Electronic Tongue for microcystin screening in waters

L. Lvova[1,2,*], C. Guanais Gonçalves [1], K. Petropoulos[1], L. Micheli[1], G. Volpe[1], D. Kirsanov[2,3], A. Legin[2,3], E. Viaggiu[4], R. Congestri[4], L. Guzzella[5], F. Pozzoni[5], G. Palleschi[1], C. Di Natale[2,6], R. Paolesse[1,2]

[1]*Department of Chemical Science and Technologies, University "Tor Vergata", Rome, Italy*

[2] *Laboratory of artificial sensory systems, ITMO University, St. Petersburg, Russia*

[3] *Institute of Chemistry, St. Petersburg State University, St. Petersburg, Russia*

[4]*Department of Biology, University "Tor Vergata", Rome, Italy*

[5]*CNR-IRSA, Brugherio (MB), Italy*

[6]*Department of Electronics Engineering, University of Rome Tor Vergata, Rome, Italy*

*corresponding author email: larisa.lvova@uniroma2.it

1. Abstract

The potentiometric E-tongue system was employed for water toxicity estimation in terms of cyanobacterial microcystin toxins (MCs) detection. The data obtained from E-tongue were correlated to the MCs content detected by the standard chromatographic technique UHPLC-DAD (Ultra High Performance Liquid Chromatography with Diode Array Detector), as far as by the colorimetric enzymatic approach. The prediction of MCs released by toxic *Microcystis aeruginosa* strains was possible with Root Mean Squared Error of Validation (RMSEV) lower or very close to 1 μg/L, the provisional guideline value of WHO for MCs content in potable waters. The application of E-tongue system opens up a new perspective offset for fast and inexpensive analysis in the field of environmental monitoring, offering also the possibility to distinguish toxin producing and non-toxic *M. aeruginosa* strains present in potable water.

1. INTRODUCTION

Freshwater monitoring is extremely important for public health, human activities, and animal life and it is strictly related to national security issues. Besides the pollutants from anthropogenic activity, the water potability should also consider the contamination caused by naturally occurring microorganisms. The water poisoning caused by cyanobacteria is nowadays a worldwide serious problem (Chorus and Bartram , 1999; Svrcek and Smith, 2004; Merel et al., 2010), since massive cyanobacterial proliferations, when dominated by toxic strains, can be a source of potent neurotoxins and hepatotoxins responsible for gastroenteritis and liver damages in humans (Metcalf et al., 2000; Carmichael et al., 2001).

Among cyanotoxins, microcystins (MCs) are the most hazardous (Rivasseau et al., 1998; Dawson, 1998; Pearson et al., 2010). MCs belong to a family of hepatotoxic cyclic heptapeptides, and, being protein phosphatase inhibitors, are recognized as tumor promoters, even in nanomolar concentrations (Fawell et al., 1993), with Adda (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) and Mdha (N-methyldehydro-alanine) moieties as the most toxic ones (Scheme 1). The main structural differences between MCs arise from the substitution of single amino acids (Chorus and Bartram, 1999). MCs are very soluble in water and present in over 80 reported isoforms, varying by degree of methylation, hydroxylation, epimerization, peptide sequence and toxicity (Botes et al., 1985; Furey et al. 2008). However, four MCs (LR, RR, LA, and YR), are of special concern to the US Environmental Protection Agency (US EPA) and are on US EPA Contaminant Candidate List III (Westrick et al. 2010). MC-LR, containing L-leucine (L, moiety 2 in Scheme 1) and L-arginine (R, moiety 4 in Scheme 1) fragments, is considered the most toxic and often the total amount of MCs is represented relative to MC-LR content (Pearson et al., 2010). The World Health Organization (WHO) has set a provisional drinking water guideline for MC-LR to 1 μg/L (Chorus and Bartram, 1999). The management of surface and drinking water is essential to protect both human and fauna health, and the development of cheap, reliable, and non-time consuming analytical procedures to detect MCs is becoming very important.

Insert Scheme 1.

One of the first screening methods for MCs detection is the "Mouse Bio-assay" (MBA) (Campas et al., 2007). This assay has several disadvantages, such as low specificity and sensitivity, high cost of the animal maintenance and ethical problems related to the living creature's involvement. For this reason, detection systems non-involving tests on animals are preferable. Nowadays a wide range of analytical methods have been developed to detect and quantify MCs in natural and controlled environments, including water bodies, drinking water treatment plants, and in associated matrices, such as clinical and pathological materials (Metcalf and Codd, 2003; Kaushik and Balasubramanian, 2013). Besides well-known instrumental methods, such as HPLC coupled to fluorescence detection (HPLC-DAD) (Meriluoto et al., 2000), and liquid chromatography coupled to mass spectrometry (LC-MS or LC-MS/MS) (Zweigenbaum et al., 2000; Barco et al., 2002), the plenty of enzyme-linked immunosorbent assays (ELISA) applications (Metcalf and Codd, 2003; Zeck et al., 2001), and other types of enzymatic (Zhang et al., 2007; Han et al., 2013; Ruiyi et al., 2013), DNA aptamers (Eissa et al., 2014), and cell-based (Huang et al., 2009) sensory systems have been reported. In particular, enzymatic assays based on inhibitory effect of PP2A and PP1 protein enzymes have been widely applied for the determination of MCs (MacKintosh et al., 1990). The colorimetric detection of enzyme inhibition has been shown to provide sufficient specificity and sensitivity (Rivasseau et al., 1999; Wong et al., 1999; Heresztyn and Nicholson, 2001; Bouaicha et al., 2002). However, the above-mentioned instrumental chromatographic methods require expensive equipment, skilled personnel, are laborious, time-consuming and not suitable for routine and outdoor analysis, while the ELISA assays often give "false positives" and require a quite long response time.

