Metal removal capability of two cyanobacterial species in autotrophic and mixotrophic mode of nutrition

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Abstract

Background: Cyanobacteria are ecologically significant prokaryotes that can be found in heavy metals contaminated environments. As their photosynthetic machinery imposes high demands for metals, homeostasis of these micronutrients has been extensively considered in cyanobacteria. Recently, most studies have been focused on different habitats using microalgae leads to a remarkable reduction of an array of organic and inorganic nutrients, but what takes place in the extracellular environment when cells are exposed to external supplementation with heavy metals remains largely unknown.

Methods: Here, extracellular polymeric substances (EPS) production in strains Nostoc sp. N27P72 and Nostoc sp. FB71 was isolated from different habitats and then the results were compared and reported.

Result: Cultures of both strains, supplemented separately with either glucose, sucrose, lactose, or maltose showed that production of EPS and cell dry weight were boosted by maltose supplementation. The production of EPS (9.1 ± 0.05 μg/ml) and increase in cell dry weight (1.01 ± 0.06 g/l) were comparatively high in Nostoc sp. N27P72 which was isolated from lime stones. The cultures were evaluated for their ability to remove Cu (II), Cr (III), and Ni (II) in culture media with and without maltose. The crude EPS showed metal adsorption capacity assuming the order Ni (II) > Cu (II) > Cr (III) from the metal-binding experiments. Nickel was preferentially biosorbed with a maximal uptake of 188.8 ± 0.14 mg (g cell dry wt) −1 crude EPS. We found that using maltose as a carbon source can increase the production of EPS, protein, and carbohydrates content and it could be a significant reason for the high ability of metal absorbance. FT-IR spectroscopy revealed that the treatment with Ni can change the functional groups and glycoside linkages in both strains. Results of Gas Chromatography-Mass Spectrometry (GC–MS) were used to determine the biochemical composition of Nostoc sp. N27P72, showed that strong Ni (II) removal capability could be associated with the high silicon containing heterocyclic compound and aromatic diacid compounds content.

Conclusion: The results of this study indicated that strains Nostoc sp. N27P72 can be a good candidate for the commercial production of EPS and might be utilized in bioremediation field as an alternative to synthetic and abiotic flocculants.

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**Introduction**

Cyanobacteria are an extremely diverse groups of prokaryotes whose adaptive capacity along with the ability to tolerate extreme conditions makes them ubiquitous in aquatic [1], and terrestrial environments [2]. They are well known as producers of a wide range of natural compounds which are in turn recognized as toxins that can be potential and useful in pharmaceutical industry [3–10]. Genus *Nostoc* is a large and morphologically diverse group of phototrophic cyanobacteria which has found in various habitats. They have drawn more attention because of the presence of outermost polysaccharidic envelopes, often coupled with the capability to release exocellular polysaccharides (RPS) in the culture medium during cell growth [11, 12]. Most of these polymers are characterized by an anionic nature, owing to the presence of uronic acids and/or of other charged groups [13]. As a result, polysaccharides typically have very high affinity to metallic ions and can be considered as a very promising chelating agents for the removal of heavy metals from water [14–18].

Many factors affect cell growth, metabolite accumulation and extracellular polymeric substances (EPSs) including nutrients, such as phosphate and nitrogen, temperature, light intensity, aeration rate, and mixotrophic condition, in microalgal cultures [19, 20]. Although the presence of EPSs are extremely preserved among cyanobacteria, there is not much information about factors that maximize the biosynthesis of EPSs and affects the biosorption capacity [15, 21–24]. Several trace elements such as copper, cobalt, and nickel are essential cofactors in cyanobacteria strains. Unlike organic contaminants, heavy metals such as copper and lead are the main pollutants of freshwater due to persistent, toxicity, recalcitrant, and non-biodegradable nature [25]. On the other hand, heavy metal ions concentration at low concentrations are known to be toxic to the organisms because they inhibit many enzymes irreversibly. Heavy metal uptake capacity of algal biomass has proved to be the highest due to the presence of polymers containing functional groups (which can act as binding sites for metals) such as amino, hydroxyl, carboxyl, and sulfate. Polysaccharides, proteins, or lipids on the cell wall surface which are good examples for these polymers [11]. Several studies have been conducted on phytoremediation investigation and several authors have established the fact that treatment of wastewaters using algae, and particularly microalgae decrease organic and inorganic nutrients, such as toxic chemicals remarkably [26–30]. Our previous study indicated that cell growth and the production of EPSs are highly culture conditions dependent. There is no correlation between cell growth and the production of EPSs in cultures with different sources of nitrogen. In contrast, light intensity and cell growth in mixotrophic conditions have had a highly positive effect on the production of EPSs. In salt-grown cultures, thick layers of ASN_M strain supports the cells from NaCl stress hence its growth is maintained without the NaCl stress inhibition [31].

This study evaluates the metal removal capability of two *Nostoc* species isolated from different habitats in autotrophic and mixotrophic media cultures. We hypothesize that the strain of *Nostoc* sp. N27P72 that is isolated from lime stones of Khuzestan province have many features including high tolerance to different abiotic stress such as drought, and high light intensity that make it an ideal candidate for the selective removal and concentration of heavy metals, compared to aquatic strain.

The main aim of this study is utilizing the mixotrophic conditions to optimize the biosorption controllable factors to achieve the maximum heavy metal removal efficiency of algal biomass. Lead, Nickel and copper were selected because of their contrasting toxicity and essentiality. Furthermore, cell dry weight, carbohydrates content, total soluble proteins, analysis of functional groups have been and Chemical composition of the lyophilized EPSs has been investigated.

