



Fakulta rybnářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice
Czech Republic

Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Daniel Červený, Dipl.-Ing.	First name(s), surname, titles of supervisor: Assoc. prof. Dipl.-Ing. Tomáš Randák, Ph.D.
Title of PhD thesis: New approaches in biomonitoring of extraneous substances in aquatic environment	
REVIEWER:	
Surname: Vrana	Institution: Masaryk University Research Centre for Toxic Compounds in the Environment
Name: Branislav	
Titles: Assoc. prof. Dipl.-Ing., Ph.D.	E-mail: vrana@recetox.muni.cz
Please describe your professional relationship to the PhD student: none	Please describe your field of expertise: environmental chemistry -fate of organic pollutants in the aquatic environment, passive sampling

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

The thesis presents a systematic approach in investigation of novel approaches to monitoring of priority pollutant pollutants in the aquatic environment using chemical monitoring in biota. The research in this field is very important since in Europe, the process of implementation of Water Framework Directive prescribes chemical monitoring in biota as one of the crucial monitoring approaches applied in the assessment of chemical status in European waters. Moreover, it is equally important to know the risks associated to pollutants in fish that is used for human consumption. With the increasing demand for chemical monitoring using biota, ethical issues arise whether sacrificing large quantities of fish is an appropriate method for monitoring, when the overall goal of the monitoring is actually the protection of the environment. Therefore, I very much appreciate candidate's striving for application of non-invasive methods, e.g. passive sampling, non-invasive sampling, or sampling of abundant organisms, which will help to minimise the harm to the environment by monitoring activities. The high quality of scientific work presented in the thesis is sufficiently demonstrated by the fact that a number of papers has been published in renowned peer-reviewed scientific journals with the candidate as the first author.



Elaboration of the PhD thesis, objectives of the work and deliverables

The title of the thesis is well chosen and reflects well the contents. The abstract/summary is informative and summarises the most important issues described in the presented work. The thesis is fully acceptable with regard to grammar and syntax. I would appreciate a list of abbreviations which would simplify orientation in the text. The work is formally divided into seven self contained chapters. In Chapter 1, motivation of the research and objectives of the thesis are formulated. Chapter 2 –Chapter 6 are presented as published papers or submitted manuscripts to renowned international scientific journals. Finally, Chapter 7 contains concluding remarks and recommendations for future research. The work gives a proper recognition to previous studies. Each chapter contains citations of most relevant references in the research area according to the standards expected in scientific journals. The proper citing of publications in the whole thesis avoids unnecessary repetition of known facts and allows the author to describe own research work. The list of references according to the standards expected in scientific journals is provided at the end of each chapter. The scientific problem is well-defined and clearly formulated. The data interpretation is correct and the argumentation consistent throughout the thesis, with a few minor exceptions, which do not affect the overall quality of the work. The presented results fully correspond with objectives of the PhD thesis. Overall, the scientific content of the work corresponds to the standard of a doctoral dissertation. The thesis shows evidence of a substantial independent scientific achievement by the candidate. I recommend to accept the presented dissertation and propose to award to the scientific-academic degree philosophiae doctor (PhD).

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

I would encourage the PhD candidate to clearly define and state the term *biomonitoring* he often uses in the thesis, since several possible definitions of the term can be found in the literature: e.g. a) the measurement of chemicals in humans (or in human blood or urine); b) the use of biological responses to assess changes in the environment, where biochemical, genetic, morphological, and physiological changes in certain organisms or even their presence/absence are being related to particular environmental stressors and c) the chemical monitoring in biota as a tool for the assessment of the status of environment. To avoid this ambiguity, in current EU legislation, such as the WFD (2000/60/EC) daughter directives 2013/39/EU and related WFD guidance documents the term *biota monitoring* or *chemical monitoring in biota* is used instead of the much broader term *biomonitoring*.

