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ABSTRACT

Biocrusts and adjacent sediments were collected from the so-called loess plateau of Northern Iran and the loess deposits on the foothills of Alborz Mountains. The mineralogical and granulometric analyses characterized sediments as quartz-rich clayey or sandy silts. Cyanobacterial diversity, colony morphology and grain stabilization were studied by light and scanning electron microscopy, which showed glue-like layers of cyanobacterial EPS and dense networks of filamentous cyanobacteria immobilizing finer and bigger grains, respectively. Amorphous, tuft and globular cyanobacterial colony structures were also detected. 15 cyanobacterial genera were identified in the biocrust samples including *Microcoleus vaginatus* as the common species in all samples, *Aphanocapsa*, *Aphanothece*, *Calothrix*, *Chroococcus*, *Chroococcidiopsis*, *Cyanosarcina*, *Hassallia*, *Homeothrix*, *Nostoc*, *Oculatella*, *Schizothrix*, *Scytonema*, *Tolypothrix* and *Trichocoleus*. Two biocrust samples showed traces of toxicity in a protein phosphatase inhibition assay targeting microcystins and similarly acting compounds. The *Artemia salina* assay revealed an elevated toxic response in one sample.

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1. Introduction

Most of the scientific attention concerning the western part of Eurasian loess belt has been related to loess-paleosol sequences of south-eastern Europe and Central Asia (Frechen and Dodonov, 1998; Ding et al., 2002; Bronger, 2003; Marković et al., 2008, 2009; Buggle et al., 2009). However, recent investigations of northern Iranian loess-soil sequences (Kehl et al., 2005, 2010; Frechen et al., 2009; Lauer et al., in this issue; Vlaminck et al., in press) improve the understanding of how European, Central Asian and Chinese loess provinces are linked to each other (e.g. Marković et al., 2012, 2015).

According to the newest hypothesis on loess formation (Smalley et al., 2011; Svirčev et al., 2013), the origin of loess is partly biogenic,

and it is therefore crucial to understand what kind of biological crust communities are found in the loess sites. Biocrusts occur throughout the world in arid and semi-arid biotopes (West, 1990; Büdel, 2002), where they can cover up to 70% of the soil surface (Belnap et al., 1994; Buis et al., 2009). They are particularly significant ecosystem components in arid and semi-arid conditions (Belnap, 2006). Biocrusts are composed of cyanobacteria, algae, fungi, lichens and/or mosses in association with soil particles (Evans and Johansen, 1999; Belnap, 2006). In addition, secreted metabolites consisting mainly of extracellular polymeric substances (EPS) (Lan et al., 2012) and microfauna (Pócs et al., 2006) are components of biocrusts.

Biocrusts are known to trap airborne dust as a part of the life strategy of crust organisms (Zaady and Offer, 2010; Williams et al., 2012; Pietrasiak et al., 2014). According to the BLOCDUST hypothesis (Svirčev et al., 2013) loess formation is a partly biogenic process dependent on biocrust microorganisms, and one type of biocrusts is biological loess crusts (BLC). The wet phase of microorganisms on the loess surface, known as biological loess mat (BLM), captures and

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accumulates airborne particles while the crusted surface of the BLC phase protects the accumulated material from erosion by wind and water, and thus prevents deflation of the dust deposited.

The dominance of cyanobacteria as BLC constituents is the result of several cyanobacterial properties including xerotolerance and the ability to withstand moisture fluctuations and high irradiance as well the ability to survive active-dormant-active cycles (Raanan et al., 2015). The characteristic ability of cyanobacteria to grow photoautotrophically and the capacity of some species to fix nitrogen make cyanobacteria primary colonizers of loess surfaces. The production of EPS further enhances the surface colonization and the formation of biofilms and microbial mats, leading to the formation of more complex multilayered structures. The stable hydrated microenvironments provided by cyanobacteria, together with necessary nutrients obtained by captured particles, enable microalgae, mosses and other organisms to become part of the biological structures (Zhang et al., 2015).

The cyanobacteria of BLC can, however, produce toxins which may jeopardize human health either through exposure to dust from eroded biocrusts or through toxins which have entered the food web. Biocrusts are very sensitive to compressional and shear forces generated by off-road traffic and other human activities as well as trampling and grazing by livestock (Belnap et al., 2007; Williams et al., 2008). The affected biocrusts cannot offer full sediment surface protection in disturbed semi-arid and arid areas (Danin et al., 1989; Eldridge and Leys, 2003). The sensitivity to anthropogenic and natural disturbance has resulted in a degraded state of biocrusts in many regions, and these areas are affected by widespread wind erosion (Belnap and Gillette, 1998). Crust removal by wind influences human and animal health in two ways and compromise human occupation, health and livelihood in loess environments (Derbyshire, 2001). A major concern is the inhalation of dust particles released in the absence of the protective crust cover. Another possible health risk in arid and semi-arid areas is the inhalation of wind-carried crust particles containing metabolites produced by cyanobacteria in BLM during the wet phase and preserved in BLC during the dry phase. Cyanobacteria of terrestrial or aquatic origin produce an array of potent cyanotoxins (Metcalf and Codd, 2012) which have relevance from both ecotoxicological and human health point of view. The ecotoxicological significance of cyanotoxins in the terrestrial environment has not been extensively studied but can be extrapolated from the ecotoxicology of cyanotoxins in the aquatic settings which is better known. Microcystins are common cyanotoxins which exert their effects through inhibition of protein phosphatases. These enzymes are ubiquitous and any organism in any environment might be affected provided there are mechanisms of exposure and uptake (Metcalf and Codd, 2012). Inhalation of cyanotoxin-containing dust and aerosolized cyanotoxins released by (damaged) crusts can be seen as relevant cyanobacterial risks for human health in the arid and semi-arid environments. Rather low but still toxicologically important quantities of microcystins ($3-56$ µg/m^2) were found in desert crusts in Qatar (Metcalf et al., 2012). Cyanobacterial neurotoxins are also present in these desert crusts. BMAA, β-N-methylamino-L-alanine, identified in cyanobacteria, has been suggested as an environmental cause of sporadic amyotrophic lateral sclerosis (ALS) and other human neurodegenerative diseases (Cox et al., 2005; Bradley and Cox, 2009). For instance, BMAA has been discussed as a factor behind the increased frequency of ALS among Gulf war veterans exposed to cyanobacteria and their toxins in the desert dust generated from crusts damaged by vehicles and military activities (Cox et al., 2009). Anatoxin-a(S), a cyanobacterial organophosphorous compound with a rapid toxic action, has also been found in the Qatar biocrusts and this toxin has been associated with mortalities of

dogs drinking cyanotoxin-containing water (Metcalf et al., 2012; Chatziefthimiou et al., 2014). The full extent of cyanotoxin production in loess is still poorly known. The obvious potential for cyanotoxin production in terrestrial cyanobacteria, as shown by the studies of Qatar biocrusts (Metcalf et al., 2012, 2015; Richer et al., 2015) and common toxin production by aquatic cyanobacteria (Metcalf et al., 2012), motivate cyanotoxin analyses in loess biocrusts and sediments.

Human exposure to cyanotoxins can occur through a variety of routes: consumption of contaminated drinking water, fish/seafood, agricultural products (e.g. vegetables irrigated by toxin-containing water) or dietary supplements; inhalation of aerosols containing cyanotoxins; mucosal or dermal contact with cyanotoxins during recreational or professional activities; and intravenous contact with cyanotoxins during hemodialysis treatment with contaminated water (Drobac et al., 2013). One further exposure route that has not been discussed in scientific literature till now, might be also human exposure through contaminated meat prepared from animals after grazing cyanobacterial biocrusts. As a result of ingestion of cyanotoxin-containing biocrusts by cows, goats and sheep, toxin accumulation in tissues and milk may pose a risk to human health. Grazing of biocrusts is a common phenomenon on the Iranian Loess Plateau in Golestan province. Whether this practice has any consequences on human health remains to be examined through epidemiological and other studies concerning human health in Iran.

Much of the research on biocrusts is taking place in the deserts of Southwestern USA (Williams et al., 2012), Israel (Hagemann et al., 2015), Southern Africa (Dojani et al., 2014), India (Kumar and Adhikary, 2015), China (Zhang et al., 2007), Australia (Williams et al., 2014) and polar regions (Pointing et al., 2015). Research on biocrusts in arid and semi-arid regions is gaining more interest recently primarily because biocrusts are becoming recognized as a potential solution against aridization and desertification (Li et al., 2010; Wu et al., 2013; Lan et al., 2014).

Although more than 70% of Iran is covered with semi- and real deserts, almost no research on biocrusts has been conducted in this region. To the authors' knowledge, the only data are provided by the research of Moghtaderi et al. (2009, 2011) that specifically deals with the microbial community of cyanobacteria in biocrusts of Chadormalu desert, Yazd Province of Iran.

In this paper we report the cyanobacterial biodiversity, colony morphology, sediment stabilization and toxicity of cyanobacteria-dominated biocrusts and adjacent sediments from North Iranian Loess Plateau for the first time. This work is an innovative opening in loess research in general.

2. Materials and methods

2.1. Sampling sites

In Northern Iran, the loess deposits of the Caspian Lowland form smooth hills covering the northward facing slopes of the Alborz mountain range (Fig. 1). The landscape is dissected by the several river catchments, draining the Alborz Mountains. In this area, the loess deposits are up to 30 to more than 70 m thick (Kehl et al., 2005).

