ELEMENTAL CONTENT OF INDIGENOUS BACTERIA UNDER DIFFERENT CHROMIUM LOADINGS

N.Ya. Tsibakhashvili[1,2], L. Mosulishvili[1], E. Kirkesali[1], T. Kalabegishvili[1,2], S. Kerkenjia[2]

[1]*Andronikashvili Institute of Physics, Tbilisi, Georgia,*
[2]*Chavchavadze State University, Tbilisi, Georgia*

M.V. Frontasyeva, I. Zinicovscaia

FLNP JINR, e-mail: marina@nf.jinr.ru, zing@nf.jinr.ru

Introduction

Anthropogenic activity is a source of continual influx of heavy metal contamination into the environment. A complex variety of abiotic and biotic processes affects their speciation and distribution. Some of these processes can be applied to removing environmental pollutants. Indigenous bacteria can be successfully used to either detoxify or immobilize toxic heavy metals.

These bacteria are under continuous investigation, and in-depth molecular understanding has been developed for some of them. However, up to date the dependence between the ability of bacteria to reduce or immobilize metals and their elemental compositions is not clear yet.

This work is a continuation of our studies (Tsibakhashvili et al., 2004; 2006) where epithermal neutron activation analysis (ENAA) had been applied to study elemental content of Cr(VI)-reducer basalt-inhabiting bacteria.

In the present study the effect of Cr(VI) on the elemental content of these bacteria has been examined. Specifically, we tested three Gram-positive bacterial strains of *Arthrobacter* genera – *A. oxydans* (isolated in the USA from polluted Columbia basalt rocks), *Arthrobacter* sp. (61 B), and *A. globiformis* (151 B) (isolated from the most polluted regions in the Republic of Georgia).

Materials and Methods

Sample cultivation and preparation for analysis. The bacteria were grown aerobically in the following nutrient medium: 10 g of glucose, 10 g of peptone, 1 g of yeast extract, 2 g of caseic acid hydrolysate, 5 g of NaCl, and 1 liter of distilled water. To provide the chromium concentration of 35 and 200 mg/L, Cr(VI) [as K_2CrO_4] was added to the nutrient medium at an early stationary phase of growth.

After being cultivated for 4 days the cells were harvested by centrifugation (10,000 rpm, 15 min, 4 °C), rinsed twice in a 20 mM phosphate buffer and analyzed by NAA method.

To prepare bacterial samples for NAA, wet biomass was placed in an adsorption-condensation lyophilizer, dried, and pelletized to 5 mm pieces (~0.5 g) by means of titanium press form.

Instrumental Neutron Activation Analysis (INAA) at the reactor IBR-2. Bacterial samples of about 0.5 g were packed in aluminum cups for irradiation.

Samples were irradiated for 100 h and gamma-spectra of induced activity measured for 30 min – 2 hrs to provide sufficient counting statistics.

Quality assurance was achieved by relevant certified reference materials Lichen-336 and Bottom Sediments SDM-2T.

Results and discussions

The concentrations from 12 to 19 elements of the following set: Na, Al, Cl, K, Fe, Co, Zn, As, Br, Rb, Sr, Sb, Ba, Tb, Th, U was determined in the bacterial cells.

Data on chromium shows the high rate of Cr accumulation in tested bacterial cells (Fig. 1). The chromium content in the control cells was less than 10 μg/g, while the same values in the treated cells were much higher. For example, in *Arthrobacter* sp. it reached to 3 $.10^3$ μg/g after exposure to 35 mg/L of Cr(VI) for 4 days. According to our recent studies (Codd et al., 2006; Tsibakhashvili, Kalabegishvili et al., 2008), reduction of Cr(VI) to Cr(III) begins at the surface of bacteria with the formation of Cr(V)-diols and the main part of reduced chromium (Cr(III hydroxide) is tightly bound to bacterial cells. Our current ENAA data provide evidence that one part of chromium penetrates inside cells as well.

Really, in bacteria treated with chromate some similarity in the behaviour of the following essential elements- potassium, sodium, chlorine was observed. First, exposure to Cr(VI) caused a lower potassium concentrations in cells and the decrease of K content was almost equal at both low (35 mg/L) and high (200 mg/L) concentrations of Cr(VI). Second, concentrations of sodium and chlorine changed in a parallel way to each other, but in an opposite way to that of potassium. Potassium is known to play an important role in maintaining cellular osmotic pressure; it is involved in nonspecific activation of many enzymes, in bacterial energy metabolism (as the coupling ion), and in the regulation of intracellular pH (Hoghes et al., 1989). Decrease of K content, in other words extrusion of K from cells to maintain the acidity of their cytosol, concomitant with increase of Na (and correspondingly Cl) content, suggests that, one part of Cr(V)-diols (as well as Cr(VI) ions (via HPO_4^{2-} channels)) were able to penetrate inside bacterial cells. NAA measurement of iron content in bacteria supports this conclusion. As is known, iron is the most important metal biologically (Hoghes et al., 1989). It is a many-functional constituent of complex molecules. Figure below demonstrates that in the tested bacteria the Fe content significantly increased in response to Cr(VI) loading, indicating that the bacterial protective system was activated significantly against chromium toxic impact.

