Regulatory Guidelines for Microcystins in Drinking and Surface Waters require update

Ryerson U: Ching Lo, James Y. Li, Rehana Shabnam, Brian Sunga, Omar Ahmed**, Nankai U:** Jeanne Huang

International Water Association 4th Symposium, Shanghai 25May2017


Arctic
Ocean
GREENLAND
Hudson
Bay
CANADA
Toronto
USA
Pasific
Ocean
Atlantic
Ocean

Satellite image of Cyanobacterial blooms over the Great Lakes (21% of the world's fresh water)

Source: http://en.wikipedia.org/wiki/Great_Lakes

Microcystin-LR guidelines for drinking water

Microcystins has 90+ Variants

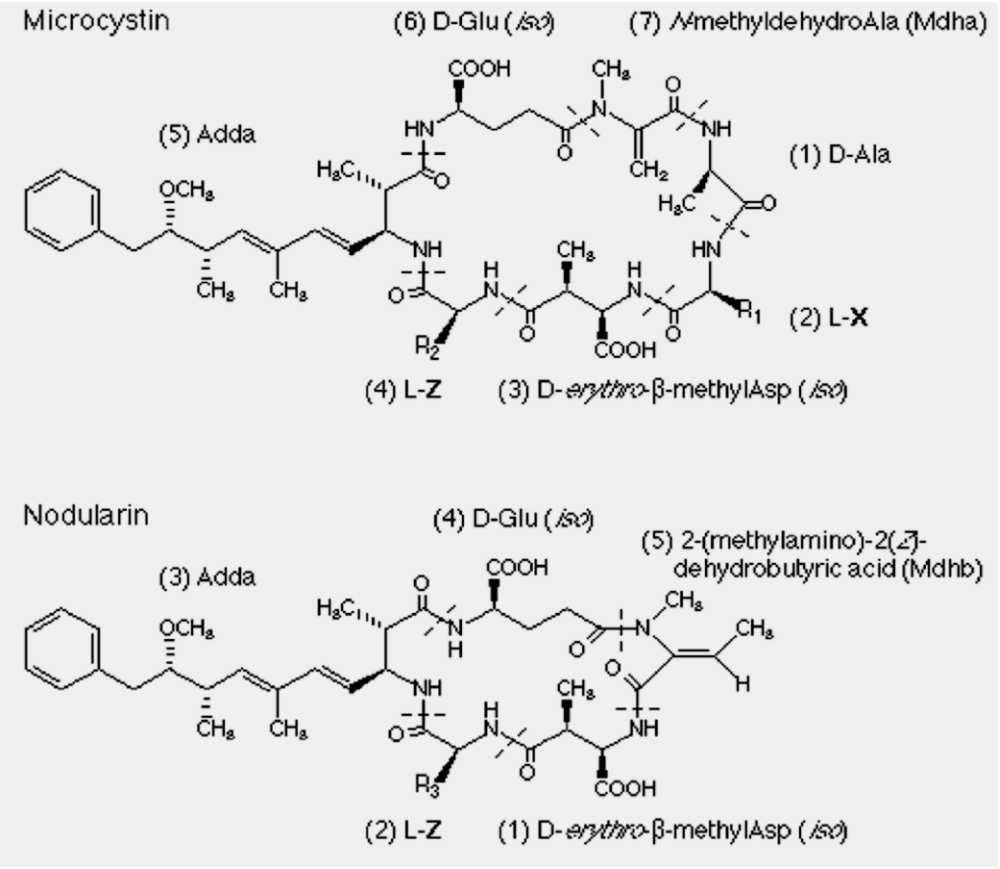
- **WHO Provisional Guideline for Microcystin-LR**