The use of non-enzymatic chemical sensors for MCs analysis has been sparsely exploited and only few applications of molecularly imprinted polymers (MIPs) have been reported (Queiròs et al., 2011; Chen et al., 2012; He et al., 2015). In these works, different transducers have been exploited, showing in general sensitivities reaching the μg/L range. Recently the multisensory approach involving the application of low-selective sensor arrays, so-called Electronic tongue (E-tongue) devices, was satisfactory applied for the

environmental monitoring and in particular for water quality evaluation and toxins detection (Heras et al., 2010; Kirsanov et al., 2014; Zadorozhnaya et al., 2015).

In this study, we present a first application of E-tongue, based on potentiometric sensors, for the analysis of potable water contamination by MCs. Moreover, the possibility to use the developed E-tongue to predict the amount of MC-LR, released by toxic *Microcystis aeruginosa* strain, has been investigated. The satisfactory PLS correlations between E-tongue data, and MCs amount determined by chromatographic UHPLC-DAD technique, and by colorimetric protein phospharase-2A (PP2A)-based assay, have been found. The application of developed E-tongue system for MCs assessment in standard solutions and in spiked tap water and natural water samples has also been performed.

3. Materials and Methods

Details on solvents, reagents, UHPLC and colorimetric analyses have been reported in the Supporting Information.

Potentiometric E-tongue system

Several types of sensors were included in the potentiometric E-tongue system: PVC-based solvent polymeric membranes doped with Co(TPP)Cl (sensor A1) and nonactin (sensor C1) ionophores, and ion-exchangers TpClPBK (sensor C2) and $TDANO_3$ (sensor A2); chalcogenide glass sensors (CG-Cu, CG-Pb, CG-Ag) and polycrystalline sensor based on LaF_3 (sensor A3). The PVC-based solvent polymeric membrane cocktails were formed according to the standard method reported elsewhere, see SI section for more details (Lvova et al., 2013). Chalcogenide glass and polycrystalline sensors were from Sensor Systems (St. Petersburg, Russia. The response of the E-tongue to MC-LR was tested in 10^{-10} - 10^{-8} mol/L aqueous calibration solutions, prepared by addition of calculated amounts of 10^{-6} mol/L stock solution of MC-LR in methanol to tap water (Tor Vergata zone, Rome, Italy).

Cyanobacteria growth, sampling and pretreatment conditions

Two strains of *Microcystis aeruginosa* (Kützing) from the SAG Culture Collection of Algae, Gottingen, Germany were cultured in laboratory at 23 °C in 1 L flask in batch culture supplied with 350 mL of inorganic Bold Basal Medium

(BBM) (Bischoff and Bold, 1963). One is a toxic strain SAG 17.85 (referred to as TOX) and the other non-toxic SAG 18.85 (referred to as NTOX). The BBM composition is reported in Table 1S. During the experiments *M. aeruginosa* strains were maintained in exponential phase by periodic (3–5 days) inoculum of fresh BBM medium. This light acclimation phase was done prior to the measurements, and the cultures were acclimated for at least 3 days to the 40 μmol photons (PAR) (m^{-2} s^{-1}) with a continuous light (OSRAML 30w/956). More details on *M. aeruginosa* strains growth are reported in SI section.

The two cyanobacterial growth experiments were performed in order to monitor hepatotoxins release over the time. In both experiments the same strains, TOX and NTOX, were involved. These strains were cultivated in BBM respectively for one month (16.09.2013 – 21.10.2013, cycle I) and three months (24.03.2014 - 23.06.2014, cycle II) periods. During these periods, 15 mL aliquots of BBM media, containing TOX and NTOX strains, were sampled from the respective incubation bath twice a week, and the cyanobacteria cells were separated by filtering on 1.2 μm pore size Whatman borosilicate filter (1822-025). The filtrates were analyzed with a standard cell population count method, UV-Vis spectroscopy, UHPLC-DAD technique, colorimetric enzymatic method based on PP2A enzyme inhibition and by multisensory E-tongue system. The samples were tested on the same day with the potentiometric E-tongue system, while the smaller aliquots were stored at -20^{0}C for further enzymatic and UHPLC-DAD analysis.