The Caspian Lowland itself represents a basin of subsidence (Brunet et al., 2003), providing an extensive accommodation space for marine and alluvial sediments such as the fluvial deposits. Depositional changes in the context of Quaternary climate shifts (Frechen et al., 2009), and base-level variations due to fluctuations of the Caspian Sea-level (Forte and Cowgill, 2013) provide many available silty material for loess deposition. This might be a reason for high resolution loess-paleosol sequences in the investigated
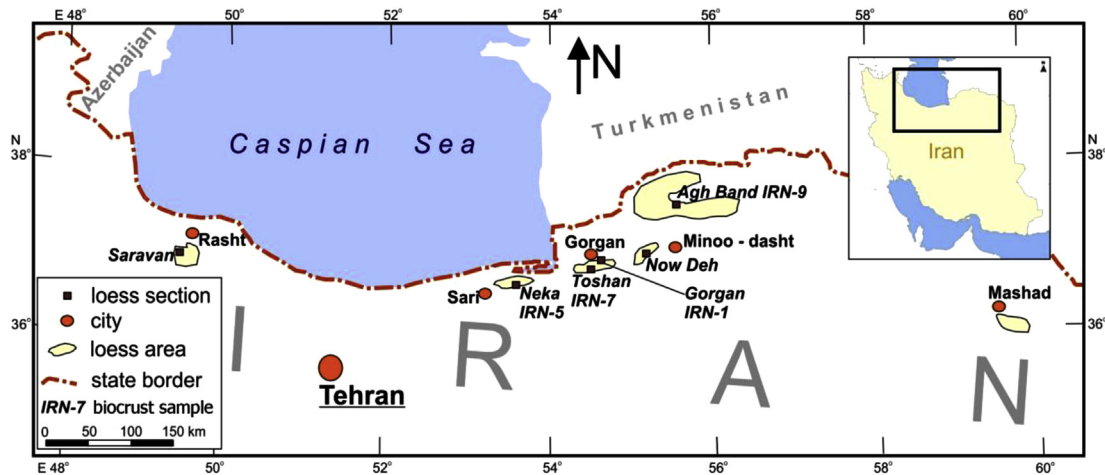
Fig. 1. Sample sites of biocrusts in North Iran (according to Lauer et al., in this issue and Vlaminck et al., in press, modified).

area. Several loess spots have been preserved between the arid regions of Central Asia and the sub-humid foreland of the Alborz Mountains (Kehl, 2010).

The present climate at Gorgan is sub-humid with a mean annual precipitation of 600 mm as measured at the meteorological station at Gorgan. Towards the Turkmen steppe, precipitation rates quickly decline to an annual mean of 200 mm. Near to the section at Neka, situated to the west of Gorgan, the average precipitation is 750 mm per year. The annual mean temperature in the Caspian Lowland is approx. 17 °C (Kehl et al., 2005). The main wind patterns in Iran are largely governed by seasonal pressure differences between the Caspian Sea and the Iranian Highlands. In the Iranian plateau a high pressure cell develops during the cold winter months, whilst the surface water of the Caspian Sea exhibits relatively low pressure, owing to its thermal inertia (Ganji, 1968). The distribution of pressure causes air masses to flow from the Iranian plateau towards the Caspian Sea. During the hot summer months the high pressure cell in the Iranian plateau is replaced by a strong heat depression, whereas a relative center of high pressure forms above the Caspian Sea, leading to an opposite wind direction (Ganji, 1968).

During the glacial intervals of the Quaternary, northern Iran represented an extensive depositional environment for windblown dust and loess formation (Frechen et al., 2009), covering particularly the northern foot slopes of the Alborz Mountains. The profiles Neka, Now Deh and Agh Band are located along a climatic gradient, ranging from sub-humid conditions in the west near Neka to semi-arid conditions towards the north and north-east at Agh Band (Fig. 1).

The profiles represent a climosequence including climatophytomorphic modern soils, which show differential degrees of soil formation, as expressed by their respective morphology, clay mineralogy and their physicochemical properties corresponding to decreasing humidity from W to E (Khormali and Kehl, 2011). Following these different environmental dynamics we sampled biocrusts from loess section near Gorgan (IRN-1), loess walls uncovered at brickyards at Neka (IRN-5) and Toshan (IRN-7), as well from the loess plateau around Agh Band (IRN-9).

2.2. Mineralogy analyses

Simultaneously with the preparation of samples for the pipet method, sandy fraction i.e. fraction greater than 0.063 mm was collected as a residue using a sieve with the pore size of 0.063 mm. In the case of IRN 5 sample, this fraction was separated into five

granulometric fractions (0.063–0.125 mm, 0.125–0.25 mm, 0.25–0.5 mm and greater than 0.5 mm) applying the dry method. Mineralogical and petrographic composition on these fractions was determined using optical methods (binocular microscope and polarization microscope). The grain size classification was performed according to Wentworth (1922).

2.3. Granulometric analysis

Granulometric tests were used in order to define sediments and their structural characteristics. Determination of sediments was carried out on the basis of three component diagram sand-silt-clay (Konta, 1969).

Since the samples belong to the fine-grained sediments (fraction smaller than 0.063 mm), granulometric analysis was performed by combining two methods: method of sifting and pipette method. The material was sieved through a 0.063 mm pore size sieve, giving the initial sample weight of about 25 g.

Pipet method required a pre-treatment of each sample that included immersion in distilled water for 24 h. Afterwards, the samples were boiled for 1 h. The cooled suspensions were sieved by a sieve of 0.063 mm pore size. The fractions larger than 0.063 mm were dried and measured, and the filtrates were treated in an ultrasonic bath for 30 min. After cooling they were individually poured into a graduated cylinder of 1 L. The fractions were extracted by 20 ml pipette, after the relevant time (Obradović and Vasić, 1988).

2.4. Determination of species composition and colony morphology

2.4.1. Stereo microscopy

Cyanobacterial colony morphology of superficial layers of crusts was described from the pieces of undisturbed crusts. Cyanobacteria-dominated biocrusts were observed under binocular stereomicroscope (Leica MZ), both dry and after wetting the surface. Pictures were taken using a Leica DFC320 digital camera and the AS Software, Version V2.4.0 R1.

2.4.2. Light microscopy

Determination of species composition was performed on samples isolated directly from the crusts after wetting. Different morphological features observed on the surface by stereo microscope were sampled and examined using Olympus BX 51 microscope. Pictures of observed cyanobacterial species were taken using

an Olympus DP 26-DKTB digital camera, with Olympus cellSens Entery CS-EN-V1,7 software.

2.4.3. Scanning electron microscopy (SEM)

SEM micrographs were taken on Jeol JSM-6610LV. Crust samples were coated with gold − thickness (nm): 15.0, density (g/cm^3): 19.32. The element used for optimization was copper. Additional conditions were: source of electrons: W wire; acceleration voltage of electrons: 20 kV; recording under ultra-high vacuum conditions: 10^{-9} Pa.

2.5. Evaluation of toxicity

2.5.1. Preparation of crust material

Crust samples were prepared in the following manner: Crust material was separated from the sediment by scraping it off, and approximately 10 mg samples were weighed in Eppendorf tubes. One ml 80% methanol was added per 10 mg crust, and microcystins in the samples were extracted by vigorous vortexing, sonication in an ultrasound bath for 15 min and shaking the samples overnight on orbital shaker at 30 °C in dark. The extracts were clarified by centrifugation at 11,000 × g for 10 min. The supernatants were collected and evaporated to 20% of the original volume by air flow at 50 °C. The toxicity in the remaining extracts was analyzed with protein phosphatase inhibition assay (MicroCystest kit) and Artemia salina assay.

2.5.2. MicroCystest

The extracts were assessed for protein phosphatase inhibition using the MicroCystest kit (Zeu-Inmunotec S.L., Spain) in the 96-well microtiter plate format. The MicroCystest kit is based on the measurement of protein phosphatase inhibition by microcystins and nodularins or similarly acting substances in the samples. The kit was used according to the manufacturer's instructions. Measurements of absorbance at 405 nm were made by a SPECTROstar Nano microplate reader (BMG Labtech, Germany).

negative control (Chroococcidiopsis sp.) was prepared the same way as the crusts. Deaths were recorded after 24 h and the surviving animals were killed by the addition of 100 μl methanol to each well and counted. Toxicity was expressed as the percentage of dead larvae minus the mortality in control samples. Triplicate aliquots of each sample were analyzed.

3. Results

3.1. Mineralogy

Mineralogical and petrological analyses confirmed the uniform distribution of mineral content with quartz being the most common component among all the samples analyzed. Some grains of quartz were coated either with iron hydroxides or secondary carbonates. The second dominant fraction was rock fragments characterized by the highest level of roundness. Feldspars were present in the classic forms of terrigenous sediments with a varying degree of secondary alternations. Fragments of fossils were observed in all analyzed samples, containing primarily debris of mollusk shells, mostly belonging to continental snails. Poorly carbon fragments of plants, originating from the root system, were also detected in all samples, notably IRN 7. Micas were present with muscovite and biotite forms in characteristic colors such as silvery white, green and dark brown. Carbonates, mostly comprised of calcite, were also observed in all samples. Some present terrigenous grains could not be mineralogically and petrologically defined since they were coated with microcrystals of calcite or iron hydroxide.

### 3.2. Granulometry, species composition and colony morphology

3.2.1. Sample IRN 1

According to the share of sand (14.3%), silt (62.0%) and clay (23.7%) fractions, the IRN 1 sediment was classified as a sandy clayey silt. The histogram was unimodal with the predominant fraction ranging from 0.016 to 0.063 mm. The sediment was poorly sorted with an average grain size of 0.026 mm (Table 1).

Table 1
Granulometric properties of the sediment below cyanobacteria-dominated crusts.

