2019

Volume : 8
Issue : 3
Page : 189 - 281

e-ISSN : 2147-4249

Eurasian Journal of Soil Science

Editor(s)-in-chief
Dr.Rıdvan KIZILKAYA
Dr.Evgeny SHEIN
Dr.Coskun GULSER

www.fesss.org

Published by Federation of Eurasian Soil Science Societies

EURASIAN JOURNAL OF SOIL SCIENCE

(Peer Reviewed Open Access Journal)

Published by Federation of Eurasian Soil Science Societies

ABOUT THIS JOURNAL : Eurasian Journal of Soil Science is the official English language journal of the Federation of Eurasian Soil Science Societies. Eurasian Journal of Soil Science peer-reviewed open access journal that publishes research articles, critical reviews of basic and applied soil science in all related to soil and plant studies and general environmental soil science.

ABSTRACTING AND INDEXING: SCOPUS, CABI, FAO-Agris, EBSCOhost, ProQuest, DOAJ, OAJI, TR Dizin, TURKISH Journalpark, CrossRef, CiteFactor, CAS, etc.

Year: 2019, Volume: 8, Issue: 3, Page: 189 - 281 **e-ISSN 2147-4249**

EURASIAN JOURNAL OF SOIL SCIENCE

(Peer Reviewed Open Access Journal)

Published by Federation of Eurasian Soil Science Societies

YEAR: 2019 **VOLUME : 8** **ISSUE : 3** **PAGE : 189 – 281**

CONTENTS

Changes in biological soil quality indicators under saline soil condition after amelioration with alfalfa (Medicago sativa L.) cultivation in meadow Solonchak **189**

Zhainagul Yertayeva, Rıdvan Kızılkaya, Sagynbay Kaldybayev, Nurzikhan Seitkali, Nurgul Abdraimova, Aigul Zhamangarayeva

Pedo-transfer functions with multiple linear regressions to predict solute-transport parameters **196**

Md. Abdul Mojid, A.B.M. Zahid Hossain, Guido C.L. Wyseure, Md. Ali Ashraf

Field suppression of Fusarium wilt and microbial population Shifts in tomato rhizosphere following soil treatment with two selected endophytic bacteria **208**

Rania Aydi Ben Abdallah, Hayfa Jabnoun-Khiareddine, Ahlem Nefzi, Fakher Ayed, Mejda Daami-Remadi

Mitigation of salinity in chickpea by Plant Growth Promoting Rhizobacteria and salicylic acid **221**

Aneela Riaz, Munazza Rafique, Muhammad Aftab, M. Amjad Qureshi, Hina Javed, Fakhar Mujeeb, Saleem Akhtar

Wheat growth and nitrogen use efficiency under drip irrigation on semi-arid region **229**

Mohamed Said Awaad, Tarek H. M. A. Deshesh

Soil fertility assessment and mapping spatial distribution of Agricultural Research Station, Bijayanagar, Jumla, Nepal **237**

Dinesh Khadka, Sushil Lamichhane, Rita Amgain, Sushila Joshi, Shree P. Vista, Kamal Sah, Netra H. Ghimire

Determination of heavy metal risk and their enrichment factor in intensive cultivated soils of Tokat Province **249**

Betül Bayraklı, Orhan Dengiz

Measurement and estimation of evapotranspiration in semi-arid grassland during the summer season in southwest Siberia **257**

Lisa Haselow, Holger Rupp, Andrej A. Bondarovich, Ralph Meissner

Physical and hydraulic properties of soils under a long-term tillage practices in Hadejia Local Government Area, Jigawa State, Nigeria **267**

Abba Nabayi, Abubakar Halilu Girei, Mohammed Saleh Abubakar

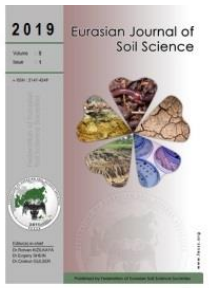
The effect of NPK foliar fertilization on yield and macronutrient content of grain in wheat under Kostanai-Kazakhstan conditions **275**

Coşkun Gülser, Zhenis Zharlygasov, Rıdvan Kızılkaya, Niyazbek Kalimov, Izzet Akça, Zhaksylyk Zharlygasov

Eurasian J Soil Sci 2019, 8 (3) 189- 195

Eurasian Journal of Soil Science

Journal homepage : http://ejss.fesss.org



Changes in biological soil quality indicators under saline soil condition after amelioration with alfalfa (*Medicago sativa* L.) cultivation in meadow Solonchak

Zhainagul Yertayeva [a,*], Rıdvan Kızılkaya [b,c], Sagynbay Kaldybayev [a] Nurzikhan Seitkali [a], Nurgul Abdraimova [a], Aigul Zhamangarayeva [a]

[a] Kazakh National Agrarian University, Almaty, Kazakhstan

[b] Ondokuz Mayıs University, Faculty ofAgriculture, Department of Soil Science and Plant Nutrition, Samsun, Turkey

[c] Agrobigen Research & Development Trade Ltd.Co, Samsun Technopark, Samsun, Turkey

Article Info

Received : 16.06.2018
Accepted : 10.04.2019

Abstract

Land use and amelioration practices are considered as main drivers in change of biological soil quality indicators in meadow Solonchaks. To gain insight into the impact of amelioration with alfalfa (*Medicago sativa* L.) cultivation on the underlying soil microbiological and biochemical properties, the objective of this study was to determine the effect of alfalfa (*Medicago sativa* L.) cultivation on biological soil quality indicators such as microorganisms counts, microbial biomass, basal soil respiration and enzyme activities (dehydrogenase, catalase, β-glucosidase, protease, urease, alkaline phosphatase and arylsulphatase) in meadow Solonchak. Post-amelioration with alfalfa cultivation influenced the soil microbiological and biochemical properties and increased soil organic matter content and improved biological soil quality indicators. The results of this study may contribute to future researches for soil microbial communities in different type of amelioration practices in soil quality and sustainable productivity meadow Solonchaks.

Keywords: Amelioration, microorganisms, saline soil, soil quality.

Introduction

Soil salinity is one of the most serious abiotic factors restricting productivity of field, plant diversity and plant growth in arid and semi-arid regions, where soil salt content is high and precipitation is insufficient (Kazemi and Eskandari, 2011) Worldwide, about 30 million ha are severely affected by salinity and an additional almost 80 million ha are estimated to be affected to some extent (Umali, 1993). The area of saline soils in Kazakhstan, including Solonetz, alkaline soils, and complexes of Solonetz with other soils, is 111.55 million ha, or 41% of the national territory (FAO, 2015). Saline soils are present everywhere in the country except in mountainous areas. They are common in the steppe zone, where they cover about 30% of the area. In dry steppe, semi-desert and desert zones these soils occupy up to 50% of the area. Salt-affected soils are represented mainly by Solonetz and alkaline soils. Solonchaks cover only 1–3% of the area of salt-affected soils in the steppe zone, and 7–13% of the area of salt-affected soils in the semi-desert and desert zones (FAO, 2015).

The amelioration of problem soils is a very important goal throughout the world, especially with saline or saline-sodic soils (Mady, 2011). Sodic and saline-sodic soils possess poor physical properties and fertility problems that adversely affect the growth and yield of most crops (Sumner 1993; Grattan and Grieve, 1999). Saline-sodic soils reclamation is one of the main problems for humans in the future. The reclamation of

* Corresponding author.
Kazakh National Agrarian University, Almaty, Kazakhstan
Tel.: +7 702 5050404
E-mail address: jain_0404@mail.ru
e-ISSN: 2147-4249
DOI: 10.18393/ejss.552563

saline soils uses many different methods such as physical amelioration (deep ploughing, subsoiling, sanding, profile inversion), chemical amelioration (amending of soil with various reagents: gypsum, calcium chloride, limestone, sulphuric acid, sulphur, iron sulphate) and biological amelioration (Oad et al., 2002). The biological amelioration methods using living (sowing new forms of leguminous plants) or dead organic matter (crops, stems, straw, green manure, barnyard manure, compost, sewage sludge) (Matsumoto et al., 1994) have two principal beneficial effects on the saline and alkaline soils reclamation: the improvement of the soil structure and permeability, thus enhancing salt leaching, reducing surface evaporation, and inhibiting salt accumulation in the surface layers; and the release of carbon dioxide during respiration and decomposition. For saline or sodic soils, the addition of organic matter can accelerate the leaching of Na, decrease the exchangeable Na percentage and electrical conductivity, and increase water infiltration, water-holding capacity, and aggregate stability (Lax et al., 1994; Qadir et al., 2001). Thus, the increase in the yield and quality of agricultural cultures, particularly in arid areas and on saline soils, can be achieved through the high-culture farming by scientifically sound ecologically safe use of new types of bio-fertilizer and biologics, sowing new forms of leguminous plants (especially leguminous vegetable), bio-amelioration with halophytes that have a positive effect on the biological soil quality indicators such as microbiological and enzymatic activities, soil respiration and the processes of humus-forming in soil, and, finally, soil fertility and productive capacity (Mady, 2011).

Soil quality has been defined as “the capacity of a soil to function within ecosystem and land-use boundaries, to sustain biological productivity, maintain environmental quality and promote plant and animal health” (Karlen et al., 1997) and can be assessed using a wide variety of biological, physical and chemical indicators (Doran and Parkin, 1994). Biological indicators typically include microbial biomass carbon (Jordan et al., 1995; Karlen et al., 1997) and microbial enzyme activities (Bandick and Dick, 1999; Eivazi et al., 2003). Microbial enzyme activities reflect metabolic factors and may serve as early indicators of soil quality improvement or degradation in agroecosystems (Dick, 1994). The purposes of the present work were to compare biological soil quality indicators such as microbial biomass carbon, enzyme activities and basal soil respiration between saline soil conditions and ameliorated soil with alfalfa (*Medicago sativa* L.) cultivation in meadow Solonchak of South Kazakhstan.

Material and Methods

Study site description

Experimental studies were carried out at the Teskensu (Kaz.Teskensu) village in the Enbekshikazakh district (43°32' N, 77°51' E) of the Almaty region of Kazakhstan (Figure 1). The study area is situated between 601 m elevation from sea level.

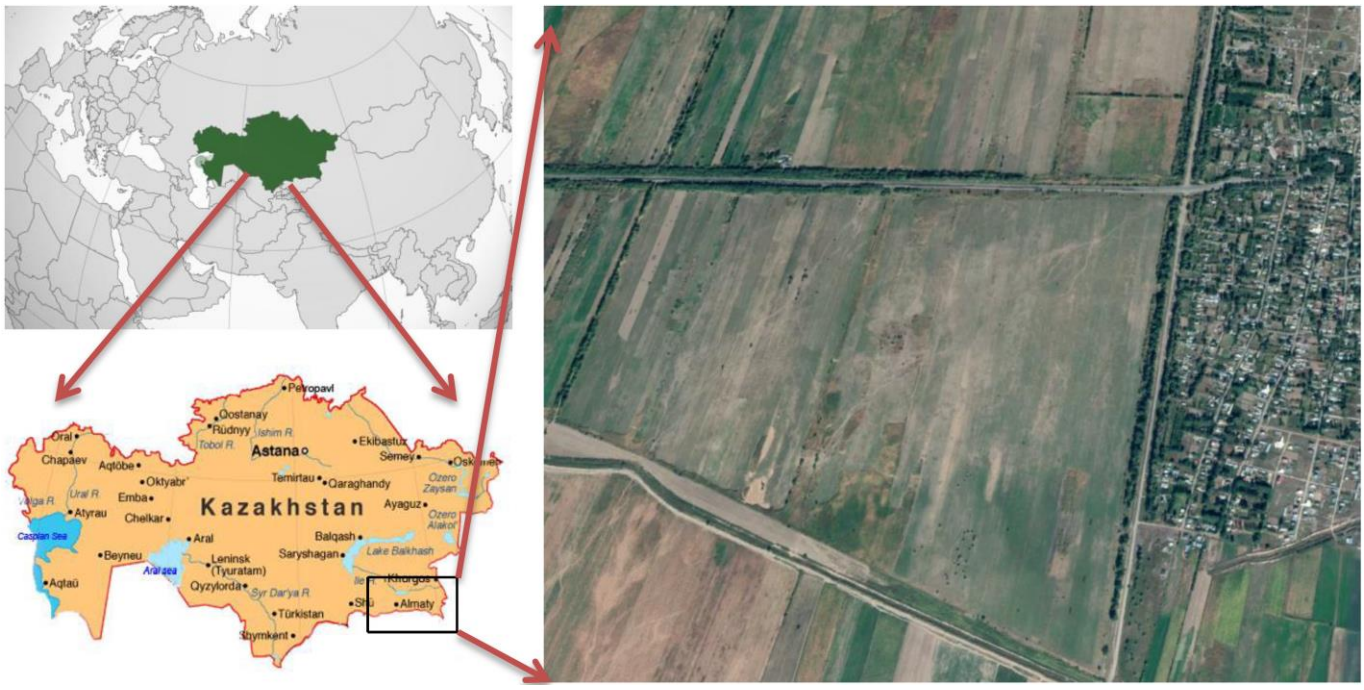


Figure 1. Location map of the study area

The climate type of study area is "Continental Climate" which can be described as low humidity, plenty of sunlight, a short but rather cold winter. The average annual air temperature is 12.3°C, total rainfall is 275.7 mm. The average long-term sum of precipitation for a period with temperature above 10°C is 198-245 mm.

The field experiment

Between 1985 and 1989, the field experiment established by S. Kaldybayev to ameliorate with alfalfa (*Medicago sativa* L.) cultivation soils in some parts of meadow Solonchaks (Beketova et al., 2017; Yerteyeva et al., 2018). From 1989 until 2017, alternating leguminous plants (especially leguminous vegetable and alfaalfa) were grown integrated production without other amelioration methods in bio-ameliorated meadow Solonchaks. Other parts in meadow Solonchak is virgin land.

Soil sampling

Soil samples were taken on 27 May 2017 as a bulked sample from ameliorated soils and virgin lands from 0 to 20 cm soil depth (plough layer). The soil samples had 43.56% clay, 40.29% silt and 16.15% sand. Soil samples were dried under atmospheric condition and passed through a 2 mm sieve to prepare for laboratory analysis. In soil samples, $CaCO_3$ content by Scheibler calsimeter, pH in 1:1 (w/v) soil:dH_2O suspension by pH-meter, electrical conductivity (EC) in the same soil suspension by EC-meter, exchangeable cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) were measured using a 1 N NH_4OAc (pH 7) extraction, water soluble exchangeable cations pH in 1:1 (w/v) soil:dH_2O. All soil samples were sieved through a 150 µm mesh before determining the total organic carbon content (C_{org}) by the wet oxidation method (Walkley–Black) with $K_2Cr_2O_7$ (Rowell, 1996).

Measurement of biological soil quality indicators

Microbiological indicators

Microbiological counts were expressed as a number of colony forming units (CFUs) per g of dry soil. The total number of bacteria was determined by the dilution method on agarized soil extract. The total number of fungi was determined on the Martin's medium (Martin, 2003). Ten grams of each soil sample were added to 95 mL of 0.1% (w/v) solution of sodium pyrophosphate. After homogenization for 30 min, this solution was decimally diluted (10−1 to 10−7) and aliquots of the resulting solutions plated on appropriate culture media. After incubation at 25 or 30°C, for up to 10 days, the colony forming units (CFU) were counted. Data were expressed as colony forming units (CFU) g^{-1} dry soil.

Microbial biomass carbon (C_{mic}) was determined by the substrate-induced respiration method of by Anderson and Domsch (1978). A moist sample equivalent to 10 g oven-dry soil was amended with a powder mixture containing 40 mg glucose. The CO_2 production rate was measured hourly using the method described by Anderson (1982). The pattern of respiratory response was recorded for 4 h. Microbial biomass carbon (C_{mic}) was calculated from the maximum initial respiratory response in terms of mg C g^{-1} soil as 40.04 mg CO_2 g^{-1} + 3,75. Data were expressed as mg C g^{-1} dry soil.

Basal soil respiration (BSR) at field capacity (CO_2 production at 22ºC without addition of glucose) was measured, as reported by Anderson (1982); by alkali ($Ba(OH)_2.8H_2O$ + $BaCI_2$) absorption of the CO_2 produced during the 24h incubation period, followed by titration of the residual OH^- with standardized hydrochloric acid, after adding three drops of phenolphthalein as an indicator. Data were expressed as µg CO_2-C g^{-1} dry soil.

Enzyme activities

Dehydrogenase activity (DHA) was determined according to Pepper et al. (1995). To 6 g of sample 30 mg glucose, 1 ml of 3% TTC (2,3,5-triphenyltetrazoliumchlorid) solution and 2.5 ml pure water were added and the samples were incubated for 24h at 37ºC. The formation of TPF (1,3,5 triphenylformazan) was determined spectrophotometrically at 485 nm and results were expressed as µg TPF g^{-1} dry sample. Catalase activity (CA) was measured by the method of Beck (1971). Ten ml of phosphate buffer (pH, 7) and 5 ml of a 3% H_2O_2 substrate solution were added to 5 g of sample. The volume (ml) of O_2 released within 3 minutes at 20ºC was determined. Three replicates of each sample were tested and controls were tested in the same way, but with the addition of 2 ml of 6.5% (w/v) NaN_3. Results were expressed as ml O_2 g^{-1} dry soil. β-glucosidase activity (GA) was measured according to Eivazi and Tabatabai (1988). 0.25 ml toluene, 4 ml TRIS (hydroximethyl) aminomethane buffer (pH, 12) and 1 ml of 0.05 M *p*-nitrophenyl β-D-glucopyranoside solution were added to the 1 g sample and the samples were incubated for 1 h at 37ºC. The formation of *p*-nitrophenol was determined spectrophotometrically 410 nm and results were expressed as µg *p*-nitrophenol g^{-1} dry soil. Protease activity was measured according to Ladd and Butler (1972). Using casein as a substrate, soil samples were incubated for 2 h at 50°C at pH 8.1. Amino acids released during the

incubation period were extracted, and the remaining substrate was precipitated after the addition of trichloroacetic acid. Aromatic amino acids react with Folin-Ciocalteu phenol reagent in an alkaline solution to form a blue complex, which was determined spectrophotometrically and results were expressed as µg Tyrosn g^{-1} dry soil.

Urease activity (UA) was measured by the method of Hoffmann and Teicher (1961). 0.25 ml toluene, 0.75 ml citrate buffer (pH, 6.7) and 1 ml of 10% urea substrate solution were added to the 1 g sample and the samples were incubated for 3 h at 37ºC. The formation of ammonium was determined spectrophotometrically at 578 nm and results were expressed as µg N g^{-1} dry soil. Alkaline phosphatase activity (APA) was determined according to Tabatabai and Bremner (1969). 0,25 ml toluene, 4 ml phosphate buffer (pH,8.0) and 1 ml of 0,115 M *p*-nitrophenyl phosphate (disodium salt hexahydrate) solution were added to the 1 g sample and the samples were incubated for 1 h at 37^{0}C. The formation of *p*-nitrophenol was determined spectrophotometrically at 410 nm and results were expressed as µg *p*-nitrophenol g^{-1} dry soil. Arylsulphatase activity (ASA) was measured according to Tabatabai and Bremner (1970). 0.25 ml toluene, 4 ml acetate buffer (pH,5.5) and 1 ml of 0.115 M *p*-nitrophenyl sulphate (potassium salt) solution were added to the 1 g sample and the samples were incubated for 1h at 37ºC. The formation of *p*-nitrophenol was determined spectrophotometrically 410 nm and results were expressed as µg *p*-nitrophenol g^{-1} dry soil. All determinations of biological soil quality indicators were performed in triplicate, and all values reported are averages of the three determinations expressed on an oven-dried soil basis (105ºC).

Results and Discussion

Some chemical properties of ameliorated and virgin land soils are given in Table 1. As shown in Table 1; soil pH, EC, $CaCO_3$, water soluble cations and exchangeable cations were higher in virgin land than in the ameliorated land.

Table 1. Some soil chemical properties of ameliorated and virgin land of meadow Solonchack.

Soil chemical properties	Ameliorated land	Virgin land
pH	8.35	9.02
EC, dSm^{-1}	0.76	3.32
Organic matter, %	2.60	0.56
$CaCO_3$, %	12.19	18.22
Water soluble cations, dSm^{-1}		
Na^+	1.09	5.89
K^+	0.39	0.42
Ca^{2+}	1.02	18.59
Mg^{2+}	0.76	6.59
Exchangeable cations, dSm^{-1}		
Na^+	1.05	3.79
K^+	0.70	0.72
Ca^{2+}	15.24	58.11
Mg^{2+}	4.19	5.17

Biological soil quality indicators

In this study two categories of biological soil quality indicators were used: microbiological indicators and enzyme activities. Biological soil quality indicators of ameliorated and virgin lands are presented in Table 2.

Table 2. Biological soil quality indicators in ameliorated and virgin land of meadow Solonchack.

Biological soil quality indicators	Ameliorated land	Virgin land
Microbiological indicators		
Bacteria count, CFU g^{-1} dry soil	$5x10^8$	$2x10^6$
Fungi count, CFU g^{-1} dry soil	$3x10^3$	$5x10^2$
C_{mic}, mg C g^{-1} dry soil	326,36	162,72
BSR, µg CO_2-C g^{-1} dry soil	75,41	36,37
Enzyme activities		
DHA, µg TPF g^{-1} dry soil	58,15	25,11
CA, ml O_2 g^{-1} dry soil	5,69	5,11
GA, µg *p*-nitrophenol g^{-1} dry soil	12,69	3,58
UA, µg N g^{-1} dry soil	8,68	4,29
PA, µg Tyrosn g^{-1} dry soil	89,54	36,72
APA, µg *p*-nitrophenol g^{-1} dry soil	27,36	5,96
ASA, µg *p*-nitrophenol g^{-1} dry soil	19,56	8,75

Microbiological indicators

Soil microorganisms play an important role as regulators of major biogeochemical cycles and can significantly affect the ecosystem functioning (Tiedje et al., 1999), being involved in organic matter dynamics, nutrient cycling and decomposition processes (Nannipieri et al., 2003). Because of these reasons, microbial populations are important in soil fertility and quality. The anthropogenic activities affect the diversity of natural habitats modifying the number of species occurring in the environment at the landscape scale. In this study, it was determined that melioration practices strongly influenced bacteria and fungi populations in soils, and the virgin land soil had the lower bacteria and fungi population than ameliorated land soil (Table 2). Therefore, microbial population and their activities in response to amelioration practices is a fundamental indicator for sustainability of ecosystem processes.

We accept that C_{mic} gives an idea of the potential microbial activity of a soil (Nannipieri et al., 1990). The incorporation of organic matter by amelioration with alfalfa (*Medicago sativa* L.) cultivation in soil raised the C_{mic} level significantly, which reflects the increased number of microorganisms (Table 2). The general increase in C_{mic} noted can be attributed to the incorporation of easily biodegradable organic materials (Perucci, 1992). In addition, favorable conditions in ameliorated land and the higher content of organic matter (Table 1), which acts as energy source for the microorganisms, thus contributing to an increase in their activity and biomass. Anderson (1982) defined BSR as a useful parameter in measuring a soil's biological activity. High contents of organic matter and microbial populations significantly raised the BSR in ameliorated land (Table 2). Hence, favorable conditions result in increase in the size of the microbial biomass and the efficiency of C substrates degradation, conducting to an increase in respiration rate per unit of microbial biomass.

Enzyme activities

Enzyme activity is essential in both mineralization and transformation of organic C and plant nutrients. Hence, enzyme measurements have been used to determine effects of agricultural and/or amelioration practices on soil microorganisms as Biological soil quality indicators. As presence of dehydrogenases, which are intracellular to the microbial biomass, is common throughout microbial species and they are rapidly degraded following the cell death, the measurement of microbial DHA in soils has been used extensively (Bolton et al., 1985; Rossel and Tarradellas, 1991; Obbard, 2001). Therefore, usage of DHA as an index of microbial activity has been suggested (Benefield et al., 1977; Nannipieri et al., 1990; Tabatabai, 1994; Masciandaro et al., 2000). The CA is based on the rates of oxygen release from the added hydrogen peroxide, and may be related to the metabolic activity of aerobic organisms (Kızılkaya et al., 2004). The virgin land soil had the lower DHA and CA enzyme activity than ameliorated land soil (Table 2).

The GA, PA, UA, APA and ASA are good markers of biological fertility since they are involved in microbial cycling of C, N, P and S. The GA catalyzes the hydrolysis of β-D-glucopyranoside and is one of the three or more enzymes involved in the saccharification of cellulose (Bandick and Dick, 1999; Turner et al., 2002). The PA hydrolyze proteins to polypeptides, oligopeptides, and amino acids. As most N compounds in mineral soils are organically bound, these transformations are necessary to release N for plant uptake (Okur et al., 2009). The UA is involved in the hydrolysis of urea to carbondioxide and ammonia, which can be assimilated by microbes and plants. It acts on carbon-nitrogen (C-N) bonds other than the peptide linkage (Bremner and Mulvaney, 1978). The APA hydrolyzes compounds of organic phosphorus and transforms them into basically phosphate ions of inorganic phosphorus, which are assimilate by plants (Amador et al., 1997). The ASA is the enzyme involved in the hydrolysis of arylsulphate esters by fission of the oxygen-sulphur (O-S) bond. This enzyme is believed to be involved in the mineralization of ester sulphate in soils (Tabatabai, 1994). In this study, activity of all the studied extracellular enzymes was significantly higher in the ameliorated land soil than in the virgin land soil samples (Table 2). Higher extracellular enzyme activity in ameliorated land soil is related higher content of organic matter content (Table 1).

Several studies (Kızılkaya and Bayraklı, 2005; Aşkın and Kızılkaya, 2006; Kızılkaya and Hepşen, 2007) showed that the soils including high organic matter content have higher enzyme activities such as; intracellular (DHA and CA) and extracelluar-hydrolytic enzymes (GA, PA, UA, APA and ASA) than the soils including low organic matter content. During the biological amelioration period with alfalfa, post harvesting material might have decomposed, resulting in higher enzymes in ameliorated land soil. In addition, increased content of organic carbon and nutrients may increase enzymes in ameliorated land soil. Some research were reported that increasing organic matter and available nutrients were increased to enzyme activity, (Leirós, et al., 2000; Kızılkaya, 2005). It is possible that the increasing organic material had stimulated microbial production of enzymes (GA, PA, UA, APA and ASA) in soil, or supported more enzymes accessible to substrate.

Conclusion

Overall, data obtained in this work revealed an important effect of amelioration practices on biological soil quality indicators. Soil microbial communities and their enzymatic activities exhibited compositional shifts that tracked with changes in land amelioration. This study, combining the microbiological and biochemical data furnishes a good methodological approach to describe the influence of bio-amelioration with alfalfa (*Medicago sativa* L.) cultivation on biological soil quality indicators. In fact, the results demonstrate that in the same pedological conditions, amelioration activities that influence the microbiological properties and their activities in soils, showed a more stable and higher microbiological and biochemical soil composition as well as intra and extracellular enzyme activities compare with virgin land soil. Further researches are required to determine whether the observed shifts in microbial community composition produce parallel changes in the functional attributes of these communities across soil types under different type of amelioration practices. The use of culture-independent approaches, like metagenome sequencing, will make it possible to identify the specific drivers of land amelioration and land use dynamics exhibited by soil microbial communities and to give a complete picture of the microbial communities in a typical meadow Solonchaks.

References

Amador, J.A., Glucksman, A.M., Lyons, J.B., Görres, J.H., 1997. Spatial distribution of soil phosphatase activity within a riparian forest. *Soil Science* 162(11): 808-825.

Anderson, J.P.E., 1982. Soil respiration. In. Methods of soil analysis, Part 2- Chemical and Microbiological Properties, Page, A.L., Keeney, D. R., Baker, D.E., Miller, R.H., Ellis, R. Jr., Rhoades, J.D. (Eds.). ASA-SSSA, Madison, Wisconsin, USA. pp. 831-871.

Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantative measurement of microbial biomass in soils. *Soil Biology and Biochemistry* 10(3): 215 – 221.

Aşkın, T., Kızılkaya, R., 2006. Assessing spatial variability of soil enzyme activities in pasture topsoils using geostatistics. *European Journal of Soil Biology* 42(4): 230-237.

Bandick, A.K., Dick, R.P., 1999. Field management effects on soil enzyme activities. *Soil Biology and Biochemistry* 31(11): 1471–1479.

Beck, T.H., 1971. Die Messung derkKatalasen aktivität Von Böden. *Zeitschrift für Pflanzenernährung und Bodenkunde* 130(1): 68-81.

Beketova, A.K., Kaldybaev, S., Yertayeva, Z., 2017. Changes in the Composition and Properties of Meadow Solonchaks of the Ili Alatau Foothill Plain in the Republic of Kazakhstan during a Long Postmeliorative Period. *OnLine Journal of Biological Sciences* 17(4): 290.298.

Benefield, C.B., Howard P.J.A., Howard, D.M., 1977. The estimation of dehydrogenase activity in soil. *Soil Biology and Biochemistry* 9(1): 67-70.

Bolton, Jr.,H., Elliott, L.F., Papendick, R.I., Bezdicek, D.F., 1985. Soil microbial biomass and selected soil enzyme activities: effect of fertilization and cropping practices. *Soil Biology and Biochemistry* 17(3): 297-302.

Bremner, J.M., Mulvaney, R.L., 1978. Urease activity in soils. In: Soil enzymes, Burns, R.G. (Ed.). Academic Press, New York, USA.pp.149-196.

Dick, R.P., 1994. Soil enzyme activity as an indicator of soil quality. In: Defining soil quality for a sustainable environment. Doran, J.W., Coleman, D.C., Stewart, B.A., Bezdicek, D.F. (Eds.). SSSA Special Publication No. 35. Madison, Wisconsin, USA. pp. 107–124.

Doran, J.W., Parkin, T.B., 1994. Defining and assessing soil quality. In: Defining soil quality for a sustainable environment. Doran, J.W., Coleman, D.C., Stewart, B.A., Bezdicek, D.F. (Eds.). SSSA Special Publication No. 35. Madison, Wisconsin, USA. pp. 3–21.

Eivazi, F., Bayan, M., Schmidt, K., 2003. Select soil enzyme activities in the historic Sanborn Field as affected by longterm cropping systems. *Communications in Soil Science and Plant Analysis* 34(15/16): 2259–2275.

Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry* 20(5): 601-606.

FAO, 2015. Status of the World's Soil Resources (SWSR) – Main Report. Chapter II Regional assessment of soil changes in Europe and Eurasia. Food and Agriculture Organization of the United Nations and Intergovernmental Technical Panel on Soils, Rome, Italy. 363p. http://www.fao.org/3/a-bc600e.pdf

Grattan, S.R., Grieve, C.M., 1999. Salinity–mineral nutrient relations in horticultural crops. *Scientia Horticulturae* 78(1-4): 127–157.

Hoffmann, G.G., Teicher, K., 1961. Ein Kolorimetrisches Verfahren zur Bestimmung der Urease Aktivitat in Böden. *Zeitschrift für Pflanzenernährung und Bodenkunde* 95(1): 55–63.

Jordan, D., Kremer, R.J., Bergfield, W.A., Kim, K.Y., Cacnio, V.N., 1995. Evaluation of microbial methods as potential indicators of soil quality in historical agricultural fields. *Biology and Fertility of Soils* 19(4):297–302.

Karlen, D.L., Mausbach, M.J., Doran, J.W., Cline, R.G., Harris, R.F., Schuman, G.E., 1997. Soil quality: a concept, definition, and framework for evaluation. *Soil Science Society of America Journal* 61(1): 4–10.

Kazemi, K., Eskandari, H., 2011. Effects of salt stress on germination and early seedling growth of rice (Oryza sativa) cultivars in Iran. *African Journal of Biotechnology* 10(77): 17789-17792

Kızılkaya, R., 2005. The role of different organic wastes on zinc bioaccumulation by earthworm Lumbricus terrestris L. (Oligochaeta) in successive Zn added soil. *Ecological Engineering* 25(4): 322-331.

Kızılkaya, R., Aşkın, T., Bayraklı, B., Sağlam, M., 2004. Microbiological characteristics of soils contaminated with heavy metals. *European Journal of Soil Biology* 40(2): 95-102.

Kızılkaya, R., Bayraklı, B., 2005. Effects of N-enriched sewage sludge on soil enzyme activities. *Applied Soil Ecology* 30(3): 192-202.

Kızılkaya, R., Hepşen, Ş., 2007. Microbiological properties in earthworm Lumbricus terrestris L. cast and surrounding soil amended with various organic wastes. *Communication in Soil Science and Plant Analysis* 38(19-20): 2861-2876.

Ladd, J.N., Butler, J.H.A., 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. *Soil Biology and Biochemistry* 4(1): 19-39.

Lax, A., Diaz, E., Castillo, V., Albaladejo, J., 1994. Reclamation of physical and chemical properties of a salinized soil by organic amendment. *Arid Soil Research and Rehabilitation* 8(1): 9–17.

Leirós, M.C., Trasar-Cepeda, C., Seoane, S., Gil-Sotres, F., 2000. Biochemical properties of acid soils under climax vegetation (Atlantic oakwood) in an area of the European temperate–humid zone (Galicia, NW Spain): general parameters. *Soil Biology and Biochemistry* 32(6): 733-745.

Mahdy, A.M., 2011. Comparative effects of different soil amendments on amelioration of saline-sodic soils. *Soil and Water Research* 6(4): 205–216.

Martin, J.P., 2003. Use of acid, rose Bengal, and streptomycin in the plate method for estimating soil fungi. *Soil Science* 69(3): 215–232.

Masciandaro, G., Ceccanti, B., Ronchi, V., Bauer, C., 2000. Kinetic parameters of dehydrogenase in the assessment of the response of soil to vermicompost and inorganic fertilisers. *Biology and Fertility of Soils* 32(6): 479-483.

Matsumoto, S., Zhao, Q., Yang, J., Zhu, S., Li, L., 1994. Salinisation and its environmental hazard on sustainable agriculture in East Asia and its neighbouring regions. 15th World Congress of Soil Science, Mexico, pp.236–255.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. *European Journal of Soil Science* 54(4): 655–670

Nannipieri, P., Ceccanti, B., Grego, S., 1990. Ecological significance of the soil biological activity in soil. In: Soil Biochemistry. Bollag, J.M., Stotzky, G. (Eds.). Marcel Dekker, New York, USA. pp. 415-471.

Oad, F.C., Samo, M.A., Soomro, A., Oad, D.L., Oad, N.L., Siyal, A.G., 2002. Amelioration of salt affected soils. *Journal of Applied Sciences* 2(1): 1-9.

Obbard, J.P., 2001. Measurement of dehydrogenase activity using 2-p-iodophenyl-3-p-nitrophenyl- 5-phenyltetrazolium chloride (INT) in the presence of copper. *Biology and Fertility of Soils* 33(4): 328-330.

Okur, N., Altındişli, A., Çengel, M., Göçmez, S., Kayıkçıoğlu, H.H., 2009. Microbial biomass and enzyme activity in vineyard soils under organic and conventional farming systems. *Turkish Journal of Agriculture and Forestry* 33: 413-423.

Pepper, I.L., Gerba, C.P., Brendecke, J.W., 1995. Environmental microbiology: a laboratory manual. Academic Press, New York, USA. 197p.

Perucci, P., 1992. Enzyme activity and microbial biomass in a field soil amended with municipal refuse. *Biology and Fertility of Soils* 14(1): 54-60.

Qadir, M., Schubert, S., Ghafoor, A., Murtaza, G., 2001. Amelioration strategies for sodic soils: A review. *Land Degradation and Development* 12(4): 357–386.

Rossel, D., Tarradellas, J., 1991. Dehydrogenase activity of soil microflora: Significance in ecotoxicological tests. *Environmental Toxicology* 6(1): 17-33.

Rowell, D.L. 1996. Soil Science: Methods and Applications. Longman, London, UK. 368p.

Sumner, M.E., 1993. Sodic soils: New perspectives. *Australian Journal of Soil Research* 31(6): 683–750.

Tabatabai, M.A., 1994. Soil enzymes. In: Methods of soil analysis. Part 2- Microbiological and biochemical properties. Mickelson, S.H., Bighan, J.M. (Eds). (Eds.). ASA-SSSA, Madison, Wisconsin, USA. pp. 775-826.

Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry* 1(4): 301-307.

Tabatabai, M.A., Bremner, J.M., 1970. Arylsulphatase activity of soils. *Soil Science Society of America Journal* 34(2): 225-229.

Tiedje, J.M., Asuming-Brempong, S., Nüsslein, K., Marsh, T.L., Flynn, S.J., 1999. Opening the black box of soil microbial diversity. *Applied Soil Ecology* 13(2): 109–122.

Turner, B.L., Hopkins, D.W., Haygarth, P.M., Ostle, N., 2002. β-Glucosidase activity in pasture soils. *Applied Soil Ecology* 20(2): 157-162.

Umali, D., 1993. Irrigation-induced salinity. World Bank Tech. Paper No. 215, Washington, USA. 79p. Available at [access date : 16.06.2018]: http://documents.worldbank.org/curated/en/486611468766779740/pdf/multi-page.pdf

Yertayeva, Z., Kaldybaev, S., Beketova, A., 2018. The scientific basis of changes in the composition and properties of meadow saline soil of the foothill plains of the Ili Alatau during a long postmeliorative period. *Ecology, Environment and Conservation* 24(2): 715-720.

Eurasian J Soil Sci 2019, 8 (3) 196 - 207

Eurasian Journal of Soil Science

Journal homepage : http://ejss.fesss.org

Pedo-transfer functions with multiple linear regressions to predict solute-transport parameters

Md. Abdul Mojid [a,*], A.B.M. Zahid Hossain [b], Guido C.L. Wyseure [c], Md. Ali Ashraf [d]

[a] Department of Irrigation and Water Management, Bangladesh Agricultural University, Mymensingh, Bangladesh
[b] Irrigation and Water Management Division, Bangladesh Rice Research Institute, Gazipur, Bangladesh
[c] Division of Soil and Water Management, Department of Earth and Environmental Sciences, K.U. Leuven, Belgium
[d] Department of Farm Structure and Environmental Engineering, Bangladesh Agricultural University, Bangladesh

Abstract

Transport parameters of soluble chemicals through soils are needed to assess the pollution risks of soil and groundwater resources. But, it is time consuming, laborious, expensive and, practically, impossible to experimentally measure such parameters for a wide range of solutes and soil types. So, indirect estimate of the parameters by pedo-transfer function is becoming popular. The aim of this study was to develop and evaluate pedo-transfer functions (PTFs) for solute-transport parameters by multiple linear regression (MLR) analysis. For this, transport parameters of three heavy metal /metalloid compounds ($NaAsO_2$, $Pb(NO_3)_2$, $Cd(NO_3)_2$), a pesticide (carbendazim) and an inert salt ($CaCl_2$) through 14 agricultural soils of Bangladesh were determined. The transport experiments were done in repacked soil columns under unsaturated steady-state water flow conditions. Breakthrough data of the solutes were measured with time-domain reflectometry (TDR), and velocity (V), dispersion coefficient (D) and retardation factor (R) of the solutes were determined by analyzing the data by a transfer-function method. Bulk density (γ), organic carbon (OC) content, clay (C) content, pH, median grain diameter (D_{50}) and uniformity coefficient (C_u) of the soils were determined. Regression models for V, D and R were developed with γ, OC, C, pH, D_{50} and C_u as the input variables. Bulk density and clay content were found the most sensitive input variables to the MLR models. The MLR models fairly predicted V, D and R, and thus provide a way of significantly enhancing prediction of reactive solute transport through agricultural soils.

Keywords: Soluble chemicals, soil properties, solute movement, indirect estimate.

Article Info

Received : 11.10.2018
Accepted : 15.04.2019

Introduction

Pollution of agricultural soils by heavy metals and pesticide residues occurs through the application of chemical fertilizers, especially phosphate fertilizers, and pesticides. The residues of these chemicals contaminate the soil and water (both surface and groundwater), enter the food chain and cause threat to human and animal health. Industrial effluents and irrigation with wastewater further degrade soil and water quality. The characterization of soluble-chemical transport through soils is an important aspect to assess the pollution of soil and groundwater resources (Porro et al., 1993). Usually, simulation models are used to quantify solute transport through subsurface as tools to implement improved agricultural management. Solute-transport parameters, such as velocity of transport, dispersion coefficient, dispersivity and retardation factor, are among the most crucial inputs for the simulation models. Success of these models depends on our ability to properly quantify the input solute-transport parameters.

* Corresponding author.
Department of Irrigation and Water Management, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
Tel.: +880 91 67401 E-mail address: ma_mojid@yahoo.com
e-ISSN: 2147-4249 DOI: 10.18393/ejss.554942

The estimation of solute-transport parameters in soils is generally done by fitting measured breakthrough curves (BTC) of solutes to analytical solutions of the convection-dispersion equation. BTCs are constructed with the concentrations of effluent or a proxy measurement of concentration like TDR-measured electrical conductivity from leaching experiments. Such measurements are however time consuming, laborious, expensive and, practically, impossible to obtain BTCs for a wide range of solutes and soil types to sample temporal and spatial variations. So, indirect approaches are needed to predict solute-transport parameters with pedo-transfer functions (PTFs), which use basic soil properties that are often routinely available from soil survey information (Bouma, 1989). The general purpose of developing PTFs is to establish predictive models using databases of soil properties, which contain suitable predictors (basic soil properties) and desired predictands (estimated less available soil properties).

Although widely used to predict unsaturated hydraulic properties of soils (e.g., Vereecken, 1992; Gonçalves et al., 1997), PTFs are yet not well-developed and very familiar to predict solute-transport parameters. Recent developments in this field include parameterizations of solute transport, heat exchange, soil respiration, organic carbon content, root density and water uptake by vegetation (Van Looy et al., 2017). Since it is difficult to relate and compare the physical meaning of specific model parameters, there are only limited PTFs for predicting solute-transport parameters from basic soil characteristics. Several studies on PTFs for adsorption isotherm parameters mostly concern contaminants such as heavy metals (e.g., Horn et al., 2006) or excess pesticides (e.g., Kodešová et al., 2011; Moeys et al., 2011) and fertilizers (e.g., Achat et al., 2016). All these studies include soil organic carbon content as a predictor. Soil pH and clay content were reported as the other common predictors for PTFs of adsorption properties. Perfect et al. (2002) predicted dispersivity using PTFs across a range of soil textures and could explain 50% of its total variation by the parameters of soil-water retention curves using step-wise multiple regressions. Alibuyog (2007), by using PTFs from multiple linear regressions, showed great potential in predicting pore-water velocity, dispersion coefficient and dispersivity from soil physical properties than from water retention parameters. In his observations, using soil properties as predictors, the PTFs could account for more than 50% of the total variation of pore-water velocity, dispersion coefficient and dispersivity.

Predictions of solute-transport parameters, instead of direct measurements, may be accurate enough for many applications. It is therefore worthwhile to analyze databases in such a way that solute-transport parameters can be predicted from easily-measured soil properties. But, extrapolation of PTFs in different agropedoclimatic contexts limits their performance (Touil et al., 2016). So, attempts to develop solute transport PTFs have, so far, been mostly kept to small, local data sets and specific models since the local PTFs are important to properly investigate the relation between the predictors and predictands, and they could be useful in meeting the local agricultural requirements for modeling with reasonable accuracy. The prediction of transport parameters at the local scale is also a first step to simulate subsurface solute movement over larger areas (Gonçalves et al., 2001).

Regression technique is widely used to determine the relationship between predictors and predictands because of its simplicity. It can use linear regressions or nonlinear regressions depending on the expected relationship among the variables (Mojid et al., 2018). The advantage of regression analysis is that it is straightforward to carry out and easy to employ. The disadvantage is that the regression equations (e.g., linear, logarithmic or exponential) and predictors must be determined as a priori and that the relationships between soil properties and predictors may be different in different portions of the database (Van Looy et al., 2017). However, improved multiple linear regression (MLR) can be an efficient and reliable method (Touil et al., 2016) if the relationship between the dependent and independent variables is not complex.

Studies of pedo-transfer function may undertake one of two primary purposes: research or application. Investigators who intend to advance research knowledge may find it more desirable that the model is flexible and can work efficiently with various data sizes and types. Or they may intend to help mine auxiliary information (e.g., importance of input variables) given the structure or features of the model (Van Looy et al., 2017). Our study addressed the research issue. The objectives were: (i) to develop pedo-transfer functions for velocity, dispersion coefficient and retardation factor of four reactive solutes and a non-reactive solute with basic soil properties by multiple linear regression analysis and (ii) to evaluate performance of the pedo-transfer functions.

Material and Methods

Soil sampling and solute-transport measurement

Fourteen (14) soil samples of adequate quantity were collected from different locations of Bangladesh under intensive agricultural activities. The plowed upper soil layers (0–15 cm) were used in solute-transport experiments to reduce variability due to heterogeneity. The soil samples were air dried and sieved to pass through a 2-mm mesh sieve after crushing. Sub-samples from the sampled soils were analyzed for particle size distribution, gradation, pH and organic carbon (*OC*) by employing standard methods. Details of soil sampling and analysis of the samples are reported in Mojid et al. (2018).

The procedures of solute-transport experiments are described here in brief. For details, the readers are referred to Mojid et al. (2016). The experiments were done in four PVC columns (hereafter called experimental columns), each 34 cm long and 15 cm in inner diameter. These columns were filled with four of the air-dried and sieved soils under investigation in the first batch. Each column was packed to 32 cm depth. The soil columns were conditioned by leaching sufficient quantity of tap water (EC = 17 mS m^{-1}) following six wetting and drying cycles during a nine-month period. The soil columns were transferred and placed vertically and axially on four 1.2-m high supporting soil columns to simulate a thick natural soil profile. Two 3-wire TDR sensors (10 cm long and 3 cm spacing with 0.2 cm wire diameter) were inserted horizontally to each column during preparing the soil columns. One sensor was at 8 cm and the other at 28 cm below the top of the upper column; the vertical distance between the two sensors (Z) was 20 cm. A cartridge pump applied tap water through fine needles at constant rate (0.32 ± 0.02 cm h^{-1}), which was considerably lesser than the saturated hydraulic conductivities (≥0.64 cm h^{-1}) of the soils to ensure unsaturated flow. The pump distributed the applied water uniformly over the soil surface of each column through nine fine needles uniformly spaced with a PVC cap on each soil column. Water flow continued until equilibrium between the applied and drainage water was attained. A constant hanging water table, maintained at 20 cm above the base of the supporting (lower) columns, created suction in soils of the experimental (upper) columns.