The PhD thesis addresses a very topical problem since for many persistent bioaccumulative toxic chemicals including many chlorinated and brominated POPs, PFOS and mercury, the EQS directive (2013/39/EU) recently set environmental quality standards (EQS) values as concentrations in biota. I fully agree with the author that chemical monitoring in biota allows a much better assessment of chemical exposure and associated toxicity to aquatic organisms than the analysis of whole water. This is not only because of higher and thus better quantifiable concentrations, but also because levels in organisms reflect levels in the environment in which they live. However, many new problems have appeared that are associated with the inherent variability of sampled biota and the fact that absence of a particular priority substance in biota does not automatically mean it is not

present in the environment (i.e. biota may have been exposed but the substance disappears due to metabolism).

One of the possibilities to reduce the data variability (especially for long term contaminant trend assessment) would be to normalise the measured concentrations by expressing them as normalised concentration in an appropriate matrix, in which they most likely accumulate in fish—e.g. lipid (for POPs), protein (for PFCs) or dry weight (mercury). Although in Chapter 2 the author found a significant correlation between concentrations of PCBs in fish muscle tissue of different fish species and its lipid content, I am missing an attempt to compare lipid based concentrations between the fish from different species from individual sites. If such differences were observed, these could be possibly related to the trophic magnification of POPs in the monitored fish species. Can the PhD candidate discuss this issue?

Further to the previous issue, in order to obtain comparable data when lipid normalisation is chosen, a consensus lipid extraction method should be applied. In Chapter 2, paragraph 2.3 Chemical analysis a lipid content determination method is stated without a reference. Can this be specified?

For hexachlorobenzene, mercury, and PFOS and its derivatives EQS values have been set in the EQS directive 2013/39/EU as 10, 20 and 9.1 µg/kg w.w. in fish, respectively. Can the author state whether the limits have been exceeded in fish samples analysed in the papers in Chapters 2, 4 and 5?

Combining biotic and abiotic (through passive sampling) measurements is an attractive option to assess levels of contaminants in aquatic environments and should help reducing the uncertainty in using biota for water quality assessment. Passive sampling can then be used for sampling locations where relevant biota cannot be found, increase spatial coverage of monitoring networks or simply to reduce/minimize the number of organisms to be sampled. In Chapter 3 the candidate shows a very good correlation between PFAS concentrations in liver tissue with those in the POCIS passive sampler. Moreover, for PFOS a correlation was found also between levels in POCIS and muscle. This makes sense from the viewpoint of the fact that both fish and the passive sampler reflects the concentration in water to which it has been exposed. The author concludes that passive sampling (POCIS) seems to be a more effective approach for monitoring PFASs in aquatic environment than analyses of fish. However, I still would recommend carefulness with such statements for several reasons: a) the uptake of PFASs to POCIS is affected by many factors including temperature and flow velocity and thus data from POCIS should be so far considered as semiquantitative; b) POCIS exposed for 3 weeks in water may accumulate some PFASs integratively, but some (such as PFASs with a short chain) may reach a steady state/equilibrium with the surrounding water. With fish, which lives in the water body for a year or more, most of the PFASs that do not metabolise may have reached steady state/partitioning equilibrium. For derivation of levels in tissue from levels found in passive samplers, both fish and passive samplers would have to reach partition equilibrium (or steady state with the surrounding water). Does the author believe that such approach is feasible or would he recommend a different approach?



In Chapter 4, much higher concentrations of PFOS and PFASs in YOY than in adult fish samples was found at the site Kozly. The author hypothesizes that the differences are due to different periods of time needed for bioaccumulation to occur in the indicator organisms. Can the author explain this hypothesis? Does the hypothesis assume fluctuation of PFASs concentration in time?

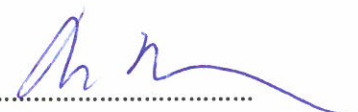
As a layman in the field of fish monitoring I would like to ask whether it is possible to remove parts of pectoral fins from the monitored fish without doing a permanent harm to the fish?

Can the correlations found between THg concentrations in from fin clips analysis be applied in other water bodies than those investigated?

