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Controlling rainfall-induced soil loss from small experimental plots through inoculation of bacteria and cyanobacteria

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ABSTRACT

Soil erosion is a major limitation for achieving sustainable development. Controlling soil detachment in initial stage of soil erosion induced by rainfall is necessary. Several organic and inorganic amendments have been used to reduce rainfall-induced erosion. Meanwhile, the impact of soil microorganisms has been approved on improving soil aggregation by previous studies. However, studying the feasibility of inoculation of soil microorganisms to reduce soil loss by rainfall-induced erosion has not been considered yet. Hence, the present study was planned to investigate the controllability of soil loss induced by rainfall-induced through the inoculation of bacteria and cyanobacteria into a study soil. Soil samples were collected from an erosion-prone area from which suitable existing bacteria and cyanobacteria for soil and water conservation were selected, purified and proliferated. The bacteria and cyanobacteria were then inoculated into 24 small 0.25 m^2 plots in individual and/or combined treatments at three time spans of 15, 30 and 60 days. The results illustrated a significant decrease in soil loss from treated plots ($p < 0.01$). Moreover, the highest effectiveness was observed in the cyanobacteria and the combined treatments at the end of the 60 days-period ($p < 0.01$) with respective reduction rate of 99 and 98% in soil loss. Based on the study results, the use of microorganisms, particularly direct inoculation of bacteria and cyanobacteria can be supposed as a new step, biologic, sustainable, environmentally-friend and economically-effective technique for soil conservation.

1. Introduction

Soil is a key component of the earth system, as the soil system determines the cycles of the water, nutrients, minerals, life, and also provides the human well-being and ecosystem health (Brevik et al., 2015; Decock et al., 2015; Smith et al., 2015). Soil health plays a decisive role in achieving sustainable development (Keesstra et al., 2016; Kheirfam et al., 2017). Nevertheless, reducing the potential of soil affected by erosion particularly water erosion is serious universal challenge in achieving sustainable development (Biswas et al., 2015; Ochoa-Cueva et al., 2015; Gessesse et al., 2015; Martínez-Murillo et al., 2013; Vaezi et al., 2016). Hence, controlling and/or decreasing of soil erosion particularly in initial first steps of its appearance on hillslopes initiated by rainfall erosion, could be an efficacious strategy to soil conservation. Therefore, various amendments have been implemented to reduce the adverse effects of rainfall-induced soil erosion. Accordingly, variety of amendments viz. gypsum, lime, sawdust and wood ash, municipal wastes (Sadeghi et al., 2016); organic composts, animal and crop manures, crop and food industries residues (Sadeghi et al., 2015); native plant residues mulching (Mwango et al., 2015); secondary vegetation succession (van Hall et al., 2017); oil mulches and types of oil and biodegradable polymers (Zohuriaan-Mehr and Kabiri, 2008) were used to controlling soil erosion.

Despite some desirable applications of aforementioned amendments in reducing rainfall-induced soil erosion, introducing new, biological, affordable, permanent, environmental friendly and applicable techniques is inevitable. Accordingly, the bacteria and cyanobacteria which construct the biological soil crusts (BSCs) known ecosystem engineers (Chamizo et al., 2012) have been introduced as biological soil amendments. The BSCs microorganisms, particularly bacteria and cyanobacteria not only build soil micro-structures through exopolysaccharides secreting (EPSs), but also create soil macro-structures by connecting together and forming micro-networks (Colica et al., 2014). These conditions lead to amend the soil surface roughness (Reynolds et al., 2001), improving soil stability (Huang et al., 2002; Dougill and Thomas, 2004), increasing soil aggregation (Bashan and de-Bashan, 2010), increasing porosity (Miralles et al., 2011) and water retention capacity (Chamizo et al., 2011), accumulating nutrients and

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increasing soil fertility (Rodríguez-Caballero et al., 2013; Kheirfam et al., 2017) and carbon sequestration (Bhattacharya et al., 2016; Kheirfam et al., 2017) as well.

Certainly, improving of above mentioned soil properties through microbial activity in soil surface may have direct and indirect effects on soil stability against erosion. In this regard, the role of rich BSCs in microorganisms has been approved in reducing soil erosion under rainfall simulation and scale plot and arid and semi-arid region conditions (Belnap et al., 2013; Rodríguez-Caballero et al., 2013; Zhao and Xu, 2013; Zhao et al., 2014; Wei et al., 2015; Chamizo et al., 2017. The effective role of enriched BSCs by inoculation of soil microorganism particularly bacteria and cyanobacteria have been reported in improving the desert soil stability (Colica et al., 2014), increasing soil resistance to wind erosion (Zaady et al., 2016), and improving quality of erodible soil (Kheirfam et al., 2017).

Reviewing of literatures showed that many studies have been conducted to assess the effectiveness of microorganisms to stabilize soil structure and improve soil quality in general. Accordingly, it has been hypothesized that increasing of cyanobacteria and bacteria population by direct inoculation on the soil would artificially improve the biological crust leading to improve soil physical, chemical and biological properties and as a consequence reduce soil loss from the small study plots with dominant rainfall-induced soil erosion. However, no document could be accessed to report the effect of inoculation of bacteria and cyanobacteria into soil in order to analyze their roles in reducing soil water erosion. The present study was therefore formulated to evaluate the role of direct and combined inoculation native bacteria and cyanobacteria to reduce soil loss by rainfall-induced erosion. In order to better control of the study conditions, the study was planned under laboratorial physical simulation. Accordingly, small plots filled by an erosion-prone soil and a standard rainfall simulator were used for experimental processes.

