Validation of SPE-TOXcontrol for the determination of metals in drinking water

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Introduction

The potential of SPE-TOXcontrol as a new early warning system to detect possible hazards in drinking water was assessed. The work was done in the framework of the WATMATIN project funded by the Spanish Ministry of Science and Innovation (CTM 2010-21182).

Objectives:

Main:

The evaluation of SPE-TOXcontrol assay for in situ drinking water toxicity determination of metals coming from the migration from distribution networks.

Specific:

a) Determination of EC_{50} (effective concentration of the tested chemical at which mortality or immobility occurs to 50 % of organisms) of metals;

b) Recovery evaluation for different sorbent materials using SPE methodology.

Figure 1 *TOXcontrol unit*

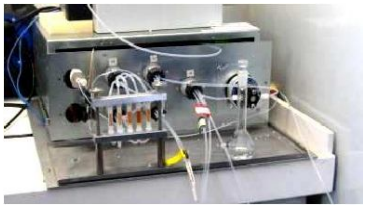

Figure 2 *SPE unit.*

Materials & Methods

Determination of EC_{50} for selected metals (Cu, Cr, Fe, Ni, Pb) was performed using TOXcontrol which allows to perform the procedure indicated in EU-EN ISO 11348 in a fully automated way (see Figure 1). System works with a marine bacteria *Vibrio fischeri.* The freeze dried *V.fischeri* was cultivated in a separate TOXbioshaker unit where automated and controlled cultivation takes at least 5 days. Afterwards freshly cultivated bacteria were placed in the bacteria module of the TOXcontrol unit, where it was maintained at 5°C during one week.

Measurement: Firstly 50 µl of bacteria is mixed with 2% NaCl solution for 5 minutes, in order to bring bacteria from 5°C to 15°C. Then the bacteria are mixed with the known concentration of toxicant, i.e. metal (incubation time 15 or 30 min). Inhibition of light emission in the presence of toxicants was observed with the incubation time of 15 min (30 min) at 15°C. Luminescence was measured with a photomultiplier placed in the light-closed housing. The resulting dose-response curve was used to determine the EC_{50} of toxicant (metal). All experiments were performed in triplicate.

The Solid Phase Extraction experiments were performed with the SPE unit shown in the Figure 2. It was chosen to test two different procedures for the SPE described in the literature (Tsogas G.Z., et. al., 2009; Otero-Romani J., et. al., 2005; Otero-Romani J., et. al., 2011; Grotti M., et. al., 2003; Abbasse G., et. al., 2002) to find out which one is better for the current study. In addition, to test the SPE method three types of cartridges were used: C-18 and Oasis and CT-1.

Optimized procedure: 1000 ml of HPLC water was spiked with a mixture of 5 (Cu, Cr, Fe, Ni, Pb) metals, giving the concentration of each metal in the sample 100 g/l or 20 g/l; then a volume of 1 ml of 0.5 M 8-HQ solution3 (giving a final 8-HQ concentration 5*10-4 M) was added to the sample. Experiments were done in triplicates, besides one blank experiment for each cartridge was performed.

Results

Determination of EC_{50}

Compound	pH	EC50 (mg/L)	
		15 min	30 min
$CuSO_4*5H_2O$	5.40	10.61	4.68
$CuSO_4$	~5.5	6.27	2.05
$NiSO_4*6H_2O$	~7	317.20	221.50
$Cr(NO_3)_2*9H_2O$	~4.5	190.40	123.00
$Fe_2(SO_4)_3*xH_2O$	~2.5	52.08	-
$Pb(NO_3)_2$	~5	70-110	80-130

Table 1 *Determined EC_{50} values for 15 min and 30 min incubation time for Copper (II) sulphate pentahydrate; Copper (II) sulphate anhydrous, Nickel (II) sulphate hexahydrate, Chromium (III)nitrate nonahydrate and Iron (III) sulphate hydrate.*

The toxicity of studied compounds is as follows:

$CuSO_4 > CuSO_4*5H_2O > Fe_2(SO_4)_3*xH_2O > Pb(NO_3)_2 > Cr(NO_3)_2*9H_2O > NiSO_4*6H_2O$

