EC FP 6 – facilitated success story how LECOTOX became visible on scientific map of Europe

LECOTOX - Laboratory for Ecotoxicology at the University of Novi Sad Faculty of Sciences, was formally established in 2006 to form a functional competitive research team ready for application of genomics based tools in ecotoxicology, together with conventional toxicity tests and traditional function-based biomarkers, which may be used to validate the toxic mechanisms of the contaminants. LECOTOX is focused on two topics: (a) endocrine disruption / reproductive toxicity and (b) identification and characterization of aquatic toxicity.

REP-LECOTOX project - INCO-CT-2006-043559-REP-LECOTOX UNSFS - Reinforcement of research potential of the laboratory for ecotoxicology funded by EC FP6 (2007-2009) presented a crucial milestone for LECOTOX and its visibility and presentation to international scientific community. The project enabled upgrading and renewal of S&T equipment, hiring new young researches to reinforce the human resources, but above all that, it facilitated extensive networking - via workshops, exchange of scientific personnel and trainings of young scientists in some of the finest research institutions in EU in the field of environmental research: Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany; School of Bioscience, University of Birmingham, UK and RECETOX, Masaryk University, Brno, Czech Republic. Literally, REP-LECOTOX project facilitated positioning of LECOTOX on Europe's map of emerging groups and laboratories, and our integration into the European research area as a promising partner in fundamental and applied research projects.

And here is the story….

Content

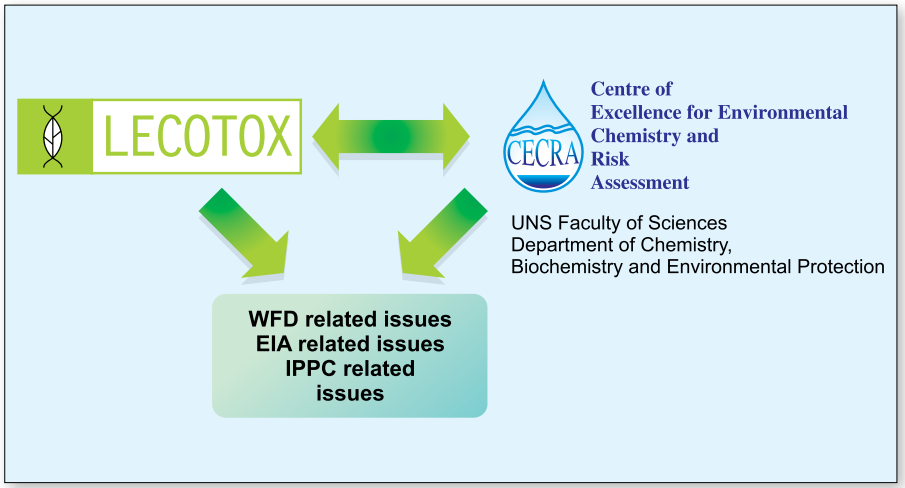
LECOTOX before REP-LECOTOX Project...

The University of Novi Sad is an educational, scientific and art institution founded in 1960 by the Republic of Serbia. It is comprised of 13 faculties, located in four main cities of the Autonomous Province Vojvodina, and has educational and scientific autonomy. One of them is Faculty of Sciences. It was founded in 1969, but teaching of Natural Sciences and Mathematics dates from 1961, when these sciences were taught at the Faculty of Philosophy. Faculty of Sciences consists of following Departments: Biology & Ecology, Chemistry, Biochemistry & Environmental Protection, Physics, Geography, Tourism & Hotel Management and Mathematics & Informatics.

Although ecotoxicological research at UNSFS dates back many years, during the 1990s it was patchy and restricted to national and regional funding, insufficient for basic consumables and chemicals needed for proper research. The overall scientific quality (and visibility) constantly failed to reach the level needed to become an equal partner in any of the European scale ecotoxicological research and networking projects, although the laboratory facilities, existing equipment, methods and level of scientific significance (in terms of papers in peer reviewed journals) already overreached the modest funding.

Activities of the LECOTOX researchers are focused on toxic impact of persistent organic pollutants (POPs) on biota with special emphases on fresh water ecosystem and specific sub-

lethal types of toxicity (endocrine disruption, reproductive toxicity, and “dioxin-like” toxicity). The aims of the LECOTOX group are identification and characterization of aquatic toxicity in accordance with modern scientific approach, environmental quality standards and criteria. Evaluation of the quality of environmental samples taken from potentially polluted area is done by bioanalyses based on the molecular and cellular biomarkers of effects (CYP enzyme induction, parameters and markers of oxidative stress and early intoxication, macrophage aggregates), and acute and chronic toxicity tests (with special emphasis on reproduction tests) on a battery of standard aquatic laboratory organisms. In close cooperation with the Department of Chemistry, Biochemistry and Environmental Protection, Laboratory of Ecotoxicology conducts aquatic toxicity tests according to standard ISO/CEN, OECD and US EPA methodology for routine ambient water quality monitoring and compliance monitoring of industrial and urban effluents (whole effluent toxicity testing - WET), under requirements of national regulations in force, as a prerequisite for remediation activities on contaminated sediments and soils and for environmental impact assessment. Laboratory is facilitated for tests aimed at setting environmental standards (according to requirements and provisions of Annex V of Water Framework Directive). Laboratory assists governmental and authorities industry in the area of investigating the effect and fate of substances in the environment. The results of the standardized ecotoxicological tests are used as required by governmental authorities in compliance monitoring and in risk assessments, i.e. for chemicals, pesticides, effluents and contaminated sediments and soils. All studies are carried out in compliance with Good Laboratory Practice (GLP).

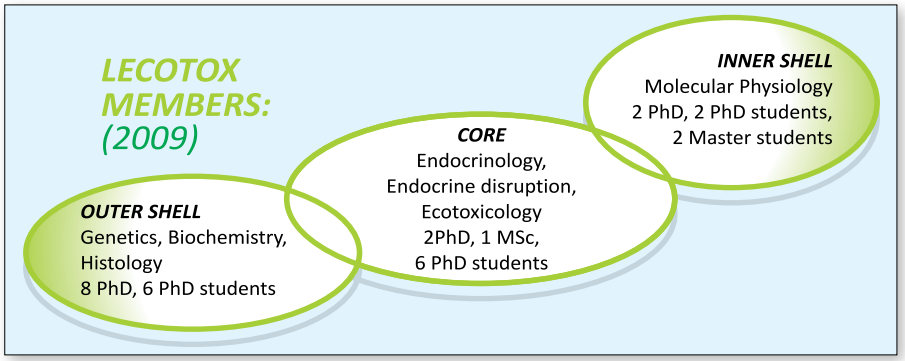


The WFD classifies the quality status of aquatic ecosystems based on traditional hydromorphological, physico-chemical, biological parameters and priority pollutant (PP) concentrations. However, biomonitoring and classical ecotoxicological studies proved not to be sufficient in understanding the overall adverse impact of toxic pollutants on aquatic ecosystems, in spite of extensive studies and enormous efforts of scientific community

worldwide. Toxicity is accompanied by changes in gene expression that are either causally linked to toxic outcomes or are downstream signalling events of toxic exposure. The measurement of gene expression levels upon exposure to a chemical can be used both to provide mechanistic knowledge of the genetic basis of toxic effect and to form a sort of «genetic signature» for the identification of toxic product. Ecotoxicogenomics, a study of gene and protein expression important in adaptive response to environmental toxicant exposure, if applied together with well established methods and techniques for environmental quality assessment, could provide a reliable diagnosis, prediction and forecasting of toxic impacts on ecosystems. Therefore, the aim of LECOTOX research group is to use gene expression as sophisticated and reliable biomarkers of exposure to environmental contaminant, together with traditional function-based biomarkers, which may be used to validate the toxic mechanisms of the contaminant.

LECOTOX – Laboratory for Ecotoxicology at the Department of Biology and Ecology at University of Novi Sad Faculty of Sciences was formally established in 2006 as a result of intention to overcome the former fragmentation of the research groups. Having recognized the great potential of "omic" methods in ecotoxicological research and risk assessment, LECOTOX core research team made an initial step towards application of genomics-based tools in ecotoxicology. Mainly focused on two topics: (a) endocrine disruption/reproductive toxicity and (b) identification and characterization of aquatic toxicity, LECOTOX decided to combine transcriptomics with established conventional toxicity tests and traditional function-based biomarkers.

FP 6 INCO Programme presented an ideal chance for established but sub-optimally equipped research groups from new, candidate and non-EU member countries to fully integrate into the international scientific community. The overall aim of the Capacities Programme is to enhance research and innovation throughout Europe by optimizing research infrastructure in Europe, enhancing research potential of European convergence and outermost regions, and building strategic R&D partnerships with non-EU countries. LECOTOX team, led by the Head of Laboratory, Prof. Dr. Radmila Kovacevic, recognized that unique chance and prepared a project proposal for **FP6 INCO-2005-C-WBC SSA** call for **Reinforcement of the WBC research capacities** launched in 2006.