2. Material and methods

2.1. Soil collection area and soil sampling

The study soil was collected from a sub-watershed of Chalusrood Watershed (Kandelus Region) located in west of Mazandaran Province in Iran with an area of 86.34 km^2. The study area has high susceptibility to erosion resulting in high sediment yield. The previous conservation measures such as exclosure, forestation and check dams had low level of success in controlling soil loss as well. The average annual precipitation and temperature based on the data collected from Kojour and Nowshahr stations (1978 to 2010) were 432 mm and 12 °C, respectively. Based on USDA Soil Taxonomy classification method (USDA Soil Taxonomy, Soil Survey Staff, 2010), the soil was classified as fine loamy, mixed, mesic, typic calcixerepts, inceptisols. The soil texture was silty clay loam with 46, 40 and 14% of silt, clay and sand contents, respectively. The bulk density of study soil was 1.10 to 1.20 g cm^{-3} with medium granular structure and fragile stability situation. The organic carbon content, humic acid, folic acid, lime percentage, pH and EC of the study soil were ≈0.18%, 0.017 g kg^{-1}, 0.03 g kg^{-1}, 27.9%, 7.42 to 7.68 and 0.17 to 0.25 ds m^{-1}, respectively. The area has been mainly covered by Mediterranean Cypress forest (*Cupressus sempervirens* var. *horizontalis*) with <25% cover density.

Soil sampling was carried out by using 5 cm-diameter coring polyvinyl chloride (PVC) from 2 cm of the surface layer (Chamizo et al., 2012) in September 2014. The collected soils were placed in polyethylene plastics bags, stored at approximately 4 °C (Kheirfam et al., 2017) and transported to the laboratory of the Natural Resource Faculty of Tarbiat Modares University. The soil samples were then air-dried and sieved by 2 mm-sized mesh (Chamizo et al., 2012) and three composite samples were ultimately prepared by mixing 100 g of soil samples for further analyses.

2.2. Preparation of culture media for bacteria and cyanobacteria isolation

In the present study, the general media were used in order to extract the various genera of bacteria and cyanobacteria in soil microorganisms' bank. Thus, a wide number of soil bacteria and cyanobacteria general media were investigated and evaluated. Finally, Chu10 and Bold's basal general media (Andersen, 2005) were selected for cyanobacteria isolation. In addition, Nutrient Agar and Tryptic Soy Agar (TSA) (Eevers et al., 2015) general media were selected for bacteria isolation. The soil bacteria were cultured and isolated according to standard protocols described by Benson (2002). Accordingly, 1 g of soil samples in three replications were mixed with 0.85% NaCl solution (physiological saline) in 1:10 ratio into Erlenmeyer flasks (10^{-1} dilution) for extraction of soil bacteria. Next, 1 ml of prepared solution was transferred into a glass bottle (10^{-2}) which it was further diluted up to 10^{-10}. Each bottle contained 9 ml saline solution. Afterwards, 1 ml solution from each of the dilutions was placed in the corresponding individual 8 cm diameter Petri-dishes for each general bacteria media and with two replications in order to increase reliability. Afterwards, number of bacteria in 1 g soil was calculated by manually colony counting and optical density factor (Awad et al., 2012).

In order to identify the bacteria, colonies of bacteria were isolated from the surface of Petri dishes by using microbiological loop. The Gram staining was then carried out followed by microscopic examination as well as morphological characteristics (Bergey and Breed, 1957).

In order to isolate and identify the cyanobacteria, 1 g of soil samples were transferred into 8 cm diameter Petri-dishes with three replications. Then, 5 ml Bold's basal and Chu10 media were also poured into Petri-dishes, separately. The cover glasses were then placed inside of Petri-dishes. The cyanobacteria were ultimately identified by using high-resolution optical microscopes based on morphological characterization at genus level (Whitton and Potts, 2012), since genus level of microorganisms identification was just needed for the present purposes. Though, molecular identification technique could also be applied for further detailed identification of microorganisms and at species level.

2.3. Selection and purification of appropriate bacteria and cyanobacteria

Various characteristics of isolated bacteria and cyanobacteria viz. power viability, activity in different soil temperatures, pH and moisture conditions, secretion of adhesive polysaccharides and network growth suggested by former scientists (e.g., Castenholz, 2001; Moore et al., 2006; Fierer et al., 2007; Kumar et al., 2007; Méjean et al., 2009; Satapute et al., 2012) were scrutinized for selection of appropriate bacteria and cyanobacteria targeting soil conservation purposes. Creation of small and large structures, converting food into usable forms for the other organisms, easy isolation and proliferation under laboratory conditions and also non-pathogenic effect on humans and other organisms were also considered. The most appropriate bacteria and cyanobacteria were ultimately selected for soil and water conservation targets. Accordingly, *Azotobacter* Agar, Modified II (Atlas, 2010) and DSMZ1 (Schrey et al., 2012) selective media were used in order to purify the selected bacteria. In addition, the colonies of selected cyanobacteria grown on the surface of coverglasses were picked by microbiological loop and transferred into a 20 ml of sterile Bold's basal and Chu10 media in 50 ml-falcon tubes. This process was repeatedly continued during several steps until complete purification. The purified bacteria and cyanobacteria were then transferred by microbiological loops into liquid Luria Broth (LB) medium series of 50, 100, 500, 1000 and 2000 ml, respectively (Garbeva et al., 2011) to achieve the suitable density of 10^{12} CFU l^{-1}.

2.4. Preparation of experimental plots

A total 36 series of 0.25 m^2-erosion plots with the depth of 0.5 m were used to conduct the study. Due to small size of the plots, the