  MAC (Maximum Accepted Concentration) = 1.0 µg/L

- **Ontario MOE Provisional Guidelines for Microcystin-LR**

  MAC (Maximum Accepted Concentration) = 1.5 µg/L

Chemical Structures of Microcystin (over 90) Variants



Cyanobacterial Toxin	L-X Position	L-Z Position
Microcystin-LR	Leu	Arg
Microcystin-RR	Arg	Arg
Microcystin-YR	Tyr	Arg
Microcystin-LA	Leu	Ala
Microcystin-LW	Leu	Trp
Microcystin-LF	Leu	Phe
Nodularin	-	Arg

Toxic moiety ADDA (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) is present in > 80% of known toxin variants

Lab support Instrument & Labour Cost

$500,000

CDN 7,000

$1,000

RYERSON UNIVERSITY

Ontario

Where did we go wrong?

Environmental Registry Policy Decision

Registry Number: PA 3E0001 ***Publish Date:*** May, 12, 2003

Ministry Reference Number: 2003011501

Title: Proposal to Establish an Ontario Drinking Water Standard for Cyanobacterial Toxins (Microcystin LR)

Policy Statement: The Ministry has adopted the Canadian Drinking Water Guideline (CDWG) for cyanobacterial toxins of 0.0015mg microcystin per litre as an Ontario Drinking Water Standard (ODWS), as part of the Ontario Drinking-Water Quality Standards Regulation (O. Reg. 168/03) under the Safe Drinking Water Act, 2002.

Source: http://www.ebr.gov.on.ca/ERS-WEB-External/displaynoticecontent.do?noticeID=MTk3MTU=&statusID=MTk3MTU=&language=en

Remedies

1. Change the legislations wordings back to the original intent "…Drinking Water Guideline for cyanobacterial toxins of 0.0015 mg microcystin per litre as a Drinking Water Standard …" The reference to Microcystin-LR should be deleted. Similar changes should be applied to surface water guidelines.
2. Implement ELISA as a standalone monitoring tool for legislative support.
3. Discontinue the use of LCMS for the purpose of legislative support.

Agreement calculation between ELISA and LC-MS/MS

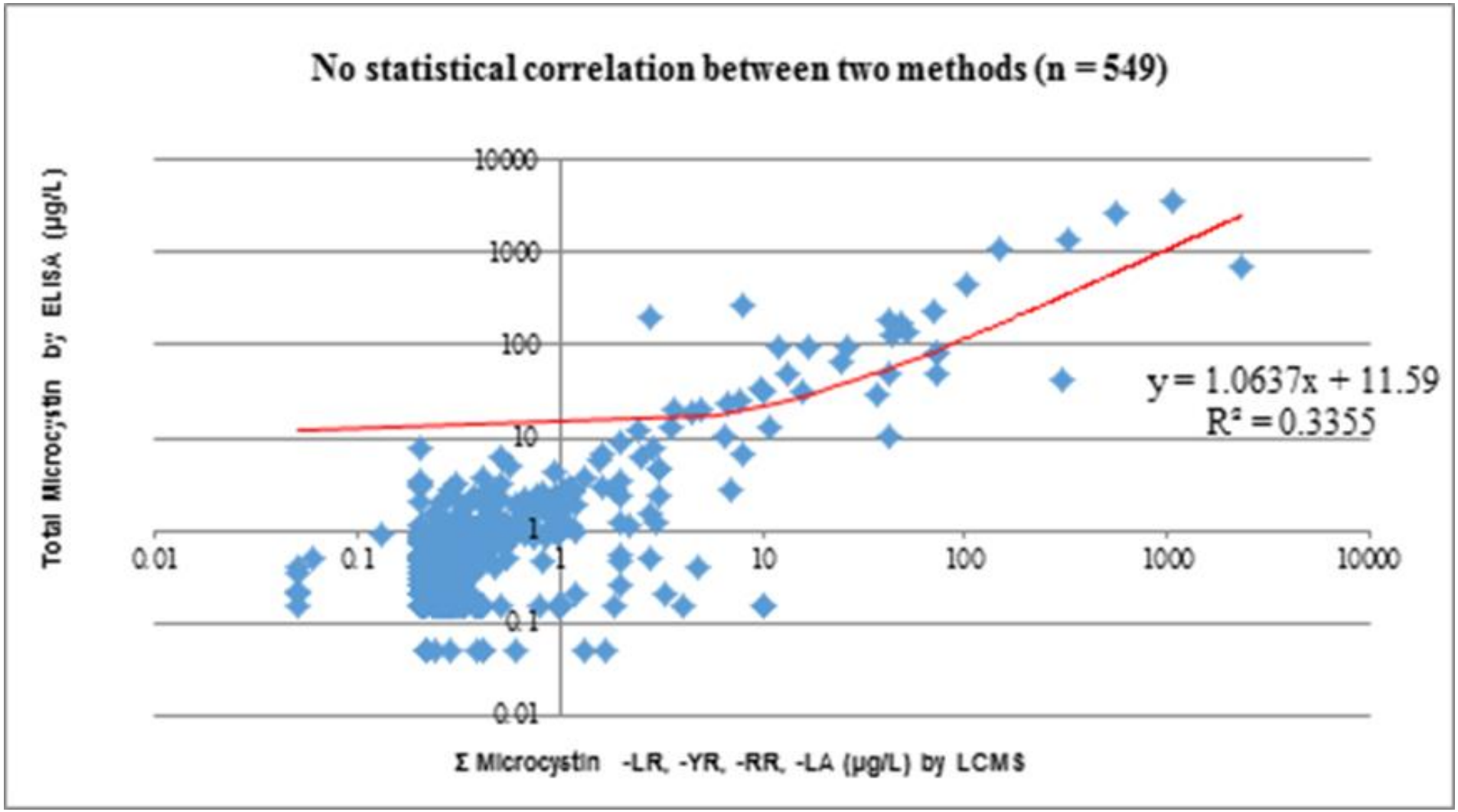
		LC-MS/MS Tested		Total
		Positive	Negative	
ELISA Tested	Positive	a	b	
	Negative	c	d	

