

CHEMICAL CHARACTERIZATION OF STROMATOLITIC "PETOLA" LAYER
(SEČOVLJE SALT-PANS, SLOVENIA) USING FT-IR SPECTROSCOPY

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ABSTRACT

At Sečovlje salt-pans (Gulf of Trieste, northern Adriatic), the stromatolitic microbial mat named "petola" is a crucial element in old mediaeval manner of salt-production, so the understanding of its chemistry is highly significant for the preservation and progress of this activity. The infrared spectra (FT-IR) of homogenized bulk samples were recorded to investigate the petola composition and modifications. The FT-IR results revealed carbonates, gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) and silicates as the major inorganic components, while the content of organic matter (OC; 4.3–6.4 wt. %) was rather low. During sampling period, including increase of brine salinity and maturation of petola, the concentration of silicates, organic carbon (OC) and total nitrogen (TN) is greatly reduced and the carbon nitrogen (OC/TN) atomic ratio increased. The present work confirmed the usefulness of FT-IR techniques for characterization of petola composition and its transformation and suggests its application for fundamental investigations of hypersaline systems.

Key words: stromatolite-petola, FT-IR, elemental composition, Sečovlje salt-pans, northern Adriatic Sea

CARATTERIZZAZIONE CHIMICA DELLO STRATO STROMATOLITICO "PETOLA" (SALINE
DI SICCIOLLE, SLOVENIA) CON L'USO DELLA SPETTROSCOPIA FT-IR

SINTESI

Alle saline di Sicciole (Golfo di Trieste, Adriatico settentrionale), lo strato stromatolitico microbico chiamato "petola" è un elemento cruciale nella produzione del sale col vecchio metodo medievale, quindi la conoscenza del suo processo chimico è molto importante per la conservazione e lo sviluppo di tale attività. Allo scopo di ricercare la composizione e le modificazioni della petola è stato registrato lo spettro infrarosso (FT-IR) di grandi campioni omogeneizzati. I risultati FT-IR hanno rivelato la presenza di carbonati, gesso ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) e silicati quali componenti inorganici principali, mentre è risultato basso il contenuto in materia organica (OC; 4,3–6,4 wt. %). Durante il periodo di campionamento, compreso l'incremento della salinità dell'acqua salmastra e la maturazione della petola, la concentrazione di silicati, carbonio organico (OC) e azoto totale (TN) si è di molto ridotta, mentre è aumentato il rapporto atomico carbonio azoto (OC/TN). La ricerca conferma l'utilità delle tecniche FT-IR per la caratterizzazione della composizione della petola e della sua trasformazione e suggerisce l'applicazione di tali tecniche per l'indagine fondamentale dei sistemi ipersalini.

Parole chiave: stromatoliti-petola, FT-IR, composizione elementare, saline di Sicciole, Adriatico settentrionale

INTRODUCTION

In the past, the littoral region along the Gulf of Trieste was greatly marked by salt-pans that are now mainly abandoned. Nowadays, the Sečovlje and Strunjan salt-pans are the only ones on our coast where salt production has survived. Sečovlje salt-pans are also known for their characteristic landscape and as an area with rich natural and cultural heritage. In 1993, they were included on the list of wetlands of international concern, as stipulated by the Ramsar Convention, and are one of the most important tourist and educational destinations. Today, the protection and preservation of natural and cultural heritage within Sečovlje Salina Nature Park and production of salt in the traditional manner are under the auspices of SOLINE Pridelava soli d.o.o. (Salt Production Co. Ltd.).