First, $CaCl_2$ was used in the breakthrough experiments; it helped retaining structure of the soils during the transport experiments. At steady-state water flow condition, a pulse of 5 ml $CaCl_2$ solution (250 g l^{-1}) was introduced uniformly on each column with a syringe attached to a fine needle. The water flow (0.32 ± 0.02 cm h^{-1}) continued until the solution was completely eluted from the upper columns. A TDR100 and CR10X data logger were programmed to record water content and bulk EC of the soils at fixed interval (40, 50 or 60 minutes depending on the solute and soil types). The measurements continued until whole of the applied solute leached out from the upper columns. Measurements of water content and bulk EC were done for $CaCl_2$, $NaAsO_2$, $Pb(NO_3)_2$, $Cd(NO_3)_2$ and carbendazim. Carbendazim is a granular organic solute, which is extensively used in Bangladesh as fungicide. The molecular weight of carbendazim is 191.2 g mol^{-1} and its solubility in water at pH 7–8 is 5–7 mg l^{-1}. The pulse volume and concentration of $NaAsO_2$, $Pb(NO_3)_2$, $Cd(NO_3)_2$ and carbendazim were the same as that for $CaCl_2$ (5 ml and 250 g l^{-1}). A solute took 5–10 days to leach completely through the soil columns depending on texture of the soils and properties of the solute. After completion of the experiments, three soil samples were collected from the surface of each column by using core samplers (5 cm × 5 cm; Eijkelkamp, The Netherlands). These samples were used to determine the basic physical and hydraulic properties of the soils. Approximately, 200 g additional soil samples were also collected from each column for determining pH, EC and *OC*. Following the whole procedures, data recording on soil-water content and bulk EC, and soil sampling were done for the remaining 10 of the 14 soils in subsequent batches of experiments. In is noted that because of very time-consuming transport experiments there was no replication on each soil.

The analysis of TDR-measured EC was based on the relation between the concentration of a solute in soil water and EC of soil water, which is linearly related to the EC of bulk soil for constant water content (Ward et al., 1994). It is noted that the applied solutes dissociated into ions in solution (e.g., $CaCl_2$ dissociated into Ca^{2+} and Cl^-) and the positive and negative ions, especially for reactive solutes, might have different behaviors and interactions with the soil solid phase and with the existing ions on the exchange complex (Rose et al., 2006). However, for a non-reactive/inert solute like $CaCl_2$, the velocity of a solute is assumed same as the velocity of pore water in most transport experiments; although the velocity of Cl^- may differ from that of the bulk solution due to anion exclusion, the possible small discrepancy was ignored for the relatively non-reactive/inert soils used in this study. The time-series of solute concentration were determined from the TDR-measured EC. Breakthrough curves (BTCs) were drawn by plotting normalized concentrations against time. The mean travel time, τ, mass-dispersion number, N (=D/ZV), and retardation

factor, R, of the solutes were fitted from the BTCs by a transfer-function method (Mojid et al., 2004; their Eqs. 5 and 7) using non-linear least-square fitting technique; the performance of the transfer-function method was reported reliable and described in detail in Mojid et al. (2004). For the physical meaning of the velocity and retardation factor of the reactive solutes ($NaAsO_2$, $Pb(NO_3)_2$, $Cd(NO_3)_2$ and carbendazim) and their differences from those of inert solutes, the readers are referred to Mojid and Vereecken (2005). For $CaCl_2$, R was fixed at unity assuming it to be a non-reactive solute. The transport velocity, V (= Z/τ), and dispersion coefficient, D (= $VZN = Z^2N/\tau$), of the solutes were calculated from τ, N and the distance, Z, between the input and response BTCs.

Soil property measurement

By determining the fractions of sand, silt and clay of the soils by Hydrometer method (Black, 1965) their textural classes were obtained from the Marshall's triangle (Soil Survey Staff, 1975). Soil pH was determined by a glass electrode pH meter following Jackson (1962). Twenty (20) grams of each air-dry soil was mixed with 50 ml distilled water in separate opaque plastic bottles. The suspensions were shaken with a horizontal electric shaker for 20 minutes and kept undisturbed in a control room at 25°C for five hours. The pH of the partly settled soil suspensions was measured by immersing the glass electrode. Soil organic matter, OM, was determined following the method of Walkey-Black (Jackson, 1962). Two grams of each soil were swirled in 10 ml of 1.0N $K_2Cr_2O_7$ solution. Then, 20 ml concentrated H_2SO_4 was added to it and mixed thoroughly. The mixture was kept undisturbed for 30 minutes and diluted to 200 ml with distilled water. It was titrated against $FeSO_4.7\ H_2O$ in presence of 0.5 g NaF and 30 drops of diphenylamine as indicator to dull green endpoint. The OM of the soil was calculated by

$$OM(\%) = 10\left(1 - \frac{K}{B}\right) \times 0.335 \tag{1}$$

where K is $FeSO_4.7\ H_2O$ (ml), which is used for titration of the sample and B is $FeSO_4.7\ H_2O$ (ml) for blank. The OC of the soil was calculated following Nelson and Sommers (1982) by

$$OC(\%) = \frac{\%OM}{1.724} \tag{2}$$

Gradation tests of the soils were done on the samples by a typical sieve analysis involving a nested column of sieves with wire mesh cloth. For each soil, a 500-g sample was poured into the top sieve, which had the largest screen openings. Each lower sieve in the column had smaller openings than the one above. There was a pan at the base. After shaking, the constituent materials retained on each sieve were weighed. The test was done in accordance with the British Standards, BS 1377 (1990) (Code of Practice). This exercise was repeated for the 14 soils. The median grain diameter (D_{50}) and uniformity coefficient (C_u) of the soils were calculated from the grain size distribution curves. C_u was calculated by

$$C_u = \frac{D_{60}}{D_{10}} \tag{3}$$

For determining bulk densities of the soils in the experimental columns, the soil samples in the core samplers, collected from each column after transport experiment, were dried in oven at 105°C for 24 h. The bulk densities were determined by dividing the oven dry weights of the soils by the inner volume of the core sampler. The average bulk density of each column, calculated from the three samples, was used in developing pedo-transfer functions. The textural class, bulk density, organic carbon content, relative pH (ratio of observed soil pH to the pH of a neutral soil (7) and denoted by pH′), clay content, median grain diameter and coefficient of uniformity of the soils are given in Table 1.

Pedo-transfer function development

Beyond some general conceptual understanding, there are no precise a priori relations that link predictors with the predictands. In addition, most pedo-transfer functions, PTFs, differ with the set of predictors (input variables) and predictands (output variables). PTFs were developed through multiple linear regression analyses to predict solute-transport parameters. SPSS 11.5 statistical program was used to construct multiple linear regression models for the velocity, dispersion coefficient and retardation factor of $CaCl_2$, $NaAsO_2$, $Pb(NO_3)_2$, $Cd(NO_3)_2$ and carbendazim. The input variables of the models were bulk density, organic carbon content, relative pH, clay content, median grain diameter, and uniformity coefficient of the soils (Table 1). The PTFs were developed through data validation, variable selection, and model calibration and verification. Performances of the PTFs were evaluated by sensitivity analysis and performance assessment.

Table 1. Textural class, particle size distribution, bulk density (γ, g cm^{-3}), organic carbon content (*OC*, %), relative pH (pH′), clay content (*C*, fraction), median grain diameter (D_{50}, mm) and uniformity coefficient (C_u) of nine soils used in developing and validating MLR models

Sl. No.	Texture	Particle size distribution (%)			γ (g cm^{-3})	*OC* (%)	pH′	*C* (fraction)	D_{50} (mm)	C_u
		sand	silt	clay						
1	Loamy sand	79.48	14.48	6.04	1.36	0.767	0.136	0.060	0.235	3.27
2	Silt loam	22.70	65.96	11.44	1.33	0.686	0.134	0.114	0.101	3.26
3	Sandy loam	54.48	37.02	8.50	1.33	0.452	0.132	0.085	0.200	3.58
4	Silt loam	37.32	52.00	10.68	1.29	0.753	0.136	0.107	0.112	3.30
5	Silt loam	17.24	70.08	12.68	1.40	0.523	0.143	0.127	0.095	3.86
6	Silt	8.68	77.96	13.36	1.34	0.787	0.141	0.134	0.069	2.98
7	Silt loam	18.48	66.04	15.48	1.37	0.372	0.158	0.155	0.066	2.63
8	Silt loam	4.92	75.96	19.12	1.32	0.840	0.146	0.191	0.062	2.78
9	Silty clay loam	2.68	70.16	27.16	1.41	0.554	1.09	0.272	0.037	2.43
10	Silt loam	16.96	59.84	23.20	1.26	0.987	0.96	0.232	0.045	2.61
11	Sandy loam	61.36	23.00	15.64	1.54	0.288	1.14	0.156	0.073	2.99
12	Sandy loam	74.59	15.91	9.50	1.61	0.245	1.16	0.095	0.134	3.35
13	Loamy sand	84.47	10.60	4.93	1.63	0.134	1.20	0.049	0.299	3.64
14	Silt loam	29.04	53.92	17.04	1.33	0.760	0.97	0.170	0.070	2.85

Data validation and variable selection

Data validation is a corrective measure that is taken at '*ab initio*' in observations. It is crucial since existence of even a single outlier can make the whole data set to a non-linear form (Draper and Smith, 1981). The purpose of selecting the appropriate regression variables is to reduce predictors to some "optimal" subset of the available regressors. This is important since a smaller set of predictors may often provide more accurate predictions of future cases, and/or identifying only the pertinent predictor variables may significantly improve the response.

An important step in data validation process is to scale up or down the observations by focusing on their units of measurements. This is because the mean change of response-dependent variables (*Y*) with measuring units controls statistical information. For a valid data set, *Y* needs to be greater than the mean change of predictors/independent variables (*X*). Such alterations of digital magnitudes in mean due to the change in measuring units do not, however, disrupt the basic theme of analysis. The yardstick of fixing the right units of measurement is called coefficient of centrality (c), which makes the mean of *Y*s (denoted by $\overline{Y}$) compatible to the mean of *X*s (denoted by $\overline{X}$) (Rashid, 1999). A typical multiple linear regression model with the means of variables is expressed by

$$\overline{Y} = b_0 \overline{X_0} + b_1 \overline{X_1} + b_2 \overline{X_2} + \cdots + b_k \overline{X_k} \quad (4)$$

where *b*s are regression coefficients. Dividing Eq. 4 by $\overline{Y}$ results in

$$1 = b_0 c_0 + b_1 c_1 + b_2 c_2 + \cdots + b_k c_k = b_0 c_0 + \sum_1^k (bc)_j \quad (5)$$

The coefficients of centrality, in all respects, are akin to the latent vectors, which are widely used in the latent-root regression analysis. These coefficients are always non-negative statistics and the unique value $c_o \geq 0$ in respect to the dummy variable X_o appears only at $\sum c_{1\to k}^2 \leq 1$. This axiom is satisfied only if $0 \leq c_0^2 \leq 1$, and the restriction could be met by expressing the respondents, *Y*s, in higher or the predictors, *X*s, in lower measuring units. The '$c^2 = 0$' implies a homogeneous model. For modeling our multiple linear regressions, the required conditions were satisfied by scaling up velocity of the solutes from 'cm h^{-1}' to 'mm h^{-1}' and dispersion coefficient of the solutes from 'cm^2 h^{-1}' to 'mm^2 h^{-1}'. Soil pH was scaled down to pH′, the unit value of which implies a neutral soil. All input variables satisfied the required conditions.

The selection of variables, done at the completion of regression analysis, was accomplished with the direction of the regression coefficients (b_j) and correlation coefficients (r_j). The predictors to be consistent, b_j must be unidirectional to r_j; their anti-directional behavior results in negative correlation coefficient (–*r*), which reveals that the variables are irrelevant and must be discarded from the regression models. Based on this criterion, irrelevant variables were discarded during model development. Acceptable probability level for the MLR analysis was fixed at 5%, that is the upper level of significance for acceptance was $p > 0.05$. Eight soils (# 1 – 8, Table 1) were used to select variables for MLR models.

Model calibration and verification

The MLR models were calibrated with experimental data of five soils (#9 – 13, Table 1); the purpose was to determine appropriate values of the regression coefficients (Eq. 4). Unlike usually employed procedure of calibrating a model by utilizing only one known data set, we utilized five known data sets and determined the overall average regression coefficients for the data sets. So, the calibration was done by comparing the model-estimated solute-transport parameters (*V*, *D* and *R*) with their measured values, while ensuring least errors between the two parameter sets. The obtained models were verified with the measured soil properties in evaluating solute-transport parameters. Data of a silt loam soil (#14, Table 1), not used in variable selection and model calibration, was used to verify accuracy of the models.

Parameter sensitivity analysis

Sensitivity analysis was done to evaluate relative importance of each input variable in the performance of the MLR models. At first, the model was run by using the measured input variables and the observed error was recorded for the solutes. Afterwards, the model was run by changing the input variables by ± 5% and ± 10%, and the error was recorded in each run. The sensitivity of an input variable was estimated by the ratio of error obtained with ± 5% or ± 10% changing of the variable to the error obtained with original (measured) value of the variable. An error ratio of less than unity implies that there is no effect of the input variable in generating output of the model. A larger error ratio, on the other hand, indicates more sensitivity of the input variable on the output of the model. The input variables were ranked in order of their degree of influence on the model output based on the error ratio.

Model performance assessment

Improving the accuracy of pedo-transfer functions, PTFs, requires studying how prediction uncertainty can be apportioned to different sources of uncertainty in inputs. The performance of the MLR models in simulating transport parameters of the five solutes in homogenous soil columns against their measured values was evaluated by using goodness-of-fit parameters following Piegorsch and Bailer (2005), Sarmah et al. (2005) and Phillips (2006). The most common metrics used to evaluate performance of the PTFs are root-mean-square errors (*RMSE*s), mean errors (*ME*s) and coefficient of determination (r^2). The *RMSE* quantifies the root of the average bivariate variance between estimated and measured quantities. It was calculated by

$$RMSE = \left[\sum_{i=1}^{n} (P_i - O_i)^2 / n \right]^{1/2} \quad (6)$$

where P_i is predicted and O_i is measured solute-transport parameters, and n is the number of observations. An *RMSE* of zero indicates no difference between the measured and simulated solute-transport parameters; the smaller an *RMSE* the better is the performance of the model. Modeling efficiency (*EF*) is a measure of accuracy of simulation and is an indicator of overall agreement between the measured and predicted results. *EF* of the model was calculated by

$$EF = \frac{\sum_{i=1}^{n} (O_i - O_m)^2 - \sum_{i=1}^{n} (P_i - O_i)^2}{\sum_{i=1}^{n} (O_i - O_m)^2} \quad (7)$$

where O_m is the average of measured values, and O_i and P_i represent the same meaning as in Eq. 6. An *EF* of unity implies a perfect match between the predicted and measured results. A negative *EF* implies that the predicted values are worse than simply using the observed mean as the best estimate of the data.

Databases used in the development of PTFs usually do not reflect the true population of soils in a region, and, as a result, PTFs tend to be biased to the database on which they are calibrated (Schaap and Leij, 1998). Mean error (*ME*) provides the size and sign of such systematic errors or bias of the prediction error. This error is computed by

$$ME = \sum_{i=1}^{n} (O_i - P_i) / n \quad (8)$$

Negative *ME* values indicate an average underestimation of the quantity being evaluated, while its positive values indicate an overestimation of the target variables. For a truly well-performing PTF, both *RMSE* and *ME* should be as low as possible.

Bias is a persistent positive or negative deviation of the measured value from the true value that arises from erroneous assumptions in the learning algorithm. This error, expressed as a percentage of overall error and denoted by *BOE*, is calculated by (Geman et al., 1992)

$$BOE = \frac{ME^2}{MSE} \times 100 \tag{9}$$

A mean square error (*MSE*), which measures the average of the square of error with the error being the amount by which the estimator differs from the quantity to be estimated, is calculated by

$$MSE = (RMSE)^2 \tag{10}$$

Results and Discussion

MLR models

Pedo-transfer functions, in the form of multiple linear regression, MLR, for predicting transport parameters of $CaCl_2$, $NaAsO_2$, $Pb(NO_3)_2$, $Cd(NO_3)_2$ and carbendazim are compared in Table 2 along with the inconsistent input variables, coefficient of determination (r^2) and probability level (p). The MLR models for predicting velocity, *V*, of the solutes were significant over probability level, p = 0.014 to 0.032. The coefficients of determination of the models, r^2 ≥0.99, revealed that over 99% variation in *V* was justified due to the contributions of organic carbon, *OC*; bulk density, γ; clay content, *C*; median grain diameter, D_{50}; and uniformity coefficient, C_u, of the soils. When relative soil reaction, pH′, was included, in addition to these input variables, the models became insignificant in predicting *V*, with probability level exceeding 0.05. It thus revealed that pH′ exerted inconsistent effects on the output of the models. The opposite signs (not shown) obtained between the regression coefficients (*b*s in Eq. 4) and correlation coefficient (*r*) of pH′ also confirmed this inconsistency. So, pH′ was ignored for modeling velocity of the solutes.

Table 2. Pedo-transfer functions of MLR models for $CaCl_2$, NaAsO2, $Pb(NO_3)_2$, $Cd(NO_3)_2$ and carbendazim with the values of the regression coefficients, inconsistent input parameters, coefficients of determination (r^2) and probability of uncertainty of the models

Solutes	MLR models	Inconsistent parameters	r^2	p-value
$CaCl_2$	$V = 11.99 + 0.561(\%OC) - 2.31(\gamma) - 21.80(C) + 4.39(D_{50}) + 0.13(C_u)$	pH′	0.990	0.025
	$D = 11.10 - 1.02(\%OC) + 2.39(\gamma) + 3.60(pH') - 14.94(C) + 33.78(D_{50})$	C_u	0.999	0.001
$NaAsO_2$	$V = 10.38 + 0.513(\%OC) - 1.31(\gamma) - 21.57(C) + 4.68(D_{50}) + 0.19(C_u)$	pH′	0.997	0.016
	$D = 23.35 + 0.31(\gamma) + 0.97(pH') - 30.63(C) + 0.54C_u$	$\%OC$, D_{50}	0.927	0.047
	$R = 1.14 + 0.021(\%OC) + 0.173(\gamma) - 0.056\ (pH') + 0.631(C) - 0.34(D_{50})$	C_u	0.998	0.005
$Pb(NO_3)_2$	$V = 12.29 + 0.489(\%OC) - 2.86(\gamma) - 21.22(C) + 4.39(D_{50}) + 0.254(C_u)$	pH′	0.998	0.032
	$D = 16.16 - 0.025(\%OC) - 19.43(C) + 3.97(D_{50}) + 0.55(C_u)$	γ, pH′	0.963	0.017
	$R = 1.16 + 0.011(\%OC) + 0.092(\gamma) - 0.098(pH') + 0.855(C)$	D_{50}, C_u	0.984	0.005
$Cd(NO_3)_2$	$V = 12.19 + 0.35(\%OC) - 3.18(\gamma) - 19.07(C) + 5.62(D_{50}) + 0.31(C_u)$	pH′	0.990	0.026
	$D = 9.90 + 3.50(\gamma) - 9.92(C) + 4.89(D_{50})$	pH′, %OC, C_u	0.939	0.007
	$R = 1.21 + 0.090(\gamma) + 0.273(C) - 0.694(D_{50})$	pH′, %OC, C_u	0.994	0.001
Carbenda-zim	$V = 12.82 + 0.433(\%OC) - 3.09(\gamma) - 21.65(C) + 4.05(D_{50}) + 0.221(C_u)$	pH′	0.994	0.014
	$D = 13.28 - 0.178(\%OC) + 0.371(\gamma) - 14.86(C) + 0.046(D_{50})$	pH′, C_u	0.983	0.005
	$R = 1.17 + 0.018(\%OC) + 0.112(\gamma) - 0.053(pH') + 0.566(C) - 0.563(D_{50})$	C_u	0.999	0.003

In modeling dispersion coefficient, *D*, all the five solutes encountered one or more inconsistent input variables. Although the MLR models for *D*, with inclusion of all input variables, were significant at p > 0.05, the uniformity coefficient, C_u, was inconsistent. Elimination of C_u from the models improved significant level of the models from 0.03 to 0.001. Modeling *D* for $NaAsO_2$ was insignificant with probability level exceeding 0.05 when all input variables were considered. Organic carbon, *OC*, and median grain diameter exerted inconsistent effects on model outputs. When these variables were discarded, the significant level of the

models improved to 0.047. For $Pb(NO_3)_2$, γ and pH′ exerted inconsistent effects on the model outputs, and their exclusion from the models reduced probability level to 0.017. Organic carbon, pH′ and C_u exhibited inconsistency in modeling *D* for $Cd(NO_3)_2$. When these variables were excluded, the models became significant with probability level improved from 0.188 to 0.007. For carbendazim, when all input variables were considered in the model, γ and pH′ appeared inconsistent, and their elimination significantly improved the probability level although C_u then became inconsistent. Consequently, C_u was also discarded. The accuracy of the models however improved surprisingly when γ was re-introduced; γ became consistent with a model probability of 0.005. The coefficients of determination of the models for the five solutes ranged from 0.927 to 0.999. The uniformity coefficient always put inconsistent influence for modeling retardation factor, *R*, of the solutes. In addition to this, pH′ and *OC* were also inconsistent in case of $Cd(NO_3)_2$, and D_{50} was inconsistent in case of $Pb(NO_3)_2$. All inconsistent input variables were eliminated from the models to obtain improved levels of model probability.

Model performance

The simulated velocity of the solutes agreed well with the measured velocity as illustrated in Figure 1. In predicting solute velocity, *V*, by the MLR models, the coefficients of determination were large (r^2 = 0.955–0.996). The RMSEs were 0.084–0.126 (Table 3). The efficiency, *EF*, of the models was 99% for all the solutes under investigation. The mean errors, *ME*s, of the models were –0.006 to –0.008 for $CaCl_2$, $Cd(NO_3)_2$ and carbendazim; the negative *ME*s imply that the models slightly overestimated *V* during validation. For $NaAsO_2$ and $Pb(NO_3)_2$, *ME*s of the models were 0.0027–0.0028, implying that the models slightly underestimated *V*. The bias components of overall error, *BOE*, were considerably small (0.051–0.99%).

Table 3. Statistical indices for performance assessment of MLR models for $CaCl_2$, $NaAsO_2$, $Pb(NO_3)_2$, $Cd(NO_3)_2$ and carbendazim

Solute solutions	Parameters	RMSE	EF	ME	BOE (%)
$CaCl_2$	*V*	0.110	0.990	–0.006	0.30
	D	0.046	0.999	0.000	0.00
	R	0.019	0.598	–0.003	3.23
$NaAsO_2$	*V*	0.091	0.993	0.003	0.09
	D	0.373	0.927	–0.004	0.01
	R	0.004	0.992	0.004	74.41
$Pb(NO_3)_2$	*V*	0.126	0.987	0.003	0.05
	D	0.213	0.963	0.012	0.34
	R	0.007	0.965	–0.005	54.82
$Cd(NO_3)_2$	*V*	0.114	0.990	–0.007	0.33
	D	0.169	0.939	0.009	0.28
	R	0.006	0.987	0.004	51.40
Carbendazim	*V*	0.084	0.994	–0.008	1.00
	D	0.074	0.983	–0.002	0.08
	R	0.003	0.997	–0.002	59.60

There were good agreements between the measured and simulated dispersion coefficients, *D*, for the solutes (Figure 2) with large coefficients of determination (r^2 = 0.982–0.997). *RMSE* of the models was 0.213 for $Pb(NO_3)_2$ and 0.373 for $NaAsO_2$ (Table 3). Models' efficiency varied from 0.927 to 0.999. *ME*s of the models for $CaCl_2$, $Pb(NO_3)_2$ and $Cd(NO_3)_2$ were positive (0.000–0.0124), but those for the other solutes were negative (–0.0021 to –0.0041). These results revealed that the MLR models for $CaCl_2$, $Pb(NO_3)_2$ and $Cd(NO_3)_2$ slightly underestimated *D*. For the other solutes, the models slightly overestimated *D*. The *BOE*s were perceptibly small (0–0.338%) for all the solutes.

The measured retardation factors, *R*, agreed well with the estimated *R* of all the solutes (Figure 3) with large coefficients of determination (r^2 = 0.971–0.993). Small RMSEs (0.003–0.019, Table 3) revealed good match between the measured and simulated *R*s of the solutes. Modeling efficiencies for *R* in case of $NaAsO_2$, $Pb(NO_3)_2$, $Cd(NO_3)_2$ and carbendazim were 0.992, 0.965, 0.987 and 0.997, respectively. For $CaCl_2$, *EF* was significantly low (0.598). The mean errors of the models, 0.0035 for $NaAsO_2$ and 0.0042 for $Cd(NO_3)_2$, imply slightly underestimation of *R* by the models. For the other solutes, *ME*s were –0.0023 to –0.0051; these small negative *ME*s indicate minimal overestimation of *R*. *BOE*s of the models were considerably large (3.23 to 74.41%), implying that the MLR models for *R* might miss the appropriate relations between the input parameters and target output (*R*).

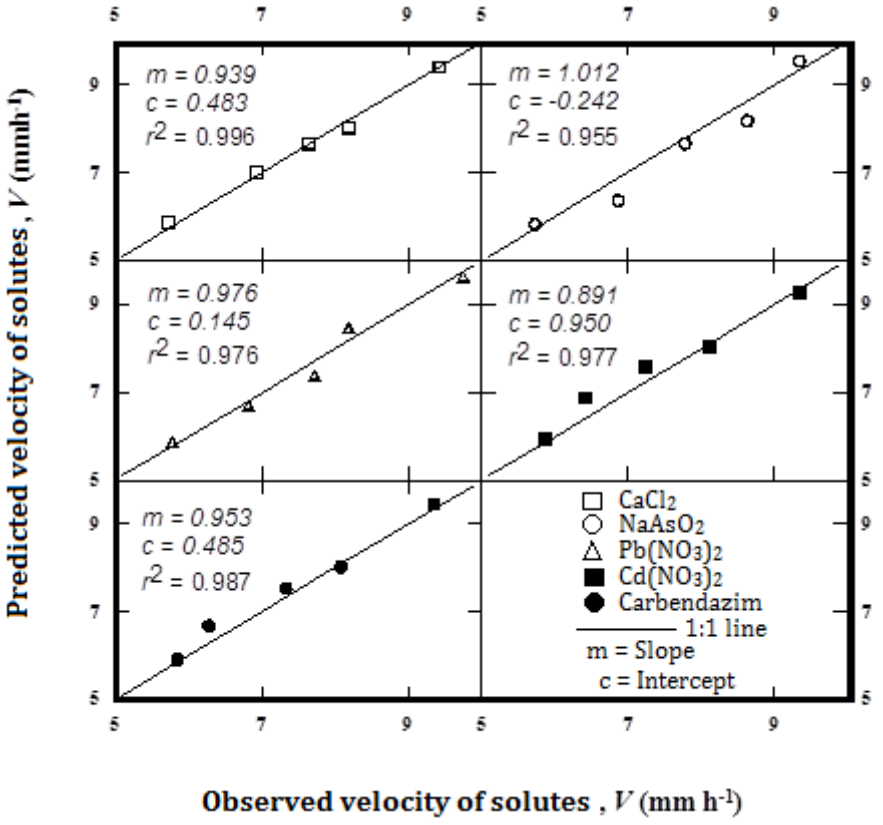


Figure 1. Predicted velocities of $CaCl_2$, $NaAsO_2$, $Pb(NO_3)_2$, $Cd(NO_3)_2$ and carbendazim by MLR models versus their observed velocities.

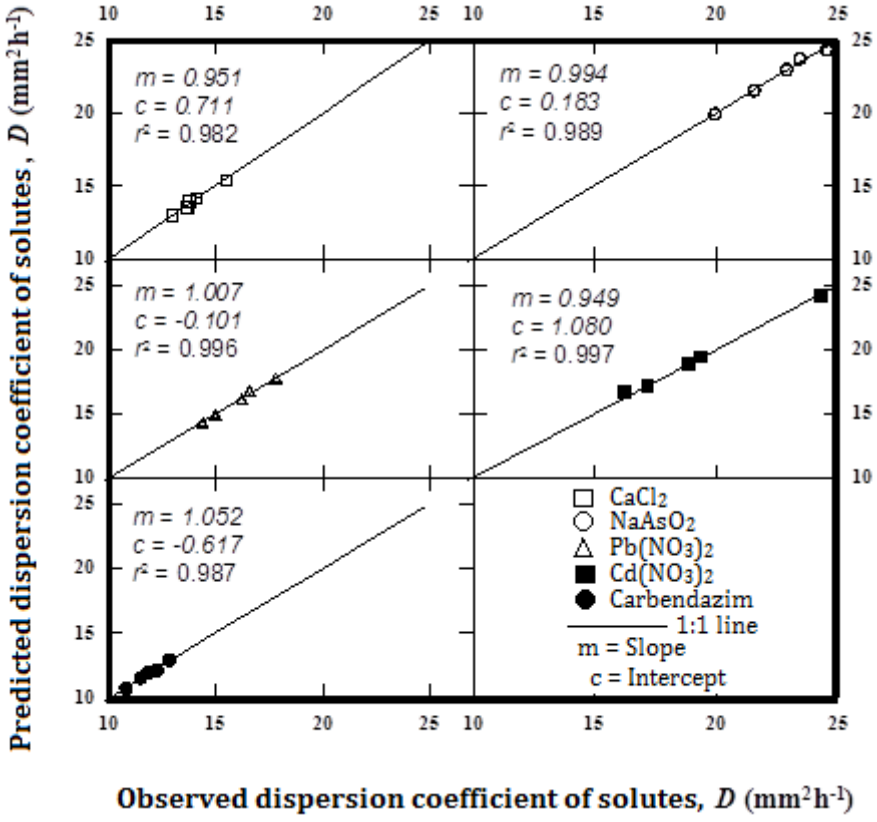


Figure 2. Predicted dispersion coefficients $CaCl_2$, $NaAsO_2$, $Pb(NO_3)_2$, $Cd(NO_3)_2$ and carbendazim by MLR models versus their observed dispersion coefficients.

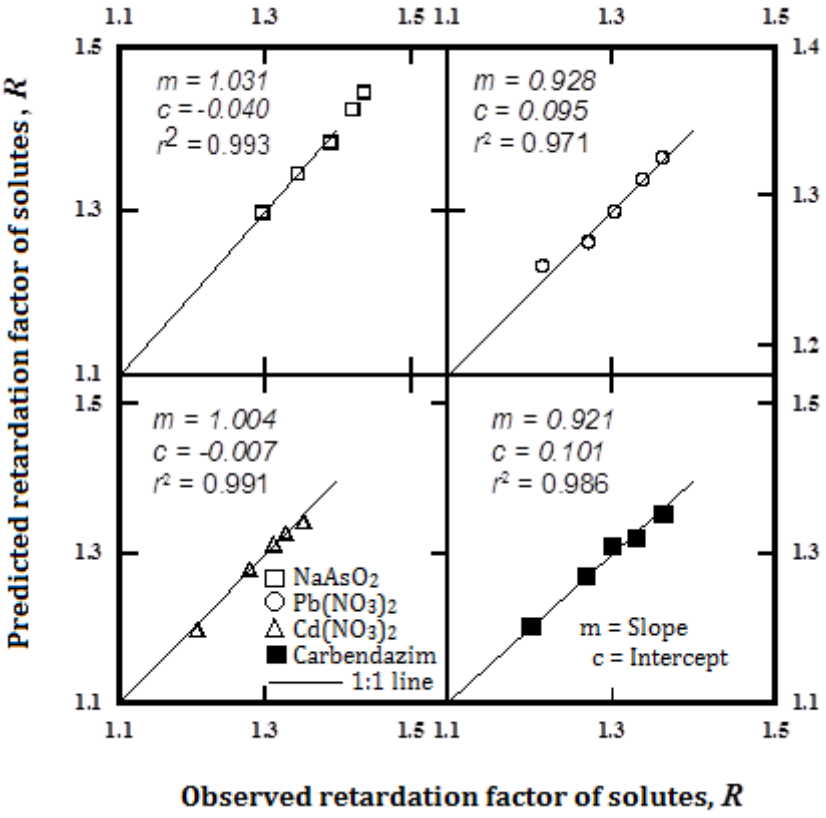


Figure 3. Predicted retardation factors of NaAsO2, $Pb(NO_3)_2$, $Cd(NO_3)_2$ and carbendazim by MLR models versus their retardation factors.

Sensitivity of input parameters

Bulk density, γ, and clay content, *C*, of the soils predominantly governed velocity of the solutes, *V*, in the MLR models, while organic carbon, *OC*, influenced them to a lesser extent. Bulk density ranked as the most impact-generating variable and clay content ranked as the second most influential variable for predicting *V* except for $NaAsO_2$. These two input variables (γ and *C*) were inversely related to *V* as was also observed by Dian-qing et al. (2010). For $NaAsO_2$, γ and *C* followed reversed ranking than for the other solutes. Median grain diameter, D_{50}, ranked as third to influence *V* except for $Cd(NO_3)_2$ for which coefficient of uniformity, C_u, was the third level and D_{50} was the fourth level influential regressors. Organic carbon ranked fifth in controlling the model output except for $CaCl_2$. Uniformity coefficient ranked as the fifth most important variable while *C* ranked fourth in controlling *V* for $CaCl_2$.

Clay ranked as the most prominent input variable in controlling dispersion coefficient, *D*, of $NaAsO_2$ and carbendazim. But, it ranked as the second, third and fourth most influential variable in controlling *D* for $Cd(NO_3)_2$, $Pb(NO_3)_2$ and $CaCl_2$, respectively. Bulk density ranked as the most influencial variable in case of $Pb(NO_3)_2$ and $Cd(NO_3)_2$. It however ranked as the second, third and fourth most influential variable in case of carbendazim, $CaCl_2$ and $NaAsO_2$, respectively. These results were in partial agreement with the findings of Bromly et al. (2007), who, by using step-wise multiple regressions, predicted *D* with pore-water velocity, clay content, silt content and bulk density with an adjusted coefficient of determination of 0.735. Since velocity of the solutes was related to *D*, γ and *C* also influenced *D*. For $CaCl_2$, D_{50} controlled the dispersion coefficient as the most prominent input variable; it ranked third in case of $Cd(NO_3)_2$ and carbendazim, and fourth in case of $Pb(NO_3)_2$. Relative soil pH, exerted second largest impact in predicting *D* for $CaCl_2$ and $Pb(NO_3)_2$, and third largest impact in case of $NaAsO_2$. Uniformity coefficient ranked as the second most dominant variable in predicting *D* for $NaAsO_2$. Organic carbon was less influential since it ranked fourth and fifth in case of carbendazim and $CaCl_2$, respectively.

Bulk density was the most impact-generating variable and exerted positive contribution in predicting retardation factor, *R*, of the reactive solutes under investigation. Clay content was the second most leading variable in controlling *R* of $NaAsO_2$ and $Pb(NO_3)$, and third most important variable in case of $Cd(NO_3)_2$ and carbendazim. For $Cd(NO_3)_2$ and carbendazim, D_{50} put the second largest impact in modeling *R*; it however ranked third and fourth in case of $NaAsO_2$ and $Pb(NO_3)_2$, respectively. Relative pH of the soils ranked third in

case of $Pb(NO_3)_2$, and fourth in case of $NaAsO_2$ and carbendazim; it however exerted only minor influence in modeling *R*. Organic carbon, *OC*, ranked as fifth main variable, also exerted less impact on the retardation factor of $NaAsO_2$ and carbendazim. The low rank of *OC* seems unexpected since it was believed to be the second most dominant factor for sorption after soil pH, and most solutes exhibit high affinities for soil organic matter (Springob and Böttcher, 1998). The low ranking of *OC* could be since most of the 13 soils contained relatively low organic carbon (0.134–0.987%, Table 1), and bulk density and clay content of the soils might dominantly controlled sorption of the solutes. Based on the ratio of *RMSE*s, the orders of sensitivity of the MLR model outputs (*V*, *D* and *R*) to the input variables were – (i) $CaCl_2$: *D*>*V*, (ii) $NaAsO_2$: *R*>*V*>*D*, (iii) Pb $(NO_3)_2$: *R*>*V*>*D*, (iv) Cd $(NO_3)_2$: *V*>*R*>*D* and (v) carbendazim: *R*>*V*>*D*.

Conclusion

Pedo-transfer functions, PTFs, in the form of multiple linear regression, MLR, models were developed for estimating transport velocity, *V*, dispersion coefficient, *D*, and retardation factor, *R*, of $NaAsO_2$, $Pb(NO_3)_2$, $Cd(NO_3)_2$, carbendazim and $CaCl_2$ in 14 Bangladeshi soils. Bulk density and clay content of the soils were the most sensitive/impact-generating input parameters to the MLR models. Based on root-mean-square error, *RMSE*, in estimating the transport parameters, the orders of sensitivity of the model outputs to the input variables for the solutes were – $CaCl_2$: *D*>*V*, $NaAsO_2$: *R*>*V*>*D*, $Pb(NO_3)_2$: *R*>*V*>*D*, $Cd(NO_3)_2$: *V*>*R*>*D* and carbendazim: *R*>*V*>*D*. The *RMSE*, mean error, *ME*, and bias components of overall error, *BOE*, were appreciably small except for the retardation factor, for which *BOE* was considerably large (3.23–74.41%,) that indicated necessity of further improvement of the model. The model efficiencies were noticeably large (0.93–1.00) for the reactive solutes. Thus, the developed MLR models could fairly predict transport velocity, dispersion coefficient and retardation factor of the reactive solutes under investigation, and hence they can be utilized for practical applications at local scales. The MLR models, however, need to be improved for predicting the retardation factor, possibly, by including additional input variable(s). Also, data of only 14 soils were used in this study and the developed MRL models were verified with the data of only one soil. This is a drawback of our study that needs to be addressed in future studies.

Acknowledgement

This study was done under the 'VLIR–Own Initiatives' program with the Bangladesh Agricultural University at Mymensingh. The authors gratefully acknowledge the funding support of the Belgian Directorate General for Development Cooperation (DGDC) through the 'Vlaamse Interuniversitaire Raad' (VLIR; Flemish Interuniversity Council).

References

Achat, D.L., Pousse, N., Nicolas, M., Brédoire, F., Augusto, L., 2016. Soil properties controlling inorganic phosphorus availability: general results from a national forest network and a global compilation of the literature. *Biogeochemistry* 127(2–3): 255–272.

Alibuyog, N.R., 2007. Development of pedotransfer functions for predicting soil hydraulic properties and solute-transport parameters using artificial neural network analysis. Ph.D. Thesis in Agricultural Engineering, University of the Philippines Los Baños, Philippines.

Black, C.A., 1965. Method of soil analysis. Part-I and II. Agronomy No. 9. American Society of Agronomy, Madison, Wisconsin, USA.

Bouma, J., 1989. Using soil survey data for quantitative land evaluation. In: Advances in Soil Science. Springer, New York, NY. pp. 177–213.

Bromly, M., Hinz, C., Aylmore, L.A.G., 2007. Relation of dispersivity to properties of homogeneous saturated repacked soil columns. *European Journal of Soil Science* 58(1): 293–301.

BS 1377, 1990. Methods of Test for Soils for Civil Engineering Purposes. British Standards Institution, London. 2004.

Dian-qing, L.V., Wang, H., Pan, Y., Wang, L., 2010. Effect of bulk density changes on soil solute transport characteristics. *Journal of Natural Science of Hunan Normal University* 33(1): 75–79.

Draper, N.R., Smith, H., 1981. Applied Regression Analysis. 2nd edn. John Wiley and Sons. New York, USA.

Geman, S., Bienenstock, E., Doursat, R., 1992. Neural networks and the bias/variance dilemma. *Neural Computation*, 4(1): 1–58.

Gonçalves, M.C., Leij, F.J., Schaap, M.G., 2001. Pedotransfer functions for solute transport parameters of Portuguese soils. *European Journal of Soil Science* 52(4): 563–574.

Gonçalves, M.C., Pereira, L.S., Leij, F.J., 1997. Pedo-transfer functions for estimating unsaturated hydraulic properties of Portuguese soils. *European Journal of Soil Science* 48(3): 387-400.

Horn, A.L., Reiher, W., Düring, R.A., Gäth, S., 2006. Efficiency of pedotransfer functions describing cadmium sorption in soils. *Water, Air and Soil Pollution* 170(1–4): 229–247.

Jackson, M.L., 1962. Soil Chemical Analysis. Prentice Hall, Inc. Englewood Chiffs, Ny, USA.

Kodešová, R., Kočárek, M., Kodeš, V., Drábek, O., Kozák, J., Hejtmánková, K., 2011. Pesticide adsorption in relation to soil properties and soil type distribution in regional scale. *Journal of Hazardous Materials* 186(1): 540–550.

Moeys, J., Bergheaud, V., Coquet, Y., 2011. Pedotransfer functions for isoproturon sorption on soils and vadose zone materials. *Pest Management Science* 67(10): 1309–1319.

Mojid, M.A., Hossain, A.B.M.Z., Cappuyns, V., Wyseure, G.C.L., 2016. Transport characteristics of heavy metals, metalloids and pesticides through major agricultural soils of Bangladesh as determined by TDR. *Soil Research* 54(8): 970-984.

Mojid, M.A., Hossain, A.B.M.Z., Wyseure, G.C.L., 2018. Relation of reactive solute-transport parameters to basic soil properties. *Eurasian Journal of Soil Science* 7(4): 326–336.

Mojid, M.A., Rose, D.A., Wyseure, G.C.L., 2004. A transfer-function method for analysing breakthrough data in the time domain of the transport process. *European Journal of Soil Science* 55(4): 699–711.

Mojid, M.A., Vereecken, H., 2005. On the physical meaning of retardation factor and velocity of a nonlinearly sorbing solute. *Journal of Hydrology* 302(1-4): 127–136.

Nelson, D.W., Sommers, L.E., 1982. Total carbon, organic carbon, and organic matter. In: Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. Page, A.L, Miller, R.H., Keeney, D.R. (Eds.). 2nd Edition. Agronomy Monograph, vol. 9. ASA and SSSA, Madison, WI, USA. pp. 539-579.

Perfect, E., Sukop, M.C., Haszler, G.R., 2002. Prediction of dispersivity for undisturbed soil columns from water retention parameters. *Soil Science Society of America Journal* 66(3): 696–701.

Phillips, I.R., 2006. Modelling water and chemical transport in large undisturbed soil cores using HYDRUS-2D. *Soil Research* 44(1): 27–34.

Piegorsch, W.W., Bailer, A.J., 2005. Quantitative risk assessment with stimulus-response data. In: Analyzing Environmental Data, Piegorsch, W.W., Bailer, A.J. (Eds.). Chichester, West Sussex, UK. pp. 171–214.

Porro, I., Wierenga, P.J., Hills, R.G., 1993. Solute transport through large uniform and layered soil columns. *Water Resources Research* 29(4): 1321–1330.

Rashid, M.A., 1999. On the linearity of multiple regression model. *Bangladesh Journal of Agricultural Engineering* 10 (1–2): 67–76.

Rose, D.A., Abbas, F., Adey, M.A., 2006. Limitations in the use of electrical conductivity to monitor the behaviour of soil solution. *Soil Research* 44(7): 695–700.

Sarmah, A.K., Close, M.E., Pang, L., Lee, R., Green, S.R., 2005. Field study of pesticide leaching in a Himatangi sand (Manawatu) and a Kiripaka bouldery clay loam (Northland). 2. Simulation using LEACHM, HYDRUS-1D, GLEAMS, and SPASMO models. *Australian Journal of Soil Research* 43(4): 471–489.

Schaap, M.G., Leij, F.J., 1998. Database-related accuracy and uncertainty of pedotransfer functions. *Soil Science* 163(10): 765–779.

Soil Survey Staff, 1975. Soil taxonomy. USDA Agriculture Handbook No. 436. Washington, D.C., U.S. Government Printing Office. p. 754.

Springob, G., Böttcher, J., 1998. Parameterization and regionalization of Cd sorption characteristics of sandy soils. I. Freundlich type parameters. *Journal of Plant Nutrition and Soil Science* 161(6): 689–696.

Touil, S., Degre, A., Chabaca, M.N., 2016. Sensitivity analysis of point and parametric pedotransfer functions for estimating water retention of soils in Algeria. *Soil* 2(4): 647–657.

Van Looy, K., Bouma, J., Herbst, M., Koestel, J., Minasny, B., Mishra, U., Montzka, C., Nemes, A., Pachepsky, Y., Padarian, J., Schaap, M.G., Tóth, B., Verhoef, A., Vanderborght, J., van der Ploeg, M.J., Weihermüller, L., Zacharias, S., Zhang, Y., Vereecken, H., 2017. Pedotransfer functions in Earth system science: challenges and perspectives. *Reviews of Geophysics* 55(4): 1199–1256.

Vereecken, H., 1992. Derivation and validation of pedotransfer functions for soil hydraulic properties. In: Indirect methods for estimating the hydraulic properties of unsaturated soils. van Genuchten, M.T., Leij, F.J., Lund, L.J. (Eds.). University of California, Riverside, CA. pp. 473–488.

Ward, A.L., Elrick, D.E., Kachanoski, R.G., 1994. laboratory measurements of solute transport using time-domain reflectometry. *Soil Science Society of America Journal* 58(4): 1031–1039.

Eurasian J Soil Sci 2019, 8 (3) 208 - 220

Eurasian Journal of Soil Science

Journal homepage : http://ejss.fesss.org

Field suppression of Fusarium wilt and microbial population Shifts in tomato rhizosphere following soil treatment with two selected endophytic bacteria

Rania Aydi Ben Abdallah *, Hayfa Jabnoun-Khiareddine, Ahlem Nefzi, Fakher Ayed, Mejda Daami-Remadi

Regional Research Centre on Horticulture and Organic Agriculture, University of Sousse, Chott-Mariem, Tunisia

Abstract

Two endophytic bacteria, *Bacillus subtilis* SV41 (KR818071) and *B. amyloliquefaciens* subsp. *plantarum* SV65 (KR818073), were assessed under field conditions for their capacity to control tomato Fusarium wilt in tomato and their effects on soil microbial activity. Six months after planting, Fusarium wilt severity, estimated through the vascular browning extent in tomato stems, was significantly reduced by 82.3 and 88.2% compared to control following bacterial treatments. The frequency of *F.oxysporum* re-isolation from roots, collars and stems was also significantly lowered in treated plants compared to controls. These effects were associated with a significant improvement, by 10.6 to 16.3%over control, in plant height and root fresh weight and an increase in fruit production by 8.4-12.5%. As for microbial activity, *F. oxysporum* population in the rhizosphere of tomato plants treated with *B. subtilis* SV41 and *B. amyloliquefaciens* subsp. *plantarum* SV65 was reduced by 87.5-91.7%compared to the initial soil (sampled before planting) and by 88.4-92.3% relative to the rhizospheric soil of untreated plants (control soil). A significant enhancement in the total culturable bacterial community was also noted in the rhizosphere of tomato plants treated with both strains compared to initial and control soils where a significant enrichment in *Pseudomonas* and actinobacteria community was recorded.

Keywords: Endophytic bacteria, Fusarium wilt, growth-promoting, microbial community, soil, tomato.

Article Info

Received : 01.10.2018
Accepted : 16.04.2019

Introduction

Plants are naturally associated with microbes in various ways (Vijayabharathi et al., 2016). To face biotic and abiotic stresses, plants interact with different members of soil biodiversity and especially microbial community. These relations involve positive and negative feedbacks between soil microbial agents, plants, and their chemical environment (Meena and Meena, 2017). Agriculturally important microorganisms can affect the efficiency of nutrient availability to grown plants and soil biodiversity, and can also regulate the interactions between plants and pathogenic microflora (Zeilinager et al., 2016). Interactions among plants and microbes can influence soil physiochemical, biochemical and microbiological properties (Dubey et al., 2016). In fact, beneficial microbial communities or microbial inoculants can offer various positive services for plants and soils including plant growth-promotion, nutrient efficiency, bioremediation, and suppression of bio-aggressors. A profound understanding of the environmental factors influencing the viability and performance of these microbial inoculants is essential of their large-scale use in sustainable agriculture production systems (Meena and Meena, 2017).