Sampling site (sample)	Grain type			Sediment category	Average grain size (mm)
	Sand%	Silt%	Clay%		
IRN 1	14.3	62.0	23.6	Sandy clayey silt	0.026
IRN 5	54.3	31.7	13.9	Clayey silty sand	0.057
IRN 7	8.6	78.0	13.3	Clayey silt	0.023
IRN 9	26.7	65.5	7.7	Sandy silt	0.045

2.5.3. Artemia salina assay

In Artemia salina assay 21 crust samples were analyzed. Toxicity of crust samples was assessed using A. salina larvae according to Kiviranta et al. (1991). A. salina was grown in medium ASW (artificial sea water), illuminated by light and gently aerated. In 200 ml of sterile medium 0.9 g of dried brine shrimp eggs were added and incubated at 30 °C, under illumination for 24−36 h. The larvae were used in tests 26 h after hatching. For toxicity tests, 96-well microtiter plate had been dosed with 50 μl batches of samples with methanol which evaporated overnight. Afterwards, 100 μl aliquots with about 15 larvae in 200 ml of ASW were transferred to a microtiter plate and then incubated for 24 h under low ambient light at 30 °C. ASW was used as a control, and as a further control, 80% (v/v) evaporated methanol in ASW with shrimps was used. Also, 10 mg of dried biomass of positive (Microcystis aeruginosa PCC 7806) and

Stereomicroscopic observations of crusts sampled at IRN 1 (Fig. 2a) confirmed the presence of two different cyanobacterial colonies. Throughout most of the biocrust superficial layer, small emerging parts of blackish filament network of the cyanobacterium Microcoleus vaginatus were observed (Fig. 2d−f). The most of the filamentous network, however, was observed in the underlying layer, with filaments surrounding and trapping the particles. The second type of the cyanobacterial colony was represented by the filamentous cyanobacterium Tolypothrix sp. whose dark brown filaments covered the wide area of the superficial layer (Fig. 2b−c).

3.2.2. Sample IRN 5

The sediment collected in this site was characterized as clayey silty sand, according to the analyzed share of sand (54.3%), silt (31.7%) and clay (13.9%) fractions. The histogram was unimodal
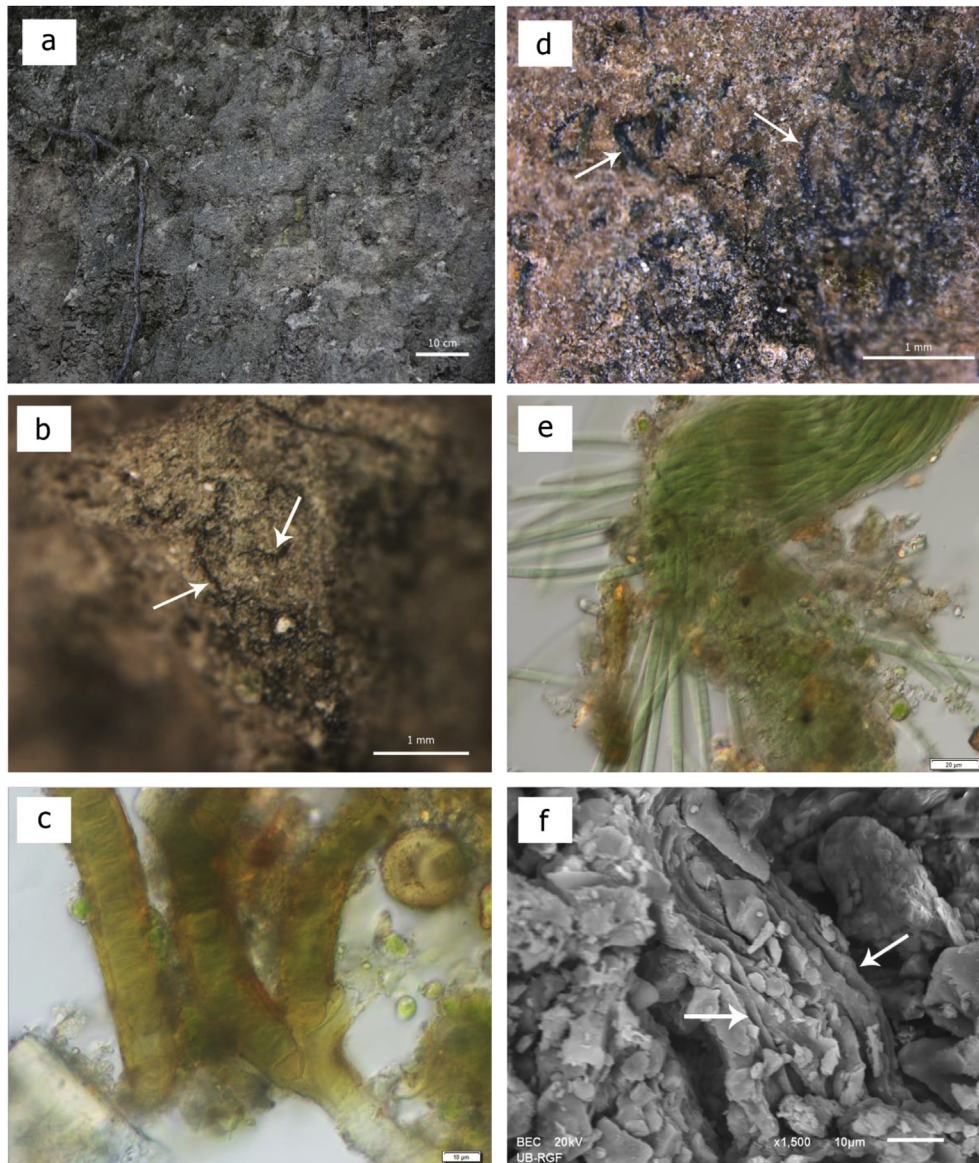
Fig. 2. Cyanobacteria-dominated crusts at sampling site IRN 1: vertical loess-paleosol sequence covered by darkish olive-green biocrusts; scale bar = 10 cm (a). Dark brown to blackish filaments of *Tolypothrix* sp. in the superficial layer of the biocrust; scale bar = 1 mm (b). Photomicrograph of *Tolypothrix* sp. trichomes in tubular EPS; scale bar = 10 μm (c). Emerging blackish parts of *M. vaginatus* filaments in the superficial layer of the crust; scale bar = 1 mm (d). Photomicrograph of *M. vaginatus* filaments partly enveloped with tubular EPS; scale bar = 20 μm (e). SEM micrograph of *M. vaginatus* EPS tubules (arrows) with finer s glued onto the surface; scale bar = 10 μm (f). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with predominant fraction above 0.063 mm. The sediment was moderately well sorted with an average grain size of 0.057 mm (Table 1).

The crusts sampled at IRN 5 (Fig. 3a) were dominated by two filamentous cyanobacteria − *Scytonema* sp. and *Microcoleus vaginatus*. The filaments of *Scytonema* sp. formed a firm, dense, dark brown filament net through the superficial layer of the crust that also penetrated the underlying layers (Fig. 3b−c,f), while the filaments of *M. vaginatus* species spread solely throughout the underlying layers of the biocrust (Fig. 3d).

3.2.3. Sample IRN 7

According to the content of sand (8.6%), silt (78.0%) and clay (13.3%) fractions, the sediment is classified as clayey silt. The histogram was unimodal with predominant fraction ranging from 0.008 to 0.016 mm. The sediment was moderately well sorted with an average grain size of 0.023 mm (Table 1).