Agreement = (a+d)/(a+b+c+d)

Qualitative agreement at the MDL between ELISA and LCMS results on surface and drinking water 2010-2012

Microcystins		LCMS MDL 0.05 ppb		Total
		Present	Absent	
ELISA Cut-off 0.15 ppb	Present	A = 312	B =226	538
	Absent	C = 11	D = 300	311
Total		323	526	849
Agreement = (A+D)/(A+B+C+D) = 72%				

Which method is correct?



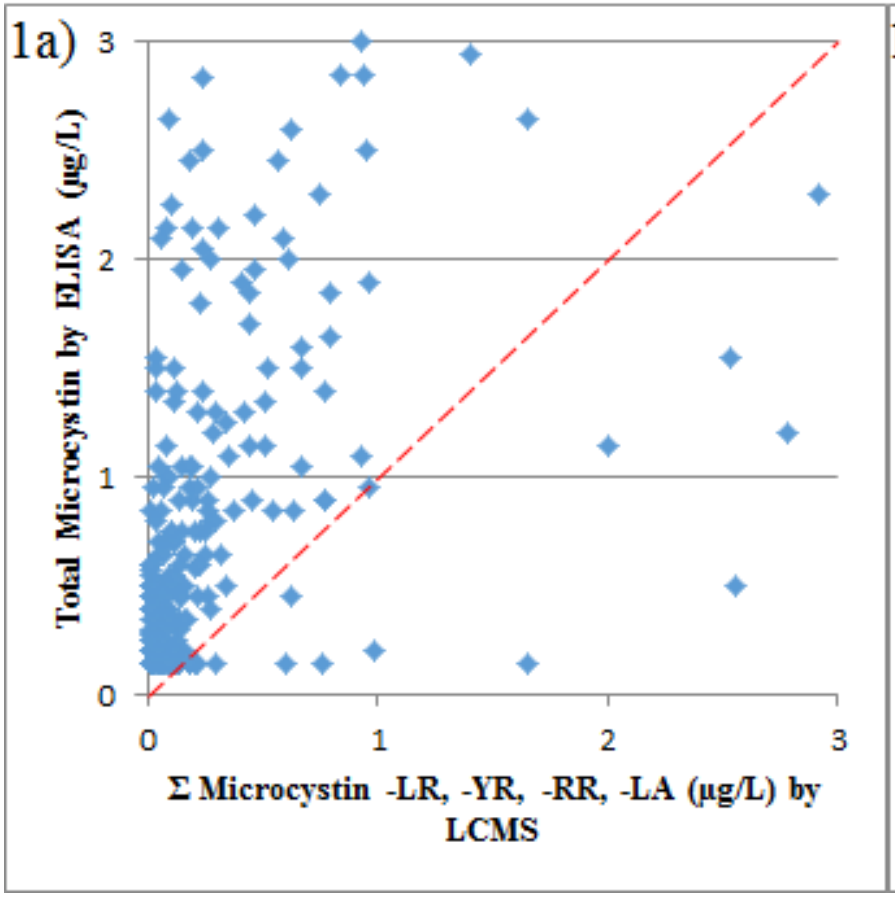
Who is right? ELISA or LC-MS/MS

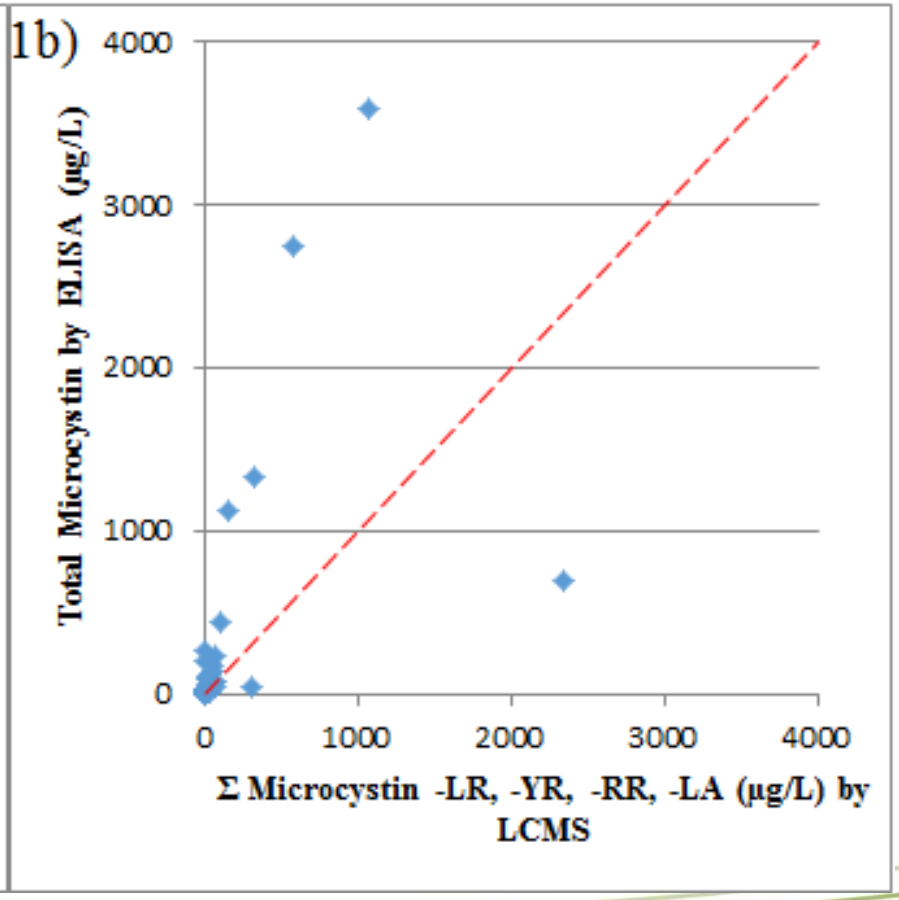
Quantitative Disagreements

Quadrant A+B+C (N= 549) the sample population were found to be in a Cauchy distribution through Tukey-lambda Probability plot correlation coefficient plot. Student's t-test has no meaning when the assumption of normality is violated. Wilcoxon signed rank test, a non-parametric test that does not assume normality, showed that there is no quantitative correlation between ELISA and LCMS at a significance level of $p < 0.0001$.