* Corresponding author.
UR13AGR09-Integrated Horticultural Production in the Tunisian Centre-East, Regional Research Centre on Horticulture and Organic Agriculture, University of Sousse, 4042, Chott-Mariem, Tunisia
Tel.: +216 96325412
E-mail address: raniaaydi@yahoo.fr
e-ISSN: 2147-4249
DOI: 10.18393/ejss.556780

Due to the increased need to reduce use of chemical fertilizers and pesticides, for sustainable agriculture and environment protection, there was a growing interest in searching for beneficial microorganisms (Figueiredo et al., 2016). Among the explored microorganisms, bacteria occurring in the rhizosphere soil, rhizoplane, and/or the internal plant tissues were widely used (Hallmann et al., 1997). Their beneficial effects include, among others, efficient systems for uptake and catabolism of organic compounds present in root exudates (Barraquio et al., 1997). Several bacteria may also help to derive maximum benefits from root exudates through their capacity to attach to root surface (rhizoplane) (Compant et al., 2005). Endophytic bacteria are able to grow inside plant tissues and to protect them from biotic and biotic stresses (Sharma and Nowak, 1998). They can stimulate plant growth via direct and/or indirect mechanisms. Directly, they act by providing essential nutrients, producing phytohormones and growth regulators, or by regulating phytohormone levels. Indirect plant growth promotion (PGP) may be achieved by suppressing pathogens and/or inactivating pollutants responsible for plant growth inhibition (Vijayabharathi et al., 2016).

Beneficial agents may adversely affect population, density, dynamics and metabolic activities of soilborne pathogens through the production of hydrolytic enzymes and antimicrobial secondary metabolites and/or through the competition for nutrients (Swarupa et al., 2016). Several microbial agents such as *Trichoderma* and *Bacillus* are able to suppress fungal inoculum in soils by producing antibiotics (Larkin and Tavantzis, 2013; Bernard et al., 2014).

The soil microbial community is thought to be responsible for biological processes that are necessary for maintaining a healthy soil and suppressing plant diseases (Mazzola, 2004). The biocontrol of soilborne diseases can be achieved by manipulating the rhizosphere microflora in favor of beneficial microorganisms acting directly against soil pathogens (Yang et al., 2001). There is a considerable interest in monitoring changes in activity and composition of soil microbial communities following the application of microbial inoculants. In fact, beneficial bacteria or fungi such as *Trichoderma*, *Hypoxylon*, *Tritirachium*, *Paenibacillus*, *Bacillus*, *Haliangium* and *Streptomyces* were more abundant in biologically treated soils as compared to control whereas *Fusarium* inoculum was markedly decreased (Qiu et al., 2012).

Two endophytic bacteria *B. subtilis* SV41 and *B. amyloliquefaciens* subsp. *plantarum* SV65 originally recovered from *Datura metel* and *Solanum nigrum*, respectively, were tested in this study under field conditions. They were previously (pot experiment) selected based on their ability to suppress Fusarium wilt and to promote tomato growth using their whole cells or their cell-free culture filtrates.

This study aimed to check, under field conditions, the ability of *B. subtilis* SV41 and *B. amyloliquefaciens* subsp. *plantarum* SV65 to enhance growth and production of tomato plants growing in soils naturally infested with *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and to suppress wilt severity. Their subsequent effects on pathogen inoculum and soil microbial community were also investigated.

Material and Methods

Plant material

Tomato cv. Sahel was used in this study. This cultivar is known to be resistant to FOL races 1 and 2 and susceptible to race 3 (Syngenta, 2015). Seedlings were grown under greenhouse conditions (16 h photoperiod, 60-70% relative humidity and 20-30°C air temperature) in alveolus plates (3×3 cm) filled with sterilized peat® (Floragard Vertriebs GmbH für gartenbau, Oldenburg) . They were watered regularly until reaching the two-true-leaf growth stage. Seedlings with approximately similar heights were used in all trials.

Bacterial material

Two endophytic bacterial strains namely *Bacillus subtilis* SV41 (Accession number KR818071) and *Bacillus amyloliquefaciens* subsp. *plantarum* SV65 (KR818073), originally recovered from the internal stem tissues of *Datura metel* and *Solanum nigrum*, respectively, were used in the current investigation.

Isolation procedure, characterization and identification using 16S rDNA sequencing genes were previously described in Aydi Ben Abdallah et al. (2015). These two strains were previously selected based on their higher efficiency, when used as cell suspensions or cell-free culture filtrates, in suppressing Fusarium wilt disease on tomato plants inoculated with FOL. Indeed, both *Bacillus* strains were harbor chitinase gene in their genome and *B. amyloliquefaciens* subsp. *plantarum* SV65 was able to produce the lipopetide antibiotic, *fengycin D*, which was detected in its genome. Also, the plant defense genes such as *PR1*, acidic *PR3* and lipoxygenase (*LOXD*) were expressed in tomato plants inoculated or not with FOL and treated with *B. amyloliquefaciens subsp. plantarum* SV65 (Aydi Ben Abdallah et al., 2017). Furthermore, the plant growth-promoting traits such as the production of indole-3-acetic acid, siderophores and organic acids in root exudates and the phosphate solubilization ability were recorded on both selected strains (unpublished data). Their plant growth-promoting properties and antagonististic mechanisms are detailed in Table 1.

Table 1. Plant growth-promoting (PGP) traits, antagonistic and inducing systemic resistance (ISR) properties of Bacillus subtilis SV41 and Bacillus amyloliquefaciens subsp. plantarum SV65 recovered from Datura metel and Solanum nigrum stems, respectively.

Strain	PGP traits (Unpublished data)					Antifungal and ISR properties (Aydi Ben Abdallah et al., 2017)						
	IAAa	Phosb	Ox, Mal acidsc	Sdd	Pece	Chitf	Protg	FenDh	SAi	LOXDj	CHI3-R3k	PR1l
B. subtilis SV41	+	-	+	+	+	+	+	-	+	-	-	-
B. amyloliquefaciens subsp. *plantarum* SV65	+	+	+	+	+	+	+	+	+	+	+	+

[a] IAA: Indole-3-acetic acid production after 48 h of incubation at 28 ± 2°C in Luria-Broth medium; +: Production of IAA.
[b] Phosphatase activity: Tested on Pikovskaya agar medium and incubated at 28 ± 2°C for 7 days; +: Presence of clear zone, -: Absence of clear zone.
[c] Organic acids (oxalic and malic acids) production detected in root exudates which are collected after 14 days from tomato plants cultivated in Hoagland solution (50 %).
[d] Siderophore production: Tested on Chrome Azurol Sulphonate (CAS) agar medium and incubated at 28 ± 2 °C for 5 days; +: Presence of zone of siderophore activity (yellow color).
[e] Pectinolytic activity:Tested on pectin-agar (0.5% w v-1) medium and incubated at 28 ± 2°C for 48 h; +: Presence of clear zone. The Polygalacturonic acid was measured at 540 nm.
[f] Chitinase activity:Tested on chitin-agar (0.5 % w v-1) medium and incubated at 28 ± 2°C for 72 h; +: Presence of clear zone; Detection of ChiA gene by PCR using 5'-TTCAYGTTCAACACTACAA-3 ' and 5'-CATTAAGGCCGCGGARTG-3' primers for B. subtilis SV41 and 5'-GATATCGACTGGGAGTTCCC-3 'and 5'-CATAGAAGTCGTAGGTCATC-3' primers for B. amyloliquefaciens subsp. plantarum SV65.
[g] Protease activity: Tested on skim milk agar (3% v v-1) medium and incubated at 28 ± 2°C for 48 h; +: Presence of clear zone.
[h] Detection of fengycin D (FenD) gene by PCR using 5'-TTTGGCAGCAGGAGAAGTTT-3' and 5'-GCTGTCCGTTCTGCTTTTTC-3'primers.
[i] Salicylic acid production after 48 h incubation at 28 ± 2°C in succinate medium; +: Production of salicylic acid.
[j] Relative expression of lipoxygenase (LOXD) gene in uninoculated tomato plants with F. oxysporum f. sp. lycopersici (FOL) using 5'-CCTGAAATCTATGGCCCTCA-3' and 5'-ATGGGCTTAAGTGTGCCAAC-3' primers for quantitative RT-PCR.
[k] Relative expression ofacidic chitinase (CHI3-PR3) gene in uninoculated tomato plants with FOL using 5'-TGCAGGAACATTCACTGGAG-3' and 5'-TAACGTTGTGGCATGATGGT-3' primers for quantitative RT-PCR.
[l] Relative expression ofPR1 gene into inoculated tomato plants with FOL using 5'-TCTTGTGAGGCCCAAAATTC-3' and 5'-ATAGTCTGGCCTCTCGGACA-3'primers for quantitative RT-PCR.

Before being used in the different bioassays, bacterial cultures were initiated from stock maintained at -20°C in Nutrient Broth (NB) supplemented with 40% glycerol and grown for 48 h at 25°C on Nutrient Agar (NA) medium.

Test of the effect of *Bacillus* spp. strains on tomato growth and Fusarium wilt severity

Bacterial treatments were prepared from cultures previously grown in NB medium for 3 days at 28 ± 2°C and under continuous stirring at 150 rpm. They were firstly applied to tomato cv. Sahel seedlings by drenching the substrate of each alveolus (3 cm in diameter) with 5 ml of a bacterial cell suspension (10^8 cells ml^{-1}). Treated and untreated tomato seedlings were transplanted into rows with a distance of 33 cm between seedlings. Planting was carried out on December 2015 under greenhouse installed in the experimental station of Teboulba, Monastir, Tunisia (N35°38'38,256'', E10°56'48,458''). Planting soil was known to be historically infected with FOL.

A second bacterial treatment was applied at planting, one week after the first treatment, by drenching the rhizospheric soil of each seedling with 100 ml of a cell suspension (10^8 cells ml^{-1}). Three treatments were tested: (i) Untreated control seedlings, (ii) seedlings treated with *B. subtilis* SV41, and (iii) seedlings treated with *B. amyloliquefaciens* subsp. *plantarum* SV65.

Two replicates of twenty seedlings each were used for each individual treatment. Tomato seedlings were grown for about six months at 24-25°C with 16/8 h photoperiod and 70% air relative humidity. They were subjected to agricultural practices commonly adopted by farmers in the region and irrigated and fertilized as needed.

Disease severity, growth and production parameters

Six months after planting, the parameters noted were the vascular browning extent (from the collar), plant height, roots fresh weight, and fruits fresh weight per plant. The frequency of colonization of roots, collars and stems of tomato plants by pathogen was determined after isolation of ten fragments per organ on PDA medium amended with 300mgl^{-1} of streptomycin sulphate. For the latest parameter and for each organ, three replicates of one plate each were used for every individual treatment. Cultures were incubated at 25°C for 4 days.

The percentage of colonization of plant tissues by various microorganisms was calculated using the formula of Moretti et al. (2008). The percentage of re-isolation from stems is the average of five counts recorded at different stem levels i.e. (0 to 5 cm), (5 to 10 cm), (10 to 15 cm), (15 to 20 cm) and (20 to 25 cm). The averages vascular browning extent, plant height, roots fresh weight were estimated from thirty plants. The average weight of fruits produced per plant was determined by calculating the total weight of fruits harvested between April and June 2016 and dividing by the total number of plants (40 plants).

Initial soil sampling and processing

Composite soil samples from each individual plot were collected twice i.e. just before planting and after the last harvest. Uprooted soil samples collected after the last harvest were removed from: (i) the rhizophere of untreated control plants, (ii) the rhizosphere of plants treated with *B. subtilis* SV41 and (iii) the rhizosphere of plants challenged with *B. amyloliquefaciens* subsp. *plantarum* SV65. At each sampling date, twenty soil cores (7cm in diameter × 15 cm in depth) collected from each treatment were combined to make one composite soil per individual treatment. Two replicates were considered for each soil sampling. Once brought to laboratory, soil samples were passed through a 2-mm sieve to remove rocks and large organic debris. They were stored in plastic bags at 10°C and processed within 1 to 4 weeks after sampling (Larkin and Honeycutt, 2006). For further assays, two subsamples were processed from each composite soil sample.

Determination of *Fusarium oxysporum* population

To confirm the presence of *F. oxysporum* in the soil and to evaluate the effect of tested bacterial treatments on the soil infectious potential, 5 g of each soil sample were placed in an Erlenmeyer flask containing 100 ml of sterile distilled water (SDW) and subjected to continuous stirring at 150 rpm for 60 min. The supernatant was filtered through a double layer of muslin followed by a series of dilutions using SDW (Daami-Remadi et al., 2009). For each sample, 200 µl of 10^{-2} dilution were plated onto selective peptone-pentachloro-nitrobenzen (PCNB) agar medium specific for *Fusarium* spp. isolation (Nash and Snyder, 1962). Plates were stirred gently by hand for homogeneous distribution of the soil extract with the culture medium. Three replicates of one Petri plate each were used for each soil sample. After incubation at 25°C for 12 days, *F. oxysporum* colonies growing on PCNB agar medium were morphologically and microscopically identified and the total number of colonies was counted.

Determination of soil properties

Soil samples were air-dried before use. Soil extracts were prepared by suspending soil in distilled water in 1:10 soil/dH_2O ratio. They were filtered through Whatman paper No 1 and analyzed for determination of their pH and conductivity using a glass electrode and conductivity meter, respectively. Water content was checked by removing 5 g of soil and weighting it before (wet weight) and after oven drying at 105°C for 24h (Larkin et al., 2006). Organic carbon was determined by drying 10 g of soil at 105°C overnight and then at 900°C for 2 h. The percentage of organic matter was calculated using the following formula:

Organic matter = (dry matter – mineralized matter) × 100 / dry matter and the percentage of organic carbon was estimated using the following formula: %Organic carbon = %Organic matter / 1.72 (Kettler et al., 2001).

Estimation of soil microbial community

General populations of culturable soil microorganisms were determined by soil dilution plating on various agar media according to Larkin and Honeycutt (2006) with some modifications. For each subsample taken from each composite soil, 10 g were added to 90 ml of sterile 0.2% water agar, vigorously stirred for 30 min, serially diluted and a-100 µl sample wasplated on 10% Tryptic Soy Agar (TSA) for total bacterial counts, selective King's B medium (KB) amended with 75 mg l^{-1} of penicillin and 75 mg l^{-1} of cyclohexamide for *Pseudomonas fluorescens* counts, Yeast Malt Agar (ISP medium No. 2) amended with 75 mg l^{-1} of nalidixic acid and 100mg l^{-1} of cyclohexamide for actinomycete counts,and Potato Dextrose Agar (PDA) amended with 300 mg $l^{-1}$of streptomycine sulphate for total fungal counts.Four replicates of one plate each were used for each soil subsample.

Bacterial and actinomycete plates were incubated at 28°C for 2 and 14 days, respectively, and fungal plates were maintained at 25°C for 7 days before counting growing colonies. Colonies of *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. were identified based on their macro- and micro-morphological traits under light microscope and counted separately.

Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) software for Windows version 16.0. Data were analyzed according to a completely randomized design. Means were separated using Duncan Multiple Range tests to identify significant pair-wise differences at $P \leq 0.05$.

Results

Effects of tested bacterial treatments on Fusarium wilt severity and tomato growth

Six months after planting, a significant decrease (at $P \leq 0.05$) in Fusarium wilt severity was achieved following tested bacterial treatments as compared to control,. Indeed, the vascular browning extent was reduced by 82.3 and 88.2% by *B. subtilis* SV41 and *B. amyloliquefaciens* subsp. *plantarum* SV65 treatments, respectively, compared to the untreated control (Figure 1).

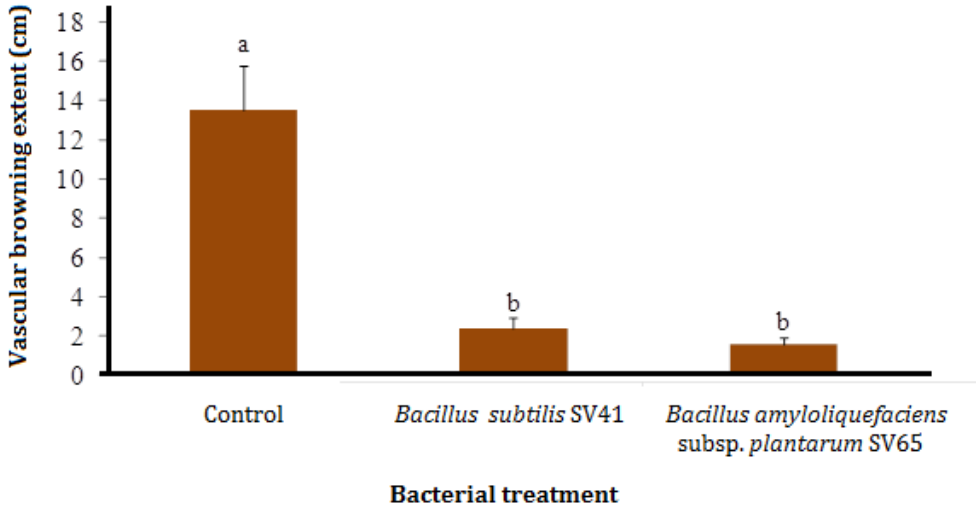


Figure 1. Effect of Bacillus subtilis SV41 and Bacillus amyloliquefaciens subsp. plantarum SV65 on Fusarium wilt severity on tomato plants cv. Sahel grown under greenhouse for six months.
Results are presented as mean ± SE (n=30, P≤ 0.05). Bars sharing the same letter are not significantly different according to Duncan Multiple Range test at P≤ 0.05.

The percentage of re-isolation of *F. oxysporum* from roots, collars and stems of plants treated with *B. amyloliquefaciens* subsp. *plantarum* SV65 and *B. subtilis* SV41 was limited to 2.6-46.6% and 10-53.3%, respectively, compared to the untreated control where the re-isolation frequency of *F. oxysporum* varied from 63.3 to 80% (Figure 2).

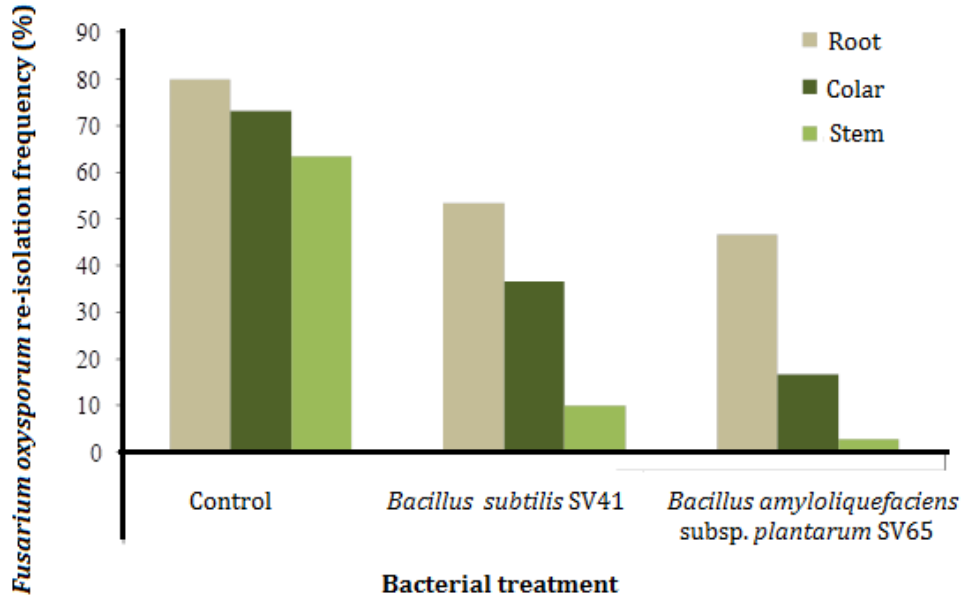


Figure 2. Effect of Bacillus subtilis SV41 and Bacillus amyloliquefaciens subsp. plantarum SV65 on Fusariumoxysporum re-isolation frequency from roots, collars and stems of tomato plants cv. Sahel grown under greenhouse for six months.

The values presented are the percentages (%) of re-isolation of F. oxysporum on Potato Dextrose Agar (PDA) medium after incubation at 25°C for 4 days. Ten fragments per organ were cultured on plates containing PDA medium supplemented with streptomycin sulfate (300 mg l-1). Each individual treatment was repeated three times (3 × 10 fragments). The percentage of re-isolation from stems is the average of five counts recorded at different stem levels i.e. (0 to 5 cm), (5 to 10 cm), (10 to 15 cm), (15 to 20 cm) and (20 to 25 cm).

As given in Table 2, growth and production parameters of tomato plants cv. Sahel (plant height, root fresh weight and fruit production per plant), noted six months after planting, varied significantly (at $P \leq 0.05$) depending on tested bacterial treatments.

Concerning plant height, a significant improvement of this parameter, of about 10.6% over control, was achieved using *B. amyloliquefaciens* subsp. *plantarum* SV65 based treatment (Table 2). The root fresh weight was significantly increased by 15.6 and 16.3% versus control in tomato plants treated with *B. amyloliquefaciens* subsp. *plantarum* SV65 and *B. subtilis* SV41, respectively. As shown in Table 2, *B. amyloliquefaciens* subsp. *plantarum* SV65 and *B. subtilis* SV41 significantly improved the fruit production by 8.4 and 12.5% compared to the untreated control where the greatest enhancement, of about 12.5% over control, was recorded in *B. subtilis* SV41 treated plants.

Table 2. Effect of two *Bacillus* spp. strains on the growth of tomato plants cv. Sahel grown under greenhouse[x] for six months.

Bacterial treatment	Plant height (cm)[a]	Root fresh weight (g)[b]	Fruit weight (g)[c]
Control	321.7 b ± 68	51.5 b ± 1.86	1622 c ± 0.73
B. subtilis SV41	326.5 b ± 67	61.5 a ± 3.15	1854 a ± 0.83
B. amyloliquefaciens subsp. *plantarum* SV65	359.17 a ± 51	61.03 a ± 12	1770 b ± 0.8

For each column, the values followed by the same letter are not significantly different according to Duncan Multiple Range test at $P \leq 0.05$; ± SE: Standard error.

[a,b] Results are noted at harvest. The values presented are the averages obtained from 30 plants for each treatment. The broken plants were eliminated from the statistical analysis.

[c] Fruit weight was expressed per plant. For each treatment, this parameter was determined by calculating the sum of the weights of all fruits harvested between April and June 2016 divided by the total number of plants (40 plants).

[x] The greenhouse is located in the experimental station at Teboulba (N35 ° 38'38.256 '', E10 ° 56'48.458 '', Monastir), The Regional Research Centre on Horticulture and Organic Agriculture of Chott-Mariem, Tunisia.

Effects of tested bacterial treatments on *Fusarium oxysporum* population

ANOVA analysis performed for the number of *F. oxysporum* colonies revealed a significant variation (at $P \leq 0.05$) in this parameter depending on sampled soils. As given in Figure3, *F. oxysporum* colonies were reduced by 87.5 and 91.7% in soils sampled from the rhizosphere of tomato plants cv. Sahel treated with *B. subtilis* SV41 and *B. amyloliquefaciens* subsp. *plantarum* SV65, respectively, as compared to those sampled before planting (initial soil state). *F. oxysporum* population decreased by 88.4 and 92.3% in tomato rhizospheric soils, compared to controlsoils, following their treatment with *B. subtilis* SV41 and *B. amyloliquefaciens* subsp. *plantarum* SV65, respectively.

Effect of tested bacterial treatments on soil properties

All collected soil samples were sandy loam. The application of bacterial treatments did not induce significant changes in soil pH which ranged between 7.3 and 7.7. However, the conductivity decreased by 26.7 and 42.1%in soils treated with *B. amyloliquefaciens* subsp. *plantarum* SV65 and *B. subtilis* SV41 compared to control and by 23.7 and 39.7% relative to the initial state, respectively (Table 3).

Table 3. Properties of sampled soils [a]

Soil sample	Sampling date	Texture	pH	EC (dS/m)	Water content (%)	Organic matter (%)	Organic carbon (%)
Initial state	December 2015	Sandy loam	7.3	0.228	15	3.16	1.83
Control	June 2016	Sandy loam	7.7	0.237	15.5	2.74	1.59
SV41-treated	June 2016	Sandy loam	7.6	0.137	15.5	3.38	1.96
SV65-treated	June 2016	Sandy loam	7.4	0.174	13.5	3.66	2.13

[a] Soil sampled from the greenhouse located in the experimental station at Teboulba (N35 ° 38'38.256 '', E10 ° 56'48.458 '', Monastir), The Regional Research Centre on Horticulture and Organic Agriculture of Chott-Mariem, Tunisia.

Initial state: Soil sampled one week before planting. Control: Soil sampled from the rhizosphere of untreated tomato plants six months after planting.SV41: Soil sampled from the rhizosphere of tomato plants treated with *Bacillus subtilis* SV41. SV65: Soil sampled from the rhizosphere of tomato plants treated with *B. amyloliquefaciens* subsp. *plantarum* SV65.

The percentage of water content varied from 13.5 to 15.5% in all soil samples. A slight increment of about 23.4 and 33.7%i n the percentage of organic matter was noted in the soil removed around roots of tomato plants treated with *B. subtilis* SV41 and *B. amyloliquefaciens* subsp. *plantarum* SV65 compared to control soil sampled on June 2016 and of about 6.9 and 15.8%relative to the initial soil state sampled on December 2015, respectively. Additionally, the percentage of organic carbon varied from 1.96 to 2.13% in soil treated separately with *B. subtilis* SV41 and *B. amyloliquefaciens* subsp. *plantarum* SV65 compared to 1.59 and 1.83% estimated on control and initial soil samples, respectively (Table 3).

Effects of tested bacterial treatments on soil microbial community

Analysis of variance revealed that the number of bacterial colonies growing from plated soil samples, after 2 days of incubation in TSA medium, varied significantly (at $P \leq 0.05$) depending on tested treatments. Soil samples treated with *B. subtilis* SV41 and *B. amyloliquefaciens* subsp. *plantarum* SV65 showed significant increase in their bacterial populations of about 78.57 10^6 and 58.12 10^6 CFU g^{-1} soil, respectively, as compared to the initial soil state (before planting) estimated at 16.75 10^6CFUg^{-1}. A significant enhancement, of about 49.9%, in the total culturable bacterial community was recorded in the tomato rhizospheric soil treated with *B. subtilis* SV41 compared to control (Figure 4A).

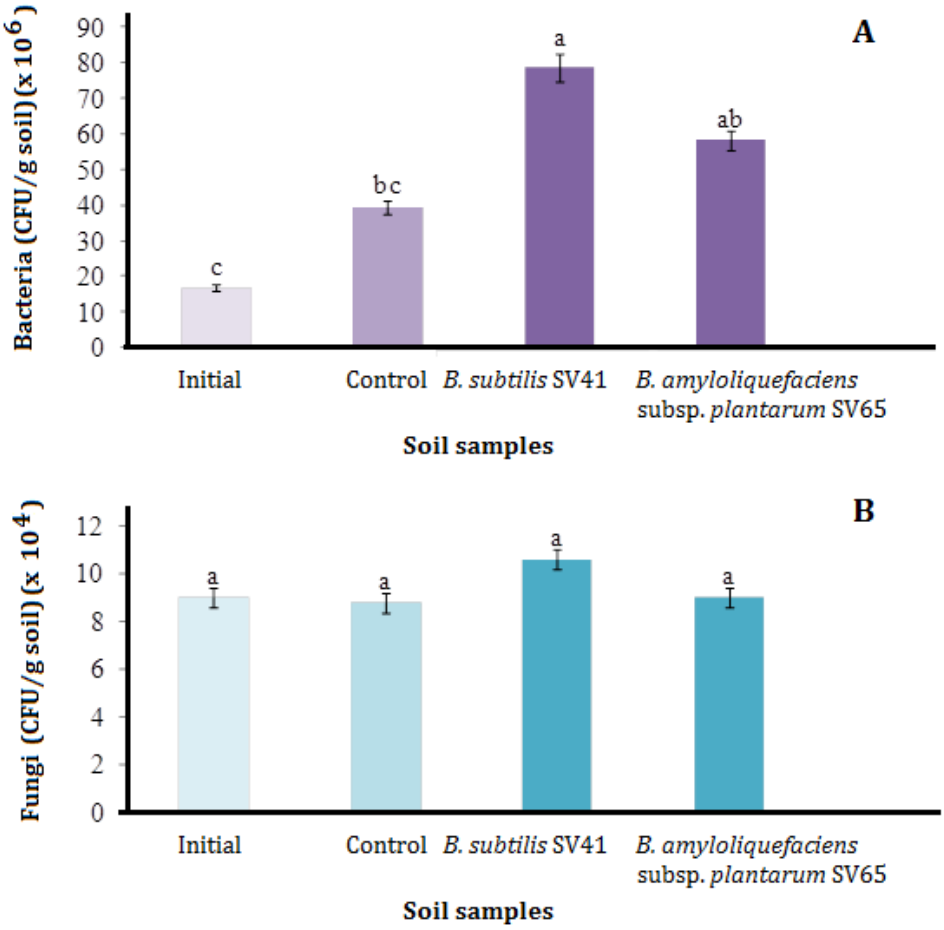


Figure 4.Effect of bacterial treatment on soil microbial population sampled from the rhizosphere of tomato plants grown under greenhouse[x] for six months.

Results are presented as mean ± SE (n = 8, $P \leq 0.05$). Bars sharing the same letter are not significantly different according to Duncan Multiple Range test at $p \leq 0.05$. Dilution was made from a concentration of 10% (w v^{-1}).Initial: Soil sampled at pre-plant (before planting). Control: Soil sampled from the rhizosphere of untreated tomato plants at the end of the trial (six months after planting). [x] The greenhouse is located in the experimental station at Teboulba (N35 ° 38'38.256 '', E10 ° 56'48.458 '', Monastir), The Regional Research Centre on Horticulture and Organic Agriculture of Chott-Mariem, Tunisia.

As for the bacterial biodiversity, the relative abundance of *Pseudomonas* spp. community was found to be relatively important in the rhizosphere of tomato plants treated with the two bacterial strains and in the untreated ones as compared to *Pseudomonas* population determined in the initial soil state (Figure 5). Indeed, both bacterial treatments significantly enriched *Pseudomonas* community in treated soils by 57.5-66% compared to the control soils and by 92.8-94.2% versus the initial soil state. Furthermore, *P. fluorescens* colonies were three times higher in the *B. amyloliquefaciens* subsp. *plantarum* SV65-treated soil than in control soil and 11.2 times more abundant than in theinitial state. Soil treated with *B. subtilis* SV41 showed significant increase in *P. fluorescens* population which was nine times higher than that ofthe initial soil (Table 4). As shown Figure5, in each soil sample, *Actinomycetes* population was lesser when compared to the remaining bacterial groups. Indeed, Actinobacteria population significantly increased by 52 and 56.2%, compared to the initial state, in soils treated with both bacterial strains. Furthermore, *B. subtilis* SV41-treated soil showed significant increment in the total of culturable Actinomycetes of about 44.9% compared to control (Table 4).

Table 4. Soil populations of selected subgroups of microorganisms isolated from the rhizosphere of tomato cv. Sahel plants treated separately with *Bacillus subtilis* SV41 and *Bacillus amyloliquefaciens* subsp. *plantarum* SV65 compared to the untreated and to the pre-plant soil samples, as determined by soil dilution plating on selective media.

Soil sample	Microorganism group (CFU g^{-1} soil)[x]					
	Actinomycetes ($\times 10^3$)	*Pseudomonas* ($\times 10^5$)	*P.fluorescens* ($\times 10^5$)	*Aspergillus* spp. ($\times 10^3$)	*Penicillium* spp. ($\times 10^3$)	*Fusarium* spp. ($\times 10^3$)
Initial state	15.00 c	15.00 b	5.00 c	13.75 b	0 b	25.00 a
Control	18.88 bc	88.75 b	18.75 bc	16.66 ab	0 b	26.66 a
SV41-treated	34.28 a	261.25 a	45.00 ab	27.50 a	6.25 a	7.50 b
SV65-treated	31.25 ab	208.75 a	56.25 a	21.25 ab	1.25 ab	2.50 b

[x] Soil samples were collected in December 2015 (before planting) and June 2016 (harvest) from the greenhouse located in the experimental station at Teboulba (N35 ° 38'38.256 '', E10 ° 56'48.458 '', Monastir), The Regional Research Centre on Horticulture and Organic Agriculture of Chott-Mariem, Tunisia.

Values following with the same letter are not significantly different according to Duncan Multiple Range test at $P \leq 0.05$. Dilution was made from a concentration of 10% (w v^{-1}). Initial: Soil sampled at pre-plant (before planting). Control: Soil sampled from the rhizosphere of untreated tomato plants at the end of the trial (six months after planting).B1: Soil sampled from the rhizosphere of tomato treated with *Bacillus subtilis* SV41. B2: Soil sampled from the rhizosphere of tomato treated with *B. amyloliquefaciens* subsp. *plantarum* SV65.

As for fungal biodiversity, there was a slight increase in the total culturable fungi, growing after 7 days of incubation in PDA medium, following bacterial treatments compared to control and initial soil samples (Figure 4B). As given in Table 3, *Aspergillus* spp. and *Penicillium* spp. colonies were more abundant in the rhizosphere of *B. subtilis* SV41-treated plants compared to the initial (before tomato planting) and control soil samples. However, a significant decrease by 71.9 and 90.6% compared to control soil and by 70 and 90% relative to the initial state, was noted in *Fusarium* spp. populations in soils treated with *B. subtilis* SV41 and *B. amyloliquefaciens* subsp. *plantarum* SV65, respectively (Table 4).

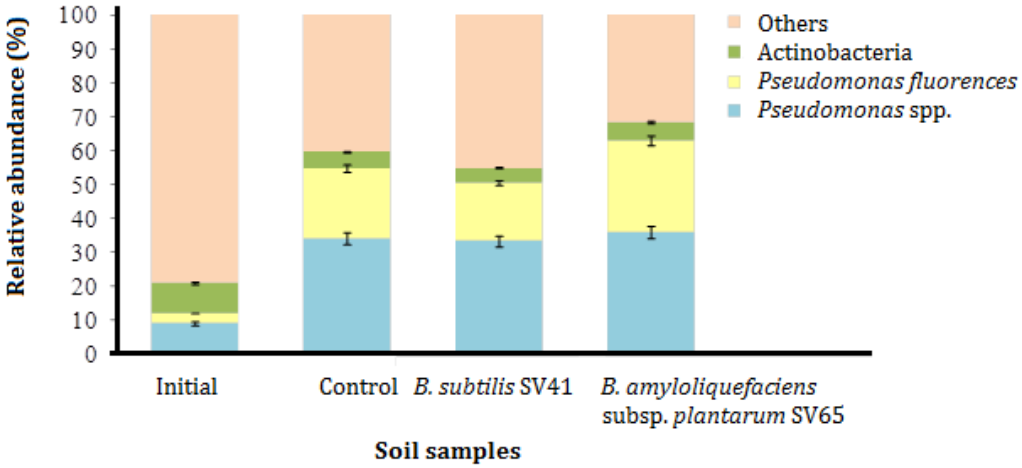


Figure 5. The bacterial community structure under bacterial soil treatment per g soil.
Results are presented as mean ± SE (n = 8, $P \leq 0.05$). The relative abundance was estimated per the total bacteria counted in each sampled soil. Dilution was made from a concentration of 10% (w v^{-1}). Initial: Soil sampled at pre-plant (before planting). Control: Soil sampled from the rhizosphere of untreated tomato plants at the end of the trial (six months after planting).

Discussion

A better understanding of what makes a plant-microbe interaction detrimental or beneficial to plants would provide an important insight into the efficient handling of microbes for agriculture production (Swarupa et al., 2016). In this study, two endophytic bacteria, *B. subtilis* SV41 and *B. amyloliquefaciens* subsp. *plantarum* SV65, were assessed for their ability to suppress Fusarium wilt disease symptoms, to enhance tomato growth and production and to reduce pathogen inoculums in soil. The effect of both bacteria on soil microbial populations was also estimated.

The exploration of endophytic bacteria as a potentially interesting and environmentally friendly alternative for the management of tomato Fusarium wilt disease has been shown to be effective in inhibiting systemic progression of the causative agent (Ramyabharathi and Raguchander, 2014). In this study, *B. subtilis* SV41 and *B. amyloliquefaciens* subsp. *plantarum* SV65 successfully reduced Fusarium wilt severity by 82-88% and limited the pathogen colonization of tomato stems compared to control. Numerous plant pathogenic *F. oxysporum* isolates were successfully controlled by *Bacillus* sp. recovered from chickpea plants (Landa et al., 1997). Disease suppressive effects were also expressed by two unidentified endophytic bacteria, recovered

from healthy wild and cultivated young oilseed rape plants, which reduced Fusarium wilt severity by 75% on tomato plants (Nejad and Johnson, 2000). Kalai-Grami et al. (2014) found that endophytic *B. mojavensis* recovered from citrus plants reduced disease severity in maize plants inoculated with *Fusarium verticillioides*. In addition, most *Bacillus* species were able to inhibit the mycelial growth of *F. oxysporumin vitro* (Idris et al., 2007; Aydi Ben Abdallah et al., 2015).

The plant growth-promoting ability was induced by the beneficial microbial agents directly by the acquisition of essential nutriments and the production of phytohormones and/or indirectly by inhibiting pathogen growth and inducing plant systemic resistance (Santoyo et al., 2016). Results from the current study clearly demonstrated that application of *B. subtilis* SV41 and *B. velezenis* SV65 in the soil reduced the population of *F. oxysporum* and enhanced the number of beneficial microbial agents leading to a significant decrease in Fusarium wilt severity and to a significant promotion of plant growth and production. This result is in agreement, in part, with previous findings related to disease-suppressive effects displayed by an endophytic *B. subtilis* EPC016, isolated from cotton plants, which decreased Fusarium wilt incidence by 68.4% and increased tomato growth and yield compared to control (Ramyabharathi and Raguchander, 2014). *B. subtilis* SV41 and *B. amyloliquefaciens* subsp. *plantarum* SV65 were previously tested with other *Bacillus* species in a pot experiment where authors indicated that Fusarium wilt severity suppression was significantly correlated to the decreaseof pathogen colonization of vascular tissues leading consequently to plant growth promotion (Aydi Ben Abdallah et al., 2017). Indeed, *B. subtilis* SV41 was found the highest salicylic acid producing agent that acts as an elicitor for the induction of systemic resistance against pathogen attack. Furthermore, Aydi Ben Abdallah et al. (2017) found that *B. amyloliquefaciens* subsp. *plantarum* SV65induced expression of the lipooxygenase gene (*LOXD*) and especially acidic PR-1 and PR-3 genes in treated plants and those uninoculated or inoculated with FOL. Therefore, the two *Bacillus* spp., tested in our study, have shown able to induce systemic resistance in treated tomato as reported in Aydi Ben Abdallah et al. (2017) leading indirectly to growth promotion. There are a large number of common mechanisms that plant growth-promoting bacteria (PGPB) use to indirectly promote plant growth which include production of antibiotics and cell wall-degrading enzymes, decrease in ethylene levels, induction of systemic resistance, reduction of iron available to pathogens, and synthesis of pathogen-inhibiting volatile compounds (Glick, 2015). In addition, PGPB directly promote plant growth through the acquisition of nutriments from environment including nitrogen, phosphorous and iron or through the production and/or regulation of various plant hormones including auxin, cytokinin or ethylene (Santoyo et al., 2016). These two *Bacillus* strains tested in the current investigation are shown able to produce the indole 3-acetic acid (IAA) and only *B. amyloliquefaciens* subsp. *plantarum* SV65 was able to solubilize the phosphate (unpublished data). Aydi Ben Abdallah et al. (2016) also demonstrated that *Bacillus* sp. SV101 and *B. tequilensis* SV104 were IAA-producing agents and only *Bacillus* sp. SV101 was a phosphate-solubilizing strain.

Many researchers have attempted the development of biocontrol agents for suppression of various soilborne pathogens (Domenech et al., 2005). In this study, pre-planting soil treatment with *B. subtilis* SV41 and *B. amyloliquefaciens* subsp*. plantarum* SV65 reduced *Fusarium* (and especially *F. oxysporum*) inoculum in treated soils as compared to control. These results are in agreement with other findings (Zhang et al., 2008; Ling et al., 2010). In fact, Qiu et al. (2012) found that following treatment with abio-organic fertilizer (composed of organic fertilizer combined with *B.subtilis* SQR-9, *Paenibacillus polymyxa* SQR-21 and *Trichoderma harzianum* SQR-T037), *Fusarium* population was reduced to one fourth as compared the organic fertilizer applied without microbial supply. Indeed, among the *Fusarium* group, *F.oxysporum* population in soils unsubjected to microbial amendment were almost ten times more abundant than in the soil treated with the bio-organic fertilizer. In this study, the abundance of fungi showed different trends. A higher frequency of *Aspergillus* and *Penicillium* colonies was detected in the rhizosphere of tomato plants treated with *B. subtilis* SV41 as compared to the initial state (before planting) and to control soil. *Aspergillus* and *Penicillium* have been reported to be antagonistic to various *Fusarium* species (Dong and Cohen, 2002; Brzezinska and Jankiewicz, 2012; Mejdoub-Trabelsi et al., 2017). In fact, Sreevidya and Gopalakrishnan (2016) demonstrated that *P. citrinum* VFI-51 is able to produce siderophores, indole 3-acetic acid, hydrocyanic acid, lipase, protease, β-1,3 glucanase, and volatile compounds and also capable to compete with other pathogenic microorganisms such as *Fusarium* species (Aydi Ben Abdallah et al., 2015; Mejdoub-Trabelsi et al., 2017).

As for their effects on bacterial community structure, the number of beneficial bacteria determined in bacterized soils was three times higher than in the initial soil state (before planting). Moreover, *B. subtilis* SV41-treated soil showed enrichment of the total bacteria populations as compared to control. These

findings are in agreement with those of Qiu et al. (2012). In the current study, the soil DNA was not extracted and pyrosequenced. The colonies number of Actinomycetes and *Pseudomonas* wereestimatedbased on their macro-morphological traits on selective media. The number of *Pseudomonas* spp. was significantly higher in treated soils than in control ones (initial state and untreated control). The application of *B. amyloliquefaciens* subsp. *plantarum* SV65 in soil enhanced the population of *P. fluorescens* as compared to the initial state and to control soil. *Pseudomonas* is also known by its ability to suppress numerous plant pathogens including *F.oxysporum* (Patel et al., 2012; Munif et al., 2012; Dalal and Kulkarni, 2013). Bibi et al. (201) and Aydi Ben Abdallah et al. (2016) showed the ability of *Pseudomonas* spp. to produce hydrolytic enzymes such as chitinase, protease, pectinase and β-1,3 glucansewhich are involved in the antifungal activity against*F.oxysporum*. Also, in the current study, Actinobacteria population was significantly increased in soils treated with both bacterial strains compared to the initial soil state. In fact, *B. subtilis* SV41-treated soil showed significant enrichment in *Actinomycetes* population which was estimated to be two times higher than to that of the untreated soil. Qiu et al. (2012) found that *Streptomyces*, an Actinobacteria, accounted for 3.2% of the total bacterial sequences on soils treated with various microbial inoculants such as *Bacillus*, *Paenibacillus* and *Trichoderma*. Its abundance is approximately three times higher than in the untreated soil. This genus can produce tubercidin, phosphlactomycin and candicidin (Hwang et al., 1994) and was found to be an effective biological control agent (Etebarian et al., 2003; Nourozian et al., 2006; Shekhar et al., 2006). Actinomycetes collected from 30 rhizospheric soils of *Catharanthus roseus* and *Withania somnifera* in different locations in Ludhiana, India, are promising biocontrol agents for *Alternaria alternata*, *Fusarium oxysporum*, *Helminthosporium oryzae*, *Macrophomina phaseolina*, *Penicillium* sp., *Rhizoctoniasolani* and *Sclerotium rolfsii* control (Kamara and Gangwar, 2015).

Fusarium wilt suppression maybe, in part, attributed to the action of microbial agents associated to treated tomato plants. In this study, soil bacterial treatment increased significantly the populations of *Pseudomonas* and *Actinomycetes* in tomato rhizosphere compared to untreated soils. Patel et al. (2012) identified an endophytic *Pseudomonas aeruginosa* HR7, recovered from cultivated tomato, as an effective agent for *F. oxysporum* biocontrol. Dalal and Kulkarni (2013) also recorded a significant inhibition of *F.oxysporum* mycelial growth using endophytic *Pseudomonas* sp. originally recovered from soybean. In Aydi Ben Abdallah et al. (2016) study, *P. brenneri* S85, shown able to inhibit FOL *in vitro* and *in vivo* growth, was capable to produce chitinase, protease and pectinase. Endophytic *Pseudomonas* species i.e *P. brenneri*, *P. koreensis*, *P. viridiflava* and *P. syringae* are also commonly known as producers of antibiotics such as ecomycins and pseudomycins (Christina et al., 2013) and/or hydrolytic enzymes (Bibi et al., 2012). Indeed, two actinomycetes species i.e*Micromonospora* sp. and *M. globosa* were successfully explored as biocontrol agents against tomato wilt and Pigeons-peas wilts caused by *F. oxysporum* f. sp. *lycopersici* and *F. udum*, respectively (Smith, 1957; Ypadhyay and Rai, 1987). Various antifungal antibiotics were released in soils by different actinomycetes (Trejo-Estrada et al., 1998; El-Tarabily and Sivasithamparam, 2006). In fact, this bacterial group produced high levels of chitinases and β-1,3-glucanses which caused extensive hyphal plasmolysis, cell-wall lysis and significantly reduced the level of disease incidence under controlled greenhouse conditions (El-Tarabily et al., 2000). Furthermore, the plant growth-promoting potential noted in treated plants may be due to the presence of beneficial microorganisms in the rihzosphere of treated plants such as *Pseudomonas* and actinobacteria. Several previous study showed that phosphate solubilization potential and IAA production ability by *Pseudomonas*are involved in plant growth promotion (Ngamau et al., 2012; Patel et al., 2012; Dalal and Kulkarni, 2013; Aydi Ben Abdallah et al., 2016). Additionally, the selected actinomycetes, identified as *Streptomyces* using 16S rDNA analysis, have good plant growth-promotion and biocontrol potentials on chickpea under *in vitro* and *in vivo* conditions and were found able to produce siderophores, cellulases, lipapses, chitinases, proteases, β-1,3-glucanses, hydrocyanic acid and IAA (Sreevidya et al., 2016).

In conclusion, soil bacterial treatment using *B. subtilis* SV41 (KR818071) and *B. amyloliquefaciens* subsp. *plantarum* SV65 (KR818073) was considered to be an effective approach to suppress Fusarium wilt of tomato through the direct inhibition of the causal agent and the suppression of its inoculum in soil and also through the enhancement of the soil biofertility via its enrichment with various beneficial microbial agents.

Acknowledgement

This work was funded by the Ministry of Higher Education and Scientific Research of Tunisia through the funding allocated to the research unit UR13AGR09-Integrated Horticultural Production in the Tunisian Centre-East, Regional Research Centre on Horticulture and Organic Agriculture of Chott-Mariem, Tunisia, and by IRESA through the funding attributed to the multidisciplinary and the multi-institutional project CleProD.

