The impact of the Sava River pollution on biomarkers response in the liver and gills of three cyprinid species

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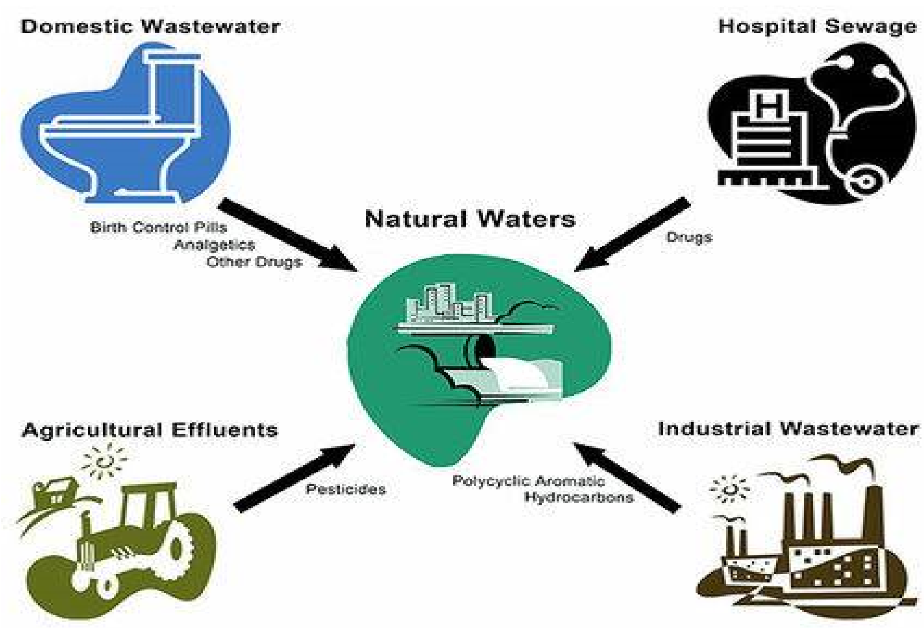
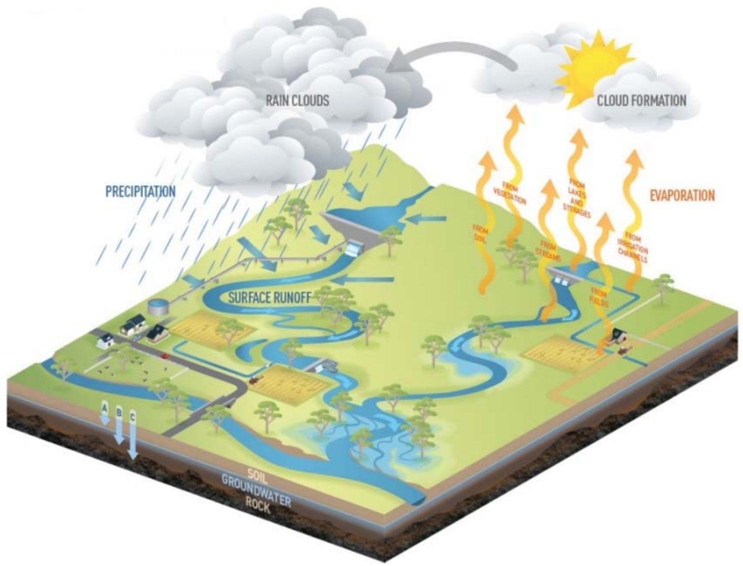
INTRODUCTION

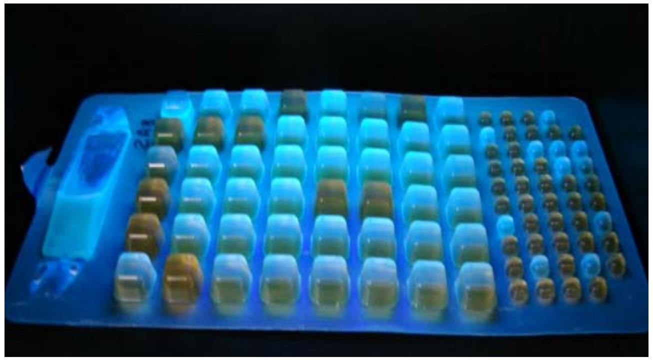
- Industrial effluents, agricultural runoff and domestic wastewaters- complex mixtures of unknown substances
- Chemical analyses- not sufficient to describe adverse effects on biota
- Climatic change- changes in the frequencies of extreme events
- Monitoring of seasonal changes in surface water quality- proper assessment of pollution impact from both anthropogenic and natural sources

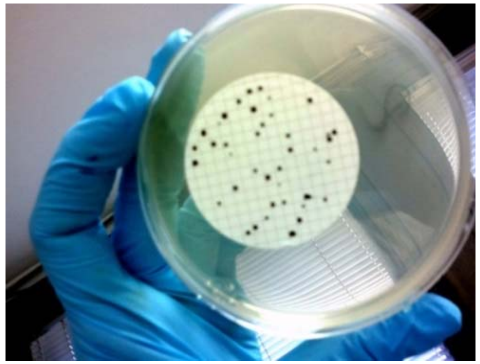




Microbiological indicators

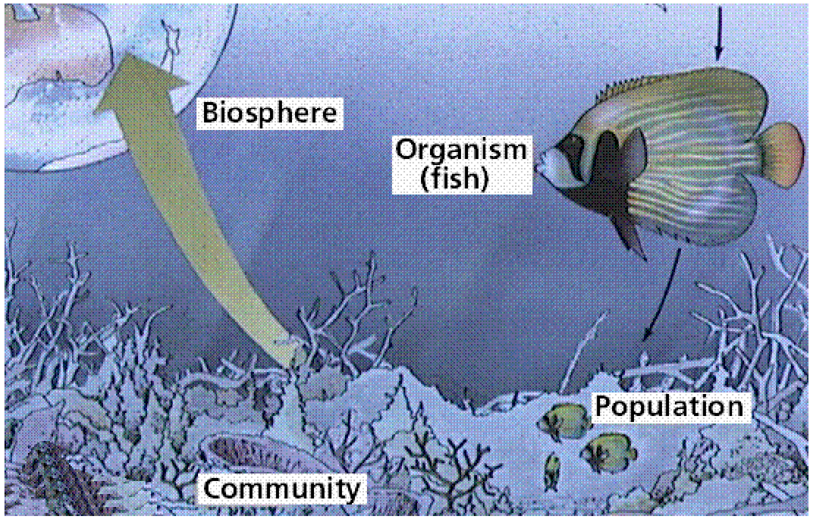
- Pollution with faecal material, represent a high health risk for all exposed organisms
- Monitoring the presence of microbiological indicators of the faecal pollution
- Coliform bacteria, *E. coli*, and enterococci, are considered as valuable indicators in the monitoring of the faecal pollution
- Faecal spore-forming bacteria *C. perfringens*- consistent faecal pollution or pollution that emerged in the past

