Importance

Besides the microcystins which are the most common cyanotoxins world wide, a new group of toxins, the so called *saxitoxins* became more relevant in aqueous medium in recent years. Up to now about eighteen (18) different structures are known. Saxitoxins are neurotoxins and can be formed by marine dinoflagellates and fresh water blue green algae. The importance of saxitoxins is in relation to red tide in shellfish and causes the paralytic shellfish poisoning (PSP).
The molecular weight of saxitoxins lies between 300 and 400 a.u.. Their structures are characterized by amino and sulfonic acid groups. This is the reason of a very high polarity which makes their extraction from the water phase quite difficult.
Besides the saxitoxins there are some other relevant biotoxins produced from marine and fresh water algae. These are also amino acids with molecular weights near 300 a.u. and lower if they are not protein associated. The most relevant compounds are ß-N-methylamino–L-alanin (BMAA), domoic acid and kainic acid.
In general all toxins occur cell bound. On the other hand, they can be released in to the water by algal cell destruction.

Approach

The approach for trace analysis of cell bound as well as dissolved nodularins, saxitoxins and amino-acid-like toxins (novel toxins) is subdivided into the following steps:

- Preparation of aqueous standard solutions of novel toxins for HPLC calibration,
- Solid phase extraction of novel toxins in water samples using a combination of pre-concentration columns,
- Liquid phase extraction of cell bound novel toxins
- Conditions for analysis novel toxins by high-performance liquid chromatography and mass detection.

Result:

Table S1: Calibration parameters of nodularin in dissolved and cell bound state

	Slope	Rel.devia-tion in % [1]	Correlation coefficient	Limit of detection in µg/L	Limit of registration in µg/L	Limit of determination in µg/L	Recov. in %
dissolved	1.075	6.2	0.997	0.03	0.06	0.10	85
cell bound	1.247	4.1	0.998	0.05	0.10	0.19	89

Table S2: Calibration parameters of saxitoxins in cell bound state

Com-pound	Slope	Rel.deviation in % [1]	Correlation coefficient	Limit of detection in µg/L	Limit of registration in µg/L	Limit of determination in µg/L	Recov. in %
SAX	0.365	4.4	0.998	0.05	0.09	0.18	83
NEO	0.049	7.1	0.996	0.09	0.18	0.31	79
GTX 2/3	0.214	4.8	0.998	0.06	0.12	0.22	87
GTX 1/4	0.233	7.2	0.996	0.09	0.18	0.31	85

Table S3: Calibration parameters of saxitoxins in dissolved state

Com-pound	Slope	Rel.deviation in % [1]	Correlation coefficient	Limit of detection in µg/L	Limit of registration in µg/L	Limit of determination in µg/L	Recov. in %
SAX	0.423	8.4	0.994	0.03	0.07	0.12	70
NEO	0.087	21.4	0.965	0.09	0.18	0.30	60
GTX 2/3	0.253	7.1	0.996	0.03	0.07	0.12	86
GTX 1/4	0.298	10.1	0.992	0.04	0.08	0.14	72

Table S4: Calibration parameters of amino-acid-like toxins

Analyte- FMOC-derivative	Q1 mass $[M+H]^+$ (m/z)	Q3 mass production (m/z)	correlation coefficient	RSD [%]	LOD [µg/L]	LOQ [µg/L]	linear range [µg/L]
BMAA 2xFMOC	563	341	0.996	7.6	0.25	0.68	0.1-2.5
Kainic acid	436	214	0.998	5.0	0.16	0.59	0.1-3.0
Domoic acid	534	312	0.998	4.6	0.16	0.58	0.1-5.0
BMAA Σ Q3-area			0.996	7.7	0.78	2.2	1.0-25
2x FMOC	563	119					
1x FMOC	341	119					

LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard deviation

More information
The overview and results have been published in the final SOP documents for HPLC based analysis of new algal toxins (dissolved state) in natural waters.

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TKI Categorisation

Classification									
Supply Chain		**Process Chain**		**Process Chain (cont'd)**		**Water Quality**		**Water Quantity (cont'd)**	
Source		**Raw water storage**		**Sludge treatment**		**Legislation/regulation**		- Leakage	
- Catchment		- Supply reservoir	x	- Settlement		- Raw water (source)	x	- Recycle	
- Groundwater		- Bankside storage		- Thickening		- Treated water	x		
- Surface water	x	**Pretreatment**		- Dewatering		**Chemical**			
- Spring water		- Screening	x	- Disposal		- Organic compounds	x		
- Storm water		- Microstraining		**Chemical dosing**		- Inorganic compounds			
- Brackish/seawater	x	**Primary treatment**		- pH adjustment		- Disinfection by-products			
- Wastewater	x	- Sedimentation		- Coagulant		- Corrosion			
Raw water storage		- Rapid filtration	x	- Polyelectrolyte		- Scaling			
- Supply reservoir	x	- Slow sand filtration		- Disinfectant		- Chlorine decay			
- Bankside storage		- Bank filtration		- Lead/plumbosolvency		**Microbiological**			
Water treatment		- Dune infiltration	x	**Control/instrumentation**		- Viruses		**Consumers / Risk**	
- Pretreatment	x	**Secondary treatment**		- Flow		- Parasites			
- Primary treatment	x	- Coagulation/flocculation	x	- Pressure		- Bacteria	x	**Trust**	
- Secondary treatment	x	- Sedimentation	x	- pH		- Fungi		- In water safety/quality	x
- Sludge treatment		- Filtration	x	- Chlorine		**Aesthetic**		- In security of supply	
Treated water storage		- Dissolved air flotation(DAF)	x	- Dosing		- Hardness / alkalinity		- In suppliers	
- Service reservoir	x	- Ion exchange		- Telemetry		- pH		- In regulations and regulators	
Distribution		- Membrane treatment	x	**Analysis**		- Turbidity		**Willingness-to-pay/acceptance**	
- Pumps		- Adsorption	x	- Chemical	x	- Colour		- For safety	x
- Supply pipe / main		- Disinfection	x	- Microbiological		- Taste	x	- For improved taste/odour	
Tap (Customer)		- Dechlorination		- Physical		- Odour	x	- For infrastructure	
- Supply (service) pipe		**Treated water storage**						- For security of supply	

- Internal plumbing		- Service reservoir				**Water Quantity**		**Risk Communication**	
- Internal storage		**Distribution**						- Communication strategies	
		- Disinfection				**Source**		- Potential pitfalls	
		- Lead/plumbosolvency				- Source management		- Proven techniques	
		- Manganese control				- Alternative source(s)			
		- Biofilm control				**Management**			
		Tap (Customer)				- Water balance			
		- Point-of-entry (POE)				- Demand/supply trend(s)			
		- Point-of-use (POU)				- Demand reduction			

TKI Categorisation (continued)

Contains		**Constraints**		**Meta data**					
Report		Low cost		*Author(s)*		Wido Schmidt		Lutz Imhof	
Database	x	Simple technology		*Organisation(s)*		TZW Branch Dresden			
Spreadsheet		No/low skill requirement		*Contact name*		Wido Schmidt			
Model		No/low energy requirement		*Contact email*		schmidt@tzw-dresden.de wido.schmidt@tzw.de			
Research	x	No/low chemical requirement		*Quality controller name*					
Literature review		No/low sludge production		*Quality controlerl organisation*					
Trend analysis		Rural location		Source					
Case study / demonstration		Developing world location		*Date prepared*					
Financial / organisational				Date submitted (TKI)					
Methodology				Date revised (TKI)					
Legislation / regulation									
Benchmarking									