Samples

Two drinking water samples (commercial mineral water and tap water, Rome) and two surface water samples collected in two different areas around Rome, Italy (Albano's Lake and Tiber river), spiked with MC standard solution or TOX and NTOX *M. aeruginosa* strains filtrates were tested. For the colorimetric enzymatic analysis, once thawed the cyanobacteria filtrates were diluted in Milli-Q water (1:50 v/v). To calibrate E-tongue, a series of additions of increasing amounts of NTOX and TOX filtrates in 50 mL of tap water (Tor Vergata zone, Rome Italy) were performed. The following relationships TOX(NTOX) filtrate/tap water were tested: 1:10000, 1:3333, 1: 2500, 1:1666, 1:1000, 1:500,

1:250, 1:100, 1:20. Subsequently, the final amount of microcystins in the samples was evaluated from UHPLC-DAD data.

Data processing

Principal component analysis (PCA) and Partial Least Squared Discriminant Analysis (PLS-DA) were applied for classification of TOX and NTOX *M. aeruginosa* strains. The PLS regression method was applied to correlate E-tongue response to MC-LR with the data obtained by both UHPLC-DAD and colorimetric enzymatic techniques. The PLS and PLS-DA models were validated using the one-leave-out cross-validation for first approximation; where the data set was enough representative, the random split test set was employed (see SI for more details). The RMSEC and RMSEV (Root Mean Square Error of Calibration and Validation respectively), and the correlation coefficient of predicted versus measured correlation line, R^2, were used to evaluate the efficiency of obtained PLS models. All chemometric treatment was performed with Unscrambler software (v. 9.7, 2007, CAMO Software AS, Norway).

4. Results and discussion

M. Aeruginosa growth monitoring

Among the different cyanobacteria genera, toxic *Microcystis* species are the most studied, due to their ability to develop bloom and to produce MCs (Chorus and Bartram, 1999). This was the main reason to employ *M. Aeruginosa* strains in this work. Although the toxicological effects of MCs are well known (Dawson et al., 1998), the factors regulating MCs content at the cellular level are not fully understood: it can vary considerably both from one bloom to another, and during the course of a single bloom (Kaebernick and Neilan, 2001; Briand et al., 2012). Moreover, natural blooms of the same cyanobacteria species do not show always the same toxicity and the toxicity of a given species can vary over the time. Generally, major toxins production is observed between half (or late exponential phase) and exponential phase of the growth (Orr and Jones,1998). The possible link between MCs production and growth rate was also reported (Long et al., 2001). Release of toxins into water can occur during cell death or senescence, but can also be due to evolutionary-derived or environmentally-

mediated circumstances, such as allelopathy or relatively sudden nutrient limitation.

In this study, the toxic strain of *M. aeruginosa* 17.85 (TOX) releases the extracellular MC-LR toxin at the initial exponential growth phase. The cell growth was monitored by UV-Vis spectroscopy and the growth curves (absorbance vs time) were plotted (Fig. 1S). No cell aggregation in *M. aeruginosa* colonies was observed during the experiments, allowing accurate optical density recordings. The increase of optical density over time was indicative of cell number increase and intracellular cyanotoxins production during the exponential growth phase. In Tables 1, 2S and on Figure 2 the concentrations of detected extracellular toxins are reported; the samples are identified by date. As it will be discussed in further sections, the quantitative toxins production, determined by potentiometric E-tongue system, chromatographic technique UHPLC-DAD and colorimetric enzymatic approach, was coherent with that reported in literature.

Insert Table 1.

Insert Figure 2.

HPLC-DAD analysis of MCs released by M. aeruginosa

The results of MCs HPLC-DAD analysis of TOX and NTOX *M. aeruginosa* strains cultivated in laboratory conditions for the first (16.09.2013 – 21.10.2013 period) and second (period from 24.03.2014 to 23.06.2014) cycles of measurements are summarized respectively in Table 1 and Table 2S. Among the eight MCs occurred into the standard solution, only two toxins were detected unambiguously: MC-YR and MC-LR. The other MCs (MC-RR, MC-LA , ect.) were not detected in the samples, being present under the limit of detection (< 1 μg/L). For these compounds no values are reported in the Table 2S. More details on MC-YR and MC-LR isomers analysis, as far as the evidence between toxic and no toxic *M. aeruginosa* strains chromatograms are reported in SI section.

The extracellular MCs concentration increase during the second measurement cycle (24.03.2014 - 23.06.2014 period) is shown in Fig. 1 (red bars). Since 50 days after beginning experiment, toxin concentrations were

stable and very low; afterwards their level began to rapidly increase till to the 80th day, when the total concentration reached about 300 μg/L. MC-YR and MC-LR showed the same pattern with the highest concentrations, 104 and 114 μg/L respectively, in 16.06.2014 sample. Afterwards, MC-YR trend decreased faster than the corresponding LR one, see Table 2S for more details. MCs are completely released from the cells into water after their death (Akin-Oriola and Lawton, 2005). The curve underlined the growth delay of MC concentrations in water solution, until the cells are alive.