The Sečovlje solar (Fig. 1) salt-pans are located in the southern part of Piran Bay (northern Adriatic) along the River Dragonja delta. Salt production proceeds by fractional salt precipitation and finally the selective recovery of a very pure NaCl through solar evaporation and concentration of seawater in crystallization basins. Due to different solubility, the evaporation process from supersaturated seawater initially results in precipitation of CaCO_3 , followed by crystallization of CaSO_4 , and finally the halite precipitates (Faganeli *et al.*, 1999). The salt is produced on "petola", *i.e.* microbial (stromatolitic) mat dominated by cyanobacteria and cemented with gypsum, calcite and clay (Faganeli *et al.*, 1999). This a few millimetres thick layer of biosediment forms the bottom of a crystallizing salt basin and enables the traditional gathering of salt. The algal mat stabilizes sediment surface (Schneider & Herrmann, 1980) and of just right thickness and undamaged provide for enough solidity

and elasticity for manual gathering of salt on the petola. At the beginning of cultivation, the petola constitutes a liable substrate that at the end of this process becomes solid and plastic. The petola also prevents the mixing of muddy floor with seawater and salt, contributing to the production of very pure, white and tasteful unrefined sea salt. For this reason, the salt-workers cultivate and maintain this basin's base throughout the year with great care. However, there is a lack of studies as far as solid (sediment) and aqueous (brine) phases of salt-pans are concerned. In the last 15 years, very few studies concerning sedimentological and biogeochemical properties of this hypersaline environment have been carried out (Dolenec *et al.*, 1994; Faganeli *et al.*, 1999; Ogorelec *et al.*, 2000). As no studies of seasonal transformations of the petola have been performed as yet and as FT-IR has been recognized as a powerful tool for chemical characterization and degradation processes of natural samples, the purpose of this study was chemical characterization and petola modification (affected by maturation processes) using FT-IR spectroscopy. In this way we also checked the applicability of spectroscopic techniques to study the diagenetic processes in this hypersaline environment.

MATERIALS AND METHODS

Sampling site and samples

Sediment samples were taken from crystallization basin in the solar salt-pans of Sečovlje, located in the Bay of Piran (northern Adriatic, Slovenia) (Fig. 1), on June 8th (Sample A), June 30th (Sample B), and July 3th (Sample C), in 2006.

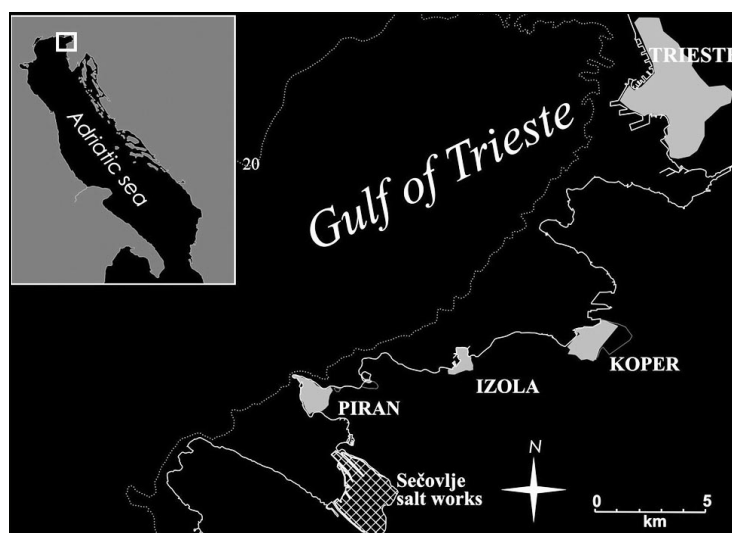


Fig. 1: Location of the Sečovlje salt-pans sampling site (Gulf of Trieste, northern Adriatic).
Sl. 1: Vzorčevalno mesto – Sečovlske soline (Tržaški zaliv, severni Jadran).

Surface sediment samples (0–2 cm) were collected using 40 mm diameter gravity core sampler (Meischner & Rumohr, 1974) and then the tiny slice of surface sediment, *i.e.* 2–3 millimetre thick layer of biosediment, was cut off and frozen. The freeze-dried samples were grounded to a fine powder and used for elemental and FT-IR analyses.

