

Інформація взята з сайту: <https://soctrade.ua>

Прискорений новий метод SingleICP-MS контролю ефективності лікування альгіцидом водойм при цвітінні токсичних водоростей

Токсичне цвітіння ціанобактерій були зареєстровані в усьому світі в різних водних системах, в тому числі в прісноводних річках, озерах, водосховищах і в прибережному морському середовищі.

1. Ціанобактеріальне цвітіння може бути викликано поєднанням декількох факторів, включаючи евтрофікацію, сонячну радіацію, температуру, закономірності течій і інші пов'язані фактори.
2. Розмноження токсичних водоростей у водному середовищі може призвести до накопичення отруйних речовин в тілі водних тварин і навіть викликати їх смерть, і тим самим побічно або прямо вплинути на здоров'я людини.
3. Цвітіння *Microcystis aeruginosa* (*M. aeruginosa*) є найбільш поширеним шкідливим цвітінням водоростей (ВГА) 4, тому його доводиться враховувати в різних областях.

Мідний купорос є альгіцидом, часто використовуваним для контролю росту водоростей. Механізм дії альгіциду на основі міді (Cu) для боротьби з цвітінням водоростей не зовсім зрозумілий. Яка кількість Cu потрібно для знищення окремої клітини водорості і морфології клітини після загибелі поки невідомо. Кількісний вимір цих явищ є складним завданням через відсутність загальноприйнятої методології.

Ця робота пропонує прискорений і точний метод одноклітинної (СК) -ICP-MS для вивчення ефективності застосування сульфату міді для контролю цвітіння *M. aeruginosa*. Запропонований метод дозволяє кількісно визначити вміст металу в окремих осередках, розподіл металу в клітинній популяції, концентрацію металу всередині осередку і концентрація позаклітинного металу в осередку одночасно, і отримати ці дані протягом декількох хвилин.

Запропонована методика може бути використана не тільки для швидкої оцінки ефективності застосування різних альгіцидів для контролю цвітіння водоростей, але також має великий потенціал застосування в багатьох інших сферах, таких як дослідження біодоступності важких металів, при розробці нових альгіцидів на основі металів, а також для швидкого моніторингу при дослідженнях токсичності металів в клітинах.

ПОСИЛАННЯ НА ДЖЕРЕЛО:

https://www.perkinelmer.com/lab-solutions/resources/docs/app_21202-nexion-sc-icp-ms-algaecidetreatmentefficiency.pdf



SocTrade постачає та обслуговує спеціалізоване й аналітичне лабораторне обладнання. Спеціалізоване - означає, що його використовують в межах однієї галузі. Ми дуже сильні в оснащенні лабораторій агро- та харчової продукції, а також лабораторій, що контролюють якість нафтопродуктів. Аналітичне обладнання — універсальне, але більш складне і використовується для високоточних аналізів у науково-дослідних або розвинутих промислових лабораторіях.

За роки роботи в цій порівняно вузькій, але дуже цікавій сфері ми накопичили величезний досвід, яким хочемо ділитися. Тому проводимо кругові випробування, розробляємо нові ДСТУ, співпрацюємо з інститутами, надаючи їм обладнання для дослідницьких і освітніх цілей, беремо участь у лабораторних виставках і конференціях та організуємо їх. Тобто з ентузіазмом долучаємося до будь-якої діяльності, спрямованої на підвищення рівня контролю якості та природничих наук в Україні.

Сайт: <https://soctrade.ua/>

**Телефон:
тел/факс, +380 (48) 757-87-88 (багатоканальний)**

ТОВ "СОКТРЕЙД" 65016, Україна, м. Одеса, вул. Літературна, 12, офіс 206

Електронна пошта: office@soctrade.ua

ICP - Mass Spectrometry

Authors:

Xing Shen^{1,2}, Honglan Shi^{1,3}, Haiting Zhang^{1,3}

1 Department of Chemistry and Center of Research for Energy and Environment Missouri University of Science and Technology, Rolla, MO, USA

2 School of Life Sciences, Central China Normal University Wuhan, China

3 Center for Single Nanoparticle, Single Cell, and Single Molecule Monitoring (CS³M) Rolla, MO, USA

Chady Stephan

PerkinElmer Inc.,
ON, Canada



A Rapid, Novel Single Cell-ICP-MS Method to Study the Treatment Efficiency of Algaecide for Toxic Algal Bloom Control

habitats.¹ Cyanobacterial blooms might be caused by a combination of multiple factors, including eutrophication, solar radiation, temperature, current patterns and other associated factors.² Their proliferation in aquatic environments can cause death or toxin accumulation in aquatic animals and then, indirectly or directly, affect human health.³ *Microcystis aeruginosa* (*M. aeruginosa*) bloom is the most common harmful algal bloom (HAB)⁴ and is widely studied in various fields.

