L) and endosulfan (546.92 ± 2.34 ng/L) were the components found at appreciably higher concentration. Meanwhile, PCB 52 (145.14±3.1 ng/L), PCB153 (623.33±4.1 ng/L) and PCB209 (203.66±2.61 ng/L) were in the highest

Table 3. Reaction Indexes and Organ Index of C. carassius fish kidneys from Seferani and D. Bulcarit Lakes

	CarassiuscarassiusSeferani Lake																														Carassiuscarassius D. Bulcarit				
Importance factors/ Indexes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5
$w_{KC1}= 1$	2/2	-	2/2	2/2	-	-	2/2	4/4	2/2	2/2	2/2	-	2/2	2/2	2/2	2/2	4/4	2/2	2/2	-	2/2	2/2	2/2	-	2/2	-	2/2	2/2	2/2	2/2	-	-	2/2	-	-
$w_{KC2}= 1$	-	2/2	-	2/2	2/2	-	-	-	2/2	-	-	-	2/2	-	-	-	2/2	-	2/2	-	-	-	2/2	-	-	-	2/2	2/2	-	-	-	-	-	-	-
I_{KC}	2	2	2	4	2	0	2	4	4	2	2	0	4	2	2	2	4	2	4	0	2	2	4	0	2	0	4	4	2	2	0	0	2	0	0
$w_{KR1}= 1$	4/4	2/2	2/2	2/2	-	2/2	2/2	4/4	2/2	2/2	2/2	2/2	2/2	4/4	2/2	2/2	4/4	2/2	2/2	2/2	4/4	2/2	-	2/2	-	2/2	2/2	2/2	-	2/2	-	2/2	-	-	-
$w_{KR2}= 1$	4/4	2/2	2/2	2/2	2/2	2/2	4/4	-	2/2	2/2	2/2	4/4	2/2	2/2	2/2	2/2	-	2/2	2/2	2/2	2/2	-	-	2/2	-	-	-	-	4/4	-	-	-	1/1	-	-
$w_{KR4}= 2$	6/12	4/8	2/4	2/4	4/8	4/8	2/4	2/4	6/12	4/8	6/12	2/4	2/4	6/12	2/4	2/4	4/8	-	2/4	-	6/12	4/8	4/8	2/4	-	2/4	2/4	2/4	2/4	4/8	-	1/2	1/2	-	-
$w_{KR5}= 2$	4/8	2/4	2/4	4/8	2/4	2/4	2/4	2/4	2/4	4/8	2/4	-	2/4	2/4	2/4	2/4	4/8	-	2/4	2/4	4/8	-	-	2/4	-	-	-	-	4/8	2/4	-	-	-	-	-
$w_{KR6}= 3$	4/12	2/6	-	2/6	2/6	2/6	-	-	4/12	2/6	4/12	-	2/6	4/12	2/6	4/12	4/12	-	2/6	-	4/12	-	2/6	2/6	-	-	-	-	2/6	2/6	-	-	-	-	-
$w_{KR7}= 1$	4/4	2/2	2/2	4/4	4/4	4/4	2/2	2/2	2/2	2/2	2/2	2/2	4/4	4/4	-	4/4	2/2	-	4/4	2/2	4/4	2/2	4/4	4/4	2/2	2/2	4/4	4/4	4/4	4/4	2/2	-	-	-	-
