Effect of Soil Physico-Chemical Characteris tics on Cyanobacterial Communities in Arid Lands

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Abstract

Cyanobacteria are a group of prokaryotes that can live under stressful environmental conditions due to their high metabolic flexibility. In this study, we examined the terrestrial cyanobacterial communities in the wheat fields adjacent to the indus trial areas of Yazd province. The physical and chemical properties of the soils were evaluated, including pH, EC, salinity, and the concentration of two heavy metals, lead, and cadmium. In addition, the diversity and abundance of cyanobacteria were investigated in the soil of the studied stations. The frequency of taxa was determined based on the colony count method. According to the results, the amount of cadmium was very low (lower than 0.1 ppm), but the lead concentration in the s tudied s tations varied between 1.602 ppm and 4.044 ppm. The sodium concentration in the soil ranged from 16.18 to 89.54 $mEqL^{-1}$. The present results show that with a slight increase in the concentration of lea, the diversity of cyanobacteria does not decrease. Still, an increase in the concentration of information from a specific range causes a reduction in their biodiversity. Moreover, the cyanobacteria abundance does not show any significant decrease in this range of lead concentration. This result may indicate the relative resistance of the dominant taxon to lead metal and the development of these taxon communities in stressful conditions.

Keywords: Cyanobacteria, Diversity, Heavy metal, Salinity, Soil texture

Introduction

Terrestrial ecosystems occupy a large part of each country's natural habitats. These ecosystems are the exclusive habitat of many microorganisms, which have developed biological communities. The abundance and diversity of soil microbial communities depend on various factors including the soil Physico-chemical properties, moisture, organic matter content, and soil texture (Barton and Northup, 2011, Saul-Tcherkas and Steinberger, 2009). In dry land and deserts, several factors such as dryness and salinity significantly affect soil microbial flora (Zhang et al., 2019; Šťovíček et al., 2017).

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Soil salinity is determined based on the concentration of soluble salts such as sodium (Na^+), potassium (K^+), chloride (Cl), and sulfate (SO_4^{-2}) (Qadir et al., 2007). Salinity and high concentration of salts are among the characteristic features of soil in dry desert areas. These factors mostly have adverse effects on the microflora of the growing medium of plants, as well as their growth efficiency and performance (Litalien and Zeeb, 2020). It should be noted that the high concentration of all salts does not necessarily reduce or stop the biological activity of plants and soil microorganisms. Some cations such as Na^+ are mainly responsible for creating salt marshes leading to biological damage to plants, while K^+ is considered an essential element for the growth of plants (Jafary, 2000; Qadir et al., 2007). Also, researchers believe K^+ is the most important essential element for plants after nitrogen (Chookalaii et al., 2020; Prajapati and Modi, 2012)

Another factor affecting the composition of soil biological communities is the presence of industrial heavy metal pollution. An increase in the concentration of heavy metals in the soil naturally causes an increase in the concentration of these metals in plant tissues. Consequently, it causes poisoning due to creating a disturbance in the absorption process and plant growth (Alam et al., 2020; Štofejová et al., 2021). Moreover, previous studies show that the high concentrations of heavy metals in the soil significantly affect soil microflora and the biodiversity of soil microorganisms (Ahlesaadat et al., 2017).

Some algae and cyanobacteria can live in habitats contaminated with heavy metals or saline soils (Hokmollahi et al. 2016; Heidari et al. 2017). These photosynthetic microorganisms grow and socialize well in damaged soils and polluted environments. These resistant taxa have received particular attention in recent years to improve the soil texture in affected and polluted soils (Rocha et al., 2020). Therefore, these microalgae and cyanobacteria are good candidates for environmental cleaning which is known as phytoremediation in biotechnology (Fawzy and Mohamed, 2017; Sarmah and Rout, 2020).

Yazd, a province located in central Iran and southeast Isfahan, is a hyperarid region with limited water resources. According to the reports, the cultivated lands of this province (agricultural lands, gardens, and tree-planted areas) occupy 40,781 hectares (Dashtakian and Baghestani, 2002). In recent years, a decrease in rainfall and an increase in evaporation caused more dryness, accumulation of salts on the surface of the soil, and the destruction of soil texture. These factors can lead to a decrease in crop yields and to the complete desertification of agricultural lands (Ghaeminia et al., 2019). Due to the limitation of water resources in this province, using wastewater or underground water sources in agriculture is one of the leading solutions for providing agricultural water. Studies show that the underground water in Yazd is contaminated with heavy metals due to the tile and ceramic industry (Rahmani, 2009). The results of research on the pollution load of the factories of this region show the presence of large amounts of lead, cadmium, chromium, and zinc in the industrial wastewater of these factories

(Morshedizadeh et al., 2009; Rahmani, 2009).