Cyanobacteria-dominated biocrusts collected at IRN 7 (Fig. 4a) were characterized by the highest cyanobacterial species diversity. Stereo microscope and microscope observations revealed the presence of three different colony types in the superficial layer, as well as the presence of the *Microcoleus vaginatus* species in the underlying layers of the biocrusts. The first colony type was characterized by the dominance of two species − *Homeothrix* sp. and *Hassallia* sp. that formed a blackish to dark olive-green filamentous tufts adhered to the surface of the sediment (Fig. 4a−b). SEM micrograph shows how thick and amorphous EPS layer was spread like a dense net over the surface (Fig. 4c,e). The second colony type was characterized by a blackish sheath of amorphous homogenous EPS dominated by *Aphanothece* sp. species (Fig. 5a−b). The EPS spreads over the surface like a thick carpet, immobilizing particles beneath. The third colony type of the microbial community was presented with small blackish glomeruli formed by *Nostoc* sp. species (Fig. 5c−d). Other species detected include *Chroococcus* sp.,
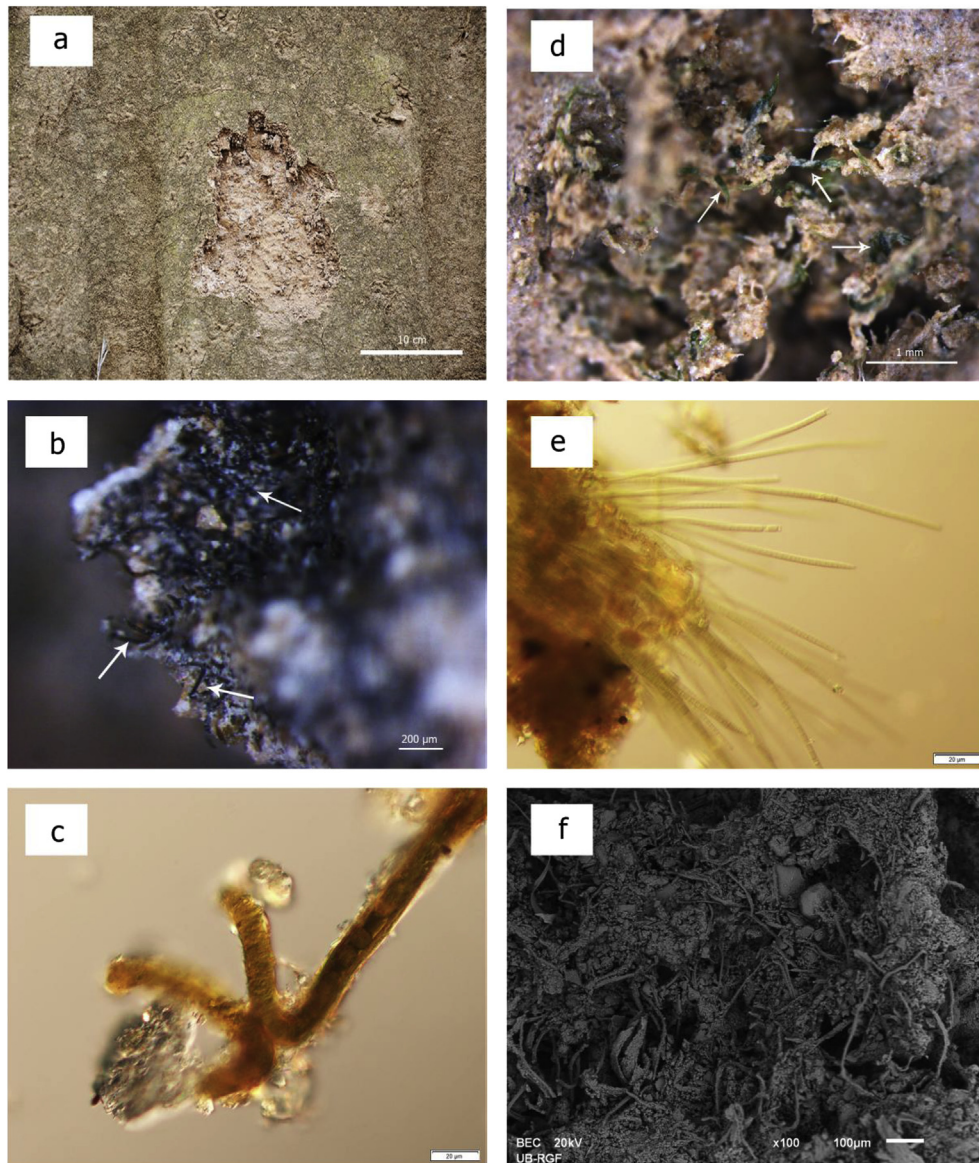
Fig. 3. Cyanobacteria-dominated crusts at sampling site IRN 5: vertical loess-paleosol sequence covered by olive-green biocrusts; scale bar = 10 cm (a). Embedded dark brown filaments of *Scytonema* sp. form the filamentous net around the particles; scale bar = 20 μm (b). Photomicrograph of *Scytonema* sp. trichomes with the characteristic scytonema-type false branching. Sediment particles are glued to the tubular EPS of filaments; scale bar = 20 μm (c). The torn crust reveals a dense net of blue−green filaments of *M. vaginatus* in the underlying layer of the crust enveloping sediment particles; scale bar = 1 mm (d). Photomicrograph of *M. vaginatus* filaments partly enveloped with tubular EPS; scale bar = 20 μm (e). BEC micrograph of *Scytonema* sp. filament network spreading between and stabilizing the particles; scale bar = 100 μm (f). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Chroococcidiopsis sp., *Cyanosarcina* sp. (Fig. 5f). *Schizothrix* sp. and cf. *Trichocoleus* (Fig. 5e) were detected in the underlying layer of the biocrust.

3.2.4. Sample IRN 9

According to the share of sand (26.71%), silt (65.57%) and clay (7.72%) fractions, the sediment was classified as sandy silt. The histogram was unimodal with predominant fraction ranging from 0.032 to 0.063 mm. The sediment was well sorted with an average grain size of 0.045 mm (Table 1).

Stereo microscope and microscope observations of crusts collected at IRN 9 (Fig. 6a) confirmed the presence of dark/blackish, thick, dense and homogenous, partly globular amorphous EPS layer that covers the whole superficial layer of the biocrust (Fig. 6b).

When wetted, the EPS layer became swollen and turned olive-green, with globules becoming more prominent (Fig. 6c). The carpet-like EPS layer was spread over a wide surface area. The species forming this type of microbial colony was determined to be the coccal cyanobacterium *Aphanocapsa* sp. (arrow 1, Fig. 6d; Fig. 6e; arrow 1, Fig. 6g). Filaments of *Oculatella* cf. *kauaiensis* (Fig. 6f) extended through the EPS layer (arrows, Fig. 6e), being the densest in the underlying layer of the biocrust (arrow 2, Fig. 6d; arrow 2, Fig. 6g). The specimens *Microcoleus vaginatus* (arrow 3, Fig. 6g), *Tolypothrix* sp (arrows, Fig. 6h). and *Schizothrix* sp (arrow 4, Fig. 6d; arrow 3, Fig. 6g). were present in the basal layer of the crust (Fig. 6d). The sectional view presented in SEM micrographs (Fig. 6g−h) clearly confirmed the conspicuous stratification in the distribution of species within the three layers.

3.3. Protein phosphatase inhibition assay

Microcystins (or other inhibitory substances) were not detected in the crust samples, or the concentrations were approaching or below the detection limit (0.25 μg/l) (Table 2).

Table 2
Toxicity of crust samples from Iran in protein phosphatase inhibition assay. Samples that exhibited any toxin content are marked with bold.

Localities	MC-LR (μg/l)
IRN 1A	<0.25
IRN 1B	<0.25
IRN 2	<0.25
IRN 3	<0.25
IRN 5A	<0.25
IRN 5B	**0.44**
IRN 5C	<0.25
IRN 5D	<0.25
IRN 5E	<0.25
IRN 5F	<0.25
IRN 5G	<0.25
IRN 5H	<0.25
IRN 5I	<0.25
IRN 5J	<0.25
IRN 5K	<0.25
IRN 6	<0.25
IRN 7	**0.39**
IRN 8	<0.25
IRN 9A	<0.25
IRN 9B	<0.25

Only two samples exhibited very low toxin content: IRN 5B (0.44 μg/l in the prepared extracts) and IRN 7 (0.39 μg/l). If these values were converted into ng toxin per g dry cyanobacteria, these values correspond to 8.8 ng/g and 7.8 ng/g, respectively, which can be interpreted as practically non-toxic samples.

3.4. Artemia salina assay

Artemia salina assay was used to assess the toxicity of crust samples from Northern Iran via mortality of larvae after 24 h (Table 3).

Table 3
Toxicity of crust samples from Iran in *Artemia salina* assay. The sample that exhibited mortality above 50% is marked with bold.

Localities	Mortality (%)
IRN 1A	4.90
IRN 1B	9.60
IRN 2	0.00
IRN 3	0.00
IRN 4	0.00
IRN 5A	17.20
IRN 5B	0.00
IRN 5C	0.00
IRN 5D	3.50
IRN 5E	11.40
IRN 5F	0.00
IRN 5G	0.00
IRN 5H	4.70
IRN 5I	0.00
IRN 5J	0.00
IRN 5K	0.00
IRN 6	0.00
IRN 7	10.02
IRN 8	39.00
IRN 9A	12.67
IRN 9B	**54.10**

Control mortality was 3.67%.

Only one crust sample from Iran IRN 9B exhibited toxicity (>50%) after 24 h in *A. salina* assay. Other samples did not show any mortality, or the mortality of *A. salina* larvae were less than 50%.

## 4. Discussion

4.1. Cyanobacterial diversity

Our research conducted on the cyanobacteria-dominated biocrusts of the North Iran Loess Plateau and the loess deposits on the foothills of Alborz Mountains gave an insight into the abundance and diversity of cyanobacteria in the biocrusts of this area, reporting taxa belonging to 15 genera (*Aphanocapsa*, *Aphanothece*, *Calothrix*, *Chroococcus*, *Chroococcidiopsis*, *Cyanosarcina*, *Hassallia*, *Homeothrix*, *Microcoleous*, *Nostoc*, *Oculatella*, *Schizothrix*, *Scytonema*, *Tolypothrix* and cf. *Trichocoleus*). These findings significantly contribute to the current knowledge on cyanobacterial diversity in biocrusts of Iran previously reported by Moghtaderi et al. (2009, 2011). In their work, six different genera were detected including *Microcoleus*, *Nostoc*, *Chroococcus*, *Chroococcidiopsis*, *Microcystis* and *Oscilatoria*. Moreover, our study reports the presence of an *Oculatella* species (Fig. 6f) morphologically similar to the *Oculatella kauaiensis* (Osorio-Santos et al., 2014). However, these two species highly differ in ecology, with *Oculatella kauaiensis* being described as a species living on damp cave walls in the biomes of tropical islands (Osorio-Santos et al., 2014). This finding gives us an opportunity to describe a new cyanobacterial species and add it to the current pool of knowledge. The undisputed taxonomic affiliation of the *Oculatella* sp. requires, however, further in-depth molecular analyses.

The difference in the morphology and diversity of the biocrusts reflects different climate regimes and, even more likely, sediment types and properties. The composition of biocrusts and their biodiversity in loess have not yet been investigated specifically, but preliminary results from Serbian loess biocrust samples show the presence of several dominant genus: *Nostoc*, *Phormidium*, *Gloeocapsa*, *Stigonema*, *Oscillatoria*, *Tolypothrix*, *Chroococcus*, *Chroococcidiopsis* and *Symplocastrum* (unpublished data).

The grain size can significantly influence development of cyanobacterial biofilm having slower and patchier biocrust development on the coarser grains, while development on the finer grains occurs more rapidly and relatively homogeneously across the surface (Rozenstein et al., 2014). In our study we observed the dominance of filamentous *Scytonema*, *Tolypothrix* and *Microcoleus* species in the samples characterized by coarse sand grains with wider grain inter-spaces between them (IRN 1, IRN 5). For these species it is typical to form an extensively branched network of filaments wrapped within firm tubular sheets that spread and grow through the grain inter-spaces. The filaments thereby surround, immobilize and stabilize sediment particles and additionally trap new airborne particles.