Copper sulfate is an algaecide frequently used for controlling algae growth. The mechanism of copper (Cu)-based algaecide for algal bloom control is not well understood. The amount of Cu required to kill an individual algae cell and the morphology of the cell after being killed is still unknown. Quantification measurement of these phenomena is even more difficult due to the lack of appropriate methodology.

Introduction

Toxic cyanobacterial blooms were reported worldwide in various aquatic systems, including freshwater rivers, lakes, reservoirs, and eutrophied coastal marine

This application note describes a rapidly emerging and accurate single cell (SC)-ICP-MS method to study the efficiency of *M. aeruginosa* control by algacide (i.e., copper sulfate). The method can quantify the metal content in individual cells, the distribution of the metal within a cell population, the metal-containing cell concentration, and the extracellular metal concentration in a cell suspension simultaneously within a few minutes. More detailed information can be found in a recent publication.⁵ This technique not only can be used to rapidly evaluate the effectiveness of different algacides in algal bloom control, but also has great application potential in many other studies, such as heavy metal bioavailability, new metal-based algacide development and evaluation studies, as well as other toxicity studies by rapidly monitoring intrinsic metal elements in cells.

Experimental

Cell Culture and Preparation

Unicellular *M. aeruginosa* was cultured in BG-11 growth medium at 22±2 °C with 3200 lux in a 12:12 h light:dark cycle. The cell size is around 2-4 μm. It takes about two weeks for the cells to reach the logarithmic phase after being sub-cultured, and the logarithmic phase could last for one week. The late exponential phase cells were used for all experiments. Five mL of fresh cells were washed three times by 0.1 mM ethylenediaminetetraacetic acid (EDTA) aqueous solution and centrifuged at 500 g for 5 min. The cell pellets were then re-suspended in 5 mL of 0.1 mM EDTA aqueous solution to make the cell stock. The cell concentration was counted in the cell stock by a hemocytometer and then diluted for SC-ICP-MS method development and treatment studies.

SC-ICP-MS Method Development

A PerkinElmer NexION® ICP-MS equipped with a single cell sample introduction kit (Part No. B8150032 for NexION 1000/2000; Part No. N8140032 for NexION 300/350) was used, and the instrument operation and data processing were handled through the Syngistix™ for ICP-MS Single Cell

Application Software Module (Figure 1). The cell integrity after nebulization was checked with different nebulization gas and sample flow rates. Four abundant intrinsic metal elements in *M. aeruginosa* cell, i.e. Cu, Mg, Mn and Zn, were detected by SC-ICP-MS. While all of these metal levels are high enough to be monitored in a single cell and result in about the same cell concentration, transport efficiencies (TE) were determined by detecting the most abundant intrinsic metal Mg-containing cell concentration. The stability of TEs with different cell concentrations and sample flow rates was then evaluated and all method parameters were optimized. Finally, the matrix effect on Mg detection was checked by spiking the Mg standard (Mg STD) into copper- and magnesium-free BG-11 modified algae cell culture medium (MM) to make sure there were no matrix effects for extracellular metal element measurement by SC-ICP-MS when cells are broken or Mg leaches out. This test also checked that there was no particulate Mg (pulse signal) formation during the cell treatment period.

M. Aeruginosa Cell Treatment by Algacide Copper Sulfate

Cells used for copper sulfate treatment experiments were prepared the same as for method development. Figure 2 shows the schematic of sample preparation and copper sulfate treatment. The cell concentration was determined with a hemocytometer, then cell stock was diluted to 1,000,000 cells/mL and treated by copper sulfate solution with 0, 30, 60, and 100 μg/L copper sulfate. After treatment for selected times, 1 mL cell suspension was removed and diluted three times with 0.1 mM EDTA aqueous solution, then analyzed directly by SC-ICP-MS to monitor cell status (i.e. cell lysis) and cell concentration. To ensure the cell concentration was not high enough to produce multi-cell coincidence, we also tested the correlation of cell concentration via pulse signal in the range from 3,000 to 1,000,000 cells/mL by the SC-ICP-MS method, and the result showed good linear correlation, indicating the pulse signals were from single cell events.