MCs colorimetric enzymatic analysis

Initially, the colorimetric enzymatic assay measurements were carried out in the same experimental conditions adopted in previous works, without obtaining satisfactory results in terms of sensitivity (Wong et al., 1999; Heresztyn and Nicholson, 2001). Although Mn^{2+} is not listed as a component of the assay buffer used in the above-cited works, previous studies have found that Mn^{2+} significantly affects the activity of PP2A (Rivasseau et al., 1999). In fact, we tested the effect of different concentrations of Mn^{2+} (0-0.5 mM) on the activity of the enzyme and we found that the optimal level was equal to 0.25 mM (data not shown). Thus, we supplemented the "Reaction buffer 2.5X" with 0.625 mM of $MnCl_2$ in order to have 0.25 mM of $MnCl_2$ in each well. In these conditions a typical sigmoid calibration curve was obtained plotting the % Inhibition versus the MC-LR concentration, expressed in μg/L. The details on % Inhibition calculus and calibration curve (Fig. 3S) are given in SI section.

The cross-reactivity studies toward other toxins such as MC-LA, MC-RR, MC-YR and NOD were carried out, and the cross-reactivity percentage varied in 64-112% range (Table 3S). Then, different drinking and surface water samples, fortified with two different known concentrations of MC-LR, were analyzed and the results showed a mean value of recovery equal to 91% for the drinking and 103% for the surface water samples, Table 4S. The effect of BBM cyanobacteria growth media on the performances of the colorimetric assay was found insignificant starting from 1:50 dilution v/v of samples (Fig. 5S). Finally, the amount of MCs in TOX and NTOX filtrates, collected during the two measurements cycles were evaluated with enzymatic colorimetric assay and the

were in correspondence with a standard chromatographic UHPLC-DAD technique (Fig. 6S). The progress of the MCs production within the culture media is comparable with both methods, during the three months growth period. The enzyme PP2A is inhibited by all the MCs and the NOD, possibly present in the sample, while the UHPLC-DAD technique is able to selectively determine only few congeners, like MC-RR, dmMC-RR, MC-LR, MC-YR, MC-LA, MC-LW, MC-LF, and the NOD. For this reason, some of the samples are overestimated by the colorimetric assay in terms of MC-LR quantification.

MCs analysis with E-tongue system

The responses of the separated potentiometric sensors in tap water background spiked with BBM inorganic buffer solution, and with filtrates of NTOX and TOX *M. aeruginosa* strains grown in BBM are shown in Fig. 3. A representative case corresponding to the 80^{th} day (the date of 09.06.2014, II cycle of measurements) of algae strains growth is considered.

Insert Figure 3.

Radar plots were built in order to compare the patterns of algae strains and BBM, in different ratios respect to tap water. The response of each singular sensor is represented as an individual axis on the plot, and for every axis the range of potential variation was chosen according to the sensor behavior, while the range of potential variation (40 mV) was kept fixed for all the axes. In this way the overall patterns, composed from all the sensors responses and corresponding to the BBM, NTOX and TOX samples, can be compared. Indeed, the clear shape variation among the patterns can be noticed. The growing amounts of just BBM spiked in tap water did not result in significant sensors potential changes, thus confirming no matrix influence on the sensors behavior (Fig. 3A). Similarly, no clear pattern variation was detected for filtrate of BBM, where NTOX *M. aeruginosa* strain was cultivated, Fig.3B. On the contrary, differentiated sensor responses to the TOX *M. aeruginosa* filtrates were obtained (Fig. 3C). Such a behavior can be attributed to the change of chemical composition of the media, and in particular to the algae MC release. Indeed, the 75.4 μg/L amount of MC-LR released by toxic *M. aeruginosa* into BBM was determined by HPLC-DAD method

(see Table 2S, date 09.06.2014), and the final toxin concentration in the samples was calculated considering the dilution, ranging from $7.8 * 10^{-11}$ to $9.3 * 10^{-9}$ mol/L. On Fig. 3C in particular it should be noticed the distinct response of polymeric membrane sensors A1, A3, C2 and the significant responses of all the chalcogenide glass sensors. It was previously demonstrated that at pH 7 MCs are rather hydrophobic, despite their neutral or anionic character (due to the possible deprotonation of the glutamic and aspartic acid side chains, fragments 3 and 6 in Scheme 1 respectively) (Rivasseau et al., 1998). Due to this high hydrophobicity, MCs will tend to partitioning from analyzed aqueous media and accumulate on the surface of the polymeric organic membrane, thus influencing the sensor potential. In the case of chalcogenide glass electrodes, some electrostatic interactions among MCs and positively (CG-Ag and CG-Cu sensors) or negatively (CG-Pb) charged membranes are occurring, upon to the exposure to TOX strain filtrate. These interactions result in sensors potential drop, which can be correlated with changes of the media chemical composition, and with the MC-LR concentration in particular (Fig. 3C). In this sense, the combined response of E-tongue sensors can be used to track the MCs presence and content through chemometric modeling.