The highest cyanobacterial diversity was described in crusts collected at IRN 7 site, characterized by 10 different genera forming three major morphologically different microbial colonies in the superficial layer. An explanation for the highest biodiversity could be based on grain size distribution. Sediment at the site IRN 7 was characterized by a high dominance of fine grained silt grains with an average grain size of 0.023 mm. For cyanobacterial species characterized by a more homogenous and amorphous EPS layer (e.g. coccal cyanobacteria), finer grained substrates are essential for successful colony development. Unlike coarse sand grains, finer silt and clay grains form a more homogenous surface with the pores significantly smaller in diameter over which the colony development through the linear amorphous sheath secretion occurs more easily, influencing a higher species diversity and the
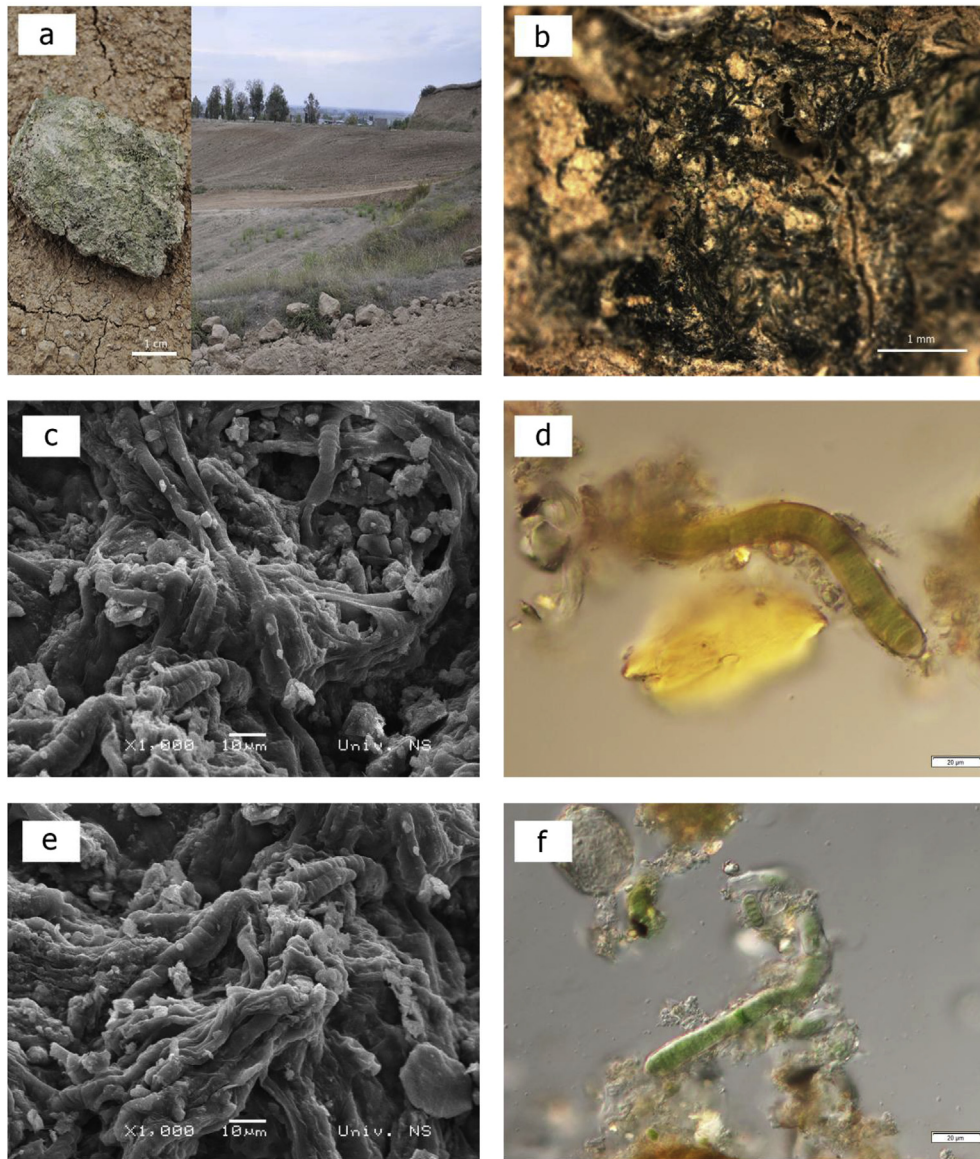
Fig. 4. Cyanobacteria-dominated crusts at sampling site IRN 7: a sediment piece from a vertical loess-paleosol sequence covered by olive-green biocrusts; scale bar = 1 cm (a); blackish to dark olive-green filamentous *Homeothrix* sp./*Hassallia* sp. microbial colony forming a net cover in the superficial layer of the biocrust; scale bar = 1 mm (b); SEM micrographs of *Homeothrix* sp./*Hassallia* sp. microbial colony; cells are enveloped in a thick amorphous EPS that spreads over the wide area of the superficial layer; finer particles stick to the surface of the EPS; scale bar = 10 μm (c, e); *Homeothrix* sp. trichome; scale bar = 20 μm (d); *Hassallia* sp. trichome; scale bar = 20 μm (f). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dominance of coccal and short trichal forms of cyanobacteria in this sample.

In the case of sample IRN 9, we observed a dense microbial colony of *Aphanothece* sp (Fig. 6b, c). in the superficial layer of the crusts even though the very sediment is characterized by a fair share of coarse sand grains (Table 1). How *Aphanothece* sp. is a coccal form of cyanobacteria, characterized by amorphous EPS surrounding the cells, the wide grain inter-space between sedimentary particles might present an obstacle negatively influencing spreading over the sediment surface. However, a dense net of filamentous *Oculatella* sp. species occurs just below the colony of *Aphanothece* sp. (Fig. 6d). It was previously shown that filamentous cyanobacterial network can significantly influence the properties of grain inter-spaces, by reducing its size and therefore enhancing the future contact between spreading filaments and neighbouring particles (Malam Issa et al., 2009).

Similar results were also presented in the work of Verrecchia et al. (1995), who showed that the proportion of small size pores was higher in sandy soils with microbiotic crusts compared to sandy soils without a microbial cover. In our case, the filamentous *Oculatella* colony influenced the grain inter-spaces of the sediment by significantly reducing their size and thus providing favorable conditions for the *Aphanothece* sp. colony to strongly develop.

Although the very micromorphology of biocrusts hasn't been fully assessed in this paper, and will be a subject of more in-depth study concerning biocrusts of Northern Iran, it is worth mentioning that the described cyanobacterial species presented vertical stratifying distribution in all the samples analyzed. In the most of the samples (IRN1, IRN 5, IRN 7) two different distribution layers were distinguished (superficial and underlying), with a notable dominance of *M. vaginatus* (all samples) and cf. *Trichocoleus* (IRN 7) in
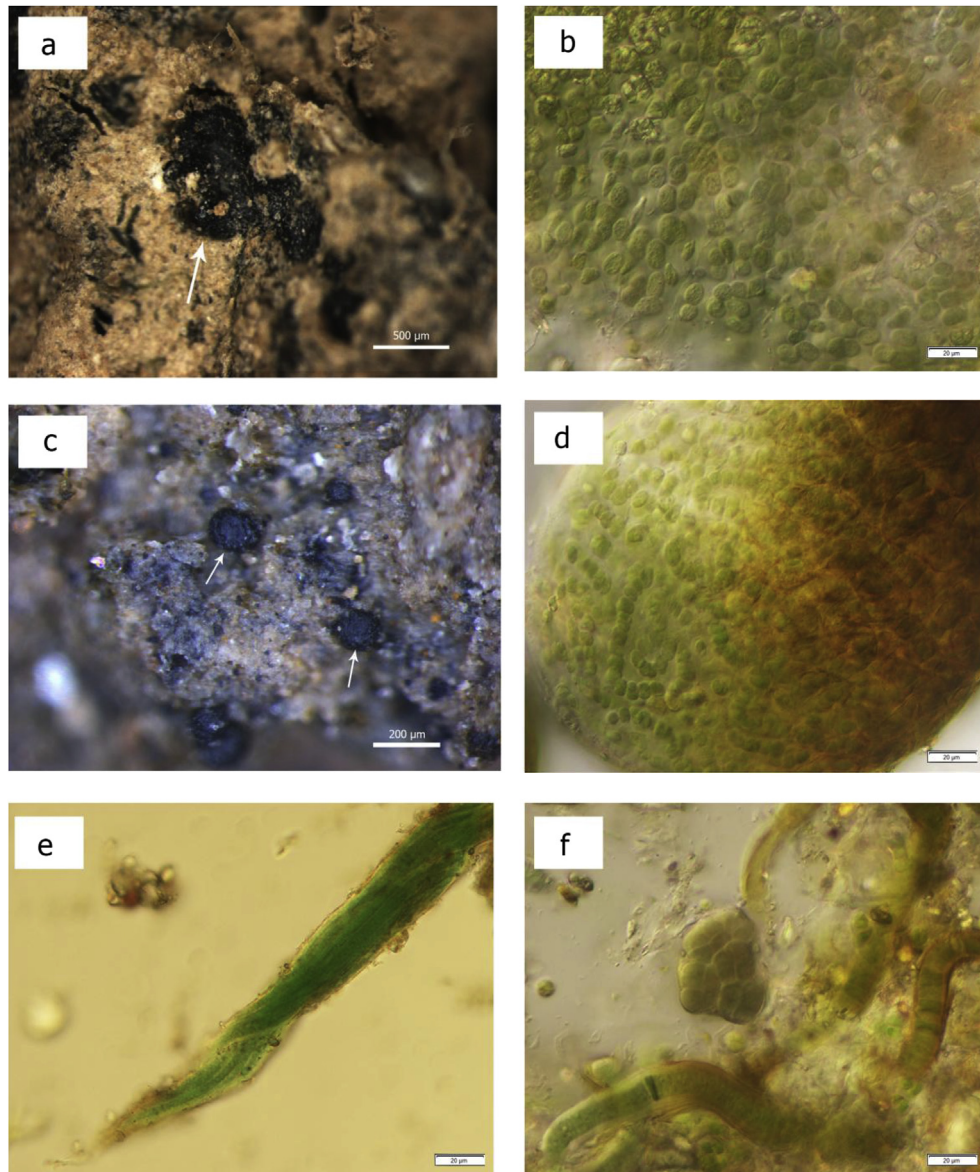
Fig. 5. Diversity of cyanobacterial crusts at sampling site IRN 7: blackish to dark brown microbial colony of *Aphanothece* sp. (arrow); thick, dense, amorphous and homogenous EPS in the superficial layer spreading over like a carpet; scale bar = 500 μm (a); photomicrograph of *Aphanothece* sp. colony in brownish amorphous EPS; scale bar = 20 μm (b); blackish globular colony of *Nostoc* sp. in the superficial layer of the biocrust (arrows) (c); microbial colony of *Nostoc* sp.; scale bar = 200 μm (d); photomicrograph of cf. *Trichocoleus* filamentous colony; scale bar = 20 μm (e); photomicrograph of coccal cyanobacterium *Cyanosarcina* sp. and cyanobacterium *Homeothrix* sp.; scale bar = 20 μm (f). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the underlying layer. The conspicuous stratification in the distribution of species within three layers was observed only in the sample IRN 9 (Fig. 6). Vertical distribution of species within biocrusts is a common phenomenon and is likely a result of gradient change of both, biotic and abiotic environmental factors including grain size properties and distribution, light intensity and nutrient availability (Malam Issa et al., 1999, 2001; Garcia-Pichel et al., 2003; Williams et al., 2012, 2014).