In A. globiformis, contrary to A. oxydans and Arthrobacter sp, the content of iron increased almost linearly with increase of Cr(VI) dose. It seems that Cr(VI) transformation mechanism is rather different in *A. globiformis* than in *Arthrobacter oxydans* and *Arthrobacter* sp., that agrees with our recent data received by NAA and ESR (Tsibakhashvili N., Mosulishvili et al., 2008).

U, Rb, Nd, As were determined in all bacteria. These non-essential elements have no beneficial function and have to be considered by cells as toxins, however behaviour of some of them (for example, U) also illustrates that the permeability of bacterial cell wall changed after treatment with chromium.

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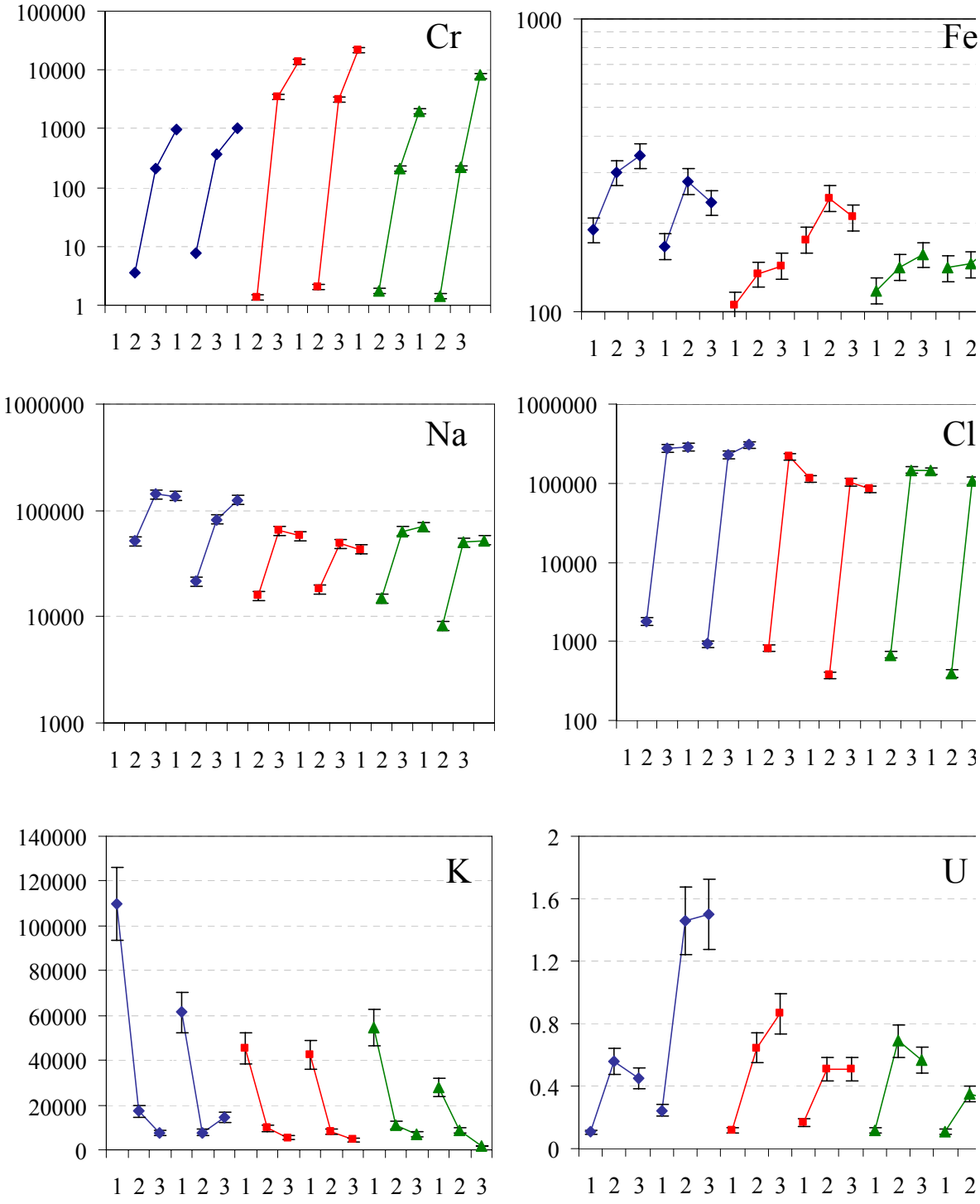

Figure: Concentration of chromium in different species of *Arthrobacter* und Cr(VI) loadings: 1 – control; 2 – 35 mg/L Cr(VI); 3 – 200 mg/L Cr(VI) and changing elemental concentrations of Fe, Na, Cl, K, and U (μg/g) in the same species as a response to

Cr(VI) impact on bacterial cell wall permeability.