At the moment of application to the call in question, research groups within LECOTOX consisted of 9 PhD researchers, 4 research assistants (M.Sc.), and 6 M.Sc./PhD students already involved in the realization of certain projects. The following senior researchers were members of LECOTOX group in 2006: LECOTOX Core group consisted of Head of LECOTOX Prof. Dr. Radmila Kovacevic & Assist. Prof. Dr. Nebojsa Andric (Physiology, Endocrinology, Endocrine disruption), and Assoc. Prof. Dr. Ivana Teodorovic (Ecotoxicology); "inner shell" senior scientists were Assist. Prof. Dr. Silvana Andric and Assist. Prof. Dr. Tatjana Kostic (Molecular Physiology), while the "outer shell" researchers were Prof. Dr. Gordana Grubor Lajsic (Biochemistry), Prof. Dr. Milica Matavulj (Histology), Prof. Dr. Ljiljana Vapa and Assist. Prof. Dr. Dragana Obreht (Genetics). However, the project was mainly implemented by the LECOTOX Core group members, and therefore, this compendium mostly presents the overview of activities, results and progress of Core LECOTOX research group.

At the moment of application to the call in question, LECOTOX Core group has just got a new national project:

2006 – 2010: Endocrine disruption chemicals – effects on reproductive and thyroid function, environmental assessment and bioanalyses, Project No 143058, Funded by Republic of Serbia Ministry of Science and Technological Development, **Project coordinator Prof. Dr. Radmila Kovacevic**. The project was (and still is) focused on: (a) impact of selected BFRs, PCBs, PAHs and pesticides on testicular and thyroid function; possible toxic effect is being studied by exploring the expression of genes involved in testicular steroidogenesis, activity of steroidogenic enzymes, structure and ultrastructure of thyroid gland, thyroid hormone levels and other biochemical indicators, and (b) the use of biomarkers as the endpoints in bioanalyses and toxicity tests to study presence of different EDCs in various environmental matrices, in parallel with chemical characterisation. Expected results are (a) better insight into the mechanisms of possible adverse effects of selected EDCs and (b) development of toxicity identification and evaluation approach where bioassays direct the chemical identification of chemicals.

Also, many of LECOTOX researchers have already had certain experience in international projects:

- **2002-2003 WUS-C.E.P. Project: "Biomarkers of exposure and of effects of persistent organic pollutants in ecosystem". Project coordinator Prof. Dr. Radmila Kovacevic.**

- **2002-2005 FP5 RTD-COPERNICUS Project: "Assessment of the selected POPs (PCBs, PCDD/Ps, POCPs) in the Atmosphere and Water Ecosystems From Waste materials Generated By Warfare in Area of Former Yugoslavia - FP5 RTD - APOPSBAL -ICA2-2002/2005. Participants in the project.**

- **2004-2005 WUS-CDP+ Project: Course Developmental programme Physiological Module. Project coordinator Prof. Dr. Radmila Kovacevic**

The implementation of the listed projects already reinforced the research capacity of LECOTOX in terms of equipment:

2002-2004: Project "Biomarkers of Exposure and of Effects of Persistent Organic Pollutants in Ecosystem", WUS-CEP, Austria, Grant No. 121/2002
Safety bench and CO2 incubator for cell structures

Project: APOPSBAL - FP5 RTD INCO FP5 ICA 2 - CT2002 - 10007
Microplate reader for fluorimetric and luminometric measurements

As a result of the research activities on realization of the listed project, LECOTOX researchers already had a significant number of publications in respectful international journals, like for example:

Andric N. L., Kostic T .S., Zoric S.N., Stanic B. D., Andric S. A., Kovacevic R. Z. (2006): Effect of a PCB-based transformer oil on testicular steroidogenesis and xenobiotic-metabolizing enzymes. *Reproductive Toxicology* 22, 102-110.

Stanic B., Andric N., Zoric S., Grubor-Lajsic G., Kovacevic R. (2006): Assessing pollution in the Danube River near Novi Sad (Serbia) using several biomarkers in sterlet (*Acipenser ruthenus* L.) *Ecotoxicology and Environmental Safety* 65, 395-402.

Dalmacija B., Prica M., Ivancev – Tumbas I., van der Kooij A., Roncevic S., Krcmar D., Bikit I, Teodorovic I. (2006). Pollution of the Begej Canal sediment – metals, radioactivity and toxicity assessment. *Environment International* 32, 606–615.

Picer M., Kovacevic R., Picer N., Kobasic V.H., Calic V., Zoric S. (2006) Characterization of Soil and Sediment Samples Collected from the Zadar Area, Croatia, by GC-ECD Analysis and Bioassay. *Bulletin of Environmental Contamination and Toxicology* 77, 687-693.

Zoric S., Andric N., Suđi J., Klanova J., Jovetic S., Kovacevic R., Vojinovic-Miloradov M. (2004) Ethoxyresorufin-O-deethylase induction potency in sediment samples from rivers Lepenica and Morava –surrounding area of Kragujevac "hot spot". *Organohalogen Compounds* 66, 598-602.

REP-LECOTOX Project

REP-LECOTOX is an acronym for the project INCO-2005-C-WBC 043559 – REP-LECOTOX "Reinforcement of Research Potential of the Laboratory for Ecotoxicology". The overall aim of the project is to strengthen research capacities and potential of the Laboratory for Ecotoxicology at the University of Novi Sad, Faculty of Sciences, Serbia and to facilitate introduction and application of genomics based tools as a prerequisite for Laboratory's long term strategic orientation towards one of the most promising fields of environmental research today – ecotoxicogenomics.

The major REP-LECOTOX objectives are to:

- **Upgrade and renew equipment necessary for successful introduction of ecotoxicogenomic techniques in the research;**
- **Hire new young researchers;**
- **Expand international collaboration by networking to:**
  - enhance the expertise of LECOTOX senior researchers;
  - provide training for young researchers;
- **Develop, launch and support information system through web portal offering access to thematic and general information in the field of ecotoxicology/ecotoxicogenomics;**
- **Organize Workshops: "Ecotoxicogenomics: the challenge of integrating genomics/proteomics/metabolomics into aquatic and terrestrial ecotoxicology", and "Trends in Ecological Risk Assessment".**

Four outstanding EU Institutions have supported the project by offering their expertise and high quality trainings for LECOTOX young researchers:

- **Centre for Environmental Research - UFZ, Leipzig, Germany with two Departments:**
  - Department of Effect-Directed Analysis, Head of the Department Dr. Werner Brack, was also one of the ISAB members of the project;
  - Department of Bioanalytical Ecotoxicology (former Department of Cell Toxicology), represented by Dr. Stefan Scholz.
- **RECETOX, Masaryk University, Brno, Czech Republic, represented by the Director of the Centre, Prof. Dr. Ivan Holoubek who also served as one of the ISAB members of the project. RECETOX also participated with two research groups:**
  - Ecotoxicology Division, AQUATOX, represented by Assoc. Professor Dr. Ludek Blaha, Head of the Ecotoxicology Division and Dr. Klara Hilscherova
  - SOILETOX research group, represented by Dr. Jakub Hofman.
- **School of Biosciences, The University of Birmingham, Edgbaston, Birmingham, UK, represented by Head of School, Prof. Dr. James Kevin Chipman, who also served as one of the ISAB members of the project**
- **Department of Animal Physiological Ecology, Zoological Institute, University of Tübingen, Germany, represented by Prof. Dr. Heinz-R. Koehler, Head of Department, who also served as one of the ISAB members of the project**

To facilitate efficient implementation of the project, activities have been divided into 5 work packages:

- **WP 1 Mobility and Training**
  - Trainings for young researchers
  - Short visits of LECOTOX senior and junior staff to partner institutions
  - Short visits of researchers from partner institutions to LECOTOX
- **WP 2 Dissemination of Information**
  - Internet portal
  - PR material
  - Scientific events

- **WP 3 Organization of the Workshops**
  - 1st REP-LECOTOX Workshop "Ecotoxicogenomics: the challenge of integrating genomics/proteomics/metabolomics into aquatic and terrestrial ecotoxicology"
  - 2nd REP-LECOTOX Workshop "Trends in Ecological Risk Assessment"
- **WP 4 Reinforcement of LECOTOX**
  - Equipment
  - Young researchers
- **WP 5 Management**
  - Management Committee: Prof. Dr. Radmila Kovacevic - project coordinator & Head of LECOTOX; Dr. Ivana Teodorovic; Dr. Silvana Andric and Dr. Tatjana Kostic

International Scientific Advisory Board (ISAB):

Dr. Werner Brack, PhD. in Environmental Chemistry. Head of the Department of Effect-Directed Analysis at the UFZ. Coordinator of the EU Integrated Project MODELKEY focusing on the assessment of the impact of environmental key pollutants on freshwater and marine ecosystems and related national projects; main research topic - the effect-directed identification of chemical key toxicants in complex environmental mixtures such as sediments, ground- and surface water and biota with focus on integrated risk assessment.

Prof. Dr. James Kevin Chipman, Ph.D., FIBiol. FRCPath. Professor of Cell Toxicology; Molecular Pathobiology, Head of School of Biosciences, The University of Birmingham, Edgbaston, Birmingham, UK. Molecular biomarkers of genotoxicity and stress responses, use of toxicogenomics in environmental risk assessment.

Prof. Dr. Ivan Holoubek, Professor of Environmental Chemistry, Director of RECETOX, Masaryk University, Brno, CZ. Research activities: the fate of persistent organic pollutants in the environment, environmental impact assessment, risks analysis - ecological risk assessment.