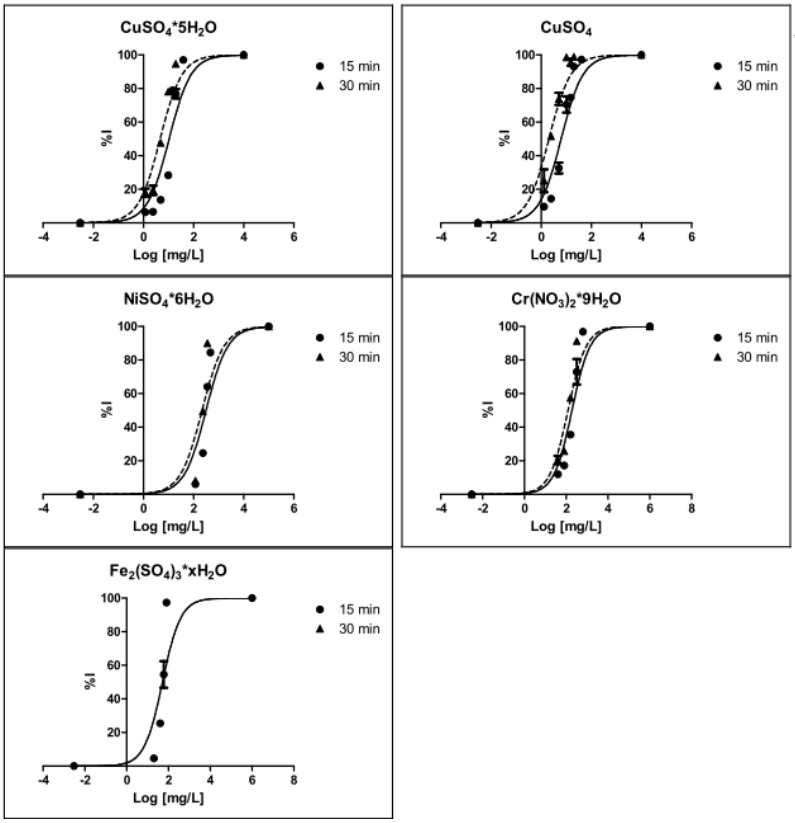

Figure 3 *Inhibition curves for 15 min and 30 min incubation time for Copper (II) sulphate pentahydrate; Copper (II) sulphate anhydrous Nickel (II) sulphate hexahydrate, Chromium (III)nitrate nonahydrate and Iron (III) sulphate hydrate (only 15 min).*

SPE: Recovery evaluation

Recoveries of three cartridges (Oasis, C-18 and CT-1) were tested. Initial experiments showed very low recoveries (up to 15%). To follow up, the procedures were optimized for the best performed cartridges, i.e. Oasis and CT-1.

According to the results of follow up experiments Oasis cartridges are able to recover more metals and in higher quantity when compared to CT-1.

Out of five tested metals CT-1 showed no recovery abilities for Cu, Cr, Ni and Pb, while the recovery of Fe was very low, and reached not more than 7%.

While Oasis (Table 2) showed no recovery ability for Cr and very low recovery of Ni (up to 1.5%), recoveries of Cu reached up to 65.3 %, of Fe up to 46.1% and Pb up to 22.6%. In addition, Oasis showed better recovery for the samples spiked with lower concentrations of metals (20 g/l). The insufficient amount of 8-HQ chelant might be the reason for lower recovery results of samples spiked with higher concentration of metals (100 g/l).

Metal	Recovery, %	SD	Recovery, %	SD
	C=100 µg/l		C=20 µg/l	
Cu	36.2	6.1	60.1	5.5
Cr	-	-	-	-
Fe	31.2	5.9	41.0	6.6
Ni	-	-	-	-
Pb	2.9	4.1	12.0	10.1

Table 2 *Recovery evaluation: Oasis cartridge*

Conclusions

The EC_{50} values of four metals (cupper, iron, chromium, nickel) salts and a concentration range in which EC50 value is expected to be (for lead nitrate) were determined. The toxicity of studied compounds is as follows: $CuSO_4 > CuSO_4*5H_2O > Fe_2(SO_4)_3*xH_2O > Pb(NO_3)_2 > Cr(NO_3)_2*9H_2O > NiSO_4*6H_2O$.

It was observed, that toxicity of tested compounds on *V.fischeri* is greater after 30 min incubation time; accordingly the toxicity of metals is lower after 15 min incubation time; although 15min incubation time is less likely to produce a 'carry over' effect.

In order to be able to detect concentrations of studied metals in the range of Drinking Water Directive requirements, TOXcontrol has to be coupled with sample pre-concentration unit, possibly SPE.

Recommendations

- Evaluate toxicity of metal mixtures.
- Assess the influence of different compound containing the same metal to the toxicity of *V.fischeri.*
- Assess influence of the pH on the toxicity.
- For optimization of SPE procedure the higher amount of chelant in samples should be tested.