In the present work, a group of cyanobacteria was studied for their resistance to soil salinity and environmental pollutants. Our purpose was to investigate the cyanobacterial biodiversity in the wheat fields of Yazd province with an emphasis on some fields adjacent to the industrial areas. In addition, the abundance and diversity of these microalgae, which were influenced by the physico-chemical characteristics of the soil and the concentration of environmental pollutants such as lead and cadmium, were investigated.

Materials and Methods

Study stations and soil sampling

The study area is located in the Yazd-Ardakanplain, the northern part of Yazd province (latitudes: 32º 17′ to 32º 24′ N; longitudes: 53º 55′ to 54º 5′ E, average elevation: 1050 m above sea level). The average amount of rainfall in many parts of this province is less than 65 milliliters per year, and the mean annual rainfall is 61.9 milliliters (Ghahraman and Taghvaeian, 2008). The dominant soil texture is loam, silt loam, and sandy loam textures in this area (Rahimian et al., 2014).

About 81% of the industries and factories of Yazd province are located in the Yazd-Ardakanplain which makes the area one of the most contaminated places with heavy metals. Also, an increase in soil salinity as a result of drought has been reported in this area (Sharifi et al., 2020). Therefore, in this study, stations were selected from wheat fields adjacent to the industrial areas of the Yazd-Ardakan plain. Five study stations were considered for the biological evaluation of farm soils in the distance between Yazd city and Ardakan (Table 1). Soil samples were collected according to the Rangaswamy method (1966).

cultivation, purification, and identification of cyanobacteria

In order to cultivate cyanobacterial samples for the morphological identification of taxa, the soil culture method was used. For this purpose, 10 gr of soil from each station was transferred to sterile plates containing liquid nitrate-free BG11 culture medium (Andersen, 2005). Then the plates were placed in the conditions of 12 hours of light, and 12 hours of darkness, with a light intensity of 74 µmol photons $m^{-2}s^{-1}$, and at a temperature of 25±2 ºC. The morphology

Table 1. Geographical details of the sampling locations

Site	Location	Latitude/Longitude
1	Torkabad	32º 20′ N/54º 58′ E
2	Sadrabad	32º 18′ N/54º 00′ E
3	Shamsi	32º 05′ N/54º 06′ E
4	Kalantar farm	32º 11′ N/54º 05′ E
5	Tabas crossroad	32º 02′ N/54º 12′ E

of cyanobacteria was studied using a light microscope (Olympus, Japan). Samples were classified according to morphological characteristics such as the shape and color of the colony, the length, and width of trichomes, the shape and dimensions of vegetative cells, heterocyst, akinete, the position of the akinete in relation to the heterocyst, the presence or absence of heterocysts, the presence or absence of mucilaginous sheath, apoheterocytic or paraheterocytic form of trichomes. In this study, we used valid identification keys to identify the taxa, such as Komárek (2013), Komárek and Anagnostidis (2005).

Counting of cyanobacterial colonies

The colony counting method was used to determine the frequency of cyanobacterial taxa. For this purpose, after identifying microalgae, one percent of soil extracts were obtained by homogenizing one gram of each station's soil in 100 mL of distilled water. One milliliter of the resulting suspension was transferred to the surface of the solid BG11 medium. After spreading the soil suspension on the surface of the culture medium uniformly, the plates were placed in the conditions of 12 hours of light, and 12 hours of darkness, with a light intensity of 74 µmol photons $m^{-2}s^{-1}$, and at a temperature of 25±2 ºC. The number of colonies was counted and calculated after 21 days.

Soil analysis

To evaluate the degree of correlation between the environmental factors and the diversity and abundance of taxa, the quantitative analysis of the physical and chemical factors of the soils were analyzed, including pH, EC, the concentration of Na^+, K^+, phosphorus, total nitrogen, salts, and the concentration of lead and cadmium. Analysis was performed by Arian FanAzma Company, Tehran, Iran. The method of measuring the factors is presented in Table 2. Soil texture was another factor evaluated in this study by the particle size analysis (PSA) method. For this purpose, the type of soil texture was determined based on the ratios of the amounts and percentages of sand, silt, and clay.

