Croatian Science Foundation

Ruđer Bošković Institute
University of Zagreb, Faculty of Science
University of Pau, France
University of Bordeaux, France

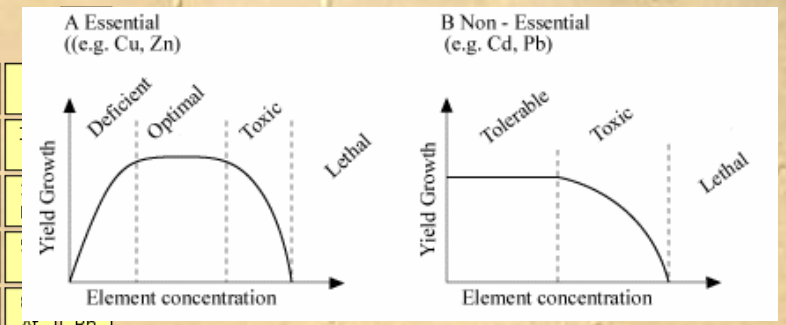
Accumulation, Subcellular Mapping and Effects of Trace Metals in Aquatic Organisms (AQUAMAPMET)

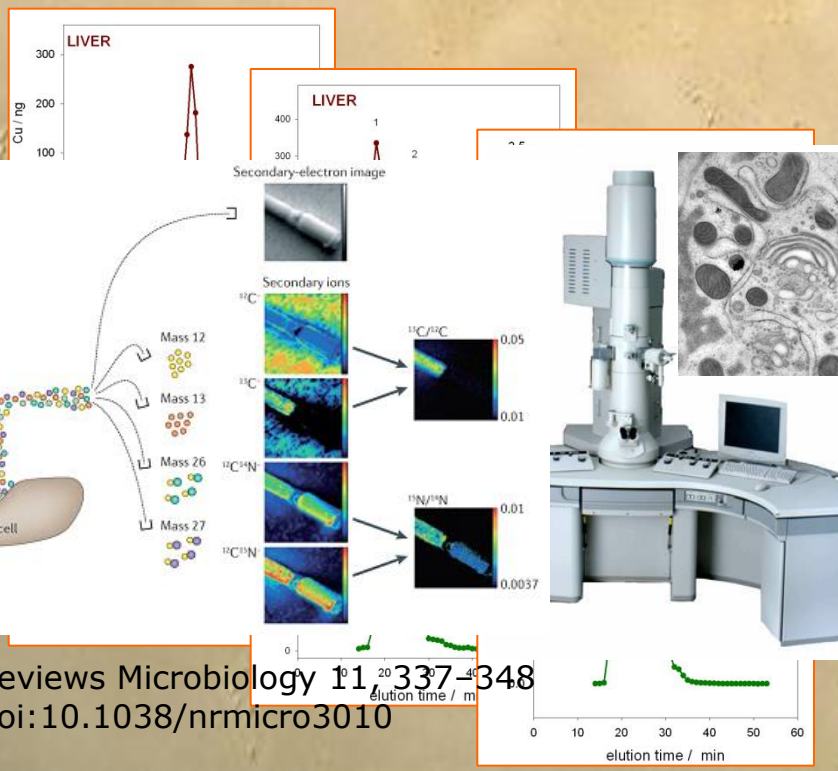
PI: Marijana Erk

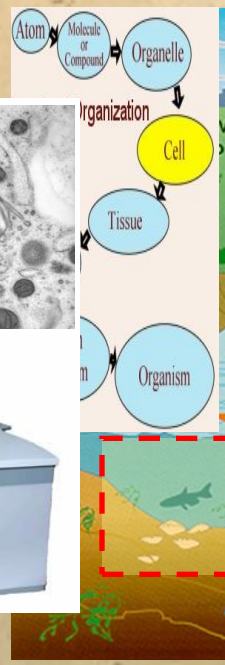
Kick-off Meeting
September 14, 2015

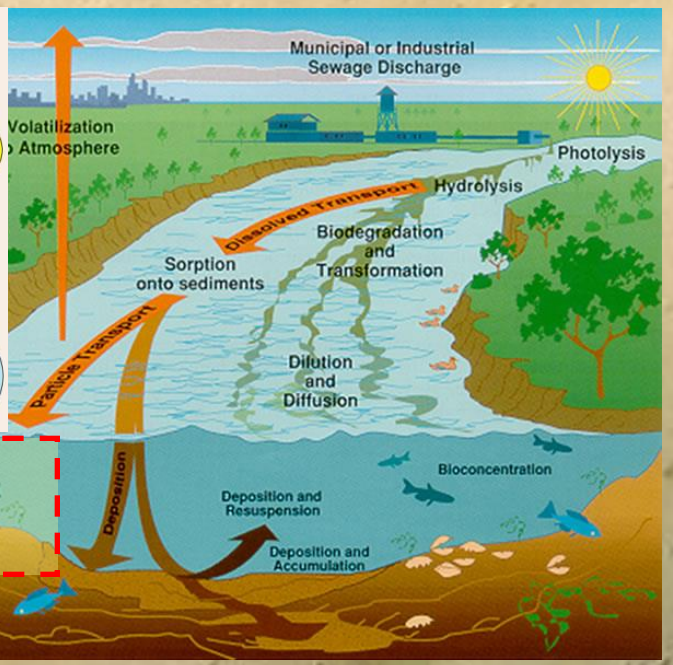
Metals

Group → / Period ↓	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1 H																	
2	3 Li	4 Be											5 B	6 C	7 N	8 O		
3	11 Na	12 Mg											13 Al	14 Si	15 P	16 S		
4	19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se		
5	37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te		
6	55 Cs	56 Ba		72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	At	Rn
7	87 Fr	88 Ra		104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 Ds	111 Rg	112 Uub	113 Uut	114 Uuq	115 Uup	116 Uuh	117 Uus	118 Uuo
Lanthanides				57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu
Actinides				89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr









