



## Exclusive partitioning of intra- and extra-cellular cyanotoxins: limitation of the conventional procedure

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Reports show that the presence of cyanotoxins in drinking water supply lakes and reservoirs causes serious public health impact (Hawkins et al. 1985; Falconer et al. 1983; Carmichael et al. 2001; Jochimsen et al. 1998). Reliable public health risk assessment associated with cyanotoxins requires accurate and exclusive determination of both the cell-bound toxins (intra-cellular) and those dissolved in water (extra-cellular) fractions. A technically sound procedure for exclusive determination of intra- and extra-cellular cyanotoxins is a key step also for any scientific study related to the dynamics of synthesis and release of cyanotoxins. This important issue, however, seems to be given little attention in many of the reported studies. The conventional procedure currently available for partitioning the intra- and extra-cellular toxins has inherent limitation particularly from field samples collected from highly turbid water bodies. The conventional procedure for separation of the two fractions currently in use involves the filtration of the samples onto 0.7 µm GF/C filter paper (Lawton et al. 1994; Merel et al. 2013; Park et al. 1998; Zheng et al. 2004). In this procedure, it is assumed that the cell-bound toxins (intra-cellular) are retained in the filter paper and the soluble cyanotoxins (extra-cellular toxin) are recovered in the filtrate. Reports show that cyanotoxins have high tendency of binding to particulate matter (Wu et al. 2012). This implies that the extra-cellular toxins bound to particulate matter above particle size of 0.7 µm including those bound to cyanobacterial cell surfaces are retained on the GF/C not recovered in the filtrate. Tilahun et al. (2019) also noted that the procedure might

overestimate the intra-cellular toxins and underestimate the extra-cellular fraction. One of the notions regarding cyanotoxins repeatedly indicated in the literature is that cyanotoxins normally remain intra-cellular unless released during cell lysis and decay (Park et al. 1998; Tsuji et al. 1994). This idea does not seem to survive the scientific scrutiny given the inherent problem of the conventional procedure for separation of the two fractions. The view that cyanotoxins normally remain intra-cellular might have resulted from a faulty procedure that caused infrequent detection of the extra-cellular toxins in the filtrate. This procedural limitation may lead to inaccurate public health risk assessment especially in thermally stratified lakes and in absence of visual cue of harmful algal bloom. This is particularly true as particulate-bound toxins may also serve as source of the toxins to the water column (Wörmer et al. 2011; Wu et al. 2012) later during mixing. Future climate scenario not only promotes the dominance of potentially toxic cyanobacteria (Paerl and Huisman 2009; Paerl and Paul 2012) but also may influence their community structure (Tilahun and Kifle 2019) which may challenge protection of public health through restoration efforts. In such situation, reliable public health risk assessment and establishing an early warning system could be one possible option which demands accurate determination of the two fractions. Furthermore, lack of accurate and exclusive partitioning procedure possibly partly contributed to the challenges for studies on the dynamics of the two fractions in relation to the environmental factors and for lack of conclusive evidence for the suggested eco-physiological roles of cyanotoxins. For instance, one of the suggested roles of cyanotoxins is their allelopathic effect against other organisms that compete for resources or against grazers that feed upon them (Engström-Öst et al. 2011; Henning et al. 2001; Kurmayer and Jüttner 1999; Merel et al. 2013). If the toxins are meant to have allelopathic effect, they are expected to be released actively and purposefully to attack the target organisms. Therefore, the idea that the toxins remain cell bound and only released during cell lysis and decay is a bit puzzling

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given the allelopathic role of the toxin. Environmental scientists and policy makers should take note of this procedural limitation before making any decisions and/or scientific conclusion. Future study should, therefore, address this procedural limitation through improving the methodology, as it is a key scientific issue for reliable public health risk assessment and understanding factors dictating the dynamics of the two fractions.

### Compliance with ethical standards

**Conflict of interests** The author declares that there are no conflicts of interest.

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