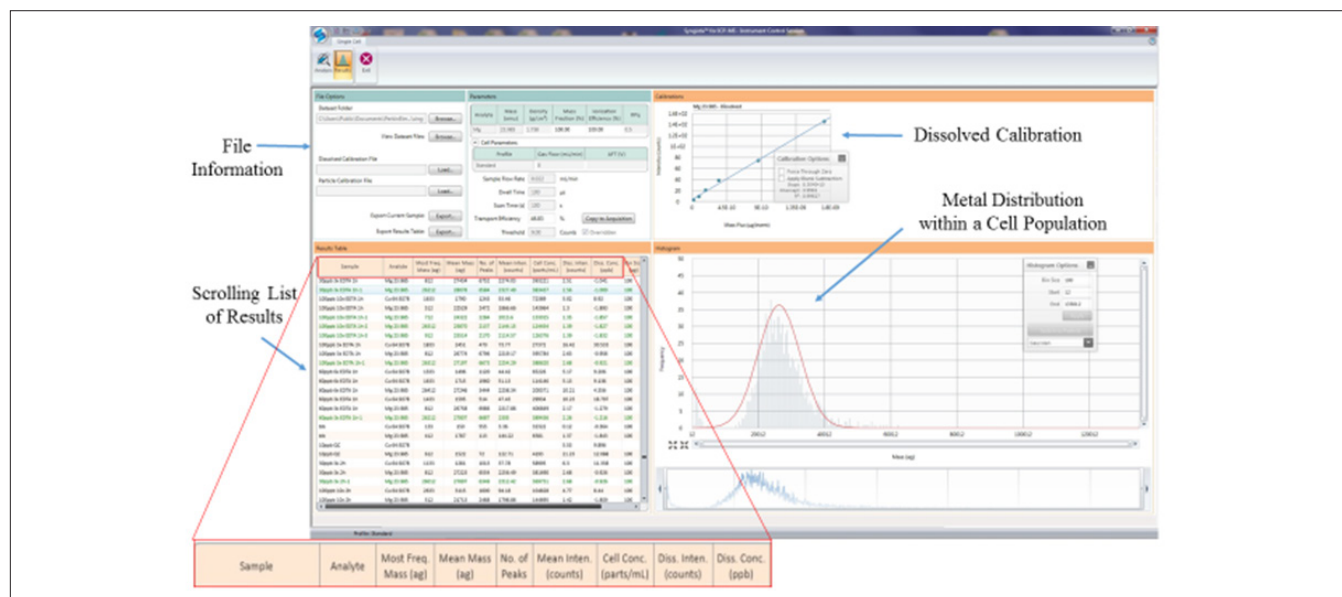


Figure 1. Example screen capture of Syngistix for ICP-MS Single Cell Application Software Module.