References

Aydi Ben Abdallah, R., Jabnoun-Khiareddine, H., Mokni-Tlili, S., Nefzi, A., Medimagh-Saidana, S., Daami-Remadi, M., 2015. Soil-borne and Compost-borne *Aspergillus* Species for Biologically Controlling Post-harvest Diseases of Potatoes Incited by *Fusarium sambucinum* and *Phytophthora erythroseptica*. *Journal of Plant Pathology and Microbiology* 6(10): 324-330.

Aydi Ben Abdallah, R., Jabnoun-Khiareddine, H., Nefzi, A., Mokni-Tlili, S., Daami-Remadi, M., 2016a. Biocontrol of Fusarium wilt and growth promotion of tomato plants using endophytic bacteria isolated from *Solanum elaeagnifolium* stems. *Journal of Phytopathology* 164(10): 811-824.

Aydi Ben Abdallah, R., Jabnoun-Khiareddine, H., Nefzi, A., Mokni-Tlili, S., Daami-Remadi, M., 2016b. Endophytic bacteria from *Datura metel* for plant growth promotion and bioprotection against Fusarium wilt in tomato. *Biocontrol Science and Technology* 26(8): 1139-1165.

Aydi Ben Abdallah, R., Stedel, C., Garagounis, C., Nefzi, A., Jabnoun-Khiareddine, H., Papadopoulou, K.P., Daami-Remadi, M., 2017. Involvement of lipopeptide antibiotics and chitinase genes and induction of host defense in suppression of Fusarium wilt by endophytic *Bacillus* spp. in tomato. *Crop Protection* 99:45-58.

Barraquio, W.L., Revilla, L., Ladha, L.K., 1997. Isolation of endophytic diazotrophic bacteria from wetland rice. *Plant and Soil* 194(1-2):15-24.

Bernard, E., Larkin, R.P., Tavantzis, S., Erich, M.S., Alyokhin, A., Gross, S.D., 2014. Rapeseed rotation, compost and biocontrol amendments reduce soilborne diseases and increase tuber yield in organic and conventional potato production systems. *Plant and Soil* 374(1-2): 611-627.

Bibi, F., Yasir, M., Song, G.C., Lee, S.Y., Chung, Y.R., 2012. Diversity and characterization of endophytic bacteria associated with tidal flat plants and their antagonistic effects on Oomycetous plant pathogens. *The Plant Pathology Journal* 28(1): 20-31.

Brzezinska, M.S., Jankiewicz, U., 2012. Production of antifungal chitinase by Aspergillus niger LOCK 62 and its potential role in the biological control. *Current Microbiology* 65(6): 666-672.

Christina, A., Christapher, V., Bhore, S.J., 2013. Endophytic bacteria as a source of novel antibiotics: An overview. *Pharmacognosy Reviews* 7: 11-16.

Compant, S., Reiter, B., Sessitsch, A., Nowak, J., Clément, C., Ait Barka, E., 2005. Endophytic colonization of Vitis vinifera L. by plant growth-promoting bacterium Burkholderia sp. strain PsJN. *Applied and Environmental Microbiology* 71: 1685-1693.

Daami-Remadi, M., Souissi, A., Ben Oun, H., Mansour, M., Nasraoui, B., 2009. Salinity effects on Fusarium wilt severity and tomato growth. *Dynamic Soil, Dynamic Plant* 3: 61-69.

Dalal, J., Kulkarni, N., 2013. Antagonistic and plant growth promoting potentials of indigenous endophytic bacteria of soybean (Glycine max (L) Merril). *Current Research in Microbiology and Biotechnology* 1(2): 62-69.

Domenech, J., Reddy, M.S., Kloepper, J.W., Ramos, B., Gutierrez-Mañero, J., 2005. Combined application of the biological product LS213 with Bacillus, Pseudomonas or Chryseobacterium for growth promotion and biological control of soil-borne diseases in pepper and tomato. BioControl 51:245-258.

Dong, H., Cohen, Y., 2002. Induced resistance in cotton seedlings against Fusarium wilt by dried biomass of Penicillium chrysogenum and its water extract. *Phtoparasitica* 30(1): 77-87.

Dubey, R.K., Tripathi, V., Dubey, P.K., Singh, H.B., Abhilash, P.C., 2016. Exploring rhizospheric interactions for agricultural sustainability: the need of integrative research on multi-trophic interactions. *Journal of Cleaner Production* 115: 362-365.

El-Tarabily, K.A., Sivasithamparam, K., 2006. Non-streptomycete actinomycetes as biocontrol agents of soil-borne fungal plant pathogens and as a plant growth-promoters. *Soil Biology and Biohemistry* 38(7): 1505-1520.

El-Tarabily, KA., Soliman, M.H., Nassar, A.H., Al-Hassani, H.A., Sivasithamparam, K., McKenna, F., Hardy, G.E.St.J., 2000. Biological control of Sclerotinia minor using a chitinolytic bacterium and actinomycetes. *Plant Pathology* 49: 573-583.

Etebarian, H.R., Scott, E.S., Wicks, T.J., 2003. Evaluation of Streptomyces strains as potential biological agents of Phytophthora erythroseptica. *Iranian Journal of Plant Pathology* 49: 49-63.

Figueiredo, M.D.V.B., Bonifacio, A., Rodrigues, A.C., de Araujo, F.F., Stamford, N.P., 2016. Beneficial microorganisms: Current challenge to increase crop performance. In: Bioformulations: for Sustainable Agriculture, Arora et al., (Eds.). Springer India, pp. 53–70.

Glick, B.R., 2015. Beneficial Plant-Bacterial Interactions: Biocontrol mechanisms. Springer, Cham.

Hallmann, J., Quadt-Hallmann, A., Mahaffee, W.F., Kloepper, J.W., 1997. Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology* 43(10): 895-914.

Hwang, B.K., Ahn, S.J., Moon, S.S., 1994. Production, purification, and antifungal activity of the antibiotic nucleoside, tubercidin, produced by Streptomyces violaceoniger. *Canadian Journal of Botany* 72(4): 480-485.

Idris, H.A., Labushagne, N., Korsten, L., 2007. Screening rhizobacteria for biological control of Fusarium root and crown rot of sorghum in Ethiopia. *Biological Control* 40(1): 97-106.

Kalai-Grami, L., Saidi, S., Bachkouel, S., Ben Slimene, I., Mnari-Hattab, M., Hajlaoui, M.R., Limam, F., 2014. Isolation and characterization of putative endophytic bacteria antagonistic to Phoma tracheiphila and Verticillium albo-atrum. *Applied Biochemistry and Biotechnology* 174(1): 365-375.

Kamara, V., Gangwar, M., 2015. Antifungal activity of actinomycets from rhizospheric soil of medicinal plants against phytopathogenic fungi. *International Journal of Current Microbiologyand Applied Sciences* 4(3): 182-187.

Kettler, T.A., Doran, J.W., Gilbert, T.L., 2001. Simplified method for soil particle-size determination to accompany soil-quality analyses. *Soil Science Society of American Journal* 65(3): 849-852.

Landa, B.B., Hervfis, A., Bettiol, W., Jimnez-Diaz, R.M., 1997. Antagonistic activity of Bacteria from the chickpea rhizosphere against Fusarium Oxysporum f. sp. Ciceris. *Phytoparasitica* 25(4): 305-318.

Larkin, R.P., Tavantzis, S., 2013. Use of biocontrol organisms and compost amendments for improved control of soilborne diseases and increased potato production. *American Journal of Potato Research* 90(3): 261-270.

Larkin, R.P., Honeycutt, C.W., 2006. Effects of different 3-year cropping systems on soil microbial communities and rhizoctonia diseases of potato. *Phytopathology* 96(1): 69-79.

Larkin, R.P., Honeycutt, C.W., Griffin, T.S., 2006. Effect of swine and dairy manure amendments on microbial communities in three soils as influenced by environmental conditions. *Biology and Fertility of Soils* 43(1): 51-61.

Ling, N., Xue, C., Huang, Q., Yang, X., Xu, Y., Shen, Q., 2010. Development of a mode of application of bioorganic fertilizer for improving the biocontrol efficacy of Fusarium wilt. *Biological Control* 55(5): 673-683.

Mazzola, M., 2004. Assessment and management of soil community structure for disease suppression. *Annual Review of Phytopathology* 42: 35-59.

Meena, S.K., Meena, V.S., 2017. Importance of soil microbes in nutrient use efficiency and sustainable food production. In: Agriculturally important microbes for sustainable agriculture: Application in crop production and protection, Meena, V.S., Mishra, P.K., Bisht, J.K., Pattanayak, A., (Eds.). Springer Nature Singapore, pp. 3-23.

Mejdoub-Trabelsi, B., Aydi Ben Abdallah, R., Ammar, N., Daami-Remadi, M., 2017. Antifungal potential of extracellular metabolites from Penicillium pp. and Aspergillus spp. naturally associated to potato against Fusarium species causing tuber dry rot. *Journal of Microbial and Biochemistry Technology* 9(4): 181-190.

Moretti, M., Gilardi, G., Gullino, M.L., Garibaldi, A., 2008. Biological control potential of Achromobacter xylosoxydans for suppressing Fusarium wilt of tomato. *International Journal of Botany* 4:369-375.

Munif, A., Hallmann, J., Sikora, R.A., 2013. The influence of endophytic bacteria on Meloidogyneincognita infection and tomato plant growth. *International Society for Southeast Asian Agricultural Sciences* 19(2): 68-74.

Nash, S.M., Snyder, W.C., 1962. Quantitative estimations by plate counts of propagules of the bean root rot Fusarium in field soils. *Phytopathology* 52(6): 567-572.

Nejad, P., Johnson, P.A., 2000. Endophytic bacteria induce growth promotion and wilt disease suppression in oilseed rape and tomato. *Biological Control* 18(3): 208-215.

Ngamau, C.N., Matiru, V.N., Tani, A., Muthuri, C.W., 2012. Isolation and identification of endophytic bacteria of bananas (Musa spp.) in Kenya and their potential as biofertilizers for sustainable banana production. *African Journal of Microbiology Research* 6: 6414-6422.

Nourozian, J., Etabarian, H.R., Khodakaramian, G., 2006. Biological control of Fusarium graminearum on wheat by antagonistic bacteria. *Songklanakarin Journal of Science Technology* 28: 29-38.

Patel, H.A., Patel, R.K., Khristi, S.K., Parikh, K., Rajendran, G., 2012. Isolation and characterization of bacterial endophytes from Lycopersicon esculentum plant and their plant growth promoting characteristics. *Nepal Journal of Biotechnology* 2(1): 37-52.

Qiu, M., Zhang, R., Xue, C., Zhang, S., Li, S., Zhang, L., Shen, Q., 2012. Application of bio-organic fertilizer can control Fusarium wilt of cucumber plants by regulating microbial community of rhizosphere soil. *Biology and Fertility of Soils* 48(7): 807-816.

Ramyabharathi, S.A., Raguchander, T., 2014. Efficacy of secondary metabolites produced by Bacillus subtilis EPCO16 against tomato wilt pathogen Fusarium oxysporum f. sp. lycopersici. *Journal of Mycology and Plant Pathology* 44(2): 148-153.

Santoyo, G., Moreno-Hagelsieb, G., Orozco-Mosqueda, M.D.C., 2016. Plant growth-promoting bacteria endophytes. *Microbiological Research* 183: 92-99.

Sharma, V.K., Nowak, J., 1998. Enhancement of verticillium wilt resistance in tomato transplants by in vitro co-culture of seedlings with a plant growth promoting rhizobacterium (Pseudomonas sp. strain PsJN). *Canadian Journal of Microbiology* 44(6): 528-536.

Shekhar, N., Bhattacharya, D., Kumar, D., Gupta, R.K., 2006. Biocontrol of wood-rotting fungi with Streptomyces violaceusniger XL-2. *Canadian Journal of Microbiology* 52(9): 805-808.

Smith, G.E., 1957. Inhibition of Fusarium oxysporum f. sp. lycopersici by a species of Micromonospora isolated from tomato. *Phytopathology* 47: 429-432.

Sreevidya, M., Gopalkrishnan, S., 2016. Penicillium citrinum VF1-51 as biocontrol agent to control charcoal rot of Sorghum (Sorghum bicolor (L.) Moench). *African Journal of Micobiology Research* 10(19): 669-674.

Sreevidya, M., Gopalkrishnan, S., Kudapa, H., Varshney, R.K., 2016. Exploring plat growth-promotion actinomycetes from vermicompost and rhizosphere soil for yield enhancement in chickpea. *Brazilian Journal of Microbiology* 47(1): 85-95.

Swarupa, V., Pavitra, K., Shivashankara, K.S., Ravishankar, K.V., 2016. Omics-driven approaches in plant-microbe interaction. In: Microbial inoculants in sustainable agricultural productivity: Research perspectives, Singh, D.P., Singh, H.B., Prabha, R., (Eds.). Springer India,pp. 61-84.

Syngenta, 2015. Tomato Sahel: Crops & Products. Available at [Access date : 01.10.2018]: http://www3.syngenta.com/country/es/sp/productos/Documents/ft/ft-tomate-sahel.pdf.

Trejo-Estrada, S.R., Sepulveda, I., Crawford, D.L., 1998. In vitro and in vivo antagonism of Streptomyces violaceusniger YCED9 against fungal pathogens of turfgrass. *World Journal of Microbiology and Biotechnology* 14(6): 865-872.

Vijayabharathi, R., Sathya, A., Gopalakrishnan, S., 2016. A renaissance in plant growth-promoting and biocontrol agents by endophytes. In: Microbial inoculants in sustainable agricultural productivity: Research perspectives, Singh, D.P., Singh, H.B., Prabha, R. (Eds.). Springer India. pp.37-60.

Yang, C., Crowley, D.E., Menge, J.A., 2001. 16S rDNA fingerprinting of rhizosphere bacterial communities associated with healthy and Phytophthora infected avocado roots. *FEMS Microbial Ecology* 35(2):129-136.

Ypadhyay, R.S., Rai, B., 1987. Studies on antagonism betweenFusarium udum Butler and root region microflora of pigeon-pea. *Plant and Soil* 101(1): 79-93.

Zeilinager, S., Gupta, V.K., Dahms, T.E.S., Silva, R.N., Singh, H.B., Ypadhyay, R.S., Gomes, E.V., Tsui, C.K.M., Chandra, S., 2016. Friends or foes? Emerging insights from fungal interactions with plants. *FEMS Microbiology Reviews* 40(2): 182-207.

Zhang, S., Raza, W., Yang, X., Hu, J., Huang, Q., Xu, Y., Liu, X., Ran, W., Shen, Q., 2008. Control of Fusarium wilt disease of cucumber plants with the application of a bioorganic fertilizer. *Biology and Fertility of Soils* 44: 1073-1080.

ˈ Eurasian J Soil Sci 2019, 8 (3) 221 - 228

Eurasian Journal of Soil Science

Journal homepage : http://ejss.fesss.org

Mitigation of salinity in chickpea by Plant Growth Promoting Rhizobacteria and salicylic acid

Aneela Riaz [a,*], Munazza Rafique [a], Muhammad Aftab [b], M. Amjad Qureshi [a], Hina Javed [a], Fakhar Mujeeb [a], Saleem Akhtar [a]

[a] Soil Bacteriology Section, Agricultural Biotechnology Research Institute, AARI, Faisalabad, Pakistan
[b] Soil Chemistry Section, Institute of Soil Chemistry and Environmental Sciences, AARI, Faisalabad, Pakistan

Article Info

Received : 10.08.2018
Accepted : 18.04.2019

Abstract

For growth or development of pulses, biotic and abiotic environmental factors are more conspicuous under stress conditions. For the survival against abiotic stresses, salicylic acid (SA) is reported a universal remedy. At the Soil Bacteriology Section, Ayub Agricultural Research Institute, Faisalabad, a pot study was conducted to monitor the role of Plant Growth Promoting Rhizobacteria (PGPR) and Salicylic acid in chickpea under salt stress. Eight treatments including control PGPR inoculation and Salicylic acid with their different combination were used. Results revealed that positive response of PGPR on productivity of chickpea but more enunciated response about grain yield was observed with the combined application of SA and PGPR compared to control. Growth parameters i.e root length, root mass, number of nodules and shoot mass were highly affected where SA was applied along with PGPR. From the study, it is proposed that under salt stress the combination of SA + PGPR can be a suitable practice for more production of chickpean Pakistan.

Keywords: Pulses, SA salt stress, microbes pulses.

Introduction

Salinity stress causes the major reduction in agricultural production of Pakistan by declining the crop yield through distressing the balance of water and micro and macronutrients of plants (Munns and Tester, 2008). There are a number of rhizospheric microorganisms which are known as plant growth promoting rhizobacteria (PGPR), when they develop strong relationship with a host plant improve the plant growth (Vessey, 2003). Nowadays, concentration of salts tremendously increasing which causes reduction in the agricultural output in major parts of the world (Hasanuzzaman et al., 2013).

Plant growth is suppressed under mainly by two ways; firstly by osmotic effects of salt stress and second by salt-specific effects (Sobhanian et al., 2010). However, salt tolerance can be enhanced in crops by various physiochemical pathways (Babu et al., 2012). To reduce the negative effects of salt stress on the growth and yield of plant a lot of research work has been conducted. Different techniques of biology like seed/plant treatments with plant growth promoting bacteria (PGPR) and exogenous application of growth hormones which make defense under stress conditions are previously studied (Hayat et al., 2010; Ullah et al., 2017; Qureshi et al., 2019). Among plant growth regulators salicylic acid is one of them which play a role in defensive system against different stresses either biotic or abiotic (Szalai et al., 2000). Among naturally existing phenolic compound, salicylic acid (SA) is one of them. A lot of evidences has been found that externally applied SA increased plant's tolerance to several abiotic stresses, including drought (Azooz and Youssef, 2010), osmotic stress (Al-Hakimi, 2006), heavy metal stress (Moussa and El-Gamel, 2010) and

* Corresponding author.
Soil Bacteriology Section, Agricultural Biotechnology Research Institute, AARI, Faisalabad, Pakistan
Tel.: +920419201807 E-mail address: aneelariaz2009@gmail.com
e-ISSN: 2147-4249 DOI: 10.18393/ejss.560745

salinity (Gunes et al., 2007). Externally applied SA reduced transpiration and increased flower longevity, nitrate reductase activity as well as the yield of some plants which overall suggest that SA may enhance the multiple types of stress tolerance in plants through which interactive effects on several functional molecules or other signaling molecules participating in more complex stress response.

Biological Nitrogen Fixation is the biological mechanism where rhizobia symbiotically fix of atmospheric nitrogen into form which is available for plants in the presence of enzyme nitrogenase (Mohammadi and Sohrabi, 2012). The biological catalyst which is present in the bacteriod and mediates the reaction is a nitrogenase enzyme. BNF is cost effective and ecologically sound source of nitrogen and helps to decrease the dependence on external inputs

In pulses chickpea ranked at 3rd portion in overall world production. In many countries of the world chickpea (*Cicer arietinum* L.) is a key leguminous crop which is vital for its nutritional value. In arid areas chickpea is locally grown crop that has ability to adopt different biotic and abiotic environmental stress conditions (Rao et al., 2002). Through symbiotic nitrogen fixation chickpea fulfills its 70% of its N requirement (Siddique et al., 2005). So current study was planned to check the response of PGPR along with various concentrations of Plant growth regulators (Salicylic acid) under salinity stress.

Material and Methods

Isolation of PGPR

For the isolation of PGPR, rhizospheric soil of chickpea was collected from pulses research Institute, Faisalabad, Pakistan. Screening of microbes were carried out through auxin biosynthesis. Identification and characterization of selected isolate were done by using morphological, physiological, biochemical testing methods as described in Bergey's manual of Systematic Bacteriology.

For experiment, these PGPR isolates were grown on Luria Bertani (LB) media. A single loop was shifted to flasks of 250 mL which contains LB broth. Flasks are placed on a rotating shaker (95 rpm) for 24 h at 27ºC. The bacterial suspension concentration of 10^6 CFU ml^{-1} was maintained. 1 mL of log culture (110 cells) of each bacterial isolates was transferred as inoculum in the corresponding treatments. Auxin biosynthesis potential of screened isolates was determined in the terms of IAA equivalents (Sarwar et al., 1992).

Isolates	IAA Equivalents (µg mL^{-1})	Isolates	IAA Equivalents (µg mL^{-1})
Rhizobium sp. (ChickPea)		Azotobacter sp.	
CP-1		AZ-6	4.36
CP-2	5.89	AZ-7	5.56
CP-3	6.84	AZ-8	1.03
CP-4	4.36	AZ-9	5.04
CP-5	4.28	Azospirillum sp.	
		AS-1	2.08

Pot experiment

A glass house study in pots was carried out at the Soil Bacteriology Section, Ayub Agricultural Research Institute, Faisalabad, to test the function of beneficial microorganisms (PGPR) along with Salicylic acid (SA) on chickpea crop in salinity stress conditions. Treatments were control (without inoculation), PGPR inoculation, Salicylic acid and their various combinations (T_1: Control, T_2: PGPR inoculation, T_3: Salicylic acid @ 10^{-3} M, T_4: Salicylic acid @ 10^{-4} M T_5: Salicylic acid @ 10^{-5} M, T_6: PGPR + Salicylic acid @ 10^{-3} M, T_7: PGPR + Salicylic acid @ 10^{-4} M, T_8: PGPR + Salicylic acid @ 10^{-5} M). Pots were filled with 10 kg soil having sandy clay loam texture, ECe 1.16 dSm^{-1}, pH 8.8 and saturation percentage age (33%). Used the chickpea (*Cicer arietinum* L.) seeds as plant materials. Chickpea seeds were sterilized in 1% $HgCl_2$ for 2 minutes, for surface sterilization and then, washed with sterilized distilled water at least 10 times to remove traces of toxic $HgCl_2$. After air drying Chickpea seeds were sown into sterilized pots (eight seeds per pot). All nitrogen and phosphorus doses were applied at time of sowing.According to treatment plan, seeds of chickpea were coated with peat based inoculum of selected strains of PGPR in a completely randomized design. Before pot filling salinity was developed by NaCl @ 5 dS m^{-1}. After 15days of seedling emergence foliar application of SA was carried out. All physiochemical parameters were recorded at the time of harvesting and after harvesting. N and P analysis was carried out by using method described by Jones (2001).

Chlorophyll and cartenoids concentration

To determine the chlorophyll and cartenoids, leaf sample of chickpea plant was taken in pre-weighed clean glass vials And add 10 mL of 80%. Bleached the leaf material and then decant off. Spectrophotometer (Spectronic Genesys-5, Milton Roy) was used to read the optical density at λ = 663, 646 and 470 nm using 80% acetone as a blank. Concentration of chlorophyll a, chlorophyll b and carotenoids (µg g^{-1}) was calculated according to Lichtenthaler and Wellburn (1983). Data was analyzed statistically by using statistics 8.1 (Steel et al., 1997).

Results

Physical parameters

Results revealed that positive response of microbes on growth and productivity of chickpea under salinity stress but distinctive effect was observed, in all growth characteristics of chickpea, where the p SA was applied along with co inoculation of PGPR. Maximum length of shoot was found in the treatment where PGPR and SA were applied. However, length of root and shoot was more in the treatment where only PGPR was applied as compare to un inoculated treatment but its effect was less as compare to combine treatment (PGPR and Salicylic acid) shown in Figure 1 and 2. Highest shoot and root length was recorded when PGPR and Salicylic acid 10^{-3} M was used. Maximum numbers of nodules were observed when combination of plant growth hormone (SA @ 10^{-5} M) and PGPR was applied (Table 1). Data regarding grain yield also showed that higher yield was recorded in treatment where PGPR + Salicylic acid 10^{-5} M was applied (Figure 3).

Table 1. Effect of treatments on yield parameters of chickpea

Treatments	Number of pods $plant^{-1}$	Pod yield (g pot^{-1})	Number of seed pod^{-1}	Branches $plant^{-1}$	Number of flowers $plant^{-1}$
T_1- Control	20.66 C	12.39 E	2	5 DE	26.00 BC
T_2- PGPR	24.66 C	14.63 D	2	6 CD	27.67 BC
T_3 - SA 10^{-3} M	20.66 C	15.40 D	2	3 E	22.67 C
T_4 - SA 10^{-4} M	21.33 C	16.01 CD	2	3 E	23.67BC
T_5 - SA 10^{-5} M	25.33 BC	17.36 BC	2	6 CD	27.33 BC
T_6 - PGPR + SA 10^{-3} M	30.33 AB	15.87 CD	2	7 BC	31.00 B
T_7 - PGPR + SA 10^{-4} M	31.00 A	18.61 B	2	9 AB	40.33 A
T_8 - PGPR + SA 10^{-5} M	25.66 ABC	21.33 A	2	10 A	47.67 A
LSD	5.519	1.702	N.S.	2.234	7.908

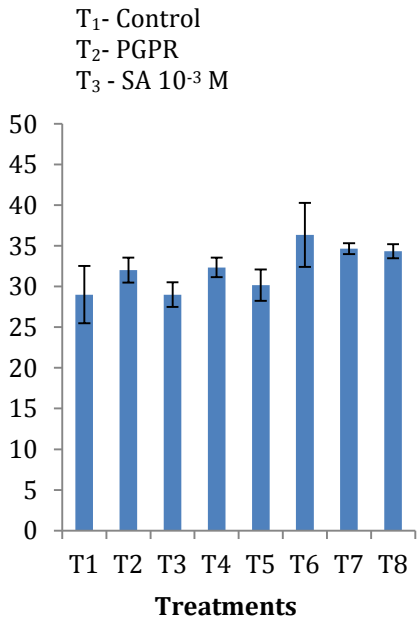


Figure 1. Effect of treatments on Plant Height (cm)

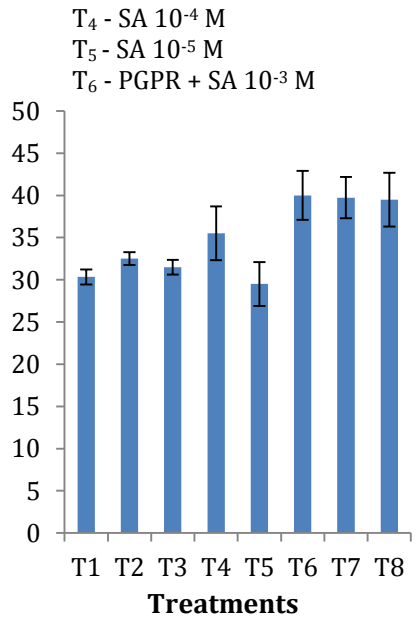


Figure 2. Effect of treatments on Root length (cm)

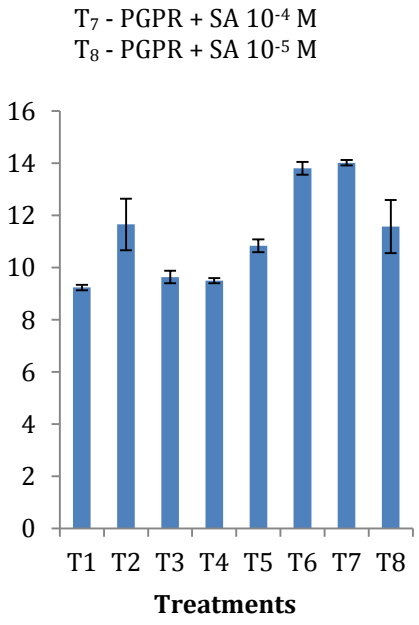


Figure 3. Effect of treatments on grain yield, g pot^{-1}

Data regarding chlorophyll and carotenoids, nodular mass, number of nodules revealed that maximum chlorophyll contents (a&b) were observed where SA@10^{-5} M along with PGPR present followed by other

treatments as shown in Figure 4 and 5. But carotenoids were almost at par in all the treatments where SA and PGPR are combined as shown in Figure 6. It means efficiency of PGPR was enhanced in the presence of salicylic acid. In case of number of nodules maximum nodules were present in T_7 (SA@10^{-4}M+PGPR) followed by T_8 (SA@10^{-5} M+PGPR). Maximum nodular mass was observed in both the treatments T_7 & T_8 as compare to other treatments as shown in (Table 2).

Table 2. Effect of treatments on growth parameters of chickpea

Treatments	Fresh shoot wt. (g pot^{-1})	Dry shoot wt. (g pot^{-1})	Fresh root wt. (g pot^{-1})	Dry root wt. (g pot^{-1})	Number of Nodules Plant^{-1}	Nodular Mass (g)
T_1- Control	40.70 F	8.06 E	32.71 C	3.06 E	35.00D	0.08 BCD
T_2- PGPR	42.50 EF	8.43 DE	41.00 B	5.23 BC	37.00 CD	0.10 ABCD
T_3 - SA 10^{-3} M	41.50 EF	8.40 DE	43.22 B	3.96 DE	39.66 C	0.07 CD
T_4 - SA 10^{-4} M	43.50 DE	8.83 CD	43.67 B	3.88 DE	47.00 A	0.06 D
T_5 - SA 10^{-5} M	45.16 CD	9.23 BC	48.50 A	5.50 BC	39.00 C	0.10 ABCD
T_6 - PGPR + SA 10^{-3} M	47.30 BC	9.40 ABC	48.93 A	6.27 B	47.66 A	0.11 ABC
T_7 - PGPR + SA 10^{-4} M	48.83 AB	9.83 AB	50.51 A	7.86 A	49.00 A	0.12 AB
T_8 - PGPR + SA 10^{-5} M	51.00 A	10.10 A	43.71 B	4.72 CD	43.66 B	0.12 A
LSD	2.298	0.728	3.483	1.174	3.238	0.037

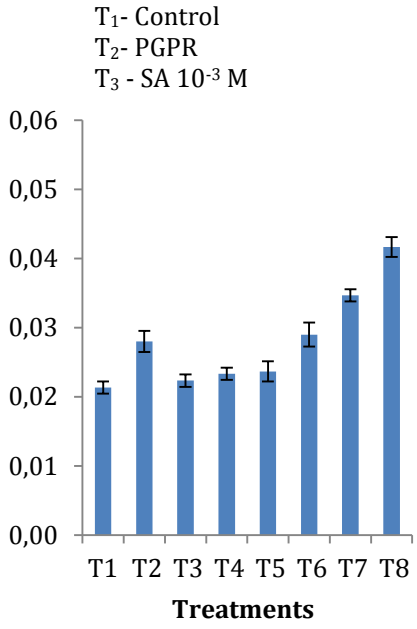


Figure 4. Effect of treatments on Chlorophyll a (µg g^{-1}) concentration

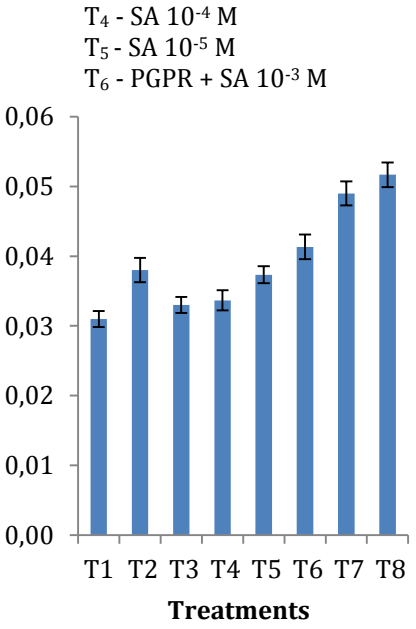


Figure 5. Effect of treatments on Chlorophyll b (µg g^{-1}) concentration

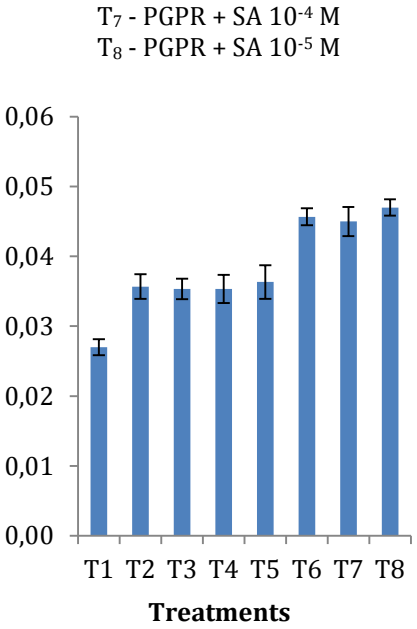


Figure 6. Effect of treatments on Cartenoids (µg g^{-1}) concentration

Agronomic parameters

Data regarding grain yield revealed that maximum grain yield (14.08 g pot^{-1}) was observed in T_7 treatment where PGPR was applied along with salicylic acid @ 10^{-4} M, following by T6 treatment the exogenous application of plant growth hormone@10^{-3} in combination with PGPR (13.81g) as compared to control (9.23g). Similarly maximum plant height (34.67cm), number of nodules pot^{-1} (49.0) was observed in T_7 treatment. Results regarding different agronomic parameters revealed that maximum pod yield (21.33 g pot^{-1}), number of flowers (47.67 plant^{-1}), fresh shoot mass (51.0g), number of branches (9.67 plant^{-1}), root length (40.0 cm) were obtained in T_6. Maximum root mass (51.51g pot^{-1}), dry root mass (7.86 g) was observed in T_7 as compared to control (32.72, 3.06 g pot^{-1}) respectively.

Chemical Parameters

In case of chemical analysis of chickpea grain for nitrogen and phosphorus the same trend was observed like the other parameters. The concentration of nitrogen in chickpea grain increased in those treatments where plant growth promoting rhizobacteria were applied as compare to other treatments. When PGPR combined with salicylic acid the nitrogen contents enhanced as compared to that treatments where only PGPR or SA applied (Figure 7). Similar trend was observed in case of % P contents in chickpea grain (Figure 8).

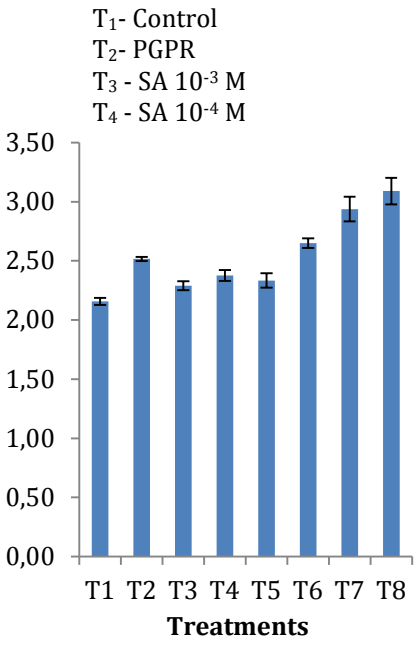


Figure 7. Effect of treatments on N (%) in chickpea grain

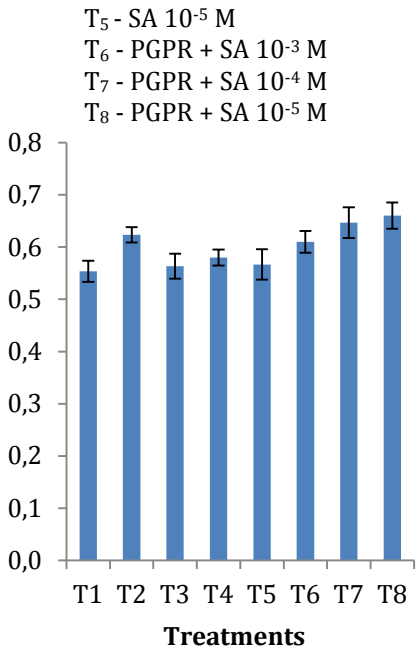


Figure 8. Effect of treatments on P (%) in Chickpea grain

Discussion

According to our results under stress conditions salicylic acid improves the growth and yield of chickpea crop. But when it combines with PGPR it not only improves the growth but also increased the grain yield. All growth and yield parameters improve by the addition of salicylic acid and PGPR. Exogenous application of salicylic acid in combination with PGPR under salt stress conditions increased growth of chickpea. Our findings were similar to Baniaghil et al. (2013) which describes that in canola plant various species of microorganisms such as Azospirillum sp. and Pseudomonas sp. enhanced growth and biomass by altering the oxidative stress enzymes and essential nutrient under saline conditions. Internal ion flux of plants severely affected under salinity stress conditions. According to the results under salinity, the control plants had less nutrient uptake as compared to PGPR and SA application, our findings are similar to the results of (Rojas-Tapias et al., 2012). During mutualistic relationship production of SA improves the growth of plants by initiating induced systemic resistance under all type of stresses whether they are biotic or abiotic (Pozo and Azcón-Aguilar, 2007). There was significant increase in number of nodules and nodular mass was observed by seed treatments with PGPR. The positive response of Rhizobium specie on nodular dry mass and number per plant in chickpea is studied by many researchers (Eusuf Zai et al 1999; Bhuiyaan et al, 2008). Rhizobium inoculation resulted in excellent nodulation in contrast to poor nodulation in control (Khattak et al, 2006). Suryawanshi et al. (2007) observed positive effect of inoculation on nodule number and nodule dry weight. Inoculation studies have showed increase nodule number and nodule dry and fresh weight per plant in chickpea (Verma et al, 2010; Sahai and Chandra, 2010). Similarly, Singh et al. (2014) observed higher number (27.6%) and dry weight (22.2%) of nodules per plant as compared to un-inoculated control in chickpea. Leghaemoglobin, red iron-containing protein, occurs in the root nodules of leguminous plants where it facilitates the diffusion of oxygen to the symbiotic bacteroids in order to promote nitrogen fixation. Moreover, Leghaemoglobin is synthesized by the symbiotic partners viz. the Rhizobia and the host plant. Rhizobium synthesizes the “haem” portion and plant synthesizes the “globine” portion. Lakshmanarao and Singh (1983) evaluated positive relationship between leghaemoglobin content and nitrogen fixation. Tagore et al. (2013) observed higher leghaemoglobin content in the nodular tissue of chickpea inoculated treatment. Further, there was a positive correlation between nitrogenase activity and both number and dry weight of nodules (Miller et al., 1986). Seed inoculation showed significant increase in the nitrogenase activity in contrast to uninoculated control (Dutta and Bandhyopadhyay, 2009). Malik and Sindhu (2011) also reported similar findings in chickpea. Such positive benefits have also been reported by Das et al (2013) which may be attributed to presence of low or useless population of indigenous rhizobia nodulating chickpea in the soil allowing the inoculant strain to form greater portion of effective nodules on plant roots (Parul and Chandra, 2009). There is positive effect observed in all yield parameters by the application of PGPR. Many researchers also reported positive effect of rhizobium inoculation and various

other yield parameters viz number of pods per plant, seeds per pod, thousand seed weight. Numbers of pods per plant were reported to be 21% higher with rhizobium over uninoculated control (Sharar et al., 2000). Further Ali et al., (2004) revealed that thousand seed weight was significantly better with inoculation. The increase in yield components through seed inoculation may be due to higher nodulation and more nutrient availability resulting in increase in plant growth and yield (Namvar et al., 2013). The present investigation suggests positive effect of salicylic acid on plant biomass and grain yield. This may be due to tolerance of plants against stressful environment. Several reports were published in the last decade that represents salicylic acid may play a vital role in signaling for plant resistance to environmental stresses (Hayat et al., 2008).

Increase in nitrogen and phosphorus uptake was observed by the application of PGPR and salicylic acid. These findings are online with those of Tagore et al. (2013) in chickpea. Kumar et al. (2014) were also found significant increase in nutrient contents by the application of rhizobium as comparison to control. İn this study exogenous application of salicylic acid and PGPR increased the chlorophyll contents and carotenoids. Our results are in agreement with the observation of Hayat et al. (2008; 2012). That describe when plants were subjected to environmental stresses chlorophyll a,b and carotenoid concentration was significantly reduced that were overcome under salicylic acid application that enhanced the activity of Rubisco and PEP carboxylase under stress.

Physiological and agronomic characteristics of plants to salinity are controlled by different types of genes, whose expressions are affected by different factors of environment (Foolad, 2004). In exogenous use of SA in the presence of salinity cause higher fresh and dry weight of plants as compared to those without SA treatment. Foliar use of SA on mustard plant results in increasing the tolerance of salinity stress (Ghoulam et al., 2002). In spite of this SA has potential to induce systemic resistance against pathogenic attack in plants.

Conclusion

From the study, it is concluded that exogenous application of salicylic acid along plant growth promoting rhizobacteria has a potential to improve the growth and yield of chickpea under salinity stress. So it is recommended that during salinity plant growth regulator (SA) along with PGPR can be an appropriate approach for better chickpea production.

References

Al-Hakimi, A.M.A., 2006. Counteraction of drought stress on soybean plants by seed soaking in salicylic acid. *International Journal of Botany* 2(4): 421–426.

Ali, H., Khan, M.A., Randhawa, S.A., 2004. Interactive effect of seed inoculation and phosphorus application on growth and yield of chickpea (Cicer arietinum L.) *International Journal of Agriculture and Biology* 6(1): 110-112.

Azooz, M.M., Youssef, M.M., 2010. Evaluation of heat shock and salicylic acid treatments as inducers of drought stress tolerance in Hassawi wheat. *American Journal of Plant Physiology* 5(2): 56–70.

Babu, M.A., Singh, D., Gothandam, K.M., 2012. The effect of salinity on growth, hormones and mineral elements in leaf and fruit of tomato cultivar PKM1. *The Journal of Animal and Plant Sciences* 22(1): 159-164.

Baniaghil, N., Arzanesh, M.H., Ghorbanli, M., Shahbazi, M., The effect of plant growth promoting rhizobacteria on growth parameters, antioxidant enzymes and microelements of canola under salt stress. *Journal of Applied Environmental and Biological Sciences* 3(1):17–27.

Bhuiyaan, M.A.H., Khanam, D., Hossain, M.F. and Ahmad, M.S., (2008). Effect of Rhizobium inoculation on nodulation and yield of chickpea in calcareous soil. *Bangladesh Journal of Agricultural Research* 33(3): 549-554.

Das, S., Pareek, B.L., Kumawat, A., Dhikwal, S.R., 2013. Effect of phosphorus and biofertilizers on productivity of chickpea (Cicer arietinum L.) in north western Rajasthan, India. *Legume Research* 36(6): 511-514.

Dutta, D., Bandyopadhyay, P., 2009. Performance of chickpea (Cicer arietinum L.) to application of phosphorus and bio-fertilizer in laterite soil. *Archives of Agronomy and Soil Science* 55(2): 147-155.

Eusuf Zai, A.K., Solaiman, A.R.M., Ahmed, J.U., 1999. Response of some chickpea varieties to Rhizobium inoculation in respect to nodulation, biological nitrogen fixation and dry matter yield. *Bangladesh Journal of Microbiology* 16: 135-144.

Foolad M.R., 2004. Recent advances in genetics of salt tolerance in tomato. *Plant Cell, Tissue and Organ Culture* 76(2): 101–119.

Ghoulam, C., Foursy, A., Farer, K., 2002. Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Environmental and Experimental Botany* 47(1): 39-50.

Gunes, A., Inal, A., Alpaslan, M., Eraslan, F., Bagci, E.G. and Cicek, N., 2007. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (Zea mays L.) grown under salinity. *Journal of Plant Physiology* 164(6): 728–736.

Hasanuzzaman, M., Nahur, K., Fujita, M., 2013. Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In: Ecophysiology and responses of plants under salt stress. Ahmad, P., Azooz, M.M., Prasad, M.N.V. (Eds.). Springer, New York, USA. pp. 25–87.

Hayat, Q., Hayat, S., Alyemeni, M.N., Ahmad, A., 2012. Salicylic acid mediated changes in growth, photosynthesis, nitrogen metabolism and antioxidant defense system in Cicer arietinum L. *Plant, Soil and Environment* 58(9): 417-423.

Hayat, R., Ali, S., Amara, U., Khalid, R., Ahmed, I., 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of Microbiology* 60(4): 579–598.

Hayat, S., Hasan, S.A., Fariduddin, Q., Ahmad, A., 2008. Growth of tomato (Lycopersicon esculentum) in response to salicylic acid under water stress. *Journal of Plant Interactions* 3(4): 297-304.

Jones, J.B., 2001. Laboratory guide for conducting soil tests and plant analyses. CRC Press, New York, USA. 363p.

Khattak, S.G., Khan, D.F., Shah, S.H., Madani, M.S., Khan, T., 2006. Role of rhizobial inoculation in the production of chickpea crop. *Soil and Environment* 25(2): 143-145.

Kumar, D., Arvadiya, L.K., Kumawat, A.K., Desai, K.L., Patel, T.U., 2014. Yield, protein content, nutrient content and uptake of chickpea (Cicer arietinum L.) as influenced by graded levels of fertilizers and bio-fertilizers. *Research Journal of Chemical and Environmental Sciences* 2(6): 60-64.

Lakshmanarao, K. and Singh, K., (1983). Effect of Phosphorus and biofertilizers on leghaemoglobin and nitrogen fixation of chickpea. *Madras Agricultural Journal* 70: 572-77.

Lichtenthaler, H.K., Wellburn, A.R., 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions* 11(5): 591–592.

Malik, D.K., Sindhu, S.S., 2011. Production of indole acetic acid by Pseudomonas sp.: effect of coinoculation with Mesorhizobium sp. Cicer on nodulation and plant growth of chickpea (Cicer arietinum). *Journal of Physiology and Molecular Bioliology in Plants* 17(1): 25–32.

Miller, J.C., Zary, K.W., Fernandez, G.C.J., 1986. Inheritance of N2 fixation efficency in cowpea. *Euphytica* 35(2): 551–560.

Mohammadi, K., Sohrabi, Y., 2012. Bacterial biofertilizers for sustainable crop production: A review. *ARPN Journal of Agricultural and Biological Science* 7(5): 307-316.

Moussa, H.R., El-Gamel, S.M., 2010. Effect of salicylic acid pretreatment on cadmium toxicity in wheat. *Biologia Plantarum* 54(2): 315–320.

Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology* 59: 651-681.

Namvar, A., Sharifi, R.S., Khandan, T., Moghandam, M.J., 2013. Seed inoculation and inorganic nitrogen fertilization effects on some physiological and agronomical traits of chickpea (Cicer arietinum L.) in irrigated condition. *Journal of Central European Agriculture* 14(3): 881-893.

Parul, B., Chandra, R., 2009. Interaction of Mesorhizobium ciceri and rhizospheric bacteria on nodulation, growth and yield of chickpea. *Journal of Food Legumes* 22(2): 137-139.

Pozo, M.J., Azcón-Aguilar, C., 2007. Unravelling mycorrhiza induced resistance. *Current Opinion in Plant Biology* 10(4): 393–398.

Qureshi, M.A, Shahzad, H., Saeed, M.S., Ullah, S., Ali, M.A., Mujeeb, F., Anjum, M.A., 2019. Relative potential of rhizobium species to enhance the growth and yield attributes of cotton (Gossypium hirsutum L.). *Eurasian Journal of Soil Science* 8(2): 159-166.

Rao, D.L.N., Giller, K.E., Yeo, A.R., Flowers, T.J., 2002. The effects of salinity and sodicity upon nodulation and nitrogen fixation in chickpea (Cicer arietinum). *Annals of Botany* 89(5): 563-570.

Rojas-Tapias, D., Moreno-Galván, A, Pardo-Díaz, S., Obando, M., Rivera, D., Bonilla, R., 2012. Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (Zea mays). *Applied Soil Ecology* 61: 264–272.