Prof. Dr. Heinz-R. Koehler, Professor of Animal Physiological Ecology, Head of Department of Animal Physiological Ecology, Zoological Institute, University of Tübingen, Germany. Research activities: assessment of small stream pollution, stress proteins, endocrine effects of xeno-hormones in aquatic biota, effects of pharmaceuticals in freshwater, implementation of ecotoxicological theories in practical application.

Mobility and Training

During the lifetime of the project, 11 trainings for young researchers were accomplished in three partner institutions. Eleven short term visits of LECOTOX senior and junior scientist to partner institutions were realized over the 3 years, while the reverse (researchers from EU institutions, mainly partner, to LECOTOX) 14 visits were realized during REP-LECOTOX Workshops.

Project year			UFZ, Leipzig	RECETOX, Brno	School of Biosciences, Birmingham
1 year	**Trainings**		**2**	**1**	**0**
	Short term visits	LECOTOX to partners	2	1	2
		Partners to LECOTOX	0	0	0
2 year	**Trainings**		**1**	**0**	**1**
	Short term visits	LECOTOX to partners	0	3	0
		Partners to LECOTOX	2	1	1
3 year	**Trainings**		**4**	**1**	**1**
	Short term visits	LECOTOX to partners	3	0	0
		Partners to LECOTOX	3	3	0
Sum per partner	**Trainings**		**7**	**2**	**2**
	Short term visits	LECOTOX to partners	5	4	2
		Partners to LECOTOX	5	4	1
Total for the project	**Trainings**		**11**		
	Short term visits	LECOTOX to partners	12 - 11 to partners and 1 visit to non-partner institution		
		Partners to LECOTOX	14 – 10 + 4 additional visits of 2 researchers from non-partner institutions		

Trainings at UFZ, Leipzig, Germany

Two Departments of UFZ Leipzig - Department of Effect-Directed Analysis (EDA), supervisor Dr. Werner Brack and Department of Bioanalytical Ecotoxicology (former Department of Cell Toxicology), supervisor Dr. Stefan Scholz provided trainings for REP-LECOTOX research trainees and hosted LECOTOX senior and junior scientists during the project implementation.

Dr. Brack with his EDA research group focuses on developing tools for precise detection of key environmental (mainly aquatic) toxic pollutants as well as methods and models for ecological risk assessment and environmental monitoring based on observed biological effects rather than on substance monitoring.

Therefore, Dept. of EDA hosted both biologist and chemists from UNSFS, as building up a competent, coherent inter and multidisciplinary team is a prerequisite for the implementation of complex, tiered EDA approach, which, as the currently best recognized method in risk assessment of complex environmental matrices is the ultimate goal for LECOTOX and UNSFS. Thus, one LECOTOX member - PhD student in Biology – **Sonja Kaisarevic** (in the photo) spent 2 months in UFZ in 2007 (thanks to the co-funding by Provincial Secretary for Science and Technological Development of Province Vojvodina, Serbia and COST Action 636) and two PhD students in Chemistry **Dejan Orcic** and **Aleksandra Tubic** (in the photo) were hosted by Dept. of EDA for one month in 2007 and 15 days in 2009, respectively.

Accelerated Solvent Extractors ASE 300 and ASE 200, followed by an automated on-line fractionation procedure on three coupled normal phase HPLC columns, enabled fractionation of sediment samples into 18 fractions, each containing different type of pollutants based on their structure, physical and chemical properties. Fractions were further analysed in LECOTOX by microEROD bioanalysis on H4IIE rat hepatoma cell culture, which enabled determination of presence of dioxin-like compounds and total toxic potency in each fraction. Apart from this, cytotoxicity tests, as well as analysis of corresponding gene expression after treatment of the cells with the investigated fractions were also carried out. Qualitative analysis of semivolatile

pollutants was performed by means of gas chromatography-mass spectrometry (GC-MS) operating in Scan mode, in conjunction with appropriate data analysis applications and chemometric tools. Quantitative analysis included determination of polycyclic aromatic hydrocarbons (PAHs), methylated PAHs, dibenzothiophene and carbazole (as these were most abundant compounds in sample fractions showing significant activity), and was also done using GC-MS, operating in SIM mode, and using external standard (ESTD) technique. More detailed fractionation of two most potent fractions was performed during 2009 by automated on-line fractionation procedure on reverse phase HPLC, which enables fractionation of contaminants into 10-13 sub-fractions.

The results were presented at the SETAC Europe 18th Annual Meeting 2008 and published:

Kaisarevic S, Lübcke-Von Varel U, **Orcic D**, Streck G, Schulze T, **Pogrmic K**, **Teodorovic I**, Brack W, **Kovacevic R** (2009). Effect-Directed Analysis Of Contaminated Sediment From The Wastewater Canal In Pancevo Industrial Area, Serbia. **Chemosphere, 77 (7), 907-913**.

Dr. Scholz and his research group within Dept. of Bioanalytical Ecotoxicology focus the research on developing alternative ecotoxicological methods which would eventually replace whole animal testing (in line with current trends) and ecotoxicogenomics - the group already has a respectful record of application of genomic-based tools (mainly transcriptomics) into research on mode-of-action of a wide range of xenobiotics. The research interest of Dr. Scholz and his group directly corroborates with the main objectives of REP-LECOTOX project, namely, the trainings organized there directly helped introduction of ecotoxicogenomics into laboratory practice of LECOTOX and, the diversification of *in vitro* methods on widely recognized biological models applied in LECOTOX research.

REP-LECOTOX research trainee, **Svetlana Fa** (in the photo) had an opportunity to spend two months (one at a time, in two successive project years) with Dr. Scholz and his research group. Her main tasks included establishment and optimization of *Danio rerio* (eng. Zebrafish) testis tissue culture. The procedure is interesting to LECOTOX as well as the Department of Bioanalytical Ecotoxicology because such system could be used for testing endocrine disrupting substances on the level of testicular steroidogenesis in fish. Having fulfilled the prerequisites (including a short term visit to Department of Biology, division of Endocrinology and Metabolism, Utrecht University, Netherlands – where the method originates from), she performed a number of experiments, testing toxicity of a brominated flame retardant hexabromocyclodoedecane (HBCDD) upon gene expression of enzymes involved in fish steroidogenesis using Real-Time PCR. The results will be incorporated into Svetlana's thesis, while the new method has been incorporated into regular research practice of both laboratories.

Two youngest REP-LECOTOX research trainees, **Jelena Hrubik** and **Branka Glisic** (in the photos with Dr. Scholz and his research group), both PhD students in Molecular Biology, spent shorter, yet very useful time at the host department in 2009. The aim of the trainings was to get familiar with embryo Toxicity Test (*Dar*T), an alternative to acute toxicity tests on fish. Also, they were trained to perform process of dechorionation of *Danio rerio* embryos, which is used for testing toxic substances whose action is blocked by chorion. During their stay in UFZ Jelena and Branka had an opportunity to learn the process of microinjection, which is used to input specific substances in unicellular stage embryos. As LECOTOX is planning to introduce *DarT* test in the next phase of the development, the experience the research trainees gained during the training would be valuable for them personally as well as to LECOTOX group.

Trainings at RECETOX, Masaryk University, Brno, Czech Republic

Ecotoxicological Division of RECETOX represents one of the research units well known in Europe for a high diversity of *in vivo* and *in vitro* ecotoxicological tests, routinely performed in research and for monitoring purposes. As the general aim of REP-LECOTOX project was to build up research potential by introduction of several new *in vivo* ecotoxicological test methods, the purpose of two trainings, accomplished by REP-LECOTOX research trainee, **Ivana Planojevic** (in the photo with Dr. Ludek Blaha and his research group) was to get skilled in several aquatic as well as terrestrial tests, which would be introduced into LECOTOX during the next developmental phase, and, as well, be useful for trainees' MSc and later PhD work.

During the first training within AQUATOX research group, real sediment samples were brought from Serbia, from highly contaminated wastewater channel of Pancevo industrial zone and subjected to tests using standardized test organisms *Vibrio fischeri*, *Pseudokirchneriella subcapitata* and *Daphnia magna*. In addition, chemical analyses for identification and quantification of metals were conducted. The results were incorporated into Ivana's master thesis and presented at SETAC Europe 18th Annual Meeting in 2008.

The second Ivana's research training in RECETOX was accomplished within SOILETOX research group under supervision of Dr. Jakub Hofman. Focus was set on methods for culturing and performing toxicity tests on nematode *Caenorhabditis elegans*. During Ivana's training various samples of soils and sediments from all over the Czech Republic and a few from Serbia were tested. Also, Ivana got familiar with other terrestrial and sediment toxicity tests with *Lactuca sativa, Folsomia candida* and *Enchytraeus albidus*. Some of the results of the research done during the second training will be presented at SETAC Europe 20th Meeting in 2010. The integrative full paper based on joint research done during trainings and poster presentation of Ivana's work at LECOTOX (in the frame of her PhD thesis) presented during 2nd REP-LECOTOX Workshop in Novi Sad in 2009 ***Planojevic I,, Teodorovic I.****, Bartova K., Machat, J., Blaha L.,* ***Kovacevic R****: Wastewater canal Vojlovica, industrial complex Pancevo, Serbia – preliminary ecotoxicological assessment of contaminated sediment* have been submitted for publication in ***Journal of Serbian Chemical Society.*** The methods learned in RECETOX, during 2007 and 2009 will help Ivana to complete her PhD thesis and eventually establish some of the selected methods into the routine practice of LECOTOX.