Nature Reviews Microbiology 11, 337–348 (2013) doi:10.1038/nrmicro3010

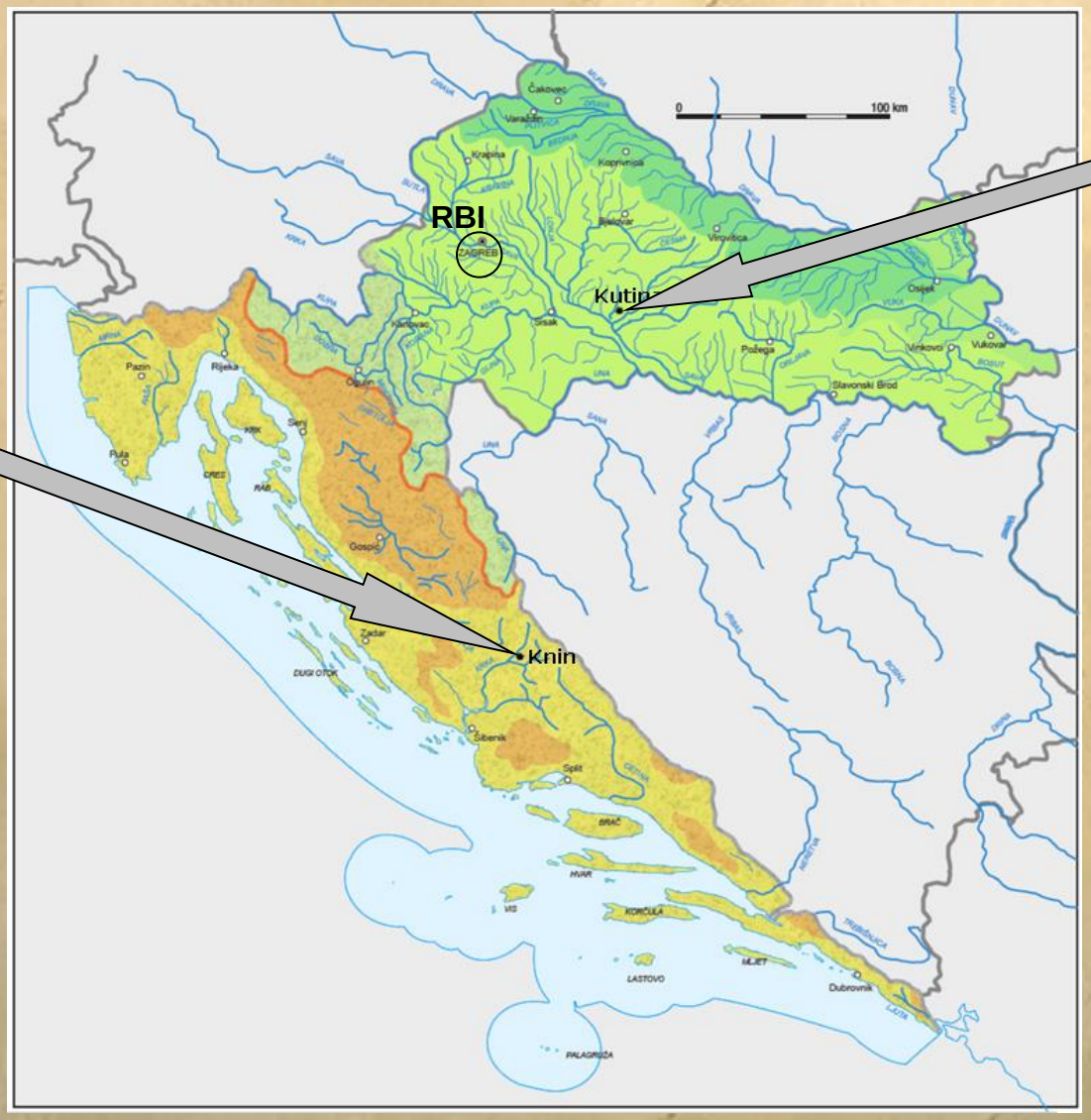
Aim of the project

- ✓ to increase our understanding and gain new knowledge on how fish, crustaceans, bivalves and fish intestinal parasites (Acantocephalans) cope with elevated metal concentrations in the freshwater ecosystems by relating data on metal concentrations in water, accumulated metals and biomarker responses in biota to data on intracellular metal mapping

Additional aim

- ✓ to evaluate anthropogenic impact on selected freshwater ecosystems referring to metal/metalloid pollution and using integrated chemical, biochemical and biological approach, as well as to evaluate the risk for human health

