TOXICITY TESTING OF RIVER AND WASTEWATER USING ALGAE AND DAPHNIDS

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SECOND MEETING

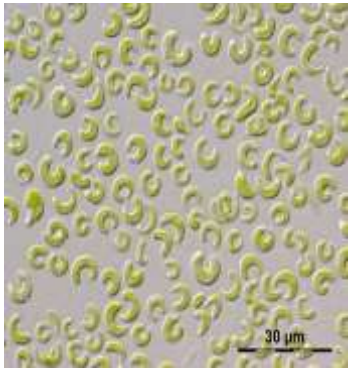
Integrated evaluation of aquatic organism responses to metal exposure: gene expression, bioavailability, toxicity and biomarker responses (BIOTOXMET)

Zagreb, 16th December 2021

Introduction

- In addition to chemical water analyses, assessment of water quality might involve toxicity testing, as a biological tool that reflects toxic impact on aquatic organisms.
- The commonly used testing organisms are:
  - crustacean *Daphnia magna* Straus,
    as primary consumers, and
  - freshwater green algae *Pseudokirchneriella subcapitata,* as primary producers,

which are sensitive to a wide range of contaminants.



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- Aims:
  - Assessment of potential toxicity of industrial and municipal wastewaters by an acute toxicity test;
  - Estimation of pollution impact on aquatic organisms and potential treath to the Krka River and its tributaries;
  - Establishment of protection plans for the Krka National Park.
- Five samples were analyzed in May and July 2021:
  - Krka River source (KRK) – reference site, and four locations downstream of the wastewater impact:
  - tributary Orašnica (TOR) and Butišnica (TBU),
  - Krka River Knin (near municipal outlet of the Town of Knin) (KRK) and
  - industrial wastewater (IWW) near screw factory,
- Sampling campaign: April and July 2021

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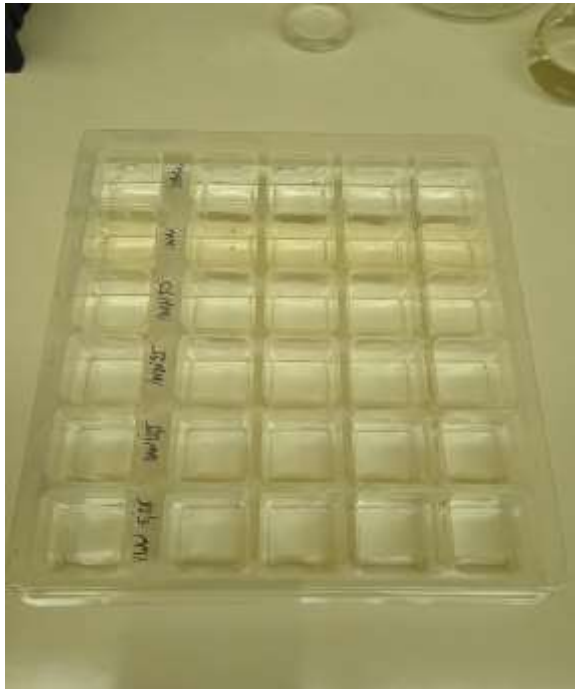
Daphnia magna immobilization test

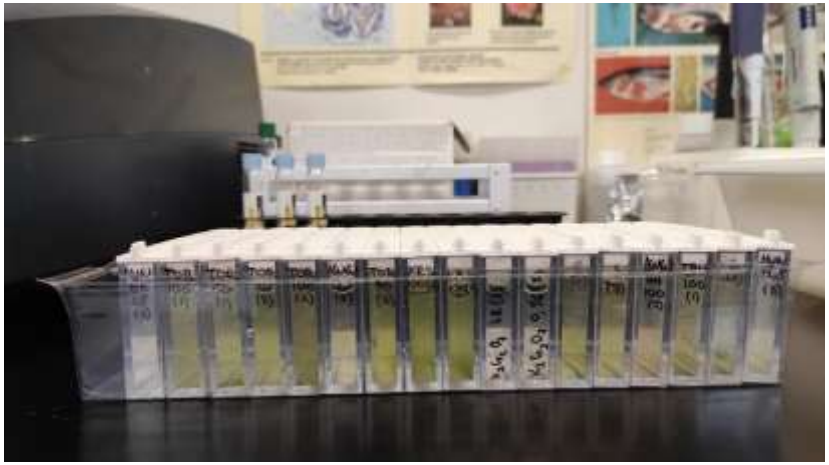
- All tests for immobilisation of daphnids were carried out under controlled conditions in an incubator at 20 °C in darkness for 24/48 hours.