Sahai, P., Chandra, R., 2010. Co-inoculation effect of liquid and carrier inoculants of Mesorhizobium ciceri and PGPR on nodulation, nutrient uptake and yields of chickpea. *Journal of Food Legumes* 23(2): 159-161.

Sarwar, M., Martens D.A., Arshad M., Frankenberger Jr., W.T., 1992. Tryptophan dependent biosynthesis of auxins in soil. *Plant and Soil* 147(2): 207-215.

Sharar, M.S. Ayub, M., Choudluy, A., Nadeem, M., 2000. Effect of NP application and inoculation on the growth and yield of gram (Cicer arietinum L.). *Pakistan Journal of Agricultural Science* 37(3-4): 155-157.

Siddique, K.H., Johansen, C., Kumarrao, J.V.D.K., Ali, M., 2005. Legumes in sustainable cropping systems. 4th International Food Legumes Research Conference on "Food Legumes for Nutritional Security and Sustainable Agriculture" 18-22 October 2005. New Delhi, India.

Singh, Y., Singh, B., Kumar, D., 2014. Effect of phosphorus levels and biofertilizer on yield attributes, yield and nutrient uptake of chickpea (Cicer arietinum L.) under rainfed condition. *Research on Crops* 15(1): 90-95.

Sobhanian, H., Razavizadeh, R., Nanjo, Y., Ehsanpour, A.A., Jazii, F.R., Motamed, N., Komatsu, S., 2010. Proteome analysis of soybean leaves, hypocotyls and roots under salt stress. *Proteome Science* 8: 19.

Steel, R.G.D., Torrie, J.H., Dicky, D.A., 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd Edition. McGraw-Hill Book International Co., Singapore. 666p.

Suryawanshi, A.V., Mandhare, V.K. and Jamadagni, B.M., 2007. Influence of Rhizobium strains on nodulation and grain yield in chickpea (Cicer arietinum L.). *Madras Agricultural Journal* 94: 124-126.

Szalai, G., Tari, I., Janda, T., Pestenácz, A., Páldi, E., 2000. Effects of cold acclimation and salicylic acid on changes in ACC and MACC contents in maize during chilling. *Biologia Plantarum* 43(4): 637-640.

Tagore, G.S., Namdeo, S.L., Sharma, S.K., Kumar N., 2013. Effect of Rhizobium and phosphate solubilizing bacterial inoculants on symbiotic traits, nodule leghemoglobin, and yield of chickpea genotypes. *International Journal of Agronomy* Article ID 581627.

Ullah, S., Qureshi, M.A., Ali, M.A., Mujeeb, F., Yasin, S., Comparative potential of Rhizobium species for the growth promotion of sunflower (Helianthus annuus L.). *Eurasian Journal of Soil Science* 6(3): 189 – 196.

Verma, J.P., Yadav, J., Tiwari, K.N., 2010. Application of Rhizobium sp. BHURC01 and plant growth promoting rhizobacteria on nodulation, plant biomass and yield of chickpea (Cicer arietinum L.). *International Journal of Agricultural Research* 5(3): 148-156.

Vessey J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil* 255(2): 571-586.

Eurasian J Soil Sci 2019, 8 (3) 229 - 236

Eurasian Journal of Soil Science

Journal homepage : http://ejss.fesss.org

Wheat growth and nitrogen use efficiency under drip irrigation on semi-arid region

Mohamed Said Awaad *, Tarek H. M. A. Deshesh

Soil, Water and Environment Research Institute (SWERI), Agricultural Research Center, Giza, Egypt

Abstract

Irrigation water is limiting factor for crop production in arid and semi-arid regions. Modern irrigation system such as drip irrigation are widely used in Egypt and also used in other countries especially have limited irrigation water resources. Drip irrigation provides the efficient use of limited water resources with increasing water productivity (WP). Application of nitrogen to wheat is needed to ensure the N availability throughout the growing season due to its important role in promoting both vegetative and reproductive growth. A field experiment was carried out during growing season of 2017/2018 at a private farm located at a newly reclaimed sandy soil at El-Sadat district El-Menofiya governorate , Egypt to study the effect of two nitrogen fertilizer types (ordinary and slow release N fertilizers) as urea 46.5%N and urea formaldehyde (38%N) with four application rates i.e., 0, 40, 60 and 100 kg N fed^{-1} (1 feddan=4200 m^2) combined with drip irrigation moisture depletion from filed capacity (FC) (I1=100% of FC) and (I2=50% of FC) on wheat crop. The results showed that application of water depletion at (I1) through drip irrigation along with 100 kg N fed^{-1}, from two sources of nitrogen recorded the highest yield of straw and grain and the nitrogen content as well as nitrogen use efficiency compared with the other rate and levels of nitrogen and irrigation, respectively. Also, water productivity increased with irrigation I1 FC and nitrogen levels and reached the highest values at 100 kg N fed^{-1} as fertigated urea compared with urea formaldehyde as slow release fertilizer.

Article Info

Received : 01.10.2018
Accepted : 05.05.2019

Keywords: Wheat, drip irrigation, nitrogen fertilization, urea formaldehyde, Nitrogen use efficiency, water productivity.

Introduction

Crops production in arid and semi-arid regions was affected by water scarcity and plants nutrients private nitrogen nutrient. About one third of the developing world's wheat (*Triticum aestivum* L.) area is located in environments that are regarded as marginal for wheat production because of drought and heat factors. Despite these limitations, the world's dry and difficult cropping environments are increasingly crucial to food security in the developing world. For example, it has been reported that 32% of the 99 million hectares of wheat grown in developing countries experiences varying levels of drought stress (Rajaram et al., 1996). In recent years, however, growing competition for scarce water resources has led to applying modified techniques for maximizing water productivity and improving crop yields and quality, particularly in arid and semi arid regions as like in Egypt. Modern irrigation systems such as drip irrigation are widely used in Egypt and also in other countries have limited irrigation water resources. Drip irrigation provides the efficient use of limited water with increasing water productivity (Viswanatha et al., 2002). Little technically, economically and environmentally feasible studies had been focused on the application possibility of the alternative drip irrigation systems (surface and subsurface drip); an evaluation and implementation consideration exists under intensive field crop conditions, which had been carried out by Alam et al. (2018). El-Boraie (2004) showed that in arid regions as Egypt, where irrigation is essential for crop production, improving

* Corresponding author.
Soil, Water and Environment Research Institute (SWERI), Agricultural Research Center, Giza, Egypt
Tel.: +201006988461 E-mail address: moayamai@yahoo.com
e-ISSN: 2147-4249 DOI: 10.18393/ejss.566537

management of irrigation water may yield substantial water saving, which can be used for agriculture horizontal expansion. One of the best approaches to achieve good water management program is knowing the amount of actual evapotranspiration (ETa) or crop consumptive use.

Wheat (*Triticum aestivum* L.) was mainly crop, occupy 75% of agricultural areas and directly contribute to the food security of the country. Recently, in Egypt cultivated the wheat crop in sandy soils, therefore the irrigation scheduling is a key factor to help farmers increase crop yield and save water regarding limited water resources. El-Rahman (2009) found that high irrigation water productivity (WP) for wheat could be achieved by saving irrigation rates under drip system. Application of nitrogen to wheat is needed to ensure the N is available throughout the growing season due to its important role in promoting both vegetative and reproductive growth. High yielding wheat, especially new varieties need high and regular supply N to develop high photosynthetic capacity and maintain the proper nitrogen concentration in the leaves so that CO_2 assimilation is not affected when large rates are required for ear growth and grain filling period Lawlor (1995). Increasing cultivated for wheat production of unit land area are the most important national objectives in Egypt for minimizing the gap between the production and population consumption. That could be achieved by improving agricultural practices especially in desert area such as irrigation and fertilizers.

The objectives of this study were to improve wheat grain yield and nitrogen use efficiency under different irrigation water depletion conditions by using the different nitrogen fertilizer type's and rates, and also to asses soil nitrate distribution in the different soil layers under drip irrigation method.

Material and Methods

A field experiment was conducted during winter growing seasons of 2017/2018 at a private farm located at a newly reclaimed sandy soil at, El-Sadate district El-Menofiya governorate, Egypt (Latitude, 31°15'25" and N latitude, 30°01'4" E longitude). The climate of the experimental site is semi-arid (Table 1). The main chemical and physical soil characteristics presented in Table 2 were determined according to Dewis and Freitas (1970) and Klute (1986).

Table 1. Weather data during the experiment period 2017- 2018.

Month	Min Temp., °C	Max Temp., °C	Humidity, %	Wind, m/s	Sun, hours	Rad, MJ/m^2/day	ETo, mm/day
October	20.80	27.10	60.00	2.40	11.30	20.20	3.95
November	16.00	25.10	59.00	2.20	10.40	16.10	3.40
December	10.60	17.80	63.00	2.90	10.00	14.40	2.80
January	6.70	17.50	67.00	2.10	11.60	16.80	2.90
February	8.90	19.00	67.00	1.90	11.00	19.00	2.94
March	13.50	21.30	61.00	2.40	11.80	23.30	4.12
April	13.50	22.90	60.00	3.70	12.80	27.50	4.70
Average	12.86	21.53	63.29	2.51	11.27	19.61	

The experiment was conducted in a split block design with eight treatments. Two moisture depletion were applied as irrigation-1 (I1) at 100% of FC and irrigation-2 (I2) at 50% of FC on the main plots with four N rates from two types of nitrogen fertilizers on the sub-plots. Each treatment was replicated three times under drip irrigation system. Distance between irrigation laterals was 1 m, distance between drippers was 35 cm, and the rate of dripper discharge was 4 liters h^{-1}, and number of drippers per feddan (4200 m^2) was 12000 (48 m^3 h^{-1}). Wheat (Triticum aestivum, CV Giza 168) was sown on 20 November 2009 and harvested 10 April 2010 a seeding rate of 70 kg fed^{-1} with 10 cm row spacing. Phosphorus and potassium were applied during land preparation in the form of single superphosphate and potassium sulfate at the rate of 15 kg P_2O_5 fed^{-1} and 24 kg K_2O fed^{-1}, respectively. Also 20 m^3 fed^{-1} farmyard manure (FYM) was incorporated in all plots during land preparation. Also, nitrogen fertilizers for ordinary or slow release types as urea 46%N and urea-formaldehyde (UF) 38% N were applied as N fertilizer sources, respectively. During time of sowing UF fertilizer was mixed with the upper 20 cm of soil surface, while urea was applied with irrigation at different stages of growth up to 75 days after sowing. The whole plot factors were two irrigation treatments (I1 and I2) in the main plots, and eight N treatments including a combination of two N sources (urea and urea-formaldehyde) with four N rates (0, 40, 60 and 100 kg N fed^{-1}) in the sub plots. At maturity, one square meter was selected randomly from each plot and subjected to determine some wheat growth parameters (number of spikes per m^2, plant height (cm), number of tillers per plant, biological, grain and straw yields and weight of 1000 grains). Nitrogen and water productivity was calculated as the ratio of grain yield, kg fed^{-1} to amount of applied N units and total water quantity concerned in m^3 per fed, respectively (Huggins and Pan, 1993; Hussain and Al-Jaloud, 1995).

Table 2. Some main physical, chemical and nutritional characteristics of the experimental soil at initial state.

Soil characteristics	Value	Soil characteristics	Value
Sand, %	92.10	Organic matter content, %	0.08
Silt, %	2.20	pH (1: 2.5)	7.50
Clay, %	5.70	ECe , dS m^{-1}	1.55
Textural class	Sand	Available N, mg kg^{-1}	18.70
Bulk Density (BD), g cm^{-3}	1.62	Available P, mg kg^{-1}	2.81
Saturation (SP), %	18.00	Available K, mg kg^{-1}	40.00
Field capacity (FC), %	5.42	Available Fe, mg kg^{-1}	3.60
Wilting point (WP), %	2.06	Available Mn, mg kg^{-1}	0.52
Available water (AW), %	3.36	Available Zn, mg kg^{-1}	0.45
$CaCO_3$, %	1.40		

Soil water content at different depths (from soil surface to 0.80 m) was measured using gravimetric method. Soil samples were taken and extracted at the harvest of wheat plants from all treatments at the depths of 0-20, 20-40, 40-60 and 60-80 cm, in order to determine NO_3. Field capacity (FC) was determined by estimating soil moisture content. for 0-20, 20-40, 40-60 and 60-80 cm soil depths. The fresh weight of the soil samples was immediately recorded with a portable weighing balance. The soil samples were placed in an oven for 24h at 105°C. The dry weight of the samples was then recorded for drip irrigation treatments. Based on the soil water balance method in which the drainage and runoff were neglected, the net irrigation depth was estimated by using soil moisture contents. Water productivity (WP, kg/mm) was calculated by dividing the total grains yield (kg $fed.^{-1}$) by total water applied.

Results and Discussion

Effect on wheat attributes

The data cited in Table 3 show a significant effect of irrigation and nitrogen fertilization on plant height, number of spikes per m^2, spike length (cm) and weight of 1000 grains. The results indicated that the highest values of the above mentioned attributes were obtained by soil moisture depletion at 100% from FC (I-1) compared with the limited available water at 50% from FC (I-2).

Table 3. Effect of different nitrogen sources and moisture content on some growth parameters of wheat plants

Treatments				Plant	Number of	Spike	Weigh of 1000
Irrigation	N-sources	N-Rate, kg fed^{-1*}		height, cm	spikes/m^2	length, cm	grain, g
100% of FC (I-1)	Urea	0		70.43	154.00	7.30	32.00
		40		75.33	180.00	8.00	34.00
		60		77.90	200.98	8.40	34.90
		100		90.00	230.98	8.50	35.87
			Mean	78.42	191.49	8.05	34.19
100% of FC (I-1)	UF	40		79.12	200.00	8.00	35.22
		60		87.14	260.76	9.90	37.12
		100		99.89	290.97	12.00	38.11
			Mean	88.72	250.58	9.97	36.82
50% of FC (I-2)	Urea	0		67.00	132.00	6.20	31.00
		40		70.39	160.00	7.30	33.00
		60		74.09	176.00	7.80	33.76
		100		75.21	190.00	8.10	34.88
			Mean	71.67	164.50	7.35	33.16
50% of FC (I-2)	UF	40		69.99	158.00	7.20	33.81
		60		71.09	160.00	7.70	33.50
		100		72.90	185.90	7.70	33.89
			Mean	71.33	167.97	7.53	33.73
$LSD_{0.05}$ (Irrigation)				0.38	0.23	0.70	0.76
$LSD_{0.05}$ (N- sources)				NS	0.65	0.24	0.01
$LSD_{0.05}$ (Irrigation x N- sources)				0.01	0.01	0.01	0.01

*1 feddan=4200 m^2

In the case of the effect of nitrogen levels and sources the results indicated that the highest values of the same parameters were obtained from the application of urea-formaldehyde at rate of 100 kg N fed^{-1} with irrigation (I-1). At the same time the lowest values were related to the lack of nitrogen and irrigation. Slow release nitrogen fertilizers were compared with the other soluble and the scientists emphasized their

superiority in increasing yield and its components of many crops Hamdallah et al. (1988). The superior of slow release than ordinary urea can be attributed to the slow release of N to meet the plant's requirement, where it has a low dissolution rate than the others which reduces nitrogen loss from soil profile and gives a chance for more uptake by plant root.

Biological, grain and straw yields and harvest index

Results presented in Table 4 indicated that straw, grain, and biological yields as well as harvest index of wheat plants were significantly affected by increasing rates of the two nitrogen types with irrigation treatments. Wheat growth in soil amended with 40 and 60 kg N fed^{-1} as slow release (urea-form) fertilizer with irrigation at 100% of FC (I-1) and 50% of FC (I-2) treatments had the highest value compared with the control (without N fertilization) and urea treatments. Also, I-1 recorded the highest value of measured yield parameters compared to I-2 treatment. These results may be reflects moisture status in root zone of plants. With increasing moisture content to near the field capacity, it may reducing the occurrence of water stress on plants especially during the reproduction stage of growth. This could be due to the increase in the available soil moisture content, which enhance nutrients uptake and increasing photosynthetic metabolic translocations from leaves to grain also, slow release nitrogen fertilizer may help reduce nitrate leaching and increase nitrogen use efficiency.

Table 4. Effect of different nitrogen source and moisture content on straw, grain, biological yields and harvest index of wheat plants

Treatments			Straw yield,	Grain ield,	Biological yield	Harvest
Irrigation	N-sources	N-Rate, kg fed^{-1*}	kg fed^{-1}	kg fed^{-1}	kg fed^{-1}	Index, %
100% of FC (I-1)	Urea	0	636.00	400.00	1036.00	62.89
		40	994.00	644.00	1638.00	64.78
		60	1271.00	890.98	2161.98	70.10
		100	1585.00	1265.00	2161.98	79.81
		Mean	1121.5	800.00	1749.49	69.40
100% of FC (I-1)	UF	40	1189.45	789.45	1978.90	66.37
		60	1200.11	987.11	2187.22	82.25
		100	2072.00	1678.09	3750.09	80.98
		Mean	1487.19	1151.55	2638.74	76.53
50% of FC (I-2)	Urea	0	564.00	332.00	896.00	58.86
		40	825.00	475.00	1300.00	57.57
		60	1099.00	690.93	1789.93	62.86
		100	1180.00	884.78	2064.78	74.98
		Mean	917.00	683.57	1718.24	65.14
50% of FC (I-2)	UF	40	598.00	390.87	988.87	65.36
		60	909.00	588.98	1497.98	64.79
		100	1034.00	743.87	1777.87	71.94
		Mean	847.00	574.57	1421.57	67.36
$LSD_{0.05}$ (Irrigation)			0.69	0.26	0.43	0.32
$LSD_{0.05}$ (N- sources)			0.01	0.46	0.56	0.01
$LSD_{0.05}$ (Irrigation x N- sources)			0.01	0.01	0.01	0.23

*1 feddan=4200 m^2

Also, the highest value of wheat yield attributes was obtained when the ultimate level of N was applied (Table 4). It is clear from these data that N fertilization to wheat enhanced plants vegetative growth which increased photosynthetic activity and the metabolites required to produce wide and heavy panicles. It worth to mentioned that at high rate (100 kg N fed^{-1}) of urea formaldehyde with I-2 deficit irrigation gave the lowest yield production compared with the same rate fertilized with urea. These results may be attributed to low soil moisture content causing slow nitrogen mineralization from urea formaldehyde due to limited microbial activity thus lowering the N availability (Paramasivam and Alva, 1997) correspondingly the emission of ammonia by volatilization was usually reduced when moisture content increased. These results may be due to that sandy soil characterized by the limited water holding capacity and high nutrient leaching losses. Hanafi et al. (2002) reported that the uncoated compound fertilizer such as urea gave significantly higher amounts of nutrients loss compared to slow release N fertilizer. Zeidan and El Kramany (2005) found that the use of slow release nitrogen fertilizer increased grain yield of wheat compared with other nitrogen sources.

Nitrogen concentration, N-uptake and protein content of grains were significantly affected by all interactions between nitrogen source and irrigation intervals treatments under study (Table 5). In general, the highest nitrogen concentration, nitrogen uptake and protein content of grains were obtained when irrigated at I-1 using urea formaldehyde as nitrogen sources and receiving 100kg N fed^{-1}. Guo et al. (2007) found that water productivity of plants may be increased by nitrogen management, as the localized ammonium application by Cultan-technique or the releasing fertilizers in combination with nitrification inhibitors.

Table 5. Effect of different nitrogen sources and moisture content on N concentration, uptake, protein content and grain nitrogen recovery(GNR).

Treatments			N-concentration, %	N-uptake by grain kg fed^{-1}	Protein content, %	GNR
Irrigation	N-sources	N-Rate, kg fed^{-1}				
100% of FC (I-1)	Urea	0	0.88	3.68	5.50	----
		40	1.10	7.08	6.87	8.50
		60	1.44	12.83	9.00	15.25
		100	1.61	20.36	10.06	16.68
		Mean	1.26	10.99	7.86	10.11
100% of FC (I-1)	UF	40	1.71	13.49	10.68	24.52
		60	1.94	19.14	12.12	25.76
		100	2.46	41.28	15.37	37.60
		Mean	2.04	24.64	12.72	29.29
50% of FC (I-2)	Urea	0	0.82	2.72	5.13	----
		40	1.00	4.75	6.25	5.07
		60	1.40	9.67	8.75	11.58
		100	1.48	13.09	9.25	10.37
		Mean	1.18	7.56	7.35	6.76
50% of FC (I-2)	UF	40	1.42	5.55	8.87	7.07
		60	1.64	9.65	10.25	11.55
		100	2.12	15.77	13.25	13.05
		Mean	1.73	10.32	10.79	10.56
$LSD_{0.05}$ (Irrigation)			0.70	0.54	0.51	0.33
$LSD_{0.05}$ (N- sources)			NS	0.01	0.01	0.01
$LSD_{0.05}$ (Irrigation x N- sources)			0.32	0.01	0.01	0.01

*1 feddan=4200 m^2

Grain nitrogen recover (GNR)

The effect of different nitrogen types and soil moisture content on nitrogen use efficiency (NUE) was shown in Table 5. Application of urea formaldehyde as a slow N fertilizer increased N-use efficiency of wheat grains as compared to the ordinary (fast release) urea. The interaction between irrigation intervals and nitrogen sources on NUE, the obtained results show that, the highest value of NUE was recorded from treatments received urea formaldehyde at rate of 100kg N fed^{-1} under irrigation by I-1 moisture content. These results are in agreement with those obtained by Amal et al. (2007). They concluded that slow release N fertilizer was long-term effects including reduced leaching losses and enhanced N-uptake as well as positive effects on both health and soil nutrient levels. Koivunen and Horwath (2005) evaluating winter wheat, as influenced by a coated urea, showed that maximum yield and NUE were grater with coated urea versus common urea.

Residual soil nitrogen

Distribution of nitrate (NO_3) in four depths (0-20), (20-40), (40-60) and (60-80) cm after harvest of wheat plants for different nitrogen sources and rates as well as two irrigation treatments can be seen in Table 6. The data show that in general high amounts of nitrogen existed in the upper depth (0-20 cm) of the soil and it decreased gradually with the soil depth up to 60-80 cm.

Results also indicated that the concentration of nitrate in different soil depths were significantly higher in the fertigated urea than in the urea formaldehyde ones at different irrigation levels. The highest value of NO_3-N was obtained at high rate of N as urea with irrigation I-1 for depth (60-80 cm), while the lowest value of NO_3-N was obtained in urea formaldehyde at a rate of 40 kg N fed^{-1} with irrigation I-1 at depth 40-60 cm. Likewise, excessive application of irrigation water can produce accelerated downward movement of NO_3 -N especially when the application of nitrogen in the form fast releases urea. Also, it's worth to notice, that the concentration of NO_3-N decreased markedly in the 80 cm of soil depth due to application of urea formaldehyde with two levels of irrigation, suggesting that there was not large amounts of NO_3-N leached to

ground water. These results are in harmony with Chikowo et al. (2004) who found that when use different mineral N concentrations at different sampling dates to determine N leaching per soil layer and for the whole soil profile. Nitrate leaching losses are directly associated with percolation of water and fertilizer application and solubility. In potatoes, controlled release nitrogen (CRN) produced less nitrate leaching, greater fertilizer-N recovery, and greater marketable yields than split applications (Zvomuya et al., 2003). It is important to note that the concentration of NO_3, besides being consistently lower under slow release fertilizer (urea formaldehyde) than under nitrogen fertilizer as urea by chemigation in the deep soil depth (60-80 cm). Zvomuya et al. (2003) found that a single application of poly coated urea improved recovery of N and reduced NO_3 leaching compared with three applications of urea. Similar results were reported by Waddell et al. (2000) comparing SCU with urea treatment. Also, Wen et al. (2001) indicated that a field study on sandy soil on peanuts yields were 81 to 137% higher with a mixture of resin-coated N fertilizers and nitrogen recovery was 79 to 94 % with the coated N and only 10 to 32% with ammonium sulfate. The highest recoveries were associated with the matched N release of coated fertilizer and the crop N uptake and the fertilizer placement.

Table 6. Effect of different nitrogen sources and moisture content on N concentration in different depths.

Treatments			Soil depths (cm)			
Irrigation	N-sources	N-Rate kg fed^{-1}	0-20	20-40	40-60	60-80
			NO_3- N (mg kg soil^{-1})			
100% of FC (I-1)	Urea	0	8.50	5.21	5.10	4.32
		40	18.90	12.45	14.99	22.21
		60	23.98	15.99	18.94	25.68
		100	33.21	21.99	23.78	44.88
		Mean	21.15	13.91	15.70	24.27
100% of FC (I-2)	UF	40	12.43	10.00	9.80	14.87
		60	15.88	12.00	10.74	18.43
		100	17.34	15.98	15.87	23.43
		Mean	15.22	12.66	12.14	18.91
50% of FC (I-1)	Urea	0	9.92	7.21	4.21	4.11
		40	24.81	20.00	15.99	11.23
		60	28.09	23.90	17.23	12.90
		100	32.11	27.90	20.11	15.99
		Mean	23.73	19.75	14.39	11.06
50% of FC (I-2)	UF	40	11.87	6.90	5.11	5.12
		60	14.21	11.56	11.00	12.11
		100	16.21	13.75	12.90	14.23
		Mean	14.10	10.74	9.67	10.49
LSD $_{0.05}$ (Irrigation)			0.30	0.24	0.21	0.33
LSD $_{0.05}$ (N- sources)			0.32	0.01	0.01	0.01
LSD $_{0.05}$ (Irrigation x N- sources)			0.32	0.01	0.01	0.01

Water productivity (WP)

The results in Table 7 showed that there were significant differences in WP of wheat plants due to soil application of nitrogen fertilizers and the highest value of WP was recorded with nitrogen application as ureaformaldhyde when compare to the urea ones. Concerning the effect of different irrigation levels, the mean values of water productivity at 50% from FC were highest when compared to the highest level of water application. Also, the interaction between amount of irrigation water and form of nitrogen fertilizers the data showed that the highest values of WP were recorded by application of urea with 50% of FC.

Conclusion

Applying nitrogen from a slow release fertilizer provides an efficient way to increase the N use efficiency and to minimize NO_3-N leaching as well as to prevent environmental pollution by the excess of nitrogen in the soil profile. Also managing soil moisture content under drip irrigation to 100% from soil FC enhanced lowering nitrogen deep movement and increased wheat productivity. It may be concluded that a slow release N fertilizer with a rate of 100 kg N fed^{-1} can be applied to cultivate wheat with using drip irrigation at 100% of FC under semi-arid conditions.

Table 7. Effect of different nitrogen sources and moisture content on WP.

Treatments			Grain Yield	Applied water, mm	WP
Irrigation	N-sources	N-Rate, kg fed^{-1}			
100% of FC (I-1)	Urea	0	400.00	3.68	1.11
		40	644.00	7.08	1.79
		60	890.98	12.83	2.47
		100	1265.00	20.36	3.51
		Mean	800.00	10.99	2.22
100% of FC (I-1)	UF	40	789.45	13.49	2.19
		60	987.11	19.14	2.74
		100	1678.09	41.28	4.66
		Mean	1151.55	24.64	3.20
50% of FC (I-2)	Urea	0	332.00	2.72	1.84
		40	475.00	4.75	2.64
		60	690.93	9.67	3.84
		100	884.78	13.09	4.92
		Mean	595.68	7.56	3.31
50% of FC (I-2)	UF	40	390.87	5.55	2.17
		60	588.98	9.65	3.27
		100	743.87	15.77	4.13
		Mean	574.57	10.32	3.19
LSD $_{0.05}$ (Irrigation)					0.14
LSD $_{0.05}$ (N- sources)					0.01
LSD $_{0.05}$ (Irrigation x N- sources)					0.01

References

Alam, M., Trooien, T., Stone, S., Rogers, D., 2018. Subsurface drip irrigation for Alfalfa. K-State Research and Extension, Irrigation Management Series. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, USA. Available at [Access date: 01.10.2018]: https://itc.tamu.edu/files/2018/05/MF2867.pdf

Amal, G.A., Zaki, N.M., Hassanein, M.S., 2007. Response of grain sorghum to different nitrogen sources. *Research Journal of Agriculture and Biological Sciences* 3(6): 1002-1008.

Chikowo, R., Mapfumo, P., Nyamugafata, P., Giller, K.E., 2004. Mineral N dynamics, leaching and nitrous oxide losses under maize following two-year improved fallows on a sandy loam soil in Zimbabwe. *Plant and Soil* 259(1-2): 315-330.

Dewis. J., Freitas, F., 1970. Physical and chemical methods of soil and water analysis. FAO Soils Bulletin No.10, Rome, Italy. 275p.

El-Boraie, F.M., 2004. Modeling of water requirement for Egyptian and Sudanese pearl millet. PhD Thesis. Institute of African Research and Studies, Natural Resources, Cairo University, Egypt.

El-Rahman, G.A., 2009. Water use efficiency of wheat under drip ırrigation systems at Al-Maghara Area, North Sinai, Egypt. *American-Eurasian Journal of Agriculture and Environmental Science* 5(5): 664-670

Guo, S., Zhou, Y., Shen, Q., Zhang, F., 2007. Effect of ammonium and nitrate nutrition on some physiological processes in higher plants - growth, photosynthesis, photorespiration, and water relations. *Plant Biology* 9(1): 21-29.

Hamdallah, G.M., Devi Prasad, J., Al-Hendi, A.A., 1988. Crop response to sulfur-coated urea (SCU) and other N-carriers in sandy soils of Saudi Arabia. *Fertilizer Research* 15(1): 47-54.

Hanafi, M.M., Eltaib, S.M., Ahmed, M.B., Omer, S.R.S., 2002. Evaluation of controlled-release compound fertilizers in soil. *Communications in Soil Science and Plant Analysis* 33(7-8): 1139-1156.

Huggins, D.R., Pan, W.L., 1993. Nitrogen efficiency component analysis: An evaluation of cropping system differences in productivity. *Agronomy Journal* 85(4): 898-905.

Hussain, G., Al-Jaloud, A.A., 1995. Effect of irrigation and nitrogen on water use efficiency of wheat in Saudi Arabia. *Agricultural Water Management* 27(2): 143–153.

Klute, A.A., 1986. Methods of soil analysis. Part 1 & 2. American Society of Agronomy & Soil Science Society of America, Madison, Wisconsin USA.

Koivunen, M.E., Horwath, W.R., 2005. Methylene urea as a slow-release nitrogen source for processing tomatoes. *Nutrient Cycling in Agroecosystems* 71(2): 177-190.

Lawlor, D.W., 1995. The effects of water deficit on photosynthesis. In: Environment and Plant Metabolism. Smirnoff, N. (Ed.). Bios Scientific Publishers, Oxford, pp. 129–160.

Paramasivam, S., Alva, A.K., 1997. Nitrogen recovery from controlled-release fertilizers under intermittent leaching and dry cycles. *Soil Science* 162(6): 447-453.

Rajaram, S., Braun, H.J., van Ginkel, M., 1996. CIMMYT's approach to breed for drought tolerance. *Euphytica* 92(1-2): 147-153.

Viswanatha, G.B. , Ramachandrappa, B.K., Nanjappa, H.V., 2002. Soil–plant water status and yield of sweet corn (*Zea mays* L. cv. Saccharata) as influenced by drip irrigation and planting methods. *Agricultural Water Management* 55(2): 85-91.

Waddell, J.T., Gupta, S.C., Moncrief, J.F., Rosen, C.J., Steele, D.D., 2000. Irrigation and nitrogen-management impacts on nitrate leaching under potato. *Journal of Environmental Quality* 29(1): 251-261.

Wen, G.T., Mori, T., Yamamoto, J., Chikushi, J., Inoue, M., 2001. Nitrogen recovery of coated fertilizers and influence on peanut seed quality for peanut plants grown in sandy soil. *Communications in Soil Science and Plant Analysis* 32(19-20): 3121-3140.

Zeidan, M.S., El Kramany, M.F., 2005. Effect of organic manure and slow – release N fertilizers on the productivity of wheat (*Triticum Aestivum* L.) in sandy soil. *Acta Agronomica Hungarica* 49(4): 379-385.

Zvomuya, F., Rosen, C.J., Russelle, M.P., Gupta, S.C., 2003. Nitrate leaching and nitrogen recovery following application of polyolefin-coated urea to potato. *Journal of Environmental Quality* 32(2): 480-489.

Eurasian J Soil Sci 2019, 8 (3) 237 - 248

Eurasian Journal of Soil Science

Journal homepage : http://ejss.fesss.org

Soil fertility assessment and mapping spatial distribution of Agricultural Research Station, Bijayanagar, Jumla, Nepal

Dinesh Khadka [a,*], Sushil Lamichhane [a], Rita Amgain [a], Sushila Joshi [a], Shree P. Vista [b], Kamal Sah [a], Netra H. Ghimire [b]

[a] Soil Science Division, NARC, Khumaltar, Lalitpur, Nepal
[b] Agricultural Research Station, NARC, Bijayanagar, Jumla, Nepal

Abstract

Knowledge about the soil fertility status and mapping their spatial distribution play a crucial role for sustainable planning of particular area. Thus, a study was conducted to assess the soil fertility status of the Agricultural Research Station, Bijayanagar, Jumla, Nepal. The farm is situated at the latitude 29.273656°N and longitude 82.180967°E as well altitude 2370masl. The total 18 samples were collected randomly at a depth of 0-20 cm by using soil sampling auger. A GPS device was used for determination of geographical position of soil sampling points. The collected samples were analyzed following standard analytical methods in the laboratory of Soil Science Division, Khumaltar. The Arc-GIS 10.1 software was used for the soil fertility distribution mapping. The observed data revealed the structure was sub-angular blocky, whereas colour were dark grayish brown and very dark brown. The sand, silt and clay content were ranged 27-47%, 33.10-61.10% and 11.90-23.90%, respectively and categorized loam and silt loam in texture. The soil pH was moderately acidic to moderately alkaline (5.45-7.66) and very low in available boron (0.01-0.28 mg/kg) and sulphur (0.59-6.23 mg/kg). Moreover, very low to very high available iron (15.90-300.50 mg/kg), very low to high available manganese (1.46-12.88) and low to high organic matter (2.07-6.53%). Similarly, medium to high total nitrogen (0.14-0.23%), available potassium (40-255 mg/kg) and zinc (1.12-8.26 mg/kg). Correspondingly, high available calcium (1632-2880 mg/kg) and magnesium (98-456 mg/kg), and very high available phosphorus (64.2-257.2 mg/kg) and copper (2.58-12.16 mg/kg). The determined soil test data can be used for sustainable soil management as well as developing future research strategy in the farm.

Keywords: ARS, Jumla, ordinary kriging, soil fertility maps, soil testing, sustainable soil management.

Article Info

Received : 27.10.2018
Accepted : 13.05.2019

Introduction

Soil is an important natural dynamic body to give life to all living things in the World (Jones, 2012). Fertile and productive soil proliferate life whereas, unfertile and unproductive soil brings hunger and famines. Soil fertility mangement have great challenge now days because of various intrinsic and extrinsic factors. In Nepal, people do not think about the sustainable management of soil for long-term benefit, while people only think for short-term benefit. During crop growth they do not think about how much element present in the soil, and how much amount should have to apply. In most of the sites of Nepal, soil organic matter is in extremely critical as well very acidic due to unmanaged farming (Khadka et al., 2016, 2017, 2018).

The soil fertility evaluation is the most basic decision making tool in order to efficient plan of a particular land use system (Havlin et al., 2010). There are several techniques for the evaluation of soil fertility status.

* Corresponding author.
Soil Science Division, NARC, Khumaltar, Lalitpur, Nepal
Tel.: +977 015521149
E-mail address: dinesh.khadka92@gmail.com
e-ISSN: 2147-4249
DOI: 10.18393/ejss.566551

Among them soil testing is a most popular everywhere, as well as more appropriate also. Soil testing provides information regarding nutrient availability in soils which forms the basis for the fertilizer recommendations for economic production of crops. Soil analysis includes physical properties (texture, structure, colour, bulk density etc.) and chemical properties (soil pH, organic matter, macro and micronutrients etc.), which symbolize prerequisite for sustainable soil management (Panda, 2010). Among them some physical parameters can be determined in the field, while most of the chemical parameters should have to analyze in the laboratory.

Soil properties vary spatially from a small to larger area might be due to effect of intrinsic (parent materials and climate) and extrinsic factors such as soil management practices, indigenous fertility status, crop rotation and nature of standing crop (Cambardella and Karlen, 1999). Describing the spatial variability of soil fertility across a field has been difficult until new technologies such as Global Positioning Systems (GPS) and Geographic Information Systems (GIS) were introduced. Collection of soil samples by using GPS is very important for preparing thematic soil fertility maps (Mishra et al., 2013). Similarly, Geographical Information System (GIS) is a potential tool used for easy access, retrieval and manipulation of voluminous data of natural resources often difficult to handle manually. It facilitates manipulation of spatial and attributes data useful for handling multiple data of diverse origin (Mandal and Sharma, 2009). Based on the geo-statistical analysis, several studies have been conducted to characterize the spatial variability of different soil properties (Huang et al., 2007; Weindorf and Zhu, 2010; Liu et al., 2013). Among the different geo-statistical methods, ordinary kriging is widely used to map spatial variation of soil fertility because it provides a higher level of prediction accuracy (Song et al., 2013).

Nepal Agricultural Research Council (NARC) was established to strengthen agriculture sector in the country through agriculture research. Agricultural Research Station, Bijayanagar, Jumla is an important wing among the research farms of NARC, in order to generate appropriate agriculture production technologies for western high hills of Nepal. This area is also the most food insecure site of Nepal (Acharya et al., 2018). However, Information on soil fertility status and mapping their spatial distribution for Agricultural Research Station, Bijayanagar, Jumla are not done yet. Therefore, it is important to investigate the soil fertility status and mapping their spatial distribution, thus may provide valuable information relating agricultural research strategy development. Considering this, the present study was initiated with the objective to assess the soil fertility status as well as their spatial distribution in the Agricultural Research Station, Bijanagar, Jumla, Nepal.

Material and Methods

Study Area

The study was carried out at Agricultural Research Station, Bijaynagar, Jumla, Nepal (Figure 1). The research station is located within the latitude 29.271627°N to 29.273656°N and longitude 82.179565°E to 82.180967°E as well altitude 2337 masl to 2370 masl. The farm is situated in the headquarter of Jumla district as well on the way of Karnali highway (Surkhet-Jumla road). The average temperature varies from 18^0C to 30^0C in summer and -14^0C to 8^0C in winter and the annual average rainfall is 1343 mm. Rice, wheat, maize, barley, bean, buckwheat, amaranthus and millets are the major crops grown in the farm (ARS, 2017).

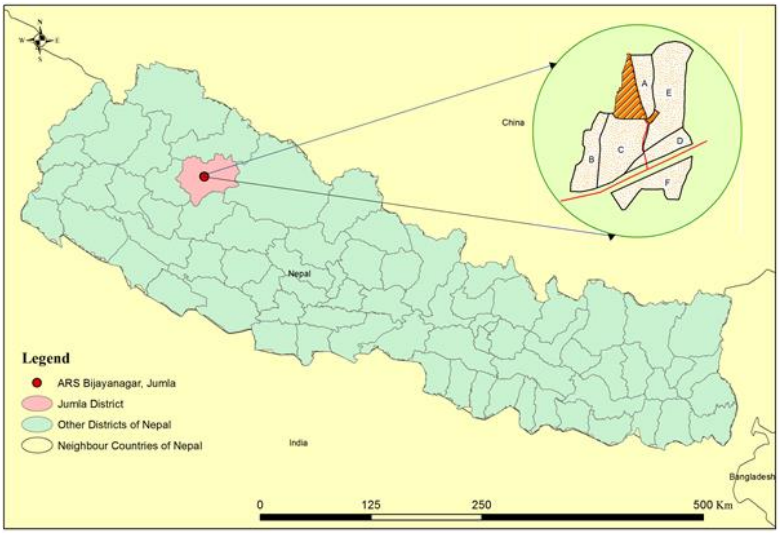


Figure 1. Location Map of Agricultural Research Station, Bijyanagar, Jumla, Nepal

Soil Sampling

Surface soil samples (0-20 cm depth) were collected from different sites of Agricultural Research Station, Bijaynagar, Jumla, Nepal during April 2017. The total 18 soil samples were collected from the research farm by using soil sampling auger (Figure 2). The exact locations of the samples were recorded using a handheld GPS receiver. The random method based on the variability of the land was used to collect soil samples.

Laboratory Analysis

The collected soil samples were analyzed at laboratory of Soil Science Division, Khumaltar. The different soil parameters tested as well as methods adopted to analyze is shown on the Table 1.

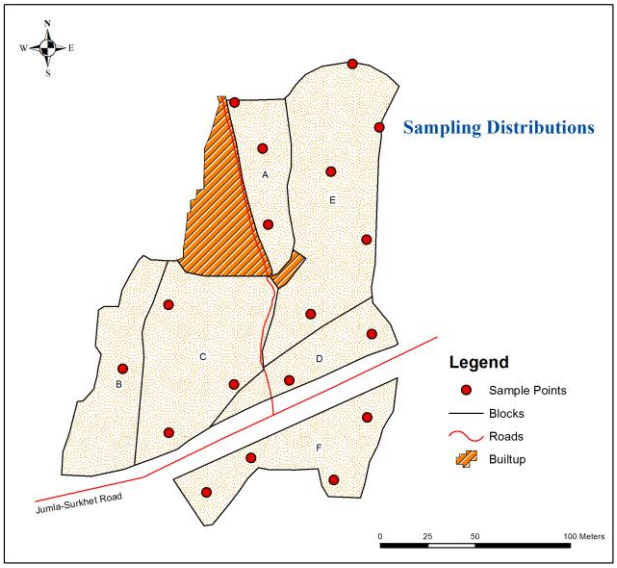


Figure 2. Soil sample distribution during soil sampling in the study area

Table 1. Parameters and methods adopted for the laboratory analysis at Soil Science Division, Khumaltar

	Parameters	Unit	Methods
Physical	Soil texture		Hydrometer (Bouyoucos, 1927)
	Soil colour		Munshell-colour chart (Munsell, 2009)
	Soil structure		Field-feel
	Bulk Density		Core (Keen and Raczkowaski, 1921)
Chemical	Soil pH		Potentiometric 1:2 (Jackson, 1973)
	Organic matter	%	Walkely and Black (Walkley and Black, 1934)
	Total N	%	Kjeldahl (Bremner and Mulvaney, 1982)
	Available P_2O_5	mg/kg	Olsen's (Olsen et al., 1954)
	Available K_2O	mg/kg	Ammonium acetate (Jackson, 1967)
	Available Ca, Mg	mg/kg	EDTA Titration (El Mahi et al., 1987)
	Available S	mg/kg	Turbidimetric (Verma et al., 1977)
	Available B	mg/kg	Hot water (Berger and Truog, 1939)
	Available Fe, Zn, Cu, Mn	mg/kg	DTPA (Lindsay and Norvell, 1978)

Statistical Analysis

Descriptive statistics (mean, range, standard deviation, standard error, coefficient of variation) of soil parameters were computed using the Minitab 17 package. Rating (very low, low, medium, high and very high) of determined values were based on Soil Science Division, Khumaltar. The coefficient of variation was also ranked for determination of nutrient variability according to the procedure of (Aweto, 1982) where, CV ≤ 25% = low variation, CV >25 ≤ 50% = moderate variation, CV >50% = high variation. Arc Map 10.1 with geostatistical analyst extension of Arc GIS software was used to prepare spatial distribution map of soil parameters, while interpolation method employed was ordinary kriging with stable semi-variogram. Similarly, the nutrient index was also determined by the formula given by Ramamoorthy and Bajaj (1969).

Nutrient index (N.I.) = $(N_L \times 1 + N_M \times 2 + N_H \times 3) / N_T$

Where, N_L, N_M and N_H are number of samples falling in low, medium and high classes of nutrient status, respectively and N_T is total number of samples analyzed for a given area. Similarly, interpretation was done as value given by Ramamoorthy and Bajaj (1969) shown on the Table 2.

Table 2. Rating Chart of Nutrient index

Nutrient Index	Value
Low	<1.67
Medium	1.67-2.33
High	>2.33

Results and Discussion

The soil fertility distribution of the studied site was assessed with respect to texture, colour, structure, bulk density, pH, organic matter, primary nutrients, secondary nutrients and micronutrients such as B, Fe, Zn, Cu, and Mn, and the results obtained are presented and discussed in the following headings.

Soil Texture

Soil texture plays important role for drainage, water holding capacity, aeration, susceptibility to erosion, organic matter content, cation exchange capacity, pH buffering capacity and soil tilth (Berry et al., 2007). The sand content of samples ranged from 27 to 47% with a mean of 33.98% and that of silt content were 33.10 to 61.10% with a mean of 47.12%, while the range of clay content was 11.9 to 23.9% with a mean of 18.91% (Table 3). This shows loam and silt loam soil texture, where distribution of loam texture is dominant (Figure 3). The loam soil inhabiting site is good for cultivation various kinds of crops, while in silt loam site care should be taken for tillage and water managment. The coefficients of variation between the soil samples were low for sand (12.14%), silt (13.06%) and clay (21.62%).

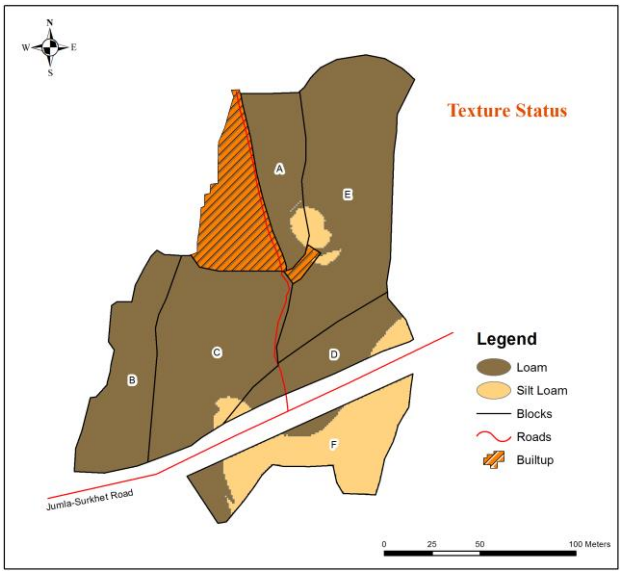


Figure 3. Spatial Distribution of Soil Texture in the Agricultural Research Station, Bijayanagar, Jumla, Nepal

Table 3. Soil Texture Status of Agricultural Research Station, Bijayanagar, Jumla, Nepal

Descriptive Statistics	Soil separates		
	Sand, %	Silt, %	Clay, %
Mean	33.98	47.12	18.91
SEM	0.97	1.45	0.96
SD	4.12	6.15	4.09
Minimum	27	33.1	11.9
Maximum	47	61.1	23.9
CV%	12.14	13.06	21.62
Class		Loam; Silt Loam	

SEM=Standard error of the mean; SD=Standard deviation

Soil Colour

Colour is most noticeable properties of soil that gives clues about the nature of the root zone (Baumann et al., 2016). Two kinds of soil colour; dark grayish brown (10YR 4/2) and very dark brown (10YR 3/2) were observed in the studied sites. The observed colour denotes optimum amount of humus in the soil.

