



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Thai Giang Pham, M.Sc.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Vladimír Žlábek, Ph.D.
Title of PhD thesis: Biological effects of anthropogenic pollutants present in recipients of treated sewage water	
REVIEWER:	
Surname: Fick	Institution: Department of Chemistry
Name: Jerker	
Titles: Assoc. Prof.	E-mail: jerker.fick@umu.se
Please describe your professional relationship to the PhD student: No relationship	Please describe your field of expertise: Analytical chemistry, fate and effects of pharmaceuticals in the environment

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):

The thesis "Biological effects of anthropogenic pollutants present in recipients of treated sewage water" by M.Sc. Thai Giang Pham focus on the effect of emerging environment contaminants and the complex mixture that is treated effluent. M.Sc. Giang apply novel state-of-the-art exposure techniques that make mimic natural settings. The thesis includes passive sampling, analysis of trace contaminants and measurements of several biomarkers in two large long-term exposure studies.

This thesis addresses effects of pharmaceuticals and treated effluent in a very comprehensive and novel set of studies and present a number of highly interesting conclusions and findings. State-of-the-art methods are used throughout.

This thesis have increased the understanding of the effects of pharmaceuticals and treated effluent. Using two common recipients and testing two native species is novel and provides relevant information, especially considering the long-term exposure used. Results presented are highly original and of high scientific importance.



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**):

This thesis reads well, with a logical and clear structure that is easy to follow. All chapters are in a logical order and the introduction and methods sections are appropriate. The general introduction gives a nice and comprehensive overview. All experiments and samplings were conducted using appropriate methods and the analyses were made with appropriate, novel and excellent analytical protocols. The thesis follows the guidelines and the included publications are all published in the top 10 percentile of the journals in the field.

References are relevant and cover the addressed field, and the abstract provides a clear and to-the-point version of the results and methodology.

All publications provide more knowledge regarding the effect of pharmaceuticals by using advanced novel exposure settings, which shows that they correspond well to the objectives of the thesis.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

This thesis is based on a general introduction and two published papers. Both published papers are in high-ranked journals and M.Sc. Giang is first author on both. M.Sc. Giang is also co-author on three additionally published papers and two submitted manuscripts.

Nice selection of relevant references, good overview of the field.

Giang, P.T., Burkina, V., Sakalli, S., Schmidt-Posthaus, H., Rasmussen, K.M., Randak, T., Grabic, R., Grabicova, K., Fedorova, G., Koba, O., Golovko, O., Turek, J., Cerveny, D., Kolarova, J., Zlabek, V., 2017. Effects of multi-component mixtures from sewage treatment plant effluent on common carp (*Cyprinus carpio*) under fully realistic condition – a real case study. Environmental Management. DOI



10.1007/s00267-017-0964-7

Excellent publication that uses a combination of long term realistic exposure, relevant biochemical protocols and state of the art analytical instrumentation. Usage of intergrate biomarkers and the long exposure (up to 360 days) makes this publication highly relevant.

Giang, P.T., Sakalli, S., Fedorova, G., Khalili, T.S., Bakal, T., Najmanova, L., Grabicova, K., Kolarova, J., Sampels, S., Zamaratskaia, G., Grabic, R., Randak, T., Zlabek, V., Burkina, V. 2017. Biomarker response, lipid composition, and intestinal microbiome in wild brown trout (*Salmo trutta m. fario* L.) exposed to a sewage treatment plant effluent-dominated stream. *Science of the Total Environment*. 625 (2018) 1494-1509

This paper presents a long term exposure done under natural conditions and long time (180 days!). This experiment is carefully planned and uses a nearly unique approach which makes the interpretation of changes in biomarker directly applicable.

Conclusion;

I think that this thesis increases our knowledge about the effect of effluents in various recipients considerably with several novel exposure settings.

FINAL RECOMMENDATION



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

PhD Thesis can be recommended for defence

PhD Thesis can be recommended with reservations for defence

PhD Thesis can not be recommended for defence

28/6-2018 Umeň

.....

Date and place

.....

Name and signature

Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Thai Giang Pham, M.Sc.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Vladimír Žlábek, Ph.D.
Title of PhD thesis: Biological effects of anthropogenic pollutants present in recipients of treated sewage water	
REVIEWER:	
Surname: Teixeira Pestana	Institution: Department of Biology and 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 ecology / 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**):

The above mentioned PhD thesis presents original work and interesting results concerning the ecotoxicological effects of STP effluents on two fish species. Results presented are already published in two scientific articles and are the result of extensive laboratory and field work.

These results are important in the context of monitoring of chemicals in freshwaters namely characterization of STP effluents and at the same time, given the use of passive samplers and the battery of sub-lethal endpoints used can contribute to a better ecological risk assessment of emerging contaminants namely some PPCP's.

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**):

Tai Giam pham PhD thesis is generally well written and clear with main conclusions correctly drawn by the results obtained. The objectives are clear and focused and chapters are organized in a logic order.

This being said, in my opinion the general introduction and the discussion sections should be improved in terms of language. Some sentences need rephrasing for clarity and some grammar and typing errors are still present.

Introduction section should end with better statements of the specific objectives of the work and approaches used. It is stated only that "*Unique experimental set up was applied to overcome the disadvantages of classical methods.*" No more details were provided.

