PREVELANCE OF CYANOTOXINS IN FOOD CROPS IRRIGATED WITH CONTAMINATED WATER

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EXTENSION
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Prevalence of cyanotoxins in food crops

Overview: harmful algae blooms (HABs) that produce cyanotoxins are increasing on a global scale and occur in water bodies throughout Utah

> **Question**: Can food crops irrigated with water contaminated with cyanotoxins pose a human health risk?

Background

The prevalence of harmful algae blooms and their associated cyanotoxins in lakes near urban centers is an increasing global issue that poses an environmental and human health hazard[1]. Cyanobacteria are a diverse assemblage of aquatic bacteria that can occur in blooms large enough to produce harmful quantities of cyanotoxins. Four groups of cyanotoxins exist, cyclic peptides, alkaloids, polyketides, and amino acids. Common cyanotoxins within these groups include microcystin, anatoxin-a, and *beta*-Methylamino-L-alanine (BMAA). These compounds can be moderately to severely toxic to humans and wildlife[2] leading to both acute and chronic neurotoxic and hepatoxic (liver-damaging) effects. Shallow water, warmer temperatures, and eutrophication promote toxin production [1]. These environmental conditions are becoming more common in water bodies [3,4] and increasing in intensity around the world[5] including Utah Lake. The Jordan river, a distributary of Utah Lake, is used by farmers and residents for crop irrigation, at times contains several globally common cyanotoxin compounds[6] .

Cyanotoxins in Utah Waters

Microcystin has been measured in Utah water bodies by the Department of Environmental Quality (DEQ) as high as 770 $\mu g\ L^{-1}$ – several orders of magnitude above the World Health Organization's 1 $\mu g\ L^{-1}$ drinking water and the US EPA 8 $\mu g\ L^{-1}$ recreational water limit for cyanotoxins[7–9]. Microcystin is produced by several cyanobacteria (e.g. *Anabaena* and *Microcystis aeruginosa)* and is one of the most common cyanotoxins present in water. Anatoxin-a (neurotoxin) and cylindrospermopsin (liver toxin) are also present in many Utah lakes and reservoirs. BMAA, which is not monitored for by the DEQ, is produced by all cyanobacteria and is likely present within all water bodies that have harmful algae blooms. BMAA can increase the likelihood to develop various neuro-degenerative diseases [10,11]. Furthermore, Utah is home to the Great Salt Lake, which allows for the production of a saltwater hepatoxic cyanotoxin, Nodularin. Fortunately, the Great Salt Lake is not used for drinking or irrigation, and is rarely used for recreation. However, dust produced off of the playa has the potential to transport nodularin from the lake into the city and farmland east of the lake[12].

Routes of Exposure

The rise of cyanobacteria and their toxins in water generates concern for environmental contamination and incidental exposure to humans. Drinking water is the most understood route for humans to be subjected to cyanotoxins, however water treatment plants help mitigate this threat. Exposure through recreation is also well studied, either via skin contact or inhalation or accidental ingestion of water.

However, due to the limited contact a person has with contaminated water during recreation, the concentrations of cyanotoxins are rarely high enough to pose a significant health threat. Because water bodies that contain cyanobacteria can be used for irrigating surrounding cropland, bioaccumulation in crop plants is a potential threat to human health. The type of plant, concentration of cyanotoxin in the water, and the type of irrigation (spray vs drip) all determine the amount of cyanotoxins taken up. Numerous cyanotoxins have been shown to accumulate in plants irrigated with water containing cyanotoxins. The presence of these toxins in water bodies like Utah Lake and the Jordan River may pose a threat to humans who irrigate with the contaminated water.

Limits on cyanotoxin exposure

Exposure limits have been created by the U.S. Environmental Protection Agency (USEPA) and the World Health Organization (WHO) for all of the cyanotoxin exposure routes. Recreation and drinking water limits are defined as concentrations in the contaminated water. Above such limits are cause for action, with beach closings for recreation and drinking water bans as seen in Toledo, Ohio in 2014[13]. Ingestion of contaminated food is a more complex route of exposure. Therefore, the WHO created a Total daily intake (TDI) limit on microcystin for humans. Microcystin is the only toxin to have sufficient toxicity data to create a TDI. Total daily intake limits are calculated by the concentration of the cyanotoxin in edible plant or animal tissue multiplied by the estimated daily intake of the plant in grams (e.g. 85 grams of lettuce consumed per day) divided by the body weight of a human in kilograms.

