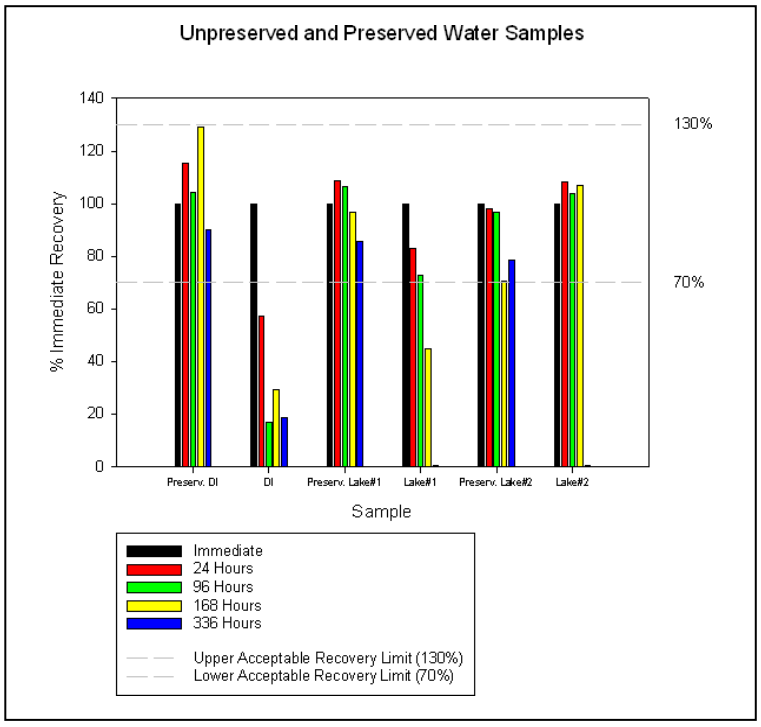
The Effects of Sample Collection and Preservation Methods on Anatoxin-a

Anatoxin-a is one of the most toxic of the cyanobacterial toxins. Because of this, accurate determinationof the presence of Anatoxin-a is critical. Due to the relatively unstable nature of the toxin itself, proper sampling, preservation, and storage techniques for water samples are vitally important to ensuring the stability of samples, and therefore the accuracy of sample results. Common issues that can produce inaccurate sample results include the following:

1. Failure to preserve samples at the time of collection

Samples must be preserved at the time of collection in order to prevent degradation of Anatoxin-a, which will cause inaccurate, biased low sample results. The addition of the appropriate preservation reagent will maintain the original toxin content present in the sample atthe time of sampling and therefore ensure accurate sample results:

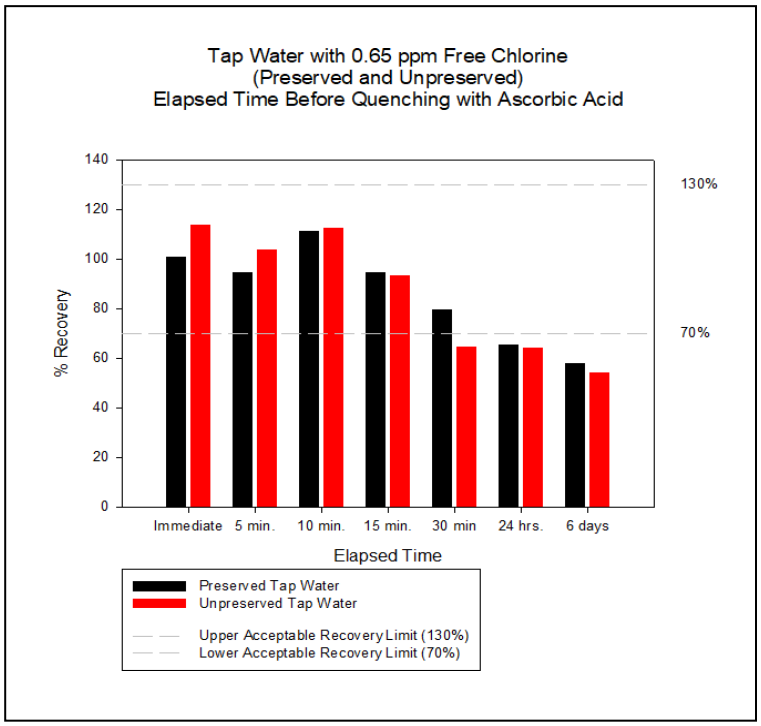


At the time of sampling, freshwater samples to be tested for Anatoxin-a must be treated with the 10X Sample Diluent Concentrate provided in the Abraxis Anatoxin-a ELISA kit (1 mL of 10X Sample Diluent Concentrate per 9 mL of water sample). Drinking water samples treated with ascorbic acid (0.1 mg/mL) and sodium bisulfate (1 mg/mL) according to EPA Method 545 do notneed to be preserved with 10X Sample Diluent Concentrate, as these reagents will effectively preserve the samples. Method 545 preserved samples will require pH adjustment to between pH 5 and pH 7 prior to ELISA analysis, however, as these reagents can decrease the pH to a level which is below the tolerance of the ELISA and may produce matrix interference. Adjustment of the pH to between 5 and 7 will eliminate potential pH induced matrix interference.