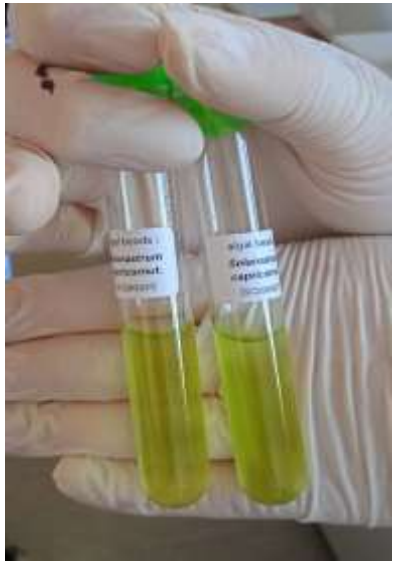


Algae inhibition test

All microalgal growth inhibition tests were conducted under controlled conditions in an incubator:

- at 23±2°C
- with continuous illumination of 10,000 lx,
- during 72 h.





Test results

- for daphnia:

- after 24 h and 48 h incubation at 20 ± 1 °C, the number of immobile daphnids was recorded.
- the Lowest Ineffective Dilution, for which at least 90% of the daphnids are mobile, is given as test result (LID_D-value).

- for algae:

- growth of the algae were analysed after 24, 48 and 72 h incubation by measuring optical density at 670 nm.
- the highest concentration at which an inhibition <20% is observed is termed the lowest ineffective dilution (LID) and reported as final result of the test (LID_A-value).

Results – May 2021
Daphnia test

24h (01.05.2021.)

		$K_2Cr_2O_7$					KRS	TOR		KRK			TBU	IWW				
	MEDIUM	0.32 mg/L	0.56 mg/L	1.0 mg/L	1.8 mg/L	3.2 mg/L	100%	50%	100%	25%	50%	100%	100%	6.25%	12.50%	25%	50%	100%
	5	4	5	3	1	0	5	5	5	5	5	5	5	4	4	4	0	0
	5	5	3	3	0	0	5	5	4	5	5	5	5	4	4	4	0	0
	5	5	5	3	1	0	5	5	4	5	5	4	5	5	4	4	0	0
	5	5	5	5	1	0	5	5	5	5	5	5	5	5	4	3	0	0
total	20	19	18	14	3	0	20	20	18	20	20	19	20	18	16	15	0	0
percentage of non-immobilized daphnia	100	95	90	70	15	0	100	100	90	100	100	95	100	90	80	75	0	0
$EC50/LID_D$		EC_{50} = 1.08 mg/L					LID_D=1	LID_D=1		LID_D=1			LID_D=1	LID_D=16				

48h (02.05.2021.)

		$K_2Cr_2O_7$					KRS	TOR		KRK			TBU	IWW				
	MEDIUM	0.32 mg/L	0.56 mg/L	1.0 mg/L	1.8 mg/L	3.2 mg/L	100%	50%	100%	25%	50%	100%	100%	6.25%	12.50%	25%	50%	100%
	5	4	4	3	0	0	5	5	5	5	5	5	5	4	4	3	0	0
	5	4	3	2	0	0	5	5	4	5	4	4	5	4	3	4	0	0
	5	5	5	3	0	0	5	5	3	5	4	4	5	4	4	4	0	0
	5	5	5	2	0	0	5	4	4	5	5	4	5	5	4	3	0	0
total	20	18	17	10	0	0	20	19	16	20	18	17	20	17	15	14	0	0
percentage of non-immobilized daphnia	100	90	85	50	0	0	100	95	80	100	90	85	100	85	75	70	0	0
$EC50/LID_D$		EC_{50} = 0.78 mg/L					LID_D=1	LID_D=2		LID_D=2			LID_D=1	LID_D=18				