FT-IR spectra were obtained on homogenized samples using a Perkin-Elmer Spectrum One spectrometer with diffuse reflectance sampling accessory. The micro-cup of the accessory was filled with the sample diluted by anhydrous KBr to give up to 5% mixture. Spectra were collected at room temperature with a resolution of 4 cm^{-1} and 8 scans have been accumulated for each spectrum in a frequency range of 4000–450 cm^{-1} .

The organic carbon (OC), total nitrogen (TN) contents of the freeze-dried and acid-washed (1M HCl) samples were determined with a Carlo Erba model 1108 elemental analyzer (Hedges & Stern, 1984).

RESULTS AND DISCUSSION

The stromatolitic algal mat indicates the specific physico-chemical environment (Golubic *et al.*, 1977). Depending on the actual/present environmental conditions, the biogenic component of microbial mats (throughout microbially-mediated processes) conversely influences the mineral precipitation (Dupraz *et al.*, *in press*). Besides the salinity conditions, a variety of factors such as nutrients, oxygen, sulphide and concentrations, temperature, pH, light, flooding and desiccation features impacts the microbial (composition and ecological strategy/functioning) communities (Sabater *et al.*, 2000) and its organic/mineral associations. To decipher such compositional differences among the petola samples sampled during crystallization processes in summer 2006, the FT-IR spectra were obtained from bulk powdered samples (Figs. 2 and 3).

In general, all spectra (Fig. 2) are dominated by strong absorptions in O-H and N-H stretching band region (3000–3600 cm^{-1}) and in the main region for carbohydrates peaks (~1150–1000 cm^{-1}) that strongly interfere with siloxanes (Si-O) stretching (Benning *et al.*, 2004a). The organic carbon is confirmed by the IR-absorbance at 2800–3000 cm^{-1} and is most pronounced in the spectrum of sample A (June 8th). The presence of CH_2 and CH_3 alkyl groups is indicated from a stretching absorption in the 2940–2800 cm^{-1} range. The other bands of aliphatic groups that usually appear around 1429 cm^{-1} (CH_3 and CH_2 asymmetric bending) and 1385 cm^{-1} (CH_3 symmetric bending) are overwhelmed by the strong signals of calcite. The band at 1641 cm^{-1} can be assigned to Amide I signals primarily consisting of the carbonyl stretching vibration of the peptidic bond. In spectra of samples B (June 30th) and C (July 3th), this band was sub-

stituted by the signals at 1621 and 1684 cm^{-1} that can be attributed to the vibration of N-H groups of the proteins amino acids. However, groups such as aromatic and olefinic C=C (around 1650 cm^{-1}) and bending vibration from adsorbed water could be contributing factors. The lipid feature was found at ~1735 cm^{-1} (>C=O ester stretching vibrations; Benning *et al.*, 2004a), probably originating from the ester linkage of the fatty acids of Cyanobacteria representing the dominating group of petola biofilm/mat. Other C=O vibrations of ketones, organic acids and esters could add to that band.

In stromatolites, the mineral component, carbonate minerals, gypsum and halite (Dupraz *et al.*, *in press*) play important role and were emphasized in FT-IR spectra (Figs. 2 and 3) of our samples. In the dry period (Faganeli *et al.*, 1999), the organic component of petola largely combines with different minerals like quartz, feldspar, gypsum, halite, high Mg-calcite and aragonite. Clay minerals are also typically present in the petola biomat (Ogorelec *et al.*, 1981) and were very clearly indicated in sample A. Clays such as illite, smectite and kaolinite contribute to the 3620 cm^{-1} band. The presence of clay minerals suggests fine sediment trapping by microbial mats originating in marine mud basement or in material used for sanitation processes of the petola. Additionally, particles from atmospheric inputs of autochthonous and allochthonous material could also be the contributing factor.