4.2. Sediment stabilization

Cyanobacteria with the EPS surrounding the cells and mineral particles were in a close association in all the samples. The role of cyanobacterial crusts in dryland surface processes is related to the production of EPS substances that act as an adhesive material between the cyanobacterial cells and the sediment particles,

enhancing the sediment stability and decreasing the effect of erosion (Thomas and Dougill, 2007). As it was presented by the SEM micrographs, two types of EPS, amorphous and tubular, significantly contributed to the grain trapping and immobilization. Tubular EPS is a characteristic feature of filamentous cyanobacteria that usually form a dense net of filaments growing and spreading through the grain inter-spaces (e.g. Fig. 3f). In this case, the EPS network acts as a framework that traps and immobilises the particles (Belnap and Gardner, 1993), causing also finer particles to adhere together (Malam Issa et al., 2009). In contrast, the most of coccal cyanobacteria in biocrusts produce an amorphous EPS layer that completely or partly covers and immobilizes particles in the superficial layer (e.g. Fig. 6b). In the process of sediment stabilization, cyanobacterial EPS layer has a two-fold function: (I) it traps, binds and immobilizes particles mitigating the effects of aeolian and fluvial erosion (Zaady and
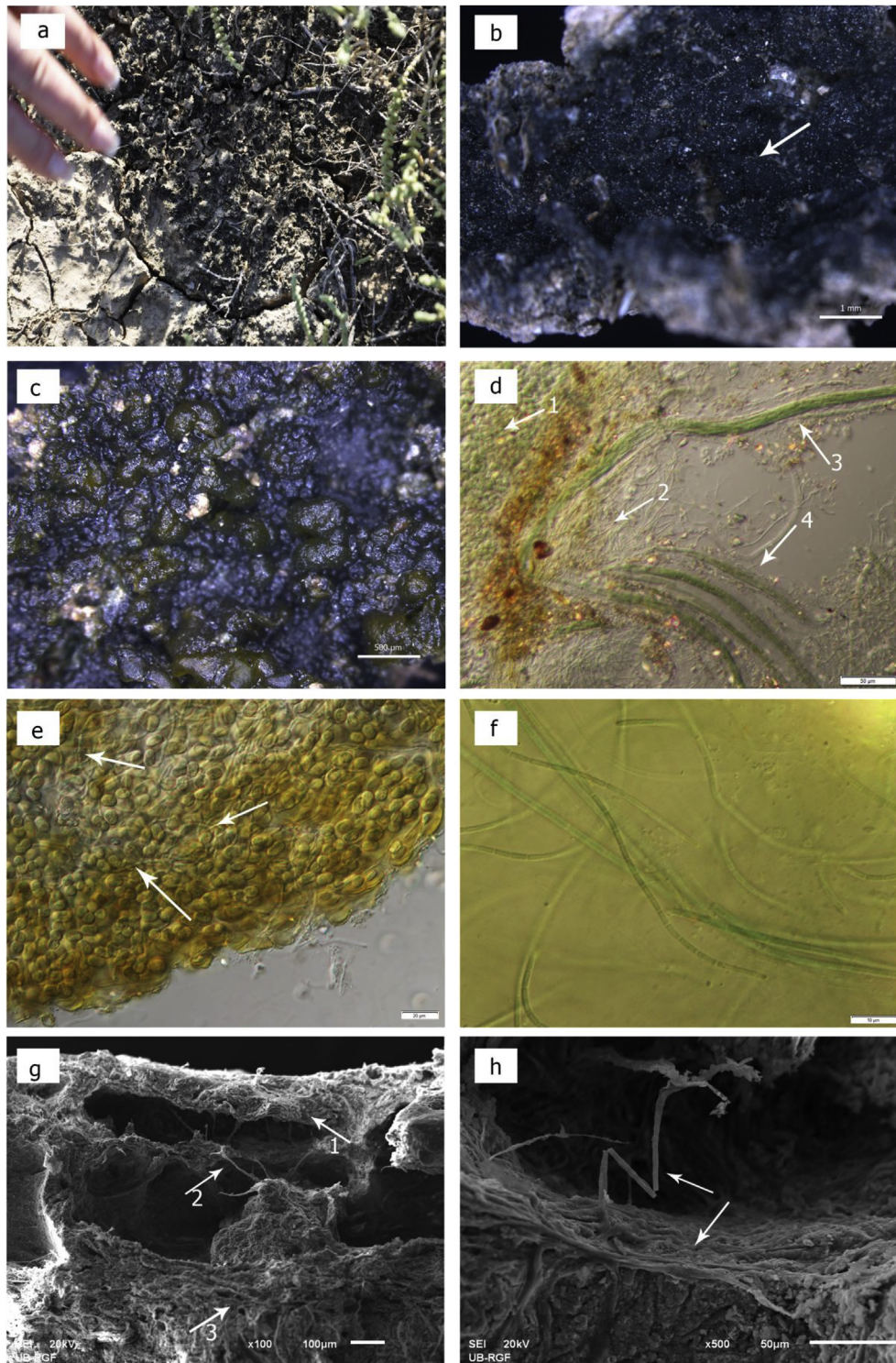
Fig. 6. Cyanobacteria-dominated crusts at sampling site IRN 9: surface of the exposed loess sediment covered with blackish biocrusts (a); blackish, thick, dense and homogenous, partly globular amorphous EPS of *Aphanothece* sp. covering a large surface area in the superficial layer of the biocrust; scale bar = 1 mm (b); prominent globules of wetted EPS; scale bar = 500 μm (c); stratified microbial community; arrow 1: coccal cells of *Aphanothece* sp.; arrow 2: filamentous net of *O*. cf. *kauaiensis*; arrow 3: EPS sheath of *M. vaginatus* filaments; arrow 4: EPS sheath of *Schizothrix* sp. sheath; scale bar = 50 μm (d); coccal cells of *Aphanothece* sp. with spreading filaments of *O*. cf. *kauaiensis* between (arrows); scale bar = 20 μm (e); isolated strain of *Oculatella* cf. *kauaiensis*; scale bar = 10 μm (f); stratified species distribution within the three layers of the biocrust; arrow 1: supperficial layer with *Aphanothece* sp. and *O*. cf. *kauaiensis* cells; arrow 2: underlying layer with *O*. cf. *kauaiensis* and *Schizothrix* sp. filamentous colonies; arrow 3: pores of *M. vaginatus* sheaths with dominance in the basal layer (g); filaments of *Tolypothrix* sp. (h).

Offer, 2010; Pietrasiak et al., 2014); (II) it permanently cements particles due to the process of EPS mineralization, when the carbonate mineral precipitates are being formed (Braissant et al., 2003). During the wet phase, cyanobacteria move up through the sediment to reclaim the surface after burial in sand/silt and the process is repeated in cycles. In this way, cyanobacteria not only influence the sediment stability, but also enhance the growth of the sediment (Garcia-Pichel and Pringault, 2001).

4.3. Toxicity of biocrusts and possible impact on human health

There was no solid evidence of the production of microcystin in the studied crust samples although at least one species with reported toxin production capability was present (*Nostoc*). The very low toxin values (a few ng/g) reported by the protein phosphatase inhibition assay could be false positives due to possible effects of sample matrix on quantification (Heresztyn and Nicholson, 2001), or a result of toxin production in a species of low abundance as toxic cyanobacterial strains typically produce several hundreds of micrograms microcystin per gram dry weight. The absence of microcystins does not exclude the presence of other potentially toxic or noxious compounds produced by cyanobacteria as shown by the *Artemia salina* results.

Since cyanobacteria might be a major component of biocrusts and dust storms can contain cyanobacteria (Griffin, 2007), it is possible that cyanotoxins are also present in airborne dust. Therefore the effects of chronic exposure to cyanobacteria via inhalation during dust storms should not be disregarded, and it is possible that inhalation of dust can lead to health problems in humans (Metcalf et al., 2012). Furthermore, anthropogenic activities in deserts can lift cyanotoxins in the air and expose humans to cyanotoxins in the inhaled air (Bener et al., 1996; Chen et al., 2004; Griffin, 2007; Cox et al., 2009; Metcalf et al., 2012). It seems that the sorption of cyanotoxins in some sediments/soils is low (Eynard et al., 2000), and could potentially result in high bioavailability to sediment/soil organisms and plants. Although the trophic connections of cyanobacteria in desert food chains are only partly understood, the potential for accumulation of cyanotoxins in such food chains exists (Metcalf et al., 2012).

