## Iron springs in Cannon River Wilderness Area

Multiple iron-rich springs can be found in the Cannon River Wilderness Area (CRWA). Right at the emergence, the spring waters are anoxic and contain dissolved ferrous iron (Fe<sup>2+</sup>). As the flowing springs are exposed to the atmosphere, ferrous iron reacts with oxygen to form orange iron oxide minerals (such as Fe(OH)<sub>3</sub>) that give the springs their characteristic rusty color.

Iron oxidation occurs by the following reaction:

$$Fe^{2+} + \frac{1}{4}O_2 + \frac{5}{2}H_2O \rightarrow Fe(OH)_3(s) + 2H^+$$

Certain microorganisms can live off the energy in this reaction, and thus the CRWA springs contain rich microbial life.

Photos and comments by Daniel Jones, Ph.D. (March 7<sup>th</sup>, 2015) U. of Minnesota BioTechnology Inst. and Dept. of Earth Sciences All images from the CRWA unless otherwise noted



Carleton College students examine one of the springs with Dr. Bereket Haileab

## Two other CRWA iron springs





Photo by B. Haileab

The springs are anoxic at the emergence, and iron oxides form as oxygen mixes in from the atmosphere. This can often be observed by a color change. For example, see how the sediments change from gray to orange in the photo below.



Arrows indicate fluffy iron oxide minerals produced by iron oxidizing bacteria (see microscope images that follow) A close up of the fluffy orange material from the CRWA springs. The fluffy material is largely composed of iron oxide sheaths and stalk-like structures created by iron oxidizing bacteria (such as *Leptothrix* spp. and *Gallionella* spp.)

Brightfield image, 100x magnification

Close up of some *Leptothrix*-like sheaths (tubular iron oxides), and a helical *Gallionella*-like stalk (arrow). These iron oxide structures are produced by the bacteria as a way to get rid of the iron oxide "waste product" of their metabolism.



Brightfield image, 1000x magnification

Iron oxide sheaths and helical stalks from the CRWA springs. (1000x magnification, black and white DIC image, z-stacked.)



Microbial sheaths and helical stalks from the CRWA springs. (1000x magnification, black and white DIC image, z-stacked.)



Microbial sheaths and helical stalks from the CRWA springs. (1000x magnification, black and white DIC image, z-stacked.)





x2.0k 30 um





Fluorescent microscopic analysis showing live *Leptothrix* cells (upper right image) in iron-oxide sheaths. Only a small fraction of the iron oxide sheaths at any location are occupied (sheaths are visible in lower left image)—the empty sheaths have been 'abandoned' by the bacteria. Both images are from the same field of view, and arrows correspond to the same locations in the two images







Fluorescence in situ hybridization (FISH)

Images from iron oxidizing bacterial mats in St. Mary's Spring, Minneapolis. D. Jones, unpublished.



Another set of photomicrographs of a *Leptothrix*-dominated mat. Both images are from the same field of view, and arrows correspond to the same locations in the two images. GAMBET

EUBMIX

DAPI

DIC (z-stacked)



Images from iron oxidizing bacterial mats in St. Mary's Spring, Minneapolis. D. Jones, unpublished.

10 uM

FISH

Different views of the previous slide. Right: iron-oxide sheaths are visible in reflected light, microbial cells are labeled with a fluorescent DNA stain. DAPI

DIC (z-stacked)



Reflected light + DAPI

10 uM

Images from iron oxidizing bacterial mats in St. Mary's Spring, Minneapolis. D. Jones, unpublished. The CRWA springs also contain photosynthetic organisms including diatoms, other algae, and cyanobacteria. Algal growth can be seen by green colors in the spring, such as the lime green material at the right (far green arrow). Air bubbles in the spring sediments (thin green arrows) are produced by photosynthetic organisms

Fluffy iron oxides produced by iron oxidizing bacteria. Leatherman tool for scale, left.

## Photosynthetic organisms in the CRWA springs include Euglenid algae (below) and diatoms (next slide)

