Initially the response of the E-tongue system towards MC-LR was evaluated in the concentration range from $1*10^{-10}$ to $1*10^{-8}$ mol/L in a tap water background. The correlation coefficient of PLS1 calibration towards MC-LR in the above-mentioned concentration range was 0.931, with a RMSEC of $1.6*10^{-9}$ mol/L. Then we passed to the analysis of hepatotoxins released by natural cyanobacteria strains during their growth. It is important to note that the aim of our tests was an identification of toxin released by algae and already present in analyzed sample. For this purpose, the cyanobacteria cells were separated from their growth media by filtration, in order to prevent any possibility of further release of toxic algae metabolites into the analyzed samples. Filtering upon water intake is a standard practice employed on most of the drinking water treatment plants, and our experiments were focused to mimic this treatment of water dedicated to human use.

The PLS1 model constructed for the I measurement cycle, in tap water background spiked with NTOX algae strain filtrate, has demonstrated a

satisfactory correlation among cells count and E-tongue response (R^2 0.923 and 0.776 for calibration and cross-validation respectively). Such a correlation can be explained by the changes of the media chemical composition upon the cells growth, which in turn can be tracked by potentiometric multisensory system. On the contrary, the initial attempt to produce PLS1 model for the prediction of the cyanobacteria cells number in toxic strain samples has returned very poor correlation, with squared correlation coefficient R^2 around 0.4. After elimination of the samples measured on two days (16.09.2013 and 30.09.2013), when the high concentrations of MCs were registered by UHPLC-DAD analysis (see Table 1 for details), the PLS modeling provided the reasonable results in coordinates "measured vs. predicted" for TOX algae cells count (R^2 0.971 and 0.863 for calibration and cross-validation respectively). This result indicates the strong influence of MCs in particular on the behavior of potentiometric multisensory system. Moreover, it is evident that the number of algae cells and the amount of released hepatotoxins are not directly correlated and the common analytical approach consisting in a count of cyanobacteria cells number is not reliable for the assessment of microcystin-related water toxicity. An application of E-tongue for the separate analysis of the above-mentioned samples (days 16.09.2013 and 30.09.2013) of TOX *M. aeruginosa* strain filtrates, spiked in potable water in different ratios (1:10000, 1:3333, 1:1666, 1:1000, 1:500, 1:100, 1:20), permitted a prediction of the released MC-LR, with a RMSEV of 0.015 μg/L, using PLS1 regression. Due to the small sample set available, a leave-one-out cross validation procedure was implemented to estimate the regression model success; the obtained correlation coefficients, R^2, were 0.994 and 0.812 at calibration and validation steps respectively. The PLS1 calibration of the E-tongue response versus amount of the other hepatotoxin MC-YR, detected by UHPLC-DAD on days 16.09.2013, 30.09.2013, 07.10.2013, and 14.10.2013 (see Table 1 for details) in toxic *M. aeruginosa* algae strain, provided RMSEV of 0.051 μg/L and calibration and validation correlation coefficients, R^2, 0.998 and 0.889 respectively. For the same days, the PLS-DA was conducted on a set composed of 28 samples (14 filtrates of TOX and NTOX strains respectively, corresponding to the higher cyanobacteria filtrate to tap water ratios 1:500, 1:100, 1:20). The confusion matrix of one-leave-out PLS-DA cross-validation is reported in Table 2.

Insert Table 2.

In the table rows indicate the expected algae strain class and columns correspond to those predicted. Two non-toxic samples were misclassified, as TOX, while another 9 samples could not be attributed to any class. The correct classification of 61% of samples was obtained and this preliminary result is satisfactory, considering the small data set available from the I cycle of algae growth monitoring.

At the next step of our studies a longer duration experiment of cyanobacteria growth was arranged, in order to better test the ability of the E-tongue to predict the amount of released extracellular MCs. During the II cycle of measurements the TOX and NTOX algae strains were cultivated in inorganic BBM media in laboratory conditions over three months, from 24.03.2014 to 23.06.2014. The result of PLS1 regression between the E-tongue output and MC-LR amount, determined by UHPLC-DAD during the II cycle of TOX *M. aeruginosa* strain growth monitoring, is shown in Fig. 3.

Insert Figure 3.

To estimate the success of E-tongue in MC-LR concentration prediction, the most representative periods, corresponding to the initial step of algae growth (dates from 24.03.2014 to 22.04.2014) with low toxin content and the final step when a sharp increase in the toxins production followed by the decline phase related to the cyanobacteria aging and death (days from 19.05.2014 to 23.06.2014, see Fig.2 for more details) were chosen. As in the previous case, a leave-one-out cross validation procedure was implemented, and the returned R^2 coefficients for logarithm of MC-LR concentration in coordinates “predicted vs measured” were 0.803 and 0.742, at calibration and validation steps respectively. Such a result is promising, considering the complexity of the analytical task. Nevertheless, since some sensors drift was observed during the three month period of II cycle cyanobacteria growth monitoring, we decide to perform the separate PLS modeling for two different phases of algae growth: the samples with low content of MCs (1.9-8.3 μg/L), collected during the first month of growth (from 24.03.2014 to 22.04.2014, see Table 1S for details) and those where the amount of released MCs was significant (28 - 300 μg/L, period from