Quadrant-A alone (n=312) Wilcoxon signed rank test revealed no correlation ($p < 0.0001$)

Bland-Altman Plot using line of idealism. Microcystin concentrations ranging from a) MDL to 3.0µg/L and b) 3.01 to 4000µg/L

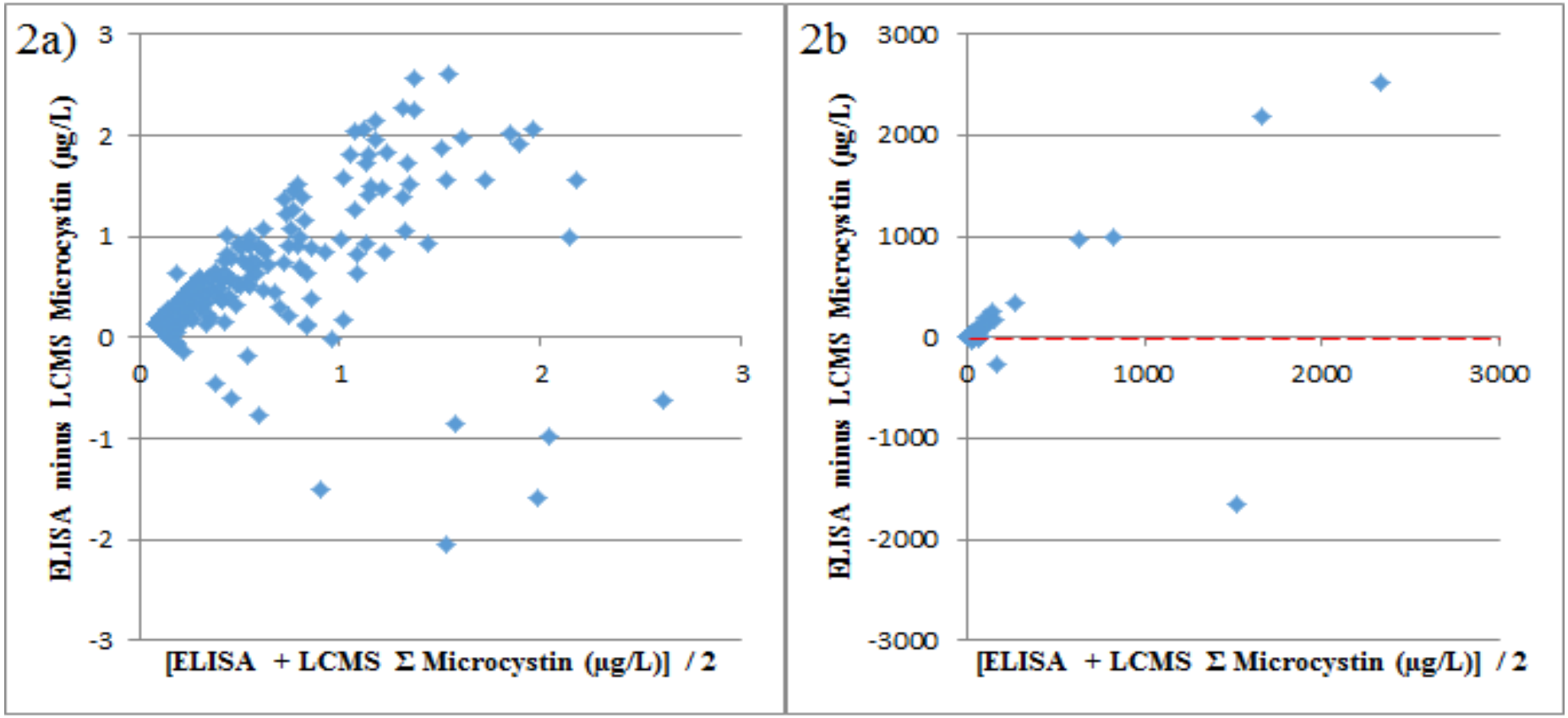




RYERSON UNIVERSITY

Ontario

Bland-Altman Plot of mean versus difference between two analytical methods of microcystins concentration