Trainings at School of Biosciences, University of Birmingham, Edgbaston, Birmingham, UK

The laboratory with sophisticated equipment and competent specialists (lead by Prof. Chipman) from the field of transcriptomics, techniques widely used in ecotoxicology and toxicology for measuring effect of stressors and understanding mechanism of their toxicity was a perfect place to gain theoretical and practical knowledge, applicable to planned research activities of LECOTOX. **Vanja Dakic** (in the photo), REP-LECOTOX research trainee was twice hosted by Prof. Chipman and his research group, so during 2008 and 2009, she spent 2 months altogether in this partner institution.

During her training she was involved in the gene expression analysis by qRTPCR of some key responding genes of *Daphnia magna* (standard aquatic test organism) after treatment with benzo (a)pyrene and sodium dichromate. As a first step, gene expression was checked upon using microarray, which is the main tool for global gene expression profiling of the response of an organism in a particular conditions. However, expression levels of key responding genes of the microarray need to be validated by Real – Time PCR. This study was a part of a bigger project aiming at development of 'omic' based methods using *Daphnia magna* as a tool for monitoring the effects of toxic agents in the environment. During

her training, Vanja got some interesting results which were already presented in a broader context during the 15th International Symposium on Pollutant Responses in Marine Organisms, Bordeaux, France, in May, 2009 but will be published in a research article in respectable scientific journal (in preparation).

Short term visits

The exchange and mobility at the level of permanent, senior and junior staff of LECOTOX and partnering institutions was very well balanced. As summarised in the table, 11 visits of LECOTOX scientists to partner institutions and 10 reverse visits were accomplished during the lifetime of the projects.

LECOTOX members' visits to partner institutions were mainly aimed at detailed planning of the young research trainings, REP-LECOTOX workshop preparations and discussions for further cooperation opportunities. Last but not least, LECOTOX scientists were able to visit the research laboratories, get familiar with the state-of-the-art equipment, laboratory organisation and see and adopt the principles of good research management. These visits also facilitated further networking and creation of very tight personal contacts with hosts.

A particular care has been taken that as many as possible LECOTOX members are given the opportunity for short term visits. Project co-ordinator, **Prof. Dr. Radmila Kovacevic** visited UFZ (together with another LECOTOX senior scientist Prof. Dr. Milica Matavulj), School of Biosciences (together with another LECOTOX senior scientist Prof. Dr. Gordana Grubor Lajsic) and non-partner institution - JRC, Ispra, Italy, while another member of REP-LECOTOX Management Committee and LECOTOX senior scientist, **Dr. Ivana Teodorovic** visited UFZ and RECETOX twice each, at different occasions. Also, one LECOTOX junior scientist – **Sonja Kaisarevic** took advantage of mobility programme and visited UFZ two years after she had accomplished her training there. In the end, even REP-LECOTOX research trainees, **Vanja Dakic** and **Svetlana Fa** were given a chance to visit RECETOX in the frame of mobility programme and attend the Summer School of Ecotoxicology there.

On the other hand, the reverse short term visits of the researchers from partner institutions to LECOTOX were mainly organized during two REP-LECOTOX workshops: the experts from UFZ (**Dr. Werner Brack, Dr. Stefan Scholz and Dr. Mikhail Beketov**), RECETOX (**Dr. Ivan Holoubek, Dr. Ludek Blaha, Dr. Klara Hilscherova and Dr. Jakub Hofman**) and School of Biosciences (**Dr. James Kevin Chipman**) participated as key note speakers and invited lecturers at the both events. Moreover, the scientists from partner institutions, particularly ISAB members used the visits to inspect the progress of the REP-LECOTOX project, to visit research facilities at LECOTOX but other research groups at UNSFS, meet other researchers and management structure and open the gate for potential further collaboration beyond REP-LECOTOX project.

In the end, additional 4 short term visits of 2 non-partner EU researchers were made possible thanks to good financial management of the project and certain co-funding from additional

financial sources. **Prof. Dr. Romana Marinsek Logar**, Biotechnical University Ljubljana, Slovenia visited LECOTOX three times: during the first project year to discuss future collaboration, which resulted with her invited lecture at the 1st REP-LECOTOX Workshop, her attendance to the 2nd Workshop, and the seminar on Comet assay she gave afterwards for LECOTOX members and interested UNSFS PhD students. **Dr. Cristina Sandu**, Institute of Biology, Romanian Academy of Science, Bucharest, Romania was partly funded to visit LECOTOX as she expressed interest for PhD students exchange with LECOTOX in near future. In addition, she was an invited lecturer at the 2nd REP-LECOTOX Workshop.

Dissemination of information

The most important milestone of WP 2 Dissemination of information was the launching of the web portal. Basic concept and design of the portal of LECOTOX can be seen at the web address www.lecotox.net. LECOTOX web site is a stand-alone bilingual (English/Serbian) portal, though linked with the home page of home institution, University of Novi Sad Faculty of Sciences Department of Biology and Ecology. The individual domain and external web administration secure independence from the home institution, as the web site is planned to be loaded with retrievable scientific information - documents, as well as links to relevant institutions and research projects.

On the other hand, all the material uploaded on the web site is prepared, edited and translated by members of LECOTOX, in order to sustain accuracy, precision and high professional level of web pages. As the web portal is planned to be used beyond the limits of the REP-LECOTOX project, its home page belongs to LECOTOX, while a clearly visible link is provided for REP-LECOTOX with a number of shared pages, files and information.

Portal has two special domains which served as the communication tools for the Workshops, as they enabled on–line registration and provided all necessary information regarding the workshops: objectives, outcomes, programme, information about accommodation facilities, travelling to and from Novi Sad and general information Novi Sad/Serbia etc. Most importantly, the pdf files of all workshop presentations had been made and still are available for download via the portal.

Other important dissemination activities included publications in scientific and popular journals, participation in various scientific meetings and other events and press releases.

The scientific report on 1st REP-LECOTOX Workshop „Ecotoxicogenomics: the challenge of integrating genomics/proteomics/metabolomics into aquatic and terrestrial ecotoxicology" held in Novi Sad in June 2008 was published in highly ranked scientific journal Environmental Science and Pollution Research in August 2009:

Kovacevic R, Teodorovic I, Kaisarevic S, Planojevic I, Fa S, Dakic V, Pogrmic K, Virijevic S. (2009): First Rep-Lecotox Workshop - Ecotoxicogenomics: The Challenge Of Integrating Genomics/Proteomics/ Metabolomics Into Aquatic And Terrestrial Ecotoxicology. **Environmental Science And Pollution Research 16 (Suppl. 1): S130–S131.**

In the same issue, another article, in the form of the review paper, based on the presentation given during the 1st REP-LECOTOX Workshop was also published:

Teodorovic I. (2009): Ecotoxicological Research And Related Legislation In Serbia. **Environmental Science And Pollution Research 16 (Suppl. 1): S123–S129.**

Danube News - Official Bulletin of International Association for Danube Research (IAD) ISSN 2070-1292, published in December 2009 (electronic version available for download from www.iad.gs) brings two articles which promote LECOTOX research group: Teodorovic, I. Ecotoxicological research and its implications for important water management issues in the Danube River Basin, Danube News, 20, pp 2-4 and Teodorovic, I. REP-LECOTOX project: An example of FP INCO project to strengthen ecotoxicological research in Eastern Europe, Danube News, 20, pp15.

The article (4 pages) presenting LECOTOX have also been published, in Serbian and English version, in a brochure "Scientific Research at University of Novi Sad Faculty of Sciences" which has been compiled for the occasion of 40 year anniversary of UNSFS.

LECOTOX members continuously promote REP-LECOTOX project, as well as the results of recent and on-going research projects of the group at the important international scientific conferences, workshops and conventions.

Promotion of REP-LECOTOX project and LECOTOX itself particularly intensified during the 2nd and the 3rd project year, as LECOTOX was presented during:

- 3 Workshops of **COST Action 636** Xenobiotics in Urban Water Cycle

- Workshop of EU funded **RISKBASE** coordination project, presentation given by **Dr. Ivana Teodorovic** "Implementing the WFD in the Danube River Basin: major risks, pressures and challenges in Middle and Lower Danube River"
- **2nd REP-LECOTOX Workshop** in 2009 – presentation – **Teodorovic, I:** From LECOTOX to REP-LECOTOX and back
- **SedNet** Round Table Discussion „Sediment management in River Basin Management Plans'', participant Dr. Ivana Teodorovic

- **EuLimnos** International Workshop "Environmental research in SEE and its implementation in the teaching process" Virpazar, Montenegro, 9-11 Oct 2009 , presentation given by **Dr. Ivana Teodorovic**: „EU Projects at UNSFS: Implications for environmental education"

- Final Conference of EU FP 6 funded **MODELKEY project**: poster and abstract in Book of Abstracts: **Kovacevic et al**. EC FP 6 REP – LECOTOX PROJECT EC FP – facilitated success story how LECOTOX became visible on scientific map of Europe, pp62.
- As a participant in the REP-LECOTOX project, Prof. Dr. Gordana Grubor-Lajsic had the opportunity to apply for participation in the EU-NMR project. The application was evaluated successfully and she spent a week (20 – 24.October, 2008) together with her teaching assistants, Danijela Kojic, MA and Jelena Purac, MA at EU-NMR Centre at the University of Birmingham, the Institute of Cancer Studies, HWB-NMR analyzing their samples at the 500 MHz Bruker spectrometer NMR. Obtained results have been presented at 3rd Annual User Meeting EU-NMR on January 27-29, 2009, Autrans, France.