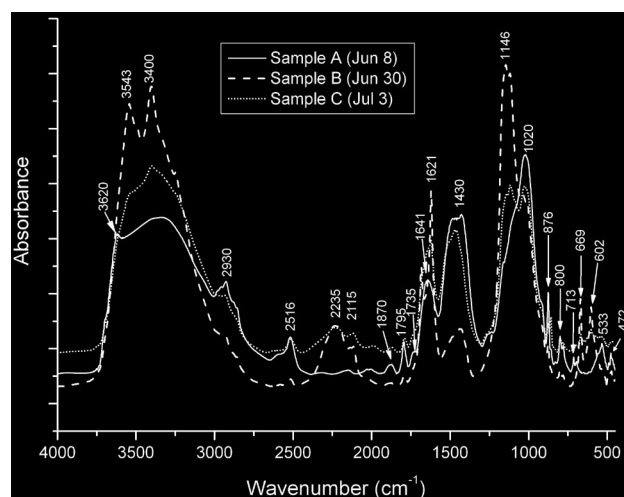


Fig. 2: FT-IR spectra of petola samples collected on June 8, June 30, and July 3, 2006. Main bands are labelled and detailed description of spectra discussed in the text.

Sl. 2: FT-IR spektri vzorcev petole vzorčenih 8. junija, 30. junija in 3. julija 2006. Označeni so glavni trakovi, podroben opis spektrov je podan v tekstu.

Spectra from all samples also display the main calcite peaks situated at 2516, 1795, 1420–1450, 876 and 712–713 cm^{-1} . Considering the previous mineralogical studies of the petola (Faganeli *et al.*, 1999; Ogorelec *et al.*, 1981, 2000), magnesium carbonate (with typical absorptions at 1482, 1420 cm^{-1} and with its much lower absorption usually located at 1120, 886, 853, 803, 719 and 593 cm^{-1} ; Jackson, 2004) and aragonite also contribute to the obtained spectra. Three major bands attributed to CO_3^{2-} for the aragonite group present at 1471 cm^{-1} and two doublets around 877–848 cm^{-1} and at 713–700 cm^{-1} are marked in the spectra of sample C (Adler & Kerr, 1962; White, 1974; Guzman *et al.*, 2008). The bands around 1160 and 472 cm^{-1} belong to the silicates. The intense bands at 1150 and 1020 cm^{-1} are those of the Si-O stretching frequencies and overlapped with C-O polysaccharide frequencies. There is also a characteristic doublet with peaks at approximately 800 and 780 cm^{-1} (Pacáková *et al.*, 2000) reflecting the presence of quartz, which is also indicated by bands at 1870, 695 and 533 cm^{-1} . Comparison of the spectra obtained during petola maturation indicates significant changes in the silicate (Si-O) stretching region (1150 to 950 cm^{-1}). Instead of the maximum at $\sim 1020 \text{ cm}^{-1}$, present in the spectrum of petola from sample A (June 8th), we observed the maximum peaks ~ 1146 and $\sim 1030 \text{ cm}^{-1}$ in the spectra of later samples. From those results, a degradation of carbohydrates concomitant with a modification/decrease of the silicate component (especially decrease of quartz content) could be inferred during petola cultivation/maturation. Some of those changes observed in the spectra of samples B and C are due to the crystallization of gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) that begins

after crystallization of CaCO_3 . The strong band centred around 1020 cm^{-1} that splits into two components at around 1146 and 1117 cm^{-1} , and the small peaks at 669 and 602 cm^{-1} are assigned to the stretching and bending mode of sulphate in gypsum. For gypsum, peaks typical of the bending vibration of O-H bond in H_2O molecule, and stretching and bending vibrations of S-O bond in SO_4^{2-} were detected at around 3543, 3407, 1683, 1621, 1141, 1118, 669 and 602 cm^{-1} (Rampazzi & Bugini, 2006; Cappitelli *et al.*, 2007). Gypsum and anhydrite sulphate could also contribute to the absorption feature at ~ 2235 and $\sim 2115 \text{ cm}^{-1}$ (Sutter *et al.*, 2005). However, the CN grouping also gives an IR signal in this region and the quartz itself as well.