RYERSON UNIVERSITY

Ontario

Accuracy of ELISA

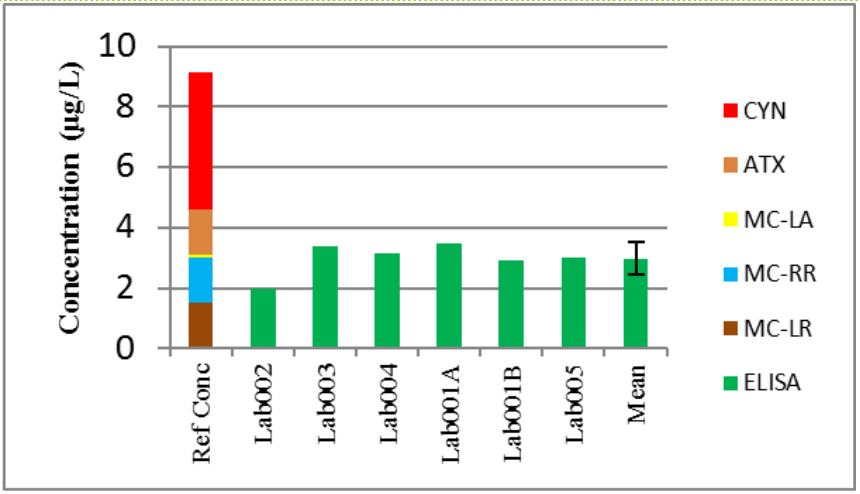
Both Bland-Altman plots unequivocally established a preponderance of higher microcystins concentration by ELISA compared to LCMS.

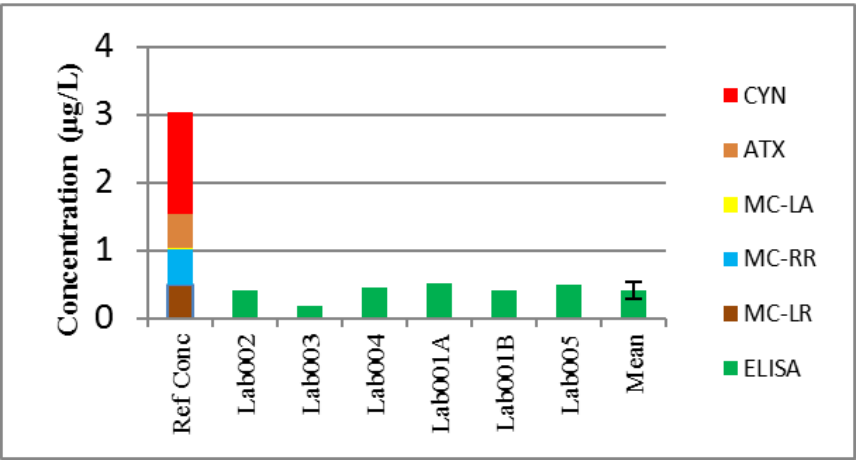
Question: Are the ELISA measurements accurately confirming our hypothesis that ELISA detect more variants or biased high?