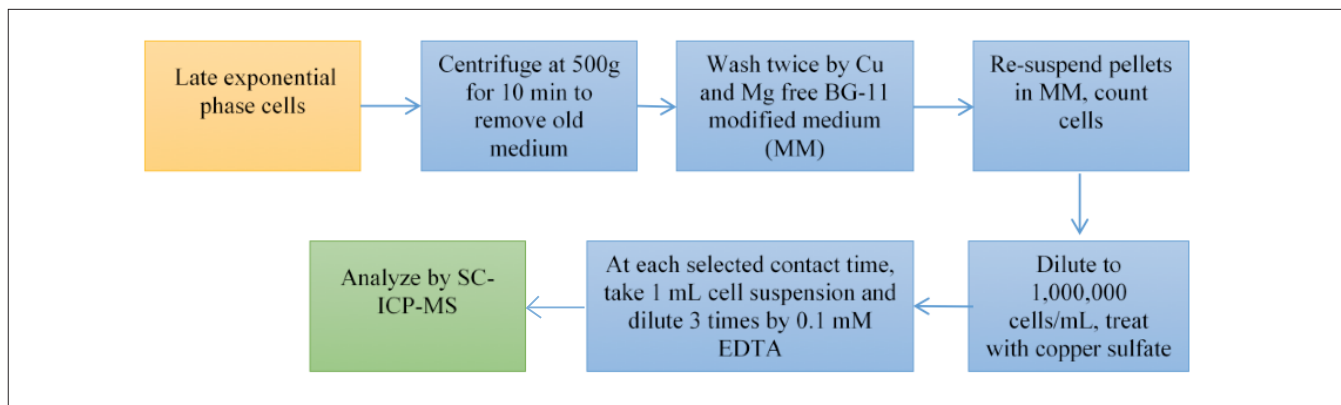


Figure 2. Schematic illustrating analysis procedure

Results

Method Development

Cells were undamaged after nebulization with sample flow rates ranging from 20 to 42 $\mu\text{L}/\text{min}$ and nebulization gas flow rate at 0 to 0.7 L/min (Figure 3). *M. aeruginosa* cells contain more Mg compared to copper, zinc, and manganese, from SC-ICP-MS analysis. Thus, the transport efficiency and cell status were monitored by detecting the intrinsic metal Mg content in each cell. The optimized analysis parameters are shown in Table 1. We also detected the Mg concentration in MM by spiking standard Mg in the MM. The spike recoveries were good and indicate no matrix effect for dissolved Mg quantification measurement and no particulate Mg formation in the MM, confirming that it is suitable to analyze post-dosing samples directly. Therefore, calibration standards were prepared as suspensions in MM with 0.1 mM EDTA to matrix match the samples. To reduce the effect of the signal of extracellular Mg to the pause signals of Mg-containing cells, we made a three-fold dilution for the post-dosing samples before analysis by SC-ICP-MS.

The Efficiency of Algacide Treatment for Toxic Algal Bloom Control

The Mg concentrations in MM, 0.1 mM EDTA solution, and copper sulfate stock solution were analyzed as controls and were found to have Mg concentrations less than the detection limit (0.2 $\mu\text{g}/\text{L}$). The Mg-containing cell concentration decreased over time in 60, 100, 200 $\mu\text{g}/\text{L}$ Cu treatment groups; changes were not significant in 0 and 30 $\mu\text{g}/\text{L}$ Cu treatment groups (Figure 4). These results suggest that cells are broken over time with high concentrations of Cu treatment, with the higher Cu concentrations breaking the cells faster. The extracellular Mg concentration (released to medium when the cells broke) in each treatment group increased with the decrease of intact cell concentration over time (Figure 5). In high Cu concentration treatment groups, Cu released when cells were broken, resulting in the increase of extracellular Mg concentration over time. The variation of mean mass of Mg in a single cell showed that the mean mass of Mg per cell did not change until most of the cells broke (Figure 6), which suggests that Mg release was caused by cells breaking. Mg mass distribution in cells exhibited cell heterogeneity.

Table 1. Optimized SC-ICP-MS analysis method parameters.⁵

Parameter	Value
RF Power (W)	1600
Nebulization Gas Flow Rate (L/min) ^a	~ 0.5
Makeup Gas Flow Rate (L/min)	0.7
Sample Flow Rate ($\mu\text{L}/\text{min}$)	21 - 22
Dwell Time (μs)	100
Scan Time (s)	100
Transport Efficiency (%) ^b	45.56 - 63.65
Analyte	⁶⁵ Cu, ²⁴ Mg

^a: Parameter is re-optimized daily.

^b: Parameter is determined daily.

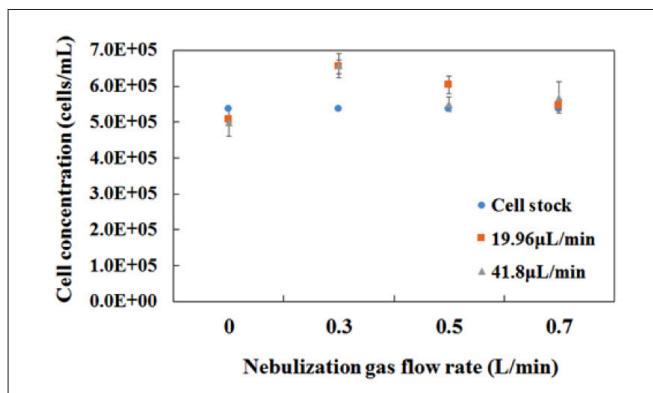


Figure 3. Cell concentration of undamaged cells before (cell stock) and after (20 $\mu\text{L}/\text{min}$ and 42 $\mu\text{L}/\text{min}$) with different nebulization gas flow rates and sample flow rates.

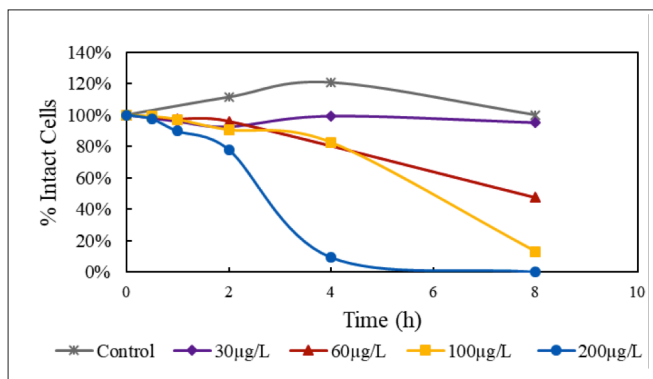


Figure 4. Variations of intact cell concentrations with different treatment times by different Cu concentrations.