Results

In this study, a total of 32 species of cyanobacteria were identified, including four orders, 10 families, and 18 genera. Among the identified taxa, the order Oscillatoriales (filamentous and non-heterocystous cyanobacteria) had the most diversity with 8 genera and 13 species while the members of the order Chroococcales (unicellular or colonial taxa), only had three genera and four species among the identified taxa. In the reported microflora, *Jaaginema* and *Oscillatoria* showed the highest species diversity with 18% and 15%, respectively (Figure 1).

Although Turkabad station had less cyanobacterial diversity than Tabas crossroad and Kalantar farm stations (Figure 2), the dominant taxa present in the soil of this station, especially members of the *Pseudanabaena*, can develop their communities under environmental stress conditions. Moreover, some members of this genus can fix nitrogen. Among the study stations, Sadrabad station also showed less diversity and abundance of cyanobacteria than other stations (Figure 2).

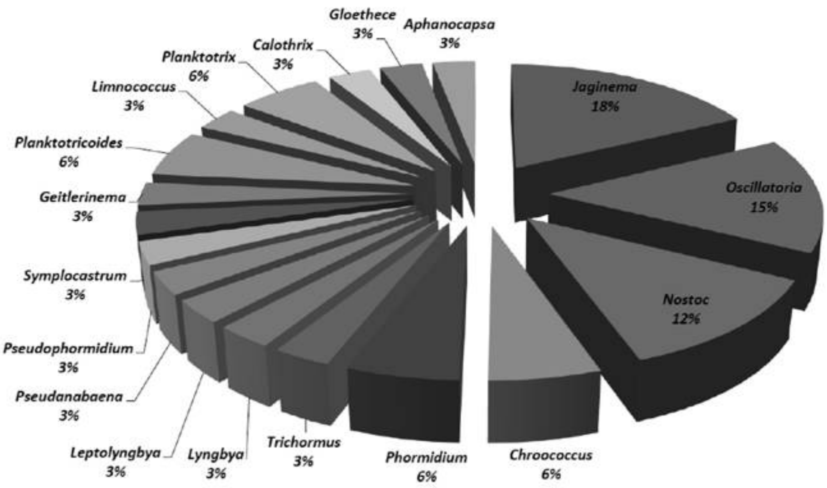

Fig. 1. Frequency percentage of cyanobacterial taxa in soil of studied stations

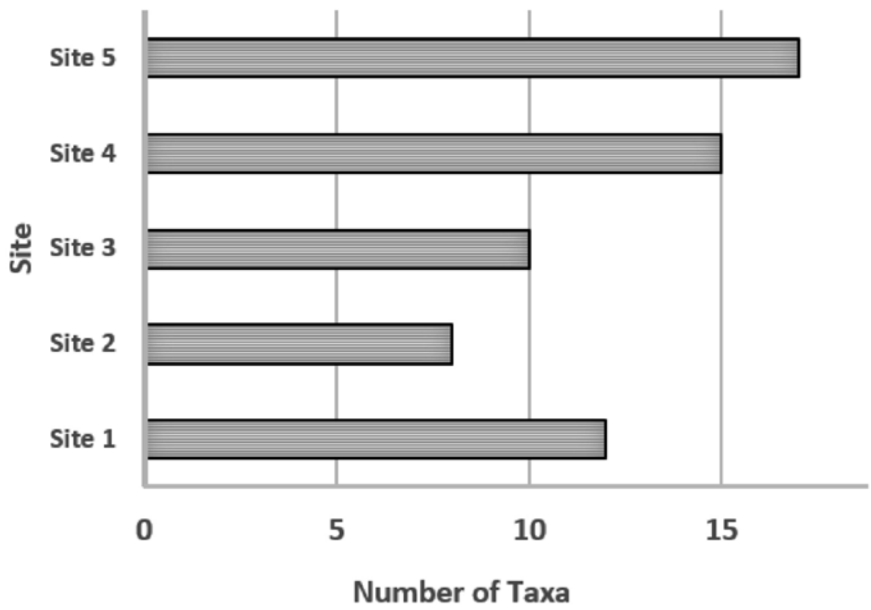

Fig. 2. Genera diversity of cyanobacteria in the studied sites (Site 1. Torkabad, Site 2. Sadrabad, Site 3. Shamsi, Site 4. Kalantar farm, Site 5. Tabas crossroad)