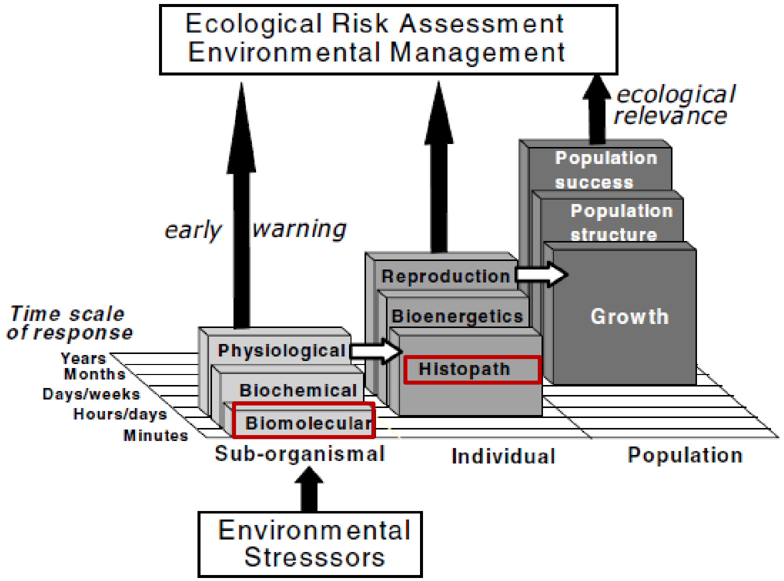




Biomarkers response

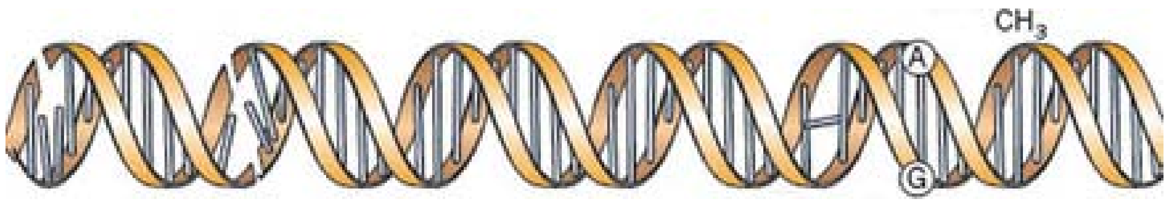
- Genotoxic effects- mutations and alterations on higher levels of biological organisation
- **Multi-biomarker approach**- combined use of different biomarkers- signal the exposure to contaminants (molecular level) and quantify their effects on the organism (cellular/tissue level)
- Insight on the mechanism of pollutant action and overall response of biota
- Water temperature, salinity, dissolved oxygen, diet, feeding behavior, gender and reproductive stage- influence on the biomarker response

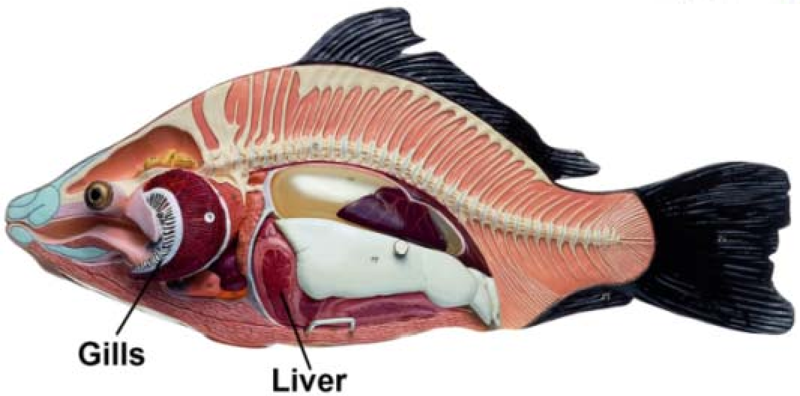


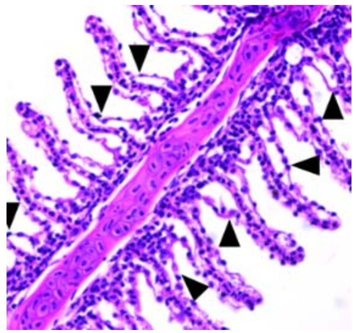


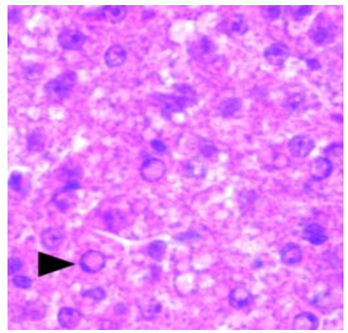
Fish as bioindicators

- Water pollution may induce many changes (biochemical alterations in single cells to changes in population)
- Fish are often used as sentinels- number of roles in the food web, bioaccumulation potential, respond to low concentrations of xenobiotics
- Gills- first organ in direct contact with water and waterborne pollutants
- Liver- metabolic breakdown of xenobiotics, controls many life functions

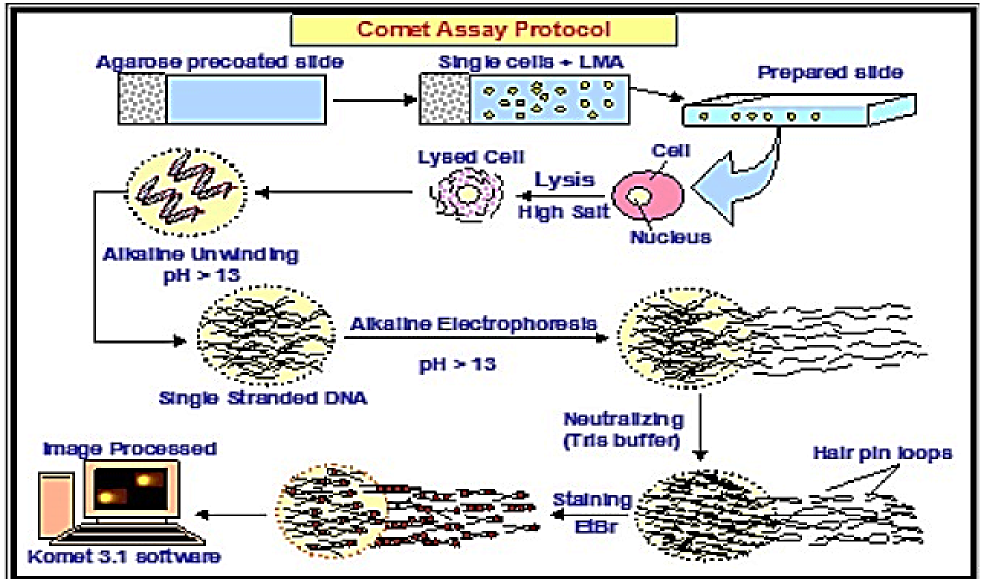








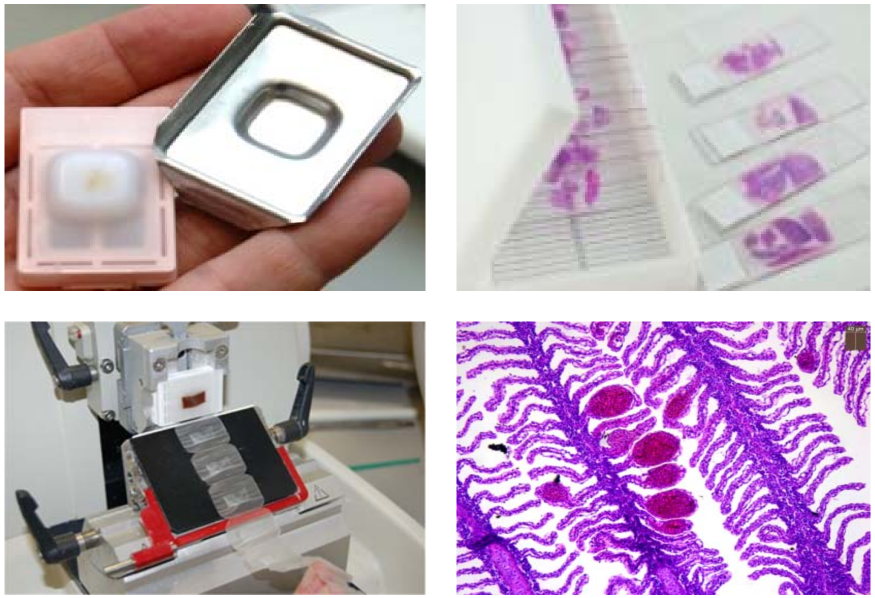
Single Cell Gel Electrophoresis (SCGE)-Comet assay