The **accuracy** of ELISA was assessed by inter-laboratory proficiency test:

- 5 laboratories: provincial, municipal & private lab
- variety of ELISA kits from different manufacturers
- 3 variants (-LR, -RR, and -LA) singly or mixed with other toxins (anatoxin-a, cylindrospermopsin)

Inter-laboratory ELISA proficiency: reference total microcystin conc = 3.105µg/L and 1.035µg/L





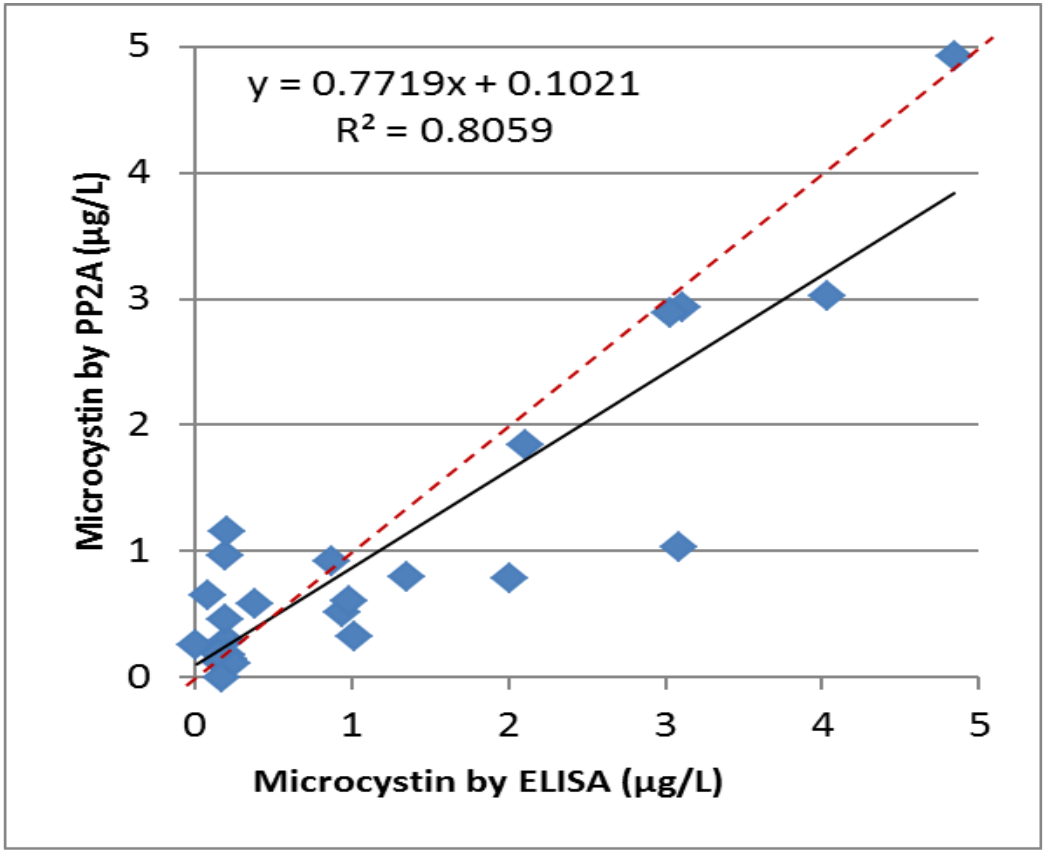
1.10	0.96	1.05	0.93	0.86	0.95

Since ELISA was found to be accurate for individual and mixture samples, the higher microcystins concentrations detected by ELISA as visualized in the Bland-Altman Plots must represent other variants that was NOT detected by LCMS

RYERSON UNIVERSITY

Ontario

Validation of no false positive ELISA by Protein Phosphatase Inhibition Assay (PPIA)



(n = 29 surface water samples)

- "ELISA-positive and LCMS-negative" samples were proven to be toxic by PP2A
- Toxicity correlated with microcystin concentration ($p < 0.001$)
- Student's t-test assumes each data set has a normal distribution, which is confirmed by probability plot correlation coefficient (PPCC) plot