According to the results, the pH of the soil in the studied sites is in the optimal range (between 6.5 and 7) (Figure 3), and only the Kalantar farm station has a pH greater than 7 (Table 2). Tabas crossroad station also showed the lowest pH among the study stations. Based on the results of the present study, the abundance of taxa showed a high correlation with the salinity of the stations (R^2=0.87). In this case, the cyanobacteria abundance increased in the EC of 3.03 to 8.85 dSm^{-1}. In the station 1, a higher EC (12.96 dSm^{-1}), a decrease in the diversity and abundance of taxa was observed (Figure 3). An increase in the diversity and abundance of cyanobacterial taxa was also observed in a specific range of sodium concentrations similar to the effect of EC (except site 1).

The soil of station 1 showed a decrease in abundance and diversity of cyanobacteria taxa in a high concentration of sodium 89.54 mEqL^{-1} (Figure 4).

According to the results, the amount of cadmium in the soil of all the studied stations was significantly lower than 0.1 ppm, but the concentration of lead metal varied between 1.602 ppm and 4.044 ppm depending on the study station (Table 2). In our studied, lead concentration up to 2.68 ppm accompanied by an increase in the diversity of cyanobacteria. According to Figure 5, growth in diversity and abundance of cyanobacteria are observed by increasing lead concentration in a specific range.

Phosphorus and nitrogen are essential nutrients, which have a significant effect on soil microflora and impact on the development of biological communities in the soil. In this study, the amount of soil nitrogen did not show a significant correlation with the abundance and diversity of cyanobacteria. Besides, based on the results, an increase in soil phosphorus up to 30 ppm increased the cyanobacterial abundance. However, in the Shamsi station an increase in phosphorus (54.48 ppm) was associated with a decrease in the abundance and diversity of cyanobacteria (Table 2). Our results show that the soil's percentage of sand and silt correlates with the species diversity of the investigated stations. The correlation was particularly high concerning the percentage of sand, and the diversity and abundance of cyanobacteria decreased in most stations with the increasing amount of sand. In contrary to the negative correlation between the diversity and abundance of cyanobacterial taxa with the percentage of sand in the soil, the diversity of cyanobacteria species increased with the increase in the silt