**Materials and methods**

**Materials**

All materials and reagents were purchased from Sigma-Aldrich unless otherwise specified.
Cyanobacterial strains

*Nostoc* sp. N27P72 and *Nostoc* sp. FB71 were obtained from the Cyanobacteria Culture Collection (CCC) and the ALBORZ Herbarium, at the Science and Research Branch, Islamic Azad University, Tehran. Originally *Nostoc* sp. N27P72 and *Nostoc* sp. FB71 was obtained from the lime stones of Khuzestan province and fresh water of Golestan province respectively.

Culture conditions

*Nostoc* sp. N27P72 was cultured on modified Z8IX medium and *Nostoc* sp. FB71 was cultured on liquid media BG11 medium (nitrate free) [32] (Rippka et al. 1979) and the pH was adjusted to 7.1.

The cultures were used to optimize the metal removal capability of the broth (25 ml in 250 ml baffled shake flasks) containing the sugars glucose, maltose, lactose, and sucrose, separately, as additional carbon sources at the concentrations of 10 g/l. *Nostoc* sp. N27P72 and *Nostoc* sp. FB71 were cultured for 48 h.

Cultures were incubated in a culture chamber at 28 °C and were provided with continuous artificial illumination of approximately 15 μmol m$^{-2}$ s$^{-1}$ for two weeks [33].

Determination of cell biomass

The cells were harvested and dried in an oven set at 100 °C. The cell dry weight was measured after 6, 12, 24, and 48 h [31, 34].

Isolation of exopolysaccharides

Strains *Nostoc* sp. N27P72 and *Nostoc* sp. FB71 were harvested after 6, 12, 24, and 48 h from the culture, then they were centrifuged at 1792 g for 30 min at 4 °C (SigmaPK). The EPSs were precipitated by adding ethanol and storing overnight at 4 °C. Precipitates were harvested and put in a fume hood to evaporate the remaining ethanol. Finally, the precipitates were dissolved in milliQ water and lyophilized (Labconco freeze dry system) to obtain the crude EPSs [31].

Selectivity in the heavy metal removal

The cyanobacterial species were cultured in Z8IX and BG11 media containing maltose and without maltose as control culture, then they were tested for their ability to remove Cu (II), Cr (III), and Ni (II). The cultures (400 ml of cell suspensions in 1000 ml Erlenmeyer flasks) were cultured for 10–15 days in an orbital Incubator (Gallenkamp, Loughborough, UK) at 30 ± 1°C under continuous illumination provided by cool white fluorescent tubes giving a mean photon flux of 100 μmol photons m$^{-2}$ s$^{-1}$ photosynthetic active radiation at the flask surface. Before their use for the experiments, aliquots of the cultures were confined in dialysis tubing and pretreated with 0.1 N HCl and then dialyzed against water. Next, the cultures were suspended into metal solutions with continuous stirring. Working solutions of 10 mg l$^{-1}$ Cr (III), Cu (II), and Ni (II) were prepared, (using dilution of 1000 mg l$^{-1}$ standard solutions (pH 5.0) for each metal). The experiments were performed in a thermostat at 25 ± 1 °C. For the determination of the kinetics of metal removal, 5 ml samples were withdrawn five-time every 30 min (0 to 90 min), centrifuged (10 min at 10 000 g), and filtered through a 0.45 μm membrane. The metal uptake was calculated via evaluating the difference between the concentrations of the metals in solution by an Atomic adsorption spectrometer (SpectrAA 10 plus, Varian, CA, USA), at the beginning and end of exposure with the cyanobacterial cell suspensions: Cu(II), Ni(II), and Cr(III) concentrations were determined at 232.0, 359.9 and 324.7 nm respectively. All the experiments were performed at least in triplicate, and the data was reported as mean values ± standard error of the mean. The metal uptake q, expressed as mg of metal removed per g of dry biomass, was calculated as $q = (C_{i} - C_{t}) / m$.

Analyzing of carbohydrates content

To confirm the affection of the metals and determine the interaction of the cations within the different functional groups of the EPSs, metal solution and all incubated EPSs were lyophilized after 24 h dialysis (lyophilized EPSs containing maltose and metal solution of Cu (II), Cr (III) and Ni (II)). Total produced EPSs, total soluble proteins, carbohydrate content, and chemical composition were measured.

Estimation of total soluble proteins

To confirm the affection of the metals and determine the interaction of the cations within the different functional groups of the EPSs, metal solution and all incubated EPSs were lyophilized after 24 h dialysis (lyophilized EPSs containing maltose and metal solution of Cu (II), Cr (III) and Ni (II)). Total produced EPSs, total soluble proteins, carbohydrate content, and chemical composition were measured.

Determination of total soluble proteins

20 mg of EPS were resuspended in 1 ml of deionized water and sonicated and successively diluted until a homogenous solution was obtained. Protein content was determined according to Lowry et al. (1951), and a calibration curve was constructed using serum albumin.
Analysis of functional groups by FT-IR

2 mg of lyophilized EPSs was grinded with 100 mg dry KBr and pressed into a mold in a uniaxial hydraulic press. FT-IR spectra of the purified EPSs fractions were recorded in the 4000–400 cm⁻¹ region using a FT-IR system (Nicolet is5, ThermoFisher Scientific). The determinations were performed in two independent replicates and were reported as the mean with a standard error of the mean [38].

Chemical composition of the lyophilized EPS

Chemical composition of extracts of *Nostoc* sp. N27P72 was evaluated by a coupled gas chromatography–mass spectrometry (GC–MS). The separation of compounds and their analysis was performed by agilent 7000 series Quadrupole GC–MS system with electron impact ionization. The total GC run time was 32 min and the carrier gas was helium. The initial oven temperature was held at 90 °C for 1 min and then reached 300 °C in 13 min, then it was held at this temperature for 20 min. the injector temperature was 300 °C. Interpretation on mass spectrum GC–MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns and Fiehn Mass Spectra Libraries. The spectrum of the unspecified component was equaled with the spectrum of the identified components stored in the NIST library. The Name, Molecular weight, and Structure of the components of the test materials were ascertained [39].