FINAL RECOMMENDATION

- PhD Thesis can be recommended for defence
- PhD Thesis can be recommended with reservations for defence
- PhD Thesis can not be recommended for defence

.....14.6.2016 in Brno.....
Date and place


.....
doc. Ing. Branislav Vrana, PhD



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Daniel Červený, Dipl.-Ing.	First name(s), surname, titles of supervisor: Assoc. prof. Dipl.-Ing. Tomáš Randák, Ph.D.
Title of PhD thesis: New approaches in biomonitoring of extraneous substances in aquatic environment	
REVIEWER:	
Surname: Teixeira Pestana	Institution: Department of Biology & CESAM, University of Aveiro, Portugal
Name: Joao Luís	
Titles: Dr.	E-mail: jpestana@ua.pt
Please describe your professional relationship to the PhD student: none	Please describe your field of expertise: Aquatic ecotoxicology

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (**extent ¼ – ½ page**):

Daniel Červený PhD thesis presents original work and interesting results concerning the monitoring of chemical substances using fish and invertebrate samples. Results presented, some of which are already published, are derived from well-planned research with appropriate methodologies.

These results are important in the context of monitoring of chemicals in freshwaters and at the same time, given the different focal species used, can contribute to a better health risk assessment related to fish consumption. Innovative aspects of the thesis are mainly related to the assessment of emerging contaminants in biota samples and the proposed method of using fin-clip samples as a non-lethal approach to measure internal concentrations of contaminants in fish. Also the use of young-of-the-year fish as an alternative to adult fish sampling is proposed and in my opinion this approach could also have significant advantages in monitoring programs and environmental risk assessment strategies.

Research within environmental sciences (including ecological and ecotoxicological basic and applied research) will greatly benefit from some of these methodologies.



Elaboration of the PhD thesis, objectives of the work and deliverables

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (**extent ¼ – ½ page**):

Daniel Červený PhD thesis is generally well written and clear. The objectives are clear and focused and chapters are organized in a logic order. Main conclusions are correctly drawn by the results obtained.

This being said, in my opinion the general introduction and discussion sections as well and the chapters 4 and 5 (submitted papers) should be improved in terms of language. Some sentences need rephrasing for clarity and some grammar and typing errors are still present. Also some sentences need a better contextualization with appropriate literature references and parts of the methods section in chapters 4 and 5 are not easily understandable. At times, some repetition of ideas was noticeable.

I agree with the structuring of the thesis (general introduction followed by chapters in the form of published or submitted scientific papers and general discussion). However, the main chapters should have been all presented in a continuous formatting and not by inserting a pdf version of published papers. This hinders reading and interpretation of figures and more importantly not all of the results are presented since supporting information of published papers is not presented in the thesis document as it should be (e.g. in the end of each chapter or as annexes). Moreover I think that chapter 6, although presenting interesting and relevant results deviates from the objectives and is only slightly linked with the overall focus of the thesis concerning approaches for monitoring internal concentrations of different contaminants in fish samples). As such the inclusion of this article as a formal chapter of the thesis is somewhat poorly justified.

Nevertheless and in general, appropriate methods were used and data analysis was done competently to address the specific objectives. The outcomes of the thesis are published /submitted to well respected journals within environmental sciences and, as stated, offer important data and approaches that can be used in a near future by researchers and environmental managers.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

Daniel Červený PhD thesis related with approaches for biomonitoring chemicals in the aquatic environment is a scientifically sound thesis. As already stated, results obtained during the PhD work are new and significant for researchers and regulators. Also, innovative methods for sampling biota were proposed and while some results and conclusions deserve further investigation and confirmation, significant advantages in those proposed methods are to be expected.

I think that the thesis document is of sufficient quality and I look forward the discussion with the candidate during the defence. This discussion will in my opinion address some details that can be improved, especially in chapters 4 and 5 which are not published yet

Chapter 1 (General Introduction):

The introduction section is clear and short but probably too short hampering a more focused review of the literature and state of the art concerning biomonitoring of contaminants in fish samples. As already mentioned, I somewhat disagree with the inclusion of chapter 6 and in line with this it is hard for me to understand the inclusion of the sub-section related with algae samples. I think that the general introduction section could have benefited if only an in-depth review of the literature concerning only fish samples, passive sampling and pros and cons of the different approaches used to measure internal concentrations of contaminants in fish and how they can be used for basic and applied research (environmental, and health risk assessment). Also some reference to freshwater contamination and ecological effects (in fish species and populations) caused by the emerging and persistent contaminants of interest (pharmaceuticals, PFAS, metals) would be helpful.