Exceeding any of the limits of exposure poses chronic and acute health risks.

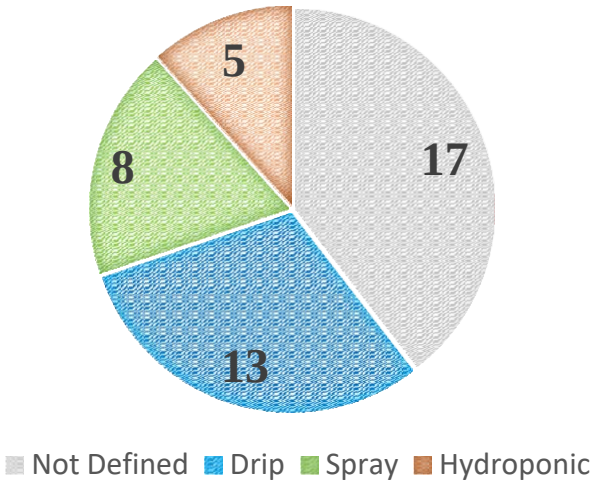
Recreation[7]	Drinking[14]	Ingestion[15]
8 µg per L	1.6 µg per L	0.04 µg per kg

Current Literature

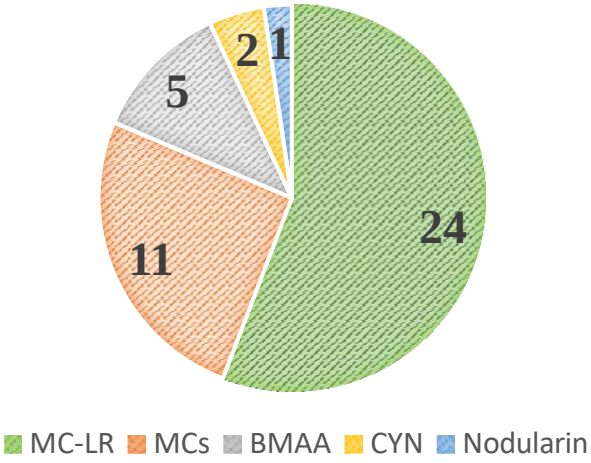
To assess the risk of cyanotoxins to humans from food, 27 publications (Pages 6-10) were reviewed that measured the ability for different food crops to take up the toxins into their tissues using assorted irrigation methods and concentrations of toxins (Figure 1, Page 5). Most studies used drip and hydroponic irrigation, which determines a plants ability to transport cyanotoxins from their roots into their potentially edible above ground tissue (e.g. fruits and leaves). Drip irrigation introduces cyanotoxins to the soil where physical characteristics (pH, cation exchange capacity, and organic matter) and environmental conditions (microbial presence, UV exposure, temperature) can influence the bioavailability of the toxins to plants[16,17]. A short experiment completed by the authors of this fact sheet examined cyanotoxin sorption capacity of Utah soils (Figure 2, Page 5). Other studies use spray irrigation, which only shows how cyanotoxins adhere to the above ground plant matter. However, many publications did not specify an irrigation method, which limits the ability of the study to define root to shoot translocation of cyanotoxins. A large majority of research has been done on one cyanotoxin, microcystin (specially its -LR congener), which is an important limitation to the knowledge available on toxin uptake in food crops. Summarized below is the distribution of research using different cyanotoxins, irrigation and crop types. Some publications included multiple toxins, irrigation and crop types – so each induvial occurrence of a toxin, irrigation or crop type in a study is counted.