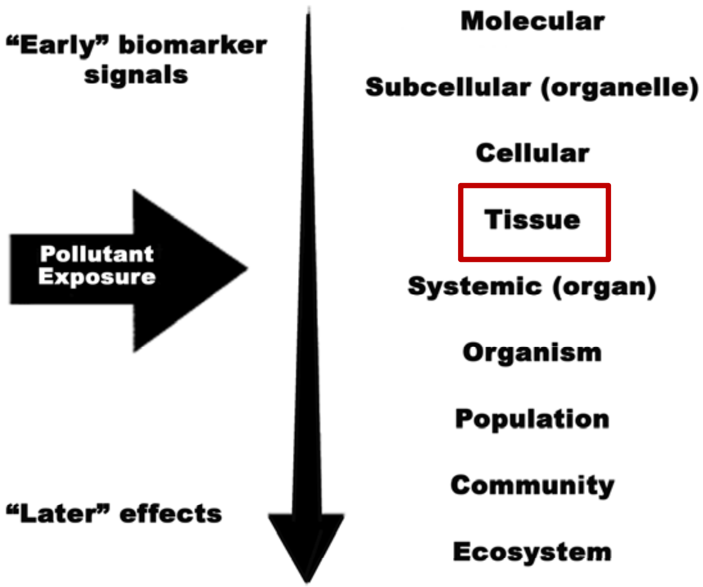


- Simple, versatile, rapid, sensitive and extensively used tool to assess DNA damage in single cells
- Widely accepted tool in ecogenotoxicology studies
- Sensitive indicator of genotoxicity and biomarker of exposure
- Cells embedded in agarose are lysed and exposed to alkaline conditions
- Single and double strand breaks, alkali labile sites, DNA-DNA crosslinks and DNA-protein crosslinks

Histopathological analyses

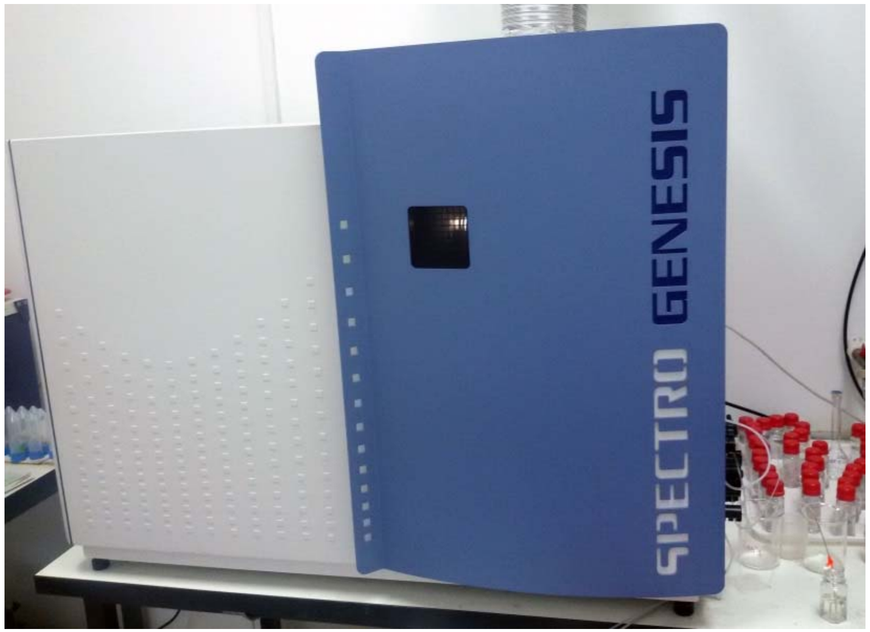
- Water pollution may induce pathological changes in fish tissues
- Endogenous and exogenous time-integrated effects on the organism
- Alterations are assessed on the middle level of biological organisation (cells, tissues, organs)- biomarker of effect

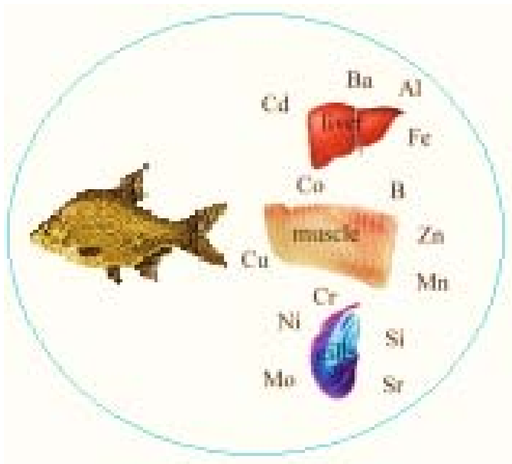




Analyses of metals and metalloids in fish tissues

- Toxicity, genotoxicity, persistance, bioaccumulation and biomagnification in the food chain
- Production of ROS- may interact with biomolecules, which could be seen as histopathological change
- Assessment of metals and metalloids in different fish tissues is extremely important





THE AIMS OF THE STUDY

- The impact of multiple stressors during different seasons on different biomarkers response in liver and gills of freshwater breams

✓ Basic chemical and physical parameters

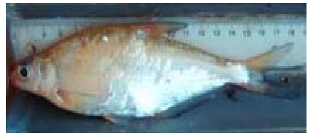
✓ Microbiological indicators of faecal pollution

✓ DNA damage- comet assay- **gills** and **liver**

✓ Histopathological alterations- **gills** and **liver**

White bream

Common bream



White-eye bream

- Four reaction patterns: circulatory, regressive, progressive and inflammatory (Bernet, 1999)
- Importance factor- pathological significance of a lesion (1-3) and score value- extent of a specific alteration (0-6)

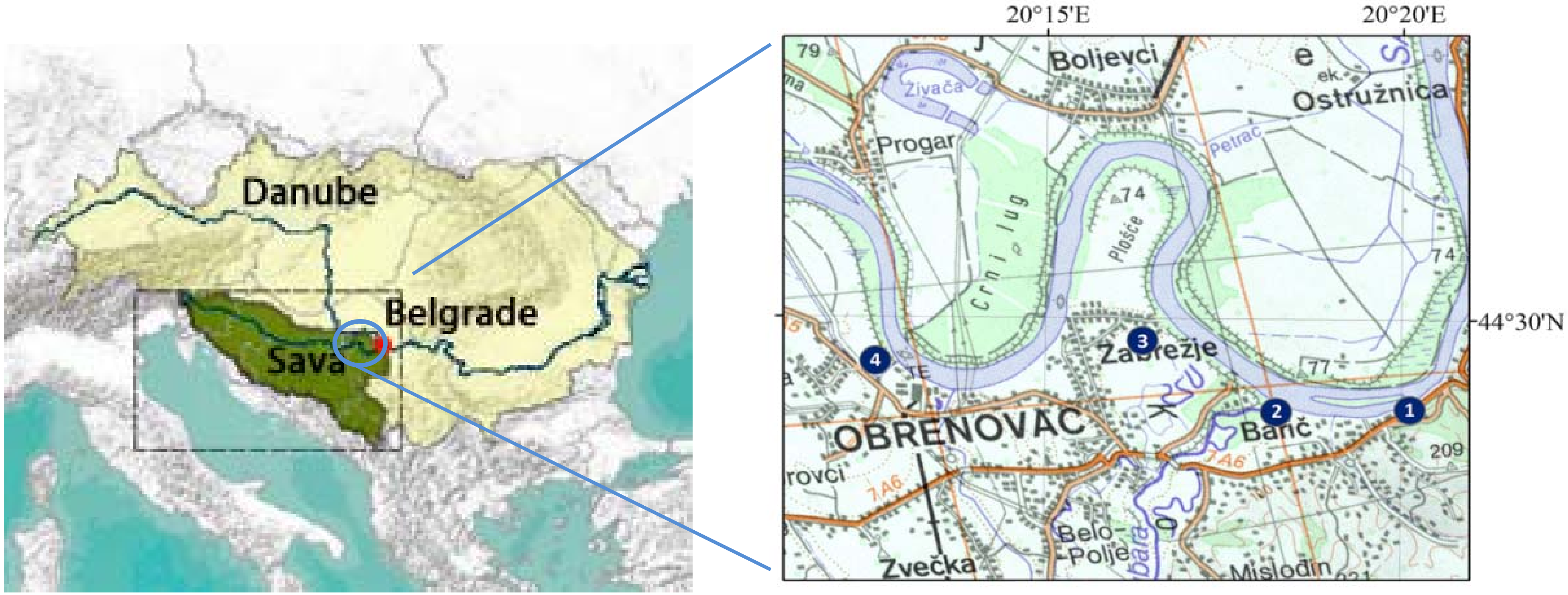
✓ Metals and metalloids concentration- ICP-OES- **gills**, **liver** and **muscle**- Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Sr, Zn