Krka River source	KRS
Tributary Orašnica River	TOR
Krka River Knin (near municipal outlet of the Town of Knin)	KRK
Tributary Butišnica River	TBU
Industrial wastewater (near screw factory)	IWW

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Results – May 2021

Algal tests

$K_2Cr_2O_7$	0 h (4.5.2021.)	24 h (5.5.2021.)	48 h (6.5.2021.)	72 h (7.5.2021.)
	Mean OD values (670 nm)			
Control	0.002	0.149	0.460	0.888
0.18 mg/L	0.002	0.149	0.453	0.786
0.32 mg/L	0.002	0.132	0.428	0.722
0.56 mg/L	0.002	0.118	0.298	0.344
1.0 mg/L	0.002	0.109	0.133	0.295
1.8 mg/L	0.002	0.078	0.085	0.099
	EC_{50} = 0.73 mg/L			

KRS	0 h (4.5.2021.)	24 h (5.5.2021.)	48 h (6.5.2021.)	72 h (7.5.2021.)
	Mean OD values (670 nm)			
Control	0.002	0.149	0.460	0.888
100%	0.002	0.019	0.178	0.834
	LID_A=1			

TOR	0 h (4.5.2021.)	24 h (5.5.2021.)	48 h (6.5.2021.)	72 h (7.5.2021)
	Mean OD values (670 nm)			
Control	0.002	0.149	0.460	0.888
50%	0.002	0.142	0.272	0.724
100%	0.002	0.145	0.271	0.713
	LID_A=1			

KRK	0 h (4.5.2021.)	24 h (5.5.2021.)	48 h (6.5.2021.)	72 h (7.5.2021.)
	Mean OD values (670 nm)			
Control	0.002	0.149	0.460	0.888
25%	0.002	0.146	0.384	0.856
50%	0.002	0.120	0.298	0.712
100%	0.002	0.118	0.211	0.639
	LID_A=2			

TBU	0 h (4.5.2021.)	24 h (5.5.2021.)	48 h (6.5.2021.)	72 h (7.5.2021)
	Mean OD values (670 nm)			
Control	0.002	0.149	0.460	0.888
100%	0.002	0.069	0.230	0.797
	LID_A=1			

IWW	0 h (4.5.2021.)	24 h (5.5.2021.)	48 h (6.5.2021.)	72 h (7.5.2021)
	Mean OD values (670 nm)			
Control	0.002	0.149	0.460	0.888
6.25%	0.002	0.112	0.245	0.359
12.50%	0.002	0.068	0.170	0.267
25%	0.002	0.020	0.061	0.192
50%	0.002	0.015	0.049	0.050
100%	0.002	0.006	0.010	0.010
	LID_A= 32			

- For samples analyzed in May 2021 the maximum LID values were observed with the IWW sample (algae LID_A = 32 (72 h), daphnia LID_D = 16 (24 h) and LID_D = 18 (48 h)).

- Testing on algae showed that for all other samples (KRS, TBU and TOR) LID_A = 1 were obtained. The only exception is the KRK sample for which the LID_A = 2 was observed.
- Testing on daphnia showed that for samples KRS, TBU, TOR and KRK, LID_D = 1 (24 h) values were observed. After 48 hours, values of LID_D = 2 were observed for TOR and KRK samples while for samples KRS and TBU obtained values remained the same LID_D = 1.

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Results – July 2021

Daphnia test

		24h (23.07.2021.)																
		$K_2Cr_2O_7$					KRS A	TOR B		KRK B			TBU B	IWW				
	MEDIUM	0.32 mg/L	0.56 mg/L	1.0 mg/L	1.8 mg/L	3.2 mg/L	100%	50%	100%	25%	50%	100%	100%	6.25%	12.50%	25%	50%	100%
	5	5	4	4	1	0	5	5	5	5	5	5	5	5	5	5	4	4
	5	5	3	2	1	0	5	5	5	5	5	5	5	5	5	5	5	5
	5	5	4	2	0	0	5	5	5	5	5	5	5	5	5	5	4	4
	5	5	5	3	2	0	5	5	5	5	5	5	5	5	5	4	5	4
total	20	20	16	11	4	0	20	20	20	20	20	20	20	20	20	19	18	17
percentage of non-immobilized daphnia	100	100	80	55	20	0	100	100	100	100	100	100	100	100	100	95	90	85
EC50/LID_D		EC_{50} = 1.08 mg/L					LID_D=1	LID_D=1		LID_D=1			LID_D=1	LID_D=2				