Study areas



Ilova River
lowland river

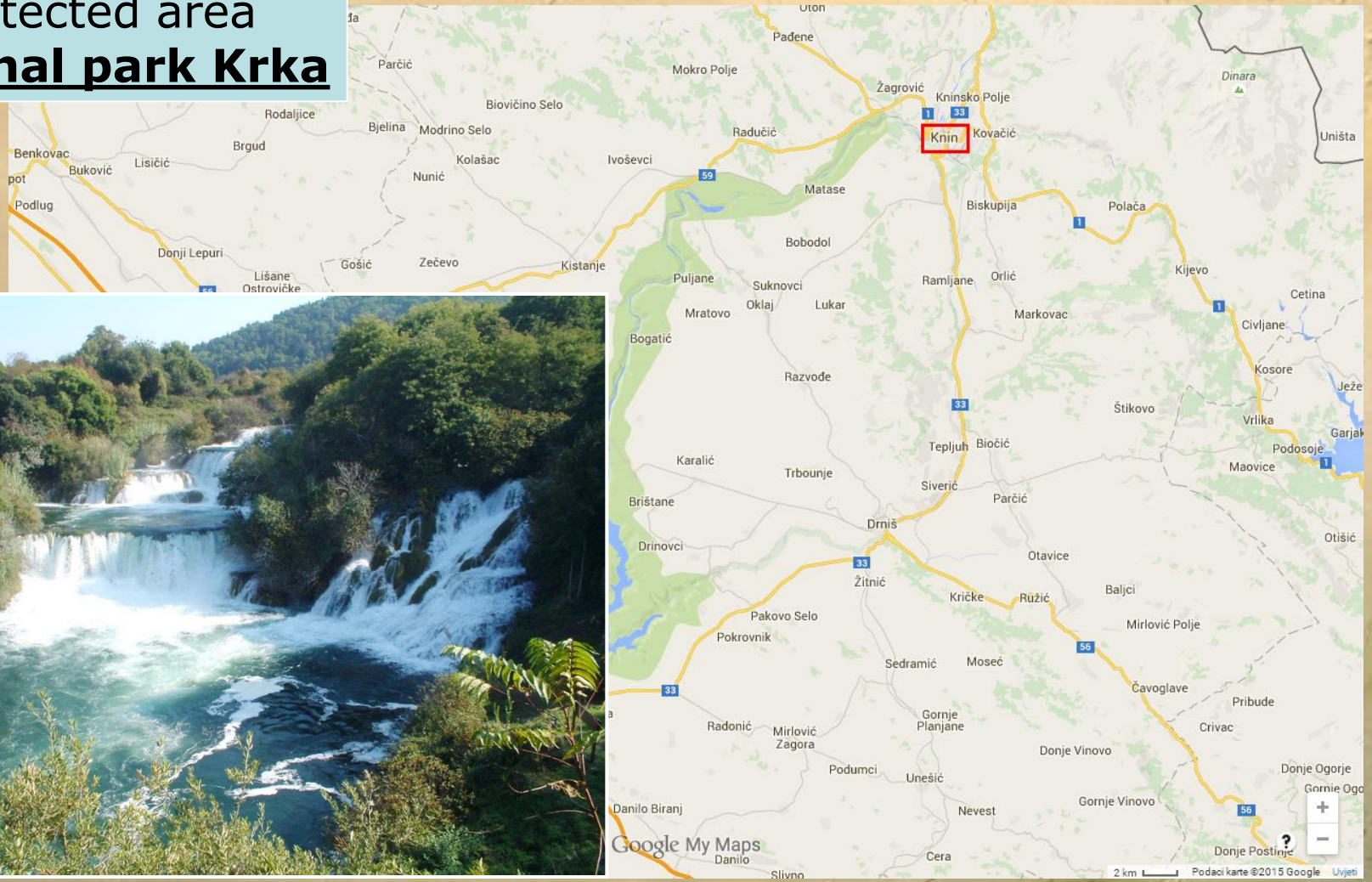
3rd project year

Krka River
karst river

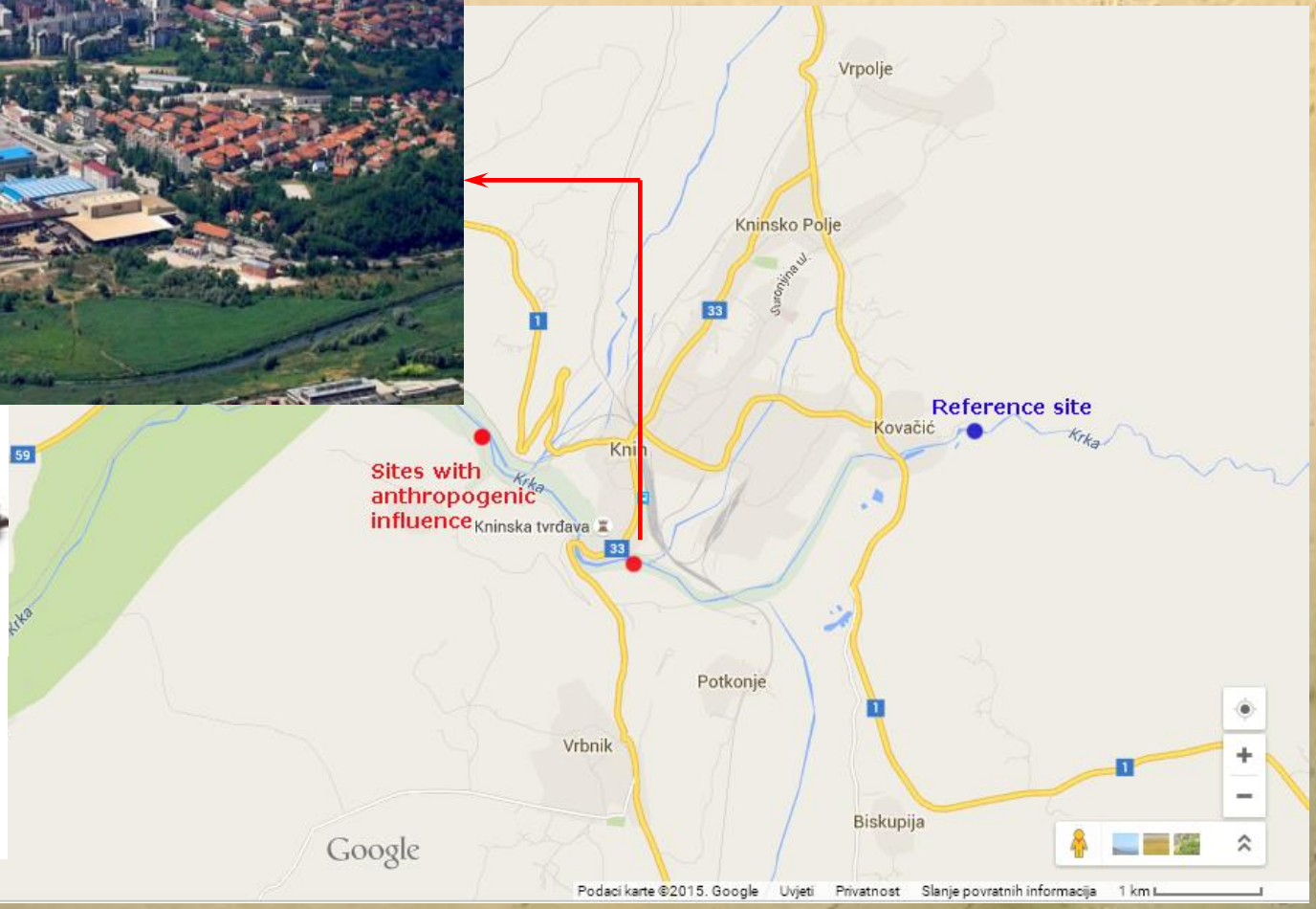
1st project year

Study area - Krka river

