Ecotoxicological research and related legislation in Serbia

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Abstract

Introduction Several hot spots of severe freshwater pollution and sediment contamination (mostly heavy metals, polyaromatic hydrocarbons and polychlorinated biphenyls) have been identified in Serbia as the consequence of outdated environmental legislation, negligible amounts of properly treated waste waters and accidental spills.

Discussion Since ecotoxicological methods have never been incorporated into risk assessment procedures, mandatory effluent discharge or ambient water monitoring programmes, ecotoxicological research, based on bioaccumulation studies, conventional ecotoxicological tests and, recently, biomarkers of exposure and effect have been restricted to independent small-to medium-scale studies, conducted, basically, to confirm, underline or oppose the results of chemical-based monitoring and to lament on inadequate environmental regulations/policy and management practice. Although hot and unresolved ecotoxicological problems still remain beyond the reach of ecotoxicological research currently conducted in Serbia, or are tackled only sporadically, it is to be expected that on-going research and institutional capacity building should, hopefully, increase the competence and competitiveness of scientific community and speed up the process of harmonisation of national environmental legislation and policy with European Union.

Keywords Biomarkers · Contaminated sediments · Danube River Basin · Ecotoxicological testing · Hazardous substances · Legislation · Research · Risk assessment

1 Background, aims and scopes

As a signatory party of The Danube River Protection Convention and a contracting party of International Commission for the Protection of the Danube River (ICPDR) since August 2003, Serbia has been committed to implementation of the Danube Convention, the objectives of which, amongst others, include the control of hazards originating from accidents involving hazardous substances and implementation of measures to reduce pollution loads entering the Black Sea from sources in the Danube River Basin. Since ICPDR got the mandate for implementation of the European Union (EU) Water Framework Directive (WFD), Serbia, although not an EU Member State yet, has been undertaking measures to contribute to fulfilment of the WFD main objective—good ecological status of all European rivers by 2015.

This review aims at presenting a comprehensive overview of recent and contemporary ecotoxicological research in Serbia. One of the objectives of this paper is to put the fundamental and applied research and the results obtained over the last decade into the context of historical and social-economic conditions, along with the current environmental legislation, in order to search for the reasons for relatively modest interest in ecotoxicology within the Serbian research community and almost complete absence of interest amongst water managers and competent authorities. In the end, perspectives and possible future trends in ecotoxicological research in Serbia will be stressed.

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2 Main features

The review is based on the peer-reviewed articles that have been published in international journals and available official reports that have been published by the competent authorities in the region.

3 Results

3.1 Current Serbian environmental regulations related to water protection and hazardous substances

For years, Serbian environmental legislation has been (and it still is) an ambient-standard based: Common national emission level values have never existed, so the regulations have been developed to ensure that the discharge does not violate numeric ambient standards and narrative criteria outside the mixing zone. Compliance monitoring exclusively includes water quality assessment based on physico-chemical parameters, while whole effluent toxicity testing (WET) is not required by any environmental regulations in Serbia. Industrial waste waters are customarily discharged (partly treated or untreated) into sewage systems, or directly into receiving waters. Yet, according to recent estimates (SWRDMP 2002), less than 10% of all waste waters generated in Serbia are treated at all, which means that effluents still present an actual hazard to aquatic environment. Oddly enough, official surface water quality monitoring programmes have relied for years mostly on physicochemical water quality parameters and to some extent on biomonitoring data (saprobic indices based on plankton communities). Ambient standards, which are rather outdated nowadays, are set for water column only, while sediment quality criteria have never been established so far. Also, ambient water and sediment toxicity evaluation have never been included into official monitoring programmes.

Important environmental laws adopted in 2004 should, eventually, enable transposition of the Environmental Impact Assessment, Strategic Environmental Assessment and Integrated Pollution Prevention and Control Directives, provided that the missing secondary legislation, i.e. bylaws (emission level values in the first place) is adopted soon enough. A new Water Law (harmonised with the WFD) and accompanying bylaws, the Law on Biocides and Law on Chemicals, are in preparation. Serbia received the contribution from the Swedish International Development Cooperation Agency for the implementation of the Project “Chemicals Risk Management in Serbia 2007–2010” as a step towards the harmonisation and approximation of national legislation with EU Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Directive.