I agree with the structuring of the thesis (general introduction followed by chapters in the form of published scientific papers and general discussion).

Nevertheless, and in general, appropriate methods were used and data analyses were done competently to address the specific objectives. The outcomes of the thesis are published in two respected journals within environmental sciences and, as stated, offer important data that can be used in a near future by researchers and environmental managers. This is a valuable work addressing effects of complex chemical effluents on freshwater biota.

Innovative aspects in this work include the experimental set up with collection and tagging of native fish (on site) and the extensive work with many sub-lethal , physiological and biochemical responses analysed.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

Tai Giam pham PhD thesis related with effects of contaminants present in STP effluents in two fish species in lentic and lotic freshwaters. Although the thesis presents only two scientific articles these convey a lot of results since besides de chemical characterization of water contamination (using passive samplers), fish were also sampled and a complete battery of endpoints were used. These sub-lethal responses range from biochemical biomarkers to morphology and histopathology and even bacteria consortia present in fish. I do have some questions/ comments regarding each section:

Chapter 1 (General Introduction):

The introduction section is clear and short for a better overview of the literature and state of the art concerning all the biomarker and responses used in fish. In detail the sections on IBR and its statistical analyses and Bacteria consortia in fish are quite incomplete. Also a brief discussion on the two fish species used (ecology, sensitivity to chemical contamination, etc.) should be present. A section on fatty acid composition of fish and its relevance for environmental studies is lacking

since it was used in chapter 3 as an indicator. In my opinion the introduction section should end with the main objectives hypothesis and outline of the thesis (it is currently in the end of the first subsection of the introduction). English revision would improve in some parts.

Chapter 2 - "Biological Effects of STP Effluent on Common Carp Living in a Biological Pond":

The paper is clear and well written and I only have questions/comments:

- A better explanation on IBR methodology should have been provided namely on why statistics were not applied (concerning possible permutations on biomarkers order in star plots that could give average area values in both control and exposed fish and how IBR values were calculated in fig 5 b) . Also why weren't these responses analysed separately between male and female fish for 180 and 360 days ?
- Evidence of chemical contamination in water is clear from passive samplers. Any idea of accumulation in fish? This could be really interesting if at least major compounds detected would have been analysed in fish tissues.
- A better characterization of both ponds namely in terms of invertebrate communities, which is what fish eat, would have allowed to better discuss and interpret the differences in fish growth health status (CF)

Chapter 3 - "Biological Effects of STP Effluent on Brown Trout Living in an STP Effluent Dominated Stream":

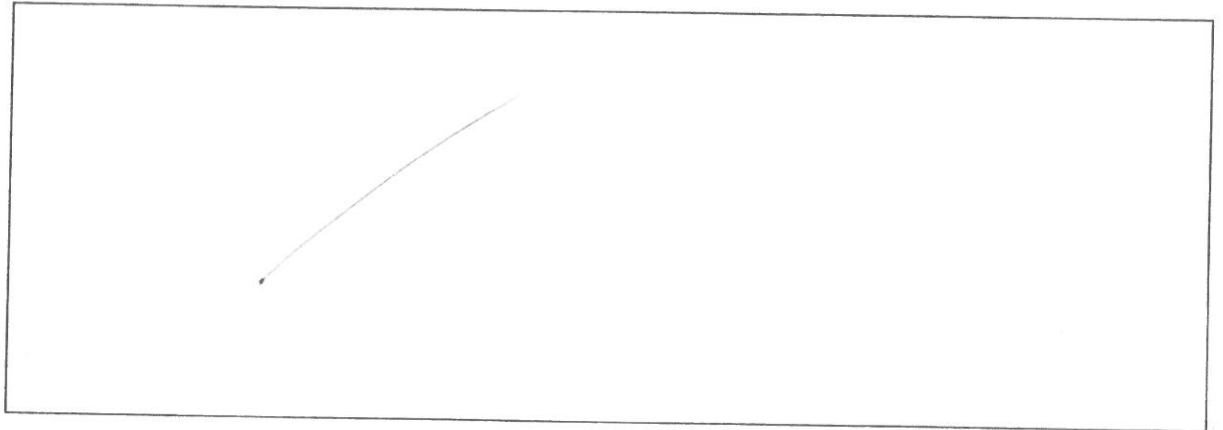
This paper is published. It's clear and sound concerning results and methods used. I only have a minor question:

- Why was the environmental microbiota analysed only in pebbles? Shouldn't it be also assessed in water and sediments?

Chapter 7 (General discussion):

This section summarizes the results from the two chapters and in my opinion is really good. It clearly resumes all main findings while discussing the advantages and challenges of the approaches used as well potential future research and perspectives. I congratulate the candidate for this discussion.

I only have a suggestion: maybe the fact that using only one contaminated pond and a control pond or in lotic systems, only one upstream site vs downstream site (fish collected downstream were pooled together) might pose some challenges. Shouldn't the work be extended to include more reference sites (reference condition approach) and more contaminated sites (dilution effect) ? In other words , can these individual fish be considered true replicates?



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

Avicino 4th July 2018

Date and place

JOÃO PESTANA

João Pestana

Name and signature