



LETTER TO THE EDITOR

**Commentary on the article entitled “Investigation of the microbial diversity of an extremely acidic, metal-rich water body (Lake Robule, Bor, Serbia)” by Srđan Stanković, Ivana Morić, Aleksandar Pavić, Branka Vasiljević, D. Barrie Johnson and Vladica Cvetković, published in the *Journal of the Serbian Chemical Society*, Volume 79, Issue 6, Pages: 729–741 (available online 27 June 2013)**

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This letter and comments within refer to the article by Srđan Stanković, Ivana Morić, Aleksandar Pavić, Branka Vasiljević, D. Barrie Johnson and Vladica Cvetković, “Investigation of the microbial diversity of an extremely acidic, metal-rich water body (Lake Robule, Bor, Serbia)” (hereinafter: **Paper**) published in the *Journal of the Serbian Chemical Society*, 2014, Volume 79, Issue 6, Pages: 729–741.

This communication highlights only the most relevant comments, while the Addendum part (given as Supplementary material to this letter) comprises all the essential and technical issues and also shortcomings of the review of this **Paper**, listed one by one, issues that could attract the attention of colleagues dealing with biogeochemical cycles, especially in extreme environments.

The results of an investigation of the microbial diversity in the said habitat are published in the **Paper** for which the authors used culture-dependant and culture-independent (T-RFLP) methods. T-RFLP analysis revealed that the dominant bacteria in the lake water samples were the obligate heterotroph *Acidiphilium cryptum* ( $\approx 50\%$  of the total bacteria) and the iron-oxidizing autotroph *Leptospirillum ferrooxidans* ( $\approx 40\%$ ).

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The authors of the **Paper** compared their results with those of another paper: V. P. Beškoski, P. Papić, V. Dragišić, V. Matić, M. M. Vrvić, *Long term studies of the impact of thionic bacteria on the global pollution of waters with toxic ions*, published in *Advanced Materials Research, Proceedings of the 18<sup>th</sup> International Biohydrometallurgy Symposium 2009*, Bariloche-Argentina, 105 (2009) p. 71.<sup>1</sup> In this study, on selective media, sulphur- and iron-oxidizing microorganisms were monitored (primarily *Acidithiobacillus ferrooxidans*), which produce sulphuric acid and  $\text{Fe}^{3+}$  and it is these which, as potent oxidants in an acid environment, contribute to the release of heavy metals into the environment. However, there is not a single reference to microbial communities or biodiversity in the cited paper, since these were not the focus of that investigation; rather, the cited study presents the results of multi-year monitoring of the impacts of thionic bacteria on water polluted by toxic metals.

The claim that the authors of the **Paper** grew *At. ferrooxidans* on solid medium is inexplicably contrasted with their inability to identify it by the use of T-RFLP method (without any reference to statistical data). This leads to the only possible conclusion, that some methodological and/or conceptual errors may have been made in this **Paper**, both during the sampling procedure and the analysis of the results, and which would normally be addressed during the scientific review process. Such misunderstandings can be seen in numerous sections of the **Paper**. The result that the obligate heterotroph *A. cryptum* comprised approximately 50 % of the total bacterial population in a habitat where the concentration of dissolved organic substance is low is particularly dubious. The result that *L. ferrooxidans* makes up about 40 % of the total bacterial population of the lake water also seems highly unlikely to be correct.<sup>2</sup> If the native preparation of sampled water was examined microscopically by the authors of the **Paper**, it would be clear to them that this bacterial species was sporadic.<sup>2,3</sup>

It is inappropriate for a reputable journal to publish, and for the reviewers to allow the publication of, statements which are notoriously false, and which is found in the 3rd paragraph of page 6: "Redox potential of the lake water was measured by calomel electrode (personal correspondence with author)." Any competent chemistry technician is aware that redox potential cannot be measured by using only a reference electrode.

Additional confusion is contributed by the fact that the **Paper** draws conclusions based on speculation. For example, the authors extrapolated meaning outside the framework of the data presented (in the context of timeframe), and made an ill-founded comparison to data in other studies (they compared their estimates of bacterial percentages in water sample(s) to bacterial most probable number (MPN) counts).

Among the flaws in the **Paper**, we highlight the analysis of water samples (probably more than 50 mL should have been taken), and that this analysis

should have been more complete and conducted at multiple times (the current analysis, as published in the **Paper**, appears to be lacking basic chemical data and is described as being “point-like”, since water samples were taken only once). Scientific doubts about the obligate heterotrophy of the habitat would, in this case, have been removed. Furthermore, talking about the presence of organic substance in the lake based only on the determined microorganisms and without conducting any basic analysis, such as determination of the chemical oxygen demand (*COD*), biological oxygen demand (*BOD*) and total organic carbon (*TOC*), should be unacceptable.