- To compare the total metal content in different tissues during different seasons metal pollution index (MPI) was calculated:

$$\mathbf{MPI = (cf1 \times cf2 \times cf3 \times \ldots cfn)^{1/n}}$$

Sampling site

- The sampling site Duboko (23 rkm), on the Sava River- untreated wastewater (town of Obrenovac-70,000 inhabitants), largest thermal power plant in Serbia (TENTA) and ash field, intensive agricultural activity

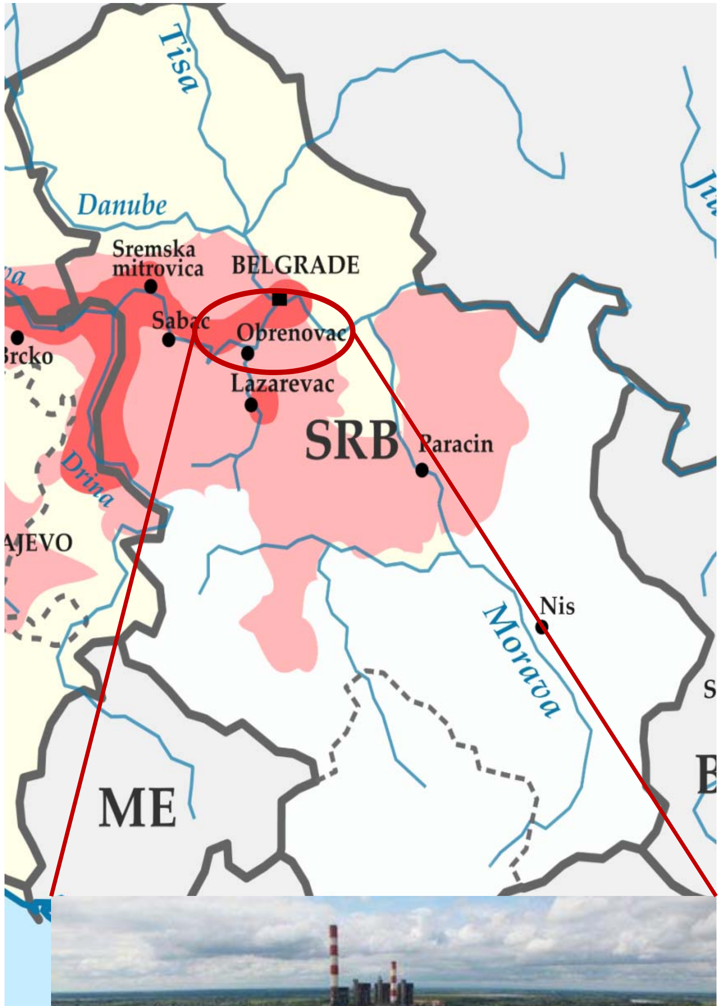


- Sampling was perfromed on monthly basis during 2014

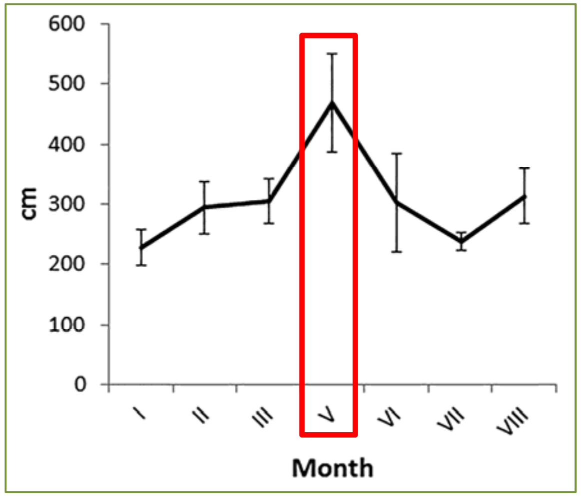
- **Winter**- January and February, **Spring**- March and early June, **Summer**- late June, July and August

Flooding event

- Extensive flooding in the mid-May 2014
- Obrenovac city most severely affected
- 90% of populated area was flooded
- Majority of inhabitants were evacuated
- Exlusion of urban wastewater discharge
- Influence of floods on the variation of measured parameters