Statistical analysis

Results of each representative experiment were analyzed by ANOVA, using the statistical software package SPSS version 24. A significance level of 95% was considered to indicate a statistical difference. The Tukey test was performed to evaluate the significance of difference among mean values when a significant variation (p < 0.05) was found by the ANOVA test. Each treatment underwent three replicated measurements for which the mean values ± standard error of mean were obtained [31].

Results

**Growth and EPS production**

The two *Nostoc* strains exhibited a distinct difference in growth behavior. As initial trials with both strains showed that use of disaccharides (maltose, lactose, and sucrose) as carbon source supplementation generally resulted in higher production of EPSs than the use of monosaccharides (Glucose), moreover the amounts of the total produced EPSs, cell dry weight, and residual sugar content were higher in *Nostoc* sp. N27P72 in comparison to *Nostoc* sp. FB71 in all treatments. Consumption of the monosaccharide glucose and the disaccharides lactose and maltose were verified (Fig. 1, 2 and Fig. S1). In all situations, the production of EPSs was started in the exponential growth phase and was shown to remain in the stationary phase. The consumption rate of glucose, maltose, lactose, and sucrose was nearly uniform in both strains and after 48 h all glucose was not consumed and the residual concentration was 6–6.5 ± 0.1 g/l in all treatments using one way ANOVA (p < 0.05). The change in cell dry weight was very obvious in both strains in cultures adding maltose and reaching 1.01 ± 0.06 g/l in *Nostoc* sp. N27P72 and 0.75 ± 0.16 g/l in *Nostoc* sp. FB71 at 48 h (Fig. 1, 2 and Fig. S1). The increase in cell dry weight might be due to the consumption of produced EPSs, which was reaching a final EPSs concentration of 9.1 ± 0.05 μg/ml in *Nostoc* sp. N27P72 and 7.04 ± 0.1 μg/ml in *Nostoc* sp. FB71 (at 48 h). The experiments using cultures without added sugar showed a slightly lower maximum cell mass (0.55 ± 0.03 g/l in *Nostoc* sp. N27P72 and 0.323 ± 0.05 in *Nostoc* sp. FB71 after 48 h) and the final EPS concentration was 3.3 μg/ml in *Nostoc* sp. N27P72 and 2.5 μg/ml in *Nostoc* sp. FB71 after 48 h. This indicates that the 10 g/L maltose addition had a small boosting effect on both cell mass and EPS production.

The addition of glucose did not stimulate cell growth and EPSs production in *Nostoc* sp. N27P72, however, it was stimulating in EPS production in *Nostoc* sp. FB71.

The consumption rate of glucose during the first 6 h was 1.5 g/l in *Nostoc* sp. N27P72 and 2 g/l in *Nostoc* sp. FB71 but decreased to 0.5 g/l (6–15 h) and reaching a cell mass of 0.59 ± 0.21 g/l in *Nostoc* sp. N27P72 and 0.25 g/l in *Nostoc* sp. FB71 at 24 h. After 48 h the cell mass finally reached 0.79 ± 0.35 g/l in *Nostoc* sp. N27P72 and 0.38 g/l in *Nostoc* sp. FB71, while the production of EPSs continued (1.7 μg/ml in *Nostoc* sp. N27P72 and 3.9 μg/ml) during the whole 48 h of cultivation. The cell mass obtained in maltose supplemented cultivations resembled that of the lactose supplementation (0.4 ± 0.28 g/l in *Nostoc* sp. N27P72 and 0.5 ± 0.1 g/l in *Nostoc* sp. FB71 after 24 h, maintained at 24 h as 0.94 ± 0.14 g/l in *Nostoc* sp. N27P72 and 0.66 ± 0.09 g/l in *Nostoc* sp. FB71. The EPSs concentration reached to 6.41 ± 0.13 μg/ml in *Nostoc* sp. N27P72 and 5.10 ± 0.26 μg/ml in *Nostoc* sp. FB71 (after 48 h). Sucrose supplemented cultivations showed that the maximum cell concentration was 1.15 ± 0.21 g/l and the concentration of produced EPSs was 2.01 ± 0.15 μg/ml in *Nostoc* sp. N27P72 and 4.32 ± 0.19 μg/ml in *Nostoc* sp. FB71 after 48 h. The results displayed that while the effects on cell mass were slightly small, production of EPSs amplified upon adding disaccharides such as maltose and lactose at stationary and exponential phases.
Optimization of metal removal capability

The time course of specific metal removal (q), expressed as mg of metal removed per g of biomass dry weight, by *Nostoc* sp. N27P72 and *Nostoc* sp. FB71 cultivated in media culture containing (10 g/l) maltose, copper, chromium, and nickel in single-metal solutions. The results showed the kinetics of sorption were always rapid for all the metals tested by the cyanobacterial cultures; the saturation of the metal removal capacity of each strain was achieved within the first 10 min in the metal solution.

The metal affinity of the two *Nostoc* strains generally decreased Ni > Cu > Cr respectively. The specific metal uptake (q) was very high, in particular for Ni (Fig. 3 and Fig. S2), which generally were removed in larger amounts compared to Cu and Cr. Among the strains tested, the highest q values towards Ni was $188.8 \pm 0.14$ mg Ni (g cell dry wt)$^{-1}$ shown by *Nostoc* sp. N27P72, while the highest values of uptake was $185.5 \pm 0.24$ by *Nostoc* sp. FB71. In single ion solutions, both *Nostoc* strains tested showed the lowest affinity for Cr namely $105.65 \pm 0.34$ $104.5 \pm 0.1$ mg metal for *Nostoc* sp. N27P72 and *Nostoc* sp. FB71 (g cell dry wt)$^{-1}$ respectively.