5. Conclusions

The results reported in this paper support the BLOCDUST hypothesis describing the partly biogenic origin of loess and the integral role of cyanobacteria in the formation of loess. Layers of EPS and networks of filamentous cyanobacteria in the biocrusts were observed by microscopic techniques to immobilize finer and bigger silt particles, respectively, which is a necessary step for loess formation.

A total of 15 different cyanobacterial genera were observed in the biocrusts originating from the Iranian Loess Plateau and the loess deposits on the foothills of Alborz Mountains, with *Microcoleus vaginatus* as the most abundant species, being detected in all the samples. One new cyanobacterial species (*Oculatella* cf. *kauaiensis* but differing in ecology) was tentatively identified. These finding shed new light on cyanobacterial diversity of biocrusts in Iran, and encourage further in-depth studies of this region.

Toxicity assays suggest there is a potential for cyanotoxin production in the biocrusts and further assessment of this phenomenon, as well as of human health risks is necessary.

References

Belnap, J., 2006. The potential roles of biological soil crusts in dryland hydrologic cycles. Hydrological Processes 20 (15), 3159–3178.

Belnap, J., Gardner, J.S., 1993. Soil microstructure in soil of the Colorado Plateau: the role of the cyanobacteria *Microcoleus vaginatus*. Great Basin Naturalist 53 (1), 40–47.

Belnap, J., Gillette, D.A., 1998. Vulnerability of desert biological soil crusts to wind erosion: the influences of crust development, soil texture, and disturbance. Journal of Arid Environment 39, 133–142.

Belnap, J., Harper, K.T., Warren, S.D., 1994. Surface disturbance of cryptobiotic soil crusts: nitrogenase activity, chlorophyll content and chlorophyll degradation. Arid Soil Research and Rehabilitation 8, 1–8.

Belnap, J., Phillips, S.L., Herrick, J.E., Johansen, J.R., 2007. Wind erodibility of soils at Fort Irwin, California (Mojave Desert), USA, before and after trampling disturbance: implications for land management. Earth Surface Processes and Landforms 32, 75–84.

Bener, A., Abdulrazzaq, Y.M., Al-Mutawwa, J., Debuse, P., 1996. Genetic and environmental factors associated with asthma. Human Biology 68 (3), 405–414.

Bradley, W.G., Cox, P.A., 2009. Beyond Guam: cyanobacteria, BMAA and sporadic amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis 10 (Suppl. 2), 5–6.

Braissant, O., Cailleau, G., Dupraz, C., Verrecchia, A.P., 2003. Bacterially induced mineralization of calcium carbonate in terrestrial environments: the role of exopolysaccharides and amino acids. Journal of Sedimentary Research 73 (3), 485–490.

Bronger, A., 2003. Correlation of loess-paleosol sequences in East and Central Asia with SE Central Europe - towards a continental Quaternary pedostratigraphy and paleoclimatic history. Quaternary International 106–107, 11–31.

Brunet, M.F., Korotaev, M.V., Ershov, A.V., Nikishin, A.M., 2003. The South Caspian basin: a review of its evolution from subsidence modelling. Sedimentary Geology 156, 119–148.

Büdel, B., 2002. Diversity and ecology of biological crusts. Progress in Botany 63, 386–404.

Buggle, B., Hambach, U., Glaser, B., Gerasimenko, N., Marković, S.B., Glaser, I., Zöller, L., 2009. Magnetic susceptibility stratigraphy and spatial and temporal paleoclimatic trends in East European loess paleosol sequences. Quaternary International 196 (2–3), 86–106.

Buis, E., Veldkamp, A., Boeken, B., van Breemen, N., 2009. Controls on plant functional surface cover types along a precipitation gradient in the Negev Desert of Israel. Journal of Arid Environments 73 (1), 82–90.

Chatziefthimiou, A.D., Richer, R., Roles, H., Powell, J.T., Metcalf, J.S., 2014. Cyanotoxins as a potential cause of dog poisonings in desert environments. Veterinary Record 174, 484–485.

Chen, Y.S., Sheen, P.C., Chen, E.R., Liu, Y.K., Wu, T.N., Yang, C.Y., 2004. Effects of Asian dust storm events on daily mortality in Taipei, Taiwan. Environmental Research 95 (2), 151–155.

Cox, P.A., Banack, S.A., Murch, S.J., Rasmussen, U., Tien, G., Bidigare, R.R., Metcalf, J.S., Morrison, L.F., Codd, G.A., Bergman, B., 2005. Diverse taxa of cyanobacteria produce β-*N*-methylamino-L-alanine, a neurotoxic amino acid. Proceedings of the National Academy of Sciences 102, 504–507.

Cox, P.A., Richer, R., Metcalf, J.S., Banack, S.A., Codd, G.A., Bradley, W.G., 2009. Cyanobacteria and BMAA exposure from desert dust: a possible link to sporadic ALS among Gulf War veterans. Amyotrophic Lateral Sclerosis 10, 109–117.

Danin, A., Bar-Or, Y., Dor, I., Yisraeli, T., 1989. The role of cyanobacteria in stabilization of sand dunes in southern Israel. Ecologia Mediterranea 15, 55–64.

Derbyshire, E., 2001. Geological hazards in loess terrain, with particular reference to the loess regions of China. Earth-Science Reviews 54, 231–260.

Ding, Z.L., Ranov, V., Yang, S.L., Finaev, A., Han, J.M., Wang, G.A., 2002. The loess record in southern Tajikistan and correlation with Chinese loess. Earth and Planetary Science Letters 200 (3–4), 387–400.

Dojani, S., Kauff, F., Weber, B., Büdel, B., 2014. Genotypic and phenotypic diversity of cyanobacteria in biological soil crusts of the Succulent Karoo and Nama Karoo of Southern Africa. Environmental Microbiology 67 (2), 286–301.

Drobac, D., Tokodi, N., Simeunović, J., Baltić, V., Stanić, D., Svirčev, Z., 2013. Human exposure to cyanotoxins and their health effects. Archives of Industrial Hygiene and Toxicology 64 (2), 305–316.

Eldridge, D.J., Leys, J.F., 2003. Exploring some relationships between biological soil crusts, soil aggregation and wind erosion. Journal of Arid Environment 53, 457–466.

Evans, R.D., Johansen, J.R., 1999. Microbiotic crusts and ecosystem processes. Critical Reviews in Plant Sciences 18, 183–225.

Eynard, F., Mez, K., Walther, J.L., 2000. Risk of cyanobacterial toxins in Riga waters (Latvia). Water Research 34 (11), 2979–2988.

Forte, A.M., Cowgill, E., 2013. Late Cenozoic base-level variations of the Caspian Sea: a review of its history and proposed driving mechanisms. Palaeogeography, Palaeoclimatology, Palaeoecology 386, 392–407.

Frechen, M., Dodonov, A., 1998. Loess chronology of the Middle and Upper Pleistocene Tadjikistan. Geologische Rundschau 87 (1), 2–20.

Frechen, M., Kehl, M., Rolf, C., Sarvati, R., Skowronek, A., 2009. Loess chronology of the Caspian Lowland in Northern Iran. Quaternary International 198 (1–2), 220–233.

Ganji, M.H., 1968. Climate. In: Frye, N. (Ed.), The Cambridge History of Iran. Cambridge University Press, Cambridge, pp. 212–249.

Garcia-Pichel, F., Pringault, O., 2001. Cyanobacteria track the water in desert soils. Nature 413, 380–381.

Garcia-Pichel, F., Johnson, S.L., Youngkin, D., Belnap, J., 2003. Small-scale vertical distribution of bacterial biomass and diversity in biological soil crusts from arid lands in the Colorado Plateau. Microbial Ecology 46, 312–321.

Griffin, D.W., 2007. Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. Clinical Microbiology Review 20 (3), 459–477.

Hagemann, M., Henneberg, M., Felde, V.J., Drahorad, S.L., Berkowicz, S.M., Felix-Henningsen, P., Kaplan, A., 2015. Cyanobacterial diversity in biological soil crusts along a precipitation gradient, Northwest Negev Desert, Israel. Microbial Ecology 70, 219–230.

Heresztyn, T., Nicholson, B.C., 2001. A colorimetric protein phosphatase inhibition assay for the determination of cyanobacterial peptide hepatotoxins based on the dephosphorylation of phosvitin by recombinant protein phosphatase 1. Environmental Toxicology 16 (3), 242–252.

Kehl, M., 2010. Quaternary Loesses, Loess-like Sediments, Soils and Climate Change in Iran. Gebr. Borntraeger Science Publishers, Stuttgart.

Kehl, M., Sarvati, R., Ahmadi, H., Frechen, M., Skowronek, A., 2005. Loess paleosol sequences along a climatic gradient in Northern Iran. Eiszeitalter und Gegenwart 55, 151–175.

Khormali, F., Kehl, M., 2011. Micromorphology and development of loess-derived surface and buried soils along a precipitation gradient in Northern Iran. Quaternary International 234, 109–123.

Kiviranta, J., Sivonen, K., Niemelä, S.I., Huovinen, K., 1991. Detection of toxicity of cyanobacteria by *Artemia salina* bioassay. Environmental Toxicology and Water Quality 6 (4), 423–436.

Konta, J., 1969. Quantitative analytical petrological classification of sedimentary rocks. Acta Universitatis Carolinae Geologica 1, 175–253.

Kumar, D., Adhikary, S.P., 2015. Diversity, molecular phylogeny, and metabolic activity of cyanobacteria in biological soil crusts from Santiniketan (India). Journal of Applied Phycology 27, 339–349.

Lan, S., Wu, L., Zhang, D., Hu, C., 2012. Successional stages of biological soil crusts and their microstructure variability in Shapotou region (China). Environmental Earth Sciences 65, 77–88.