The results of LECOTOX research activities were presented at:

- **37thConference of IAD** (International Association for the Danube Research) 2008 by Ivana Teodorovic and Ivana Planojevic
- **33rd FEBS Congress** in 2008: attended by Kristina Pogrmic

- **SIL Congress** (International Society for Fundamental and Applied Limnology): attended by Ivana Teodorovic

- **SETAC Europe** 18th Annual Meeting (Society of Environmental Toxicology and Chemistry) in 2008: attended by Sonja Kaisarevic and Ivana Planojevic

- **PRIMO** 15th International Symposium on Pollutant Responses in Marine Organisms in 2009: attended by Vanja Dakic

- **ICCE** 12th EuCheMS International Conference on Chemistry and the Environment in 2009: attended by Sonja Kaisarevic

- **21st IUBMB and 12th FAOBMB** International Congress of Biochemistry and Molecular Biology: attended by Kristina Pogrmic

- **2nd REP-LECOTOX Workshop** in 2009.

All the mentioned events were used for further networking, gaining invited speakers and participants for REP-LECOTOX workshops and promotion of LECOTOX as a suitable competitive and competent partner for future research projects within the FP and beyond.

Two traditional events, National Fair of Higher Education and Career Days, in Novi Sad and Belgrade, Serbia, were the perfect opportunities for LECOTOX to present itself to future PhD students interested in higher education within the wide field of ecotoxicology. LECOTOX presented itself with leaflets on National Fair of Higher Education in Novi Sad and Belgrade, in November 2008.

In order to provide eye-catching, but comprehensive information about LECOTOX capacities, activities and researchers, our involvement in national, regional and international projects, with the main focus on REP-LECOTOX project, flyer was designed and printed in 450 copies. The flyer has been distributed during the important international meetings attended by LECOTOX staff.

One of the major activities, though limited in scope, is internal dissemination of knowledge gained through trainings of young and short term study visits of senior researchers to REP-LECOTOX partner institutions. Upon their return, young trainees applied the knowledge and skills gained trough their training activities into their own research. Also, trainees gave oral presentations to all LECOTOX members during internal brainstorming meetings, so the knowledge acquired and information gathered are disseminated throughout the lab staff. The written reports of the trainees are available via web pages to interested public.

On the other hand, REP-LECOTOX project and other activities of LECOTOX were presented to the wide national public via press releases in printed (3 articles in regional newspapers)

and electronic media (interviews of senior LECOTOX members on radio and TV, talk show on regional TV, footage from the openings of the REP-LECOTOX Workshops, web resources).

REP-LECOTOX Workshops

The 1st REP-LECOTOX Workshop: "Ecotoxicogenomics: the challenge of integrating genomics/proteomics/metabolomics into aquatic and terrestrial ecotoxicology", Novi Sad, 15-18 June 2008

Objectives and outcomes of the First Workshop "Ecotoxicogenomics: the challenge of integrating genomics/proteomics/metabolomics into aquatic and terrestrial ecotoxicology"

- To bring together experts in the field of genomics, transcriptomics, proteomics, metabolomics and bioinformatics as invited speakers to the workshop in order to get comprehensive but at the same time introductory view about the implementation of "omic" technologies in the field of ecotoxicology;
- To give opportunity to participants from Serbia, neighbouring countries and others to get impression about the state of the art in this field and orientation to such propulsive field of investigation as ecotoxicogenomics, and introduction and application of genomics based tools to tackle ecotoxicological questions;
- Specific relevance to end users and regulators in Serbia;
- Focusing of LECOTOX on implementation of "omic" technologies to tackle ecotoxicological questions (mainly transcriptomics). Better understanding of importance of option of suitable test organisms and adequate assays;
- Gained knowledge should be integrated in ecotoxicology courses at Master and PhD studies at the Faculty of Science;
- Impact on recognition of LECOTOX as a centre for ecotoxicological research;
- Impact on ecotoxicogenomic research in Serbia.

Workshop participants had opportunity to learn about trends in ecotoxicogenomics

from five plenary and six specialized lectures which were given by recognized experts in each specific field. Last two days of the Workshop were devoted to laboratory demonstrations of 7900HT Fast Real-Time PCR system (Applied Biosystems) and 2100 Bioanalyzer (Agilent).

Five introductory lectures were:

- Overview of ecotoxicogenomics – by Prof. Dr. James Kevin Chipman, School of Biosciences, University of Birmingham, UK;
- Environmental transcriptomics – by Dr. Stefan Scholz, Department for Cell Toxicology, UFZ, Leipzig, Germany;
- Environmental proteomics – by Dr. Thomas Knigge, Laboratoire d'Ecotoxicologie - Milieux Aquatiques, Université du Havre, France;
- Environmental metabolomics – by Dr. Jake Bundy, Biomolecular Medicine, Sir Alexander Fleming Building, Imperial College, UK;
- Environmental Bioinformatics – by Renzo Kottmann, PhD student, on behalf of Microbial Genomics Group, Department of Molecular Ecology, Max Planck Institute for Marine Microbiology, Bremen, Germany.

Six lectures on specialised topics were:

- Biomarkers of aquatic toxicology: past, present and future – by Dr. Ron van der Oost, Waternet, Research & Engineering, Toxicology, Amsterdam, Netherlands;
- Reporter gene assays and specific gene expression in biomonitoring of the contaminated environmental matrices by Dr. Klara Hilscherova, RECETOX, Brno, Czech Republic;
- Introduction of toxicoproteomic approach with *Tetrahymena thermophila* in environmental monitoring by Prof. Dr. Romana Marinsek-Logar, University of Ljubljana, Slovenia;
- Bacterial community structure analysis in terrestrial and aquatic environments - an ecotoxicogenomic approach by Dr. Rok Kostanjsek, University of Ljubljana, Slovenia;

- Immunotoxicity of xenobiotics as an aspect of their ecotoxicity – Prof. Dr. Milena Kataranovski, Department of Ecology, Institute for Biological Research "Sinisa Stankovic", Belgrade, Serbia;

- State of ecotoxicology research and legislation in Serbia – Dr. Ivana Teodorovic, LECOTOX, University of Novi Sad, Serbia.

All lecturers agreed to have their presentation available on the web, as protected pdf files, for public download. According to Workshop plan, 50 participants from Montenegro, Croatia, Romania, Bulgaria, Slovenia, Portugal and Serbia registered and attended an event. In the

end of the Workshop, the participants were asked to fill in the evaluation forms. Analysis showed that they were extremely satisfied with the workshop, as all elements (key note lecture, lectures on specialized themes, laboratory demonstration as well as organisation itself) were rated very highly.

The 2nd REP-LECOTOX Workshop: "Trends in Ecological Risk Assessment", Novi Sad, 21-23 September 2009.

Workshop objectives and expected outcomes were

- To present comprehensive information about the current state, challenges and trends in the field of ecological risk assessment, with special reference to application into environmental management practice.

- To provide local and regional professionals, decision makers, different stakeholders (industry, regulatory agencies, academia...) with the comprehensive overview of current EU regulations and future trends regarding risk assessment of chemicals and ecological risk assessment (with special reference to REACH and WFD).

- To serve as a forum for knowledge and information exchange and dissemination.

- To make a substantial contribution to Serbian/WBC societies in the processes of transition and harmonisation with EU environmental regulation, policy and practice with EU.

- To enable further networking of Serbian/WBC and EU professionals and institutions.

- To promote LECOTOX as a centre for ecotoxicological research.

The workshop scientific and organizing committee structured the workshop into 2 large sessions. Session 1) Risk assessment of chemicals with special attention to REACH was divided according to topics into 3 sub-sessions: 1.A Legislative aspect; 1.B Emerging substances and nano-materials: analyses, environmental fate, effects, ERA and 1.C Incorporating "omic" Information into Risk Assessment and Policy. Session 2) Ecosystem Risk Assessment was also divided into 3 sub-sessions: 2.A Aquatic ecosystems, ERA in WFD and GD; 2.B Special ERA topic – up-scaling and 2.C Terrestrial Ecosystems.

As the main objective of the Workshop was to present comprehensive information about the current state, challenges and trends in the field of ecological risk assessment, 2 distinguished key note lecturers (Prof. Dr. Ivan Holoubek, RECETOX, Masaryk University, Brno, Czech Republic and Dr. Werner Brack, UFZ, Leipzig, Germany) and even 18 invited speakers from well known European research institutions were invited to give talks on extremely diverse ERA topics, ranging from case studies to conceptual frameworks and opinion statements. It is worth mentioning that among the invited lecturers, there were 2 Serbian researchers and one presentation given by the representative of Serbian Ministry of Environmental Protection and Sustainable Development. To enable further exchange of experiences and information about the research potential and capacities of local (Serbian) and regional research institutions and teams, 7 registered workshop participants were invited to present the results of their current work in form of short oral presentations, while the others were invited to present posters. Still, enough room was envisioned for discussions, which was best accepted and highly appreciated feature of the first REP-LECOTOX workshop, so the second one was designed to have even more discussion-oriented structure.