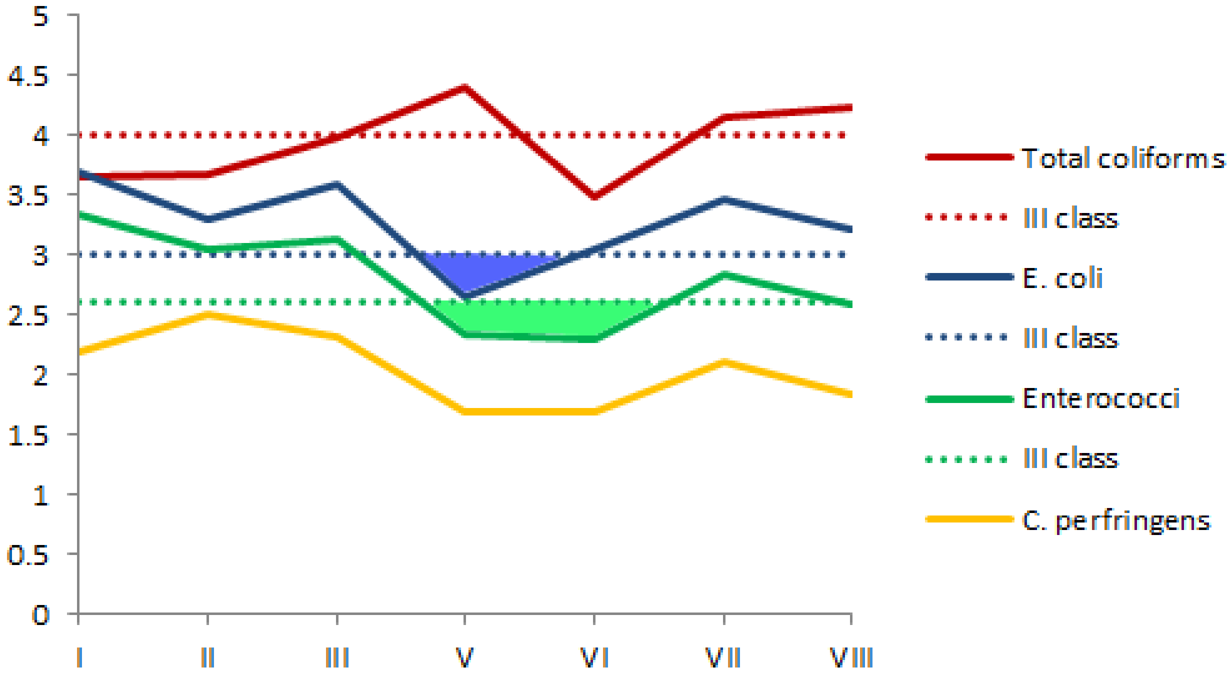


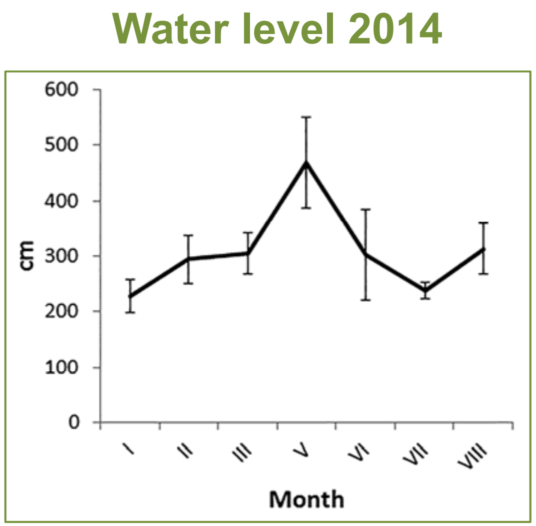
Water level 2014



RESULTS

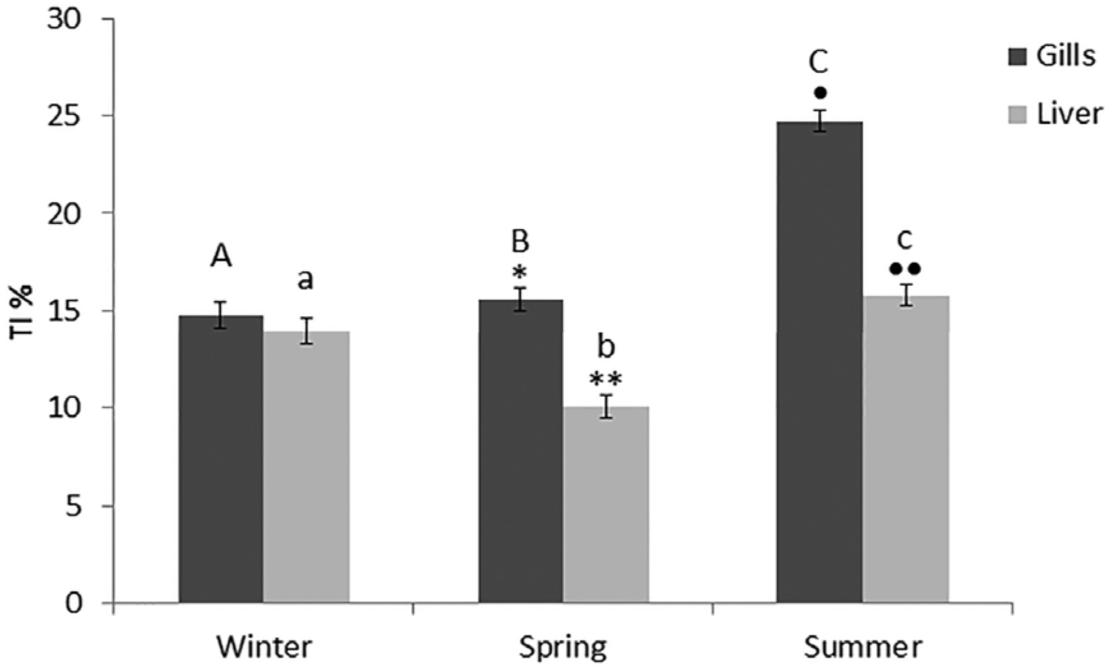
Microbiological indicators

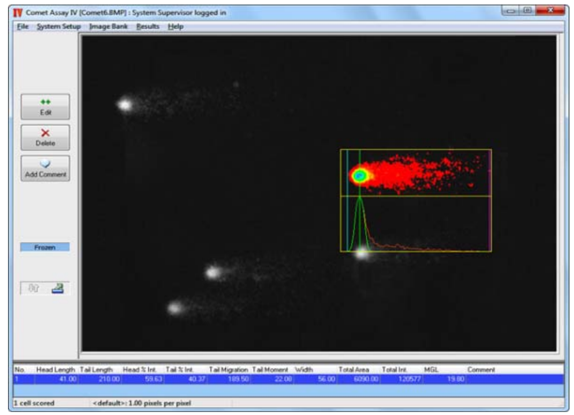




- Critical faecal pollution- present during most of the months in 2014
- *E. coli*- moderate pollution during May, Enterococci- moderate pollution during May and June
- *E. coli* and enterococci concentrations related to domestic wastewater discharge
- Total coliforms- not strictly dependent on the urban wastewater discharge

DNA damage level- comet assay





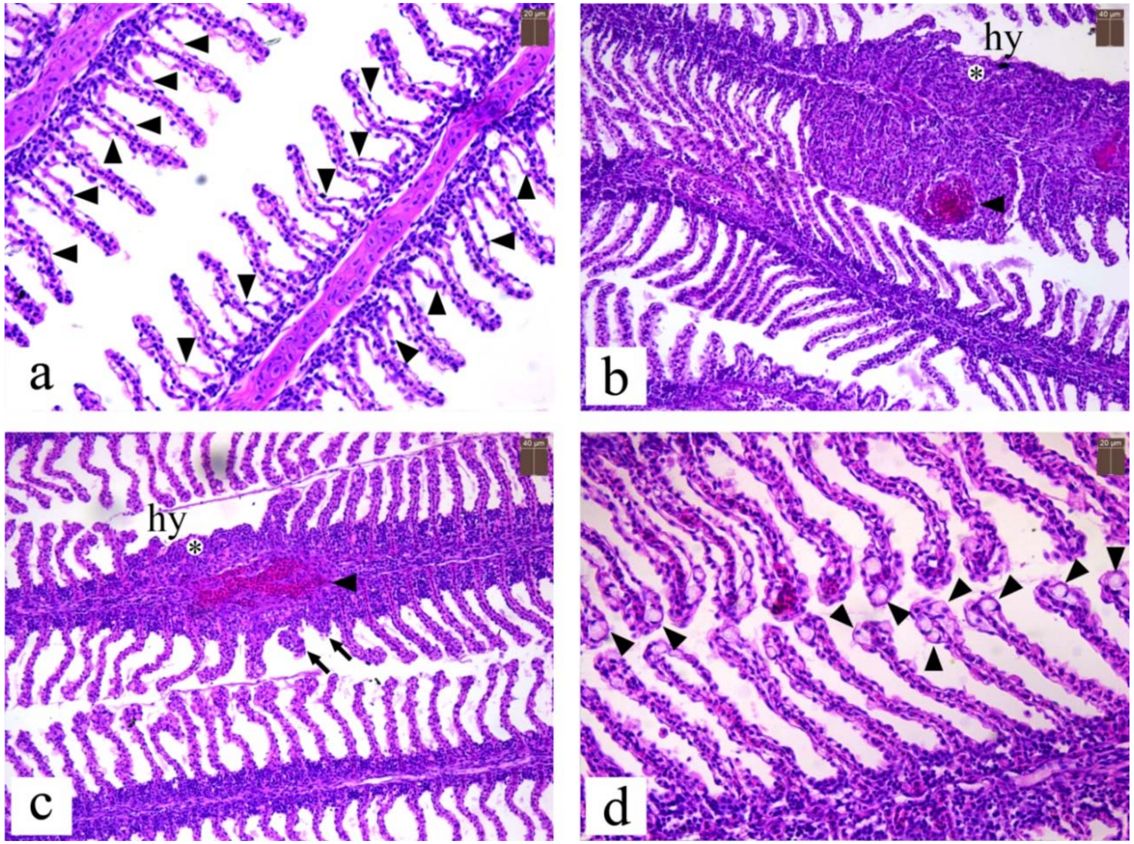
• Significant seasonal difference in DNA damage level was observed for both tissues

• Gills had the lowest level of DNA damage during winter, and liver during spring

• Both tissues had the highest level of DNA damage during summer (gills in June and liver in August)