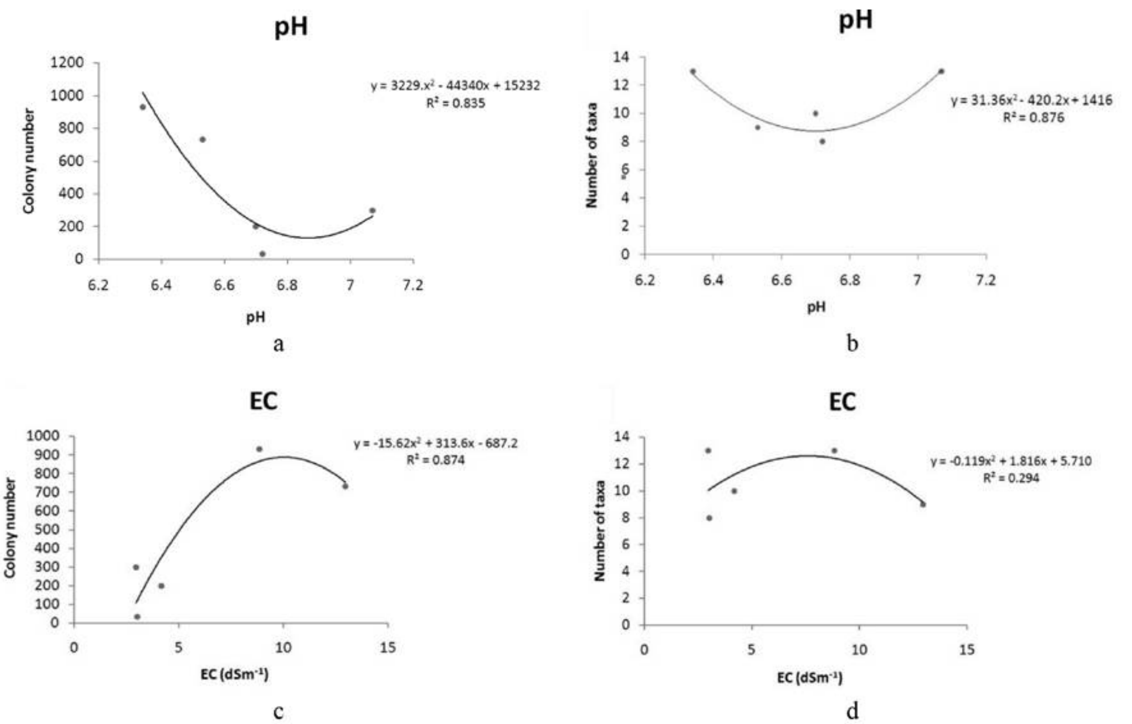

Fig. 3. a, b. The correlation curve between pH and colony number and number of taxa; c, d. The correlation curve between EC and colony number and number of taxa

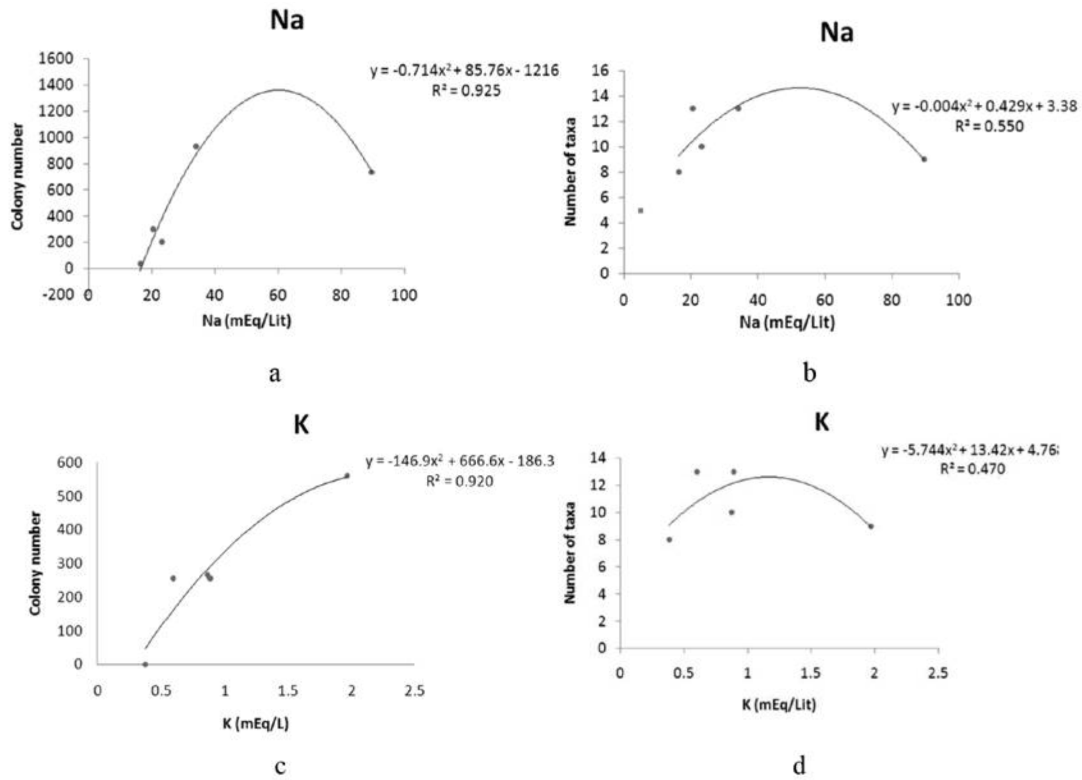

Fig. 4. a, b. The correlation curve between Na^+ concentration and colony number and number of taxa; c, d. The correlation curve between K^+ concentration and colony number and number of taxa