All lecturers and workshop participants were kindly asked to provide extended abstracts of their talks before the workshop, so that the proceedings - Book of Abstracts could be printed and disseminated to all participants at the registration. The abstracts were not available in electronic form, only in print. The Proceedings also contain the Workshop programme as well as the full list (with contact information) of all lecturers and participants. Also, all lecturers agreed to have their presentation available on the web, as protected pdf files, for public download.

All invited lecturers and workshop participants selected for oral presentations were invited to prepare full papers for peer-reviewed SCI journal – ***Journal of Serbian Chemical Society*** by the end of the year. After the regular peer-review procedure, the accepted manuscripts will be published in several successive volumes of the journal, within session Environment, with the note that the material was originally presented at 2nd REP-LECOTOX Workshop. At the moment of submission of this report, several papers have been submitted, 3 papers have undergone the reviewing procedure and have been accepted for publication, but the first volume with the Environment session devoted to 2nd REP-LECOTOX workshop has not been published yet.

Both Workshops successfully fulfilled many of the foreseen objectives. Apart from giving the comprehensive overview of application of genomics-based tools in ecotoxicology and trends in ecological and chemical risk assessment, both events contributed to popularization of ecotoxicogenomic and ecotoxicological research in general in Serbia as well as in the region which, due to specific historical problems and current socioeconomic conditions, can be recognized as the region with the most enhanced problems of environmental pollution, including hazards from numerous hot spots of severe terrestrial and aquatic contamination. Above all, the workshops offered the unique opportunity for networking between some of the best European institutions and scientists with researchers and professionals, including

the end users in the field of environmental protection from the Western Balkan Region. The workshops surely represent milestones in contemporary approach to ecotoxicological research and risk assessment in the region.

Reinforcement of LECOTOX

The measurement of gene expression levels upon exposure to a chemical can be used both to provide mechanistic knowledge of the genetic basis of toxic effect and to form a sort of «genetic signature» for the identification of toxic product. Real-time PCR allow accurate quantification of starting amounts of DNA, cDNA, and RNA targets. Fluorescence is measured during each cycle, which greatly increases the dynamic range of the reaction, since the amount of fluorescence is proportional to the amount of PCR product. PCR products can be detected using either fluorescent dyes that bind to double-stranded DNA or fluorescently labeled sequence-specific probes. This is in contrast with endpoint detection in conventional PCR, which does not enable accurate quantification of nucleic acids.

Real-time - quantification polymerase chain reaction (qPCR) detection system consists of three components: the optical detection system; data collection and analysis software; and, what is very important, a reliable and robust functional molecular biological assay. It is very useful to have good instrumentation and functional assay design.

We decided to purchase 7900HT FAST REAL - TIME PCR SYSTEM: consists of standard hardware configuration (peltier based thermal cycler, standard 96-well block, argon iron laser excitation source, CCD camera) and computer with Windows XP OS, Sequence Detection Software, Primer Express Oligonucleotide Design Software, and monitor. The upgrade of system configuration consists of TaqMan low density array, 384-well micro fluidic cards designed for analyzing gene expression patterns in many samples across a defined set of gene targets. For centrifugation of fluidic cards we purchased centrifuge (Heraeus Multifuge 3S + Centrifuge, Thermo Scientific) supplied with rotor and adapter for fluidic cards. We also obtained additional fast thermal cycler with 96-well block module for parallel running of simple PCR when necessary. The equipment was placed into newly redecorated air conditioned laboratory exclusively prepared for 7900HT Fast Real - Time PCR system. Installation by authorized serviceman was accompanied by technical operational instructions during installation.

Two senior researchers from LECOTOX, Dr. Silvana Andric and Dr. Tatjana Kostic, attended two days professional training at the Applied Biosystem's Centre in Holland, and upon their return, disseminated information and knowledge to junior scientists.

Two days training at the LECOTOX was performed by professional representative of Applied Biosystems settled in Belgrade. Apart from introductory theoretical fundamentals and technical instructions on system operation, the training also included practical work on gene expression analysis using TaqMan microarray fluidic cards. As the real samples were analysed, training participants were given the opportunity to discuss the results with the lecturer and get valuable information necessary for proper operation of Sequence Detection Software for data interpretation.

Another important task of WP 4 was selection of young research trainees to be hired for REP-LECOTOX project. Taking into consideration legal issues (certain constrains deriving from provisions of national regulation on employment and research) and thorough analyses of available suitable MSc and PhD students enrolled in UNSFS, MC made a selection and in the first project year hired one PhD student in Biochemistry already engaged into on-going research activities of LECOTOX and therefore with certain experience with molecular methods in biology, and one Master student at the UNSFS in Molecular biology, from the first generation of the undergraduate students in Molecular biology at FSUNS. The third research trainee was hired, as planned, in the second year of the project, and again, the candidate is a PhD student of Biochemistry. Apart from 3 research trainees hired according to the plan, other 3 part – time research trainees - PhD students who had been granted the full scholarship by Ministry of Science and Technological Development of Republic of Serbia had been co-funded by REP-LECOTOX project and included into all on-going activities during the course of the project. All 3 full time and 3 part time research trainees progressed as planned during the lifetime of the project. Their achievements and current status are summarized below:

Kristina Pogrmic, Research Assistant, has finished her PhD thesis **"The mechanism of atrazine action on steroidogenesis in peripubertal rat Leydig cells"** which should be defended by March 2010. The scientific paper based on the results of her PhD thesis

using the newly introduced real-time PCR methods acquired via REP-LECOTOX project has already been published:

Pogrmic K., Fa S., Dakic V., Kaisarevic S. And Kovacevic R. (2009): Atrazine Oral Exposure Of Peripubertal Male Rats Down-Regulates Steroidogenesis Gene Expression In Leydig Cells. Toxicological Sciences, 111(1), 189 -197.

Kristina's main tasks within project REP-LECOTOX were: standardisation of real-time PCR methods using 7900HT Fast Real - Time PCR; organization of 2nd Workshop and work on PhD thesis

Svetlana Fa, Research Assistant, is PhD student in Biochemistry at UNSFS. During the project, she passed all the exams on her PhD studies and at the moment she is working on her PhD thesis. Her main tasks within project REP-LECOTOX were:

- Member of the Organizing Committee of the 1st and the 2nd Workshop: responsible for communication with workshop participants and for the logistics;
- Updating and maintaining LECOTOX web site;
- Standardization of real-time PCR methods using 7900HT Fast Real - Time PCR system;
- Accomplishment of the training for young researchers in REP-LECOTOX partner institution: Helmholtz Centre for Environmental Research in Leipzig, Germany, under supervision of Dr. Stefan Scholz where she got familiar with ecotoxicological methods using *Danio rerio* (zebrafish) as model organism;

- Work on PhD thesis: **Potential toxic effects of brominated flame retardants on steroidogenesis in rats**

Vanja Dakic, research trainee, PhD student in Molecular Biology. Her main tasks within project REP-LECOTOX during were:

- Member of the Organizing Committee of the 1st and the 2nd Workshop;
- Work on web portal;
- Accomplishment of the training for young researchers in partner institution - School of Biosciences, The University of Birmingham, Birmingham, UK, under the supervision of Prof. Dr. Kevin Chipman, where she gained important knowledge in techniques in molecular biology, using *Daphnia magna* as model organism;
- Standardisation of real-time PCR methods used on 7900HT Fast Real - Time PCR system;
- Accomplishment of MSc studies and defending of the MSc thesis;
- PhD studies

Ivana Planojevic, part time research trainee, currently PhD student in Ecology. Her main tasks within project REP-LECOTOX were:

- Accomplishment of MSc studies and defending of the MSc thesis;
- Member of the Organizing Committee of the 1st and the 2nd Workshop responsible for providing the logistics for key note and invited lecturers;
- Accomplishment of the training for young researchers in partner institution RECETOX, Masaryk University, Brno, Czech Republic, under the supervision of Dr. Ludek Blaha during the first and Dr. Jakub Hofman during the third project year;
- Introduction of sediment contact *in vivo* toxicity tests into LECOTOX;
- PhD studies - with approval of Head of LECOTOX and UNSFS Dean, Ivana will continue her PhD studies and begin the work on her PhD thesis at University of Antwerp, Belgium in February 2010

Branka Glisic and Jelena Hrubik, part-time research trainees, enrolled into PhD studies at University of Belgrade Faculty of Biology - Molecular biology of eukaryote in September 2009.