Soil Structure

Soil structure refers to the pattern of spatial arrangement of soil particles in a soil mass (Brady and Weil, 2004). The sub-angular blocky kind of structure was observed in all the sites. The observed soil structure is good for agriculture point of view because in such structure soil aggregates are separated by elongated continuous pores, which allows good water and nutrient movement, and facilitates root growth (Pagliai and Vignozzi, 2002).

Bulk Density

Soil bulk density is a basic dynamic soil property that influenced by various physical and chemical properties (Chaudhari et al., 2013). The bulk density ranged from 1.16-1.20 g/cm^3 with a mean of 1.18 g/cm^3. This shows the bulk density is ideal for the plants growth. The satisfactory conditions of organic matter as well as fine texture might be the cause of ideal bulk density in the farm. The optimum condition of bulk density indicates less compaction inside the soil system, which helps to make water and nutrient movement easier; hence root growth also becomes easier.

Soil pH

Soil pH is one of the most important characteristics of soil fertility, because it has a direct impact on nutrient availability and plant growth (Brady and Weil, 2002). The soil pH varied from 5.45 to 7.66 with a mean of 6.54 (Table 4). The distribution soil pH varied from moderately acidic to moderately alkaline, but majority area contained nearly neutral (6.5-7.0) range (Figure 4). The variation on the soil management practice as well as crop allocation in the different sites of the farm from the longer period of time might be the cause the high variation of soil pH (moderately acidic to moderately alkaline). High acidity in the soil reduces most of the nutrient availability, as well as directly affects root structure also (Havlin et al., 2010). Application of agricultural lime in the acidic inhibiting site as shown in the figure 4 is important for their amelioration. Soil pH showed low variability (10.02%) among the soil samples.

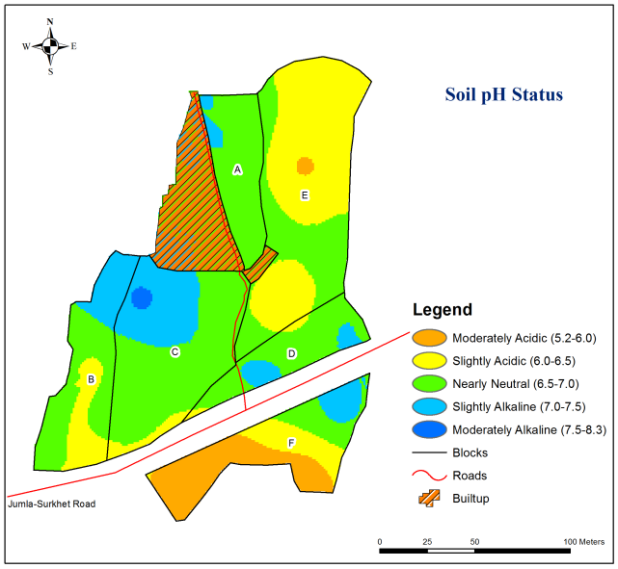


Figure 4. Spatial Distribution of Soil pH in the Agricultural Research Station, Bijayanagar, Jumla, Nepal

Table 4. Soil fertility status of agricultural research station, Bijayanagar, Jumla, Nepal

Descriptive Statistics	Soil Fertility Parameters				
	pH	OM, %	N,%	P_2O_5, mg/kg	K_2O, mg/kg
Mean	6.54	4.20	0.16	144.00	131.30
SEM	0.15	0.29	0.01	11.40	12.80
SD	0.66	1.21	0.04	48.40	54.50
Minimum	5.45	2.07	0.10	64.20	40.40
Maximum	7.66	6.53	0.23	257.20	255.20
CV%	10.02	28.80	21.81	33.64	41.52

SEM=Standard error of the mean; SD=Standard deviation

Organic Matter

Organic matter is a vital parameter for making soil alive, because it improves different physical, biological and chemical properties (Hoyle et al., 2011). The organic matter varied from 2.07 to 6.53% with a mean of 4.20% (Table 4). The distribution of organic matter ranged from low to high, but medium status was prevalent (Figure 5). The study area, being high altitude northern sites of Nepal nearly all around year have low temperature (Figure 1). The low temperature reduces the rapid microbial degradation of organic substances; hence organic matter is optimum in the study area. Organic matter showed moderate variability (28.80%) among the soil samples.

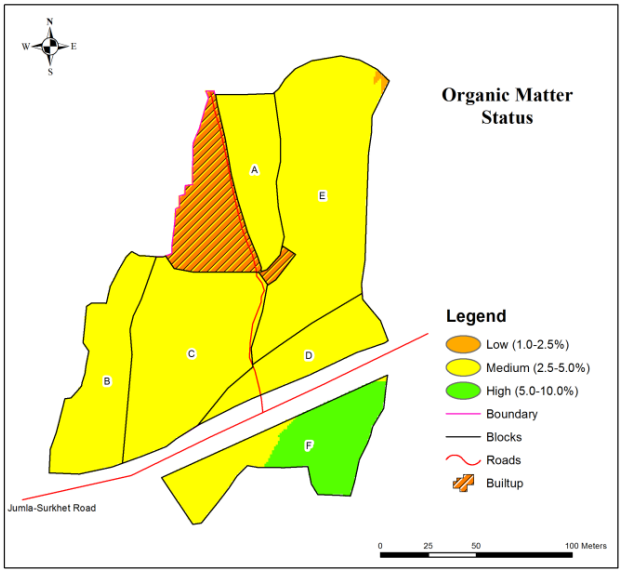


Figure 5. Spatial Distribution of Organic Matter in the Agricultural Research Station, Bijayanagar, Jumla, Nepal

Total Nitrogen

The total nitrogen ranged from 0.10 to 0.23% with a mean of 0.16% (Table 4). The distribution of nitrogen varied from medium to high, whereas medium status is common (Figure 6). The optimum organic matter status as well as continuous application of nitrogenous fertilizer before starting of every crop might be the cause of optimum total nitrogen status. The area having medium and high distribution, 75% and 50%, respectively of the total recommended nitrogen dose is requires for adequate supply of nitrogen for crops in the farm (Joshy and Deo, 1976). Total nitrogen showed low variability (21.81%) among the soil samples.

Available Phosphorus

Phosphorus is the second most limiting nutrient after nitrogen, and has negative impacts on crop yield if found to be deficient (Sharma et al., 2017). The available phosphorus varied from 64.20 to 257.20 mg/kg with a mean of 144 mg/kg (Table 4). The distribution of available phosphorus was very high singly (Figure 7). The higher content of available phosphorus in the farm might be due to the continuous application of phosphatic fertilizers for every crop without knowing phosphorus supplying capacity of soil. Being very high status, 40% of recommended phosphorus dose should be sufficient for the crops in the farm (Joshy and Deo, 1976). Available phosphorus showed moderate variability (33.64%) among the soil samples.

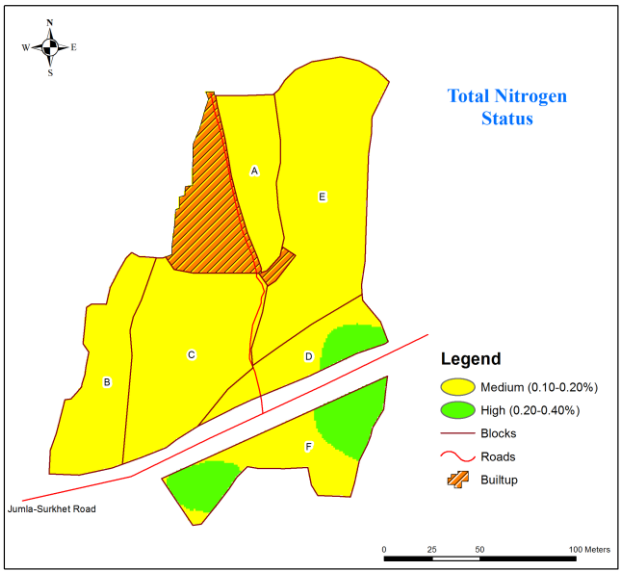


Figure 6. Spatial Distribution ofTotal Nitrogen in the Agricultural Research Station, Bijayanagar, Jumla, Nepal

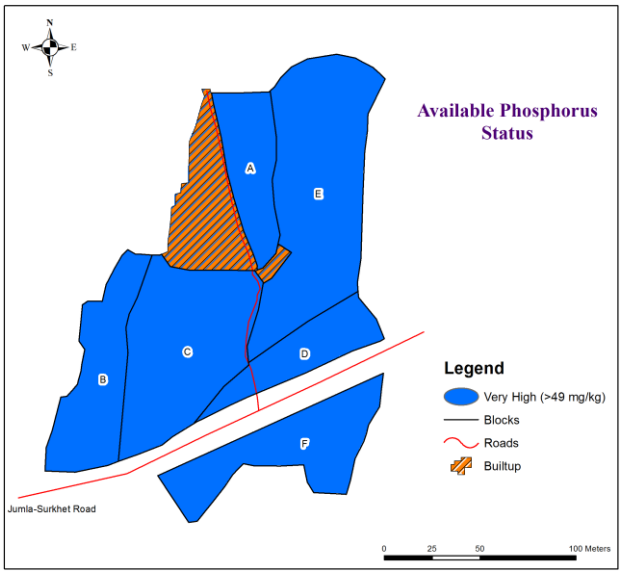


Figure 7. Spatial Distribution of Available Phosphorus in the Agricultural Research Station, Bijayanagar, Jumla, Nepal

Available Potassium

Potassium is one of the three major nutrients needed by plants, the others being nitrogen and phosphorus (Havlin et al., 2010). The available potassium varied from 40.4 to 255.2 mg/kg with a mean of 131.30 mg/kg (Table 4). The distribution of available potassium ranged from medium to high, but high is more (Figure 8). The different minerals such as muscovite, biotite, feldspars, orthoclase, microcline, mica etc. are major K-bearing minerals found in the earth (Sparks, 1987). The occurrence of their different minerals, optimum organic matter status andcomparative low content of sand separates among others might be the cause of satisfactory conditions of available potassium in the farm. The area having medium and high status, 60% and 40%, respectively of recommended potassium dose should be sufficient for the crops in the farm (Joshy and Deo, 1976). Available potassium showed moderate variability (41.52%) among the soil samples.

Available Calcium

Calcium is a secondary plant macronutrient, and is vital for running different living process in plants (Medvedev, 2005).The available calcium ranged from 1632 to 2880 mg/kg with a mean of 2166.9 mg/kg (Table 5). The distribution of available calcium was high solitary (Figure 9). The occurrence of nearly neutral soil pH in the majority of the sites might be the cause of high status of available calcium. Available calcium showed low variability (15.48%) among the soil samples.

Available Magnesium

Magnesium is the second most abundant cation in living plant cells, and involved in many metabolic process (Tanol and Kobayashi, 2015). The available magnesium varied from 98.4 to 455.5 mg/kg with a mean of 267.6 mg/kg (Table 5). The distribution of available magnesium was solitary high (Figure 10). Similar to magnesium, occurrence of nearly neutral soil pH in the majority of the sites might be the cause of high status of available magnesium. Available magnesium showed moderate variability (43.48%) among the soil samples.

Available Sulphur

Sulfur is an essential nutrient for plant growth due to its presence in proteins, glutathione, phytochelatins, thioredoxins, chloroplast membrane lipids, and certain coenzymes and vitamins (Takahashi et al., 2011). The available sulphur ranged from 0.59 to 6.23 mg/kg with a mean of 1.56 mg/kg (Table 5).The distribution of available sulphur was very low merely (Figure 11). Khadka et al. (2016, 2017, 2018) also reported very critical status of available sulphur in the different sites of Nepal. The intense cultivation of crops without application of sulphur containing fertilizer might be the cause of deficient status of available sulphur in the field. Being critical sulphur status, application at the rate of 15-30 kg S/ha is mandatory for reducing sulphur deficiency stress for crops (Khatri-Chettri, 1991). During sulphur fertilizer application care should have to take in acidic inhibiting site, as they have acidity causing behavior. Available sulphur showed high variability (84.4%) among the soil samples.

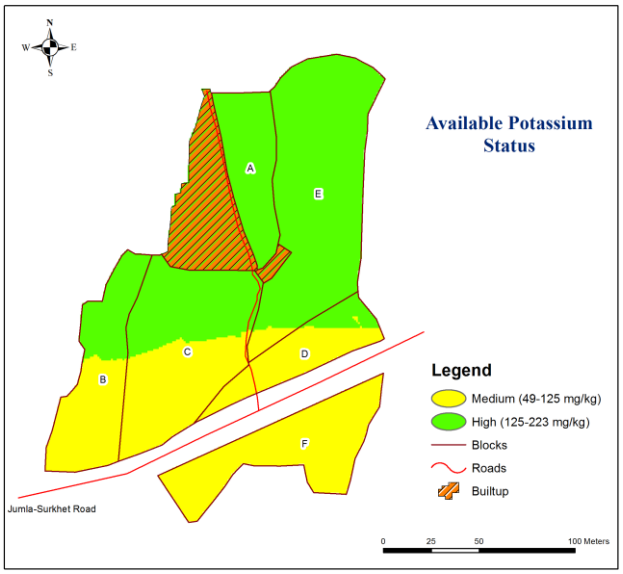


Figure 8. Spatial Distribution of Available Potassium in the Agricultural Research Station, Bijayanagar, Jumla, Nepal

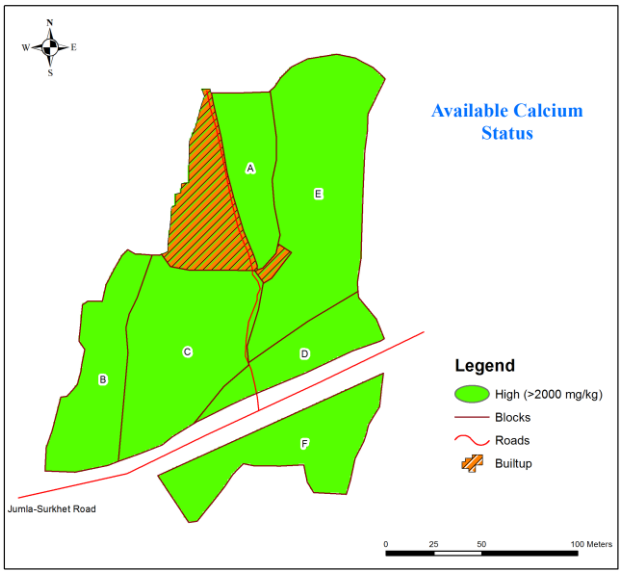


Figure 9. Spatial Distribution of Available Calcium in the Agricultural Research Station, Bijayanagar, Jumla, Nepal

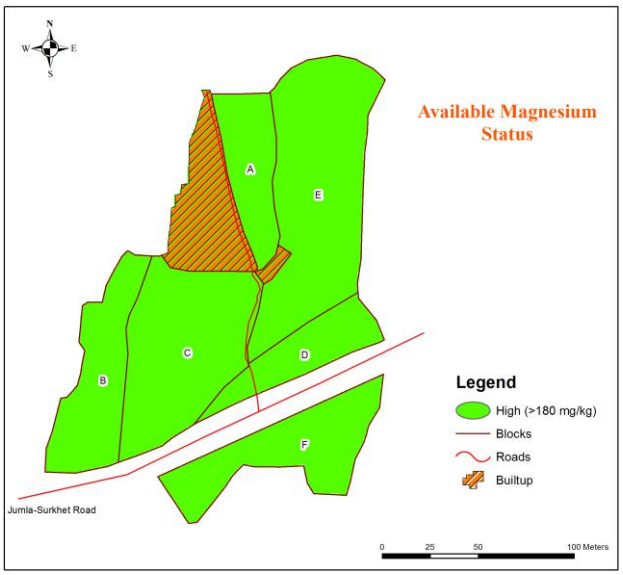


Figure 10. Spatial Distribution of Available Magnesium in the Agricultural Research Station, Bijayanagar, Jumla, Nepal

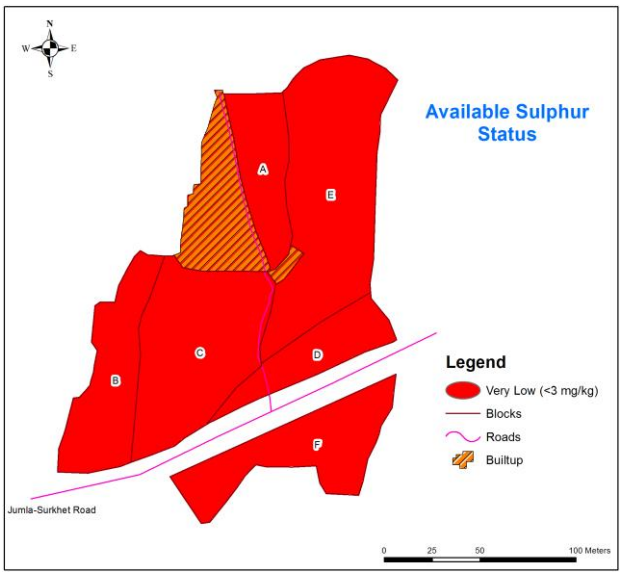


Figure 11. Spatial Distribution of Available Sulphur in the Agricultural Research Station, Bijayanagar, Jumla, Nepal

Table 5. Soil Fertility Status of Agricultural Research Station, Bijayanagar, Jumla, Nepal

Descriptive Statistics	Soil Fertility Parameters			
	Ca, mg/kg	Mg, mg/kg	S, mg/kg	B, mg/kg
Mean	2166.9	267.6	1.56	0.25
SEM	79	27.4	0.31	0.03
SD	335.4	116.4	1.31	0.13
Minimum	1632	98.4	0.59	0.01
Maximum	2880	455.5	6.23	0.38
CV%	15.48	43.48	84.4	53.44

SEM=Standard error of the mean; SD=Standard deviation

Available Boron

Boron deficiency is documented as second most important micronutrient constraints after zinc in the world (Ahmad et al., 2012). The available boron ranged from 0.01 to 0.38 mg/kg with a mean of 0.25 mg/kg (Table 5). The distribution of available boron was very low only (Figure 11). The very deficient status of available boron was also reported by Khadka et al. (2016, 2017, 2018) during their study in the different sites of Nepal. The intense cultivation of crops without application of boron containing fertilizer might be the cause of deficient status of available boron in the field. Being inadequate boron, application of 2-3 kg B/ha is advisable for reducing boron deficiency stress for crops (Khatri-Chettri, 1991). Available boron showed high variability (53.44%) among the soil samples.

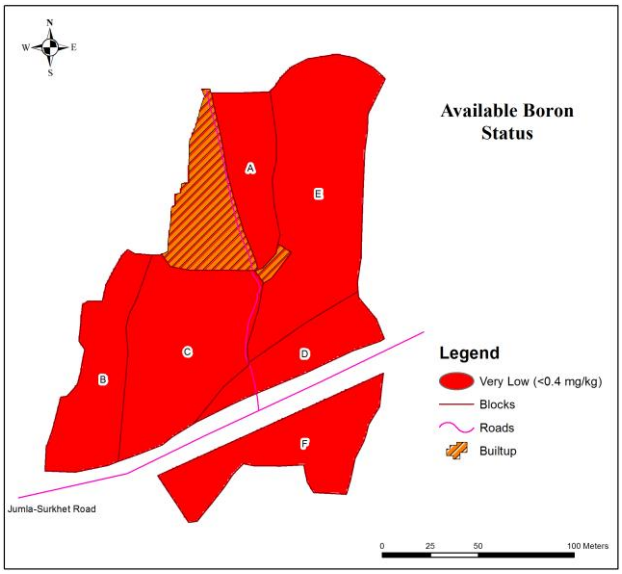


Figure 12.Spatial Distribution of Available Boron in the Agricultural Research Station, Bijayanagar, Jumla, Nepal

Available Iron

Iron is an important micronutrient in the life cycle of plant, and plays important role for their various metabolic process (Rout and Sahoo, 2015). The available iron varied from 15.9 to 300.5 mg/kg with a mean of 121.6 mg/kg (Table 6). The distribution of available iron ranged from very low to very high, although very high status was prevalent (Figure 13). Similar very high status was also determined by Khadka et al. (2016, 2017, 2018) during their study in the different sites of Nepal. The occurrence of primary and secondary iron minerals such as hematite, olivine, siderite, goethite, magnetite etc. might be the cause of high content of available iron, because these are the major mineral containing iron element (Havlin et al., 2010). The high iron availability reduces the uptake of different nutrients such as P, K, Mn and Zn; thus shows deficiency stress of these elements in the plants (Fageria et al., 2008). Therefore, proper care should be taken for reducing deficiency stress of these antagonistic elements. Available iron showed high variability (80.94%) among the soil samples.

Table 6. Soil Fertility Status of Agricultural Research Station, Bijayanagar, Jumla, Nepal

Descriptive Statistics	Soil Fertility Parameters			
	Fe, mg/kg	Zn, mg/kg	Cu, mg/kg	Mn, mg/kg
Mean	121.6	2.93	5.59	5.55
SEM	23.2	0.44	0.64	0.88
SD	98.5	1.86	2.72	3.74
Minimum	15.9	1.12	2.58	1.46
Maximum	300.5	8.26	12.16	12.88
CV%	80.94	63.57	48.64	67.31

SEM=Standard error of the mean; SD=Standard deviation

Available Zinc

Zinc deficiency is a major micronutrient constraint for food production in every parts of the world and found positive response of their application in almost all crops (Welch, 2002). The available zinc varied from 1.12 to 8.26 mg/kg with a mean of 2.93 mg/kg (Table 6). The distribution of available zinc ranged from medium

to high, while medium status was common (Figure 14). Available zinc showed high variability (63.57%) among the soil samples.

Available Copper

Copper is an essential micronutrient for plant growth and development, although it is also potentially toxic (Yruela, 2005). The available copper ranged from 2.58 to 12.16 mg/kg with a mean of 5.59 mg/kg (Table 6). The distribution of available copper was very high only (Figure 15). The very high level of available copper may showed different kinds of toxic effects in the life cycles of plants. Reduced seed germination, inhibition of root and shoot growth, disturbance on photosynthetic apparatus and pigments etc. are the toxic effects of copper (Adrees et al., 2015). Being very high status of available copper in the soil, care should have to take during fungicide, pesticides, herbicides application in the field because these chemical already contains copper element (Husak, 2015). Available copper showed moderate variability (48.64%) among the soil samples.

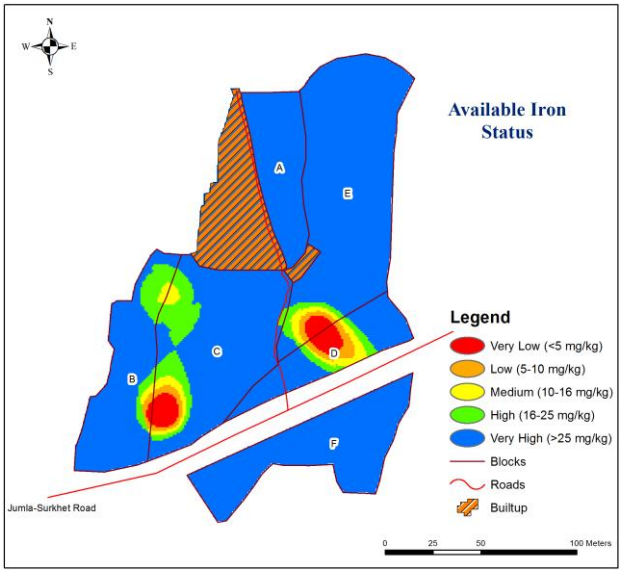


Figure 13. Spatial Distribution of Available Iron in the Agricultural Research Station, Bijayanagar, Jumla, Nepal

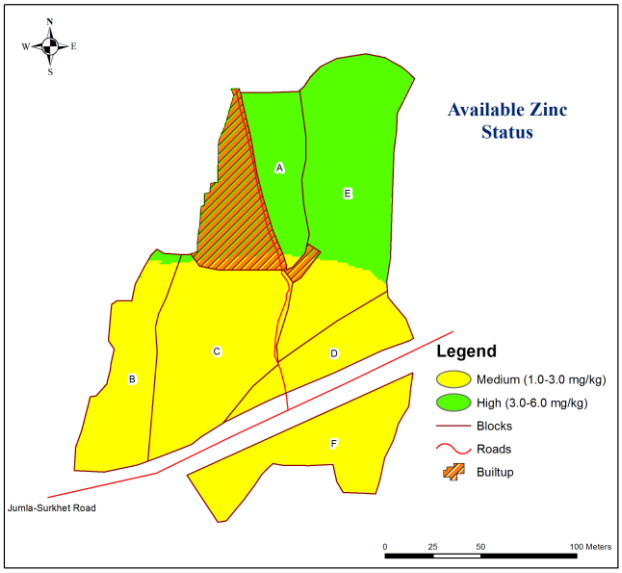


Figure 14. Spatial Distribution of Available Zinc in the Agricultural Research Station, Bijayanagar, Jumla, Nepal

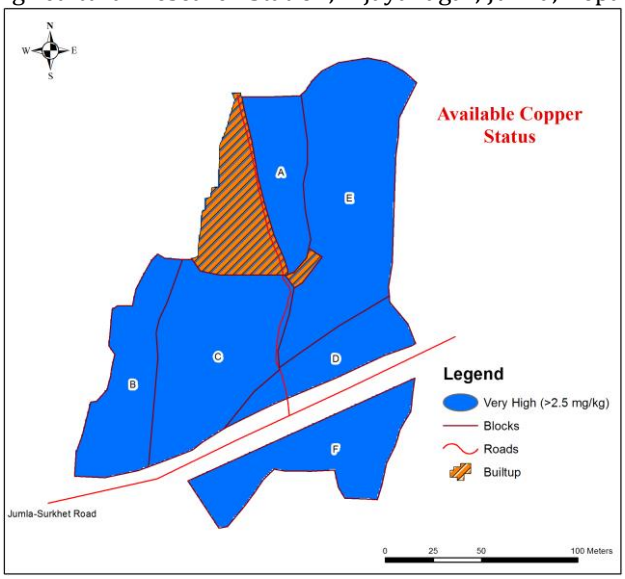


Figure 15. Spatial Distribution of Available Copper in the Agricultural Research Station, Bijayanagar, Jumla, Nepal

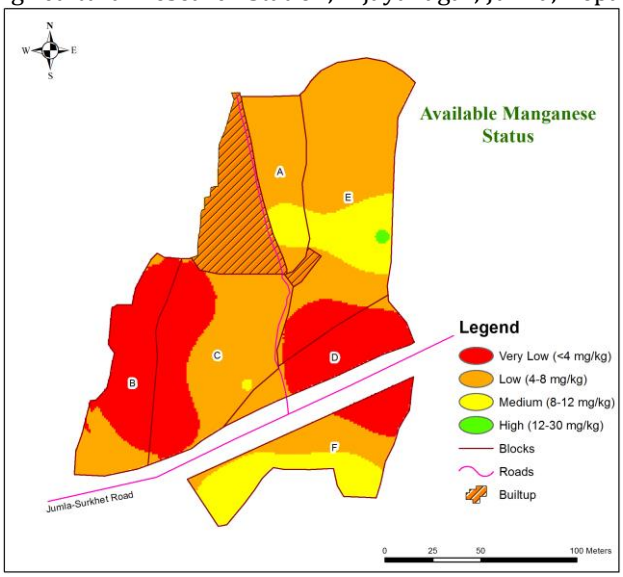


Figure 16. Spatial Distribution of Available Manganese in the Agricultural Research Station, Bijayanagar, Jumla, Nepal

Available Manganese

Manganese is also an important micronutrient for plants, prevailing in several metabolic process as photosynthetic and enzyme antioxidant-cofactor (Millaleo et al., 2010). The available manganese varied 1.48 to 12.88 mg/kg with a mean of 5.55 mg/kg (Table 6). The distribution of available manganese ranged from very low to high, whereas low status was common (Figure 16). Manganese mining due to intense cultivation

of crops without application of manganese containing fertilizer might be the cause of deficient status of available manganese in the field. Moreover, difference in cropping system, farming practices and fertilization might be the cause of high spatial distribution of available manganese. Being inadequate available manganese, application of 8-16 kg Mn/ha is advisable for reducing manganese deficiency stress in plants (Khatri-Chettri, 1991). Available manganese showed high variability (67.31%) among the soil samples.

Table 7. Nutrient indices of studied parameters of Agricultural Research Station, Bijayanagar, Jumla, Nepal

Parameters	% distribution of samples					Nutrient index	Remarks
	Very Low	Low	Medium	High	Very High		
OM	0	11	61	20	0	2.17	Medium
N	0	6	78	17	0	2.11	Medium
P_2O_5	0	0	0	0	100	3.00	High
K_2O	0	6	44	39	11	2.44	High
Ca	0	0	28	72	0	2.72	High
Mg	0	0	28	72	0	2.72	High
S	94	6	0	0	0	1.00	Low
B	100	0	0	0	0	1.00	Low
Fe	0	0	6	11	83	2.94	High
Zn	0	0	67	22	11	2.33	Medium
Cu	0	0	0	0	100	3.00	High
Mn	39	28	28	6	0	1.39	Low

Conclusion

The determined soil test data can be used mainly in two aspects. First one is for sustainable soil management, while another for developing research strategyas being a farm of research station. The studied physical properties symbolize the current status is satisfactory for agricultural purpose. The organic matter is optimum in the farm. For maintaining existing status, current organic matter management practice should be continued. The fertilizer should be applied for each crops based on the determined nutrient distribution status shown in the prepared maps of farm. The plants may suffer from deficiency stress of low, and toxicity stress of very high status of nutrients. The proper care should be taken for such types of nutrients. For enhancing research efficacy of the station, future research strategy should be built based on the determined soil fertility status and their distribution. This farm can be used as a pocket research site, especially for high altitude iron and copper toxicity as well as sulphur and boron deficiency tolerant study.

Acknowledgements

The authors would like to acknowledge Nepal Agricultural Research Council for funding this research. We are very much thankful to Agricultural Research Station, Bijayanagar, Jumla, Nepal for their cooperation. Similarly, support of the Soil Science Division, Khumaltar for providing laboratory facilities to analyze the soil samples and preparation of soil fertility maps as well as other technical support is highly acclaimed.

References

Acharya, A.K., Paudel, M. P., Wasti, P.C., Sharma, R.D., Dhital, S., 2018. Status Report on Food and Nutrition Security in Nepal. Ministry of Agriculture, Land Management and Cooperatives, Kathmandu, Nepal.

Adrees, M., Ali, S., Rizwan,M., Ibrahim, M., Abbas, F., Farid, M., Bharwana, S.A., 2015. The effect of excess copper on growth and physiology of important food crops: a review. *Environmental Science and Pollution Research* 22(11): 8148-8162.

Ahmad, W., Zia, M.H., Malhi, S. S., Niaz, A., Saifullah, 2012. Boron Deficiency in soils and crops: a review. In: Crop Plant. Goyal, A. (Ed.). IntechOpen.

ARS, 2017. Annual Report 2073/74 (2017). Agriculture Research Station, NARC, Bijayanagar, Jumla, Nepal.

Aweto, A.O., 1982. Variability of upper slope soils developed under sandstones in South-western Nigeria. *Georgian Journal* 25: 27-37.

Baumann, K., Schöning, I., Schrumpf, M., Ellerbrock, R.H., Leinweber, P., 2016. Rapid assessment of soil organic matter: Soil color analysis and Fourier transform infrared spectroscopy. *Geoderma* 278: 49–57.

Berger, K.C., Truog, E., 1939. Boron determination in soils and plants. *Industrial and Engineering Chemistry Analytical Edition* 11(10): 540-545.

Berry, W., Ketterings, Q., Antes, S., Page, S., Russell Anelli, J., Rao, R., DeGloria, S., 2007. Soil Texture. Agronomy Fact Sheet Series, Fact Sheet 29. Cornell University Cooperative Extension. Available at [Access date: 19.08.2018]: http://nmsp.cals.cornell.edu/publications/factsheets/factsheet29.pdf

Bouyoucos, G.J., 1962. Hydrometer method improved for making particle size analyses of soils. *Agronomy Journal* 54(5): 464-465.

Brady, N. C., Weil, R., 2002. The nature and properties of soils. 13th Edition, Pearson Education, New Jersey. USA. 980p.

Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen total. In: Methods of soil analysis. Part 2 Chemical and microbiological properties. 2nd edition. Page, A.L. (Ed.). American Society of Agronomy, No. 9, Madison, WI, USA. pp. 595-624.

Cambardella, C.A., Karlen, D.L., 1999. Spatial analysis of soil fertility parameters. *Precision Agriculture* 1(1): 5-14.

Chaudhari, P. R., Ahire, D. V., Ahire, V. D., Chkravarty, M., Maity, S., 2013. Soil bulk density as related to soil texture, organic matter content and available total nutrients of Coimbatore soil. *International Journal of Scientific and Research Publications* 3(2): 1-8.

El Mahi, Y.E., Ibrahim, I.S., Abdel Magid, H.M., Eltilib, A.M.A., 1987. A simple method for the estimation of calcium and magnesium carbonates in soils. *Soil Science Society of America Journal* 51(5): 1152-1155.

Fageria, N.K., Santos, A.B., Filho, M.P., Guimaries, C.M., 2008. Iron toxicity in lowland rice. *Journal of Plant Nutrition* 31(9): 1676-1697.

Havlin, H.L., Beaton, J.D., Tisdale, S.L., Nelson, W.L., 2010. Soil Fertility and Fertilizers: An Introduction to Nutrient Management. 7th Edition, PHI Learning Private Limited, New Delhi. India. 516p.

Hoyle, F.C., Baldock, J.A., Murphy, D.V., 2011. Soil organic carbon – role in rainfed farming systems: with particular reference to Australian conditions. In: Rainfed farming systems. Tow, P., Cooper, I., Partridge, I., Birch, C., (Eds.). Springer, New York, USA. pp. 339–361.

Huang, B., Sun, W., Zhao, Y., Zhu, J., Yang, R., Zou, Z., Ding, F., Su, J., 2007. Temporal and spatial variability of soil organic matter and total nitrogen in an agricultural ecosystem as affected by farming practices. *Geoderma* 139(3-4):336-345.

Husak, V., 2015. Copper and copper containing pesticides: metabolism, toxicity and oxidative stress. *Journal of Vasyl Stefanyk Precarpathian National University* 2(1): 38-50.

Jackson, M.L., 1973. Soil chemical analysis (Indian Edition). Prentice Hall of India Pvt. Ltd., New Delhi, India.

Jones, Jr, J.B., 2012. Plant nutrition and soil fertility manual. 2nd Edition, CRC press. New York, USA. 299p.

Joshy, D., Deo, G.P., 1976. Fertilizers Recommendations for Major crops of Nepal. Division of Soil Science and Agricultural Chemistry, Department of Agriculture, HMG/Nepal.

Keen, B.A., Raczkowski, H., 1921. The relation between clay content and certain physical properties of soil. *The Journal of Agricultural Sciences* 11(4): 441-449.

Khadka, D., Lamichhane, S., Bhurer, K.P., Chaudhary, J.N., Ali, M.F., Lakhe, L., 2018. Soil Fertility Assessment and Mapping of Regional Agricultural Research Station, Parwanipur, Bara, Nepal. *Journal of Nepal Agricultural Research Council* 4(28): 33-47.

Khadka, D., Lamichhane, S., Malla, R., Joshi, S., 2016. Assessment of soil fertility status of oilseed research program, Nawalpur, Sarlahi, Nepal. *International Journal of Advanced Research* 4(6): 1472-1483.

Khadka, D., Lamichhane, S., Tiwari, D.N., Mishra, K., 2017. Assessment of soil fertility status of National Rice Research Program, Hardinath, Dhanusha, Nepal. *International Journal of Agricultural and Environmental Research* 3(1): 86-105.

Khatri-Chettri, T.B., 1991. Introduction to Soils and Soil fertility. Tribhuvan University Institute of Agricultural and Animal Science, Rampur, Chitwan, Nepal. 233p.

Lindsay, W.L., Norvell, W.A., 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal* 42(3): 421-428.

Liu, Z.P., Shao, M.A., Wang, Y.Q., 2013. Spatial patterns of soil total nitrogen and soil total phosphorus across the entire Loess Plateau region of China. *Geoderma* 197-198: 67-78.

Mandal, A.K., Sharma, R.C., 2009. Computerized database of salt affected soils for Peninsular India using GIS. *Geocarto International* 24(1): 64-85.

Medvedev, S.S., 2005. Calcium signaling system in plants. *Russian Journal of Plant Physiology* 52(2): 249–270.

Millaleo, R., Reyes-Díaz, M.,Ivanov, A.G., Mora, M.L., Alberdi, M., 2010. Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. *Journal of Soil Science and Plant Nutrition* 10(4): 470-481.

Mishra, A., Das,D., Saren, S., 2013. Preparation of GPS and GIS based soil fertility maps for Khurda district, Odisha. *Indian Agriculturist* 57(1): 11-20.

Munsell., 2009. Standard soil colour charts. Munsell color company, Inc. Baltimore, Maryland, USA.

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Department of Agriculture, Circular No 939, USA, 19p.

Pagliai, M., Vignozzi, N., 2002. Image analysis and microscopic techniques to characterize soil pore system. In: Physical Methods in Agriculture. Blahovec, J., Kutilek, M., (Eds.). Kluwer Academic Publishers, New York. USA. p. 13-38.

Panda, S.C., 2010. Soil Management and Organic Farming. Agrobios, Bharat Printing Press, Jodhpur, India. 462p.

Ramamoorthy, B., Bajaj, J.C., 1969. Available nitrogen, phosphorus and potassium status of Indian soils. *Fertilizer News* 14: 25-36.

Rout, G. R., Sahoo, S., 2015. Role of iron in plant growth and metabolism. *Reviews in Agricultural Science* 3(1): 1-24.

Sharma, L.K., Bali, S.K., Zaeen, A.A., 2017. A case study of potential reasons of ıncreased soil phosphorus levels in the Northeast United States. *Agronomy* 7(4): 85.

Song, G., Zhang,L. Wang,K., Fang, M., 2013. Spatial simulation of soil attribute based on principles of soil science. 21st International Conference on Geoinformatics. 20-22 June 2013. Kaifeng, China.

Sparks, D.L., 1987. Potassium dynamics in soils. In: Advances in Soil Science. Stewart, B.A. (Ed.) Vol 6, Springer, New York, USA. pp. 1- 63.

Takahashi, H., Kopriva, S., Giordano, M., Saito, K., Hell, R., 2011. Sulfur assimilation in photosynthetic organisms: molecular functions and regulations of transporters and assimilatory enzymes. *Annual Review of Plant Biology* 62: 157-184.

Tanol, K., Kobayashi, N.I., 2015. Leaf senescence by magnesium deficiency. *Plants* 4(4): 756-772.

Verma, B.C., Swaminathan, K., Sud, K.C., 1977. An improved turbidimetric procedure for the determination of sulphate in plants and soils. *Talanta* 24(1): 49-50.

Walkley, A.J., Black, I.A., 1934. Estimation of soil organic carbon by the chromic acid titration method. *Soil Science* 37(1): 29-38.

Weindorf, D.C., Zhu, Y., 2010. Spatial variability of soil properties at Capulin volcano, New Mexico, USA: Implications for sampling strategy. *Pedosphere* 20(2): 185-197.

Welch, R.M., 2002. The impact of mineral nutrients in food crops on global human health. *Plant and Soil* 247(1): 83-90.

Yruela, I., 2005. Copper in plants. *Brazilian Journal of Plant Physiology* 17(1): 145-156.

Eurasian J Soil Sci 2019, 8 (3) 249 - 256

Eurasian Journal of Soil Science

Journal homepage : http://ejss.fesss.org

Determination of heavy metal risk and their enrichment factor in intensive cultivated soils of Tokat Province

Betül Bayraklı [a,*], Orhan Dengiz [b]

[a] Republic of Turkey Ministry of Agriculture and Forestry, Black Sea Agricultural Research Institute, Samsun, Turkey
[b] Ondokuz Mayıs University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Samsun, Turkey

Abstract

Heavy metal contamination has caused serious environmental and health-related problems around the world. This research was conducted in arable lands of some basins located on Tokat province. The aim of this present study was to determine I-) some physico-chemical properties of soils, ii-) to find heavy metal (HM) content and their enrichment factor (EF) and iii-) to detect relationship between some physico-chemical properties and HM concentration. To identify the concentrations and sources of heavy metals, 280 soil samples (0-20 cm) were collected from the study area. Subsequently, in order to evaluate natural or anthropogenic sources of heavy metal content and their EF in agricultural fields, the concentrations of some HMs (Cd, Co, Cu, Cr, Ni, Pb and Zn) and some physico-chemical properties of soil samples were analyzed. The results showed that mostly the concentration of Ni followed by Cr exceeded their threshold levels. The local pollutions from Ni and Cr were attributed to the natural influences (particularly due to parent material). The concentrations of the other HMs are relatively lower than the critical values. The mean values of the HMs contents arranged in the following decreasing order: Ni>Cr>Cu>Zn>Co>Pb>Cd in the studied soil sample. In addition, it was found significantly positive relation between Pb and OM while the same relation was also found clay content and Cd and Pb. On the other hand, according to EF of HMs in total soil samples, Cd, Ni and Cr have found 16%, 10% and 6% soil samples as moderate enrichment class, whereas 55% and 1% of the total soil samples were determined significant enrichment class in terms of Cd and Ni elements. Besides, all other HM elements did not exceed minimal enrichment level. However, in some regions of the study area, the Cu, Cd and Pb contents were also slightly raised, this case possibly stems from anthropogenic effects such as excessive P fertilization, field traffic and pesticide using.

Keywords: Heavy metal risk, enrichment factor, micro basin, Tokat.

Article Info

Received : 09.12.2018
Accepted : 15.05.2019

Introduction

The heavy metal pollution in agricultural lands has been very important in recent years, especially due to the detrimental effects on food safety and ecosystem. The source of this pollution in agricultural soils can be geogenic and/or anthropogenic. Geogenic pollution is entirely due to the composition of the main material that forms the soil. Anthropogenic pollution may be caused by excessive use of fertilizers and pesticides in agricultural areas, use of fossil fuels, mining activities, rapid population growth and related urbanization, uncontrolled wastewater discharge, atmospheric accumulation, traffic density and increased industrial activities (Manta et al., 2002; Borůvka et al., 2005; Sivry et al., 2008; Zhang et al., 2009; Meena et al., 2011, Bilge and Çimrin, 2013).

Heavy metals, which are added to the environment by natural and artificial means, are defined as dangerous pollutants because they easily accumulate and form complex structures in the soil. These heavy metals cause

* Corresponding author.
Republic of Turkey Ministry of Agriculture and Forestry Black Sea Agricultural Research Institute, Samsun, Turkey
Tel.: +90 362 2560514 E-mail address: bbetul25@gmail.com
e-ISSN: 2147-4249 DOI: 10.18393/ejss.567357

decrease in microbial activity, soil fertility, biodiversity and yield, and can also cause poisoning in animals and humans through the food chain (Karaca, 2001; Oliveira et al., 2006; Yang et al., 2006; D'Ascoli et al., 2009; Peralta-Videa, 2009; Mudgal et al., 2010; Yadav, 2010; Kaplan et al., 2011; Jaishankar et al., 2014; Liu et al., 2017.). Some physical and chemical properties of soils such as cation exchange capacity, pH and organic matter are effective in the accumulation of these metals in soil. Especially heavy clay soils can absorb heavy metals in their bodies due to their high cation exchange capacity. Also in soils with high organic matter content, heavy metals are absorbed more and low solubility compounds occur (Bakış and Bilgin, 1998).

Tokat Province, selected area for this reseach, has been shown to be an important settlement center throughout its history with the advantage of being established on the fertile valley of Yeşilırmak. The fertile plains where all kinds of agriculture can be made are distributed all over the province. The most important of them are Kazova, Omala Plain, Turhal Plain, Niksar Plain, Erbaa Plain, Artova and Zile Plain. All kinds of fruits, vegetables and sunflowers, especially cereals, sugar beet and tobacco are grown in these plains. Approximately 16.5% of the province is composed of meadow and pasture areas and sparse plant areas, while 31.0% of them constitute forest areas. The agricultural activities area covers 38.2%. In addition some researcher performed about heavy metal concentration in arable lands such as Arda et al. (2015), in their study, took 42 soil samples from the Ipsala District and its villages and stated that the nickel concentrations determined in the Ipsala Region were significantly higher than the limit values (0.25-109.5 mg/kg). Moreover, Metin (2010) indicated that in the study it was determined the amount of trace elements and heavy metals in the agricultural lands of the Aluvial, Colluvial and Vertisol group in the western part of the Bursa plain, there was no pollution exceeding the limit values in terms of Cd, Co, Cr, Ni and Pb in the studied agricultural soils. Akyıldız and Karataş (2018) reported also that in the soil samples taken from Adana city center and its surrounding areas, Fe, Mn and Pb were found to be below the standards and Cu, Hg, Co, Cd and their elements were found to be above a few sample standards. In the analysis results of Ni, As, Cr and Al, samples were mostly above the standards. It is stated that the parameters that cause pollution are generally caused by environmental factors, and that the geological structure in the region may contribute in the increase of Cr and Ni elements.

In the study, which investigated the heavy metal contents and pollution characteristics in the water and sediments of the Khoshk River in southwestern Iran, it was stated that the amount of heavy metal in the sediments decreased as Mn>Cr>Pb>Ni>Zn>Cu>Cd respectively. Based on the enrichment factor and geoaccumulation index values, it was determined that the sediments were filled with Cr, Zn, Pb, Cu and Cd. According to the results obtained from the basic component analysis with sediment samples, it was stated that the high Ni concentration is related to the composition of the main rocks, whereas Cr, Zn, Pb, Cd and Cu values may be due to anthropogenic activities (Salati and Moore, 2010).

In order to determine the amount of Cd, Co, Cu, Ni, Pb, Zn in the Bafra deltaic plain and to analyze the spatial distribution of the heavy metal, 108 soil samples were taken from 0-20 cm depth from an area of approximately 100 thousand ha. In order to reveal the source of heavy metal pollution (natural or anthropogenic), the enrichment factor was calculated. The highest enrichment factor was found for Cd (12.826), while the smaller enrichment factor values for Pb, Ni, Co and Cu were calculated. The concentrations of Cd, Cu and Zn in some regions of the study area were determined to be slightly higher due to the high percentage of phosphorus fertilizers and intensive agricultural applications. It is stated that the values exceeding the criterium for Ni are due to the high content of this element in the main material (Kızılkaya et al., 2011).

The aim of this present study conducted in arable lands of some basins located on Tokat province was to determine i) some physico-chemical properties of soils, ii) to find heavy metal (HM) content and their enrichment factor (EF) and iii) to detect relationship between some physico-chemical properties and HM concentration.

Material and Methods

Field description

This study was carried out in Tokat province in the Central Black Sea region of Turkey. The Province of Tokat is coordinated between 4200000-4520000 North and 210000-360000 East (WGS84, UTM-37 Zone m) (Figure 1). Total area of the Tokat is about 10272.58 km^2. However, arable land selected for this study covers approximately 38.2% of the total area. On the other hand, 47.5% of the study area is covered by forest and pasture lands.