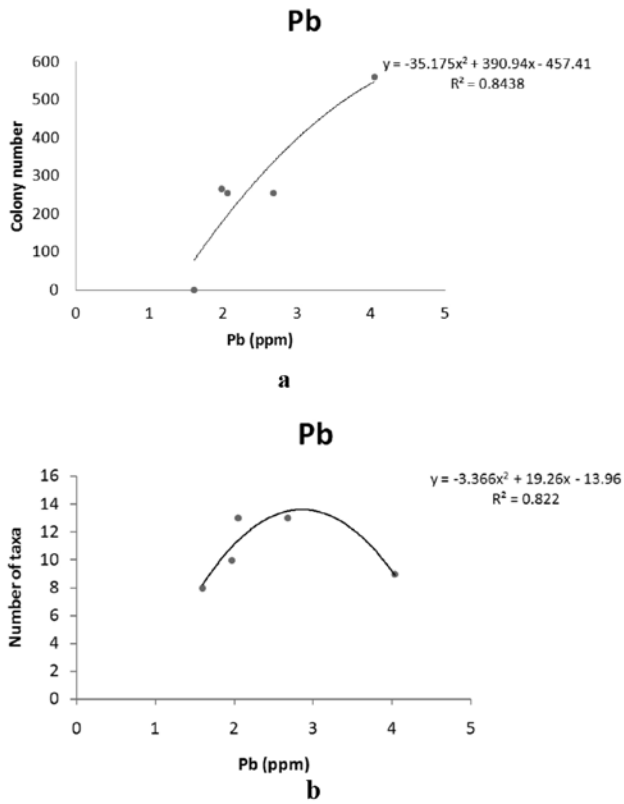

Fig. 5. a, b. The correlation curve between Pb concentration and colony number and number of taxa

Table 2. Physical and chemical data of soils collected from five stations

Parameters	Method	Sites				
		Site 1	Site 2	Site 3	Site 4	Site 5
pH	Electrometric	6.53	6.72	6.70	7.07	6.34
EC (dSm^{-1})	Platinum Electrode	12.96	3.03	4.18	2.98	8.85
Cd (ppm)	Atomic absorption	0.1>	0.1>	0.1>	0.1>	0.1>
Pb (ppm)	Atomic absorption	4.04	1.60	1.97	2.68	2.05
Na^+ ($mEqL^{-1}$)	Flame Emission Photometric	89.54	16.18	22.98	20.30	33.95
K^+ ($mEqL^{-1}$)	Flame Emission Photometric	1.97	0.38	0.87	0.60	0.89
Total nitrogen (mgL^{-1})	Macro kjeldahl	0.05	0.04	0.08	0.07	0.02
phosphorus(ppm)	Vanadomolybdophosphoric	14.50	4.96	54.48	10.76	35.40
Sand (%)	-	42.16	58.16	48.16	38.16	78.16
Silt (%)	-	34.00	26.40	26.00	40.40	10.40
Clay (%)	-	23.84	15.44	25.84	21.44	11.44
Texture	PSA method	L	SL	SCL	L	SL

Site 1. Torkabad, Site 2. Sadrabad, Site 3. Shamsi, Site 4. Kalantar farm, Site 5. Tabas cross road, L:Loam, SL:Sandy Loam, SCL:Sandy Clay Loam

in most of the studied stations, except for station 5. However, the abundance of taxa did not show a high correlation with the amount of silt in the soil (Figure 6).

Discussion

Microalgae, especially cyanobacteria, are an important part of soil microbial communities. The high adaptability of these microorganisms allows them to be present in many habitats such as the soil of terrestrial ecosystems. Soil is a complete ecosystem affected by the interaction of various elements, including biological, physical, and chemical factors. This tripartite interaction creates many vital relationships in nature. In other words, just as the microorganisms in the soil are able to influence the structure of the soil and its compounds, the physical and chemical properties of the soil, including soil texture, pH, EC, and minerals can also influence the formation of biological communities (Sneha et al., 2021; Santra, 1993).

Among the physical properties of soil, pH is the most critical factor affecting the diversity and growth of cyanobacterial communities (Nayak and Prasanna, 2007). The neutral to slightly alkaline pH range is the most suitable pH for the optimal growth of these microalgae (Shariatmadari et al., 2013). On the other hand, the acidic pH range (between 4 and 5) is considered a stressful environment for cyanobacteria. Based on the results of the present study, the investigated soil samples did not show any noticeable difference in pH, and the pH of the soil of the studied sites is in the optimal range (between 6.5 and 7) (Figure 3). Therefore in our study, pH cannot be considered a determining factor in relation to species abundance and diversity.