The majority of experiments were done on lettuce, followed by carrots, parsley, spinach, tomatoes and wheat. The remaining 24 experiments consist of cabbage, clover, green beans, rape, rice, and ryegrass each with two instances. Arugula, broccoli, corn, cucumber, dill, duckweed, lentils, mustard, spring onion, peas, radish and water spinach make up the remainder with one instance each. Of the 27 publications, 10 found plant uptake values of Microcystin-LR higher than the 0.04 ug/kg limit.

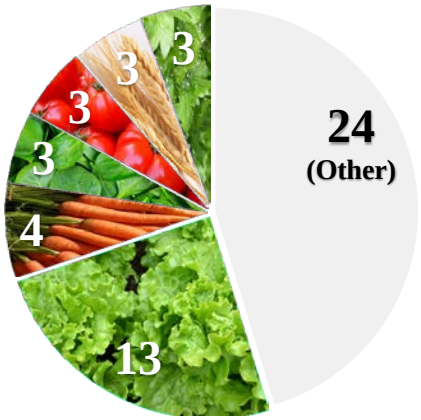
Irrigation Type



Cyanotoxin Type



Crop Type



Knowledge Gaps

Of the 27 publications examined, the methods varied drastically between irrigation (type, amount and duration), toxins and plants used, and growth conditions (substrate, field versus greenhouse). The current volume of research indicates a relationship between toxin dose and plant uptake, with fruit uptake having a strong positive relationship with toxin concentration (Figure 1, Page 5). However, trends in uptake within other plant parts is not obvious due to the low number of studies and limited range of toxin concentrations.

The diversity of the publications makes it difficult to draw comparisons on how plants bioaccumulate cyanotoxins. Standard methods on crop exposure to cyanotoxins needs to be established in order to generate a baseline for how various crops bioaccumulate toxins. Even with a better understanding of the mechanism behind cyanotoxins bioaccumulation, toxicity data is severely lacking for all cyanotoxins. The literature focuses on microcystin and BMAA, however the effects of chronic exposure is still not understood. Finally, more research needs to be done on depuration. Only two studies have been found on depuration, which could affect the long-term potency of cyanotoxins within plants [18,19].

Summary

As HABs continue to increase in frequency and intensity due to climate and anthropogenic changes to the environment, the risk for human exposure to cyanotoxins rises. Current research indicates crops irrigated with water contaminated with cyanotoxins are a potent route of exposure to humans. The ability for plants to bioaccumulate toxins makes them a vector for both chronic and acute exposure to cyanotoxins. Presently, world governments have no known restrictions or limits on cyanobacteria in irrigation water. For example, the US FDA only has guidelines for E. Coli and microbial density[20]. Therefore, further research is necessary to develop safe guidelines on cyanobacteria and their toxins in water, food, and in humans in order to reduce the risk of exposure through crops.