$w_{KR10}= 2$	2/4	2/4	2/4	2/4	2/4	2/4	-	2/4	4/8	2/4	2/4	-	2/4	2/4	2/4	2/4	-	-	4/8	-	2/4	-	2/4	4/8	-	-	2/4	2/4	2/4	2/4	-	-	-	-	-
$w_{KR11}= 2$	2/4	-	2/4	2/4	2/4	2/4	-	2/4	2/4	2/4	2/4	-	2/4	-	-	2/4	-	-	2/4	-	2/4	-	2/4	-	-	-	2/4	2/4	2/4	2/4	-	-	-	-	-
$w_{KR12}= 3$	2/6	2/6	2/6	2/6	2/6	2/6	-	2/6	2/6	2/6	2/6	-	-	-	-	-	-	-	2/6	-	2/6	-	2/6	-	-	-	-	2/6	2/6	2/6	-	-	-	-	-
$w_{KR14}= 1$	2/2	-	2/2	2/2	2/2	2/2	-	-	4/4	2/2	2/2	-	2/2	2/2	2/2	-	2/2	2/2	2/2	-	4/4	-	2/2	2/2	2/2	-	-	2/2	-	-	-	-	-	-	-
I_{KR}	60	34	30	42	40	42	18	28	56	44	50	12	32	44	24	34	36	6	42	10	60	12	32	32	4	8	18	26	40	38	2	4	3	0	0
$w_{KP1}= 1$	4/4	2/2	2/2	2/2	2/2	2/2	-	2/2	4/4	2/2	4/4	-	4/4	4/4	-	4/4	2/2	-	4/4	-	4/4	-	4/4	4/4	-	-	-	4/4	2/2	4/4	1/1	-	1/1	-	-
$w_{KP2}= 2$	-	2/4	2/4	4/8	4/8	2/4	-	2/4	4/8	4/8	4/8	-	4/8	2/4	2/4	2/4	2/4	2/4	2/4	-	4/8	-	2/4	2/4	-	-	-	-	2/4	2/4	-	-	-	-	-
$w_{KP3}= 1$	2/2	2/2	2/2	4/4	2/2	-	-	4/4	4/4	4/4	4/4	-	2/2	2/2	2/2	2/2	2/2	2/2	2/2	-	4/4	-	-	2/2	-	-	-	4/4	2/2	2/2	-	-	-	-	-
$w_{KP4}= 2$	2/4	2/4	2/4	2/4	2/4	-	-	2/4	2/4	-	-	-	-	4/8	4/8	-	-	-	4/8	-	2/4	-	-	-	-	-	-	2/4	2/4	2/4	-	-	-	-	-
$w_{KP6}= 2$	2/4	2/4	-	2/4	-	-	-	-	2/4	-	2/4	-	-	2/4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I_{KP}	14	16	12	22	16	6	0	14	24	14	20	0	14	22	14	10	8	6	18	0	20	0	8	10	0	0	0	12	12	14	1	0	1	0	0
$w_{KI3}= 2$	4/8	2/4	-	2/4	-	-	-	-	4/8	2/4	4/8	-	-	2/4	-	-	-	-	4/8	-	4/8	-	-	-	-	-	-	-	2/4	4/8	-	-	-	-	-
I_{KI}	8	4	0	4	0	0	0	0	8	4	8	0	0	4	0	0	0	0	8	0	8	0	0	0	0	0	0	0	4	8	0	0	0	0	0
I_K	84	56	44	72	58	48	20	46	92	64	80	12	50	72	40	46	48	14	72	10	90	14	44	42	6	8	22	42	58	62	3	4	6	0	0
Mean I_K	47.2																														2.6				