		48h (24.07.2021.)																
		$K_2Cr_2O_7$					KRS	TOR B		KRK B			TBU B	IWW				
	MEDIUM	0.32 mg/L	0.56 mg/L	1.0 mg/L	1.8 mg/L	3.2 mg/L	100%	50%	100%	25%	50%	100%	100%	6.25%	12.50%	25%	50%	100%
	5	5	4	4	1	0	5	5	5	5	5	5	5	5	5	5	4	5
	5	5	5	2	0	0	5	5	4	5	5	5	5	5	5	5	5	3
	5	5	4	2	0	0	5	5	5	5	5	5	5	5	4	4	5	4
	5	4	5	1	0	0	5	5	5	5	5	5	5	5	5	5	4	4
total	20	19	18	9	1	0	20	20	19	20	20	20	20	20	19	19	18	16
percentage of non-immobilized daphnia	100	95	90	45	5	0	100	100	95	100	100	100	100	100	95	95	90	80
EC50/LID_D		EC_{50} = 0.89 mg/L					LID_D=1	LID_D=1		LID_D=1			LID_D=1	LID_D=2				

Krka River source	KRS
Tributary Orašnica River	TOR
Krka River Knin (near municipal outlet of the Town of Knin)	KRK
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Results – July 2021

Algal tests

$K_2Cr_2O_7$	0 h (27.7.2021.)	24 h (28.7.2021.)	48 h (29.7.2021.)	72 h (30.7.2021.)
	Mean OD values (670 nm)			
Control	0.020	0.194	0.447	0.830
0.18 mg/L	0.020	0.141	0.388	0.629
0.32 mg/L	0.020	0.115	0.292	0.608
0.56 mg/L	0.020	0.109	0.268	0.553
1.0 mg/L	0.020	0.035	0.239	0.398
1.8 mg/L	0.020	0.025	0.178	0.295
	EC_{50} = 0.74 mg/L			

KRS	0 h (27.7.2021.)	24 h (28.7.2021.)	48 h (29.7.2021.)	72 h (30.7.2021.)
	Mean OD values (670 nm)			
Control	0.020	0.194	0.447	0.830
100%	0.020	0.188	0.420	0.829
	LID_A=1			

TOR	0 h (27.7.2021.)	24 h (28.7.2021.)	48 h (29.7.2021.)	72 h (30.7.2021.)
	Mean OD values (670 nm)			
Control	0.020	0.194	0.447	0.830
50%	0.020	0.180	0.391	0.823
100%	0.020	0.163	0.325	0.826
	LID_A=1			

KRK	0 h (27.7.2021.)	24 h (28.7.2021.)	48 h (29.7.2021.)	72 h (30.7.2021.)
	Mean OD values (670 nm)			
Control	0.020	0.194	0.447	0.830
25%	0.020	0.184	0.434	0.815
50%	0.020	0.151	0.418	0.760
100%	0.020	0.111	0.296	0.448
	LID_A=2			

TBU	0 h (27.7.2021.)	24 h (28.7.2021.)	48 h (29.7.2021.)	72 h (30.7.2021.)
	Mean OD values (670 nm)			
Control	0.020	0.194	0.447	0.830
100%	0.020	0.173	0.393	0.828
	LID_A=1			

IWW	0 h (27.7.2021.)	24 h (28.7.2021.)	48 h (29.7.2021.)	72 h (30.7.2021.)
	Mean OD values (670 nm)			
Control	0.020	0.194	0.447	0.830
6.25%	0.020	0.141	0.212	0.665
12.50%	0.020	0.120	0.178	0.484
25%	0.020	0.085	0.150	0.363
50%	0.020	0.067	0.136	0.249
100%	0.020	0.042	0.104	0.128
	LID_A= 16			

- Testing on daphnia in July showed that in samples KRS, TBU, TOR and KRK also LID_D = 1 (24h and 48 h) were obtained.
- The only exception is the IWW sample for which the value LID_D = 2 was obtained both after 24 and 48 h. Thus, this sample showed significantly lower toxicity compared to May when the values were LID_D = 16 (24 h) and LID_D = 18 (48 h), respectively.

