Characterization of bacteria in nickel-rich kaolinite, montmorillonite and saponite mixture from Gornje Orešje, Croatia

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INTRODUCTION

A variety of minerals were detected within the serpentine body near Gornje Orešje, Croatia[1]. This association was a result of the hydrothermal alteration of former peridotitic rock. Later weathering processes produced a layer of lateritic crust which contained goethite that is rich in Upper Cretaceous fossils. Millerite was common inside green clay pockets and magnezite was an alteration product of peridotite. Siderite was preserved as a subparallel group of crystals on contact with Cretaceous limestone and montmorillonite clay, while nontronite was found between serpentine and laterite. Calcite was widespread in Cretaceous limestone as part of preserved fossils, as sediment, but also as an alteration product of serpentine. Pyrite was located within cracks and vuggs of Cretaceous limestones, as well as in fossils. Quartz, a final alteration product, was found inside the serpentine body. Sepiolite veins are up to 10 cm wide and up to a few meters long. The aim of this work was to mineralogically and bacteriologically characterise the nickel (Ni)-rich clay from Gornje Orešje in Croatia.

MATERIALS AND METHODS

The clay was collected from an abandoned quarry in Gornje Orešje in May 2018 (Fig. 1). For ultrastructural analysis, a sediment sample was processed according to standard scanning electron microscopy (SEM) processing procedures. In addition, for energy dispersive spectroscopy (EDS) analysis, a sediment sample was embedded in epoxy resin, polished and carbon coated. The samples were viewed and analysed with the Zeiss Ultra PLUS FEG SEM and Oxford Instruments EDS detector, respectively.

Figure 1: The abandoned quarry in Gornje Orešje from where the Ni-rich clay (greyish-green part) was collected.

Total heterotrophic bacteria were aerobically grown on Nutrient agar (Biolife) at 22ºC/72 h, whereas Carbapenem-resistant bacteria were established on CHROMagar Acinetobacter supplemented with CR102 (CHROMagar) at 35ºC/72 h[2]. The numbers of bacteria were expressed as log Colony Forming Units (CFU) per 1 mℓ of wet clay. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) identified the colonies of carbapenem-resistant bacteria.

RESULTS

The light greyish-green sample of alteration product of peridotite mother rocks was recognized as a mixture of kaolinite, montmorillonite, saponite and negligible quantity of quartz and calcite, as described in Posilović (2009). The EDS analysis (Fig. 2A) confirmed the presence of Ni in the sample (Fig. 2B).

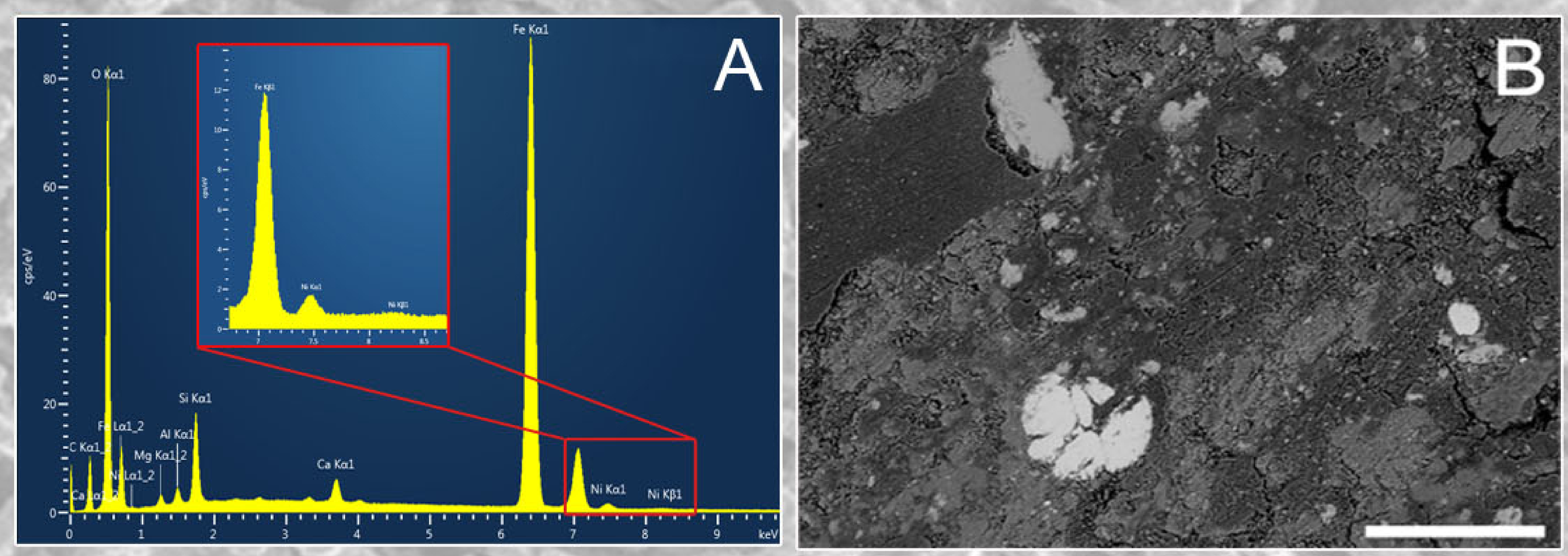

Figure 2: **A:** EDS analysis (25kV) confirming the presence of Ni (insert) in the clay. **B:** Backscatter electron micrograph of Ni-rich area analysed (Scale bar: 500 µm).

