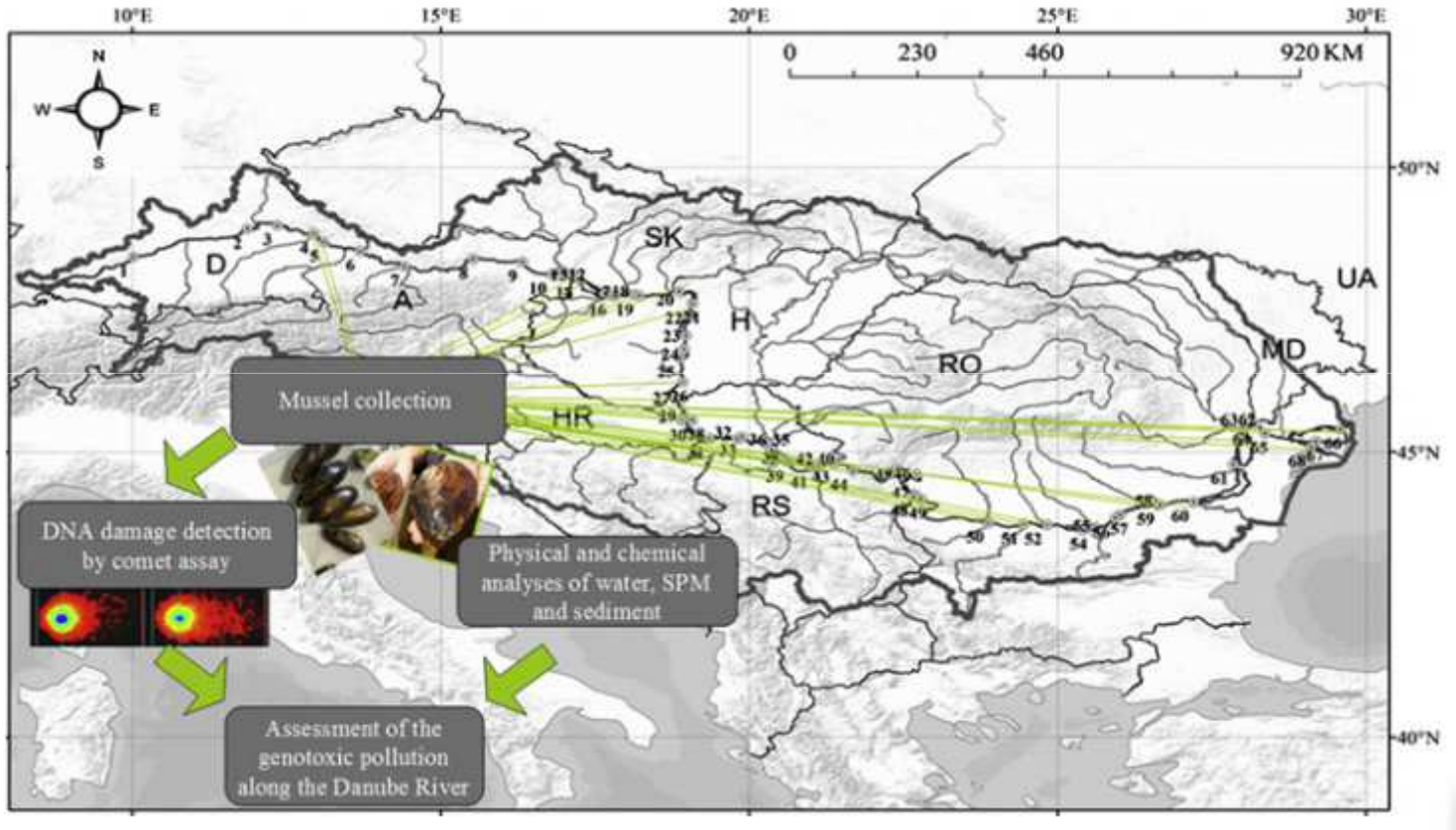
GENOTOXICOLOGICAL ASPECT OF THE JOINT DANUBE SURVEY

Program proposed by:

Center for Genotoxicology and Ecogenotoxicology
Faculty of Biology/Institute for Biological Research, University of Belgrade, Serbia