Chapter 2 - "Contamination of Fish in Important Fishing Grounds of the Czech Republic":

Chapter two conveys important results concerning fish contamination within different catchments /reservoirs in Czech Republic and is an important contribution for health risk related to fish consumption. This chapter was already published and only minor issues related to wording would have deserve clarification; e.g.- "predatory fish" vs. "piscivorous fish"

Chapter 3 - "Perfluoroalkyl substances in aquatic environment - comparison of fish and passive sampling approaches":

This chapter was published in Environmental Research and presents an interesting study on how passive samplers can be effectively used to replace continuous sampling of biota (fish) for determination and quantification of different pollutants in the freshwater environment.

Chapter 4 - "Young-of-the-year fish as a prospective bioindicator for aquatic environmental contamination monitoring":

This chapter is focused on a new and interesting alternative to adult fish sampling. It is concluded that whole fish homogenates from multispecies samples accurately reflected local contamination with advantages in terms of variability and sensitivity of measurements. Concerning this chapter it is arguable that only muscle was sampled in adult fish for comparison with whole body homogenates of YOY samples which might have affected the results (I think that conclusions in terms of correlation between adult fish and YOY pools are based mainly by results only for Hg). Also the process of pooling of YOY samples (RS/DS) is hard to understand and this part of the methods section should be improved for clarity. I also suggest to separate data for Cd and Pb for a better interpretation of figures and I am not sure if the statistical analysis are appropriate (Use of one way ANOVA to compare contamination levels in adult fish - two species from two different sites). Moreover it is not clear if average values for pooled samples are calculated based true replicates (and not sub-replicates /technical replicates).

Chapter 5 - "Fish fin-clips as a non-lethal method for biomonitoring of mercury contamination in aquatic environments":

Chapter 5 presents an interesting study where fin clips are validated as a sensitive method to predict fish muscle concentration of Hg. Several issues deserve attention. Firstly the first sentence and second paragraph of the introduction are almost identical to Chapter 2 (introduction) and this should be corrected. It is important to discuss the reasoning behind site selection and also the reasoning behind group division (based on Hg content in fins) and calculation of median PF values (I might be mistaken but in my opinion data presented in Fig 2 seem contradictory to the setting of these median PF's). It is also arguable that concentrations of muscle total Hg are better predicted by this approach....

The higher variability observed in fin-clip Hg concentrations is not discussed and it is also curious not to see levels of Hg measured in sediments of the different sites. Wouldn't this help to discuss the issues related to effects of age and migration on Hg internal concentrations?

Chapter 6 - "Presence of pharmaceuticals in benthic fauna living in a small stream affected by effluent from a municipal sewage treatment plant":

This last chapter, although not related to fish samples is scientifically sound and presents new and important results concerning accumulation of emerging contaminants (pharmaceuticals) in invertebrates which is not common in environmental studies. I would be interested in discussing the reasoning for species selection (what should be considered an ideal invertebrate species for such biomonitoring studies?) Again assessment of sediment contamination could have helped the discussion of results....

Chapter 7 (General discussion):

This section summarizes the results from the different chapters and my only comment is that it could have been more specific in terms of future research and perspectives (e.g. tests to validate the proposed fin-clip and YOY sampling or for instance the selection of an invertebrate species and conditions (size, time of collection) that can be used in similar biomonitoring programs). Finally in the general conclusion results from chapter 4 concerning the evaluation of pharmaceuticals in YOY samples and adult fish are briefly discussed. However these results were not presented in the respective chapter as such this should be removed from the general discussion as well.



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FINAL RECOMMENDATION

- PhD Thesis can be recommended for defence
 PhD Thesis can be recommended with reservations for defence
 PhD Thesis can not be recommended for defence

Aveiro, 13th June 2016

.....
Date and place

JOÃO LUÍS TEIXEIRA PESTANA
João Luís Teixeira Pestana

.....
Name and signature