Results of metal removal capability optimization showed that adding maltose in culture media resulted in higher EPSs production, protein, and carbohydrates content in comparison to control in both strains. Moreover, the amounts of the total produced EPSs, protein, and carbohydrates content were significantly higher in *Nostoc* sp. N27P72 in comparison to *Nostoc* sp. FB71 using one-way ANOVA ($p<0.05$). Exopolysaccharides concentration
Fig. 2  Growth profile and the production of EPSs of *Nostoc* sp. FB71 cultivated in media culture without additional sugars as a control a and media culture containing (10 g/l) maltose b, lactose c, sucrose d and glucose e. Symbols indicate (●) for cell dry weight (g/l), (□) for total EPS concentration (μg/ml) and (∆) for sugar concentration (g/l) in the media. Results are the mean of duplicated measurement.

Fig. 3  Time course of specific metal removal (q), expressed as mg of metal removed per g of biomass dry weight, by *Nostoc* sp. N27P72 a and *Nostoc* sp. FB71 b cultivated in media culture containing (10 g/l) maltose, with copper, chromium, and nickel in single-metal solutions. Symbols represent the mean of at least three replicates and bars represent the standard error of the mean, if larger than the dimensions of the symbols, using one-way ANOVA (p < 0.05). Symbols indicate () for nickel, (-) for copper, and (▲) for chromium in the media.
in medium containing maltose and metal solution of Ni (II) was 2.87 and 2.57 μg/ml, while it was 1.72 and 1.8 μg/ml in control for Nostoc sp. N27P72 and Nostoc sp. FB71 respectively. Protein content in medium containing maltose and Ni (II) was 5.64 and 5.16 μg/ml, while it was 2.85 and 1.74 μg/ml in control for Nostoc sp. N27P72 and Nostoc sp. FB71 respectively. Nickel removal rate was significantly higher in both strains, which means this metal is more absorbed by polysaccharide envelopes. The reason for removing more nickel (based on the results of the diagrams) is the higher amount of EPSs, proteins, and carbohydrates content compared to other elements. More EPSs, proteins, and carbohydrates content can effectively sequester dissolved metal ions from dilute aqueous solutions (Fig. 4 and Fig. S3).

Analysis of functional groups

Fourier Transformed Infrared (FT-IR) spectroscopy provided useful information about active functional groups that can be used in the determination of polysaccharide composition. So, the FT-IR spectra was used to evaluate both Nostoc sp. N27P72 and Nostoc sp. FB71 cultivated in media culture containing (10 g/l) maltose, with copper, chromium, and nickel in single-metal solutions. So, the FT-IR spectra was used to evaluate both Nostoc sp. N27P72 and Nostoc sp. FB71 respectively. Nickel removal rate was significantly higher in both strain, which means this metal is more absorbed by polysaccharide envelopes. The reason for removing more nickel (based on the results of the diagrams) is the higher amount of EPSs, proteins, and carbohydrates content compared to other elements. More EPSs, proteins, and carbohydrates content can effectively sequester dissolved metal ions from dilute aqueous solutions (Fig. 4 and Fig. S3).

The GC–MS analysis showed cyanobacteria in the presence of heavy metals change aliphatic compound (2-Ethoxyethanol, 3,3-dimethylhexane, Undecane, Dodecane, 2,6,10-trimethyl, 3,3-dimethylhexane, Undecane, Dodecane, Tetradecane, Nonadecane, Nonadecane, Propionic acid, Dotriacontane, and Carbohydrates). The non-asymmetric stretching vibrations for NH and OH and asymmetric stretching vibrations for CH was identified at 2854 and 2924 cm\(^{-1}\) only in Nostoc sp. N27P72 in presence of nickel. A peak at about 1000 cm\(^{-1}\) region was associated with C-O polysaccharide and was not observed at Nostoc sp. FB71 in presence of nickel. Furthermore, this peak shifted to lower wavenumber at Nostoc sp. N27P72 that could be related to polysaccharide conformational changes in both strains. Peaks at 1040 and 1029 cm\(^{-1}\) were correlated to polysaccharides skeletal and C-O-C and C-O groups of the anomeric region. Aliphatic esters can be observed at 1103 cm\(^{-1}\) and this peak was removed due to the interaction of Ni, Cr, and Cu to both strains. The 800–900 cm\(^{-1}\) region depicts several vibrational modes corresponding to the type of glycosidic linkages which were removed after treatments of Cr and Cu with both strains. Peaks in 840–860 cm\(^{-1}\) region corresponding to \(\alpha\)-glucan and 890–910 cm\(^{-1}\) corresponding to \(\alpha\) and \(\beta\) glycosidase and these peaks were removed in all samples except Ni treatment at Nostoc sp. FB71. Peak at 600 cm\(^{-1}\) was related to The C-N stretching band (600 cm\(^{-1}\)) which was detected in both samples but shifted to lower wavenumbers in both strains. Collectively, after treatment of both strains with Ni, Cu, and Cr, the FT-IR patterns for both strains changed obviously (Figs. 5 and 6).

Chemical composition of the lyophilized EPS by GC–MS

The GC–MS analysis showed cyanobacteria in the presence of heavy metals change aliphatic compound (2-Ethoxyethanol, 3,3-dimethylhexane, Undecane, Dodecane, 2,6,10-trimethyl hydroxyamine, O-decyl tetradecane, Nonadecane, Nonadecane, Propionic acid, Dotriacontane, Eicosane, 2-Methyldecane) and alkanes compounds (Dotriacontane, Dodecane, 2,6,10-trimethyl, 3,3-dimethylhexane, Eicosane) to a rich variety of phytochemical
compounds which are effective in heavy metal removal. The active compounds with their retention time (RT), molecular formula, molecular weight, nature of the compound, composition percentage, and quality in the hexane extract are presented in Tables 1, 2, 3 and 4.

