



## Using Stripping Voltammetry to Analyse the Interactions of Metals with the Biomass of Acidophilic Algae

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### ABSTRACT

Interactions of metals with the biomass of plant organisms, including algae, are of interest for biology, biogeochemistry and biotechnology. This work studies the interactions of the unique thermophilic red algae *Galdieria sulphuraria* (Class: Rhodophyta; Family: Cyanodiaceae) with copper and lead in the aquatic environment. This extremophilic, acidophilic organism is found in such ecosystems as hot springs and geothermal habitats. This paper presents the results of experiments with the biomass and mortmass of this organism. The results indicate that the biomass of this organism immobilises copper after incubation in aquatic medium with heavy metals. Lead was also added to the incubation environment, but no immobilisation of lead from the aquatic environment was observed. The mortmass of *G. sulphuraria* immobilised neither copper nor lead.

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### Introduction

Scientific issues related to biogeochemistry, biology and related sciences continue to attract researchers' attention.

V.I. Vernadsky proved the importance of scientifically analysing the migration of chemical elements in the biosphere and the elemental composition of the biosphere components (Vernadsky, 1924, 1926, 1998a,b, 2006; Dobrovolsky, 2007).

Instrumental methods of analysing the elemental composition make it possible to analyse the elemental composition of biogenic material samples, including the biomass of plant bodies. We previously identified a number of examples where samples of the biomass of aquatic plants immobilised some chemical elements (Ostroumov and Kolesov, 2010; Johnson et al., 2011). This paper presents further results in this area using the biomass of the unicellular eukaryotic organism *Galdieria sulphuraria* (Galdieri) Merola. This organism has unique features, including the ability to grow in an environment with extremely low pH and high temperature (hot springs and geothermal habitats), and it is of interest for biotechnology (Minoda et al., 2015; Jain et al., 2014; Carfagna et al., 2014; Selvaratnam et al., 2014; Allen, 1959). The goal of this work is to test the hypothesis that the biomass of the thermophile, acidophile *G. sulphuraria* can immobilise certain heavy metals using the stripping voltammetry method (Ostroumov and Shestakova, 2009a).

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## Methods

Cultivation of algae. The culture of the thermophilic algae *G. sulphuraria* was cultivated in Allen medium (Allen, 1959) in a shaker (90 rpm) at 34 °C under white light illumination (60  $\mu\text{E}/\text{m}^2$ , photoperiod: 10 h light, 14 h dark). Allen medium was acidified with sulphuric acid to pH = 2.6 prior to sterilisation.

The initial concentration of cells in the medium was 1 million cells per 1 mL. The cells were spun down from suspension by centrifugation at 4 thousand rpm for 15 min.

## Incubation

Incubation was carried out in an aquatic medium with added metals for 90 min at 24 °C. The multi-element solution for the incubation was prepared using double-distilled water and the following certified reference samples: zinc (state standard reference sample No. 7770-2010, concentration of 1 mg/mL in 1 M hydrochloric acid), cadmium (state standard reference sample No. 7773-2008), copper (state standard reference sample No. 8210-2008), and lead (state standard reference sample No. 778-2008, concentration of 1 mg/dm<sup>3</sup> in 1 M nitric acid).

Sodium hydrogen carbonate was used to neutralise the excess acidity, resulting in a final solution pH of 2.4.

The calculated concentrations of metals in the solution are as follows, in mg/L: Cu – 2; Pb – 0.1; Zn – 2; Cd – 0.1.

The resulting solution was analysed by mass spectrometry with inductively coupled plasma (ICP-MS). The analytical results were as follows, in ppm: Cu – 1.854; Pb – 0.102; Zn – 2.174; Cd – 0.092.

The metal concentrations were chosen based on the relative degree of toxicity of metals and the possible presence of these metals in the polluted water of aquatic ecosystems.

## Sample Preparation and Measurement

The experiment yielded 4 biomass samples, which were dried to a constant weight at 80 °C in a drying oven. After that, they were ashed with 2 drops of concentrated nitric acid, first on a hot plate, and then in a muffle at 450 °C for 2 h. Table 1 presents sample descriptions and the weight of the dry matter and ash.

Ash of solid samples was dissolved by acid leaching (concentrated HCl, concentrated HNO<sub>3</sub> and 1:1 H<sub>2</sub>SO<sub>4</sub>). After decomposition, the samples were transferred into sterile centrifugal volumetric tubes and brought to the appropriate volume with double-distilled water (2.7  $\mu\text{S}/\text{cm}$ ).

Mortmass. Samples were obtained by drying the biomass at 90 °C for 4 h, before which the samples were stored in a freezer at –15 °C for 1.5 months. Dried samples were in the form of a dark brown vitrified mass. The mass was ground into a powder with a pestle in a porcelain mortar prior to incubation.

The stripping voltammetry method was previously described (Ostroumov and Shestakova, 2009). The concentration of copper in prepared solutions was determined after the decomposition of biomass by the stripping voltammetry method using the AKV – 07 MK analyzer (OOO “Akvilon”, Russia) with a three-electrode sensor (rotating measuring carbosital electrode AKU-1, auxiliary electrode and reference electrode). Measurements of copper and lead were performed according to the copper programme at an accumulation potential of –0.9 V against 0.05 M HCl and  $1 \cdot 10^{-4}$  M Hg(NO<sub>3</sub>)<sub>2</sub>. Automated measurements and results processing were carried out in the “Polar-4.1” programme. The detection limit is 2 ppb ( $\mu\text{g}/\text{l}$ ), and the convergence (relative root-mean-square standard deviation) is 10%. This method allows for the detection of electrochemically active forms of copper that can be reduced to metal under these conditions to form the amalgam.

## Results

Table 2 presents the lead and copper contents measured by stripping.

Table 2 shows that the concentration of copper in the biomass is significantly increased after incubation. No increases were detected in the concentration of copper (immobilisation) in the mortmass or of lead in either the biomass or mortmass. Other metals (zinc and cadmium) were also not immobilised in these samples (data not shown in the table).

These results are comparable with data from similar experiments conducted on other biological objects. Previous studies reported an increase in the metal content after incubation of another biogenic material in aquatic medium containing added metals (for example, (Ostroumov and Kolesov, 2010; Johnson et al., 2011; Ostroumov, 2011)).

**Table 1**

Characteristics of the analysed samples.

Sample	Dry weight, g	Ash weight, g	Ash content, %
Sample 1 – biomass after incubation	0.6265	0.0493	7.9
Sample 2 – biomass without incubation	0.6590	0.054	8.2
Sample 3 – dead biomass (mortmass) after incubation	0.3962	0.0201	5.1
Sample 4 – dead biomass (mortmass) without incubation	0.5276	0.0249	4.7

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