The salinity data (brine) during sampling supported the presence of major salts identified by FT-IR analyses. On June 8th, the seawater concentration was approx. 5.1 $^\circ\text{Be}$, which led to precipitation of CaCO_3 , and on June 30th concentration of brine was 22.5 $^\circ\text{Be}$ when $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ crystallizes (it begins at about 13.2 $^\circ\text{Be}$; Korovessis & Lekkas, 2006). Evaporation of seawater finally proceeds to the halite precipitation. In the period of last sampling (sample C; July 7th, 2006), the seawater concentration in the crystallization pan was approx. 26 $^\circ\text{Be}$ (data from SOLINE Pridelava soli d.o.o./Salt Production Co. Ltd.), which is consistent with the crystallization of NaCl in the system. Unfortunately, the halite is known as spectrally featureless in the infrared spectra because it is transparent over much of the infrared region of the electromagnetic spectrum. Samples of halite usually give the near 100% flat absorption line, so its precipitation cannot be followed by FT-IR analyses.

During sampling the decrease of organic carbon in petola was observed. Spectra of samples sampled after 8th June obviously show decreasing absorption intensities of aliphatic CH_3 and CH_2 stretching in the 2940–2800 cm^{-1} range. The other organic absorption peaks were overlapped by main mineral absorption. The elemental analyses of petola showed similar organic carbon values as previously reported by Faganeli *et al.* (1999) (3.6–6.2%) and confirmed the above mentioned trend detected by FT-IR spectroscopy (Tab. 1).

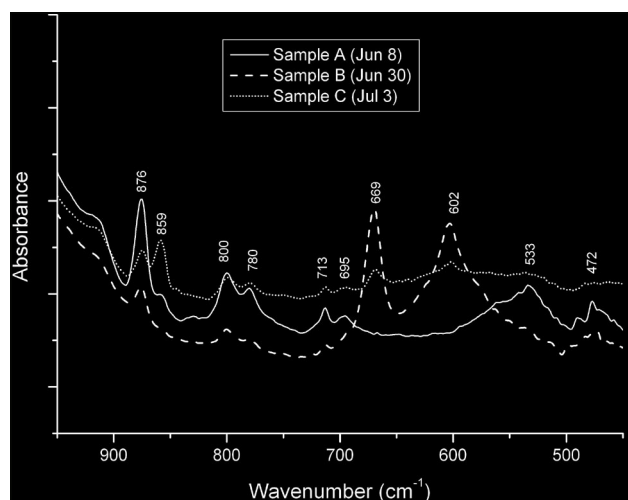


Fig. 3: FT-IR spectra of petola samples (spectral range 950–450 cm^{-1}).

Sl. 3: FT-IR-spektri vzorcev petole (spektralno območje 950–450 cm^{-1}).

Tab 1: Organic carbon (OC) and total nitrogen (TN) contents (wt. %), and OC/TN ratios (atomic) in the petola samples.

Tab 1: Vsebnost (ut. %) organskega ogljika (OC) in celotnega dušika (TN), in atomska razmerja OC/TN v vzorcih petole.

	Jun 8, 2006	Jun 30, 2006	Jul 3, 2006
OC	6.35	5.99	4.26
TN	0.28	0.22	0.11
OC/TN	26.96	31.75	46.01