Readers are left with the impression that the speed with which this **Paper** was reviewed and accepted contributed to such flaws.

The main aim of publishing scientific work in peer-reviewed journals is to ensure quality standards are maintained in science. Good peer-review also serves to improve studies in the natural sciences, and must necessarily encompass these points: 1) Does the work express a clear aim which has been achieved?; 2) Are the methods and materials used suitable for purpose?; 3) Is the work able to be repeated by other experts in the field, and is enough detail given in the Experimental to allow this without hindrance?; 4) Have quantifiable results been collected impartially in a suitable manner?; 5) Are data analysis methods correctly applied to all the data?; 6) Is the data analysis valid and are the datasets of an appropriate size?; 7) Does the work fairly and correctly represent and discuss the relevant work of other experts in the field, and other findings?; 8) Does the work compare the results obtained with those of other studies in a suitable manner?; 9) Are the conclusions based on the results presented and the discussion?; 10) Are there any novel findings in the work, or does the work contribute to the body of knowledge? The review of this **Paper** seems to be lacking in several of these fundamental areas.

The authors believe that the publication of these well-meaning comments will be of benefit not only to the authors of the **Paper**, but also to all who work or intend to work in this complex and difficult multidisciplinary scientific field, which requires a substantial understanding of all aspects of biogeochemical cycles.

#### SUPPLEMENTARY MATERIAL

*Adendum* to this Commentary is available electronically from <http://www.shd.org.rs/JSCS/> or from the corresponding author on request.

## ИЗВОД

КОМЕНТАРИ НА РАД ПОД НАСЛОВОМ „ИСТРАЖИВАЊЕ МИКРОБИОЛОШКОГ ДИВЕРЗИТЕТА ЕКСТРЕМНО КИСЕЛЕ ВЕШТАЧКЕ АКУМУЛАЦИЈЕ ВОДЕ СА ВИСОКИМ САДРЖАЈЕМ МЕТАЛА (ЈЕЗЕРО РОБУЛЕ, БОР, РЕПУБЛИКА СРБИЈА), АУТОРА СРЂАН СТАНКОВИЋ, ИВАНА МОРИЋ, АЛЕКСАНДАР ПАВИЋ, БРАНКА ВАСИЉЕВИЋ, D. BARRIE JOHNSON И ВЛАДИЦА ЦВЕТКОВИЋ, ОБЈАВЉЕНОГ У *JOURNAL OF THE SERBIAN CHEMICAL SOCIETY*, VOLUME 79, ISSUE 6, PAGES: 729–741 (ЕЛЕКТРОНСКИ ОБЈАВЉЕНОГ 27. ЈУНА 2013)

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Ова кратка комуникација са припадајућим коментарима се односи на рад објављен у *Journal of the Serbian Chemical Society*, а који је био објављен електронски 27. јуна 2013 године, под насловом „Истраживање микробиолошког диверзитета екстремно киселе вештачке акумулације воде са високим садржајем метала (језеро Робуле, Бор, Србија) чији су аутори Срђан Станковић, Ивана Морић, Александар Павић, Бранка Васиљевић, D. Barrie Johnson и Владица Цветковић, doi: 10.2298/JSC130605071S. Комуникација и припадајући додаток истичу најрелевантније коментаре, суштинска и техничка питања, а такође и недостатке процеса рецензије овог рада, питања која би могла привући пажњу колега који се баве биогеохемијским циклусима, посебно у екстремним условима. Аутори ове комуникације верују да ће објављивање ових добронамерних коментара бити од користи не само ауторима предметног рада, већ и свима који се баве или намеравају да раде у овој комплексној и тешкој мултидисциплинарној научној области, која изискује суштинско разумевање свих аспеката биогеохемијских циклуса.

(Примљено 28. децембра 2013, прихваћено 17. октобра 2014)

## REFERENCES

1. V. P. Beškoski, P. Papić, V. Dragišić, V. Matić, M. M. Vrvic, in *Proceedings of the 18<sup>th</sup> International Biohydrometallurgy Symposium 2009*, Bariloche, Argentina, 2009, *Adv. Mater. Res.* **105** (2009) 71
2. M. M. Vrvic, *PhD Thesis*, Faculty of Chemistry, University of Belgrade, Belgrade, 1991, pp. 319–360 (in Serbian)
3. M. M. Vrvic, in *Proceedings of UNEP International Seminar on Dump and Underground Bacterial Leaching of Metals from Ores*, G. I. Karavaiko, G. Rossi, Z. A. Avakyan, Eds., Centre for International Projects, USSR State Committee for Environment Protection, Moscow, 1990, pp. 59–73.