22.05.2014 to 19.06.2014). Considering the consecutive dilutions of *M. aeruginosa* strain filtrates with potable water (in ratios 1:3333, 1:1666, 1:1000, 1:500, 1:100, 1:20), data set with low and high MCs content included 45 and 60 samples respectively. One third of the samples in each set (15 or 20 samples) was randomly chosen as test set for PLS modeling, while the rest of the samples was employed for calibration. The random splitting procedure was repeated 20 times for both low and high MCs content data sets. To evaluate the constructed PLS models, RMSEV values were averaged over the splits and resulted values were 0.231 and 0.035 μg/L of MCs, determined with enzymatic and UHPLC-DAD methods for "low" and 1.181 and 0.297 μg/L for high sample sets correspondingly. The PLS results MCs prediction by means of potentiometric E-tongue system are summarized in Table 3. The satisfactory correlation coefficients and RMSEV lower than or very close to 1 μg/L, the provisional guideline value of WHO for MCs content in potable waters, open up a new perspective in the field of environmental monitoring, by means of fast and inexpensive potentiometric multisensory system.

Insert Table 3.

5. Conclusions

The application of a potentiometric E-tongue for the qualitative and quantitative detection of MCs contamination of different water matrices has been investigated and the results obtained have been compared with those obtained from the standard chromatographic technique UHPLC-DAD, and a colorimetric enzymatic analysis. The experiments demonstrate the the success of the E-tongue in the MCs concentration prediction, showing also the possibility to evaluate the amount of MCs released by cyanobacteria during their growth. Based on obtained results, Electronic tongue system can be considered a promising easy-to-handle tool for express water toxicity assessment.

6. Acknowledgements

The research leading to these results has received funding from the Italian Ministero dello Sviluppo Economico, under Grant Agreement "Industria

2015 – New Technologies for the Made In Italy", No. MIOl 00223 (project ACQUASENSE) and partially financially supported by Government of Russian Federation, Grant 074-U01. The work of C. C. Guanais Gonçalves is sponsored by CNPq – Brazil. Dr. L. Lvova acknowledges A. Gattaino and S. Autieri for the part of performed work. R. Congestri acknowledges CyanoCOST, "An international network for Cyanobacteria and Cyanotoxins in water resources" (COST Action ES 1105).

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Table 1:
Extracellular concentration (µg/L) of MCs and unknown compounds related to MCs measured with UHPLC-DAD during I cycle of *Microcystis aeruginosa* growth *(*period from 16.09.2013 to 21.10.2013)

Analyte (µg/L)	Retention Time (min)	Date						
		TOX					NTOX	
		16/09	23/09	30/09	07/10	14/10	07/10	21/10
MC-RR	1.53; 1.57	-	-	-	-	-	-	-
NOD		-	-	-	-	-	-	-
MC-YR	1,73; 1.76; 1.80	10	<1	29	6	5	7	-
MC-LR	1.84; 1.88	6	<1	11	<1	<1	-	-
MC-LA	2.20	-	-	-	-	-	-	-
MC-LW	2.63	-	-	-	-	-	-	-
MC-LF	2.70	<1	<1	30	<1	<1	-	-
Cells x 1000 (million/ml)		560	1240	2183	5417	4600	8463	9483

Table 2.
PLS-DA confusion matrix of classification TOX and NTOX *M. aeruginosa* strains during I cycle algae growth monitoring.

Expected	Predicted	
	TOX	NTOX
TOX	**9**	2
NTOX	0	**8**
non classified	5	4

Table 3.
The E-tongue system performance in MCS content PLS prediction

Correlation vs E-tongue	MCs content	Correlation coefficient, R2	RMSEC (μg/l)	RMSEV (μg/l)
UHPLC-DAD	1.9-8.3 μg/L	0.880	0.019	0.035
	28 - 300 μg/L	0.954	0.164	0.297
enzymatic approach	1.9-8.3 μg/L	0.924	0.148	0.231
	28 - 300 μg/L	0.917	1.140	1.181

Figure captions

Scheme 1: The chemical structure of MC-LR

Figure 1: Extracellular concentration (μg/L) of MC-LR and MCs sum measured with UHPLC-DAD during II cycle of *M. aeruginosa* growth (period from 24.03.2014 to 23.06.2014).

Figure 2: The responses of potentiometric sensors in tap water spiked with BBM inorganic buffer solution (A), NON TOX (B) and TOX (C) *M. aeruginosa* strains grown in BBM.

Figure 3: PLS correlation result for MCs content determined by means of potentiometric E-tongue system.

Scheme 1.