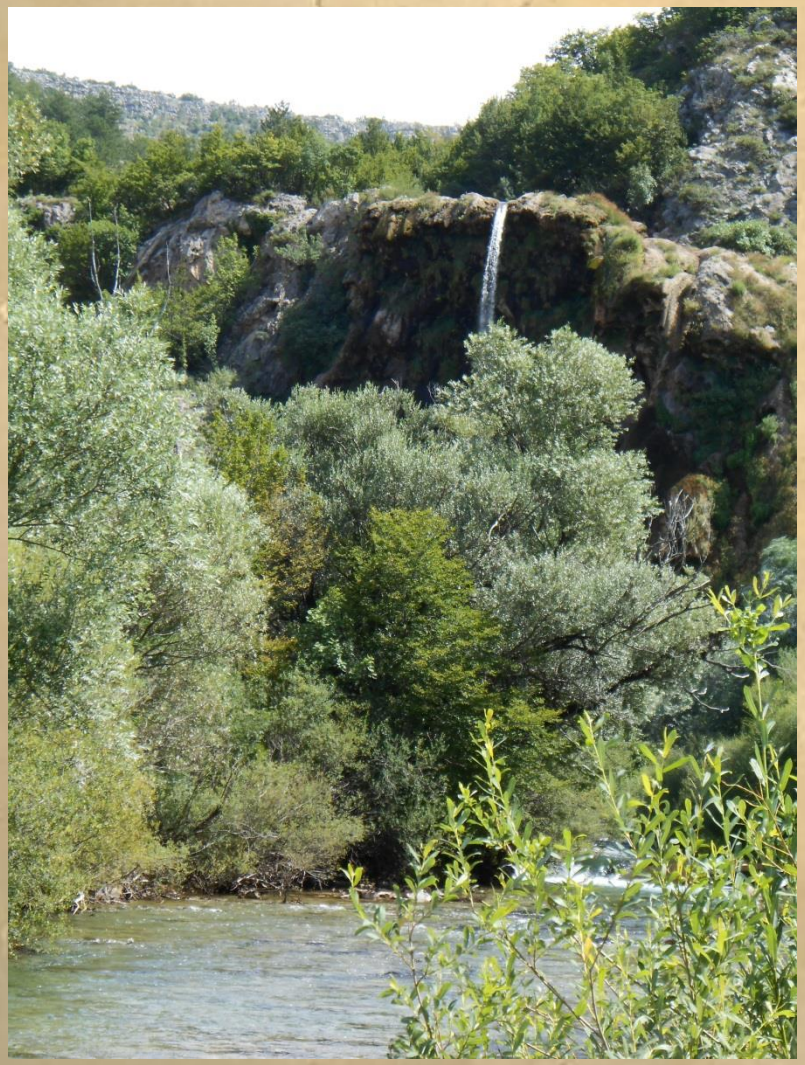
Protected area
National park Krka



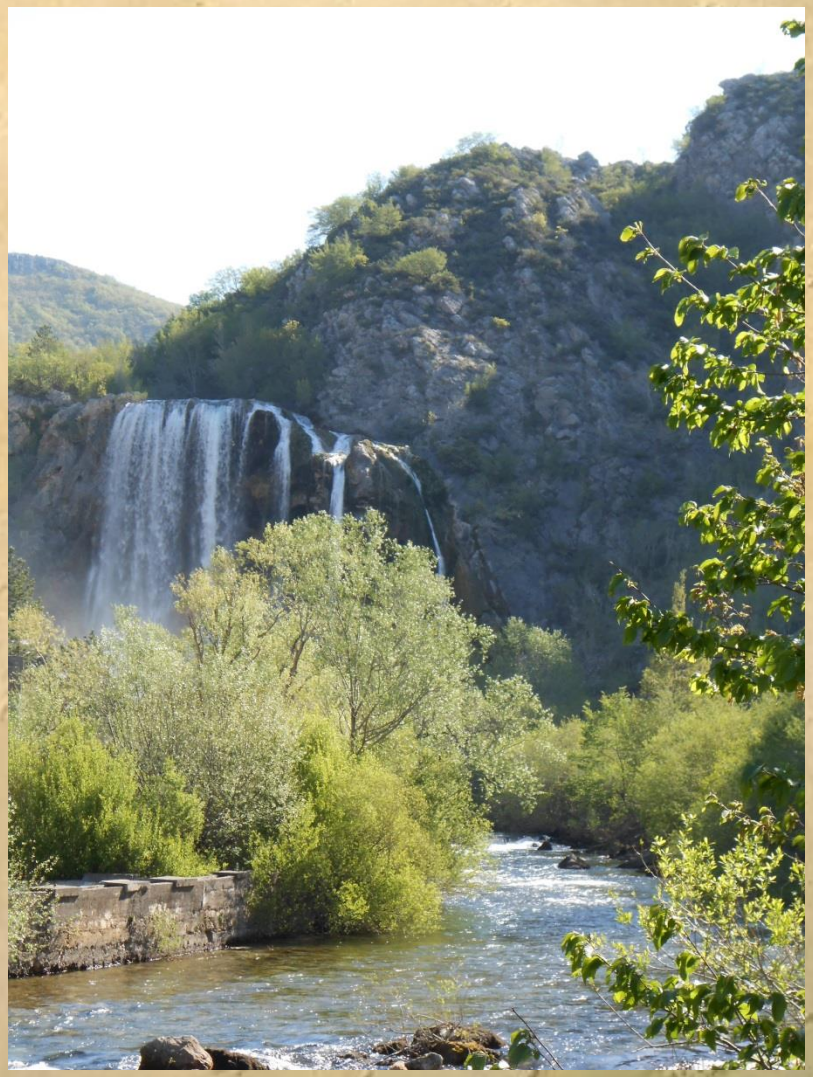
Study area - Krka river



Krka river – reference site



August 7, 2014

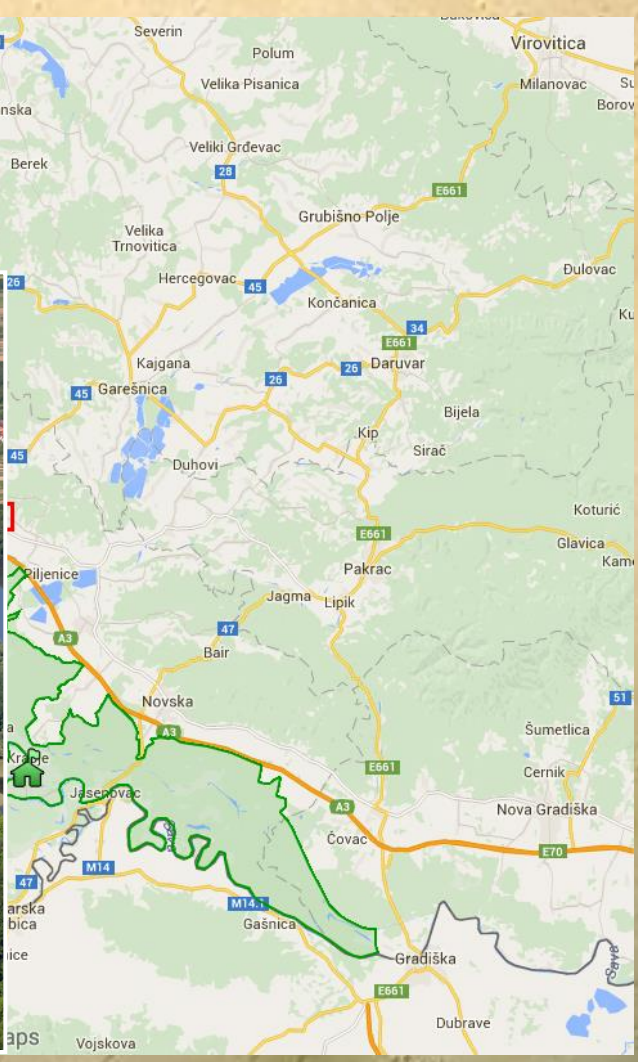


April 22, 2015

Krka river – site with anthropogenic influence

Study area - Ilova River

Protected area
Nature park Lonjsko polje



Ilova River - site with anthropogenic influence

Kutina – Petrokemija Plc.
Production of:

