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Cyanobacterial diversity and toxicity of biocrusts from the Caspian Lowland loess deposits, North Iran

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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.

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 $\mu g/m^2$) 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