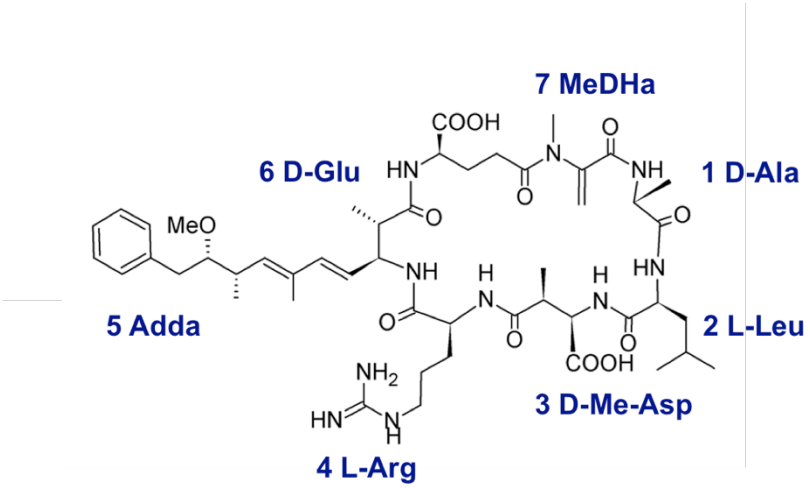

7 MeDHa
6 D-Glu
1 D-Ala
5 Adda
2 L-Leu
3 D-Me-Asp
4 L-Arg
COOH
HN
NH
MeO
O
N
H
NH2

Figure 1

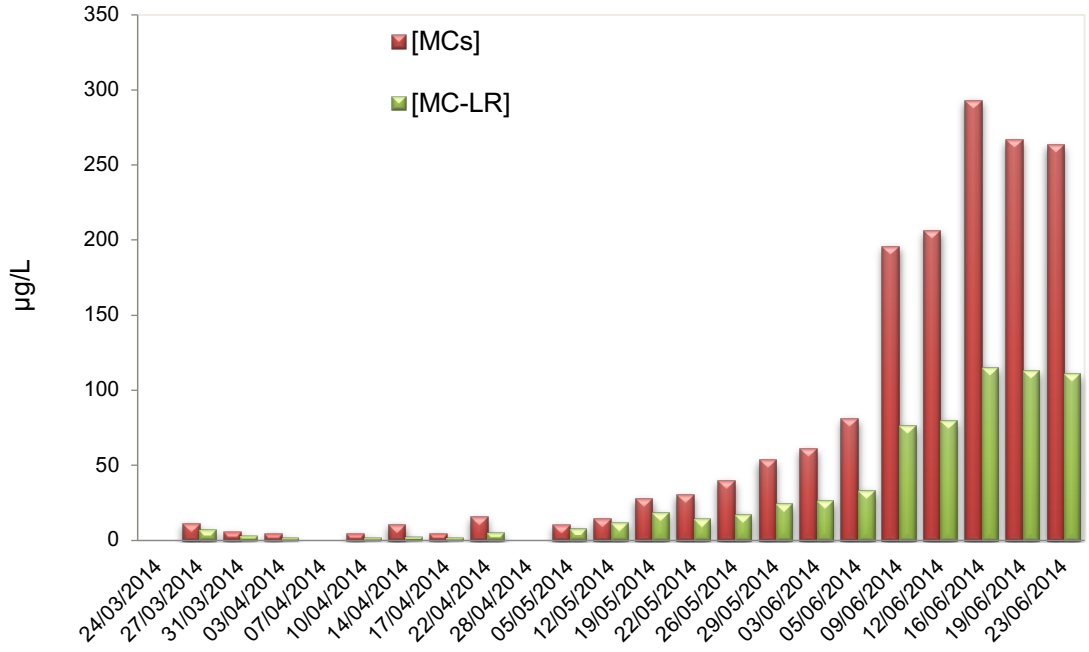

350
300
250
200
150
100
50
0
µg/L
[MCs]
[MC-LR]
24/03/2014
27/03/2014
31/03/2014
03/04/2014
07/04/2014
10/04/2014
14/04/2014
17/04/2014
22/04/2014
28/04/2014
05/05/2014
12/05/2014
19/05/2014
22/05/2014
26/05/2014
29/05/2014
03/06/2014
05/06/2014
09/06/2014
12/06/2014
16/06/2014
19/06/2014
23/06/2014