Salinity is another environmental factor that can affect the diversity and development of cyanobacterial communities in different

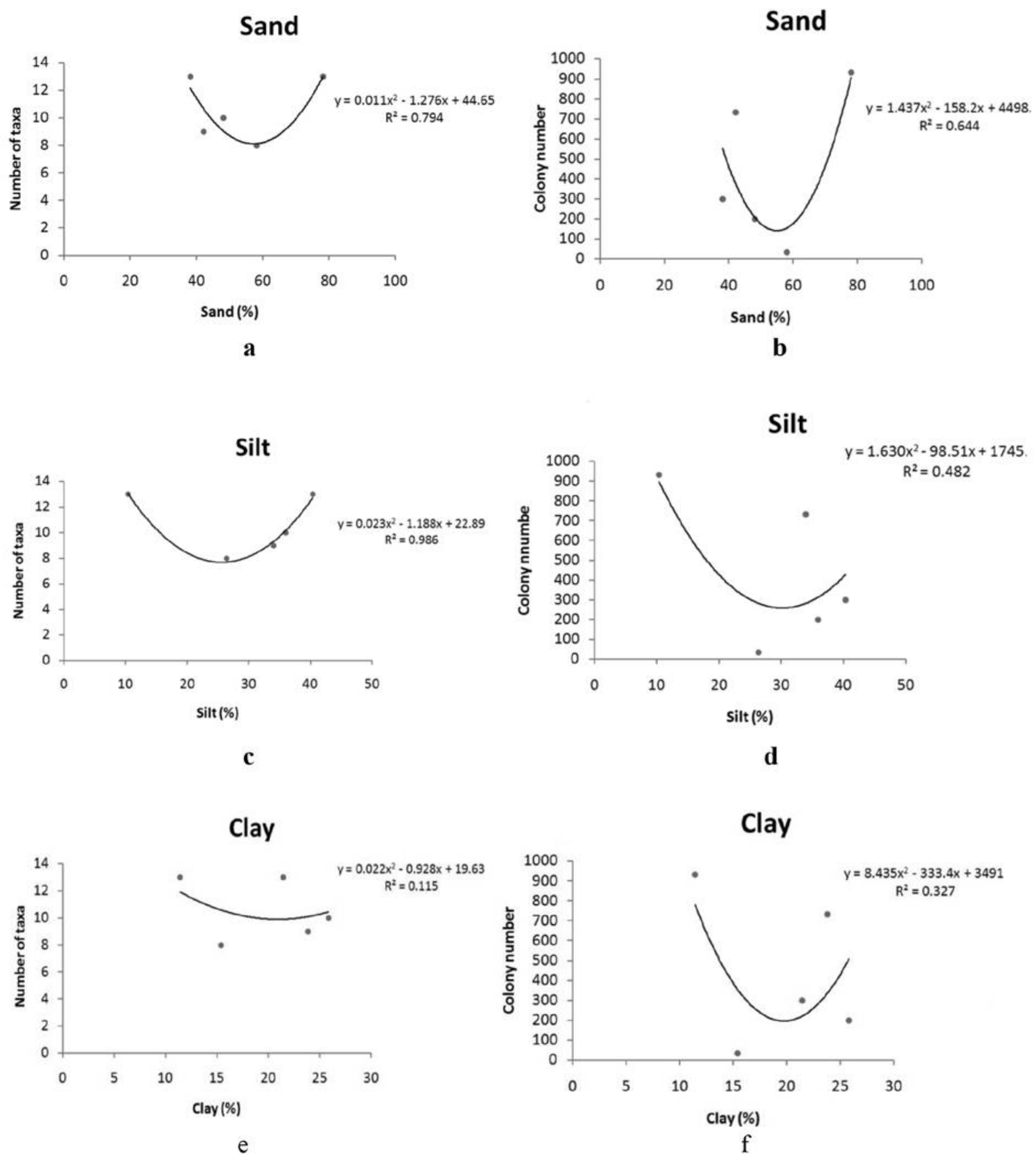

Fig. 6. a-f. a, b. The correlation curve between sand (%) and number of taxa and colony number; **c, d.** The correlation curve between silt (%) and number of taxa and colony number; e, f. The correlation curve between clay (%) and number of taxa and colony number

habitats by influencing the physiological activities of cyanobacteria, such as photosynthesis, growth, and nitrogen fixation (Srivastava et al., 2009). In the present study, the soil salinity of the studied farms is moderate to non-saline (Table 2). Although a decrease in the diversity and abundance of algae was expected with increasing salinity levels, the reduction was not necessarily observed.