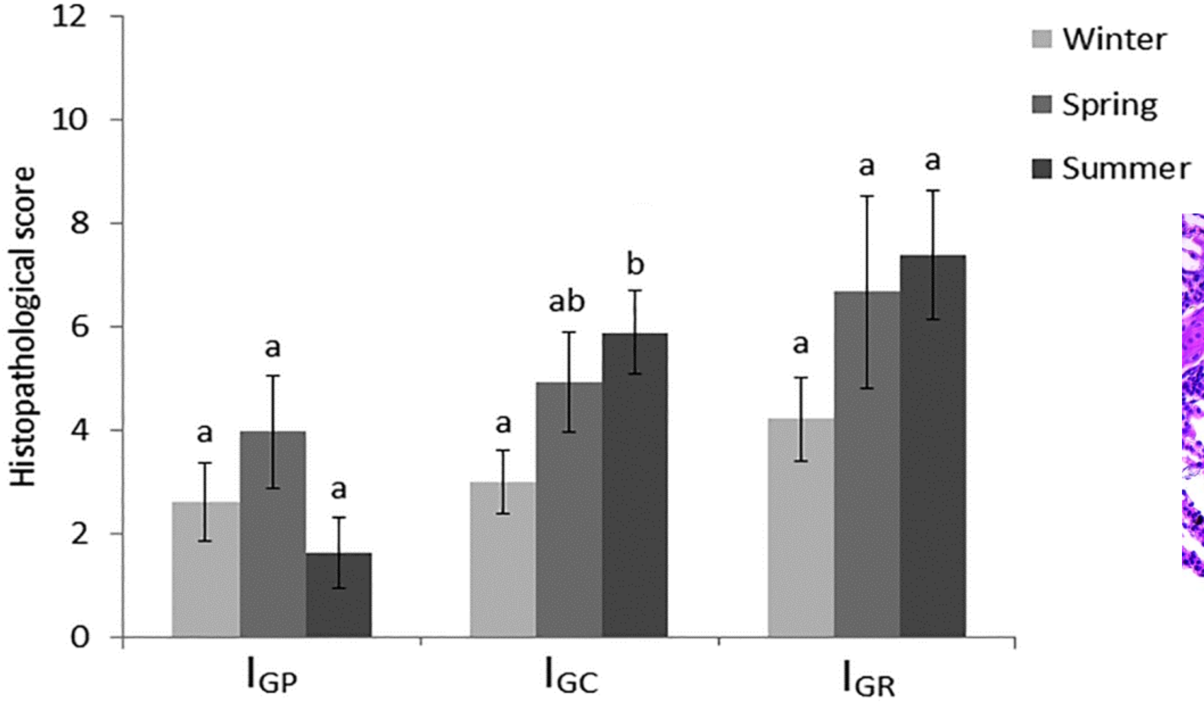
• During spring and summer DNA damage in gills was significantly higher in comparison to liver

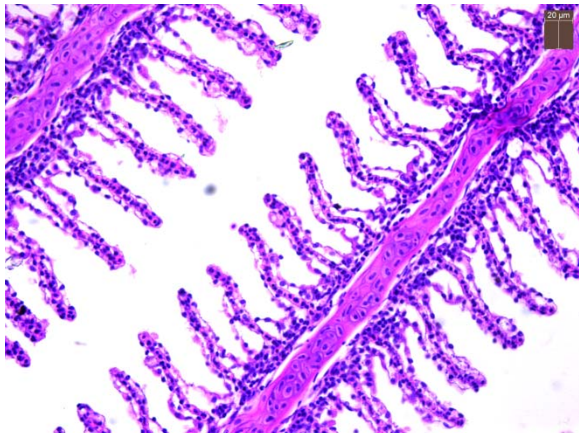
Specific histopathological alterations in gills



a) Epithelial lifting [1]- **R**
b) Hyperplasia of epithelial cells leading to complete lamellar fusions [2]- **P**, with rupture of blood vessel forming hematoma [1]- **C**
c) Hyperplasia of epithelial cells [2]- **P**, shortening of secondary lamellae [1]- **R**, stasis in the central venous sinus [1]- **C**
d) Presence of goblet cells in secondary lamellae [1]- **R**

Categorization of alterations in gills



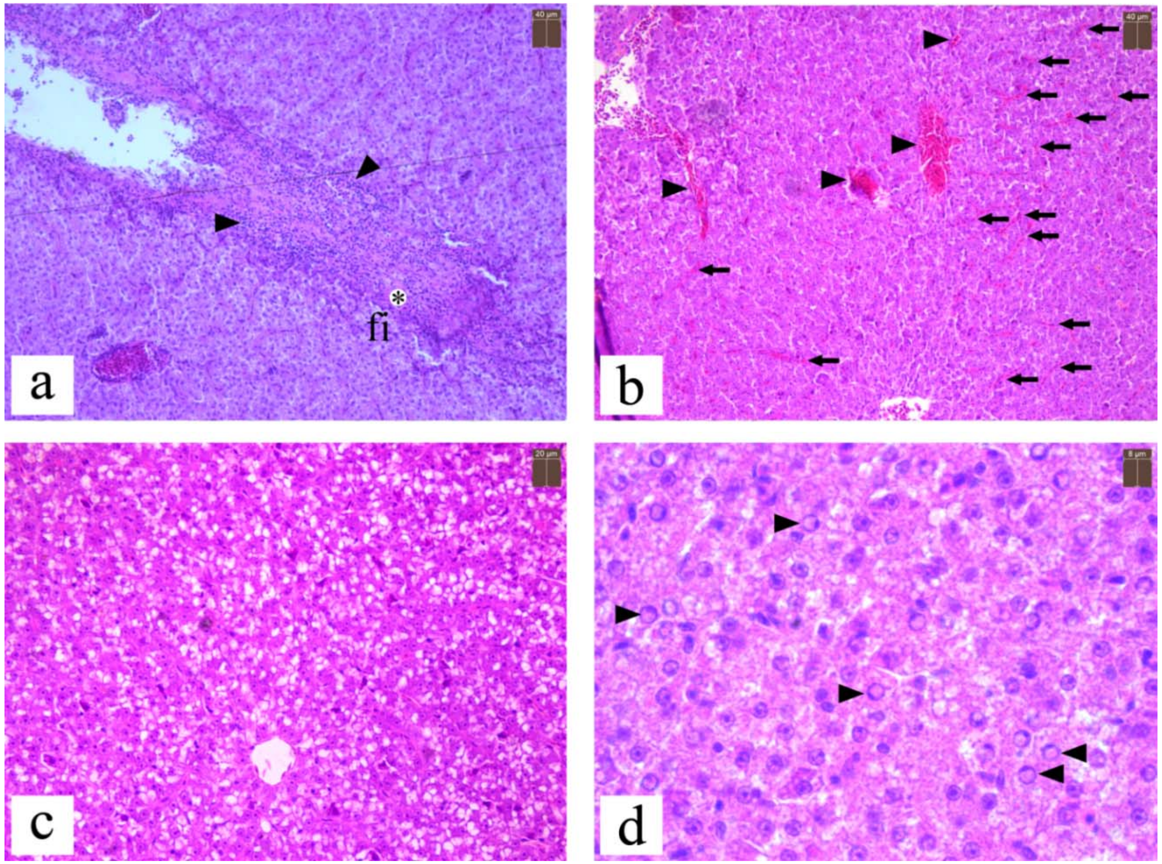


- Regressive alterations were dominant during all three sampling seasons, following circulatory and progressive

- Significant correlation was observed between regressive and circulatory alterations ($r = 0.5472$, $p = 0.0018$)