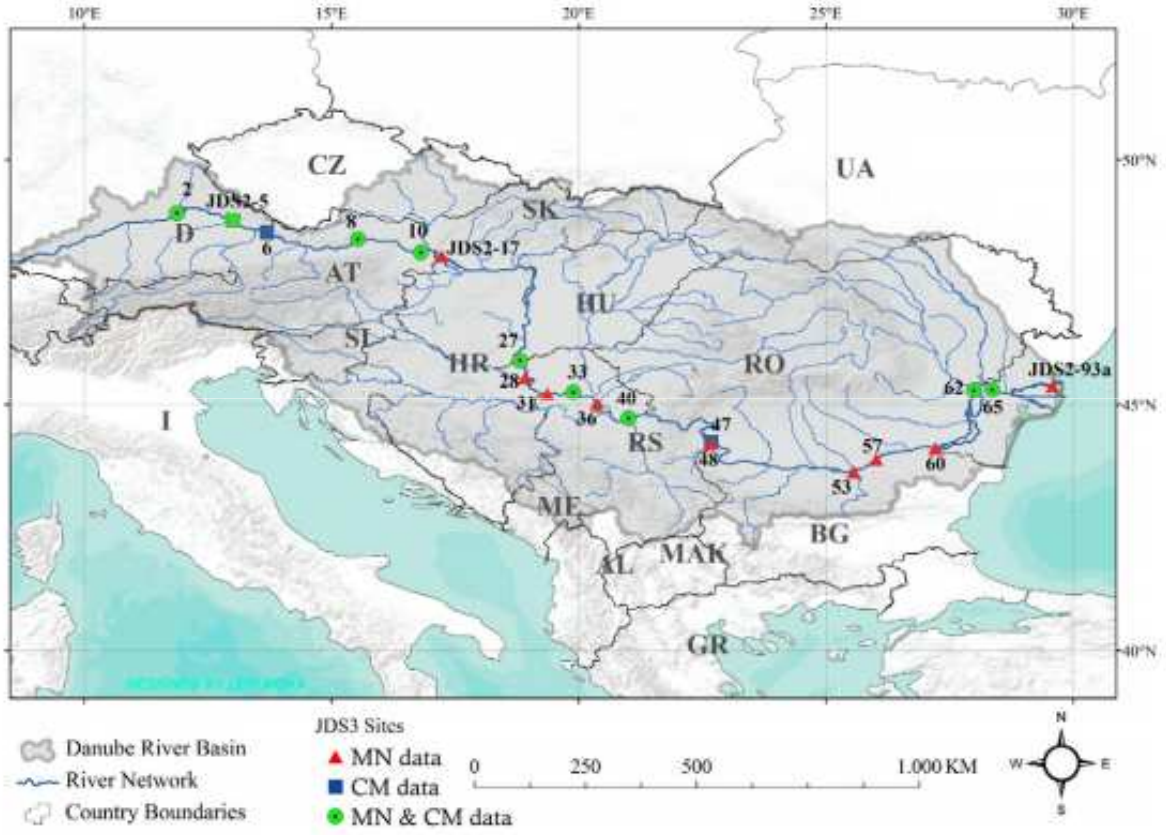
National Institute of Biology, Ljubljana, Slovenia