3.2 Water quality monitoring programmes and the current state of the aquatic environment in Serbia with respect of contamination with hazardous substances

The Republic Hydro Meteorological Service is the competent authority in charge of official water quality monitoring in the Republic of Serbia. The daily, weekly and annual reports, as well as sampling station network and a list of water quality parameters (with environmental standards and criteria) are available for public download (<http://www.hidmet.gov.rs/eng/zivotnasredina/index.php>). Serbia is also a part of Danube Trans-National Monitoring Network—annual reports are available at ICPDR official web site <http://www.icpdr.org>.

Official monitoring programmes and independent studies performed recently (UNEP 2004; ICPDR 2005 Ivančev-Tumbas et al. 2004, 2008; Terzić et al. 2008; Sakan et al. 2007) identified several hot spots of severe freshwater pollution and soil and sediment contamination that are attributed to outdated environmental policy, regulations and management practice, heavy pollution caused by insufficient wastewater treatment as well as major accidental spills (as the result of North Atlantic Treaty Organisation (NATO) campaign in 1999, or Baya Mare cyanide accident in 2000, for instance). They include certain sections of the Danube–Tisa–Danube Hydrosystem canal network (the biggest canal network in the Danube River Basin), Iron Gate Reservoirs and the Tisa River sediments. Heavy metals appear as the most emphasised problem, although the elevated contents of organic pollutants (polychlorinated biphenyls (PCBs), organochlorine pesticides, polyaromatic hydrocarbons (PAHs), organic micro pollutants, even emerging substances) have also been registered in some areas. Yet, the obvious lack of knowledge on organic xenobiotics (sources, fluxes, environmental fate and risks) should be stressed. The largest individual hot spot is the industrial complex near the city of Pančevo—effluents are discharged into the wastewater canal entering the River Danube (Jovančević et al. 2005). Many reservoirs in the central part of Serbia encounter the problem of contaminated sediments (Ivančev-Tumbas et al. 2004), while the south of the country, which was affected by the NATO campaign, has been recognised as a hot spot of depleted uranium contamination (Radenković et al. 2008).

3.3 Recent and contemporary ecotoxicological research in Serbia

3.3.1 Laboratory scale ecotoxicological research

Studies on relative sensitivity of standard test species and/or endpoints to various toxic substances, as well as studies on effects of priority pollutants, began in the late 1970s, with pioneer studies of effects of heavy metals on survival and

respiration rate of tubificid worms (Brković-Popović and Popović 1977a, b), *Daphnia magna* (Brković-Popović 1990), acute toxicity of heavy metals and phenols to *Allium cepa*, *Lepidium sativum* and *D. magna* (Arambašić et al. 1995) and, recently, study on impact of culturing patterns on chronic toxicity testing with *D. magna* (Teodorović and Planojević 2008). The initial steps in application of histological, biochemical and physiological biomarkers in laboratory scale ecotoxicological research, mainly in laboratory studies on modes of toxic effects of heavy metals and certain pesticides on aquatic species, date back to the 1990s. These include the study on activities of superoxide dismutase (SOD) and catalase (CAT) in erythrocytes and transaminases in the plasma of carps and goldfish exposed to cadmium (Žikić et al. 1997, 2001), study on acute and sub-acute toxicity of atrazine and 2,4-D herbicide to carp (Nešković et al. 1993, 1994); research on functional enzymes activity and gill histology of carp after glyphosate, copper sulphate and trifluralin exposure and recovery (Nešković et al. 1996; Karan et al. 1998; Poleksić and Karan 1999). The results of all cited studies on effects of pesticides to carp, as well as previously mentioned studies on toxicity of metals to *D. magna*, are included into the ECOTOX database (http://cfpub.epa.gov/ecotox/ecotox_home.cfm).

3.3.2 Ecotoxicology in environmental monitoring

The lack of reliable ecotoxicological data due to rather conservative official monitoring programmes based on traditional set of physicochemical parameters stimulated

the implementation of small to regional scale field ecotoxicological research projects that are based on (a) bioaccumulation studies, mostly on heavy metals in fish (Teodorović et al. 2000) or mosses and lichen (Popović et al. 2008) and PCBs in aquatic biota (Adamov et al. 2003) and (b) conventional ecotoxicological tests, such as the study on chronic toxicity of the Sava River (water and sediment), based on algal bioassay (Kallqvist et al. 2008). Sediment toxicity evaluations, undertaken as a part of feasibility studies for remediation activities on transboundary watercourses (Dalmacija et al. 2006; Prica et al. 2008), showed that although heavy metal concentrations were high, bioavailability and consequently toxicity to aquatic biota was low, due to the high content of clay, iron and sulphides (Table 1). The study on the relationship between WET and mandatory effluent monitoring across a range of industry types (Teodorović et al. 2009) showed that even effluents fulfilling discharge requirements are quite often moderately to highly toxic (Fig. 1).