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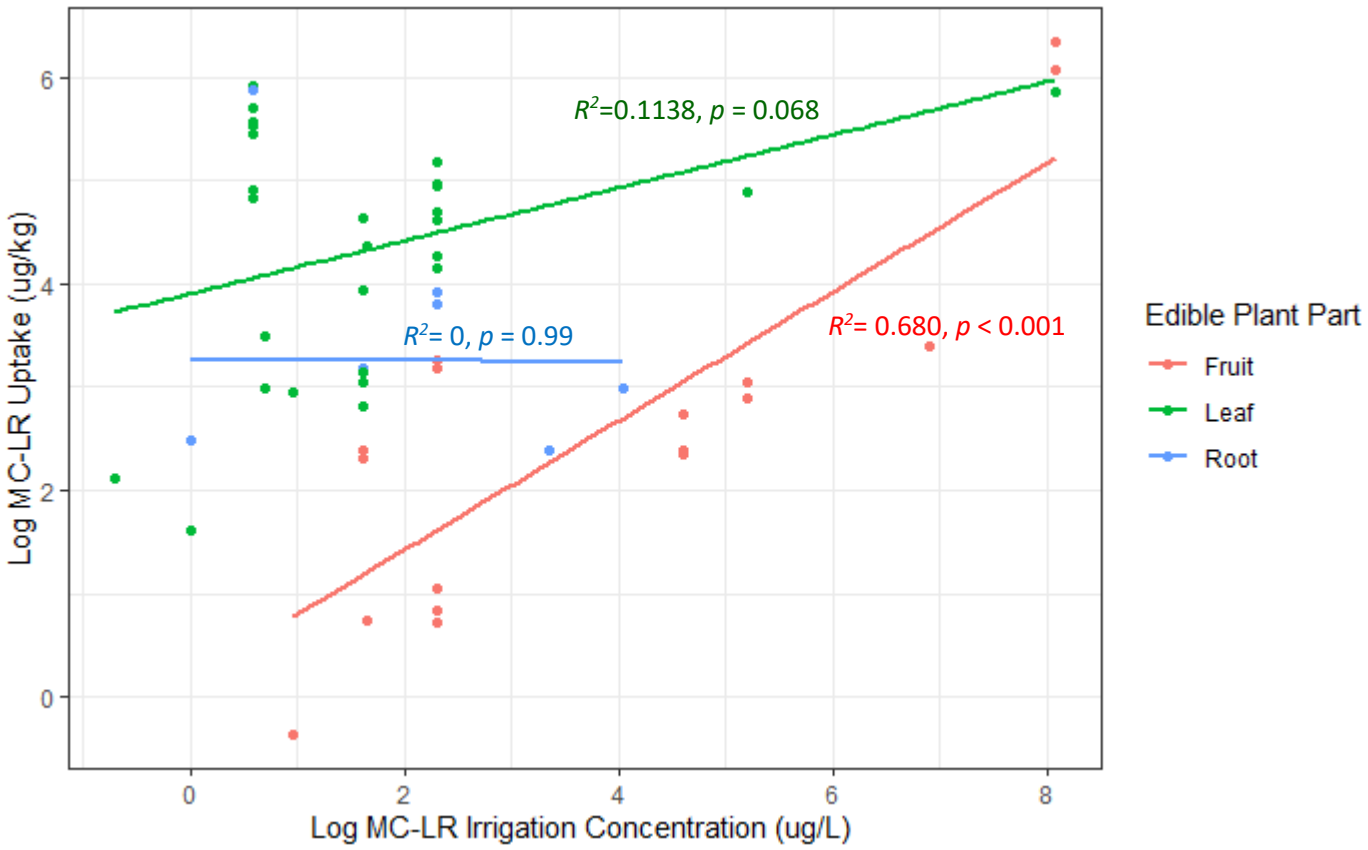

Figure 1: Bioaccumulation of microcystin-LR in various plant parts and regression for relationship between toxin irrigation concentration and toxin uptake