1. Fertilizers
2. Carbon black
3. Clay based production and liquid fertilizers

Photos: Google Earth

Ilova River – reference site

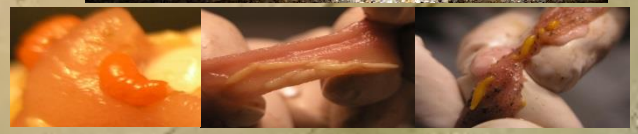
Photos: Google Earth

Organisms selected for study

- fish – brown trout (*Salmo trutta*)
  - European chub (*Squalius cephalus*)
- amphipod crustaceans – *Gammaridae*
- bivalves – *Unionidae*
- fish intestinal parasites (*Acantocephala*)





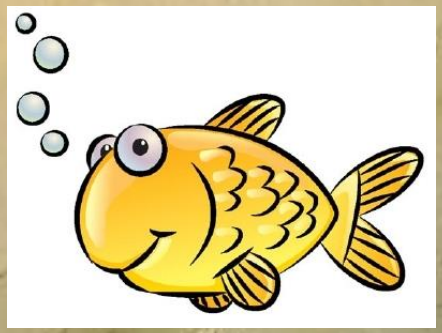


Organisms selected for study

- target tissues – **fish:** gills, liver, intestine
- target tissues – **bivalve:** gills, digestive gland
- whole organism – crustaceans, acantocephalans

Main objective

- ✓ to examine the fate of metals/metalloids at the cellular level, after their bioaccumulation in the target tissues of selected aquatic bioindicator organisms as a result of increased metal exposure in the river water under anthropogenic impact, and to compare it with aquatic organisms from the reference locations



Specific objectives

Evaluation of metal/metalloid accumulation in fish, acanthocephalans, crustaceans and bivalves

Evaluation of biological effects of contamination on biota

Evaluation of subcellular partitioning of metals/metalloids in fish, acanthocephalans, crustaceans and bivalves

Intracellular mapping of trace metals in fish tissues, acantocephalans and bivalve tissues, and correlation between two imaging techniques – NanoSIMS and TEM

Integration of chemical, biochemical and cellular imaging approach

Evaluation of

River water chemical quality status

- physico-chemical properties
- nutrient concentrations
- contamination with metals/metalloids

River water biological quality status

- biodiversity of macrozoobenthos and periphyton
- characteristics and abundance of drift fauna
- presence of specific invasive amphipod species