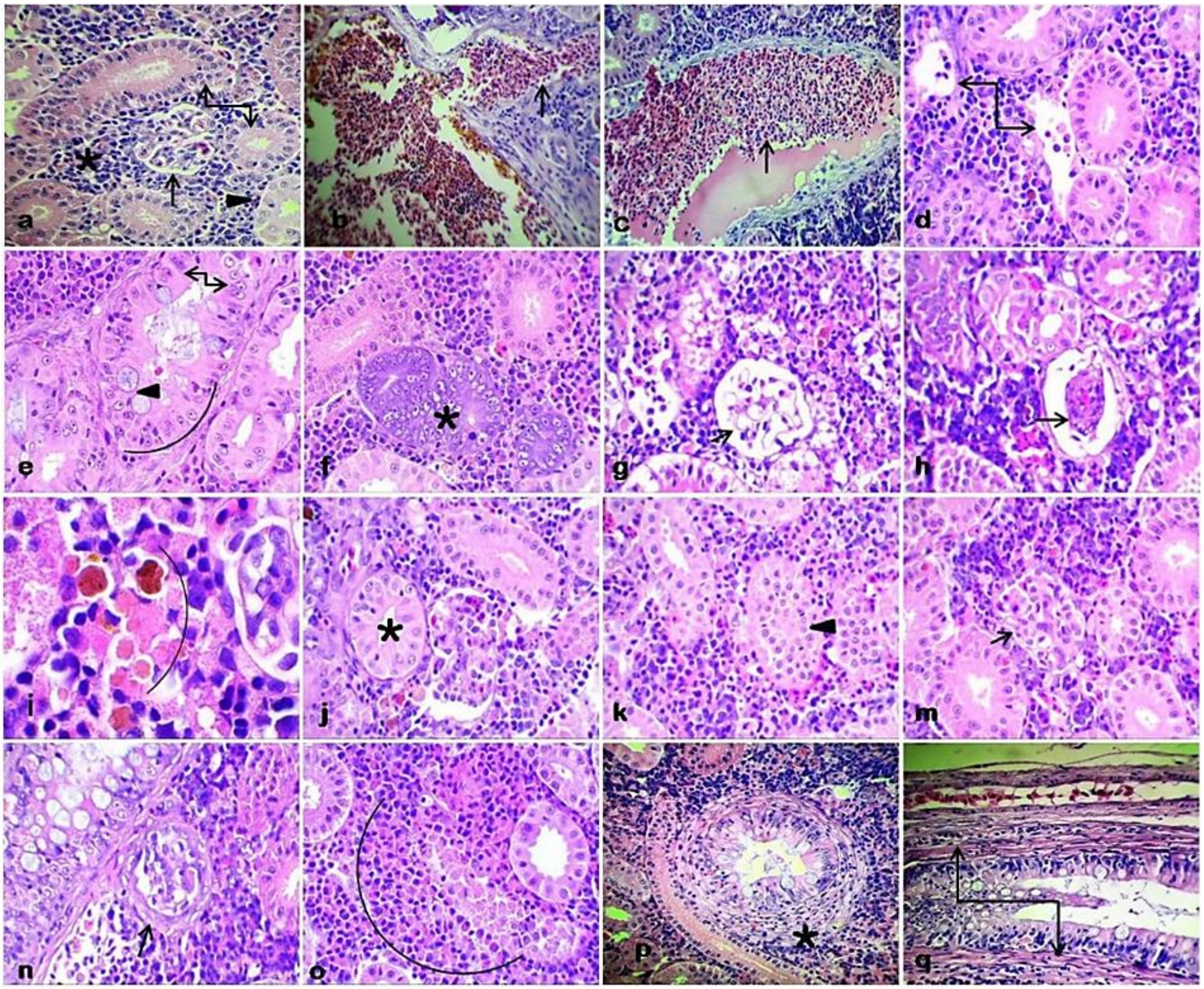

Figure 1. Photomicrographs of kidneys of *Carassius carassius* fishes caught in Bulcarit and Seferan Lakes. All sections are stained with H&E. (a) Normal kidney tissue showing glomerulus and Bowman's space well defined (arrow), hematopoietic tissue (asterisk), proximal tubules (double arrow), and distal tubule (arrow head), (b) Rupture of an artery and hemorrhage (arrow), (c) Blood congestion, hyperemia (arrow), (d) Intercellular edema of hematopoietic tissues (double arrow), (e) Tubule with architectural and structural cell alterations (arc), plasma alterations (arrow head), and nuclear alterations (double arrow), (f) Tubules showing atrophy and necrosis (asterisk), (g) Glomerulus with architectural and structural variations, alterations of nucleuses of epithelial endothelial cells and podocytes (arrow), (h) Atrophy and necrosis of glomerular cells (arrow), (i) plasma alterations, hyaline degeneration of interstitial tissue (arc), (j) Hypertrophy of tubular cells (asterisk), (k) Increase in number of tubule cells known as hyperplasia (arrow head), (m) Hypertrophy of glomerulus cells occupying all renal corpuscle (arrow), (n) Thickening of Bowman's capsular membrane (hyperplasia), (o) Hyperplasia of hematopoietic leukocyte cells (arc), and (p-q) Infiltration of fibroblasts, neutrophils, monocytes and macrophages known as peritubular fibrosis (asterisks and double arrow).