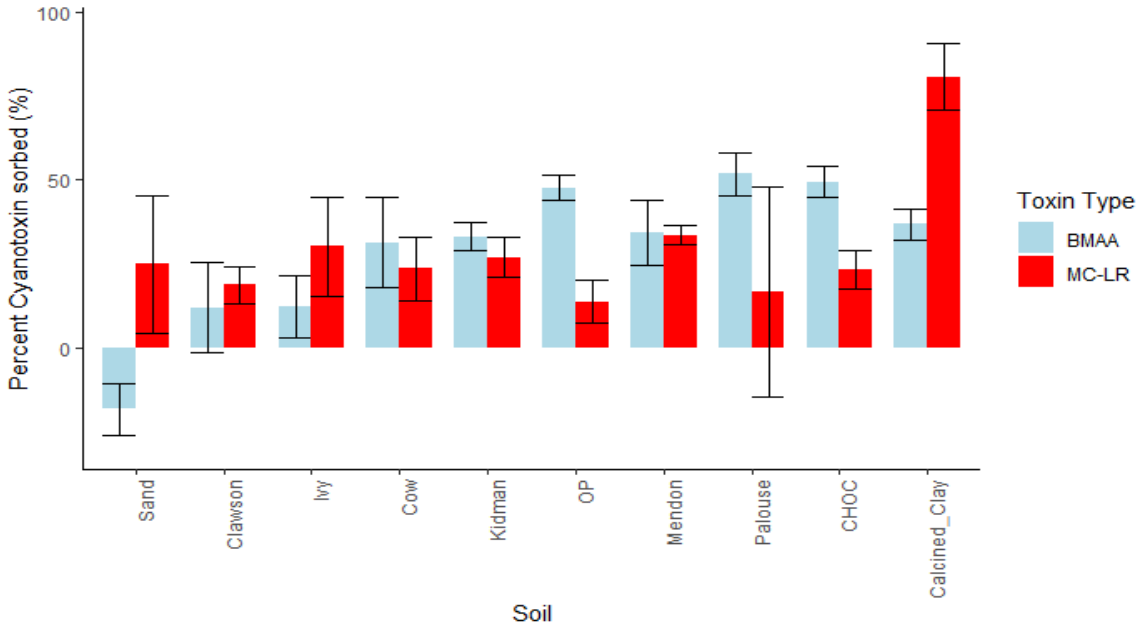

Figure 2: Percent cyanotoxin sorption to Utah soils at a dose of 250 μg L^{-1}. Calcined clay and sand are positive and negative controls respectively. Utah soils sorbed 33.9 ± 8.1 and 19.3 ± 11.0 percent of BMAA and MC-LR respectively

Irrigation Dose (ug/L)	Uptake & Analysis	Std.Dev.	Watering Conditions	Publication
Leafy Greens				
Arugula, *Eruca vesicaria*				
MCs, leaf 0.3-1.8	ug/kg FW, ELISA & HPLC-UV ~185	 33	Irrigation type not specified - field harvest	Mohamed & Al Shehri 2009
Broccoli, *Brassica oleracea*				
MC-LR, leaf 2.6 10	ug/kg FW, LC-MS BDL BDL		Irrigation type not specified. 20 days exposure	Jarvenpaa et al. 2007
Cabbage, *Brassica rapa*				
BMAA, leaf 4 ug	ug/g DW, HPLC/MS/MS 1.9 (Free) 12.2 (Protein-bound)	 0.15 1.6	100 g of potting soil dosed with 4 ug of BMAA, growth for 15 days	Li et al. 2018
MCs, leaf 0.3-1.8	ug/kg FW, ELISA & HPLC-UV ~218	 48	Irrigation type not specified - field harvest	Mohamed & Al Shehri 2009
Lettuce, *Lactuca sativa*				
MC-LR, leaf 2100	ug/kg FW, ELISA & LC-MS/MS BDL		Both spray and drip 100 mL 6x over 15	Crush et al. 2008
MC-LR, leaf 2 5 10	ug/kg FW, LC-MS/MS 32.99 103.24 143.35	 5.23 13.09 12.19	Spray 100 mL/day for 15 days	Bittencourt-Oliveria et al. 2016
MC-LR, leaf 2.61 5.22	ug/kg FW, UHPLC/MS 19.3 78	 7.5 24.6	Drip 500 mL/day for 60 days	Cao et al. 2018
MC-LR, leaf 10	ug/kg FW, HPLC 110	 22	Drip 200 mL/day for 12 days	Cao et al. 2019
MC-LR, leaf 5 10	ug/kg FW, LC-MS/MS 51.25 101	 5.25 2	Spray 100 mL/day for 7 days	Cordeiro-Araújo et al. 2016
BMAA, leaf 50	ug/kg DW, LC-MS 0		Irrigation type not specified. 10 mL once a week for 9 weeks	Esterhuizen Londt & Pfaugmacher 2019
MC-LR, leaf 0.5 2 5 10	ug/kg FW, ELISA 8.31 19.8 16.8 177.8	 0.2 4.1 6.3 3.4	Spray 100 mL/day for 15 days	Hereman et al. 2012

