

2001-F201-3

Review of “Use of microbial indicators for selenium hazard assessment and for management of real-time electrical conductivity and dissolved oxygen sensor biofouling” submitted to CALFED by Prof. Terrance Leighton et al.

1. Scientific merit

a) Objectives

The proposal has clearly-stated objectives that address Se and water quality issues in the San Joaquin River basin: 1) to develop a microbial-ecology technique to assess site-specific selenium hazards and 2) to investigate biofouling of water quality sensors and determine how best to solve this problem so electrical conductivity and dissolved oxygen measurements may be made accurately and continuously.

b) Approach

The first objective will be addressed by characterizing cultured and uncultured microbes in Mud Slough and the San Joaquin River and determining Se transfer from microbes to crayfish and mosquito fish. BIOLOG plates and limited 16S rDNA sequencing will be used for microbial identification. X-ray absorption spectroscopy will be used to determine how cultured microbes transform Se. BIOLOG profiles will be used to determine how microbial populations respond to changes in drainage parameters such as Se concentration and salinity, which will vary with seasons. Selenium transfer up the food chain will be assessed by feeding Se-containing microbes and pure Se compounds to crayfish and mosquito fish.

The second objective will be addressed by examining the physical and microbial characteristics of biofilms in the San Luis Drain and the San Joaquin River; by growing biofilms under laboratory conditions that will then be characterized; and developing management strategies to limit biofilm growth.

Comment 1:

There are significant problems associated with the primary approach that addresses Objective 1, i.e., the use of BIOLOG and BIOLOG community-level physiological profiles (CLPP) to provide information on the identities, functions and variations in microbial populations as a function of drainage operating parameters. In fact, molecular methods, such as denaturing gradient gel electrophoresis (DGGE), are much more useful (van Elsas et al., 1998, J. Microbiol. Methods 32: 133-154).

In addition to some of the methodological problems with BIOLOG methods (e.g., inoculum levels which affect the results) I have listed some important reasons why this method is inappropriate for the proposed research with San Joaquin River Basin samples.

- 1) BIOLOG is only useful for looking at *aerobic heterotrophic microbes* (Garland and Mills, 1991, Appl. Environ. Microbiol. 57: 2351-2359), and will exclude the contributions of all other microbes (e.g., autotrophic or anaerobic microbes) that may be involved in Se transformations.
- 2) If the investigators only use Gram negative BIOLOG plates for their CLPP analysis (page 3) they will miss organisms like their selenite-reducing *Bacillus subtilis* strain (page 9), which is Gram positive.
- 3) A large proportion of microbes in the environment are dormant and will very likely not respond to BIOLOG fingerprints. Although the investigators have used this method successfully in a carbon-rich algal-bacterial bioreactor it is highly unlikely that BIOLOG will provide meaningful data under the very different environmental conditions found in the San Joaquin River Basin.

Comment 2:

Regarding the isolation and identification of microbes for Objectives 1 and 2, there is a very low probability of isolating environmentally relevant organisms that may be used to develop realistic Se concentration objectives for drainage water. This is because approximately 1% of existing environmental microbes can be cultured (Amann et al., 1995. Microbiological Reviews 59:143). 16S rDNA sequencing is the state of the art method for microbial identification and is perfectly adequate to identify microbial populations and isolates. Rather than it being used to verify the results of the BIOLOG identifications (as they state), it would have been better to use this as the primary method of identification and characterization, and use BIOLOG to corroborate the results from the 16S rDNA sequencing.

Comment 3:

The x-ray absorption spectroscopy analysis will be very useful to determine selenate transformations in cultured bacteria. However, it is not clear how the information from this work will be used to formulate seasonal site-specific selenium objectives for the field sites.

Comment 4:

The results of the food chain transfer experiments will depend on the types of microbes that are cultured (see Comment 2). If Se-accumulating microbes that are not dominant in the water are cultured in the lab (a very likely scenario), and then used in the food transfer experiments, the results will be irrelevant. The proposed research will be carried out on crayfish and mosquito fish (page 4) so **direct** effects on other species will not be obtained (Attachment H claims that the proposal primarily addresses various salmon, trout, and birds).

Comment 5:

Regarding the biofilm experiments, the same comments that are stated in Comments 1 and 2 apply. For example, the biofilms that exist in the San Luis Drain and the San Joaquin River may

consist of microbes that cannot be cultured in the laboratory. Thus, achieving the other parts of this study (growing and studying biofilms with culturable microbes and algae) may not be relevant.

c) Adaptive management approach:

The proposal does not adequately link the results from the experimental work to ecosystem restoration in the San Joaquin River Basin.

2. Adequacy of monitoring, information assessment, and reporting plans:

The BIOLOG profiles will be performed as a function of season. Although the method of monitoring is definitely not appropriate (see above), the number of monitoring points (page 9 of the proposal) and data handling would be adequate if better methods were used.

3. Technical Feasibility:

The approach is feasible but not appropriate to the proposed work or objectives (see above).

4. Qualifications:

The project team has the necessary training and experience to carry out the proposed research, and the institutions involved have adequate facilities to perform the work.

Overall Evaluation: Fair

Summary statement:

The proposed research contains some interesting ideas relevant to the overall goals of the program. However, a very serious concern arises from the fact that the proposal lacks an adequate method to evaluate microbial community profiles in environmental samples from the San Joaquin River Basin. This is an essential component because microbial community profiles and microbial isolation provide the basis for both of the stated objectives. In addition, the food chain Se transfer experiments could well be performed with environmentally-irrelevant microbes because only 1% of microbes can be cultured in the laboratory. There is also an inadequate link between the expected results and the water quality objectives for the San Joaquin River Basin. Most importantly, given the lack of methodology appropriate to the specific environment under study, it is unlikely that the research will produce results that will be relevant to the water quality and restoration goals set by CALFED ERP and CVPIA.