concentrations among PCBs detected in the sites. All pesticide and PCBs values are considered higher than the reported values of their toxicity for cyprinid fish (EPA, 2001; Helfrich et al., 2009; Jayraj et al., 2016).

Kidney tissue morphology: The histopathological observations of *C. carassius* are shown in Figure 1, and calculated indexes in Table 3. Circulatory disturbances, inflammation, regressive and progressive changes of tubules, glomerulus and interstitial tissue were the important histopathological alternations found in the fish of the study site, compared to the reference Lake. *Carassius carassius*' kidneys have displayed various alterations related to circulatory viz. hemorrhage and hyperemia, tubular

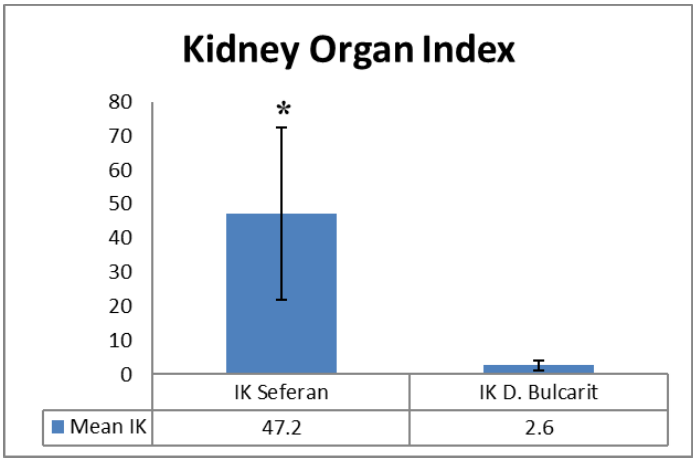

Figure 2. Mean kidney index differences between Seferani and D. Bulcarit lake fishes. The values given are Mean±STD. Significance * = P<0.05.

and glomerular alterations like nuclear and plasma alterations, atrophy, hypertrophy and hyperplasia of tissue cells, even tubular and glomerular focal necrosis. (Fig. 1; b-q). The fish of Dushku Bulcarit i.e. reference Lake, showed a normal kidney structure, consisting of normal glomerulus, a well-defined Bowman's capsule, and renal tubules (Fig. 1).

Organ index for all crucian carp fish from Seferani Lake revealed significantly higher values ranging 6 to 92, with a mean of 47.2, indicating a moderate occurrence (Table 3). Meanwhile, that of Dushku Bulcarit i.e. reference site, had a mean organ index of 2.6 (Fig. 2). A straightforward correlation of kidney hitopathological status and pesticide and PCBs concentrations analysed for Seferani Lake, established a weak correlation of r=0.0346 (P<0.05).

Discussions

The present study revealed that crucian carp inhabiting the Seferani Lake is affected by combined toxic effects, such as moderate hypoxia and chronic sub-lethal pesticide and polychlorinated biphenyl (PCBs) exposure. Intensive and long-term use of pesticides in agriculture, especially in developing countries, have increased due to their persistence in nature. Additionally, the fast urbanization can contribute to increase influent of urban wastes to the lake, decreasing the water quality. Chemical analyses are essential to evaluate water quality as they provide information for types of xenobiotics like organochlorines that influence water biota. It has been estimated that only a small amount, about 0.1% of pesticides reach the target organisms for which they are used; the remaining contaminates the surrounding environment (Carriger et al., 2006). Compounds such as pesticides and PCBs are among those substances which are capable to cause endocrine, genetic, or severe reproductive problems in fauna and also qualified for inclusion under the broad heading of substances which possess carcinogenic properties which all together lead to death of organisms (EPA, 2001).