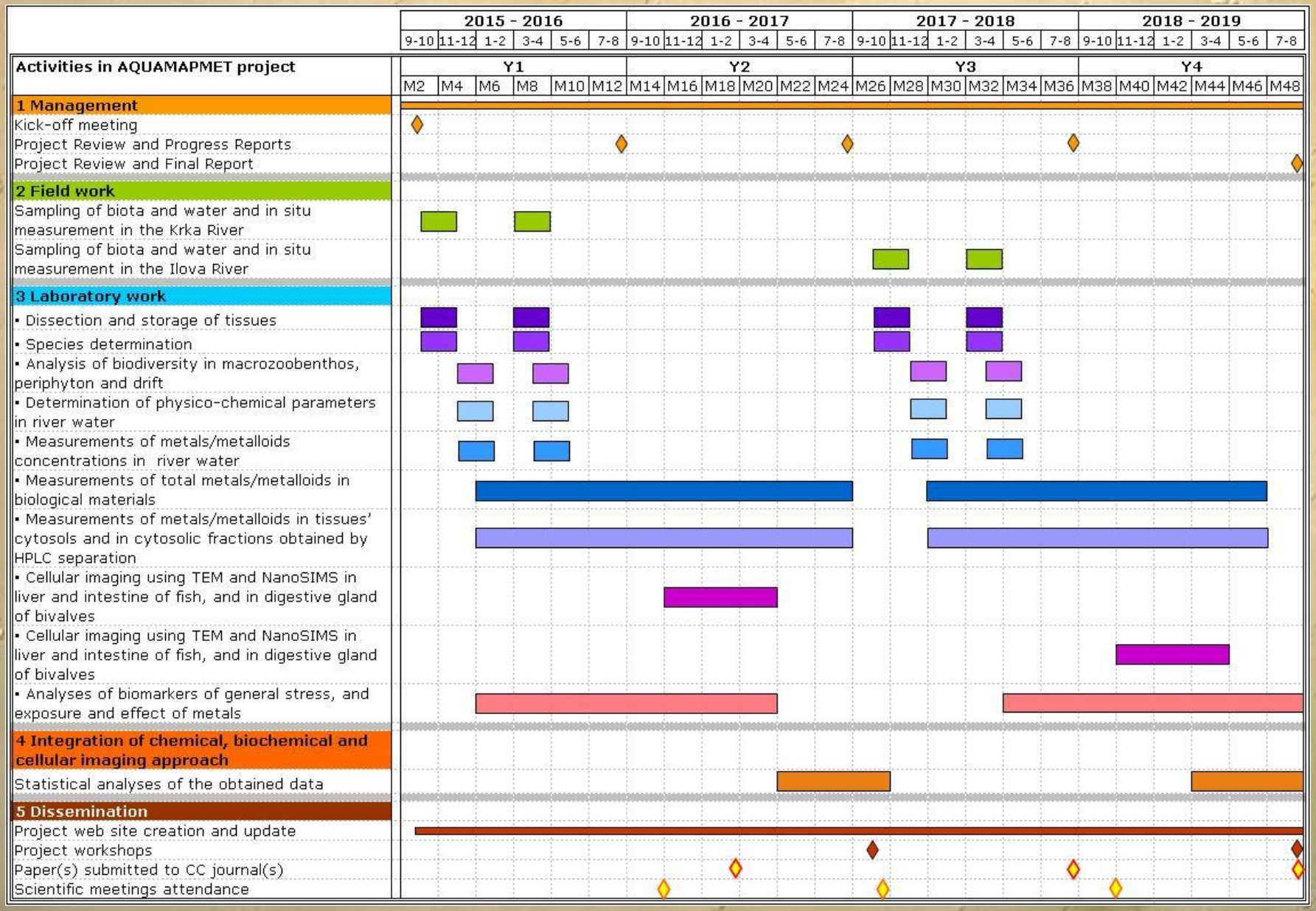
Acanthocephalans as bioindicators

- application as bioindicators of metal exposure
- their possible role in protecting the fish against metal accumulation

Possible risk to human health

- comparison of metal / metalloid concentrations in the trout and chub muscle with the permissible metal concentrations for human consumption

Timing of activities in the AQUAMAPMET project



Detailed work plan – 1st year

Objective 1: The overall running of the project		
Activities	**Duration of activities**	**Team members**
<u>Office work</u> A1.1. Project managing	Sep 2015 - Aug 2016	M. Erk Z. Dragun V. Filipović Marijić D. Ivanković N. Krasnići R. Matoničkin Kepčija S. Gottstein M. Sertić Perić M. Miliša J. Lajtner D. Schaumlöffel J. Malherbe E. Gontier
A1.2. Kick-off meeting	Sep 2015	
A1.3. Preparation of project reviews and progress reports	Aug 2016	

Detailed work plan – 1st year

Objective 2: Sampling in the area of the Krka River		
Activities	**Duration of activities**	**Team members**
<u>Field work</u> A2.1. Sampling of water and biota (electrofishing - service of LAPAO, RBI) A2.2. In situ measurements of physico-chemical parameters in water	Oct-Nov 2015, Mar-Apr 2016	Z. Dragun V. Filipović Marijić D. Ivanković N. Krasnići PhD student R. Matoničkin Kepčija S. Gottstein M. Sertić Perić M. Miliša J. Lajtner
<u>Laboratory work</u> A2.3. Dissection and storage of tissues A2.4. Species determination, detection of invasive species (performed at Biology Department of the Faculty of Science)	Oct-Nov 2015, Mar-Apr 2016	

Detailed work plan – 1st year

Objective 3: Evaluation of river water quality status (biological and chemical)		
Activities	**Duration of activities**	**Team members**
<u>Laboratory work</u> A3.1. Analysis of biodiversity in macrozoobenthos, periphyton and dift (performed at Biology Department, Faculty of Science)	Dec 2015 - Jan 2015	R. Matoničkin Kepčija M. Sertić Perić M. Miliša
A3.2. Determination of physico-chemical parameters in river water (performed at Biology Department, Faculty of Science)	May-Jun 2016	R. Matoničkin Kepčija M. Sertić Perić
A3.3. Measurements of metals/metalloids concentrations in river water		Z. Dragun N. Krasnići

Detailed work plan – 1st year

Objective 4: Evaluation of metal/metalloid accumulation in fish organs, acanthocephalans, crustaceans and bivalves		
Activities	**Duration of activities**	**Team members**
<u>Laboratory work</u> A4.1. Measurements of metals/metalloids in tissue cytosols of fish, bivalves and crustaceans	Jan-Mar 2016	Z. Dragun V. Filipović Marijić D. Ivanković N. Krasnići PhD student
A4.2. Measurements of metals/metalloids in digested tissue homogenates of fish and bivalves	Apr-Jun 2016	
A4.3. Measurements of total metals/metalloids in digested acanthocephalans	Jul-Aug 2016	

Detailed work plan – 1st year

Objective 5: Evaluation of subcellular partitioning of metals/metalloids in fish, acantocephalans, crustaceans and bivalves		
Activities	**Duration of activities**	**Team members**
<u>Laboratory work</u> A5.1. Preparation of cytosolic samples for the separation of cytosolic proteins by HPLC	Jul-Aug 2016	Z. Dragun V. Filipović Marijić D. Ivanković N. Krasnići