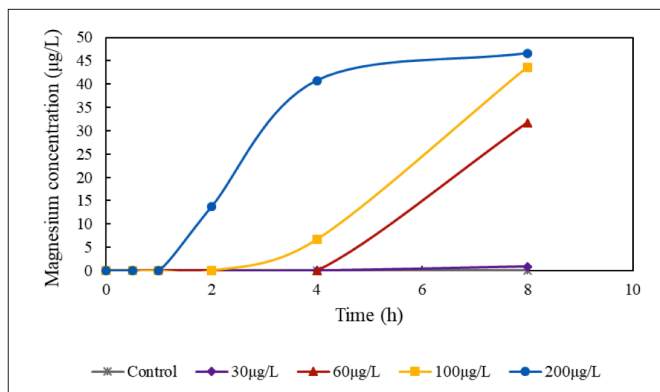


Figure 5. Variations of extracellular Mg concentrations with different contact times by treatment with different Cu concentrations.⁵

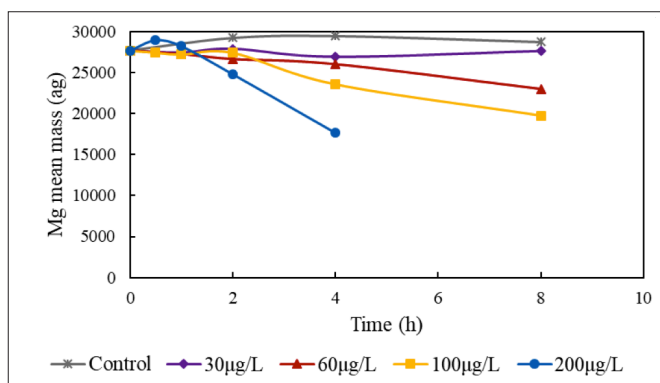


Figure 6. The variation of magnesium mean mass in a single cell with different treatment times and different copper concentrations.

Conclusions

This work has shown the ability of SC-ICP-MS to monitor cell status by rapidly detecting abundant intrinsic metal in a single cell without complex post-dosing sample preparation procedure. This method can be used to evaluate the effectiveness of various algaecides for HAB control and monitor heavy metal ion bioavailability of algal cells. The SC-ICP-MS method can be used to rapidly monitor cell status (broken/unbroken) for any types of cell treatment, such as drug treatment efficiency for drug development and evaluation, as well as nanotoxicity studies.

References

1. B.A. Neilan; L.A. Pearson; J. Muenchhoff; M.C. Moffitt; E. Dittmann, Environmental conditions that influence toxin biosynthesis in cyanobacteria. *Environ. Microbiol.*, 2013, 15, 5, 1239-1253.
2. S.K. Sarkar, Algal Blooms: Basic Concepts. In: *Marine Algal Bloom: Characteristics, Causes and Climate Change Impacts*. Springer, Singapore. 2018.
3. E. Berdalet; L.E. Fleming; R. Gowen; K. Davidson; P. Hess; L.C. Backer; S.K. Moore; P. Hoagland; H. Enevoldsen, Marine harmful algal blooms, human health and wellbeing: challenges and opportunities in the 21st century. *J. Mar. Biol. Assoc. U. Kingd*, 2015, p1–p31.
4. T. Preston; W.D.P. Stewart; C.S. Reynolds, Bloom-forming cyanobacterium *Microcystis aeruginosa* overwinters on sediment surface. *Nature*, 1980, 288, 365-367.
5. X. Shen, H. Zhang, X. He, H. Shi, C. Stephan, H. Jiang, C. Wan, T. Eichholz, Evaluating the Treatment Effectiveness of Copper-based Algaecides on Toxic Algae *Microcystis Aeruginosa* Using Single Cell-Inductively Coupled Plasma-Mass Spectrometry. *Analytical and Bioanalytical Chemistry*, 2019, 411(21): 5531–5543, DOI: 10.1007/s00216-019-01933-9, 2019.samples.

Consumables Used

Description	Part Number
Sample Uptake Tubing; Orange/Red (0.19 mm id), PVC, Flared	N0773111 (NexION 300/350)
	N8145146 (NexION 1000/2000)
Drain Tubing; Gray/Gray (1.30 mm id), Santoprene	N0777444 (NexION 300/350)
	N8145173 (NexION 1000/2000)
Copper Standard, 1000 ppm	N9300183 (125 mL)
	N9300114 (500 mL)
	N9300179 (125 mL)
Magnesium Standard, 1000 ppm	N9300131 (500 mL)