Non-conventional tests and modern effect directed analyses (EDA) approach are being gradually applied, as evidenced by the sediment contact test with *Myriophyllum aquaticum* and *Lemna* sp. applied to assess the sediment quality (Stešević et al. 2007), application of *Vibrio fischeri* Flash test in the search for the optimum test battery for assessment of contaminated sediments (Planojević et al. 2008) and EDA approach in contaminated sediments of Pancevo hot spot (Kaišarević et al. 2008). Biomarkers of exposure and effect are also being applied of late, e.g. the study on effects of acute cyanide poisoning on the fish gills

Table 1 Parameters determining bioavailability/toxicity of metals in the sediment of the Begej Canal (Vojvodina, Serbia; from Dalmacija et al. 2006, with permission): The ratio $\Sigma[\text{SEM}_i]/[\text{AVS}] > 1$ was recorded

at several locations that were already recognised as places of high risk based on Dutch standards

Sample	[SEM _i]/R/%					[AVS]	$\Sigma[\text{SEM}_i]$	R ₁	R ₂	R ₃	R ₄
	Ni	Cd	Zn	Pb	Cu						
1 (top)	0.05/0.01/4.5	0.08/0.02/40	6.6/1.8/55	0.05/0.01/6.1	1.05/0.3/35	3.73	7.83	2.1	4.1	115	3.02
1 (bottom)	0.06/0.02/7.5	0.03/0.01/32	4.0/1.2/42	0.07/0.02/5.8	0.94/0.3/27	3.42	5.13	1.5	1.7	110	2.77
4 (top)	0.03/0.01/5.0	0.004/0.001/20	1.6/0.5/30	0.03/0.01/2.9	1.1/0.3/33	3.45	2.76	0.8	<0	82.9	1.69
6 (top)	0.03/0.01/3.0	0.06/0.01/20	2.2/0.5/20	0.04/0.01/4.9	0.56/0.1/20	4.13	2.89	0.7	<0	86.7	1.8
6 (bottom)	0.08/0.04/13	0.001/0.0007/18	0.71/0.3/42	0.07/0.003/5.5	0.21/0.1/30	4.18	1.01	0.2	<0	266	5.67
9 (top)	0.14/0.02/8.4	0.21/0.03/35	9.4/1.2/47	0.02/0.002/1.4	1.59/0.2/30	7.57	11.36	1.5	3.79	66.2	1.42
9 (bottom)	0.08/0.02/5.6	0.16/0.04/38	3.74/1.01/33	0.08/0.02/5.7	0.77/0.2/22	7.71	4.83	0.6	<0	125	1.72
13 (top)	0.07/0.01/4.5	0.15/0.02/32	6.29/0.94/3.0	0.07/0.01/6.0	1.48/0.22/38	6.72	8.06	1.2	1.34	82.3	0.81
13 (bottom)	0.01/0.002/1.0	0.037/0.008/34	4.7/1.02/42	0.36/0.08/11	1.37/0.3/28	6.83	6.48	0.9	<0	92.9	1.57
14 (top)	0.02/0.02/3.5	0.003/0.003/26	0.33/0.3/22	0.002/0.0002/1.0	0.08/0.08/17	1.10	0.44	0.4	<0	260	13

The other samples have the ratios $\Sigma[\text{SEM}_i]/[\text{AVS}] < 1$, despite the high class based on the Dutch evaluation. A low ratio of [SEM_i] to the corresponding total average concentration (<55%) might indicate that metals are bound to a relatively inert phase and point out to a limited bioavailability. [SEM_i] (μmol g⁻¹); R = [SEM_i]/[AVS]; %—percentage of [SEM_i] to the total concentration of *i*th metal in the sediment; [AVS] (μmol g⁻¹); $\Sigma[\text{SEM}_i]$ = SEM (Cd) + SEM (Cu) + SEM (Ni) + SEM (Pb) + SEM (Zn) (μmol g⁻¹); R₁ = $\Sigma[\text{SEM}_i]/[\text{AVS}]$; R₂ = $\Sigma[\text{SEM}_i] - [\text{AVS}]$; R₃ = [Fe]/[AVS] and R₄ = [Mn]/[AVS]