The total ion chromatograms (TICs) of all samples demonstrated a strong signal, large peak capacity, and reproducible retention time, indicating the reliability of the metabolomic analysis. However silicon containing heterocyclic compound retention time was not in the same range for the Ni (II) and Cu (II) media culture (11.63 to 23.22), while aromatic diacid compounds retention time for Ni (II) and Cu (II) media culture between 23.26 to 25.14. Moreover, Ni(II) and Cu(II) culture revealed a high composition of cyclic and Ester compounds (Silicon containing heterocyclic compound, Bis(2-ethylhexyl) phthalate and aromatic diacid compounds), while in Cr(III) cultures there were aldehydes compounds (Decanal, 2,3-dimethylbenzaldehyde), ketones compounds (6-Bromo-2-hexanone), Ester compounds (2-piperidinone), alcoholic compounds (3,5-Hexadien-2-ol, 2-Hexanol, 1,4-pentanediol), ether compounds (2H-pyran, 3,4-dihydro-6-methyl, 2-Butoxyethanol) and cyclic compounds (Silicon containing heterocyclic compound). The strong Ni (II) removal capability of the *Nostoc* sp. N27P72 was attributed to the abundance of Silicon containing heterocyclic compound (91%) and aromatic diacid compounds nds (91%) characterizes by GC–MS (Fig. 7) (Tables 1, 2, 3 and 4).

**Discussion**

The possible use of exopolysaccharides producing cyanobacteria for the recovery of valuable metals from industrial wastewater seems to be more promising than most of the other microorganisms [18, 40–42]. The use of cyanobacterial EPSs for biotechnological applications depends on the identification of culture parameters that influence the maximum production of the EPSs [43–45]. Factors such as the amounts of C: N ratio, as well as growth parameters such as light intensity, salinity, and temperature, have been largely disregarded, and very few exhaustive studies on factors influencing the production of cyanobacterial EPSs are available in the literature [23]. Though, several elements that can stimulate the

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**Fig. 5** Fourier transforms infrared (FTIR) spectra of EPSs from *Nostoc* sp. FssB71 against control or exposure to a solution containing 400 ml of cell suspensions in 1000 ml Erlenmeyer flasks of Cu (II), Cr (III), and Ni (II)
production of EPSs, especially pH, dilution rate, growth phase, presence/absence of magnesium, calcium, potassium, and heavy metals, as well as the addition of glyoxylate, glucose, citrate, acetate, valerate, and EDTA have been sporadically studied [23]. Moreover, the responses of cyanobacteria to changes in culture conditions appear to be frequently strain-dependent, making the optimization of EPSs production even more difficult [46]. It was

![Fig. 6 Fourier transforms infrared (FTIR) spectra of EPSs from Nostoc sp. N27P72 vs control or exposure to a solution containing 400 ml of cell suspensions in 1000 ml Erlenmeyer flasks of Cu (II), Cr (III), and Ni (II)](image)

Table 1  Chemical composition of Nostoc sp. N27P72 (control) extracts as revealed by gas chromatography mass spectrophotometry (GC–MS)

| Name of Compound                        | Molecular Formula | Molecular weight | RT (Mins) | Nature of the compound | Area % |
|-----------------------------------------|-------------------|------------------|-----------|------------------------|--------|
| 2-Ethoxyethanol                         | C_4H_{10}O_2      | 90.12            | 8.77      | hydroxyether            | 83%    |
| Phenol, 2,4-bis-(1,1-dimethylethyl)     | C_{17}H_{30}OSi   | 278.5            | 26.29     | Phenolic ester          | 96%    |
| Dodecane, 2,6,10-trimethyl              | C_{12}H_{24}      | 212.41           | 26.41     | alkane                 | 64%    |
| 3,3-dimethylhexane                      | C_{9}H_{18}       | 114.23           | 27.15     | alkane                 | 40%    |
| Undecane                                | C_{11}H_{24}      | 156.31           | 28        | alkane                 | 78%    |
| Hydroxylamine, O-decyl                  | C_{19}H_{34}      | 173.2957         | 29.75     | alkane                 | 78%    |
| Tetradecane                             | C_{20}H_{44}      | 198.39           | 29.95     | alkane                 | 59%    |
| Nonadecane                              | C_{19}H_{44}      | 268.5            | 30.20     | alkane hydrocarbon     | 83%    |
| Propionic acid                          | CH_{3}CH_{2}CO_{2}H | 74.08           | 30.66     | Organic acid           | 35%    |
| Dotriacontane                           | C_{32}H_{66}      | 450.8664         | 30.68     | alkane                 | 64%    |
| Eicosane                                | C_{20}H_{42}      | 282.5            | 31.69     | alkane                 | 83%    |
| 2-Methyldecane                          | C_{11}H_{24}      | 156.31           | 31.99     | alkane                 | 53%    |
suggested that some additives, including amino acids, vitamins, and precursors, may also play an important role in EPSs production during the growth process of cyanobacteria [47].