Irrigation Dose (ug/L)	Uptake & Analysis	Std.Dev.	Watering Conditions	Publication
MC-LR, leaf	g/kg FW, ELISA & HPLC		Spray or drip 100 mL, 3x a week for 4 weeks	Lee et al. 2017
1	5 (Drip), 5 (Spray)			
5	21 (Drip), 23 (Spray)			
10	64 (Drip), 72 (Spray)			
MC-LR, leaf	ug/kg FW, ELISA		Drip 100 mL, 3x week for 2 months Began irrigation at 4 different growth stages	Levizhou et al. 2017
1.81	370 (Seed)	22		
	365 (Cotyledon)	27		
	251 (2 leaves)	23		
	135 (4 leaves)	12		
MCs, leaf	ug/kg FW, ELISA & HPLC-UV		Irrigation type not specified - field harvest	Mohamed & Al Shehri 2009
0.3-1.8	~126	16		
Spinach, *Spinacia oleracea*				
MC-LR, leaf	ug/kg FW, HPLC		Drip 200 mL/day for 12 days	Cao et al. 2019
10	140	18		
Nodularin, leaf	ug/kg FW, LC-MS		Drip 30 mL 2x week, 5 weeks	Jokela et. al 2010
8.414	54.44	4.53		
MCs, leaf	ug/kg FW, LC-MS/MS-MRM		Field sprayed with contaminated water two times over 2 weeks	Spoof et al. 2020
3.8	BDL			
Water Spinach, *Ipomoea aquatica*				
MC-LR, leaves	ug/kg FW, HPLC		Hydoponic, field samples harvested in October 2016 and laboratory experiment lasted 4 months	Wijewickrrama & Manage 2019
180 (Field)	132.86	0.26		
3197.37 (Laboratory)	350.82	2.86		
Fruting Plants				
Cucumber, *Cucumis sativus*				
MCs, fruit	ug/kg FW, ELISA		Hydroponic, 7-day exposure starting at various growth stages	Zhu et al. 2018
1	BDL (All)			
10	2.87 (Seedling)			
	2.32 (Flower)			
	2.07 (Fruiting)			
100	No fruit (Seedling)			
	No fruit (Flower)			
	15.43 (Fruiting)			
1000	No fruit (Seedling)			
	No fruit (Flower)			
	29.64 (Fruiting)			

Irrigation Dose (ug/L)	Uptake & Analysis	Std.Dev.	Watering Conditions	Publication
Green Bean, *Phaseolus vulgaris*				
MC-LR, fruit	g/kg FW, ELISA & HPLC		Spray or drip 100 mL, 3x a week for 4 weeks	Lee et al. 2017
1	BDL			
5	BDL (Drip), 1 (Spray)			
10	3 (Drip), 3 (Spray)			
Lentil, *Lens culinaris*				
MC-LR, leaves	ug/g FW, HPLC/MS		Irrigation type not specified, 200 mL every 3 days for 30 days	Saqrane et al. 2009
0.5	BDL			
1.05	2.33			
4.2	36.61			
Pea, *Pisum sativum*				
MC-LR, leaves	ug/g FW, HPLC/MS		Irrigation type not specified, 200 mL every 3 days for 30 days	Saqrane et al. 2009
0.5	BDL			
1.05	BDL			
4.2	1.17			
Tomato, *Solanum lycopersicum*				
ug eq. MC-LR, leaf	ug/kg DW, UHPLC/MS		Irrigation type not specified, daily for 90 days	Corbel et al. 2016
5	BDL			
20	0.29	0.06		
50	0.33	0.01	No MC-LR measured in fruits	
100	0.55	0.19		
MC-LR, fruit	ug/kg FW, LC-MS/MS		Irrigation type not specified, 500 mL every 3 days for 1 week	Gutierrez-Praena et al. 2014
100 (reagent)	10.52	6.48		
100 (bloom extract)	10.83	0.94		
Grains				
Corn, *Zea mays*				
MC-LR, leaves	ug/g FW, HPLC/MS		Irrigation type not specified, 200 mL every 3 days for 30 days	Saqrane et al. 2009
0.5	BDL			
1.05	1.29			
4.2	7.65			
Rape, *Brassica napus*				
MC-LR, leaf	ug/kg FW, ELISA & LC-MS/MS		Both spray and drip 100 mL 6x over 15	Crush et al. 2008
2100	BDL			
Rice, *Oryza sativa*				
MC-LR, grain	ug/kg FW, UHPLC-MS		Hydroponic, 1500 mL per day for 120 days	Cao et al. 2018
2.61	0.7	0.67		
5.22	2.1	1.1		
MC-LR, grains	ug/kg FW, HPLC		Hydroponic, field samples were harvested in October 2016 and laboratory experiment lasted 4 months	Wijewickrrama & Manage 2019
180 (Field)	20.97 (BG358)	0.31		
	18.19 (Suvandel)	0.2		
3197.37 (Laboratory)	567.52 (BG358)	4.9		
	429.83 (Suvandel)	4.4		