Fig. 1 Percentage of agreement between the whole effluent toxicity test and the chemical characterisation based on compliance monitoring (from Teodorović et al. 2009, with permission)

(Poleksić and Jeremić 2003), genotoxicity screening of the river Rasina in Serbia based on *Allium* anaphase–telophase test (Vujošević et al. 2008), assessment of pollution in the Danube River near Novi Sad based on several biomarkers (SOD, catalase, glutathione peroxidase (GSH-Px), glutathione S-transferase (GST), induction of CYP1A1 in liver) in sterlet (Stanić et al. 2006) and the study on antioxidant defence enzyme activities (SOD, CAT, GSH-Px, glutathione reductase, GST) in hepatopancreas, gills and muscle of Spiny cheek crayfish from the River Danube (Borković et al. 2008). Although the applied biomarkers are rather unspecific, the toxicity identification evaluation is missing in all the mentioned studies, so stress confirmed by the results could not be attributed to a specific group of toxic substances. Toxicity of samples from Kragujevac hot spot (Zorić et al. 2004) and soil from oil refineries in Vojvodina (after the NATO campaign spills; Kaišarević et al. 2007), determined by micro-ethoxyresorufin-*o*-deethylase analysis, could not be attributed exclusively to the contents of measured PCBs and PAHs (Table 2), but they should be attributed also to the presence of unknown dioxin-like compounds, and/or positive interactions among similarly acting chemicals, proving that biotests, when applied in ecotoxicological assessments, should be used as a screening tool or initial step in effect-directed analyses.

3.3.3 Recent and on-going national, regional and international research projects in the field of ecotoxicology

A recent turbulent history and a period of sanctions during the 1990s lead to severe isolation of Serbia from the rest of the world, over a long period of time. Research institutions and individual scientists were not eligible for participation in international projects so that cooperation between the

Serbian research community and the rest of the world was kept on a personal basis. The research was supported only by national funding mechanisms which, due to a rather limited capacity, proved to be insufficient to keep pace with contemporary research. However, Serbian researchers and institutions have recently become eligible partners to the EU Framework Programme (FP) and other EU funding programmes. A big step forward related to ecotoxicological research was reflected, for instance, in participation of the University of Novi Sad, Faculty of Sciences in the APOPSBAL project (Assessment of the selected POPs (PCBs, PCDD/Fs, POCPs) in the Atmosphere and Water Ecosystems from Waste Materials Generated by Warfare in the Area of Former Yugoslavia, FP5-ICA2-CT-2002-10007, 2002–2005). Still, Serbian research institutions are not included in any of the major, recent and on-going EU-funded ecotoxicological projects (e.g. Modelkey—<http://www.modelkey.org>; AquaTerra—<http://www.attempto-projects.de/aquaterra/>; Liberation—<http://www.liberation.dk/>; NoMiracle—<http://nomiracle.jrc.ec.europa.eu>). During the last couple of years, a significant number of research laboratories in Serbia have been upgraded with state-of-the-art laboratory equipment, quality training in and networking with the respectful European research centres via the National Investment Fund of Serbia, regional programmes (e.g. biomarkers of exposure and of effects of persistent

Table 2 Bio-TEQ values (pg PCB126/g d.w.), PCB and PAH levels (ng/g d.w.) in soil samples from Oil Refinery Novi Sad (from Kaišarević et al. 2007, with permission)

Sample	Refined hexane extract		PAH fraction	
	Bio-TEQ ^a	ΣPCBs ^b	Bio-TEQ ^a	ΣPAHs ^c
NS 1	13.72	1.47	NA	NA
NS 2	241.22	0.94	742.30	175.9
NS 3	76.99	4.95	NA	NA
NS 4	444.97	581.44	924.72	3,928.6
NS 5	224.74	0.55	NA	NA
NS 6	911.22	<0.05	18,701.49	507.1
NS 7	127.18	1.84	NA	NA
NS 8	15.22	1.48	425.47	<5
NS 9	235.74	18.14	244.54	<5
NS 10	25.70	1.93	47.31	<5

NA sample was not analysed on the presence of PAHs and corresponding bio-TEQ value

^a Bio-TEQ = PCB126EC25/SampleEC25PCB126

^b Riedel-de-Haen standard of seven PCB congener nos. 28, 52, 101, 138, 153, 180 and 209