- Testing on algae in July showed that in samples KRS, TBU and TOR LID_A = 1 (72 h) were obtained.
- The only exception is the KRK sample for which the value LID_A = 2 was obtained and IWW sample for which the value LID_A = 16 was obtained after 72 h. Thus, IWW sample showed lower toxicity compared to May when the value was LID_D = 32 (72 h).

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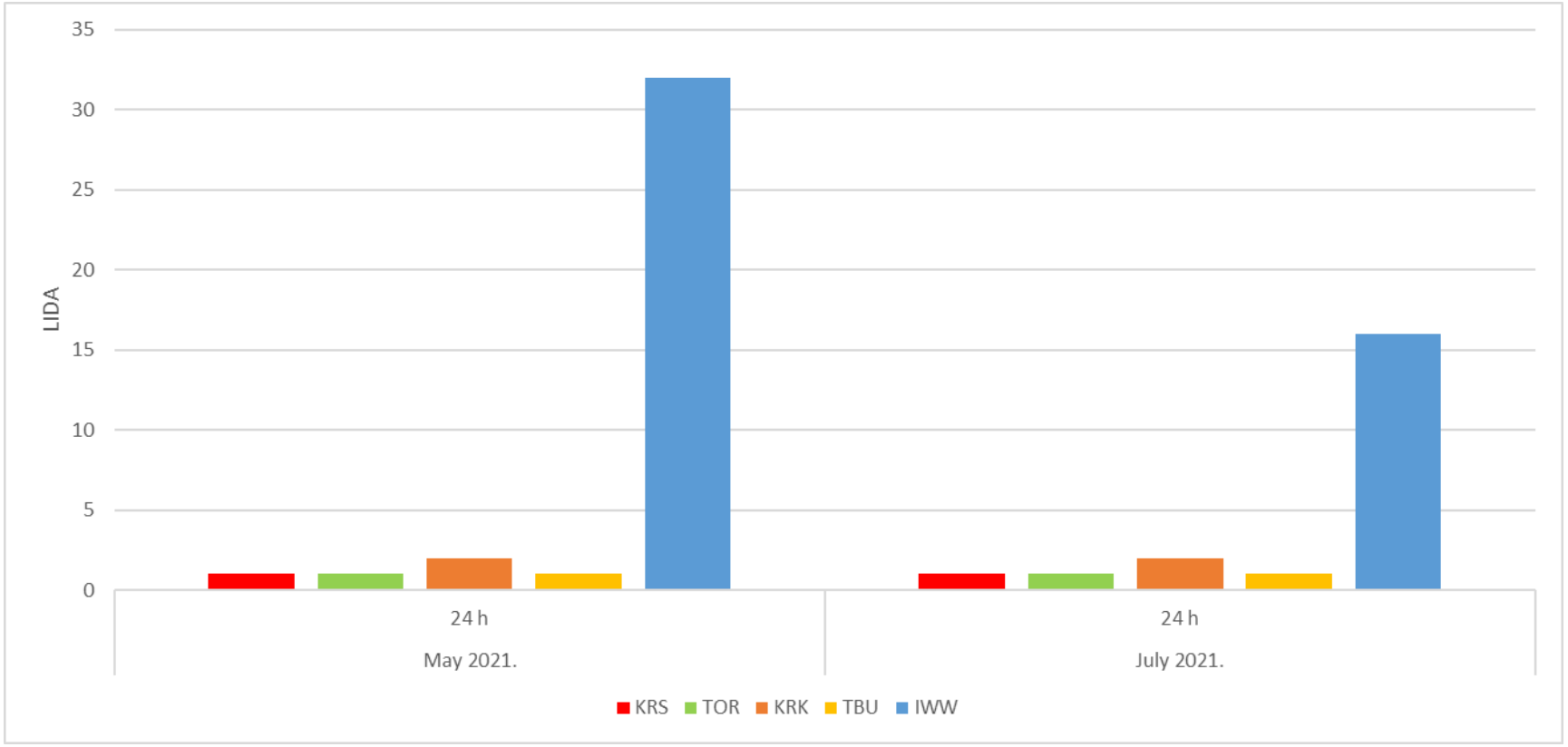
