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Feasibility of Predicting Microcystin Poisoning on Animals via Non-Toxin Assays

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In fresh waterbodies, not only are cyanobacteria a group of photoautotrophic prokaryotes, but they are also producers of cyanotoxins posing a grave threat to animals that consume toxin-contaminated water. What's even worse, the toxins can rapidly accumulate over the level of lethal dozes when cyanobacteria proliferate explosively and eventually form a harmful algal bloom (HAB). HABs usually occur when the waterbody is eutrophic (i.e., nutrients are overrich) and weather is hot, and summer is the best season when these requirements are fulfilled. However, animals sizably increase their consumption of water in the season, and thus the risk of cyanotoxin poisoning escalates a lot especially for the wild and livestock animals that directly drink the unprocessed water from the environment [1]. As a matter of fact, pets are killed even if they just swim in and accidentally swallow the contaminated water in recreational waterbodies. Therefore, it is necessary to rapidly and accurately detect the cyanotoxins in water for timely and effective veterinary diagnostics and therapies.

Among the numerous cyanotoxin species is the most prevalent microcystin, which is a potent non-ribosomal heptapeptide hepatotoxin and inhibits protein phosphatases PP1 and PP2A. It can kill a nursery pig at a concentration as low as four ppb [2] and is the culprit for most dog deaths when they swim in contaminated water where visible algal aggregations are seen (i.e., HABs) or not. Microcystin production can be sporadically found in different intergeneric species, meaning it is not the unique product of a single cyanobacterial genus despite the fact that *Microcystis* spp. primarily account for its production. Although the multiple producers are able to make over 100 microcystins isoforms, they have in common the ten clustered synthetase genes (*mcyA-J*) to synthesize the circular seven-peptide backbone that are chemically modified to finally make different isoforms. It should be noted that the toxicity of microcystin is determined by its component 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (ADDA), so any modification does not eliminate its toxicity without dramatically changing the ADDA.

Hitherto, direct toxin detection methods have been maturely developed for microcystin. Protein phosphatase inhibition assay is established based on the inhibitive function of microcystin on PP1 and PP2A, in which the decrease of catalytic efficiency is calculated by the consumption rate of enzymatic substrates. As a result, the potency of microcystin in samples is estimated reciprocally to the decreased efficiency. In addition, mouse bioassays are used as well, in which the median lethal dose (LD_{50}) is used as an indicator for toxicity. Nonetheless, results from the two methods are not direct measurement of microcystin concentration. Nowadays, immunology and analytic chemistry are involved in measurement of microcystin with satisfactory sensitivity. The enzyme linked immunosorbent assay (ELISA) is well utilized to detect microcystin quantitatively by capturing the ADDA as an antigen, and the high-performance liquid chromatography (HPLC) and mass spectrometry (MS) are the ultimate analytical technologies with the highest precision. ELISA, HPLC, and MS have sensitivity up to 0.1 ppb which is much lower than the suggested warning level for microcystin poisoning (e.g., 1 ppb by the World Health Organization).

Because direct toxin tests may not be prompt and cost-effective, scientists attempt to find out ways for the prediction

of microcystin toxicity by correlating it to related factors. However, it seems a mission impossible thus far. Limnologists failed in seeking for a correlation between microcystin concentration and abundance of a toxic *Microcystis* sp., because the toxic strains of the species usually coexist with the non-toxic strains but their proportions are indefinitive. They also thought there could be a correlation intuitively between microcystin concentration and nitrogen level (e.g., nitrate and nitrite) as nitrogen is an indispensable element for the peptide toxin. Unfortunately, no correlation was found probably due to the intricate utilization of nitrogenous compounds. These are corroborated by my studies. In an investigation of four farm ponds serving as water supplies to nearby swine facilities in 2015, 60 water samples were collected temporally in the ponds, and microcystin concentration, *Microcystis* abundance, and level of nitrate/nitrite were measured. The Pearson's *r* linear regression regarding microcystin versus either of the rest two factors did not convey an eligible correlation ($R^2 < 0.5$). Moreover, *Microcystis* abundance was even not able to indicate the presence/absence of microcystin because it had no significant differences in toxin positive/negative samples as disclosed by a logistic regression ($p >> 0.05$).

While no correlation was discovered for microcystin with nitrate/nitrite, it might be worthwhile to introduce more nitrogen related compounds (e.g., urea) because they are likely to be the critical precursors for toxin synthesis. Additionally, some microbiologists assumed there would be a good correlation between microcystin and *mcy* genes, now that genes are fundamental for toxin synthesis. On one hand, my 2015 study demonstrated via polymerase chain reaction (PCR) that the assumption is qualitatively true by the McNemar's test ($p << 0.05$), meaning presence/absence of genes is closely associated to presence/absence of toxin. It should be noted that although this conclusion is statistically valid, not all samples conform to it, which denotes some samples are tested microcystin positive but gene negative and vice versa. Then prediction of microcystin toxicity by existence of *mcy* genes is not 100% trustworthy. On the other hand, their quantitative correlation is arguable due to the facts that scientists have made controversial conclusions that microcystin concentration is or not connected with abundance of *mcy* genes. Our investigation of the same four ponds in 2016 with 100 water samples manifested that the two factors barely had a correlation ($R^2 < 0.5$ by Pearson's *r*), in which gene abundance was estimated by real-time quantitative PCR. In conclusion, it is not reliable to assess the microcystin concentration from a molecular perspective. Some scientists tried more factors for accurate prediction of microcystin such as pigments (e.g., chlorophyll) and other nutrients (e.g., phosphate) but turned out with no successful outcomes.

Alternative methods for predicting microcystin rather than measuring it may seem cost-efficient but lose precision partly or utterly and thus have a poor feasibility. Diagnosticians can employ them (e.g., existence of *mcy* genes by PCR) as a makeshift approach for the sake of immediacy, whereas they are absolutely NOT the eventual options. Toxin testing is with no doubt the best selection, even though a few experimental factors should be taken into consideration such as the cost and instrumentation.

References

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