In the present study, water analysis revealed presence of various toxic organochlorine pesticides and PCBs such as DDD, Dieldrin, Endosulfan-sulfat and PCB 153 in sub-lethal concentrations. The retention of PCBs in tissues, especially those with high lipid concentration is linked to the degree of chlorination, higher chlorinated compounds persist for long periods of time. PCBs induce interactive effects like increased kidney toxicity of trichloroethylene (EPA, 1999). DDT and Dieldrin are banned in United States for survival of fish and protection of water quality (Helfrich et al., 2009). Dieldrin has extreme nonpolar properties that result in a high affinity for organic matter with strong tendencies to adsorb sediments and accumulate in the tissue of aquatic biota, where they can persist for long time (ATSDR, 1993; Nowell et al., 2000). The results showed a great concentration of dieldrin, approximately 0.66 µg/l compared to HRL (Health Reference Level) i.e. 0.002 µg/l (based on estimated excess lifetime cancer risks of 10^{-6}) (EPA, 2003). Occurrence of dieldrin is high in fishes, which highlight the potential of bioaccumulation for this pesticide (Kolpin et al., 1998).

Endosulfan sulfate is banned in many countries (Wan et al., 2005); it is a derivate of endosulfan but more stable in environment (Hose et al., 2003). According to Helfrich (2009), endosulfan is classified at category "Super" with LC50 <10 µg/l (Lethal Concentration) related to its toxicity. In the present study, Endosulfan-sulphate value was 0.00055 mg/l that is half of 96-hour LC_{50} (0.001 mg/l) to for rainbow trout and Bluegill (Helfrich et al., 2009). Endosulfan is extremely toxic to aquatic organisms, especially fish. It has a relatively high potential to bio-accumulate in fish tissues (UNEP and GEF, 2002). The subacute and chronic toxicity studies of endosulfan in animals suggest that liver, kidneys and immune system are the main target organs (CalEPA, 2008).

Teleost kidney act as an excretory route for all xenobiotic metabolites which fish may be exposed and consequently are one of the first organs to be affected (Hinton et al., 1992; Thophon et al., 2003; Fazio et al., 2015; Moyson et al., 2016). The exposure of fish to sub-lethal concentration of organic compounds such as pesticides may result in different histopathological alterations in specific tissues (Altinok and Capkin, 2007; Ghelichpour and Mirghaed, 2019). Important changes related to circulatory disturbances, inflammation, regressive and progressive changes of tubules, glomerulus and interstitial tissue are found on fish from the polluted site, compared to the reference one. In our case, *C. carassius*' kidneys have displayed various alterations that corroborate with findings of Pandey et al. (1993), who described histopathology of estuarine mullet *Liza parsia* exposed to DDT kidney with eosinophilic material in cell plasma, edema, hypertrophy of epithelial cells, karyopyknosis, karyolysis and fibrosis, shrinkage of glomeruli and focal necrosis. McHugh et al. (2011) also showed hyaline degeneration, vacuolation of the renal tubule and dilation of the glomerulus capillaries in tiger fish, *Hydrocynus vittatus,* kidneys exposed to DDT in a natural habitat.