SEM analysis confirmed that the Ni-rich clay is a habitat for dense bacterial population (Fig. 3) as the bacteria were strongly attached to the clay particles with signs of biofilm formation. The bacteria were mostly restricted to the edges of montmorillonite sheets, which probably represents the sites of easy access of bacteria to the cations present in mineral.

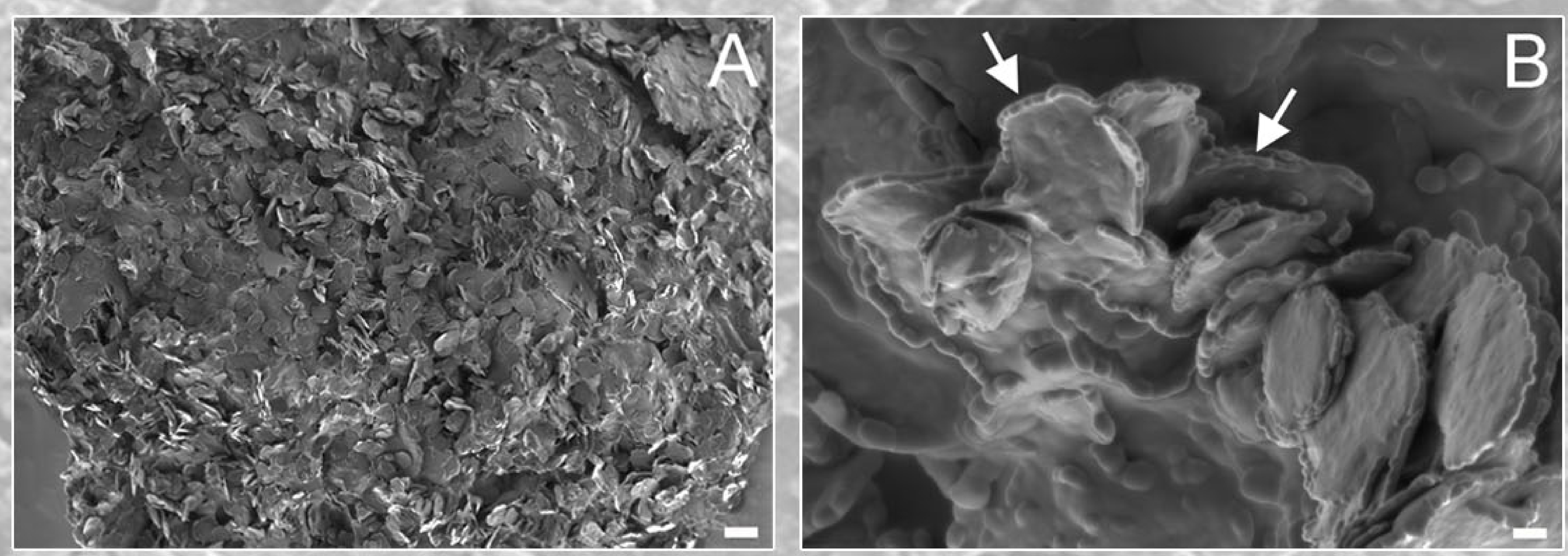

Figure 3: Scanning electron micrographs of **A:** The Ni-rich clay and **B:** The bacteria (arrows) that was mostly restricted to the edges of montmorillonite sheets (Scale bars: A: 2 µm and B: 200 nm).

The abundance of total heterotrophic bacteria grown at 22°C was 7.0±0.8 log CFU/mℓ of wet clay (Fig. 4). MALDI-TOF MS analysis verified that the population of carbapenem-resistant bacteria was represented by *Stenotrophomonas* sp. and *Pseudomonas* sp. Although both bacterial genera are normally found in the environment, including soil, *Stenotrophomonas* sp. is intrinsically resistant, while *Pseudomonas* sp. is acquired resistant to carbapenems[4].

Figure 4: Heterotrophic bacteria grown on Nutrient agar plate inoculated with clay sample diluted 100,000 times in peptone water.

CONCLUDING DISCUSSION

The Ni-rich clay from Gornje Orešje in Croatia represents a mixture of kaolinite, montmorillonite and saponite. The clay is a habitat for dense bacterial population, including the clinically relevant species with acquired resistance to last-resort antibiotics. Bacteria resistant to carbapenems represent a worldwide problem, because carbapenems are used as last-resort antibiotics to treat human infections caused by antibiotic-resistant bacteria[5]. While the intrinsic chromosomally encoded carbapenem-resistance genes are of low epidemiological relevance, the acquired plasmid-located genes raises public health concern.

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