



Přírodovědecká
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Faculty
of Science

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

OPPONENT'S REVIEW ON BACHELOR/DIPLOMA* THESIS

Name of the student: František Jiřík
Thesis title: The effect of selected pharmaceuticals on growth, biomass and activity of soil microorganisms
Supervisor: MSc. Zuzana Frková, Ph.D., RNDr. Alica Chroňáková, Ph.D.
Referee: RNDr. Jarmila Štěrbová
Referee's affiliation: University of South Bohemia, Institute of Chemistry & Institute of Parasitology, Biology Centre of CAS

	Point scale ¹	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), balanced length of the thesis parts (recommended length of the theoretical part is max. 1/3 of the total length), logical structure of the thesis	0-3	3
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
Graphic layout of the text and of the figures/tables	0-3	3
Quality of the annotation	0-3	2
Language and stylistics, complying with the valid terminology	0-3	2
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	2
Formal requirements – points in total		17
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	2
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	3
Logic in the course of the experimental work	0-3	2

* Choose one

¹ Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Completeness of the description of the used techniques	0-3	3
Experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	2
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2
Practical requirements – points in total		21
POINTS IN TOTAL (MAX/AWARDED)		48
		38

Comments of the reviewer on the student and the thesis:

The bachelor thesis presented by František Jiřík aimed to study effect of pharmaceuticals on growth, biomass and activity of soil microorganisms. The thesis is focused on the influence of irbesartan on the microorganisms in arable soil sampled in Czech University of life Sciences campus in Prague. The topic is actual due to the increasing contamination of soil by pharmaceuticals caused by human activities. Student chose the commonly used drug irbesartan, since the information about microbial responses to the pollution of the soil by irbesartan is missing.

The thesis has 51 pages and it is divided into Abstract, The aim of the work, Theoretical background, Materials and methods, Results, Discussion, Conclusion, References and Supplementary information. The extent of the thesis seems to me satisfactory and text is coherent and mostly clearly written.

Despite the very good writing skills, several mistakes are unfortunately present. Some abbreviations are missing in the list of abbreviations, for example WWTP, LC-MS/MS, DMSO.

Despite these minor mistakes, the formal side of the thesis is on a very good level.

Abstract is well written with appropriate length.

Aims of the work clearly stated, filled in three hypotheses. Experimental procedures done by the student himself are distinguished from those that were done in cooperation.

Theoretical background covers ten pages with three figures, and it is divided into four subchapters. Text is written understandably, and the reader gets the information in the chapters about Soil function on Earth, Role and structure of microbiota, Pharmaceutical pollution in soil, and Irbesartan. From 48 articles being cited, about 30 citations are not older than ten years, which shows that this scientific field belongs to those currently highly interesting.

Materials and methods cover 15 pages with two figures and seven tables. Student himself prepared stock and spiking solution, an optimal protocol for soil extract preparation and tried to assess optimal dilution factor for enumerating Total cell counts in soil. He learned to work with microscope, to use CARD-FISH and L-[¹⁴C]-leucine incorporation technique and statistically evaluate measured data. Every step and procedure are described in detail, often complemented with explanation why is this particular procedure necessary.

The text is well written but in some subchapters it is quite difficult to follow the **text** due to the long sentences covering several lines (for example in page 18 - A combination of the CARD-FISH and Enumeration of Total cell counts methods enables the comparison of the development of the population of a particular group of bacteria with the development of the population of the whole bacterial community).

I also think that several parts are unnecessary, for example, in page 20 the information, that the student attempted to enumerate abundance of different groups of bacteria, but the measurements were not completed due to the technical problems – this part should be deleted from the text because the experiment was not repeated.

On the other hand, I appreciate that samples were carried out in triplicates and that the results were confirmed using statistical methods.