Irrigation Dose (ug/L)	Uptake & Analysis	Std.Dev.	Watering Conditions	Publication
Wheat, *Triticum aestivum*				
MC-LR, leaves 0.5 1.05 4.2	ug/g FW, HPLC/MS BDL BDL 1.17		Irrigation type not specified, 200 mL every 3 days for 30 days	Saqrane et al. 2009
BMAA, grain 10	ug/kg FW, HPLC BDL (Free) 207 (Protein-bound)	 150	Drip 273 mL/week for 26 weeks	Contardo-Jara et al. 2018
BMAA, shoots 100	ug/kg FW, LC-MS/MS 100	 15	Irrigation type not specified, 2x/week for 4 weeks (400 mL total)	Contrado-Jara et al. 2014
Root Vegetables				
Carrot, *Daucus carota*				
MC-LR, taproot 1 5 10	ug/kg FW, HPLC 12 (Drip), 18 (Spray) 72 (Drip), 74 (Spray) 220 (Drip), 204 (Spray)		Spray or drip 100 mL, 3x a week for 4 weeks	Lee et al. 2017
MC-LR, taproot NA, 20% WTR NA, 40% WTR	ug/kg FW, ELISA 11 20	 1.5 0.4	Plants grown for 3 months in soil mixed with water treatment residuals containing microcystin (20 & 40% by mass)	Ai et al. 2019
MCs, taproot 50	ug/kg FW, ELISA 5.23	 0.47	Drip 40 mL 2x a week for 4 weeks	Machado et al. 2017
Radish, *Raphanus raphanistrum*				
MCs, taproot 0.3-1.8	ug/kg FW, ELISA & HPLC-UV ~235	 45	Irrigation type not specified - field harvest	Mohamed & Al Shehri 2009
Spring Onion, *Allium fistulosu*				
BMAA, shoot 50	ug/g DW, LC-MS 0.5 (Free)	 0.2	Irrigation type not specified, 10 mL/week for 9 weeks	Esterhuizen Londt & Pfaugmacher 2019
Herbs & Other				
Clover, *Trifolium repens*				
MC-LR, leaf 2100	ug/kg FW, ELISA & LC-MS/MS BDL		Both spray and drip 100 mL 6x over 15 days	Crush et al. 2008
Dill, *Anethum graveolens*				
MCs, leaf 0.3-1.8	ug/kg FW, ELISA & HPLC-UV ~189	 18	Irrigation type not specified - field harvest	Mohamed & Al Shehri 2009

Irrigation Dose (ug/L)	Uptake & Analysis	Std.Dev.	Watering Conditions	Publication
Parsley, *Petroselinum crispum*				
MCs and CYN, leaf	ug/kg FW, HPLC		Drip 30 mL, every other day for 10 days	Pereira et al. 2017
100	BDL			
500	BDL			
1000	BDL			
MCs, leaf	ug/kg FW, ELISA & HPLC-UV		Irrigation type not specified - field harvest	Mohamed & Al Shehri 2009
0.3-1.8	~264	41		
Mustard, *Sinapis alba*				
MC-LR, leaf	ug/kg FW, LC-MS		Irrigation type not specified. 19 days exposure	Jarvenpaa et al. 2007
1	BDL			
10	BDL			
Ryegrass, *Lolium perenne*				
MC-LR, leaf	ug/kg FW, ELISA & LC-MS/MS		Both spray and drip 100 mL 6x over 15 days Crush et al. 2008	Crush et al. 2008
2100	BDL			

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