Response to Comment on "Fluorescence Inner-Filtering Correction for Determining the Humification Index of Dissolved Organic Matter"†

I thank Zsolnay (*1*) for his comments regarding my paper (*2*) and will address his concerns. Regarding his concern for working with only three samples, I used a limited number of materials because my focus was on methodology refinement rather than the application of the method itself. As noted in the paper, the three materials that I utilized represented organic matter in the early, mid, and late stages of the humification continuum. While the specific chemical properties of these three samples may differ from those of other materials, I believe the general conclusions regarding changes during humification are valid.

Zsolnay also questions the relevancy of a base-extracted sample due to such fractions not existing in situ. Base-extracted humic substances are generally accepted to represent the highly humified fraction of natural organic matter. Zsolnay et al. (*3*) evaluated the ability of the fluorescence-based humification index to differentiate between dissolved organic matter (DOM) and mature soil organic matter using an aqueous (4 mmol L^{-1} $CaCl_2$) extract and a NaOH−pyrophosphate-extracted fulvic acid, respectively. His extractants were chemically similar to the deionized water and NaOH extract used in my study to isolate the water-soluble fraction of organic matter and the more humified material. Clearly, as Zsolnay and I have demonstrated in our papers, the fluorescence-based humification index has the ability to quantify the extent of humification from materials that range from the precursors of organic matter to the highly humifed fulvic acids.

I disagree with his assessment that making a dilution series for samples is not practical. With modern pipetting equipment, the ability to make a series of dilutions takes little more effort than making one dilution to a fixed absorbance value. Indeed, the point that I wished to make with this paper was that, with a standard diode-array UV−vis spectrophotometer and a PC spreadsheet program, a researcher can easily calculate a concentration-invariant humification index. The use of the exact correction requires only one additional experimental step, the determination of the UV−vis absorption spectra. The calculations to correct the fluorescence intensity for absorption can be easily performed on a PC.

Zsolnay also points out that I did not discuss physicochemical interactions that can affect the humification index value and that crop residue-derived DOM may be particularly susceptible. Any interaction that alters the absorptivity at a given wavelength will alter the index value. Unwanted artifacts introduced by physicochemical interactions such as light scattering are a concern with the use of UV−vis or fluorescence spectroscopy. Previous work done with a colleague demonstrated that light scattering of corn-derived DOM was not significant at pH values above 5 (*4*). It is interesting to note that this study (*4*) showed that light scattering by DOM isolated from a forest soil was greater than that of the corn-derived organic matter at the native soil pH of the soil. This suggests that care needs to be taken to assess the role of light scattering by DOM in determining the humification index. I thank Zsolnay for his histogram that shows that both indicies produce similar distribution of calculated values.

In conclusion, while I agree that the corrections are usually less than 10% if absorbance at 254 nm is less than 0.1 unit, I disagree with his final conclusion that this method refinement makes DOM research more complicated than necessary. I am in total agreement that DOM is a complex fraction to study, characterize, and understand (*5*). It is this complexity that makes DOM research so interesting. While the exact correction of fluorescence inner-filtering will not single-handedly increase our understanding of DOM characteristics, it may make a small contribution to lessening the uncertainty surrounding DOM chemical characterization. It is through these small steps that the generalized structure and function of DOM in terrestrial ecosystems becomes clearer.

Literature Cited

(1) Zsolnay, A. *Environ. Sci. Technol.* **2002**, *36*, 4195.
(2) Ohno, T. *Environ. Sci. Technol.* **2002**, *36*, 742.
(3) Zsolnay, A.; Baigar, E.; Jimenez, M.; Steinweg, B.; Saccomandi, F. *Chemosphere* **1999**, *38*, 45−50.
(4) Ohno, T.; Cronan, C. S. *Int. J. Environ. Anal. Chem*. **1997**, *66*, 119−136.
(5) Zsolnay, A. www.esw.org/generic/163/0020L.pdf; 2001.

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