In this article, we investigated the effect of adding four different sugars to the culture medium (glucose, maltose, lactose, and sucrose) as carbohydrate sources and their effect on the amount of EPSs and cell dry weight in two strains of Nostoc. The results of the present investigation indicated that the optimal medium for the production of EPSs by the isolated Nostoc sp. N27P72 strain was boosted by maltose supplementation. Previous studies on several bacterial EPSs production reported that amino acids and precursor supplements showed a stimulating effect on bacterial growth and EPSs synthesis, while others demonstrated that neither additional carbohydrates nor amino acids supplementation affects the EPS level [48]. Moreover, among the different nature of carbon sources, carbohydrate sugars are the preferred ones for EPSs production. In this study, maltose was found to be the most efficient carbon source. Nowruzi et al., 2013 showed that light intensity and cell growth in cultures experiencing mixotrophic conditions at either 150 or 50 μmol photon m⁻² s⁻¹ had a strong positive effect on EPSs production [31]. A similar result has been previously reported for other species in this genus [19, 49–51].

| Name of Compound                  | Molecular Formula | Molecular weight | Nature of the compound               | RT   | Area % |
|-----------------------------------|-------------------|------------------|--------------------------------------|------|--------|
| pyran                             | C₅H₆O             | 82.1             | six-membered heterocyclic            | 11.55| 49%    |
| Cyclotrisiloxane                  | H₂O₃Si₃           | 138.3            | Heterocyclic compound                | 12.02| 90%    |
| Cyclotrisiloxane                  | H₂O₃Si₃           | 138.3            | Heterocyclic compound                | 15.97| 91%    |
| Cyclotrisiloxane                  | C₅H₆O             | 84.12            | Heterocyclic compound                | 19.62| 91%    |
| Cyclotrisiloxane                  | H₂O₃Si₃           | 138.3            | Heterocyclic compound                | 23.22| 91%    |
| 1,2-Benzenedicarboxylic acid      | CBH6O4            | 166.1308         | Quinoline Ester                      | 23.51| 90%    |
| 1,2-Benzenedicarboxylic acid      | CBH6O4            | 166.1308         | Quinoline Ester                      | 24.01| 91%    |
| 1,2-Benzenedicarboxylic acid      | CBH6O4            | 166.1308         | Quinoline Ester                      | 24.13| 91%    |
| 1,2-Benzenedicarboxylic acid      | CBH6O4            | 166.1308         | Quinoline Ester                      | 24.35| 91%    |
| 1,2-Benzenedicarboxylic acid      | CBH6O4            | 166.1308         | Quinoline Ester                      | 24.55| 91%    |
| 1,2-Benzenedicarboxylic acid      | CBH6O4            | 166.1308         | Quinoline Ester                      | 24.63| 91%    |
| 1,2-Benzenedicarboxylic acid      | CBH6O4            | 166.1308         | Quinoline Ester                      | 25.04| 91%    |
| 1,2-Benzenedicarboxylic acid      | CBH6O4            | 166.1308         | Quinoline Ester                      | 25.14| 91%    |

| Name of Compound                  | Molecular Formula | Molecular weight | Nature of the compound               | RT   | Area % |
|-----------------------------------|-------------------|------------------|--------------------------------------|------|--------|
| Tranlylcypromine, pentafluorobenzoyl| C_{16}H_{10}F_{5}NO | 327.25           | ester                                | 10.5 | 37%    |
| 2-Hexanol                         | C₆H₁₄O            | 102.17           | Six carbon alcohol                   | 10.85| 43%    |
| Cyclotrisiloxane                  | H₂O₃Si₃           | 138.3            | Heterocyclic compound                | 11.73| 64%    |
| Cyclotrisiloxane                  | H₂O₃Si₃           | 138.3            | Heterocyclic compound                | 11.83| 87%    |
| 2H-pyran, 3,4-dihydro-6-methyl    | C₄H₅O             | 98.14            | enol ether                           | 12.70| 60%    |
| 2-Butoxyethanol                   | C₄H₈O₂            | 118.17           | glycol ether                         | 13.35| 72%    |
| 1,4-pentanediol                   | C₅H₁₀O₂           | 104.15           | diol                                 | 15.12| 40%    |
| Cyclotetrasiloxane                | C₂₃H₃₂O₄Si₄       | 482.8            | Heterocyclic compound                | 15.97| 91%    |
| 3,5-Hexadien-2-ol                 | C₅H₁₀O            | 96.13            | alcohol                              | 16.29| 9%     |
| 6-Bromo-2-hexanone                | C₅HₐBrO           | 179.05           | ketone                               | 18.40| 9%     |
| 2-piperidinone                    | C₅H₉NO            | 99.13            | lactam                               | 18.55| 9%     |
| Cyclopentasiloxane                | H₁₀O₅Si₅          | 230.5            | silicone                             | 19.63| 91%    |
| 2,3-dimethylenzaldehyde           | (CH₃O)₂C₆H₃CHO    | 166.17           | aldehyde                            | 20.5 | 20.5   |
| Succinic acid                     | C₄H₉O₂            | 118.09           | Organic acid                         | 20.64| 20.64  |
| Decanal                           | C₆H₁₀O₂            | 156.26           | aldehyde                            | 23.04| 23.04  |
The prominent feature of mixotrophic cultures is the presence of two energy sources: organic carbon sources and the light. The former is controlled by the concentration of organic carbon sources, and the latter is influenced by light intensity. This offers the possibility of remarkable increase in the microalgal cell concentration, and hence the EPSs productivity, in batch systems [31].

A study conducted by Fabregas et al. (1999) have shown the ability of Porphyridium cruentum to produce 0.33 g L\(^{-1}\) of EPS when grown mixotrophically with 15% potato extract as an organic carbon source, an amount greater compared to the photoautotrophic conditions that were used in the same study [52].

A study conducted by Fabregas et al. (1999) have shown the ability of Porphyridium cruentum to produce 0.33 g L\(^{-1}\) of EPS when grown mixotrophically with 15% potato extract as an organic carbon source, an amount greater compared to the photoautotrophic conditions that were used in the same study [52].