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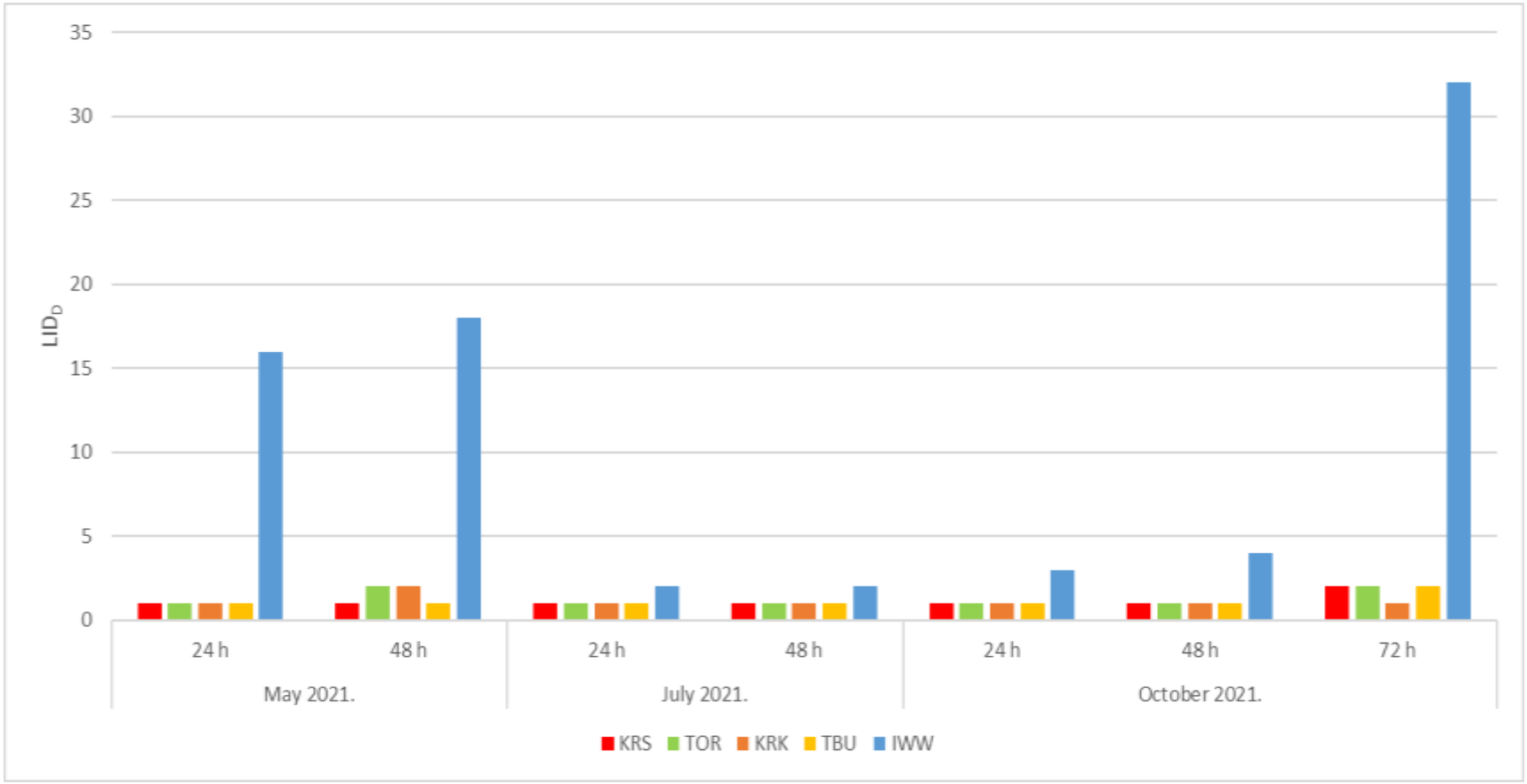
May 2021.
24 h
July 2021.
KRS
TOR
KRK
TBU
IWW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May 2021.
July 2021.
October 2021.
KRS
TOR
KRK
TBU
IWW

CONCLUSIONS

- Testing on daphnids and algae confirmed them as sensitive bioindicators and pointed to toxic influence of the industrial wastewater from the screw factory and the importance of proper purification before discharging it in the environment.