Lan, S., Zhang, Q., Wu, L., Liu, Y., Zhang, D., Hu, C., 2014. Artificially accelerating the reversal of desertification: cyanobacterial inoculation facilitates the succession of vegetation communities. Environmental Science and Technology 48, 307–315.

Lauer, T., Frechen, M., Vlaminck, S., Kehl, M., Lehndorff, E., Shahriari, A., Khormali, F., 2016. Luminescence-chronology of the loess palaeosol sequence Toshan, Northern Iran a highly resolved climate archive for the last glacial-interglacial cycle. Quaternary International (in this issue).

Li, X.R., Tian, F., Jia, R.L., Zhang, Z.S., Liu, L.C., 2010. Do biological crusts determine vegetation changes in sandy deserts? Implications for managing artificial vegetation. Hydrological Processes 24, 3621–3630.

Malam Issa, O., Trichet, J., Défarge, C., Coute, A., Valentin, C., 1999. Morphology and microstructure of microbiotic soil crusts on a tiger bush sequence Niger, Sahel. Catena 37, 175–196.

Malam Issa, O., Le Bissonnais, Y., Défarge, C., Trichet, J., 2001. Role of a cyanobacterial cover on structural stability of sandy soils in the Sahelain part of western Niger. Geoderma 101, 15–30.

Malam Issa, O., Défarge, C., Trichet, J., Valentin, C., Rajot, J.L., 2009. Microbiotic soil crusts in the Sahel of Western Niger and their influence on soil porosity and water dynamics. Catena 77, 48–55.

Marković, S.B., Bokhorst, M., Vandenberghe, J., Oches, E.A., Zöller, L., McCoy, W.D., Gaudenyi, T., Jovanović, M., Hambach, U., Machalett, B., 2008. Late Pleistocene loess-paleosol sequences in the Vojvodina region, North Serbia. Journal of Quaternary Science 23, 73–84.

Marković, S.B., Hambach, U., Catto, N., Jovanović, M., Buggle, B., Machalett, B., Zöller, L., Glaser, B., Frechen, M., 2009. The middle and late Pleistocene loess-paleosol sequences at Batajanica, Vojvodina, Serbia. Quaternary International 198, 255–266.

Marković, S.B., Hambach, U., Jovanović, M., Stevens, T., O'Hara-Dhand, K., Basarin, B., Smalley, I.J., Buggle, B., Zech, M., Svirčev, Z., Milojković, N., Zöller, L., 2012. Loess in Vojvodina region (Northern Serbia): the missing link between European and Asian Pleistocene environments. Netherlands Journal of Geosciences 91, 173–188.

Marković, S.B., Stevens, T., Kukla, G.J., Hambach, U., Fitzsimmons, K.E., Gibbard, P., Buggle, B., Zech, M., Guo, Z.T., Hao, Q.Z., Wu, H., O'Hara-Dhand, K., Smalley, I.J., Ujvari, G., Sümegi, P., Timar-Gabor, A., Veres, D., Sirocko, F., Vasiljević, Dj.A., Jari, Z., Svensson, A., Jović, V., Kovács, J., Svirčev, Z., 2015. The Danube loess stratigraphy – new steps towards the development of a pan-European loess stratigraphic model. Earth Science Reviews 148, 228–258.

Metcalf, J.S., Codd, G.A., 2012. Cyanotoxins. In: Whitton, B.A. (Ed.), Ecology of Cyanobacteria II. Their Diversity in Space and Time. Springer, Dordrecht, pp. 651–675.

Metcalf, J.S., Richer, R., Cox, P.A., Codd, G.A., 2012. Cyanotoxins in desert environments may present a risk to human health. Science of the Total Environment 421–422, 118–123.

Metcalf, J.S., Banack, S.A., Richer, R., Cox, P.A., 2015. Neurotoxic aminoacids and their isomers in desert environments. Journal of Arid Environment 112, 140–144.

Moghtaderi, A., Taghavi, M., Rezaei, R., 2009. Cyanobacteria in biological soil crusts of Chadormalu Area, Bafq Region in Central Iran. Pakistan Journal of Nutrition 8 (7), 1083–1092.

Moghtaderi, A., Moore, F., Taghavi, S.M., Rezaei, R., 2011. The application of ASTER imageries and mathematical evaluation method in detecting cyanobacteria in biological soil crust, Chadormalu Area, Central Iran. Iranian Journal of Science and Technology A1, 13–28.

Obradović, J., Vasić, N., 1988. Metode ispitivanja sedimentnih stena. Rudarsko-geološki fakultet, Univerzitet u Beogradu.

Osorio-Santos, K., Pietrasiak, N., Bohunická, M., Miscoe, L.H., Kovácik, L., Martin, M.P., Johansen, J.H., 2014. Seven new species of *Oculatella* (Pseudanabaenales, Cyanobacteria): taxonomically recognizing cryptic diversification. European Journal of Phycology 49 (4), 450–470.

Pietrasiak, N., Drenovsky, R.E., Santiago, L.S., Graham, R.C., 2014. Biogeomorphology of a Mojave Desert landscape – configurations and feedbacks of abiotic and biotic land surfaces during landform evolution. Geomorphology 206, 23–36.

Pócs, T., Gánti, T., Horváth, A., Bérczis, Z., Kereszturi, A., Sik, A., Szathmáry, E., 2006. Comparison of the cryptobiotic-crusts and surface mineral crusts according to their main characteristics in helping life support mechanisms and their implied role for Martian living organisms. In: Proceedings of the 30th Conference on Antarctic Meteorites. National Institute of Polar Research, Tokyo, pp. 99–100.

Pointing, S., Büdel, B., Convey, P., Gillman, L., Körner, C., Leuzinger, S., Vincent, W., 2015. Biogeography of photoautotrophs in the high polar biome. Frontiers in Plant Science. http://dx.doi.org/10.3389/fpls.2015.00692.

Raanan, H., Felde, V.J.M.N.L., Peth, S., Drahorad, S., Ionescu, D., Eshkol, G., Treves, H., Felix-Henningsen, P., Berkowicz, S.M., Keren, N., Horn, R., Hagemann, M., Kaplan, A., 2015. Three-dimensional structure and cyanobacterial activity within a desert biological soil crust: biological soil crust structure and activity. Environmental Microbiology. http://dx.doi.org/10.1111/1462-2920.12859.

Richer, R., Banack, S.A., Metcalf, J.S., Cox, P.A., 2015. The persistence of cyanobacterial toxins in desert soils. Journal of Arid Environment 112, 134–139.

Rozenstein, O., Zaady, E., Katra, I., Karnieli, A., Adamowski, J., Yizhaq, H., 2014. The effect of sand grain size on the development of cyanobacterial biocrusts. Aeolian Research 15, 217–226.

Smalley, I.J., Marković, S.B., Svirčev, Z., 2011. Loess is [almost totally formed by] the accumulation of dust. Quaternary International 240, 4–11.

Svirčev, Z., Marković, S.B., Stevens, T., Codd, G.A., Simeunović, J., Obreht, I., Dulić, T., Pantelić, D., Hambach, U., 2013. Importance of biological loess crusts for loess formation in semi-arid environments. Quaternary International 296, 206–215.

Thomas, A.D., Dougill, A.J., 2007. Spatial and temporal distribution of cyanobacterial soil crusts in the Kalahari: implications for soil surface properties. Geomorphology 85, 17–29.

Verrecchia, E., Yair, A., Kidron, G.J., Verrecchia, K., 1995. Physical properties of the psammophile cryptogamic crust and their consequences to the water regime of sandy soils, north-western Negev desert, Israël. Journal of Arid Environments 29, 427–437.

Vlaminck, S., Kehl, M., Lauer, T., Shahriari, A., Sharifi, J., Eckmeier, E., Lehndorff, E., Khormali, F., Frechen, M., 2016. Loess-soil sequence at Toshan (Northern Iran): insights into late Pleistocene climate change. Quaternary International. http://dx.doi.org/10.1016/j.quaint.2015.04.028 (in press).

Wentworth, C.K., 1922. A scale of grade and class terms for clastic sediments. Journal of Geology 30, 377–392.

West, N.E., 1990. Structure and function of microphytic soil crusts in wild land ecosystems of arid to semi-arid regions. Advances in Ecological Research 20, 179–223.

Williams, W.J., Eldridge, J.D., Alchin, M.B., 2008. Grazing and drought reduce cyanobacterial soil crusts in an Australian acacia woodland. Journal of Arid Environment 72, 1064–1075.

Williams, A.J., Buck, B.J., Beyene, M.A., 2012. Biological soil crusts in the Mojave Desert, USA: micromorphology and pedogenesis. Soil Science Society of America Journal 76, 1685–1695.

Williams, W.J., Büdel, B., Reichenberger, H., Rose, N., 2014. Cyanobacteria in the Australian northern savannah detect the difference between intermittent dry season and wet season rain. Biodiversity and Conservation 23, 1827–1844.

Wu, Y., Rao, B., Wu, P., Liu, Y., Li, G., Li, D., 2013. Development of artificially induced biological soil crusts in fields and their effects on top soil. Plant and Soil 370 (1), 115–124.

Zaady, E., Offer, Z.Y., 2010. Biogenic soil crusts and soil depth: a long-term case study from the central Negev desert highland. Sedimentology 57, 351–358.

Zhang, Y.M., Chen, J., Wang, L., Wang, X.Q., Gu, Z.H., 2007. The spatial distribution patterns of biological soil crusts in the Gurbantunggut Desert, Northern Xinjiang, China. Journal of Arid Environments 68 (4), 599–610.

Zhang, B.C., Zhou, X.B., Zhang, Y.M., 2015. Responses of microbial activities and soil physico-chemical properties to the successional process of biological soil crusts in the Gurbantunggut Desert, Xinjiang. Journal of Arid Land 7, 101–109.