Their main tasks within project REP-LECOTOX were:

- Accomplishment of MSc studies and defending MSc theses;
- Members of the Organizing Committee of the 2nd Workshop;
- Accomplishment of the trainings for young researchers in partner institution Helmholtz Centre for Environmental Research in Leipzig, Germany, under supervision of Dr. Stefan Scholz where they got familiar with ecotoxicological methods using *Danio rerio* (zebrafish) as model organism
- PhD studies

Main project outcomes

During the lifetime of the project, main achievements of the REP-LECOTOX project were:

- **Acquisition and installation of the 7900HT Fast Real - Time PCR system; by the end of the project, the platform has been fully operational, in regular laboratory use, the results obtained have been incorporated into PhD theses in progress as well as into scientific papers**
- **Successful networking with partner institutions through trainings of young researchers and short study visits of senior scientists**
- **Three research-trainees have been hired and co-funding has been secured for other 3 PhD students/research trainees**
- **Two Workshops had been organized**
- **Web portal www.lecotox.net had been launched, maintained and utilized with the main goal to improve visibility of LECOTOX**

Fully operational 7900HT Fast Real - Time PCR system has already but is still expected to further raise overall scientific performance and activities of LECOTOX in on-going and future research projects. The knowledge gained via trainings in partner institutions had been transferred to LECOTOX and incorporated into individual research activities of young researchers in form of 4 PhD theses in the progress and peer reviewed publications in scientific journals, although further publications are still expected to come in near future. Intensive networking with partner institutions and individual researchers facilitated, in the first place, highly professional organization of two workshops of outstanding scientific performance, while the workshops themselves had been used not only for getting acquainted with state-of-the-art techniques in ecotoxicogenomics and risk assessment, but for further networking and scientific exchange.

Realization of this project has a significant impact on research activities of LECOTOX, which has become the recognized centre in Serbia capable of applying modern bioanalysis as tools for detection of early-warning signals of environmental contamination. Upgraded and strengthened, LECOTOX will in future, besides its scientific goals, have important task, but also the capability and capacity to offer its expertise and advice to policy and decision makers as well as various stakeholders. The direct impact of the project has already been reached by enhancing professional skills of young and propulsive researchers and their orientation towards application of their excellent knowledge of basic sciences into environmental problems. Facilitation of EU – WBC exchange of the ideas and approaches both in basic sciences and

applied problem treatment will stimulate further appearance of new cooperative efforts in basic and environmental sciences, and regional applications funded from international and national sources.

Given the opportunity to have access to information and being able to use sophisticated equipment and information technologies, LECOTOX researchers have promising carrier and work conditions. They have become members of the international research community no matter where they live and work, which reduces “brain-drain” phenomena Serbia had undergone during the previous decade and gives new impulses to stabilization and reinforcement of S&T potential in Serbia.

LECOTOX beyond REP-LECOTOX Project

LECOTOX Members:

Prof. Dr. Radmila Kovacevic, BSc, MSc and PhD in Biology (Endocrinology)

Full professor of Biochemistry at University of Novi Sad Faculty of Sciences

Head of LECOTOX

Research interest: Endocrine disruption/testicular steroidogenesis, biomarkers and bioanalyses in environmental quality assessment

Dr. Ivana Teodorovic, BSc in Biology, MSc and PhD in Environmental Engineering

Associate professor of Environmental Sciences (Ecotoxicology) at University of Novi Sad Faculty of Sciences

Research interest: Ecotoxicology (Aquatic, Ecosystem), Ecological risk assessment, Water management

Kristina Pogrmic, BSc in Biology, PhD student in Biochemistry

Research Assistant in Animal Physiology at University of Novi Sad Faculty of Sciences

Research interest: Mechanisam of action of endocrine disruptors on testicular steroidogenesis.

Laboratory skills: Primary and continual cell cultures, PCR, qRT-PCR, RIA, ELISA, AOE activity assays, microEROD analysis, H4IIE-*luc* analysis, cell cytotoxicity/proliferation assays.

Trainings: Research Centre for Environmental Chemistry and Ecotoxicology (RECETOX), Ecotoxicology Division, Brno, Czech Republic - July 2006; Course "Frontiers in Reproduction 2008", Marine Biological Laboratory (MBL), Woods Hole, Massachusetts, USA - May-June 2007

Sonja Kaisarevic, BSc in Biology, MSc in Ecophysiology

Teaching Assistant in Animal Physiology University of Novi Sad Faculty of Sciences, currently finishing PhD thesis

Research interest: Implementation of biomarkers and bioanalyses in environmental monitoring of POPs, and investigation of their toxic and endocrine-disrupting effects

Laboratory skills: Primary and continual cell cultures, microEROD analysis, H4IIE-*luc* analysis, cell cytotoxicity/proliferation assays, PCR, qRT-PCR, RIA, AOE activity assays, FRIEDA, some experience in effect-directed extraction and fractionation of environmental samples (ASE, NP-HPLC).

Trainings: Research Centre for Environmental Chemistry and Ecotoxicology (RECETOX), Ecotoxicology Division, Brno, Czech Republic - July 2006; the Helmholtz Centre for Environmental Research, Department for Effect-Directed Analysis, Leipzig, Germany - April-June 2007

Svetlana Fa, BSc in Biology, PhD student in Biochemistry

Research Assistant in Animal Physiology at University of Novi Sad Faculty of Sciences

Research interest: Endocrine-disrupting effects and mechanisms of action of certain Brominated Flame Retardants (BFR) upon testicular and ovarian steroidogenesis; *Danio rerio* (zebrafish) toxicity tests

Laboratory skills: Primary testicular Leydig and ovarian granulosa cell cultures, *Danio rerio* testis tissue culture, *Dar*T toxicity test, cytotoxicity/proliferation assays, PCR, qRT-PCR, RIA, ELISA.

Trainings: Helmholtz Centre for Environmental Research, Department for Bioanalytical Ecotoxicology, Leipzig, Germany – September-October 2008 and July-August 2009; Utrecht University, Department for Endocrinology and Metabolism, Utrecht, Netherlands - July 2009

Ivana Planojevic, BSc and MSc in Ecology

Research trainee in Environmental Sciences (Ecotoxicology) at University of Novi Sad Faculty of Sciences

In February 2010 starting PhD at University of Antwerp, Belgium

Research interest: Ecotoxicology, aquatic toxicology, ecological risk assessment of contaminated sediments, aquatic ecology

Laboratory and field skills: Culturing and conducting toxicity tests with *Daphnia magna, Vibrio fischeri, Myriophyllum aquaticum, Caenorhadbitis elegans*, field (aquatic) sampling and measurements

Trainings: Research Centre for Environmental Chemistry and Ecotoxicology (RECETOX), Ecotoxicology Division, Brno, Czech Republic – July-August 2007; Bavarian Environment Agency, Munich, Germany – February-March 2009; Research Centre for Environmental Chemistry and Ecotoxicology (RECETOX), Ecotoxicology Division, Brno, Czech Republic – November-December 2009

Vanja Dakic, BSc and MSc in Molecular Biology

Research trainee in Animal Physiology at University of Novi Sad Faculty of Sciences, PhD student in Molecular Biology at University of Belgrade Faculty of Biology

Research interest: Pharmaceuticals as environmental contaminants and investigation of their toxic and endocrine-disrupting effects

Laboratory skills: Primary and continual cell cultures, PCR, qRT-PCR, RIA, ELISA, culturing of *Daphnia magna*

Trainings: School of Biosciences, The University of Birmingham, UK, October-November 2008 and August-September 2009.

Branka Glisic, BSc and MSc in Molecular Biology

Research trainee in Animal Physiology at University of Novi Sad Faculty of Sciences, PhD student in Molecular Biology at University of Belgrade Faculty of Biology

Research interest: Effect of endocrine disrupting chemicals on ovarian steroidogenesis; *Danio rerio* (zebrafish) toxicity tests

Laboratory skills: Primary and continual cell cultures, PCR, qRT-PCR, RIA, microEROD analysis, cell cytotoxicity/proliferation assays, some experience in *Dar*T assay

Trainings: Helmholtz Centre for Environmental Research (UFZ), Department of Bioanalytical Ecotoxicology (previously Department of Cell Toxicology), Leipzig, Germany, December, 2009.

Jelena Hrubik, BSc and MSc in Molecular Biology

Research trainee in Animal Physiology at University of Novi Sad Faculty of Sciences, PhD student in Molecular Biology at University of Belgrade Faculty of Biology

Research interest: Cytotoxic effects of plant extracts; *Danio rerio* (zebrafish) toxicity tests

Laboratory skills: Primary and continual cell cultures, PCR, qRT-PCR, RIA, microEROD analysis, cell cytotoxicity/proliferation assays, some experience in *Dar*T assay

Trainings: Helmholtz Centre for Environmental Research (UFZ), Department of Bioanalytical Ecotoxicology (previously Department of Cell Toxicology), Leipzig, Germany, December, 2009.

On-going projects:

2010 – 2012: Establishing and developing of an ecotoxicology platform in Serbia and Croatia: a focus on zebrafish (*Danio rerio*): University of Applied Sciences (FHNW) School of Life Sciences, Muttenz, Switzerland, team leader Prof. Dr. Karl Fent; Rudjer Boscovic Institute (RBI) Division for Marine and Environmental Research Molecular Ecotoxicology (LME), Zagreb, Croatia, team leader Dr. Tvrtko Smital and **University of Novi Sad Faculty of Sciences Department of Biology and Ecology, LECOTOX, Novi Sad, Serbia, team leader Prof. Dr. RadmilaKovacevic**. Funded by Swiss National Scientific Foundation (SNF) via SCOPES 2008-2012 program, Project Co-ordinator Prof. Dr. Karl Fent

The first part of the project will be directed to the transfer of knowledge and expertise that will enable the Serbian and Croatian partners to improve their overall research capacity, performing part of the research using zebrafish (*Danio rerio*) cell lines (PAC1 and ZFL) as already established but still promising models in biomedical and ecotoxicological research. The second part of the project, which is focused on research, will be directed to better understanding of critical cellular, evolutionary conserved xenobiotic defence systems in aquatic organisms, using zebrafish as a model, specifically focused on the uptake of environmental chemicals and metabolites by specific transmembrane proteins, detoxification by phase I and II biotransformation enzyme systems; and finally, active elimination of xenobiotics and metabolites through specific transmembrane proteins (ABC transporters).