According to some reports, non-heterocystous cyanobacteria grow better in saline soils (Srivastava et al., 2009). In the present study, the frequency of two genera *Jaaginema* and *Oscillatoria* were 18% and 15%, respectively. The estimation showed that these non-heterocystous taxa were the most diverse genera in the examined soil (Figure 1). In other respects, it has been shown that heterocyst-forming cyanobacteria, such as *Anabaena* and *Nostoc* species, are also resistant to salinity and play an important role in maintaining the fertility and productivity of saline soils (Zahran et al., 1992). In the present study, the members of the genus *Nostoc* had relatively high diversity and accounted for about 12% of the total cyanobacterial diversity of the study station's soil (Figure 1).

Based on the results, the abundance of taxa showed a high correlation with the salinity of the stations (R^2=0.87). The concentration of different salts, like sodium, affects the EC value. This study observed an increase in the diversity and abundance of cyanobacterial taxa in a specific range of sodium concentrations (Figure 4). Therefore, it can be said that the cyanobacterial flora of the study area is generally halophilic, and the increase in salinity level is tolerated by some native taxa in the studied sites.

Physical characteristics of soil (soil texture) and their effect on the diversity and abundance of cyanobacteria have been mentioned less in previous studies. In this research, besides studying the effect of other physical and chemical soil factors on species diversity, the effect of soil texture on the diversity and abundance of cyanobacteria was also investigated.

According to the results of our study, the percentage of sand and silt in the soil shows a correlation with the cyanobacterial species diversity. In other words, the diversity and abundance of cyanobacteria decreased in most stations with the increasing amount of sand. Some previous studies also show that the diversity of cyanobacteria in sandy soils is much lower than non-sandy soils (Bhatnagar et al., 2008). One of the reasons for the negative correlation between the percentage of soil sand and the cyanobacterial species diversity is that an increase in the percentage of sand reduces the amount of the active part of the soil and causes a decrease in the ability to retain soil moisture. Obviously, with the decrease in the humidity level, there will be the possibility of establishing more limited communities of these moisture-loving algae. Notably, the results of studies conducted in recent years show that the establishment of resistant cyanobacteria in sandy soils could gradually improve the texture and performance of the soil (Issa et al., 2001). Thus, soil inoculation with cyanobacteria has been proposed as a sustainable biotechnological technique for rehabilitating degraded areas and dry lands (Issa et al., 2001).

Other factors examined in this study include the concentration of lead and cadmium in the soil. Pinchasov et al. (2006) believed that lead toxicity is not only dependent on its concentration in the environment, but the resistance of living organisms present in that environment, the chemical form of lead and its solubility and ease of entry into the cell are the factors that matter to the subject. According to the results of our study, with the increase of lead concentration in a certain range, an increase in the diversity and abundance of algae is observed.

Although, this can be affected by other environmental factors, what is clear is that the lead concentration up to 2.68 ppm has not caused a reduction in soil cyanobacterial diversity and has not had a limiting effect on the native taxa of the region. However, a decrease in diversity has been observed at a concentration higher than this value, which can indicate inappropriate concentration range of lead for native cyanobacteria in this region.

Based on the results, despite the high lead concentration in the soil, the Turkabad station has a relatively high abundance of cyanobacteria. Unlike the abundance of algae observed in this site, the results showed low species diversity in this station compared to the Tabas crossroad and the Kalantar farm stations. The higher abundance of cyanobacteria, despite their low diversity of them in the Turkabad station, can be due to the higher resistance of the dominant taxon, *Pseudanabaena* sp. against high concentrations of heavy metals. In other words, considering the high concentration of lead in the soil of the Turkabad station, it seems that the cyanobacterial taxa present in this station are heavy metal-resistant. Despite their low diversity, they can form communities in extreme environments.

The diversity and abundance of cyanobacteria in the soil of the studied wheat fields depend on the sum of the evaluated items, including the physical and chemical factors of the soil, and the resistance level of the taxa to various environmental factors. In the studied stations, the abundance and diversity of cyanobacteria showed a high correlation with the EC, pH, as well as sodium and lead concentration of soil.

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