- Therefore, strict and continuous biomonitoring plans must be established if serious consequences want to be avoided on the whole ecosystem, biota and the national park itself.

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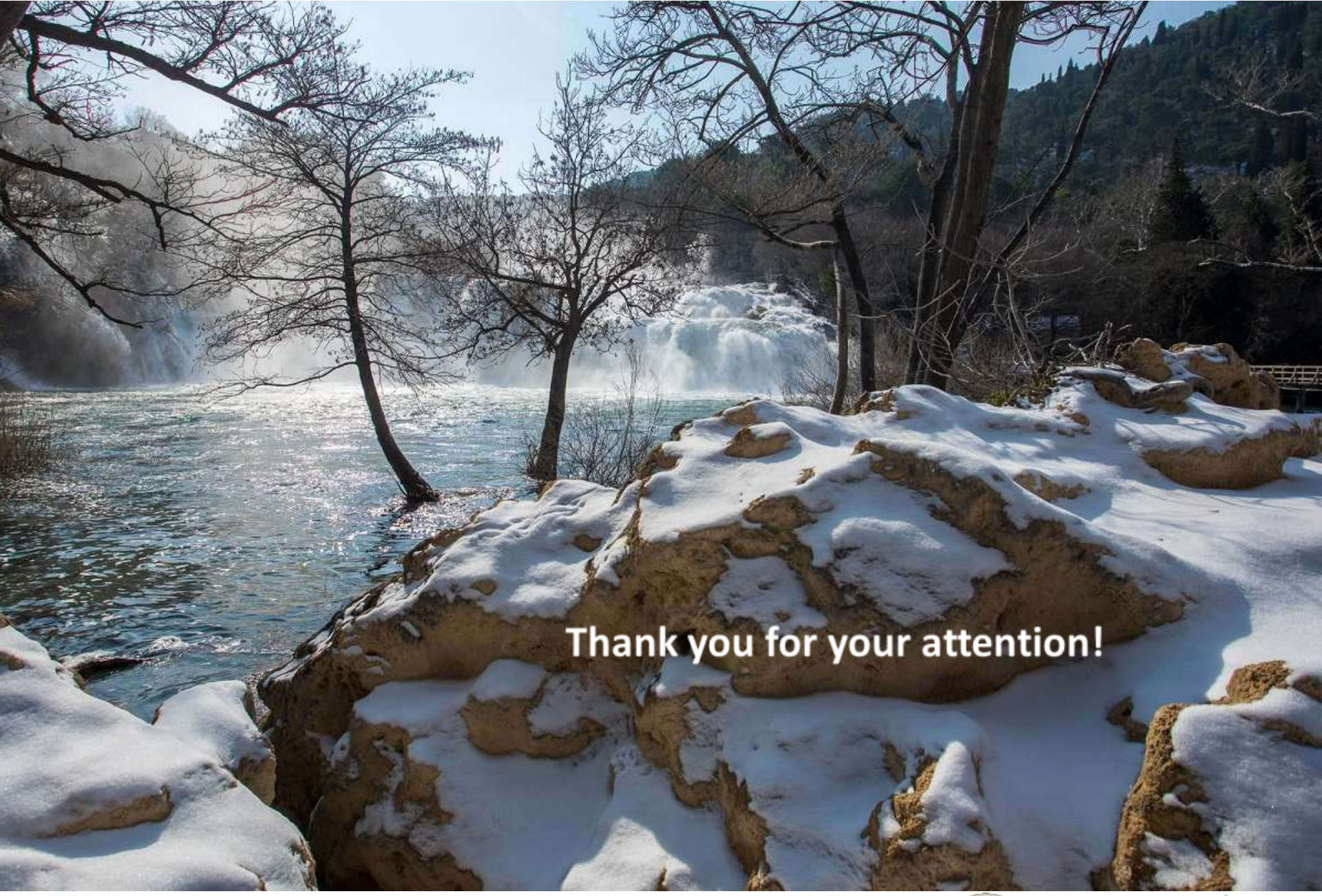

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