GENOTOXICOLOGY WITHIN JDS3-MUSSELS



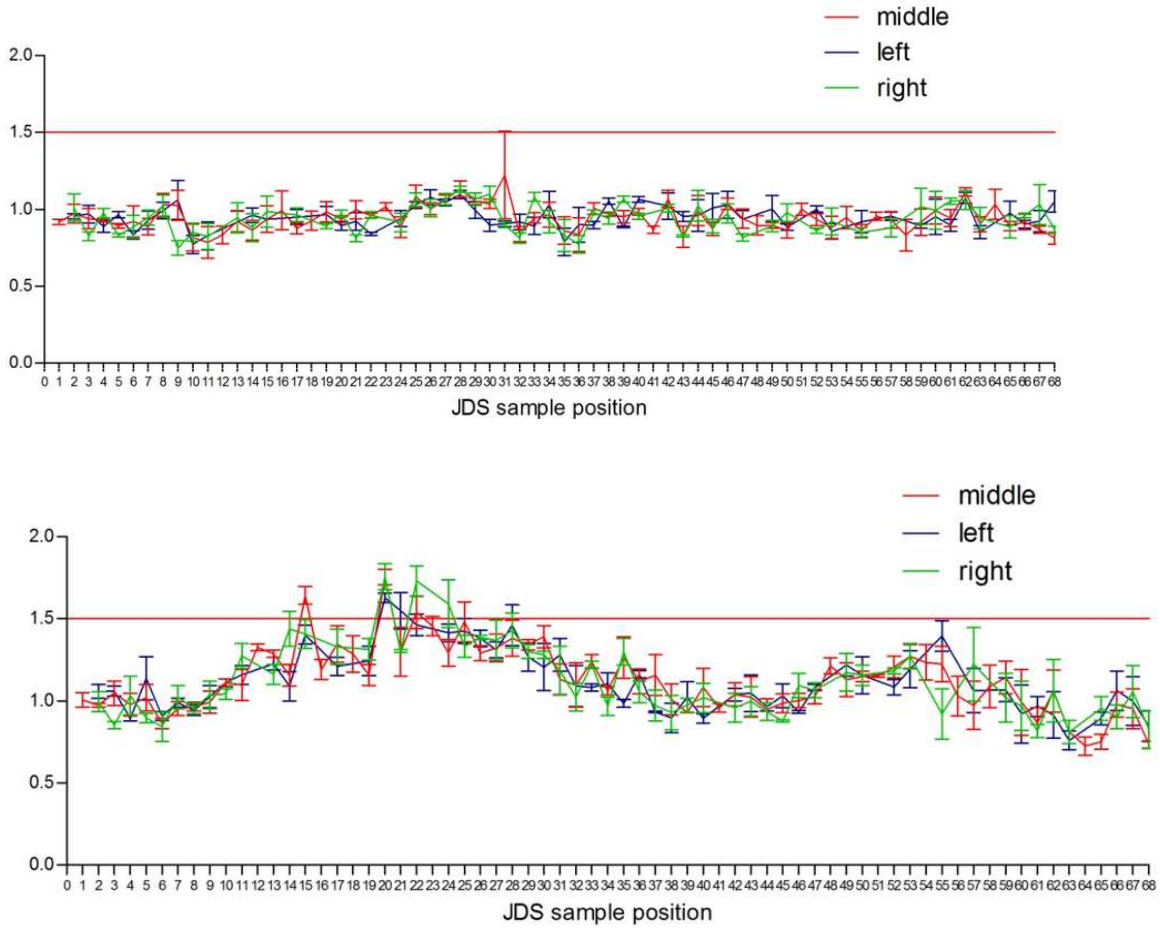
Kolarević, S., Kračun-Kolarević, M., Kostić, J., Slobodnik, J., Liška, I., Gačić, Z., Paunović, M., Knežević-Vukčević, J., Vuković-Gačić, B. (2015). Assessment of the genotoxic potential along the Danube River by application of the comet assay on haemocytes of freshwater mussels: The Joint Danube Survey 3. Science of the Total Environment, 540, 377-385

GENOTOXICOLOGY WITHIN JDS3-FISH



•Deutschmann, B., Kolarevic, S., Brack, W., Kaisarevic, S., Kostic, J., Kracun-Kolarevic, M., Liska, I., Paunovic, M., Seiler, T-B., Shao, Y., Sipos, S., Slobodnik, J., Teodorovic, I., Vukovic-Gacic, B., Hollert, H. (2016). Longitudinal profile of the genotoxic potential of the River Danube on erythrocytes of wild common bleak (*Alburnus alburnus*) assessed using the comet and micronucleus assay. Science of the Total Environment. 573, 1441-1449.