Table 4

| Name of Compound | Molecular Formula | Molecular | Nature of the compound | RT | Area % |
|------------------|-------------------|-----------|------------------------|----|--------|
| Cyclotrisiloxane  | H\(_6\)O\(_3\)Si\(_3\) | 138.3     | Heterocyclic compound  | 11.63 | 78%    |
| Cyclotrisiloxane  | H\(_6\)O\(_3\)Si\(_3\) | 138.3     | Heterocyclic compound  | 11.71 | 78%    |
| Cyclotrisiloxane  | H\(_6\)O\(_3\)Si\(_3\) | 138.3     | Heterocyclic compound  | 15.89 | 90%    |
| 4-penten-2-one    | C\(_5\)H\(_8\)O   | 84.12     | methyl ketones         | 16.31 | 7%     |
| Cyclotrisiloxane  | H\(_6\)O\(_3\)Si\(_3\) | 138.3     | Heterocyclic compound  | 19.61 | 90%    |
| 1,2-Benzenedicarboxylic acid | C\(_8\)H\(_6\)O\(_4\) | 166.1308 | mono ester             | 23.26 | 43%    |
| 1,2-Benzenedicarboxylic acid | C\(_8\)H\(_6\)O\(_4\) | 166.1308 | mono ester             | 23.42 | 91%    |
| Bis(2-ethylhexyl) phthalate | C\(_{24}\)H\(_{38}\)O\(_4\) | 390.6    | diester of phthalic acid | 23.54 | 80%    |
| 1,2-Benzenedicarboxylic acid | C\(_8\)H\(_6\)O\(_4\) | 166.1308 | mono ester             | 23.81 | 91%    |
| 1,2-Benzenedicarboxylic acid | C\(_8\)H\(_6\)O\(_4\) | 166.1308 | mono ester             | 24.03 | 91%    |
| 1,2-Benzenedicarboxylic acid | C\(_8\)H\(_6\)O\(_4\) | 166.1308 | mono ester             | 24.15 | 91%    |
| 1,2-Benzenedicarboxylic acid | C\(_8\)H\(_6\)O\(_4\) | 166.1308 | mono ester             | 24.57 | 91%    |
| 1,2-Benzenedicarboxylic acid | C\(_8\)H\(_6\)O\(_4\) | 166.1308 | mono ester             | 25.05 | 91%    |
| 1,2-Benzenedicarboxylic acid | C\(_8\)H\(_6\)O\(_4\) | 166.1308 | mono ester             | 25.54 | 91%    |

Fig. 7 GC–MS chromatogram of the extract of Nostoc sp. N27P72, in media culture containing the maltose and without maltose as control culture, were tested for their ability to remove Cu(II), Cr(III), and Ni(II)
media culture as an additional sugar source exopolysaccharides to evaluate its efficiency in adsorbing various toxic heavy metals (Cu, Ni, and Cr) [57]. We found that using maltose as a carbon source was reported to produce a higher amount of EPSs, protein, and carbohydrates content and it could be a reason for the high ability of metal absorbance. This statement has been underlined by Wong and Tam (1984) who stated that algal cells cultivated in the media with very high metal contents also gathered higher metal contents. Despite this, in several samples, the metal uptake was independent of the external metal concentration, this point was coincided with the conclusion of Wetton et al. (1976). N. muscorum could grow in wastewater. Interestingly high concentrations of Cu and Mn could not only affect the growth of the microorganism but also promote its growth. This event might happen as a result of the resistance nature of the cyanobacterium to Cu and Mn beside the occurrence of a high content of organic matter, which might detoxify Cu and Mn [58].

The quantity and compactness of different kinds of carbohydrates can help to sort the polysaccharidic layer surrounding the cells which inhibits direct interaction between the cells and toxic heavy metals. Recent studies suggested that high viscosity of the cultures, can, delayed the free diffusion of copper ions into the media culture [36], the presence of negatively charged polysaccharidic layers surrounding cyanobacterial cells such as uronic acids, sulfate, and ketal-linked pyruvate groups may play an important role in the sequestration of metal cations, and informing an environment improved in those metals that are crucial for cell growth but are existing at very low concentrations in some environments [23].

We found that strain of Nostoc sp. N27P72 that was isolated from lime stones of Khuzestan province have many features which include a high amount of EPSs and high tolerance to drought and high density of light that make it an ideal candidate for selective removal and concentration of heavy metals, compared to aquatic strain. Actually, in all of the possible systems in which cyanobacteria were involved, the synthesis of EPSs provides a structurally resistant and hydrated microenvironment, as well as a putative supportive characteristic toward several risk factors, both chemical and physical. This environment represent a boundary between cells and the immediate outer environment, and supports the cells from toxic heavy metal [59].

The experiments showed that Nostoc sp. N27P72 seems to be efficient in metal removal, and its q max (maximum amount of nickel removed per biomass unit) was reported to be 188.8 ± 0.14 mg Ni (g cell dry wt)−1 nickel removed in compare to the value of 185.5 ± 0.24 mg Ni (g cell dry wt)−1 of Nostoc sp. FB71 biomass. the metal uptake capacity of EPSs under study (especially for nickel) was remarkable in compare with other biosorbent efficiencies and the lowest affinity seems to be Cr. Our results contradicted the result of Chan et al. (1991) who found the possibility Ni removal from the mixture of 90% electroplating effluent and 10% raw sewage by using two species of Chlorella was comparatively low (below 20%) [60]. While Kazy et al. (2002) declared that the production of EPSs was considerably higher in a copper-resistant isolate of Pseudomonas aeruginosa compared to its copper-sensitive counterpart [61]. Moreover, Ozturk and Aslim, 2008 found Cr(VI) is an important stress factor that increases EPSs concentration in cyanobacteria [62].

Optimized culture conditions elevate the capillitate of the Nostoc sp. N27P72 EPSs, consequently enhance the chances of its commercial-scale production and makes it more acceptable for specific environmental industry applications. However, finding a general pattern for the special effects of metals on EPSs synthesis is very difficult, the effects on EPSs synthesis are metal-specific. In some cases, the shortage of Mg2+ and Ca2+ elicited the production, whereas there were no effects in other cases. The increase in EPS synthesis appears to develop toxic metals resistance. A study carried out by Ozturk and Aslim 2008, showed that Chroococcus and Synechocystis strains resistant to Cr (VI) created larger amounts of EPSs compared to the Cr (VI)-sensible isolates [62]. Pereira et al. 2013 suggested that there is a promising view that a greater EPSs concentration inspired by the metal, played a role in increasing its immobilization [13]. Though, it was later reported that in Cyanothecae sp. CCY 0110, the existence of heavy metals expressively affected its protein profile but did not improve the amount of RPS released by the cells [38].