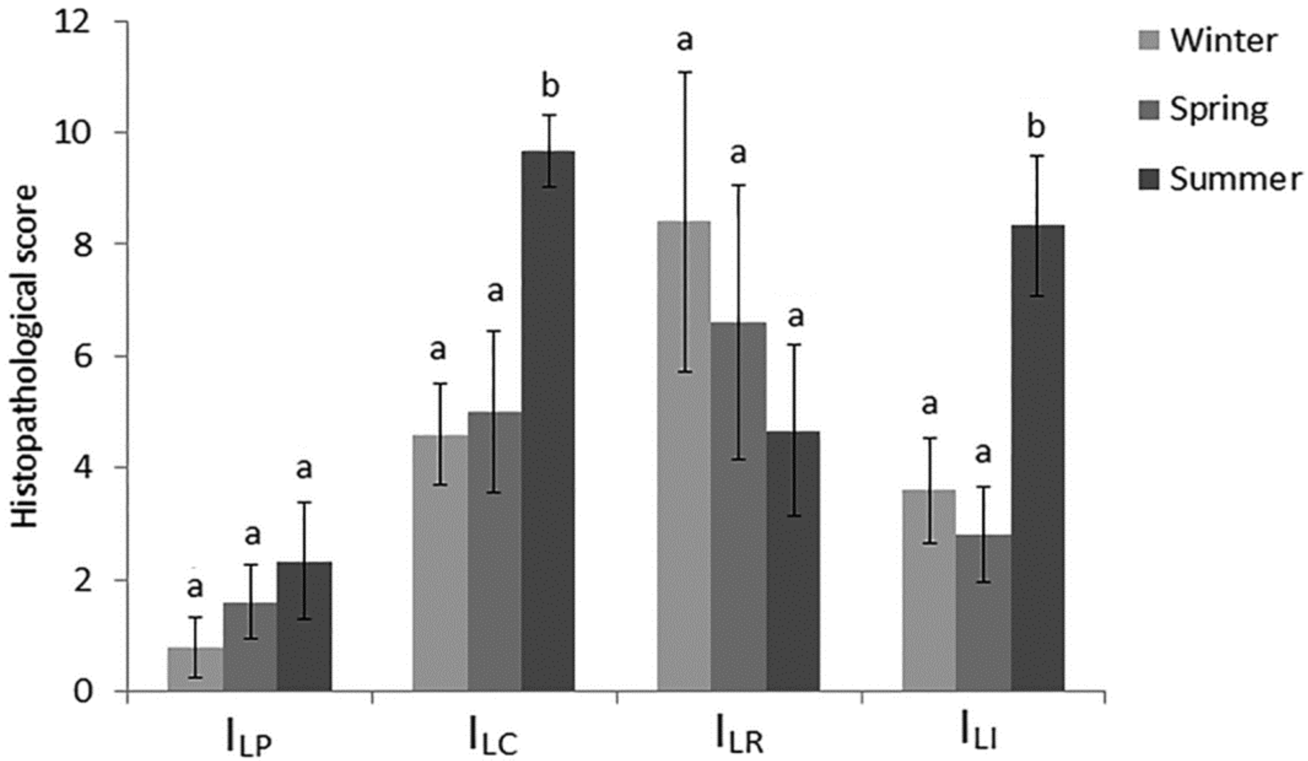
- Significant seasonal variation was observed only between winter and summer within circulatory disturbances

Srecific histopathological alterations in liver



a) Leukocyte infiltration into liver parenchyma and especially around blood vessels [2]- **I**; extensive fibrosis of blood vessels [2]- **R**
b) Congestion of sinusoids and presence of stasis inside the blood vessels [1]- **C**
c) Vacuolation of hepatocytes [2]- **R**
d) Vaculation of nuclei in hepatocytes [2]- **R**

Categorization of alterations in liver

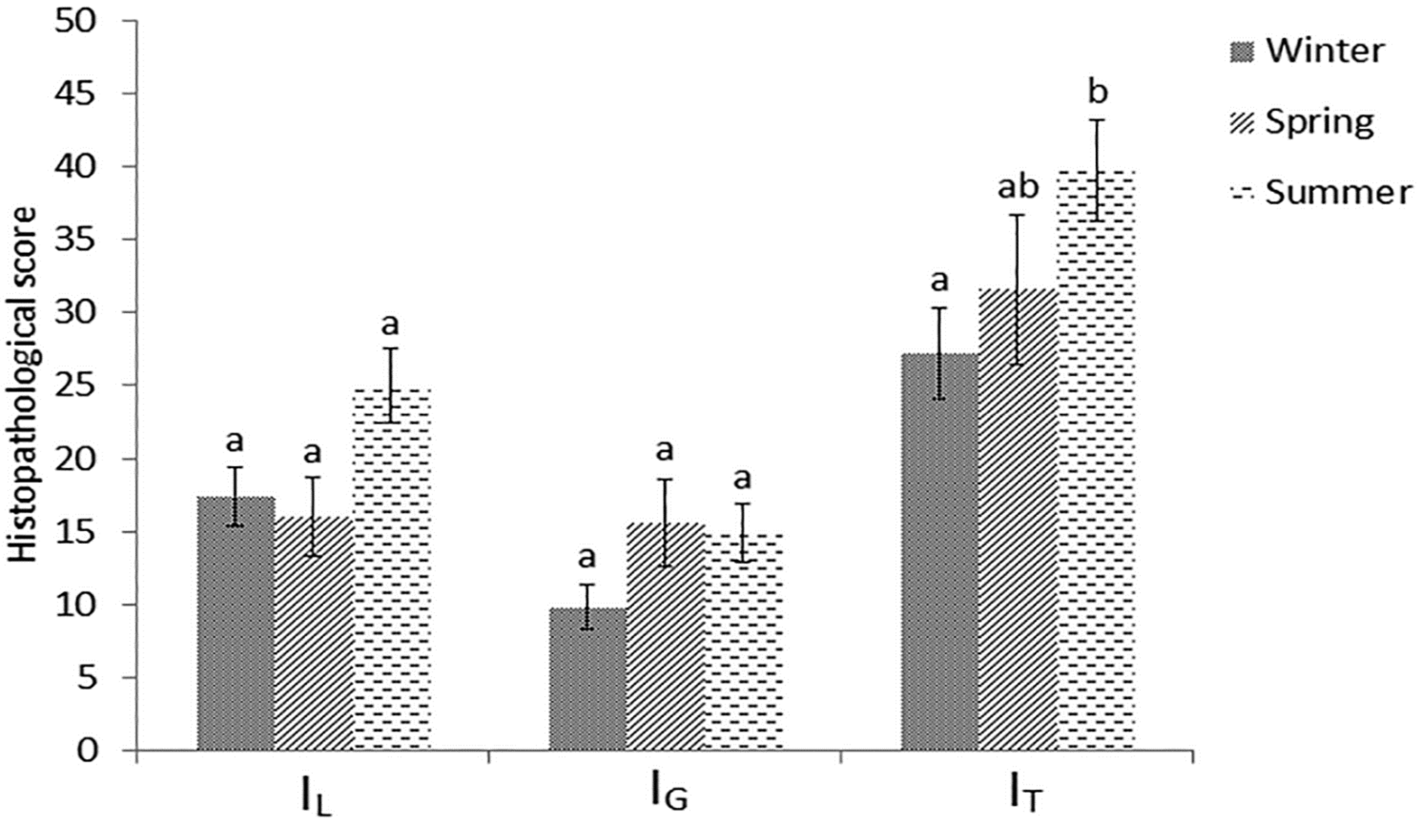


- Circulatory and inflammatory disturbances dominated during the summer with significant differences in comparison to both winter and spring

- During winter and spring the most prevalent in liver were the regressive changes