The values for organic carbon and total nitrogen in the petola showed considerable decline. About 33% lowering of the OC content, from 6.35 to 4.26% and ~60% reductions in total nitrogen concentrations, was determined. Comparison of the OC/TN values of the petola samples during the summer indicates that bulk elemental compositions have been progressively altered by the maturation of petola and increasing brine salinity. Petola organic matter mostly derives from cyanobacteria (dominating group), diatoms and other microalgae (Faganeli *et al.*, 1999) formed *in situ* and organic matter originating in seawater (brine) above the petola biomats. Microbial community in solar salterns is a dynamic system, responding to changes in salinity and chemical environment (Litchfield *et al.*, 2009). The increasing brine salinity alters the biological composition (different microbial community structure) switches from halotolerant (stenohaline forms) to halophilic species (euryhaline forms) and could lead to reduction of microorganism biomass/growth. At Sečovlje salt-pans, the increasing brine salinity resulted in higher alteration of community structure and decrease of prokaryotic activity (Kračun, 2006). In such extremely saline environment, the cyanobacteria *Microcoleus chthonoplastes* was reported to be one of the most important species present in the petola (Schneider & Herrmann, 1980; Faganeli *et al.*, 1999). As the nitrogen content in cyanobacteria may amount up to 10% of their dry weight (Stal, 2000) and most plankton nitrogen is present as protein (Lourenço *et al.*, 1998), a preferential degradation of protein-N (in parallel with reduction in growth of microorganisms) can be suggested from our results. Protein pools, especially amino acid nitrogen (Blackburn, 1986; Burdige & Martens, 1988), are generally considered to be more labile fraction, whereas carbohydrate pools appeared to be more conservative in sediments (Dell'Anno *et al.*, 2000). Conversely, recent studies revealed that some proteinaceous material could be resistant to microbial degradation and that part of nitrogen may be preserved in sedimentary environments (Knicker & Hatcher, 2001; Nguyen & Harvey, 2001; Zang *et al.*, 2001). Additionally, the decrease of cyanobacteria accumulation of nitrogen (from sediment, brine, air) also contributes to the lower nitrogen content. This resulted in rather high OC/TN atomic ratios of more mature samples with low protein content and the presence of degradation products. Determined OC/TN ratio values were much higher than those reported by Faganeli *et al.* (1999), *i.e.* <10. The decrease of organic component seems to affect dropping of the silica/silicate content as the decrease of intensity of silica related bands was observed in FT-IR spectra (Fig. 3). This could relate to the lower diatom (with siliceous frustules and intracellular Si pool) and/or poorer cyanobacteria presence. Microbial surface was suggested to act as a nucleation site for silica precipitation (Konhauser, 1996; Phoenix *et al.*, 2000) and accu-

mulation. Amorphous silica could accumulate on the surface of cyanobacteria (Benning *et al.*, 2004a, b, 2005). Aggregation of inorganically nucleated silica nanoparticles into larger silica assemblages is enhanced by exopolymeric polysaccharides (Benning *et al.*, 2005). Organic exopolymeric matrix, important component of bacterial mat, contributes also to the sediment stabilization (Klock *et al.*, 2007; Paterson *et al.*, 2008; Dupraz *et al.*, *in press*). The trapping and biding capacity of stromatolite mat community impacts the strength of the sediment and the nature of the mineralization processes (Casillas-Martinez *et al.*, 2005). Organic decay processes seem to be important for petola cementation leading to formation of a very firm substrate needed for manual gathering of salt.

Parallel to the petola analyses, we also inspected the compositional changes occurring in the dark clayey sediment just below the petola. FT-IR spectra of those samples (Fig. 4) show just minor alterations during sampling period (petola maturation). Major spectra bands of this saline mud were assigned to clay minerals (3620 cm^{-1}); O-H and N-H groups (3300–3400 cm^{-1}), aliphatic C-H stretching (2940–2800 cm^{-1}); calcite (2517, 1795, 1420–1450, 876 and 713 cm^{-1}); H_2O bending vibration, aromatic and/or olefinic C=C, proteins (1630 cm^{-1}), polysaccharide and silicate vibrations (~1150–900 cm^{-1}) and quartz (1870, 800, 780, 695, 533 cm^{-1}). The peaks located at 470 cm^{-1} and around 857 cm^{-1} could be ascribed to silicates and other carbonates (such as aragonite, Mg-carbonate etc.). A few millimetres below the petola mat, there is a border between oxic and anoxic layer (Faganeli *et al.*, 1999), so some ferric oxides (with