Due to the presence of negatively charged groups, primarily carboxyl group have been indicated to have a good sorbent capacity towards positively charged metal ions [63–66]. Among the parameters that strongly affect the metal uptake, the capacity of biopolymers is associated to the metal affinity to their functional groups [67]. Shuhong et al. (2014) and Delattre et al. (2016) reported the implication of O–H, C=O, C–O–C, and C=O–C groups of the EPSs in the binding of Cu2+, Pb2+, and Cr6+ ions [67, 68].

GC–MS is an extensively used method for metabolomics researchs, particularly for enabling the identification and quantification of the metabolites involved in the central pathways of primary metabolism such as amino acids, sugar alcohols, sugars, organic acids, and polyam- ines. In this study, the metabolite profiling analysis has been performed using GC–MS analysis in two Nostoc species after 24 h exposure to Cu (II), Cr (III), and Ni (II). The GC–MS analysis of an extract of Ni(II) and Cu(II)
Nostoc sp. N27P72 revealed cyclic and ester compounds (Silicon containing heterocyclic compound, Bis(2-ethylhexyl) phthalate, and aromatic diacid compounds), compared to the control. These compounds were recorded as absorption agents in the EPSs. In this regard, Agronematal, 2013, investigated the nematicidal potential of different species of cyanobacterial, Aphanocapsa albida, Anabaena oryzae, Nostoc muscorum, and Calothrix marchica against the Meloidogyne incognita on banana plants. He found that Acetamide, 2-fluoro, Cyclotrisiloxane, hexamethyl, and Oxime-methoxy-phenyl were the major components found in algae by GC–MS. Moreover, Gheda et al., 2020, investigated the natural products from some soil cyanobacterial extracts with potent antimicrobial, antioxidant and cytotoxic activities [69]. They found the presence of Cyclotrisiloxane in phenol compounds of soil cyanobacteria of Nostoc by GC–MS. Additionally, Kanimozhi et al., 2021, found the presence of Cyclotrisiloxane in Microcystis sp. with synergistic activity against the bacterial pathogens using GC–MS [70].

Wu et al. (2006) attributed the cytotoxicity effects of fatty acids to their ability to increase the membrane permeability leading to membrane damage [71]. Mundt et al. 2003 [72], suggested that fatty acids is produced by cyanobacteria as a defense mechanism against other microorganisms might be able to change the permeability of the cell membrane through interacting with proteins and lipids of the membrane, inhibiting special enzymes by a layer around the cells. Presence compounds of Benzene derivatives in Ni and Cu cultures may be related to absorption compounds to remove the metal.

. it has been previously shown that some benzene inhibited b-ketoacyl-acyl carrier protein synthase III, a condensing enzyme that initiates fatty acid biosynthesis in most cyanobacteria, leading to absorption activity of metal. Furthermore, the cytotoxic activity of the pure compound 1, 2-benzene dicarboxylic acid, mono 2-ethylhexyl ester (DMEHE) from marine-derived actinomycete Streptomyces sp. VITSIK8 was examined against mouse embryonic fibroblast (NIH 3T3) and human keratinocyte (HaCaT) normal cell lines, human hepatocellular liver carcinoma (HepG 2), and human breast adenocarcinoma (MCF-7) cell lines by using MTT assay (Krishnan et al., 2014). 1,2-Benzenedicarboxylic acid, bis(2-methyl propyl) ester was recorded by Alghamdi et al., 2018 [73] as a plasticizer and has light and heat stability. The antibacterial properties of olive leaves extract is probablyassociated with the high cyclotrisiloxane hexamethyl content, which has been tested by Keskin et al., 2012 [74], lastly, as compared to solid wastes created from the old approach used for the heavy metal removal, polysaccharides are natural, nontoxic, and biodegradable polymers, thus reducing their polluting effects and making them attractive for potential use as metal-absorbent safe materials.

Conclusion
Base on this study, EPSs play a crucial role in protecting the environment from harmful toxic heavy metals. However, despite large number of studies claimed this role, only a few of them directly investigated the modification of metal removal capability by cyanobacteria under mixotrophic conditions. In this study, the highest EPSs production efficiency was found in cultures supplemented by maltose and biomass of Nostoc sp. N27P72 possesses a high affinity and a high specific uptake for nickel, comparable with the best performances reported by other microbial biomass, and suggest the possibility to use Nostoc sp. N27P72 for the bioremoval of heavy metals from polluted water. The FT-IR spectra showed that treatment of Ni with both strains made obvious changes in functional groups of polysaccharides and linkages. Silicon containing heterocyclic compound and aromatic diacid compounds, a major constituent of Nostoc sp. N27P72. in this study, metal removal capability of Nostoc sp. N27P72 extract was probably associated with the high Silicon containing heterocyclic compound and aromatic diacid compounds content. Although the key elements controlling the cyanobacterial EPSs production have been recognized, inclusive strain-specific studies taking into account the interaction between the variables to know the system reacts to changes, are still missing. This needs a better fact of the genes and metabolic paths complicated in the mechanism of EPS production in cyanobacteria. In conclusion, the strain Nostoc sp. N27P72 may be a suitable candidate for mass production of an ecologically attractive EPSs with a potential use in the bioremediation field.

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Authors’ contributions
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Code availability
Not applicable.

Declarations

Ethics approval and consent to participate
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Competing interests
The authors declare no competing financial interest.

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