Figure 2

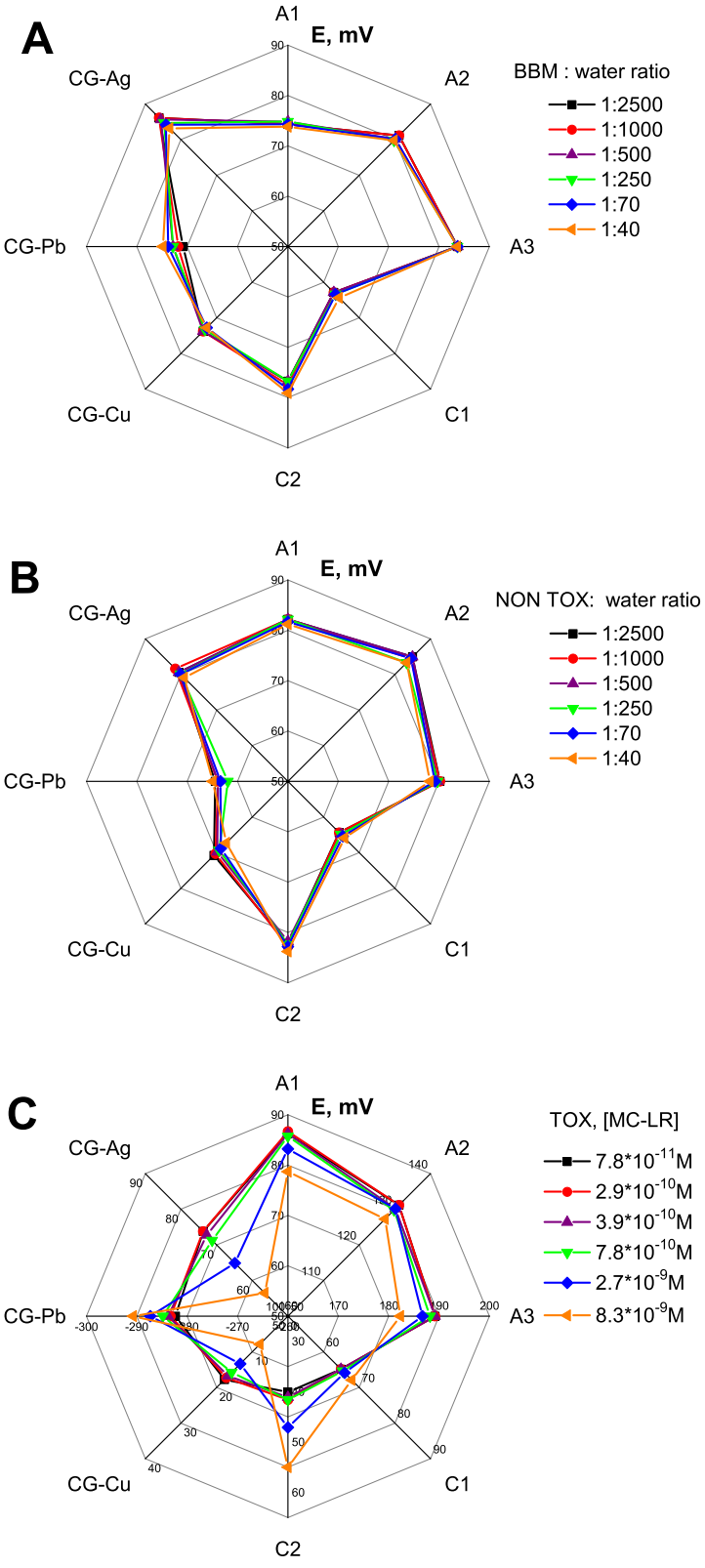
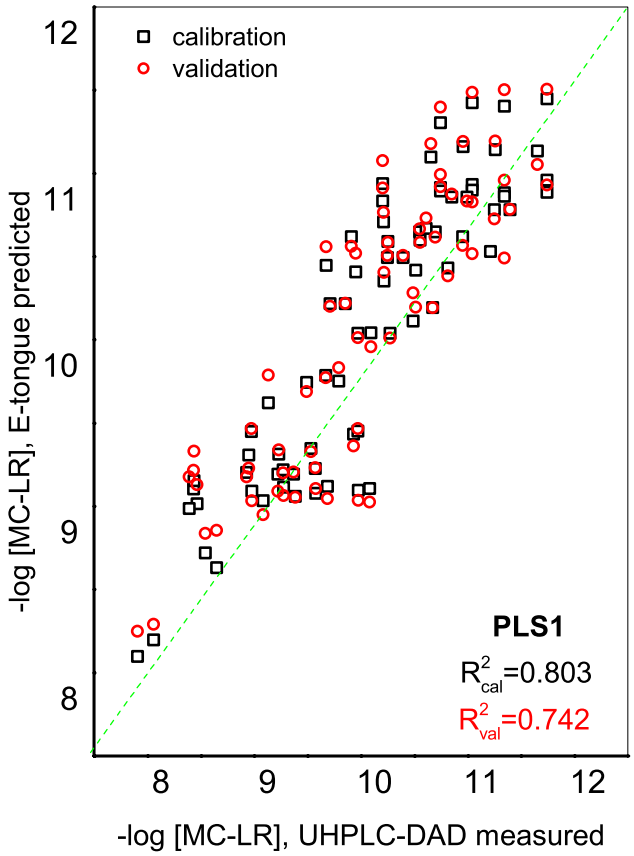

A
E, mV
A1
A2
A3
C1
C2
CG-Cu
CG-Pb
CG-Ag
BBM : water ratio
1:2500
1:1000
1:500
1:250
1:70
1:40
B
E, mV
NON TOX: water ratio
1:2500
1:1000
1:500
1:250
1:70
1:40
C
E, mV
TOX, [MC-LR]
7.8*10-11M
2.9*10-10M
3.9*10-10M
7.8*10-10M
2.7*10-9M
8.3*10-9M

Figure 3


calibration
validation
PLS1
R^2_{cal}=0.803
R^2_{val}=0.742
-log [MC-LR], E-tongue predicted
-log [MC-LR], UHPLC-DAD measured
8
9
10
11
12