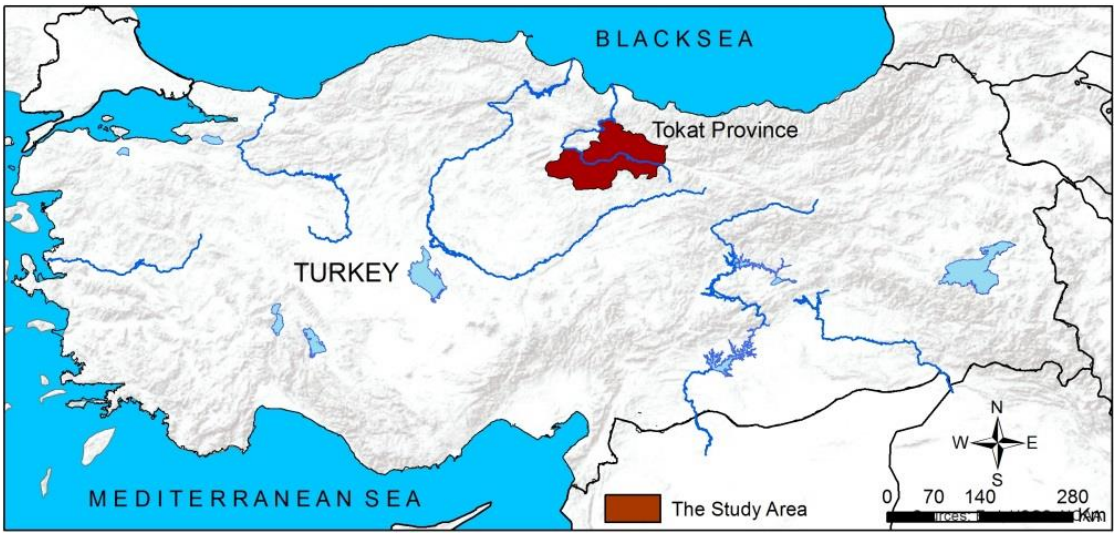


Figure 1. Location map of the study area

The climate can be described as sub-humid and according to long term meteorological data (1974-2017), average annual precipitation and temperature of the study area are 431.4 mm and 12.6 ºC, respectively. The study area lies at an elevation above the sea level from 75 to 2415 m. The region has topographically very heterogeneous topographic features such as hilly, rolling, flat, etc., but particularly hilly and rolling physiographic units are common in the study area, only 12.0% of the total area is almost flat and gentle slope and about 22.5% of it is less than 10% slope degree (Figure 2). Most of the total area corresponding with 796200.9 ha has more than 20% slope degree.

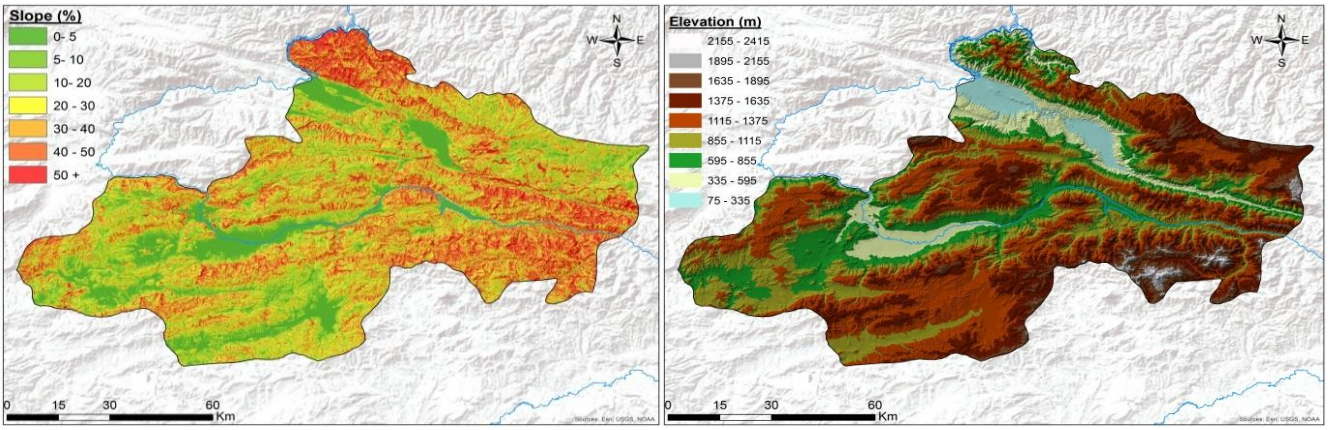


Figure 2. Elevation and slope maps of the Tokat Province

Soil Sampling and analysis

Field study was conducted in 2017. In total 280 soil samples classified as mostly alluvial, brown, brown forest, non-calcareous forest, chestnut and reddish chestnut great soil group were taken from soil surface (0-20 cm) in agricultural lands (Figure 3). The sampling was carried out after harvest in the autumn and before start of the next cropping season in order to avoid the influence of agricultural practices during the crop growing season, i.e. fertilization. In addition, their coordinates were recorded using global positioning system (GPS) tool. Samples were air-dried and sieved through a 2 mm sieve to be prepared for analyses. Soil requirements for organic farming including soil physico-chemical properties, heavy metal concentration were determined based on literatures. Table 1 shows the selected analytical protocols.

Table 1. Protocol measurements for some soil physical and chemical properties

Parameters	Unit	Protocol	Reference
Texture (Clay, Silt and Sand)	%	hydrometer method	Bouyoucos (1951)
pH	1:1	(w:v) soil-water suspension	Soil Survey Laboratory (1992)
EC	dSm^{-1}	(w:v) soil-water suspension	Soil Survey Laboratory (1992)
$CaCO_3$	%	Scheibler calcimeter	Soil Survey Staff (1993)
Organic Matter	%	Walkley-Black method	Soil Survey Laboratory (1992)
Total heavy metal (Cu,Cd,Cr,Pb,Co,Ni,Zn)	$mg\ kg^{-1}$	According to EPA 3051 sing ICP-OES detection	Kloke (1980)

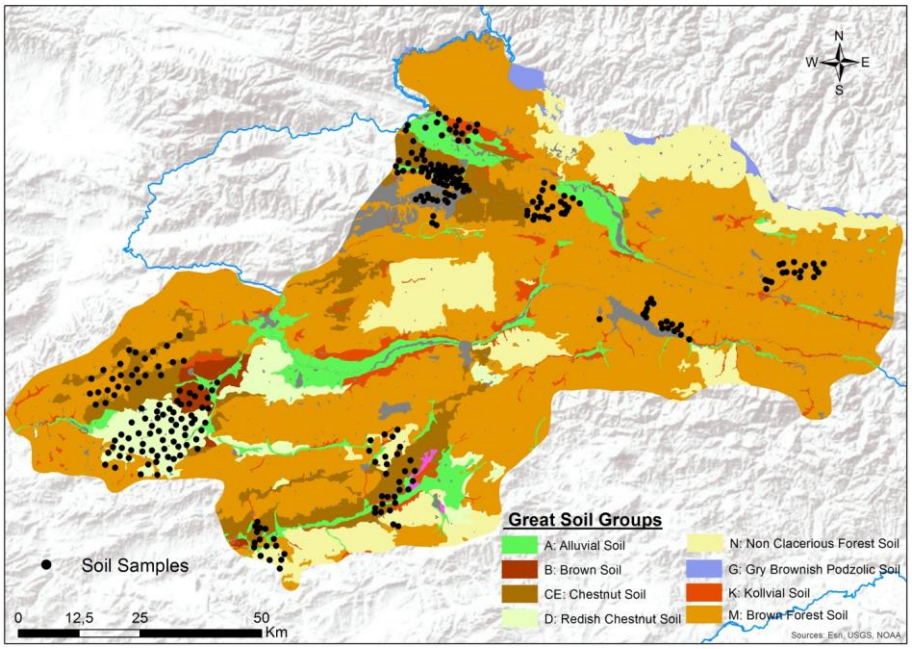


Figure 3. Soil samples pattern in great soil group map of the study area

Some physico-chemical characteristics of soil such as the organic matter, pH, and lime contents, and the particle size fractions are of great importance in the heavy metal toxicity of soils. The calculation of the enrichment factors (EF) for the heavy metals was made using an equation suggested by Sposito (1989) and Agbenin (2002).

$$EF = (HM_{soil}) / (HM_{earth})$$

where HM_{soil} is the total heavy metal concentration in the soil sample, and HM_{earth} is the mean heavy metal concentration in the earth's crust, which is 0.11 mg kg^{-1} for Cd, 50 for Cu, 100 for Cr, 20 for Co, 80 for Ni, 14 for Pb, and 75 mg kg^{-1} for Zn (Sposito, 1989). Based on the EF value, five categories of pollution were distinguished by Sutherland (2000): the absence of enrichment (<2), moderate enrichment (2–5), high enrichment (5-20), very high enrichment (20–40), and extremely high enrichment (>40).

Results and Discussion

Soil physico-chemical properties and heavy metals

The physico-chemical characteristic selected in this study showed changefulness as a result of dynamic interactions among natural environmental factors, including the degree of soil formation, leaching process, and agricultural activities such as tillage systems or fertilization (Dengiz et al., 2015). The descriptive statistical parameters such as mean, maximum, minimum, and coefficients of variation (CV) of the some basic physico-chemical properties related to 280 soil samples taken from surface (0-20 cm) of the crop lands in 21 micro catchments of the Tokat province were given in Table 2. In order to determine variability of some physico-chemical soil properties, many researchers offer to investigate coefficient of variation (CV). According to CV values, it was classified as low (<15%), medium (15-35%) and moderate (> 35%) (Mallants et al., 1996). In this case, variables of sand, silt of physico-chemical soil properties and Zn have moderate CV. On the other hand, the variables of clay, EC, $CaCO_3$ content and OM of soil physico-chemical properties and all HM (except for Zn) had a high level of variability. In addition to that, the values of pH in soil samples have low variability and ranged from moderately acid to slightly alkaline (5.22 and 8.07), whereas electrical conductivity had a mean value of 0.63 dS m^{-1}. The mean values of organic matter and $CaCO_3$ content (%) were 2.04 and 11.34. Table 2 shows also HM status of the soil samples.

As for the concentrations of heavy metals in the surface soils given in Table 2, it was determined that variables of heavy metal concentration of soils had a high level of variability. In the soils studied, the concentrations of Cu amounted to 6.83–106.11; Cr, 0.00-377.44; Pb, 0.00-19.80; Co, 0.00-27.55; and Ni, 2.79-521.92 mg kg^{-1}. The heavy metal contents such as Cu, Ni and Cr were higher than those given in Table 2. On the other hand, Pb, Co, Cd and Zn concentration not exceeded their permissible threshold level in all of the soil samples. While 51% of the soil samples was found Ni concentration exceeded its maximum permissible value and illustrated its grouping in representative micro catchments (Figure 4), Cr concentration passed maximum level of permission in 15% of soil samples. Furthermore, only in one sample an elevated Cu and Pb content was found.

Table 2. Descriptive statistical analysis of physico-chemical properties and heavy metal of soil samples

Parameters	Mean	SD	*CV	Variance	Min.	Max.	**Skewness	Kurtosis
Physico-chemical properties								
Sand, %	42.57	11.66	27.38	135.88	11.11	87.05	0.39	0.83
Clay, %	27.59	10.28	37.25	105.60	4.91	52.93	0.33	-0.22
Silt, %	29.84	8.01	26.83	64.12	7.73	67.27	1.29	3.42
pH, 1:2.5	7.45	0.46	6.19	0.21	5.22	8.07	-2.93	9.49
EC, dS m^{-1}	0.63	0.31	48.87	0.09	0.00	2.83	2.20	10.48
$CaCO_3$, %	11.34	9.41	82.99	88.56	0.00	53.05	0.90	0.95
OM, %	2.04	0.87	42.78	0.76	0.74	5.88	1.66	3.82
Heavy metal								
Cu (0-100)#	36.55	15.64	42.79	244.60	6.83	106.11	1.14	2.95
Cd (0-3) #	0.56	0.47	83.35	0.22	0.00	2.37	0.59	0.29
Cr (0-100) #	58.54	50.66	86.53	2566.18	0.00	377.44	2.61	9.68
Pb (0-100) #	6.93	3.70	53.44	13.71	0.00	19.80	0.36	-0.05
Co (0-50) #	8.63	5.59	64.71	31.22	0.00	27.55	0.80	0.61
Ni (0-50) #	67.04	67.86	101.22	4605.17	2.79	521.92	3.30	14.11
Zn (0-300) #	36.14	11.85	32.79	140.46	3.73	76.69	0.18	0.18

SD: Standard deviation, Min.: Minimum, Max.: Maximum, n: sample number, EC: Electric conductivity, OM: Organic matter.
*CV (Coefficient of Variation), **skewness:< ∓ 0.5 = Normal distribution, 0.5- 1.0 = Application of character changing for dataset, and > 1,0 → application of Logarithmic change,
#: Maximum permissible concentration (mg kg^{-1})

According to results, micro catchments coded as 2, 3, 4, 11, 8, 12, 13, 14, 16, located around Zile, Sulusaray, Niksar, and Artova Districts were determined accumulation of Ni concentration which exceeded maximum permissible concentration (MPC) in surface soil soils of the northern east and southern west parts of the Tokat province area. This relatively high Ni concentration level is not related with industrial or other anthropogenic pollution. It can be said natural case namely; it appears to be associated with the properties of the parent (volcanic) rock. As Chen et al. (2005) and Kızılkaya et al. (2011) reported, the Ni concentration in volcanic rocks is 20–40 times greater as compared to other ones. All the heavy metal concentrations determined in the soils of the test plots were lower than the threshold ones. In addition to that, high Cu heavy metal content was found in one micro catchments coded as 8 located around Niksar District. This fact is likely related to the wide application of pesticides containing copper. In addition, in some micro catchments coded as 2, 3, 4 and 11 of Sulusaray and Artova District have Cr accumulation group. On the other hand, the other micro catchments codded as 9, 15, 20, 21and located around Zile, Reşadiye and Erbağ Districts were detected less affected or no contaminated by heavy metal (Figure 4). Moreover, Cu have (106.11 mg kg^{-1}) over threshold level in each one soil sample in micro catchments coded as 8 and 11. This case can be assessed no potential risk in terms of heavy metal concentration. Because, these heavy metals don't show grouping of samples as Ni or Cr in Figure 4.

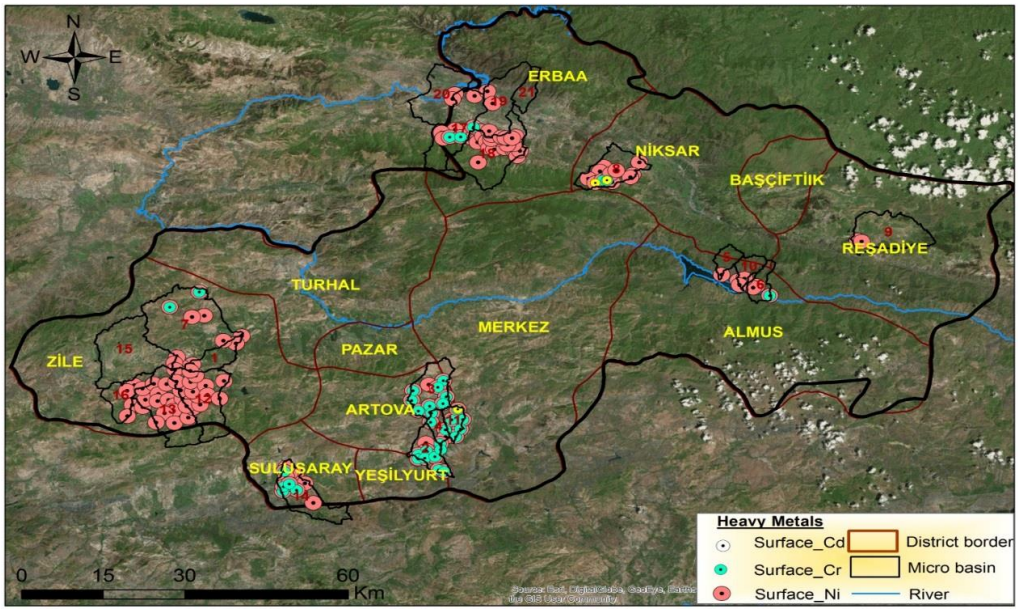


Figure 4. Exceeded heavy metals their maximum permissible concentration in representative micro catchments

A correlation analysis was done for the determination of the relationships between the physico-chemical properties of the soils and heavy metals and given in Table 3. Puschenreiter and Horak (2000) stated that the basic soil characteristics, such as the pH and texture, are of great importance in the availability of heavy metals in the soils. It was found that a significant negative correlation was revealed between the content of sand and the Pb concentrations, whereas a positive correlation was determined between the clay content and the heavy metal content such as Cd and Pb. This result confirmed the data of Temmerman et al. (2003). In addition, no significant relation was found between the content of silt and the heavy metals which are Cd, Pb, Co and Zn. The soil's pH and the $CaCO_3$ content have significant role related to the heavy metals accumulation in the soils. Negative linear correlations were found between these soil properties and some heavy metals in surface soil depth.

Table 3. Relationships between some physico-chemical properties of the soils and the heavy metals in the surface and subsurface soils

Parameters	Sand, %	Clay, %	Silt, %	pH	EC, dS m^{-1}	$CaCO_3$, %	OM, %
Clay, %	-0.740**	1					
Silt, %	-0.506**	-0.206**	1				
pH	-0.019	0.045	-0.030	1			
EC, dS m^{-1}	-0.173**	-0.026	0.285**	0.137*	1		
$CaCO_3$, %	-0.275**	0.090	0.284**	0.374**	0.170**	1	
OM, %	-0.040	0.035	0.014	-0.369**	0.246**	-0.031	1
Cu, mg kg^{-1}	0.073	0.076	-0.204**	-0.155**	-0.241**	-0.432**	-0.024
Cd, mg kg^{-1}	-0.076	0.155**	-0.088	-0.015	0.045	0.084	0.100
Cr, mg kg^{-1}	0.074	0.014	-0.125*	0.079	-0.095	-0.151*	-0.041
Pb, mg kg^{-1}	-0.181**	0.187**	0.023	-0.002	0.048	0.006	0.183**
Co, mg kg^{-1}	0.106	-0.116	-0.006	0.085	-0.092	-0.129*	-0.038
Ni, mg kg^{-1}	0.074	0.034	-0.151*	0.099	-0.075	-0.130*	-0.058
Zn, mg kg^{-1}	-0.008	0.064	-0.070	-0.054	-0.070	-0.216**	0.112

*P < 0.05, ** P < 0.01, EC: Electric conductivity, OM: Organic matter.

In addition to obtain real heavy metals' values, all the elements were additionally grouped into five levels by Sutherland (2000) in order to estimate their relative accumulation according to the enrichment factors (EF) values for surface and subsurface soil samples. Some statistical characteristics of the EF for the surface and subsurface soils are given in Table 4. In surface soil samples, the EF values for Cd and Ni attest that the soils were enriched with these elements as compared to their mean background value. , Cd, Ni and Cr have found 16%, 10% and 6% soil samples as moderate enrichment class, whereas 55% and 1% of the total soil samples were determined significant enrichment class in terms of Cd and Ni elements. The EF values for the other elements were < 2. The maximal EF value for Cd (21.6) points to the enrichment of the soil with this element; the EF values <2 for Pb, Co, and Zn pointed to the absence of the soil's enrichment with these elements (Table 4). Only a few soil samples have moderate EF classes in terms of Pb and Cu.

Table 4. Some statistical characteristics of the enrichment factors (EF) for the surface and subsurface soils, mg kg^{-1}

Heavy Metals	Mean	SD	*CV	Variance	Min.	Max.	**Skewness	Kurtosis
Cu	0.73	0.31	42.79	0.10	0.14	2.12	1.14	2.95
Cd	0,49	0,26	53,44	0,07	0,00	21.6	0,36	-0,05
Cr	0.59	0.51	86.53	0.26	0.00	3.77	2.61	9.68
Pb	0.53	0.58	110.25	0.34	0.00	9.12	11.79	174.47
Co	0.43	0.28	64.71	0.08	0.00	1.38	0.80	0.61
Ni	0.84	0.85	101.22	0.72	0.03	6.52	3.30	14.11
Zn	0.48	0.16	32.79	0.02	0.05	1.02	0.18	0.18

Conclusion

This present study was conducted in some intensive cultivated land of Tokat Province in order to determine heavy metals content and their enrichment factors. For this purpose, 280 soil samples were collected form surface soil. The results showed that mostly the concentration of Ni followed by Cr exceeded their threshold levels. The local pollutions from Ni and Cr were attributed to the natural influences (particularly due to parent material). The concentrations of the other HMs are relatively lower than the critical values. The mean values of the HMs contents arranged in the following decreasing order: Ni>Cr>Cu>Zn>Co>Pb>Cd in the studied soil sample. In addition, it was found significantly positive relation between Pb and OM while the same relation was also found clay content and Cd and Pb. On the other hand, according to EF of HMs in total

soil samples, Cd, Ni and Cr have found 16%, 10% and 6% soil samples as moderate enrichment class, whereas 55% and 1% of the total soil samples were determined significant enrichment class in terms of Cd and Ni elements. Besides, all other HM elements did not exceed minimal enrichment level. However, in some regions of the study area, the Cu, Cd and Pb contents were also slightly raised, this case possibly stems from anthropogenic effects such as excessive P fertilization, field traffic and pesticide using.

References

Agbenin, J.O., 2002. Lead in a Nigerian Savanna soil under long term cultivation. Science of the Total Environment 286(1-3): 1–14.

Akyıldız, M., Karataş, B., 2018. Investigation of heavy metal pollution in the soil at Adana City Center. *Çukurova University Journal of the Faculty of Engineering and Architecture* 33(2): 199-214 [in Turkish].

Arda, H., Helvacıoğlu, İ. A., Meriç, Ç., Tokatlı, C., 2015. İpsala ilçesi (Edirne) toprak ve pirinç kalitesinin bazı esansiyel ve toksik element birikimleri açısından değerlendirilmesi. *Tarım Bilimleri Araştırma Dergisi* 8 (1): 7-13 [in Turkish].

Bakış, R., Bilgin, M., 1998. Çöp sızıntı sularından dolayı topraklarda meydana gelen ağır metal kirliliğinin araştırılması, Kayseri I. Atıksu Sempozyumu, 22-24 Haziran 1998, Kayseri, Bildiri Kitabı s.167-170 [in Turkish].

Bilge, U., Çimrin, K.M., 2013. Heavy Metal Pollution in Soils Adjacent to the Kızıltepe – Viranşehir Road. *Journal of Agricultural Sciences* 19: 323-329 [in Turkish].

Borůvka, L., Vacek, O., Jehlička, J., 2005. Principal component analysis as a tool to indicate the origin of potentially toxic elements in soils. *Geoderma* 128(3-4): 289–300.

Bouyoucos, G.J., 1962. Hydrometer method improved for making particle size analyses of soils. *Agronomy Journal* 54(5): 464-465.

Chen, T.B., Zheng, Y.M., Lei, M., Huang, Z.C., Wu, H.T., Chen, H., Fan, K.K., Yu, K., Wu, X., Tian, Q.Z., 2005. Assessment of heavy metal pollution in surface soils of urban parks in Beijing, China. *Chemosphere* 60(4): 542–551.

D'Ascoli, R., Rao, M.A., Adamo, P., Renella, G., Landi, L., Rutigliano, F.A., Terribile, F., Gianfreda, L., 2006. Impact of river overflowing on trace element contamination of volcanic soils in south Italy: Part II. Soil biological and biochemical properties in relation to trace element speciation. *Environmental Pollution* 144(1):317-326.

Dengiz, O., M.A. Özyazıcı., Sağlam, M., 2015. Multi-Criteria assessment and geostatistical approach for determination of rice growing suitability sites in gokirmak catchment. *Paddy and Water Environment* 13(1): 1–10.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B., Beeregowda, K.N., 2014. Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology* 7(2): 60–72.

Kaplan, O., Yildirim, N.C., Yildirim, N., Cimen, M., 2011. Toxic elements in animal products and environmental health. *Asian Journal of Animal and Veterinary Advances* 6 (3): 228-232.

Karaca, A., 2001. Afşin-Elbistan termik santralı emisyonlarının çevre topraklarının fiziksel, kimyasal ve biyolojik özellikleri üzerine etkileri. *Pamukkale Üniversitesi Mühendislik Fakültesi Bilimleri Dergisi* 7(1): 95-102.

Kızılkaya, R., Dengiz, O. Özyazıcı, M. A. Aşkın, T Mikayiloy, F., Shein, E.V., 2011. Spatial distribution of heavy metals in soils of the Bafra plain in Turkey. *Eurasian Soil Science* 44(12): 1343-1351.

Kloke, A., 1980. Orientierungsdaten fur tolerierbare gesa mtgehalte einiger elemente in kulturboden. Mitt.VDLUFA 1-3: 9–11.

Liu, Y., Xiao, T., Perkins, R.B., Zhu, J., Zhu ,Z., Xiong, Y., Ning Z., 2017. Geogenic cadmium pollution and potential health risks, with emphasis on black shale. *Journal of Geochemical Exploration* 176: 42-49.

Mallants, D., Mohanty B.P., Jacques D., Feyen J., 1996. Spatial variability of hydraulic properties in a multi-layered soil profile. *Soil Science* 161(3): 167-181.

Manta, D.S., Angelone, M., Bellanca, A., Neri, R., Sprovieri, M., 2002. Heavy metals in urban soils: a case study from the city of Palermo (Sicily), Italy. *The Science of the Total Environment* 300(1-3): 229–243.

Meena, N.K., Maiti, S., Shrivastava, A., 2011. Discrimination between anthropogenic (pollution) and lithogenic magnetic fraction in urban soils (Delhi, India) using environmental magnetism. *Journal of Applied Geophysics* 73(2):121–129.

Metin, S.Ü., 2010. The determination of heavy metal pollution in the agricultural soils of alluvial, colluvial and vertisol in the Bursa Plain. Msc thesis. Uludağ University, Department of Soil Science, Bursa, Turkey. 62p. [in Turkish].

Mudgal, V., Madaan, N., Mudgal, A., Singh, R.B.S., Mishra, S., 2010. Effect of Toxic Metals on Human Health. *The Open Nutraceuticals Journal* 3: 94-99.

Oliveira, A., Pampulha, M.E., 2006. Effects of long-term heavy metal contamination on soil microbial characteristics. *Journal of Bioscience and Bioengineering* 102(3): 157–161.

Peralta-Videa, J.R., Lopez, M.L., Narayan, M., Saupe, G., Gardea-Torresdey, J., 2009. The biochemistry of environmental heavy metal uptake by plants: Implications for the food chain. *The International Journal of Biochemistry & Cell Biology* 41(8-9):1665–1677.

Puschenreiter, M., Horak, O., 2000. Influence of different soil parameters on the transfer factor soil to plant of Cd, Cu and Zn for wheat and rye. *Die Bodenkultur* 51(1): 3–10.

Salati, S., Moore, F., 2010. Assessment of heavy metal concentration in the Khoshk River water and sediment, Shiraz, Southwest Iran. *Environmental Monitoring and Assessment* 164(1-4): 677–689.

Sivry, Y., Riotte, J., Sonke, J.E., Audry, S., Schäfer, J., Viers, J., Blanc, G., Freydier, R., Dupré, B., 2008. Zn isotopes as tracers of anthropogenic pollution from Zn-ore smelters The Riou Mort–Lot River system. *Chemical Geology* 255(3-4): 295-304.

Soil Survey Laboratory, 1992. Procedures for collecting soil samples and methods of analysis for soil survey. Soil Survey Investigations Reports U.S. Govermentan Print Office, Washington D.C., USA.

Soil Survey Staff, 1993. Soil Survey Manuel. USDA Handbook. No: 18, Washington D.C. USA.

Sposito, G., 1989. The Chemistry of Soils. Oxford University Press, New York, USA. 272p.

Sutherland, R.A., 2000. Bed sediment-associated trace metals in an urban stream, Oahu, Hawaii. *Environmental Geology* 39(6): 611–627.

Temmerman, L., Vanongeval, L., Boon, W., Hoenig, M., Geypens, M., 2003. Heavy metal content of arable soils in Northern Belgium. *Water, Air, and Soil Pollution* 148(1-4): 61–76.

Yadav, S.K., 2010. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *South African Journal of Botany* 76(2): 167–179.

Yang, Q.W., Lan, C.Y., Wang, H.B., Zhuang, P., Shu, W.S., 2006. Cadmium in soil–rice system and health risk associated with the use of untreated mining wastewater for irrigation in Lechang, China. *Agricultural Water Management* 8(1-2): 147–152.

Zhang, X.Y., Lin, F.F., Wong, M.T.F., Feng, X.L.. Wan, K., 2009. Identification of soil heavy metal sources from anthropogenic activities and pollution assessment of Fuyang County, China. *Environmental Monitoring and Assessment* 154: 439–449.

Eurasian J Soil Sci 2019, 8 (3) 257 - 266

Eurasian Journal of Soil Science

Journal homepage : http://ejss.fesss.org

Measurement and estimation of evapotranspiration in semi-arid grassland during the summer season in southwest Siberia

Lisa Haselow [a,*], Holger Rupp [a], Andrej A. Bondarovich [b], Ralph Meissner [a]

[a] Department of Soil System Science, Helmholtz Centre for Environmental Research – UFZ, Falkenberg 55, 39615 Altmaerkische Wische, Germany

[b] Faculty of Geography, Altai State University, Barnaul, Russia

Abstract

This study quantifies actual evapotranspiration (ET_a) for a period from June to September 2016 measured by two weighable gravitation lysimeters in a semi-arid grassland in southwest Siberia. As part of a crop rotation system, the first lysimeter was fallow but covered with ruderal vegetation. The second lysimeter is permanently characterized by pristine steppe vegetation. In addition to ET_a measurements, the reference evapotranspiration (ET_0) is computed by a Penman-Monteith model. The estimates are related to the ET_a records and the model is evaluated with regard to its performance in a semi-arid environment. The results indicated an ET_a driven by energy but limited by water. Within 115 days the total amounts of ET_a ranged from 205 mm to 374.1 mm, and daily values varied from 0.1 to 6.9 mm day^{-1}. The large differences are caused by the different vegetation cover of the lysimeters. Due to the high and dense canopy of the pristine steppe vegetation, the transpiration term was considerably higher compared to the ruderal vegetation where soil evaporation took the major part. The daily ET_a records differed on average by -91.1% to the ET_0 estimates. The statistical analyses yielded a low correlation between ET_a of the ruderal vegetation and ET_0 but an acceptable model performance for the pristine steppe. However, it was observed that ET_a occasionally exceeds ET_0, particularly after precipitation. Due to the high water availability and the subsequent rise of ET_a, ET_0 was underestimated, whereas it was overestimated during dry periods. Finally, the quality of the Penman-Monteith model varied substantially with the water supply at the study site.

Keywords: Actual evapotranspiration, Penman-Monteith FAO-56, semi-arid, Siberia, weighable gravitation lysimeter.

Article Info

Received : 08.01.2019
Accepted : 14.05.2019

Introduction

In semi-arid areas, the availability of water is of particular importance. These environments are characterized by low precipitation and water is a limited resource, which influences vegetation density, cover, and biomass. Knowledge of soil-atmosphere exchange of energy and moisture, as well as crop water requirement, is important for the management of regional water resources. For this purpose, processes have to be identified that exhibit influence on the hydrological cycle. Actual evapotranspiration (ET_a) is often used to determine the water loss from the soil surface (evaporation) and from the growth and temperature regulation process of plants (transpiration). However, measurement of ET_a is a challenge (Wohlfahrt et al., 2010; Allen et al., 2011; Amatya et al., 2016). There are different possibilities for obtaining accurate estimates of ET_a; indirect methods such as residual energy balance, Bowen ratio energy balance, soil water balance (Shi et al., 2008; Wegehenkel et al., 2008; Meissner et al., 2016a; Martel et al., 2018), and those that

* Corresponding author.
Department of Soil System Science, Helmholtz Centre for Environmental Research – UFZ, Falkenberg 55, 39615 Altmaerkische Wische, Germany
Tel.: +49 345 558 5205
E-mail address: lisa.haselow@ufz.de
e-ISSN: 2147-4249
DOI: 10.18393/ejss.567359

include lysimetry and eddy covariance methods for direct measurements (Wohlfahrt et al., 2010; Fleischer et al., 2015; Gebler et al., 2015). Weighable lysimeters are widely used for ET_a measurements (Von Unold and Fank, 2008; Schrader et al., 2013; Wegehenkel and Gerke, 2013; Mauder et al., 2017; Oberholzer et al., 2017). Though, for lysimeter measurements a lot of requirements have to be considered (Allen et al., 2011). In summary, soil properties and vegetation cover of the lysimeter must be very similar to the surrounding area. It is usually difficult to reconstruct the original soil profile and to maintain the field conditions of soil and vegetation. Eventually, lysimeter measurements represent only point measurements that will be transferred to large areas. Nevertheless, they remain effective due to the weighing system that enables the derivation of evapotranspiration (ET) from mass records with the highest accuracy compared to the methods mentioned above (Allen et al., 2011).

Furthermore, lysimeter measurements will also be used for calibration and validation of ET models (Makkink, 1957; DehghaniSanij et al., 2004; López-Urrea et al., 2006; Wegehenkel and Gerke, 2013). As water stress become more and more to an important issue, baseline information is required for water resource planning, particularly in arid and semi-arid regions. Therefore, the estimation of ET by deterministic models has exponentially increased in recent years. These models built around climate and land surface data, provide reliable ET rates for a reference crop. From the several existing models, the Penman-Monteith FAO-56 (PM FAO) equation is the most used for estimating reference evapotranspiration (ET_0). Due to the high demand of data, the PM FAO model proved to be highly accurate (DehghaniSanij et al., 2004; López-Urrea et al., 2006; Sabziparvar and Tabari, 2010; Martel et al., 2018). Nevertheless, the data demand is also a major drawback of the model. For calculation of ET_0 high-resolution, the data that is required is limited in many regions. Large parts of Siberia, for instance, are not covered by meteorological measurement stations. Yet, water resources and ET estimations are relevant for these large areas; especially with regard to climate change, which has a direct impact on the regional hydrological cycle and agriculture (Fraser et al., 2013; Degefie et al., 2014). Moreover, they belong to the region which has the potential to become the "*bread basket*" of the world due to the large land and yield reserves (Bagley et al., 2012; Swinnen et al., 2017).

Previous studies conducted in Siberia (Yamazaki et al., 2004; Park et al., 2008; Fleischer et al., 2015), have used land surface models to investigate water and energy exchanges at forests and transition zones. The estimation of ET refers solely to the Bowen ratio method, eddy covariance, and models based on Penman formulations. However, there is an absence of studies based on ET estimation by weighable lysimeter measurements.

In the framework of the research project KULUNDA (Balikyn et al., 2016) an established monitoring network enabled the estimation of ET_a by weighable gravitation lysimeters in the Kulunda grass steppe of southwest Siberia. On this basis, the study quantifies ET_a of two lysimeters with different vegetation cover under semi-arid conditions. In addition, ET_0 is calculated by using the PM FAO equation (Allen et al., 1998), which was selected due to the crop reference of grass and the independence to the climate type.

The objectives of the study are:

i. to assess ET_a as a function of vegetation cover and climatic conditions,
ii. to compare ET_0 estimates with ET_a records,
iii. to assess the PM FAO model for a semi-arid environment.

Material and Methods

The study site and monitoring network

The study site is part of the semi-arid Kulunda steppe, southwest Siberia, and located between the Central Asian steppe and the North Asian forest-steppe (Balikyn et al., 2016). The site is 100-140 m a.s.l.; its mean annual temperature is about 0 °C with a maximum in July (up to +40 °C) and a minimum in January (down to -47 °C). The annual precipitation is about 250-450 mm, where the major part of 200 mm occurs from April to October. The global radiation is 2-3 times higher than the energy that is required to evaporate precipitation. The surrounding area is plain and dominated by natural steppe vegetation.

In the framework of the research project KULUNDA a monitoring network was established, which has consisted of a weather station and a weighable gravitation lysimeter station. The set-up of the weather station was in September 2012. Meteorological parameters such as rainfall, air temperature, wind speed and direction, air humidity and barometric pressure are measured by a multisensor at a height of 2.3 m. A pyranometer recorded the solar radiation at a height of 2 m, and a pluviometer mounting in a tipping bucket rain gauge collected precipitation at the standard height of 1 meter.

The containerized lysimeter station with two weighable soil monoliths (manufacturer "UGT-Muencheberg", Germany and Helmholtz Centre for Environmental Research – UFZ, Germany) was installed at the test farm of the project in Poluyamki (N52° 03.959' E79° 42.786'; approximately 700 km southwest of Novosibirsk) between June and August 2013 (Balikyn et al., 2016). The soil monoliths were monolithically extracted from an arable land (lysimeter 1 - LYS 1- ruderal vegetation) and a fallow site (lysimeter 2 - LYS 2 – pristine steppe), which was covered with pristine steppe vegetation since the 1950s. The different cultivation allowed comparative analyses between arable land and unconverted grassland. Thus, there was an ascertained crop rotation at LYS 1: wheat (2013), peas (2014), wheat (2015), and fallow (2016). In contrast, LYS 2 was dominated by natural feather grass (*Stípa pennáta*) between 2013 and 2016.

Each lysimeter had a surface area of 1 m^2, a depth of 2 m and was monolithically filled with a soil, which was identified as Calcic Chernozems according to the guidelines of the Food and Agriculture Organization of the United Nations (FAO). A detailed description of the lysimeters is given by Meissner et al. (2016b). The total mass of each lysimeter vessel was approximately 4000 kg and the mass changed with water input (precipitation, dew, rime, and the water equivalent of snow) and water output (ET_a). The vessels were positioned into the lysimeter station on load cells that measure the mass with high precision of ± 20 g (Xiao et al., 2009). The data were consolidated and stored in a data logger with a recording interval of one hour. Due to their geometry, a change of mass is equal to a water storage change in millimeters (1 kg ≈ 1 L/m^2 = 1 mm). Therefore, all changes of mass are given in millimeters in the following.

Data preparation and calculation of ET_a

The lysimeter measurements started in August 2013. However, reliable measurements have turned out to be a challenge at this study site. Currently, data was only available from August 2013 to September 2016, but the time series was not continuous. Due to the malfunction of the lysimeters during winter induced by sub-zero temperatures and snow, all data between October and May were nonapplicable for data analysis. Furthermore, direct access to the data was not possible. The release of the data was hindered by the Russian administration and created a time delay between measurement and receipt of data. For these reasons, data analysis took place only with a data set from June 08, 2016 to September 30, 2016.

The processing of lysimeter data was executed in several steps according to the principle of the adaptive window and adaptive threshold filter (AWAT) developed by Peters et al. (2014). First, the raw data were manually filtered, and all data during system error or noticeable outliers were removed. If the resulting gaps did not exceed a period of four hours, values were estimated by linear interpolation. In step two, the data were smoothed by using an adapted window width. The *Savitzky-Golay* filter (Savitzky and Golay, 1964) was proven as an eligible smoothing routine for the data set. The window width (ω) was set at 5 hours and a polynomial of 3rd order was used. The window width was adapted to increasing noises. Third, an adaptive threshold (δ) was applied to obtain ET_a out of mass data. The setting of threshold was optimized for lysimeter separately. For LYS 1 the lower limit for threshold (δ_{min}) was set to 0.04 mm, whereas the upper limit (δ_{max}) was set to 0.7 mm. The threshold values of LYS 2 were increased with δ_{min} = 0.05 mm and δ_{max} = 0.8 mm. At last, inconsistent values of the filter output were corrected manually.

Information about water fluxes at the pedosphere-atmosphere interface can be derived from mass changes of a lysimeter. The total mass of the system (M) is the sum of lysimeter mass (M_{lys}) and of drainage (M_{drain}). It is assumed that a mass increase is precipitation (P) and a mass decrease is ET_a. With this assumption that either ET_a or P occurs, but not both at the same time, P is equal to zero when ET_a is active, and vice versa (Schrader et al., 2013):

$$M = M_{lys} + M_{drain} \quad (1)$$

$$P = \begin{cases} \Delta M & \text{for} \quad \Delta M > 0 \\ 0 & \text{for} \quad \Delta M \leq 0 \end{cases}$$

$$ET_a = \begin{cases} \Delta M & \text{for} \quad \Delta M < 0 \\ 0 & \text{for} \quad \Delta M \geq 0 \end{cases}$$

where M_{lys} is the mass of lysimeter vessel [kg], M_{drain} is the amount of seepage water [kg], and ΔM is the total mass change of lysimeter vessel in the according time interval [kg].

Depending on the aims of data use, ET_a can be expressed at different time scales. Where daily values are required, hourly values are summed-up for one day, starting from 12:00 and follows to 24 hours. Furthermore, for lysimeter readings of ET_a, LYS 2 was considered as a reference since the canopy of the lysimeter corresponded to the surrounding field.

Estimation of ET_0 by using the PM FAO model

The measurements of ET_a by lysimeters were compared with ET_0 calculated by the PM FAO equation according to Allen et al. (1998). The Penman-based models are widely used in virtually any climate type. The recommended FAO version has proven as a highly accurate model for calculating ET_0 if the required meteorological input parameters are available. The approach assumes a surface of a uniform and actively growing grass vegetation without water stress, an approximate height of 0.12 m, a daily surface resistance of 70 s m^{-1}, and an albedo of 0.23. In connection with the original Penman-Monteith equation (Monteith, 1965) the final form is as follows:

$$ET_0 = \frac{408\Delta(R_n - G) + \gamma \frac{900}{T+273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34\, u_2)} \quad (2)$$

where ET_0 is the reference evapotranspiration [mm d^{-1}], R_n the net radiation [MJ m^{-2} d^{-1}], G the soil heat flux density [MJ m^{-2} d^{-1}], T the mean daily air temperature [°C], u_2 the mean daily wind speed at 2 m height [m s^{-1}], e_s the saturation vapor pressure [kPa], e_a the actual vapor pressure [kPa], Δ the slope vapor pressure curve [kPa °C^{-1}], and γ the psychrometric constant [kPa °C^{-1}] (Allen et al., 1998).

The calculation of daily ET_0 values was conducted by using R software. The R software package "*Evapotranspiration*" included different models to estimate ET_a, ET_0 and potential evapotranspiration (ET_p) (Guo et al., 2016). For the modeling, PM FAO required information about daily minimum and maximum temperature, incoming solar radiation, and wind speed as well as minimum and maximum relative humidity. The data were taken from the weather station of the monitoring network. Hourly data were manually calculated according to Eq. 2. In order to assess the model results related to the ET_a records the correlation of Pearson (r), the root mean square error (RMSE, Eq. 3), and the mean absolute error (MAE, Eq. 4) was used.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(X_i - Y_i)^2}{n}} \quad (3)$$

$$MAE = \frac{\sum_{i=1}^{n}|X_i - Y_i|}{n} \quad (4)$$

The variables X_i and Y_i are the i*th* observed reference and estimated values, respectively; n is the total number of data.

In addition, the ratio of ET_a to ET_0 delivers information about the water supply of the soil for a grass vegetation with an height of 0.12 m. The daily ET_a record divided by ET_0 estimate resulted in an index varying between 0 and 1. The water availability is high (moist) by an index of 1, i.e. there is no climatic risk of non-water supply (Louzada et al., 2018). Optimal water availability goes along with an index between 0.8 and 1. An index of <0.8 indicates a water deficit where only <80% of ET_0 will be satisfied (Roth et al., 2005).

Results and Discussion

Within the measuring period of 115 days, the total sum of ET_0 was considerably higher than ET_a of the lysimeters, +157.8% for ruderal vegetation and +41.3% for pristine steppe. The minimum of ET_a was 205 mm measured by LYS 1, which was 45.2% less than ET_a measured by LYS 2 (374.1 mm). A maximum of ET_0 was calculated by 528.6 mm. As the theoretical maximum estimate by ET_0 was not reached, it is assumed that ET_a was limited by water and not by energy. Table 1 lists the monthly ET_a and ET_0 records of June – September 2016. The maximum ET_a was achieved in July as well as the slightest deviation (-31%) between both lysimeters. The largest deviation was in June with -66.4%. ET_0 differs on average +235.5% (ruderal vegetation) and +163% (pristine steppe) per month, respectively.

Table 1. Monthly ET_a records of the lysimeters and ET_0 estimates calculated by PM FAO model; the data are based on daily mean values.

Month	ET_a LYS 1 ruderal vegetation	LYS 2 pristine steppe	ET_0 PM FAO
	mm month^{-1}		
Jun[a]	34.8	103.7	125.8
Jul	88.5	128.3	145.2
Aug	56.4	100.2	142.4
Sep	25.3	41.8	115.4

[a]No data from June 01 to June 07, 2016

The daily ET_a values were within the range of 0.1 to 6.9 mm day^{-1}, whereas ET_0 calculated by PM FAO model varied from 2 to 8.3 mm day^{-1} (Figure 1). The medians lay between 1.4 and 4.5 mm day^{-1}. The difference between mean ET_a of the lysimeters and ET_0 is -91.1%. The ET_a of the ruderal vegetation had the smallest median and yielded the lowest values as well as variation. The median of the ET_a of the pristine steppe was at 3.3 mm day^{-1} and it covered the widest range of values. The minimum and maximum values of ET_a of the lysimeters were very similar. ET_0 had a maximum value of 8.3 mm day^{-1} with a maximum difference of +24.5% (to ET_a of the ruderal vegetation), and a mean difference of +20.7% (to maximum ET_a of both lysimeters). The absolute deviation was greatest with 7 mm day^{-1} between ET_a of the ruderal vegetation and ET_0, which corresponds to a difference of -556.3%.

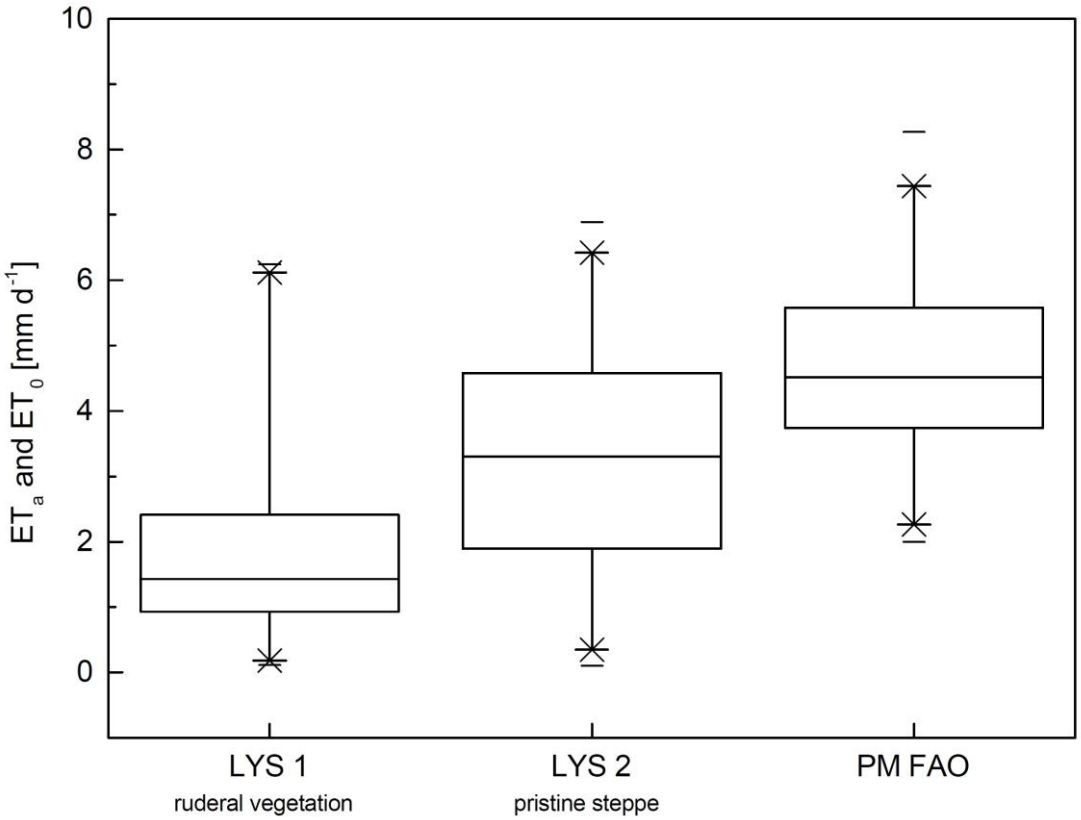


Figure 1. Comparison of ET_a measured by lysimeters and ET_0 computed by the PM FAO model. The box plots are based on daily data. The box boundaries represent the 25th and 75th percentiles, the inner lines indicate the medians, the whiskers extend to 1.5 times the interquartile range, the crosses mark the 1st and 99th percentiles, and the strokes show the minimum and maximum values.