SCOPES Kick off meeting in Swiss Alps, Braunwald 24-26 January, 2010
(From left to right: Ivana Teodorovic, Marta Popovic, Verena Christen, Roko Zaja, Karl Fent, Tvrtko Smital, Ivana Ivancev Tumbas and Radmila Kovacevic, missing from the photo Sara Zucci)

2009 - 2010: Introduction and development of fish ecotoxicity tests recommended by REACH Regulation Annex C project funded by Provincial Secretariat for Science and Technological Development of Autonomous Province of Vojvodina. **University of Novi Sad Faculty of Sciences Department of Biology and Ecology, LECOTOX, Novi Sad, Serbia, Project Coordinator Prof. Dr. Radmila Kovacevic**.

The project will be directed to the establishing a small laboratory facility that will enable LECOTOX to improve its overall research capacity by introduction of fish acute and chronic ecotoxicological tests which are recommended methods under REACH regulation (Annex C – ecotoxicological methods). The project would mainly focus on zebrafish (*Danio rerio*) as highly established and promising model in biomedical and ecotoxicological research. Apart form the classic fish tests, the ultimate goal of the project is to further develop embryotoxicity DarT and introduce Gene DarT test – the latest toxicity test on *D. rerio* based on gene expression of potential marker genes.

2006 – 2010: Endocrine disruption chemicals – effects on reproductive and thyroid function, environmental assessment and bioanalyses, Project No 143058, Funded by Republic of Serbia Ministry of Science and Technological Development, **Project coordinator Prof. Dr. Radmila Kovacevic**.

The project was (and still is) focused on: (a) impact of selected BFRs, PCBs, PAHs and pesticides on testicular and thyroid function; possible toxic effect is being studied by exploring the expression of genes involved in testicular steroidogenesis, activity of steroidogenic enzymes, structure and ultrastructure of thyroid gland, thyroid hormone levels and other biochemical indicators, and (b) the use of biomarkers as the endpoints in bioanalyses and toxicity tests to study presence of different EDCs in various environmental matrices, in parallel with chemical characterisation. Expected results are (a) better insight into the mechanisms of possible adverse effects of selected EDCs and (b) development of toxicity identification and evaluation approach where bioassays direct the chemical identification of chemicals.

2009-2010: LECOTOX members are taking part in Project "**Chemicals Risk Management in Serbia**", funded Republic of Serbia, Ministry of Environment and Spatial Planning & Swedish Chemical Agency. Activity: Revision of Translated Text of Annex to Council Regulation (EC) No 440/2008 Laying Down Test Methods Pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) – Part C Ecotoxicological methods. **Consultant Prof. Dr. Ivana Teodorovic**

2009 – 2010: LECOTOX members participate in the Neighbouring Programme Romania-Serbia: **Eco-status of the River Tamis**, Tender No 06SER02/03/007-8, Contractor City of Pancevo, Funded by EU. Joint project of 3 departments of UNSFS: Department of Biology and Ecology,

Department of Chemistry, Biochemistry and Environmental Protection and Department of Physics. **Project Coordinator Prof. Dr. Ivana Teodorovic** (in the photo, sampling trip Fall, 2009)

Recent LECOTOX Publications (2007-2009)

- **Kaisarevic S.**, Lübcke-von Varel U., Orcic D., Streck G., Schulze T., **Pogrmic K.**, **Teodorovic I.**, Brack W., **Kovacevic R.** (2009): Effect-directed analysis of contaminated sediment from the wastewater canal in Pancevo industrial area, Serbia. *Chemosphere, 77 (7), 907-913.*

- Planojevic I., Teodorovic I., Bartova K., Machat J., Blaha L., Kovacevic R.: Wastewater canal Vojlovica, industrial complex Pancevo, Serbia – preliminary ecotoxicological assessment of contaminated sediment. *Journal of Serbian Chemical Society* (submitted)

- **Teodorovic I., Planojevic I.**, Knezevic P., Radak S., Nemet I. (2009): Sensitivity of bacterial vs. acute *Daphnia magna* toxicity tests to metals. *Central European Journal of Biology, 4 (4), 482-492.*

- **Pogrmic K., Fa S., Dakic V., Kaisarevic S., Kovacevic R.** (2009): Atrazine Oral Exposure of Peripubertal Male Rats Down-Regulates Steroidogenesis Gene Expression in Leydig Cells. *Toxicological Sciences, 111(1), 189 -197.*

- **Teodorovic I.,** Becelic M., **Planojevic I.,** Ivancev-Tumbas I., Dalmacija B. (2009): The relationship between whole effluent toxicity (WET) and chemical-based effluent quality assessment in Vojvodina (Serbia), *Environmental Monitoring and Assessment, 158(1-4), 381-392.*

- **Kovacevic R., Teodorovic I., Kaisarevic S., Planojevic I., Fa S., Dakic V., Pogrmic K.,** Virijevic S. (2009): First REP-LECOTOX Workshop—ecotoxicogenomics: the challenge of integrating genomics/proteomics/metabolomics into aquatic and terrestrial ecotoxicology. *Environmental Science and Pollution Research 16 (Suppl 1), S130–S131.*

- **Teodorovic I.** (2009): Ecotoxicological research and related legislation in Serbia. *Environmental Science and Pollution Research* 16 *(Suppl 1), S123–S129.*

- Andric N., Kostic T., **Kaisarevic S., Fa S., Pogrmic K., Kovacevic R.** (2008): In vivo and in vitro effects of PCB126 and PCB153 on rat testicular androgenesis. *Environmental Toxicology and Pharmacology 25, 222-226.*

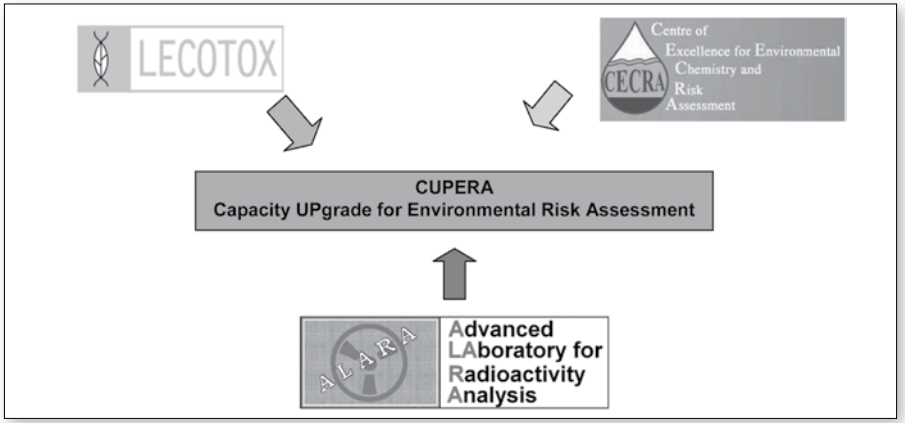
- **Teodorovic I., Planojevic I**. (2008): *Daphnia magna* culturing methods - implications on chronic toxicity tests. *Fresenius Environmental Bulletin, Vol. 17 (8a), 985-991.*

- **Kaisarevic S.**, Andric N., Bobic S., Trickovic J., **Teodorovic I.**, Vojinovic-Miloradov M., **Kovacevic R.** (2007) Detection of dioxin-like contaminants in soil from the area of oil refineries in Vojvodina region of Serbia. *Bulletin of Environmental Contamination and Toxicology 79, 422-426.*

Further plans

CUPERA: Project proposal submitted to FP 7 call REGPOT 1

Three research groups of University of Novi Sad Faculty of Sciences prepared joint project proposal: LECOTOX (Department of Biology and Ecology), CECRA (Department of Chemistry, Biochemistry and Environmental Protection) and ALARA (Department of Physics). The final decision is still pending.



Dissemination:

The results of the research activities of LECOTOX will be presented at several important national and international scientific events:

- **International Conference Wastewater, urban solid waste and hazardous waste,** 29.03 – 01.04 2010. Subotica, Serbia - Conference of the Association of Water Technology and Sanitary Engineering - 40th Anniversary of the Association

  - Invited lecture and review paper in preparation for national journal Water and Sanitary Technologies: **Teodorovic, I**. "Assessing the impact of wastewaters on chemical and ecological status: Methods for identification of key toxic pollutants"

- **SETAC Europe 20th Annual Meeting**, 23-27 May, 2010, Seville, Spain, Two posters and abstracts:

  - **Kaisarevic, S. et al**: "Contaminated sediment from industrial area in Serbia: further steps in effect-directed analysis"

  - **Planojevic, I. et al**: "Linking Ecology and Ecotoxicology in Ecological Risk Assessment. Case study The Tamis River (Danube River Basin)"

- **38th Conference of International Association for Danube Research (IAD)**, 22-25 June 2010, Drezden, Germany. Oral presentation and abstract:

  - **Teodorovic, I**. "Selecting the appropriate set of methods for evaluating ecosystem functions and services in ecological risk assessment. Lessons learned from the River Tamis (Danube River Basin)"

Instead of the conclusion....