2. Failure to quench treated tap water samples at the time of collection

Contact with chlorine and other oxidizers used in the water treatment process will degrade Anatoxin-a. Because of

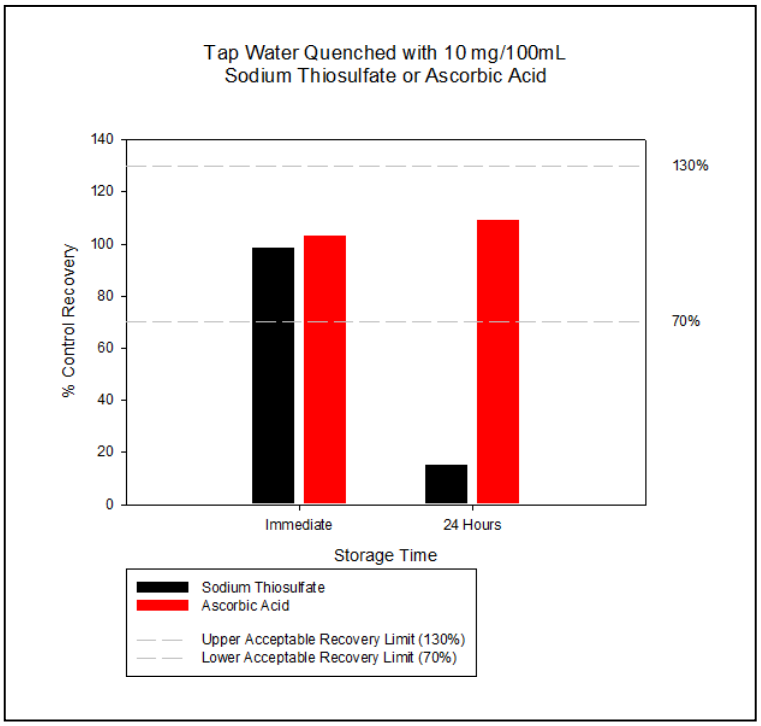
this, samples must be quenched at the time of sampling. Delays in quenching samples, even when using the appropriate sample preservation reagent(s), can result inbiased low recoveries or false negative results:



3. Use of sodium thiosulfate (instead of ascorbic acid) to quench tap water samples

Although sodium thiosulfate is one of the most commonly used reagents for quenching chlorinein water samples, it should never be used for samples to be tested for Anatoxin-a. Although it iseffective at quenching chlorine, sodium thiosulfate will degrade Anatoxin-a, producing biased low recoveries or false negative results:

Treated drinking water samples must be quenched with ascorbic acid at the time of sampling(recommended concentration of 0.1 mg/mL of ascorbic acid).



4. Failure to protect samples from light and high pH conditions

As described by Stevens et al. (1991), Anatoxin-a is light sensitive and will degrade when exposed to light. It will also undergo degradation in high pH conditions. Degradation of toxin insamples will cause inaccurate, biased low sample results. For this reason, it is necessary to use amber sample containers to protect from light and to check sample pH at the time of sampling, adjusting the pH if necessary. It is recommended that sample pH be ≤ 7 to maintain toxin stability. For testing of samples using ELISA, the recommended range for sample pH is 5 to ≤ 7,as a pH of less than 5 can produce matrix interference.

5. Not lysing samples before analysis

Algal toxins can be intracellular, meaning they are produced and contained within the algal cell, or extracellular, meaning that they are released into the surrounding water, during the life of the cell. Although some initial studies have shown the majority of toxin to be extracellular, additional research has found high levels of intracellular Anatoxin-a. For example, Heath et al. (2014) found over 85% of Anatoxin-a, Homoanatoxin-a (HTX), dihydroanatoxin-a (dhATX), anddihydrohomoanatoxin-a (dhHTX) were intracellular. The US EPA fact sheet "Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems" (September 2014) states that "Anatoxin-a and microcystin variants are found intracellularly approximately 95% of the time during the growth stage of the bloom." Testing of un-lysed water samples would provide results for free toxin (toxin that is released into the surrounding water) only. Intracellular toxin would not be detected, as it is sheltered within the algal cells. Although it is not freely circulating in the water sample and therefore is not detected during the testing of un-lysed samples, intracellular toxin poses a significant threat to those who consume the water, as stomach acid will cause the lysis of the cells, releasing the toxin. It is for this reason that samples must always be lysed before analysis, in order to provide results for total toxin concentration, which is necessary for accurate risk assessment.

References

Heath, MW, Wood, SA, Barbieri, RF, Young, RG, Ryan, KG. Effects of nitrogen and phosphorus on anatoxin-a, homoanatoxin-a, dihydroanatoxin-a and dihydrohmoanatoxin-a production by *Phormidiumautumnale*. Toxicon. 2014; 92:179-185.

Stevens, DK, Krieger, RI. Stability studies on the cyanobacterial nicotinic alkaloid anatoxin-a. Toxicon.1991;

29(2):167-179.

US EPA. Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems. 2014. EPA-810F11001. Available online at: http://www.epa.gov/sites/production/files/2014- 08/documents/cyanobacteria_factsheet.pdf.

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