An interesting finding of this study was the presence of small granules in the cytoplasm, which later, can progress to hyaline degeneration. These granules may be formed inside of cells or by the reabsorption of plasma proteins lost in the urine by glomerular damage, which can lead necrosis (Hinton and Lauren, 1990; Takashima and Hibiya, 1995; Boran et al., 2010). Altinok and Capkin (2007) studied rainbow trout exposed to endosulfan and described that kidney of fish had eosinophilic exudates on glomeruli, occluded glomerular capillaries and segregation of tubular epithelium from connective tissue. They observed also necrosis of hematopoietic, glomerular and tubular cells. Capkin et al. (2006) showed that kidney was the most affected organ of rainbow trout treated with endosulfan. Assessing the histopathology of *C. carassius* kidneys exposed to dieldrin, Satyanarayan et al. (2012) described presence of vacuolated cells in glomeruli, atrophy of glomeruli, and hematopoietic and tubular necrosis. Such feedback alterations might be due to variations in type, strength, and persistence of a stressful chemical factor to which the fish is exposed.

The high incidence of histological alterations in the kidney of *C. carassius* living in Seferani Lake, indicates their chronic expose to toxic substances in their natural habitat. Therefore, the renal lesions might be expected to be good bio-indicators of environmental pollution in this species. Inevitably, all kidney damages reported here for *C. carassius*, are expected to impair the osmoregulatory function of the fish, since their kidney is not only an excretory organ but, also an osmoregulatory one. In addition, as a long-term effect, the impaired organ structure and function can directly affect survival, growth and reproduction of the organism (Wahli, 2002). Different studies support the fact that organ histopathological changes integrate the impact of a variety of stressors (Schmidt-Posthaus et al., 2001; Au, 2004).

Histopathological assessment is subjective and often it is related to proficiency of the researcher. It is a descriptive method and quantitation is rather challenging. Usually, a histopathological assessment is done routinely using light microscopy (LM), and extend of a tissue alteration is categorized as either mild, moderate or a severe change (Cengiz, 2006) or as a percentage of altered tissue (Benli et al., 2008). For a better semi-quantitative evaluation of the severity of tissue damages, a mathematical approach of calculating organ index, named Bernet's system (1999) which sum up all detected changes in a tissue, being so a parameter that measures the importance factor and type and/or severity of the change. According to Bernet' scoring system, a definition of certain patterns of tissue response to pollutants, can be achieved. Using this mathematical approach, we calculated that the mean organ index for all fishes was 47.2 indicating moderate occurrence. Values of organ indexes ranged 6-92 indicating that in most cases kidney possess lesions of severe damages of tissues.

In the current study, a weak positive correlation between nature and intensity of kidney damages and water concentrations of pesticides and PCBs, does not necessarily mean that these pollutants are not the direct responsible actors, but can support the idea that under the complex exposure situation in the field, with the presence of other co-stressors, establishing straightforward relationships between a single stressor and a biological response can be complicated (Segner, 2005; Zimmerli et al., 2007). In this context, the importance of taking into consideration of mixture effects for biological responses has to be emphasized, including combination of chemicals (Silva et al., 2002) as well as combinations between chemicals and physical or biological stressors. In our previous studies on the same ecosystem, we found a significant increased level of cortisol, plasmatic glucose and oxidative stress enzymes accompanying liver histological alterations in this fish (Morina et al., 2013; Sula and Aliko, 2017). The results of this study suggest that crucian carp at Seferani Lake show poor health status than those of Dushku Bulcarit i.e. reference site. The conclusion on a decreasing health status of fish in Seferani Lake is based mainly on the kidney histopathological findings. However, to get a full tableau of the complex interactions between pollution and histological changes, other parameters such as sediment chemistry and bioaccumulation must be assessed in conjunction with.

As conclusion, the results of this study showed that the histopathology of kidney is a useful biomarker for monitoring fish health. Histological changes appear as medium-term responses to sub-lethal stressors, considering their intermediate location with regard to the level of biological organization, and histology represents a rapid method to detect effects of stressors, especially chronic ones, in various tissues and organs. Organ index mathematical model represents an integrative indicator of fish conditions and offers a better evaluation of the severity of tissue damages. As such, it can be successfully applied to better predict the severity of xenobiotic toxicity on fish tissue level, and in comparison, between two or more polluted sites, including different freshwater ecosystems, such as streams, rivers, lakes and fish farms.

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