Action Level

Hypothetical trigger frequencies

	Action Level at Microcystin Concentrations					
	≥1.0µg/L			≥1.5µg/L		
Quadrant A+B+C	ELISA	MC-LR	Σ MC-LR,YR, RR,LA	ELISA	MC-LR	Σ MC-LR,YR, RR,LA
n = 549	129	42	61	100	35	57

Actual trigger frequencies when Microcystins in Recreational water samples ≥ 20µg/L

Current regulations (Microcystin-LR) failed to trigger appropriate response to genuine threat in 50% (15/30) of recreational water. Within the un-triggered group, ELISA revealed microcystin at alarmingly concentrations as high as 270µg/L

RYERSON UNIVERSITY

Ontario

Matrix interference in LC-MS/MS

- Occurred in 9% (76 / 849) samples in this study.
- Matrix interference was due to the physical nature of the sample contaminated by slurry, vegetation or green algae rendering LC-MS/MS results unreliable.
- Artificially inflated MDL to cancel out the background noise.
- No matrix effect in ELISA because the particulate contaminants were removed by centrifugation whereas the soluble contaminants were removed throughout several ELISA washing steps.

Cost Comparison between ELISA and LC-MS/MS

	LC-MS/MS Cost ($)		ELISA Cost ($)	
	Line Item	**Cost per annum**	**Line Item**	**Cost per annum**
Instrument Cost	300,00/10 years	30,000	30,000/ 10 years	3,000
Maintenance & consumables		10,000		6,260
Labour Cost (annually)	2 Senior scientist (equivalent to 1 FTE/ 1 junior scientist) , 1 Technician & 1 Student	240,000	1 FTE (80% ELISA) 1 Senior Research Scientist, SRS (15% ELISA)	FTE 60,000 x 0.8 = 48,000 SRS 100,000 x 0.15 = 15,000 Total 63,000
Total cost per year		280,000		72,260
Cost Per Sample		426.18		110.00

Estimated Savings by ELISA

Based on average of 657 samples per year over 3 years 2010-2012 = 1971 samples

Total cost by LCMS alone @426.18 $ /sample = $840,000

Total cost by ELISA alone @110 $/sample = $216,810

Savings from converting to ELISA alone

= $840,000 – $216,810 = $623,190

Treated water is rarely positive and therefore a great waste to use LC-MS/MS

ELISA Empowers

- Private and local labs without LCMS capabilities considering capital investments, operation cost, labour cost for expertise
- Remote and developing countries

Why ELISA?

	ELISA (E3469)	LC-MS/MS (E3450)
Labour	1 Full Time Employee	Several
Cost per sample	$110	$426
Detection	Detects 80% variants	Limitation to 4 variants
Workload Capacity	35 Samples/plate	20-26 Samples per week
Maximum Turnaround Time	1Day	1 Week
Matrix interference	None	9% samples
Trigger Safety Action	More comprehensive	Less comprehensive
Empowerment	General	Restrictive
Client preference	1 concentration ~ total toxicity	Several variants

Recommendations

1. Change the legislations wordings back to the original intent "…Drinking Water Guideline for cyanobacterial toxins of 0.0015 mg microcystin per litre as a Drinking Water Standard …" The reference to Microcystin-LR should be deleted. Similar changes should be applied to surface water guidelines.
2. Implement ELISA as a standalone monitoring tool for legislative support.
3. Discontinue the use of LCMS for the purpose of legislative support.

Acknowledgements

- Ministry of Research & Innovation, Ideas and Innovation Fund # 07082, 08035 – Microcystin
- Natural Sciences and Engineering Research Council of Canada; Research Assistantship
- Best in Science Program; Ministry of the Environment and Climate Change
- Environmental Monitoring and Reporting Branch
- Laboratory Services Branch directors: Dr. PK Misra, Dr. Joseph Odumeru; Manager: Frank Tomassini

RYERSON UNIVERSITY

Ontario