^c Standard mixture of 16 components: acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, chrysene, dibenzo(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene and pyrene

organic pollutants in ecosystem, WUS Austria 87/2002, 2002–2005 granted to the University of Novi Sad, Faculty of Sciences) and FP INCO special programmes for Western Balkan countries (e.g. two on-going projects at the University of Novi Sad, Faculty of Sciences: Reinforcement of Research Potential of the Laboratory for Ecotoxicology (REP-LECOTOX), INCO-CT-2006-043559-REP-LECOTOX (<http://www.lecotox.net>) and Reinforcement of the Laboratory for Environmental Protection as a Centre of Excellence for Environmental Chemistry and Risk Assessment (CECRA), INCO-CT-2006-043741 (<http://www.cecra.ih.ns.ac.yu>)).

4 Discussion

As has been previously stated, quite a large number of the so-called hot spots of extreme water pollution (sediments in particular) and soil contamination have been identified over the past decade in Serbia. Consequently, it could be expected that ecotoxicology as a discipline stands rather high in the agenda of researchers, professionals and funding agencies in Serbia. However, ecotoxicological methods have never been incorporated into risk assessment, or mandatory effluent and official ambient water monitoring programmes, nor have they been applied in environmental quality standards setting. Therefore, the research has been restricted for years on small- to medium-scale laboratory and monitoring-like studies, rare fundamental and sporadic conceptual research, which mainly applied conventional ecotoxicological methods and standard aquatic test species and which have been undertaken by a few independent research teams. Moreover, the results until the mid-1990s either remained unpublished or ended up in the so-called grey literature. Only recently, Serbian researchers have begun publishing the results of their studies in international peer-reviewed, yet low- to medium-ranked, journals, but majority of articles still belong to rather descriptive report-like papers. The results that have been published are derived from the modestly funded studies where well-established standardised ecotoxicological methods are applied, basically, to confirm, underline or oppose the results of chemical-based monitoring and to lament on inadequate environmental regulations/policy and management practice. Proper scientific research and unresolved ecotoxicological problems still remain beyond the reach of ecotoxicological research currently conducted in Serbia, or they are tackled only sporadically. They are dealing mainly with (a) the contribution of individual toxicants to overall toxicity of the complex mixtures; (b) the interaction between potentially present toxicants and other compounds in complex matrices and, consequently, the bioavailability of toxicants; (c) the problem of toxicity identification

evaluation in complex mixtures; (d) extrapolation from laboratory (or species) to ecosystem scale and (e) development of alternative methods and endpoints, including the application of genomic-based tools, which would be more sensitive to priority pollutants and emerging substances.

5 Conclusions

Environmental protection in Serbia has been standing rather low on the agenda of policy and decision makers for many years now, due to numerous historic and social-economic factors. The outdated environmental legislation and poor implementation, insufficient waste water treatment, ineffective environmental monitoring programmes, limited human resources and absence of knowledge on risk assessment of hazardous substances have characterised water protection and management sector for years. Such under-prioritisation of serious environmental problems led to water pollution and sediments contamination in Serbian inland waters. Yet, ecotoxicological research, which should have been seen as a helping hand for the competent authorities, has been ignored for years and therefore remained isolated and restricted to small-scale laboratory and field monitoring, i.e. studies without or with very limited impact on environmental policy in general. Serbian scientific community has struggled, under extremely difficult conditions, to keep up with trends and progress in international research and development, by learning, adopting and applying ecotoxicological methods in locally relevant studies, which resulted with a certain number of publications, but more importantly, in valuable data easily applicable in water management and policy, once the relevant EU regulations, proper implementation strategies and environmental policy in general are fully transposed and incorporated into the Serbian legal system.

6 Perspectives

Modern approach to environmental risk assessment set forth by REACH and partly by the WFD, once fully transposed into national legal system, would certainly give additional stimulus to ecotoxicological research in Serbia. Research and institutional capacity building in Serbia should, hopefully, increase the competence and competitiveness of scientific community for participation in the EU FP and other large-scale research programmes and, on the other hand, contribute to radical changes in national environmental policy and management strategies. To catch up with trends in ecotoxicological research and risk assessment, the scientific community in Serbia should get prepared for new challenges—switching from descriptive

and monitoring-like studies to fundamental and conceptual research on unresolved ecotoxicological issues and problems.

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