Results are composed of 8 pages, 3 tables and 5 figures. Each table and figure is described sufficiently. Abundance of soil microorganisms was studied by Enumeration of Total cell counts, abundance of soil bacteria was studied using CARD-FISH technique and the effect of irbesartan on the activity of soil microorganisms was studied using the L-[¹⁴C]-leucine incorporation method. Moreover, concentrations of irbesartan in soil samples was investigated using LTDT/APCI-MS/MS with the cooperation of analytical laboratory in Vodňany.

Generally, the text seems coherent and it is relatively easy to follow it, I also appreciate links to particular chapters in the materials and methods part. Nevertheless, I am not sure that the student always understands the principles because some interpretations seem to me a little bit confusing. For example in page 28 student write that the highest total cell counts were found in both soils, but in the table 8 there is only one sample, moreover, according to the text, the highest total cell counts were found in the sample S-500 but according to the table, the highest total cell count was in the sample S-250. I have also several suggestions and question, see below.

Discussion has three pages and thirteen citation included. The extent of the text and number of citations seems to me adequate. Student goes through his results fluently, discusses them with similar published results and gives the reader possible explanations of his results.

I have no remarks except one – methylated metabolites found using MS analysis were mentioned for the first time here, in discussion and then in conclusion. If they are important, I would recommend mentioning them earlier, in the results chapter, and attach the corresponding MS spectra.

References cover seven pages and at the first sight they look comprehensively. Nevertheless, they are not uniform. For example, in many cases, the numbers of the pages are missing (Ainsworth et al., 2006, Alexandre at al., 2001, Amann et al., 1990, Baath et al., 2001, and so on), in Biel-Maeso et al., (2018), the month of the acceptance of the paper is present. For the future work I recommend using some reference manager program like EndNote or the freely available Mendeley.

Suggestions and questions, to which the student has to answer during the defense.

Mistakes, which the students should avoid in the future:

Answer please questions in bold.

The title of thesis is general – the effect of selected pharmaceuticals on growth, biomass and activity of soil microorganism even though only irbesartan and its effect on soil microorganisms was studied in thesis. A more specific title would be better.

Q1: p.14 what does it mean that soils were sampled destructively?

In Material and methods, chemical composition of majority of buffers is described in tables, the composition of maleic acid buffer (page 20) is described in brackets in the text – better would be to unify the way of writing.

Figures 4 and 5 with stained cells from microscope analysis are placed in Material and methods chapter. I suggest moving them into the Results chapter. In both figures, the scale is missing, so the reader isn't able to estimate the size of the cells, moreover, such type of the figure is inappropriate for publication purpose.

I miss also controls for the CARD-FISH staining. **Q2: Why is it important to have controls in**

microscopy techniques and what control would you prepare for microscopic analysis of cells prepared using the CARD-FISH technique? What type of control was used in publication Eickhorst 2008b?

p.19 the citation should be Eickhorst 2008b

Q3: p.20 Why the enumeration of abundance of different groups of bacteria was not repeated?

Table 8 – The student used one dilution factor for one speed. This does not seem as finding the optimal conditions. **Q4: Why you did not try various dilution factors for the same speeds (which would be the optimization)?** I also think that it is not possible to do the average from the results obtained under various conditions as you did in this table.

Q5: Chapter 5.1.2. “Based on the statistical analysis, the TCC were found influenced by the time of incubation” – I didn’t find which data and analysis was used – could you explain?

Table 9 – In two cases, the number of living bacteria is higher than total cell count – control, time 0, 3rd sample; and control, time 14, 2nd sample. **Q5: Could you explain how it is possible?**

Figure 7. **Q6: Could you please explain figure 7 with the conclusions? What does it mean when the reliability of regression is as low as yours?**

Chapter 5.4 - there should be a mention about Methylated metabolites found using MS analysis. **Q7: Could you provide MS spectra showing these metabolites?**

Conclusion:

In conclusion, I

recommend / ~~do not recommend~~*

the thesis for the defense and I suggest the grade 2 .²

In Ceske Budejovice date 23.7.2020

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signature

² You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).