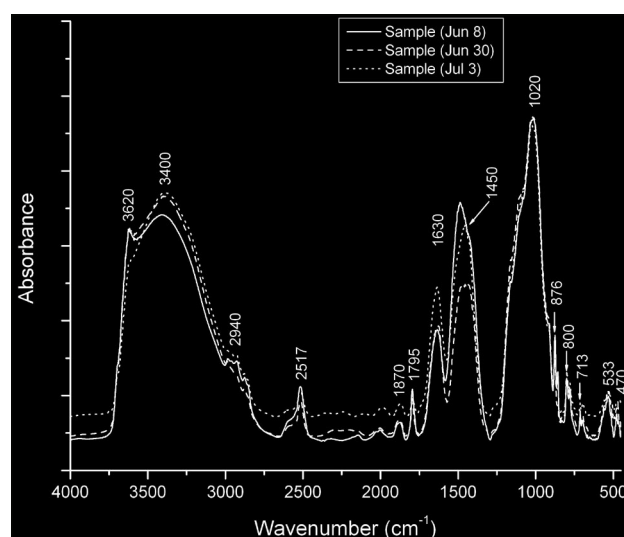


Fig. 4: FT-IR spectra of clayey sediment sampled just below petola.

Sl. 4: FT-IR-spekter glinenega sedimenta, vzorčenega pod petolo.

signals around 470 and 535–555 cm^{-1}) could contribute to those spectra. It is confirmed from these results that the studied stromatolitic mat *i.e.*, petola constitutes the most reactive surface in the salt-pans.

CONCLUSIONS

The present work confirmed the application of FT-IR spectroscopy in the studies of hypersaline system of the solar Sečovlje salt-pans. This spectroscopic technique showed modification of organic component and minerals of stromatolitic mat "petola" *i.e.*, the base layer (bio-sediment) of crystallization basins. With the increasing evaporation, calcium carbonate crystallized first, followed by calcium sulphate dihydrate (gypsum) and halite crystallizing at the end of evaporation. Unfortunately, the halite that crystallizes at the end of evaporation is transparent to the infrared light and does not show any lines in the spectra. The alteration of organic fraction of

petola was detected during its maturation and increasing salinity concentration in crystallization basin. During the summer, the decrease of organic carbon, total nitrogen and silicate content in petola was observed. The increasing degree of cementation seems to play a very important role in the stabilization of petola, allowing manual gathering of salt on this basin's base. At the millimetre scale, petola constitutes a mainly reactive, self-sustaining system and the most important surface in saline ponds.

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KEMIČNA KARAKTERIZACIJA STROMATOLITA "PETOLA" (SEČOVELJSKE SOLINE, SLOVENIJA) Z UPORABO SPEKTROSKOPIJE FT-IR

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POVZETEK

Stromatolitska plast, imenovana "petola", je v Sečovljskih solinah (Tržaški zaliv, severni Jadran) osnovni substrat za tradicionalno ročno pridelavo soli, zato je poznavanje njenega kemizma pomembno za ohranitev in razvoj te aktivnosti. Sestavo in spremembe petole smo preučevali s pomočjo spektrov FT-IR homogeniziranih celotnih vzorcev. Glavne anorganske komponente sestavljajo karbonati, sadra ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) in silikati, organska frakcija pa se pojavlja v manjšem deležu (OC; 4,3–6,4 ut. %). Med vzorčevanjem, tj. v času zorenja petole in porasta slanosti slanice, smo zasledili padec koncentracije silikatov, organskega ogljika (OC) in celotnega dušika (TN) ter porast OC/TN atomskega razmerja. Opisana raziskava potrjuje uporabnost spektroskopije FT-IR pri preučevanju sestave in pretvorb petole ter bazičnih raziskavah hipersalinih območij.

Ključne besede: stromatolit-petola, FT-IR, elementna sestava, Sečovljske soline, severni Jadran

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