Detailed work plan – 1st year

Objective 6: Intracellular mapping of trace metals in fish tissues, acantocephalans and bivalve tissues		
Activities	**Duration of activities**	**Team members**
<u>Laboratory work</u> A6.1. Preparation of tissue/organ/organism samples for TEM and NanoSIMS analyses (service of Laboratory for ichthyopathology – biological materials, RBI)	Oct-Nov 2015	service RBI training of new PhD student and N. Krasnići

Detailed work plan – 1st year

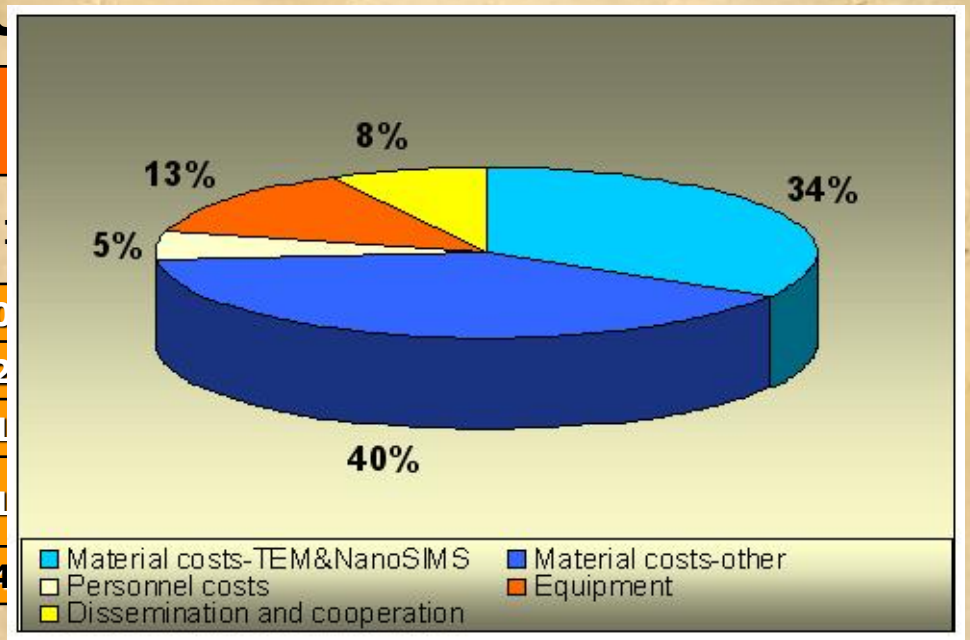
Objective 7: Evaluation of biological effects of metals		
Activities	**Duration of activities**	**Team members**
<u>Laboratory work</u> A7.1. Analyses of biomarkers of general stress	May-Jun 2016	M. Erk V. Filipović Marijić D. Ivanković N. Krasnići PhD student
A7.2. Analyses of biomarkers of exposure and effect of metals	Jul-Aug 2016	

Detailed work plan – 1st year

Objective 8: Dissemination of the project's results		
Activities	Duration of activities	Team members
<u>Office work</u> A8.1. Creating the project web site	Oct-Nov 2015	M. Erk V. Filipović Marijić
A8.2. Updating the project web site	Nov 2015 - Aug 2016	

Project budget

	1-12	
1. Material costs	154,800.00	20
2. Personnel costs	5,000.00	2
3. Equipment	65,250.00	1
4. Dissemination and cooperation	9,000.00	1
TOTAL (1+2+3+4)	234,050.00	24



1 EUR = 7.7 HRK

	Cost per months in EUR				
	1-12	13-24	25-36	37-48	Total
1. Material costs	20,104.00	26,195.00	20,130.00	25,545.00	91,974.00
2. Personnel costs	649.00	2,597.00	1,948.00	1,299.00	63494.00
3. Equipment	8,474.00	1,948.00	4,675.00	1,558.00	16,656.00
4. Dissemination and cooperation	1,169.00	1,299.00	3,766.00	3,636.00	9,870.00
TOTAL (1+2+3+4)	30,396.00	32,039.00	30,519.00	32,039.00	124,994.00

Laboratory for biological effects of metals

Expertise – technical skills and competences

- field-work: sampling and dissection of aquatic organisms
- determination of general physico-chemical parameters in river water
- preparation of biochemical samples for cytosolic metal and protein analysis
- preparation of tissues for multielemental analysis – total digestion (microwave)
- electrochemical and spectrophotometric methods for quantification of metallothioneins

Laboratory for biological effects of metals
Expertise – technical skills and competences

- determination of lysosomal membrane stability in mussel hemocytes by NRR (Neutral red retention time) method
- spectrophotometric determination of protein, lipid and carbohydrate contents; kinetic measurement of electron transport system (ETS) activity; analysis of malon dialdehyde formation
- size-exclusion HPLC for separation of cytosolic proteins
- metal determination by HR ICP-MS and AAS (flame and electrothermal techniques)

LBEM - equipment

- ICP-MS (LIEG)
- HPLC
- AAS
- UV-VIS spectrophotometer
- computerized potentiostatic instrument with multimode Hg-electrode
- microwave oven
- microscope
- portable sensors for in situ measurements
- tissue homogenizer
- lyophilizer
- centrifuges
- deep-freezers -80°C
- precision and analytical balances


START