- Progressive alterations were the least frequent hepatic lesions

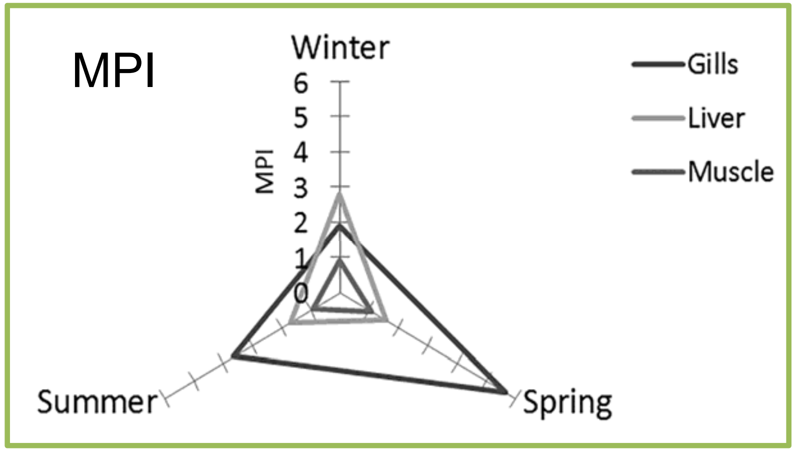
Tisssue HI and total HI



- Greater presence of alterations in liver was visible during summer, and in gills during spring, without significant seasonal differences
- A total histopathological index (IT) was significantly higher during summer in comparison to winter
- Gills degeneration could make an additional pressure on fish liver

Analysis of metals and metalloids in fish tissues

- Gills were under the highest pressure of metals, especially during spring and summer (Cr, Ba, Mn, Mo, and Sr)
- Liver was the main organ of accumulation of Cu, Pb and As
- Muscle was the least affected tissue
- None of the elements for which MAC are set (Pb, As, Cu, Fe and Zn) did not exceed prescribed values



		Muscle	Liver	Gills
Al µg/g	Winter	14.26±14.94 $^{A a}$	9.09±10.61 $^{A a}$	11.52±7.06 $^{A a}$
	Spring	19.62±18.88 $^{A a}$	42.32±77.3 1 $^{A a}$	233.95±171.8 7 $^{A a}$
	Summer	7.20±4.27 $^{A a}$	36.79±69.91 $^{A a}$	47.67±64.40 $^{A a}$
As µg/g	Winter	0.35±0.32	2.32±2.43 ^{a}	0.52*
	Spring	0.42*	1.21±1.73 ^{a}	1.54*
	Summer	0.04*	1.10±0.48 ^{a}	0.03*
Cr µg/g	Winter	0.20±0.16 $^{A a}$	0.22±0.11 $^{A a}$	0.64±0.24 $^{B a}$
	Spring	1.72±3.34 $^{AB a}$	0.23±0.18 $^{A a}$	1.44±0.3 4 $^{B b}$
	Summer	0.29±0.23 $^{A a}$	0.22±0.11 $^{A a}$	0.97±0.16 $^{B ab}$
Cu µg/g	Winter	1.05±0.67 $^{A a}$	19.18±15.83 $^{A a}$	0.31±0.44 $^{A a}$
	Spring	0.83±0.31 $^{A a}$	17.63±3.39 $^{B a}$	15.12±32.78 $^{AB ab}$
	Summer	0.55±0.23 $^{A a}$	19.38±3.61 $^{B a}$	1.22±0.52 $^{A b}$
Fe µg/g	Winter	13.64±4.20 $^{A a}$	225.89±198.85 $^{AB a}$	148.42±54.63 $^{B a}$
	Spring	16.57±12.95 $^{A a}$	223.23±151.48 $^{A a}$	331.41±215.19 $^{A a}$
	Summer	14.74±10.13 $^{A a}$	231.03±95.03 $^{B a}$	204.50±175.42 $^{AB a}$
Mn µg/g	Winter	0.80±0.21 $^{A a}$	4.90±1.11 $^{B a}$	13.63±6.30 $^{B a}$
	Spring	2.70±1.15 $^{A ab}$	6.34±2.04 $^{B a}$	81.22±35.18 $^{C b}$
	Summer	4.29±0.80 $^{A b}$	6.64±1.30 $^{B a}$	92.27±13.67 $^{C b}$
Mo µg/g	Winter	0.21±0.19 $^{A a}$	0.28±0.15	1.65±1.49 $^{A a}$
	Spring	0.33±0.13 $^{A a}$	0.56±0.57**	2.91±0.85 $^{B a}$
	Summer	0.40±0.18 $^{A a}$	0.60*	2.57±0.43 $^{B a}$
Pb µg/g	Winter	0.11*	0.53±0.25 ^{a}	ND
	Spring	0.06±0.05 ^{a}	0.30±0.21 ^{a}	1.07*
	Summer	0.07±0.04 ^{a}	0.36±0.23 ^{a}	0.28*
Sr µg/g	Winter	1.52±0.50 $^{A a}$	0.38±0.14 $^{B a}$	63.17±31.57 $^{C a}$
	Spring	2.19±1.06 $^{A ab}$	0.30±0.11 $^{B a}$	86.05±38.57 $^{C a}$
	Summer	3.04±1.03 $^{A b}$	0.49±0.18 $^{B a}$	75.41±5.53 $^{C a}$
Zn µg/g	Winter	31.09±6.92 $^{A a}$	55.20±24.52 $^{A a}$	48.12±21.33 $^{A a}$
	Spring	20.20±4.91 $^{A a}$	42.83±8.50 $^{B a}$	59.38±7.98 $^{C a}$
	Summer	22.17±6.21 $^{A a}$	58.08±14.52 $^{B a}$	69.04±5.08 $^{B a}$
Ba µg/g	Winter	2.01±0.71 $^{A a}$	0.28±0.22**	21.05±7.91 $^{B a}$
	Spring	1.39±0.49 $^{A a}$	2.56*	37.26±9.81 $^{B ab}$
	Summer	1.73±0.49 $^{A a}$	0.33±0.21	40.70±5.71 $^{B b}$

CONCLUSIONS

- Sampling season and floods influenced the variation of the biomarkers response and concentrations of metals and metalloids in the fish tissues
- Gills and liver respond differently to environmental stress
- Gills as the first organ in direct contact with water showed a higher level of DNA damage (biomarker of exposure) in comparison to liver
- Liver as the major organ for processing of xenobiotics both from water and food showed a higher degree of histopathological alterations (biomarker of effect) in comparison to gills
- The use of a battery of markers, as well as examination of different tissues was approved as an effective approach
- Seasonal variations in water quality must be considered in monitoring programs

ACKNOWLEDGEMENTS

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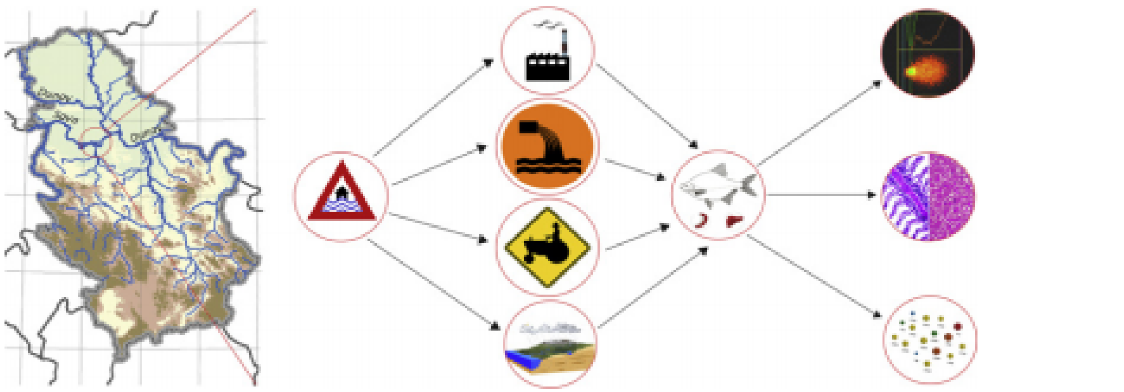
HIGHLIGHTS

- The impact of multiple stressors was studied by biomarkers response in fish.
- DNA damage, histopathology and metal accumulation were studied in gills and liver.
- DNA damage was higher in gills, changes in histopathology were prevalent in liver.
- The variation of the biomarkers response depended on the sampling season.
- Use of multibiomarker approach is essential for confident water quality assessment.

GRAPHICAL ABSTRACT



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