GENOTOXICOLOGY WITHIN JDS3-NATIVE WATER SAMPLES

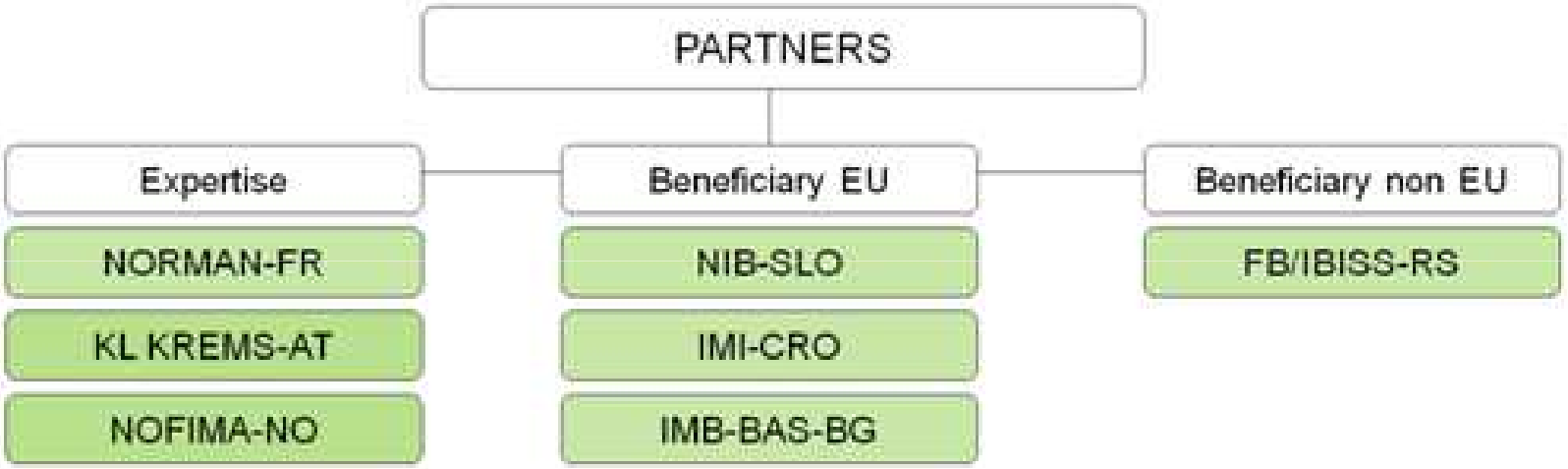


Kittinger, C., Baumert, R., Folli, B., Lipp, M., Liebmann, A., Kirschner, A., ... & Zarfel, G. E. (2015). Preliminary toxicological evaluation of the river Danube using in vitro bioassays. *Water*, *7*(5), 1959-1968.

GOALS WITHIN THE PROPOSED EEA PROJECT:

- Identification of the hotspots of genotoxic pollution along the Danube River by combined *in situ/in vitro* approach.
- Identification of the major xenobiotic stressors at the selected sites.
- Impact of the pollution on the physiological and molecular biomarkers and population genetic structure.
- Impact of pollution on population epigenetics, dynamics in epigenetic make-up.
- Estimation of gene expression and epigenetic profiles as valuable pollution indicators.
- Molecular mechanisms of adaptation to xenobiotic stressors in organisms from the sites under pressure.

GOALS ACHIEVEMENT IN COLLABORATION WITH PARTNERS:



Proposed alternative Program:

- Joint program of UB (RS) and NIB (SLO)
- Combined *in situ/in vitro* approach

UB - *IN SITU* DETECTION OF GENOTOXIC POTENTIAL (UP TO 10 SITES)

- Research will be performed in fish and mussels
- Assessment of genotoxic potential by Comet assay and MN test
- Focus will be placed on the Serbian stretch of the river (limitations because of the national team support)

NIB - *EX SITU* DETECTION OF GENOTOXIC POTENTIAL (UP TO 30 SITES)

- Program will be focused on the water extracts obtained from large volume sampling or passive samplers
- Testing will be performed in prokaryotic and eukaryotic models
- In the first phase the screening of the genotoxic potential of all samples will be performed at one concentration that will be selected according to the results of cytotoxicity tests.
- SOS/umuC without and with S9 metabolic activation (we can test native un-concentrated samples or concentrated samples- two different versions of the method)
- In vitro testing in cell cultures: human hepatoma HepG2 cells and/or in case of fish RTL-W1, which is a fibroblast-like non-transformed permanent cell line isolated from the liver of adult male rainbow trout (Oncorhynchus mykiss).

NIB - *EX SITU* DETECTION OF GENOTOXIC POTENTIAL IN CONCENTRATED WATER SAMPLES (UP TO 30 SITES)

- On cells we can apply:
- Comet assay (samples selected according to the preliminary results from the SOS/umuC assay or chemical analyses – expected positive end effects according to the presence of specific chemicals
- gH2AX- the assay measures DNA double strand breaks and we can perform the testing using flow cytometry
- CBMN assay (the method is more time consuming; so I suggest to perform the method on selected samples
- For the genotoxic endpoints on cells the same cell line should be used (it has to be decided to use human or fish cells).