In general, daily ET_a and daily values of solar radiation and air temperature were positive correlated (Table 2). Therefore, the energy was the leading factor for ET_a at the study site. In contrast to observations of other studies in which factors such as relative humidity and wind speed removed water vapor from the vegetation surface (Priestley and Taylor, 1972; Shi et al., 2008; Yang et al., 2014) their low correlation indicated no relationship. The negative correlation with wind speed was noticeable, but similar results were found in previous studies with arid and semi-arid site conditions (El Bably, 2003; Martel et al., 2018).

Table 2. Pearson correlation coefficients (r) between ET_a of the lysimeters and meteorological parameters

Lysimeter	Meteorological parameter			
	Solar radiation	Air temperature	Relative humidity	Wind speed
LYS 1 - ruderal vegetation	0.41**	0.45**	0.46**	-0.17
LYS 2 - pristine steppe	0.80**	0.69**	0.18*	-0.13

*P< 0.05; **P< 0.01

Daily ET_a rates between lysimeters and ET_0 illustrated largely negative differences (Figure 2). Lysimeter 1 recorded up to 5.2 mm day^{-1} (06/14/2016) less than LYS 2 (cf. Figure 2a). The fluctuations were high in June and became lower from August. This may be caused by the diversity of vegetation. LYS 2 exhibited a high and dense layer of grass, which has induced ET_a and interception. On the other hand, LYS 1 was fallow in June. Hence, evaporation was the exclusive process at LYS 1, as a result of the lack of vegetation. From July a convergence of ET_a of LYS 1 to ET_a of LYS 2 was observed based on the development of ruderal vegetation and the additional transpiration term at LYS 1. The conditions of LYS 1 were also reflected in the high fluctuation between ET_a of the ruderal vegetation and ET_0 during the whole period (cf. Figure 2b). The discrepancies between observed and calculated data were high with an average of -3.2 mm day^{-1}, except for July where the deviations became lower with a mean of -1.8 mm day^{-1}. ET_a of the pristine steppe demonstrated minor deviations to ET_0 between June and August, and account several days where $ET_a = ET_0$ (cf. Figure 2c). It was found that ET_a repeatedly exceeded ET_0 with amounts up to +0.8 mm day^{-1} (07/13/2016), in which ET_a of the pristine steppe tended more frequently to exceed ET_0.

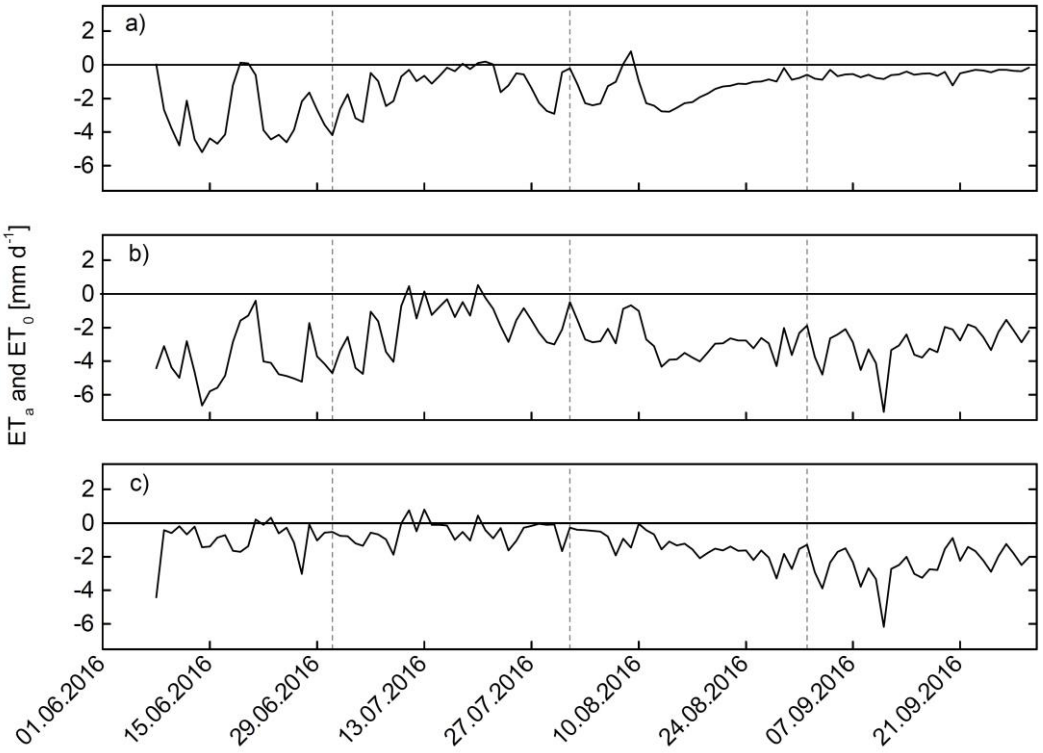


Figure 2. Daily differences of ET between June 08 and September 30, 2016; a) ET_a of ruderal vegetation – ET_a of pristine steppe, b) ET_a of ruderal vegetation – ET_0, c) ET_a of pristine steppe - ET_0.

The results of the statistical analyses showed a low correlation between ET_a of the ruderal vegetation and ET_0 (Figure 3). In addition to the high values of RMSE and MAE, the model can be assessed with poor quality for LYS 1. The PM FAO assumes an extensive surface canopy that completely covers the ground. However, the ruderal vegetation at LYS 1 did not comply with these conditions over the investigated period. ET_a of the pristine steppe and ET_0 were strongly correlated. The RMSE with 1.7 mm and MAE with 1.4 mm presented a significant performance, with a tendency to overestimation. Nevertheless, due to the dense and high steppe grass at LYS 2, the transpiration term may be larger as the model assumed. This assumption is justified by Roth et al. (2005) who pointed that the water consumption of a full developed vegetation cover may lead to an underestimation of ET_0. Similar results were also found by other studies (Wegehenkel and Gerke, 2013; Gebler et al., 2015). Indeed, a further and more widespread explanation for $ET_a > ET_0$ is the "oasis effect". It

occurs if conditions of vegetation and atmosphere of the lysimeters differ to those of the surrounding area. Actually, LYS 2 had to be preventing the oasis effect by reason of the same vegetation cover. Yet, LYS 1 was fallow; therefore, different soil water availability between the surrounding area and LYS 1 has to be considered. On the other hand, there is also the possibility that ET_a was overestimated by the pristine steppe vegetation. If the canopy of the lysimeter exceeds the rim, the effective area of the lysimeter can be larger than the original lysimeter extent. This "bloom effect" leads to higher radiation absorption of the vegetation, which eventually results in increased ET_a (Allen et al., 2011). The bloom condition can be excluded at LYS 1 because the vegetation is not tall and dense enough to exceed the lysimeter. There is a low likelihood that the oasis effect, as well as the bloom effect, occurs at both lysimeters at the same time. Thus, they cannot be responsible for $ET_a > ET_0$ in this case.

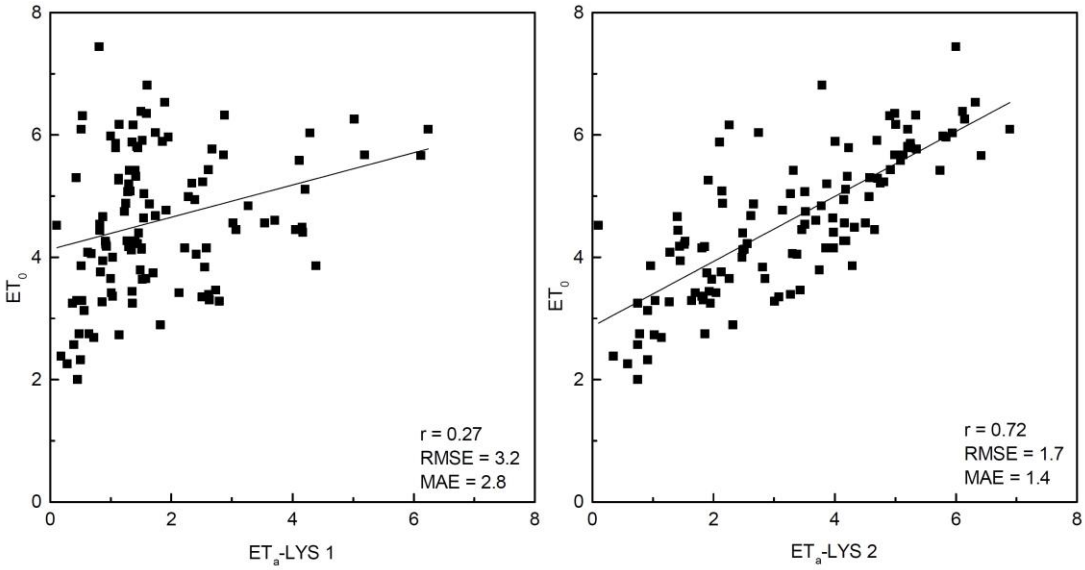


Figure 3. The relationship between ET_a and ET_0 on a daily basis (n=115), and the respective error values.

Figure 4 illustrates the daily water availability for the lysimeters. In June, the mean soil water availability index was at 0.3 for LYS 1 and at 0.8 for LYS 2. Thus, the pristine steppe showed an optimal water supply, whereas only 30% of the potential water demand was available for soil and the ruderal vegetation. However, the values for the ruderal vegetation should be treated with caution since it has already been demonstrated that the model quality for LYS 1 was poor. Thus, the lower index of the ruderal vegetation in June was less related to the soil water availability. Frequent precipitation caused a rise in water availability in July; consequently, ET_a is increased (Wever et al., 2002; Armstrong et al., 2008). On some days the water availability crossed the 100% threshold ($ET_a/ET_0 = 1$), which follows from $ET_a > ET_0$ (cf. Figure 2b, 2c). A connection was found between $ET_a > ET_0$ and precipitation events with cumulative amounts of >20 mm occurring a few days before. Between July 09 and July 11, for instance, a total rainfall of 30.9 mm was measured by the tipping bucket rain gauge. Within two days the water availability indices rose from 0.3 (ruderal vegetation) and 0.7 (pristine steppe) to 1.1, respectively. However, the virtual absence of precipitation led to a decrease of ET_a from August to September. During this period only 30 to 50% of ET_0 could be covered by ET_a. Due to the water stress, the deviations from observed data have increased. Hence, the PM FAO model overestimated ET_0 and indicated that the quality is strongly influenced by water availability. The PM FAO model is more suitable for short vegetation with permanent high water supply. As the growth stage constitutes an essential part within the ET_a process, the crop evapotranspiration may be a more appropriate approach because the crop canopy and aerodynamic resistance will be adapted to the reference crop. Though, the response to water stress cannot be reproduced since the method does not process information about soil water content. The issue of $ET_a > ET_0$ suggests that the calculated ET_0 reflects insufficiently the real water consumption of the vegetation (Roth et al., 2005). In such a case, plant-specific correction factors are necessary to derive ET_a from ET_0. This circumstance is again a proof that lysimeter measurements are qualified to improve model estimations (irrespective of model assumptions) because they provide reliable field data.

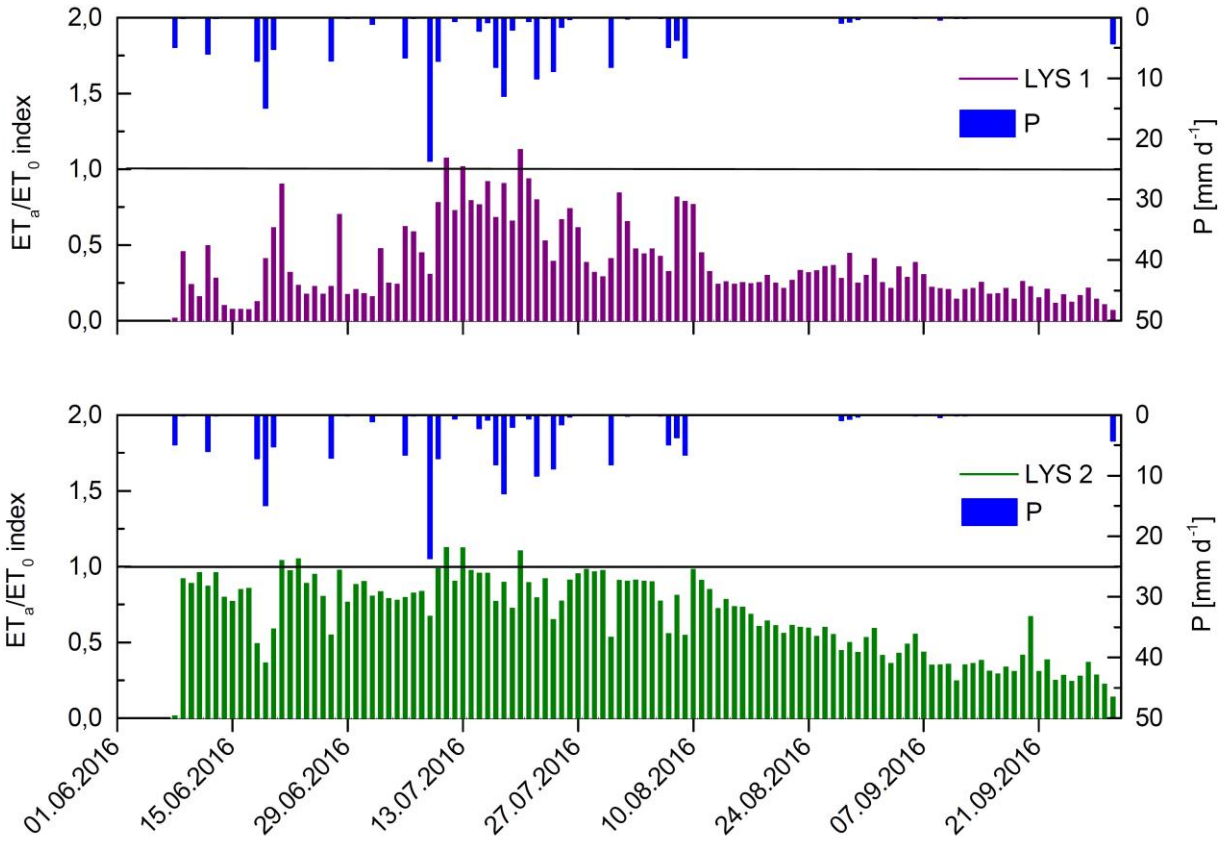


Figure 4. Illustration of the daily water availability between June and September 2016. The ET_a/ET_0 indices are related to the left axis; the upper bars are related to the right axis and show the daily sum of P measured by a tipping bucket rain gauge.

Although the study led to first scientific findings of ET_a for this site, further investigations are necessary. The major challenge is to get long-term data as measurements could not be executed so far over winter. Finally, a long-term study can enable to determine the local soil water balance and to evaluate the contribution of ET_a.

Acknowledgements

The paper was based on research studies which have been funded by the German Federal Ministry of Education and Research (BMBF) in the framework of the former project KULUNDA (FKZ 01LL0905D) and the running project ReKKS (FKZ 01LZ1704C). The intellectual content of the paper is the responsibility of the authors. We thank G. Schmidt and J. Doering from the Martin-Luther University Halle-Wittenberg for supporting this work. We also thank K. Bousi for polishing the language of this manuscript.

References

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop Evapotranspiration (guidelines for computing crop water requirements). FAO Irrigation and Drainage Paper No. 56, 300p.

Allen, R.G., Pereira, L.S., Howell, T.A., Jensen, M.E., 2011. Evapotranspiration information reporting: I. Factors governing measurement accuracy. *Agricultural Water Management* 98(6): 899-920.

Amatya, D.M., Irmak, S., Gowda, P., Sun, G., Nettles, J.E., Douglas-Mankin, K.R., 2016. Ecosystem evapotranspiration: challenges in measurements, estimates, and modeling. *Transactions of the ASABE* 59(2): 555-560.

Armstrong, R.N., Pomeroy, J.W., Martz, L.W., 2008. Evaluation of three evaporation estimation methods in a Canadian prairie landscape. *Hydrological Processes* 22(15): 2801-2815.

Bagley, J.E., Desai, A.R., Dirmeyer, P.A., Foley, J.A., 2012. Effects of land cover change on moisture availability and potential crop yield in the world's breadbaskets. *Environmental Research Letters* 7(1): 014009.

Balykin, D., Puzanov, A., Stepahn, E., Meissner, R., 2016. Using the innovative lysimeter technology in the German-Russian research project "KULUNDA". In: Novel Methods for Monitoring and Managing Land and Water Resources in Siberia. Mueller, L., Sheudshen, A.K., Eulenstein, F., (Eds.). Springer Water Switzerland, pp. 387-399.

Degefie, D.T., Fleischer, E., Klemm, O., Soromotin, A.V., Soromotin, O.V., Tolstikov, A.V., Abramov, N.V., 2014. Climate extremes in South Western Siberia: past and future. *Stochastic Environmental Research and Risk Assessment* 28(8): 2161-2173.

DehghaniSanij, H., Yamamoto, T., Rasiah, V., 2004. Assessment of evapotranspiration estimation models for use in semi-arid environments. *Agricultural Water Management* 64(2): 91-106.

El Bably, A.Z., 2003. Estimation of evapotranspiration using statistical mode. In: Local Resources and Global Trades: Environments and Agriculture in the Mediterranean region Camarda, D., Grassini, L., (Eds.). Bari, Italy. CIHEAM, pp. 441-449.

Fleischer, E., Bölter, J., Klemm, O., 2015. Summer evapotranspiration in western Siberia: a comparison between eddy covariance and Penman method formulations. *Hydrological Processes* 29(20): 4498-4513.

Fraser, E.D.G., Simelton, E., Termansen, M., Gosling, S.N., South, A., 2013. "Vulnerability hotspots": integrating socio-economic and hydrological models to identify where cereal production may decline in the future due to climate change induced drought. *Agricultural and Forest Meteorology* 170: 195-205.

Gebler, S., Hendricks Franssen, H.-J., Pütz, T., Post, H., Schmidt, M., Vereecken, H., 2015. Actual evapotranspiration and precipitation measured by lysimeters: a comparison with eddy covariance and tipping bucket. *Hydrology and Earth System Sciences* 19(5): 2145-2161.

Guo, D., Westra, S., Maier, H.R., 2016. An R package for modelling actual, potential and reference evapotranspiration. *Environmental Modelling & Software* 78: 216-224.

López-Urrea, R., Marin de Santa Olalla, F., Fabeiro, C., Moratalla, A., 2006. Testing evapotranspiration equations using lysimeter observations in a semiarid climate. *Agricultural Water Management* 85(1-2): 15-26.

Louzada, F.I.R. de O., Xavier, A.C., Pezzopane, J.E.M., 2018. Climatological water balance with data estimated by tropical rainfall measuring mission for the Doce river basin. *Engenharia Agricola* 38: 376-386.

Makkink, G.F., 1957. Testing the Penman formula by means of lysimeters. *Journal of the Institution of Water Engineers and Scientist* 11(3): 277-288.

Martel, M., Glenn, A., Wilson, H., Kröbel, R., 2018. Simulation of actual evapotranspiration from agricultural landscapes in the Canadian Prairies. *Journal of Hydrology: Regional Studies* 15: 105-118.

Mauder, M., Genzel, S., Fu, J., Kiese, R., Soltani, M., Steinbrecher, R., Zeeman, M., Banerjee, T., De Roo, F., Kunstmann, H., 2017. Evaluation of energy balance closure adjustment methods by independent evapotranspiration estimates from lysimeters and hydrological simulations. *Hydrological Processes* 32(1): 1-18.

Meissner, R., Bondarovich, A.A., Scherbinin, V.V., Ponkina, E.V., Matsyura, A.V., Puzanov, A.V., Rupp, H., Schmidt, G., Stephan, E., Illiger, P., Fruhauf, M., Harlamova, N.F., Galahov, V.P., Balykin, D.V., Rudev, N.V., 2016a. Calculation of water balance for the south desert area of Western Siberiaby international monitoring network data. *Biological Bulletin of Bogdan Chmelnitskiy Melitopol State Pedagogical University* 6(2): 223-238.

Meissner, R., Rupp, H., Bondarovich, A.A., Rinklebe, J., 2016b. Soil water management in the Siberian Kulunda-dry steppe. International Scientific Conference. Conserving Soils and Water. 31.08.2016-03.09.2016, Burgas, Bulgaria. Book of Proocedings, pp.87-91.

Monteith, J.L., 1965. Evaporation and environment. *19th Symposia of the Society for Experimental Biology* 19: 205-234.

Oberholzer, S., Prasuhn, V., Hund, A., 2017. Crop water use under Swiss pedoclimatic conditions - Evaluation of lysimeter data covering a seven-year period. *Field Crops Research* 211: 48-65.

Park, H., Yamazaki, T., Yamamoto, K., Ohta, T., 2008. Tempo-spatial characteristics of energy budget and evapotranspiration in the eastern Siberia. *Agricultural and Forest Meteorology* 148(12): 1990-2005.

Peters, A., Nehls, T., Schonsky, H., Wessolek, G., 2014. Separating precipitation and evapotranspiration from noise - a new filter routine for high-resolution lysimeter data. *Hydrology and Earth System Sciences* 18(3): 1189-1198.

Priestley, C.H.B., Taylor, R.J., 1972. On the assessment of surface heat flux and evaporation using large-scale parameters. *Monthly Weather Review* 100(2): 81-92.

Roth, D., Günther, R., Knoblauch, S., Michel, H., 2005. Wasserhaushaltsgrößen von Kulturpflanzen unter Feldbedingungen, In: Landwirtschaft und Landschaftspflege in Thüringen, Thüringer Landesanstalt für Landwirtschaft, (Ed.). Schriftenreihe Heft 1 Jena, pp. 159.

Sabziparvar, A.A., Tabari, H., 2010. Regional estimation of reference evapotranspiration in arid and semiarid regions. *Journal of Irrigation and Drainage Engineering ASCE* 136(10): 724-731.

Savitzky, A., Golay, M.J.E., 1964. Smoothing and differentiation of data by simplified least squares procedures. *Analytical Chemistry* 36(8): 1627-1639.

Schrader, F., Durner, W., Fank, J., Gebler, S., Pütz, T., Hannes, M., Wollschläger, U., 2013. Estimating precipitation and actual evapotranspiration from precision lysimeter measurements. *Procedia Environmental Sciences* 19: 543-552.

Shi, T., Guan, D., Wu, J., Wang, A., Jin, C., Han, S., 2008. Comparison of methods for estimating evapotranspiration rate of dry forest canopy: eddy covariance, Bowen ratio energy balance, and Penman-Monteith equation. *Journal of Geophysical Research* 113: D19116.

Swinnen, J., Burkitbayeva, S., Schierhorn, F., Prishchepov, A.V., Müller, D., 2017. Production potential in the "bread baskets" of Eastern Europe and Central Asia. *Global Food Security* 14: 38-53.

von Unold, G., Fank, J., 2008. Modular design of field lysimeters for specific application needs. *Water, Air, & Soil Pollution: Focus* 8(2): 233-242.

Wegehenkel, M., Zhang, Y., Zenker, T., Diestel, H., 2008. The use of lysimeter data for the test of two soil-water balance models: A case study. *Journal of Plant Nutrition and Soil Science* 171(5): 762-776.

Wegehenkel, M., Gerke, H.H., 2013. Comparison or real evapotranspiration measured by weighing lysimeters with simulations based on the Penman formula and a crop growth model. *Journal of Hydrology and Hydromechanics* 61(2): 161-172.

Wever, L.A., Flanagan, L.B., Carlson, P.J., 2002. Seasonal and interannual variation in evapotranspiration, energy balance and surface conductance in a northern temperate grassland. *Agricultural and Forest Meteorology* 112(1): 31-49.

Wohlfahrt, G., Ischick, C., Thalinger, B., Hörtnagl, L., Obojes, N., Hammerle, A., 2010. Insights from independent evapotranspiration estimates for closing the energy balance: A grassland study. *Vadose Zone Journal* 9(4): 1025-1033.

Xiao, H., Meissner, R., Seeger, J., Rupp, H., Borg, H., 2009. Testing the precision of a weighable gravitation lysimeter. *Journal of Plant Nutrition and Soil Science* 172(2): 194-200.

Yamazaki, T., Yabuki, H., Ishii, Y., Ohta, T., Ohata, T., 2004. Water and energy exchanges at forests and a grassland in eastern Siberia evaluated using a one-dimensional land surface model. *Journal of Hydrometeorology* 5: 504-515.

Yang, F., Zhang, Q., Wang, R., Zhou, J., 2014. Evapotranspiration measurement and crop coefficient estimation over a spring wheat farmland ecosystem in the Loess Plateau. *PLOS One* 9: e100031.

Eurasian J Soil Sci 2019, 8 (3) 267 - 274

Eurasian Journal of Soil Science

Journal homepage : http://ejss.fesss.org

Physical and hydraulic properties of soils under a long-term tillage practices in Hadejia Local Government Area, Jigawa State, Nigeria

Abba Nabayi *, Abubakar Halilu Girei, Mohammed Saleh Abubakar

Department of Soil Science, Faculty of Agriculture, Federal University Dutse, Jigawa State, Nigeria

Abstract

The study was conducted to determine the effects of different tillage practices on some physical and hydraulic properties of soils in Hadejia Local Government Area of Jigawa state during the 2017 cropping season. The field experiment was laid in a Randomized Complete Block (RCB) Design in factorial arrangements with 4 treatments for tillage practices-TP (Zero tillage-ZT, Minimum tillage-MT, Conventional tillage-CT and Deep tillage-DT) and sampling depth-SD (5 cm, 15 cm, 25 cm and 35 cm) all were in four replicates. Data collected were analyzed using the generalized linear model of Statistical Analyses System (SAS 9.4) for the ANOVA. The results showed that there were significant differences ($p<0.05$) in the main effect of TP and SD as well as in the interaction effect between TP and SD on soil bulk density (Bd), saturated hydraulic conductivity (Ksat), volumetric moisture content (VWC) at different soil water potentials and plant available water (PAW). Greater Bd and Ksat were observed in DT which differed significantly ($p<0.05$) from other TP while the lowest was found in ZT with 6.5% reduction than DT. The result further showed no significant difference in terms of PAW ($p>0.05$) between the TP at the average depth of 15 and 25 cm, but they (TP) differed significantly at the average depth of 5 and 35 cm with the highest in ZT. Higher soil moisture content retained at all soil water pressure was found in ZT which differed significantly ($p<0.01$) form other TP. The research concluded that the best TP to be adopted in Hadejia in terms of improvement in physical and hydraulic properties is ZT practice.

Article Info

Received : 09.11.2018
Accepted : 24.05.2019

Keywords: Conventional tillage, deep tillage, minimum tillage, sampling depth, tillage practices, zero tillage

Introduction

Tillage is the mechanical disturbance of the soil for the purpose of crop production. Over the decades, focus has been drawn to the effects of different tillage practices on some physical and hydraulic properties of soils around the globe. However, increasing demand for food and fiber by the ever-growing human population has placed high stress on farmers to produce large quantity of food to meet this demand. One of the means to increase the output is through the employment of modern Agricultural machineries like ploughs, harrows, planters, harvesters etc. These machineries lead to high output per unit area, timely and efficient operations and reduction of drudgery associated with crop production. These practices, however, have shown to be highly destructive of the soil, which resulted to about 24% of the global agricultural land degradation (Bai et al., 2008).

Irrigation management practices largely depend on accurate and timely characterization of spatial and temporal soil moisture changes in the root zone, especially in arid and semi-arid regions. Conservation

* Corresponding author.
Department of Soil Science, Faculty of Agriculture, Federal University Dutse, Jigawa State, Nigeria
Tel.: +2347031068906 E-mail address: abba.nabayi@fud.edu.ng
e-ISSN: 2147-4249 DOI: 10.18393/ejss.573914

tillage has increasingly been utilized as the agricultural best management practice to reduce soil erosion (Salem et al., 2015). The knowledge outcome of conservation tillage on soil moisture conditions, soil compaction and soil temperature, has become a major concern among producers considering adopting this tillage system (Licht and Al-Kaisi, 2005). Soil compaction is usually determined by measuring soil bulk density and cone index. Soil bulk density and cone index are also utilized to predict the depth of soil hardpans (Mehari et al., 2005; Afzalinia and Zabihi, 2014). Therefore, evaluating the effects of conservation tillage practices on soil moisture and compaction can help to explain some of the differences in plant growth and development under different tillage practices (Licht and Al-Kaisi, 2005).

Undesirable management practices cause degradation in soil health; depletion of organic matter and other nutrients, as well as decline in crop productivity (Ramos et al., 2011). Reducing disturbance of soil by reduced tillage influences several physically (López-Garrido et al., 2012), chemically (Page et al., 1986), and biologically (Bronick and Lal, 2005; Muñoz et al., 2007) interconnected properties of the natural body. Soil tillage is one of the important factors influencing soil properties and crop yield. Among the crop production factors, tillage contributes up to 20% (Khurshid et al., 2006) and affects the sustainable use of soil resources through its influence on soil properties (Lal and Stewart, 2013). Therefore, currently there is a significant interest and emphasis on the shift from extreme tillage to conservation and or no-tillage methods for the purpose of controlling erosion process and preserving soil nutrients (Iqbal et al., 2005). Conventional tillage practices cause change in soil structure by modifying soil bulk density and soil moisture content. In addition, repeated disturbance by conventional tillage produces a finer and loose-setting soil structure while conservation and no-tillage methods leave the soil intact (Rashidi and Keshavarzpour, 2007). The number, size, and distribution of pores again control the ability of soil to store and transmit water, air and agricultural chemicals and, thus, in turn, regulate erosion, runoff, and crop performance (Khan et al., 2001).

Generally, these properties are affected by land management practices associated with row crop and perennial crop production practices (Zaibon et al., 2016). A research conducted by Fuentes et al. (2004) by comparing the hydraulic properties under natural prairie, conventional tillage, and no-till management for silt loam soils (Ultic Haploxerolls), discovered that in the natural prairie, hydraulic conductivity values were about one order of magnitude larger than in the cultivated soils. They concluded that even after 27 years of continuous no-till, hydraulic properties were not restored to the original values of the prairie soils.

However, scientific researches are limited on the effect of tillage practices on the rainfed arable land in the Hadejia area. The research has been initiated to determine the best tillage practices with desirable soil properties. The soil physical and hydraulic properties investigated include bulk density, soil water retention, saturated hydraulic conductivity and pore size distribution. This research was carried out to evaluate the effect of tillage practices on bulk density, saturated hydraulic conductivity and water retention of the dryland soil under the four (4) different tillage practices. We hypothesized that differences in tillage practices would have significant effects on the physical and hydraulic properties of the soils.

Material and Methods

Experimental site

The study was conducted at four different locations (Lat. 12.46° 06¹N and Long.10.04° 08¹E, Lat. 12.46° 01¹N and Long.10.04° 09¹E, Lat. 12.47° 03¹N and Long.10.04⁰ 07¹E and Lat.12.46⁰ 04¹N and Long.10.04⁰ 03¹E) in Hadejia local govt. area. The ecological zone is Sudan savannah which comprises of scattered trees and sparse vegetation. Rainfall ranges from 500-700 mm annually and rains between the month of June and October, mean annual temperature ranges from 27°C to 38°C. The experimental sites were continuously being utilised for rainfed cultivation of cereals and legumes for a long period of time.

Experimental design and treatments

The Tillage Practices (TP) were considered as first factor and the sampling depth (SD) as the second factor, with four (4) replications each which were laid in Randomized Complete Block design in a factorial arrangement. The four tillage practices were; Zero tillage (ZT: no any tillage activity is performed), Minimum tillage (MT: using hoe manually), Conventional tillage (CT: ox-ploughed by animal to a depth of about 15 cm) and Deep tillage (DT: tillage by chisel plough up to 22–25 cm depth using a tractor). The sampling depths included; 0-10 cm (average = 5cm), 10-20 cm (average = 15 cm), 20-30 cm (average = 25cm) and 30-40 cm (average= 35 cm).

Soil sampling and analysis

Undisturbed soil samples were taken using aluminum ring core samplers at the sites in the month of October 2017. The core samplers had a dimension of 5 × 5 cm each. The samples were collected by 10 cm increment from 0 to 40 cm depth for each tillage practice, making a total of 64 soil core samples. The sample cores were trimmed, covered the top and bottom openings, labelled and sealed in plastic bags and transported to the laboratory for measurements and analyses.

Particle size analysis

Particle size analysis was conducted using Bouyoucos hydrometer method, where sodium hexameta-phosphate (Calgon) was used as a dispersant agent (Gee and Bauder, 1986). The USDA textural triangle was used to determine the textural class of the soil samples.

Bulk density (Bd) and soil moisture contents (MC)

Bulk density was determined using core method (Blake and Hartge, 1986). The soil was taken by pressing the core ring and carefully removed to preserve a known volume. Sample was then oven dried to 105 ^{o}C for 24 hours and reweighed to determine the oven dry weight. The bulk density and moisture content were calculated thus;

$$\text{Bulk Density (Mg m}^{-3}) = \frac{\text{Weight of ovendried soil sample at } 105^{o}\text{C}}{\text{Total Volume of fresh soil sample}} \quad (1)$$

$$\text{Moisture Content (g g}^{-1}) = \frac{\text{Weight fresh soil sample} - \text{Weight of ovendried soil sample}}{\text{Weight of ovendried soil sample}} \times 100 \quad (2)$$

Saturated hydraulic conductivity (Ksat)

The saturated hydraulic conductivity of the soil samples was determined by constant head method as described by Klute and Dirksen (1986). To determine the hydraulic conductivity, calibrated measuring cylinder was placed on the stand and followed by funnel on top of the cylinder, then a brass ring covered one side with white cloth and tighten with rubber ring was placed in the funnel and the required amount of the soil sample was placed inside the brass ring. Volumetric flask with the required amount of water was tilted upside down by the help of clamp and then the water was released by removing the stopper of volumetric flask to the brass ring with already contained soil sample, then the water drops into the measuring cylinder. The quantity of dropped water has been measured as well as the time taken. Hydraulic conductivity was calculated using the formula below;

$$\text{Ksat (cm hr}^{-1}) = \frac{\text{QL}}{\text{ATH}} \quad (3)$$

where Ksat is the saturated hydraulic conductivity (cm/h), Q is the volume of water collected (cm^3), T is the time taken (hr), A is the cross-sectional area of the sample (cm^2), H is the hydraulic head difference (cm), and L is the length of the soil column (cm)

Soil water retention

Water retention of the soils under different tillage practices at different sampling depth was determined using the pressure plate and pressure membrane described by Richards (1947). The soil of a known weight was put into the core ring and placed on porous ceramic plates and was placed inside a pressure chamber for 7 days. The applied pressure was 0.1, 1, 10, 33, and 1500 kPa. The samples were then oven dried at 105 ^{o}C for 24 hours which was weighed and multiply by the soil bulk density to obtain the volumetric moisture content (VMC).

Data analysis

Minitab 16, was used to test the normality for the parameters studied, which was conducted using Anderson–Darling at P=0.05. All data collected were analyzed using Statistical Analyses System (SAS 9.4 SAS system for windows by SAS Institute Inc., Cary, NC, USA). Analysis of Variance (ANOVA) and Proc GLM were used to determine the significant treatment effect on the measured properties with the significant difference of $p<0.05$. Least significant difference (LSD) was used for mean separation to detect significant difference between the means.

Results and Discussion

The results of main and interactive effects of various tillage practices and sampling depth on bulk density (Bd) and saturated hydraulic conductivity were presented in Table 2. The results showed significant effect of tillage practices and sampling depth as well as their interaction on soil Bd ($p<0.05$). The mean values of the bulk densities differed significantly from one tillage practice to another in the order DT>CT>MT>ZT with DT having 3.3%, 5.2% and 6.5% higher bulk densities than CT, MT and ZT respectively. Similar result was reported by Alam et al. (2014) who also recorded highest bulk density reduction (6.41%) in ZT followed by MT (3.95%). Higher Bd obtained in DT could be due to soil compaction attributed to regular machinery used for ploughing. The lower Bd observed in ZT may be attributed to the maximum utilization of the soil vegetative cover year-in year-out. Many researchers (Rachman et al., 2004; Liebig et al., 2005; Mudgal et al., 2010) acknowledged the influence of vegetative cover on soil bulk density; the higher the vegetative cover the lower the bulk density of a soil. The sampling depth also differed from one another statistically ($P<0.05$) in terms of Bd. Higher bulk density values were recorded in the lower depth whereas the lowest Bd values were obtained in the upper depths. There was 1.4% increment in Bd in the lower depth (20-40 cm) over the upper depth (0-20 cm). the Lower Bd values in the upper depth was due to the organic residues that are being left behind after harvest which decomposes and translocated up to some certain levels of the soil profile. The results agree with Seobi et al. (2005) who reported concomitant increase in Bd with an increase in sampling depth. The interaction effect between tillage practices and soil depth on Bd is presented in Figure 1a. The interaction showed that the Bd values increases with increase in depth in virtually all the tillage practices, with the exception of ZT which remain constant at the average depth of 5, 15 and 25 cm depth and then increases with 2.7% at the lower average depth of 35 cm. Bd of CT also remain unstable throughout the different sampling depth, which increases and decreases alternately as the depth progressed.

Table 2. Analysis of variance (ANOVA) and main effect means for bulk density and saturated hydraulic conductivity. Main effects are Tillage practices and sampling depth.

	ANOVA P>F	
Source of Variation	Bulk Density (Bd)	Sat. Hydraulic Conductivity (SHC)
Replication (R)	0.1573	0.0596
Tillage practices (T)	<0.0001	<0.0001
T*R	0.0493	0.2274
Sampling depth (D)	<0.0001	<0.0001
T*D	<0.0001	<0.0001
Tillage practices	Means	
ZT	1.43d	0.75d
MT	1.45c	1.84b
CT	1.48b	1.02c
DT	1.53a	2.90a
LSD	0.02	0.14
Sampling depth (cm)		
0-10 cm	1.46b	1.76b
10-20 cm	1.46b	1.41c
20-30 cm	1.48a	1.43c
30-40 cm	1.49a	1.91a
LSD	0.02	0.14

Mean comparisons were made only when *P* values for the main effects were ≤0.05. Means followed by same letters within the same column (for a given Bd and SHC) are not significantly different from one another at 5% level of significance.

The result presented in Table 2 and Figure 1b showed significant difference ($P<0.05$) in the main effect of tillage practices, sampling depth and the interaction of the two on saturated hydraulic conductivity (Ksat). The highest Ksat was recorded under DT which differed significantly from that of other tillage practices in the order of DT>MT>CT>ZT with DT having an increment of 36.5%, 64.8% and 74% over MT, CT and ZT respectively. Despite having wide margin in Ksat values between the tillage practices, ZT, CT and MT all fall under the saturated hydraulic conductivity class of moderately slow (Teh and Jamal, 2006), with only DT having different class of moderate among the tillage practices. However, in terms of the sampling depth, Ksat differed from one another but they all fall under moderate slow class. Particle size distribution plays a vital role in determining the saturated hydraulic conductivity of soils. Higher Ksat in DT was due to the

proportion of sand, silt and clay content of the soil under tillage practice possessed. DT had higher proportion of sand and lower percentage of clay across the sampling depths (Table 1) when compared with the soils under other TP, and sandy soils had very rapid conductivity of water than silt and clay soils. The results of Ksat disagrees with Seobi et al. (2005) and Mudgal et al. (2010) but concurred with Zaibon et al. (2016) who indicated that normally, Ksat decreases with sampling depth due to the overburden pressure of the overlying soil, the downward movement of fine particles, and fewer roots, which reduce the proportion of interconnected pores and the pore size distribution. Seobi et al. (2005) reported that Ksat at 0-10 cm soil depth was 36 times greater than at the 30-40 cm soil depth. They found that the lowest value of Ksat at the 30-40 cm soil depth was due to the higher concentration of smectite clay in that soil horizon (Seobi et al., 2005), however, in this study it was found contrary because of higher proportion of sand content as shown in Table 1.

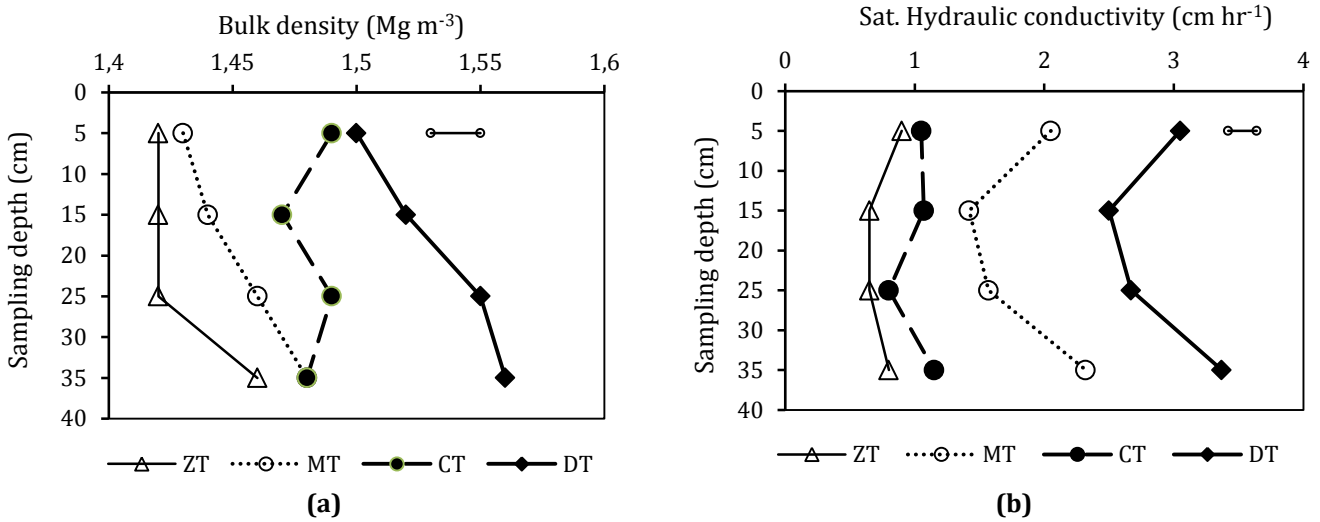


Figure 1. Effect of (a) Tillage practices and sampling depth on Bulk density (Bd) and (b) Tillage practices and sampling depth on saturated hydraulic conductivity (SHC). Bars indicate LSD values at 5% level of significance.
ZT: Zero tillage, MT: Minimum tillage, CT: Conventional tillage, DT: Deep tillage

The interaction effect between TP and SD (Figure 1b) on Ksat followed the same trend for all the tillage practices. They all decrease from 5 cm average depth to 15 cm and then increases slightly at 25 cm and finally increases considerably at the final average sampling depth (35 cm) greater than the initial sampling depth. Higher Ksat values observed at the 5 cm depth compared to 15 and 25 cm was due to the greater percentage of organic matter content at the surfaces and lower clay content than at the deeper depth (Table 1), while at the same time significant Ksat values at the deeper depth (35 cm) than its upper depths counterparts was because of the significant proportion of the sand content in the depth.

Table 1. Particle size analyses of the experimental soils under different tillage practices at different sampling depth.

Tillage practices	Sampling depth (cm)	Clay (%)	Silt (%)	Sand (%)	Textural class
ZT	0-10	12	19	69	Sandy loam
	10-20	12	22	66	Sandy loam
	20-30	13	22	65	Sandy loam
	30-40	14	22	64	Sandy loam
MT	0-10	14	19	67	Sandy loam
	10-20	14	19	66	Sandy loam
	20-30	15	21	64	Sandy loam
	30-40	16	21	63	Sandy loam
CT	0-10	22	14	64	Sandy clay loam
	10-20	23	15	62	Sandy clay loam
	20-30	22	16	62	Sandy clay loam
	30-40	23	17	60	Sandy clay loam
DT	0-10	16	13	71	Sandy loam
	10-20	17	13	70	Sandy loam
	20-30	16	14	70	Sandy loam
	30-40	18	14	68	Sandy loam

ZT: Zero tillage, MT: Minimum tillage, CT: Conventional tillage, DT: Deep tillage

The ANOVA results for soil water retention (Table 3) showed that tillage practices had a significant effect (P< 0.05) on volumetric moisture content retained at all soil water pressures. The result showed that ZT had higher moisture content at all the soil water pressures than other tillage practices. This result suggests that conservation tillage practices and soil management can affect soil water retention at all soil water pressure range. Consequently, this range of soil water pressure is affected by soil structure and root distribution effects. Soil water retention differed significantly with sampling depth (P<0.05), and the interactions between TP and SD were also significant (P<0.05) which is presented in Figure 2. Volumetric moisture contents (VMC) in the ZT practice was significantly different from all TP at all soil water pressure except at 0.1kpa which indicated no significant difference among all the TP (Figure 2a). Similar trend was observed in Figure 2c but it did not differ significantly from CT. At 15 and 35 cm depth (Figure 2b and 2d), similar trend was observed with ZT having higher moisture content which differed significantly (P<0.05) from other TP at 1, 33 and 1500 kpa only while DT had the lowest moisture content at these soil water pressure. Soil water retention is also a function of soil structure; total porosity and aggregates distribution. Allowing land under ZT to be covered with residues and vegetation during raining season is an important activity towards aggregate stability and increasing soil pore spaces which added a greater below ground biomass as compared with other tillage practices. Bulk density results were numerically lower at virtually all the depth for the ZT practice, which perhaps contributed to higher water retained in the ZT practice. Root of crops and weeds also played a significant role towards having lower Bd values and greater water retention of the soil under ZT practice. Mudgal et al. (2010) reported that because of the shallowest topsoil in the upper depth (4 cm), it had more water content than deeper depth.

Table 3. Analysis of variance (ANOVA) and main effect means for volumetric moisture content (VMC) across a range of soil water pressures. Main effects are Tillage practices and sampling depth

	ANOVA P>F				
Source of Variation	**0.1kpa**	**1kpa**	**10kpa**	**33kpa**	**1500kpa**
Replication (R)	0.5925	0.9116	0.4200	0.4362	0.1253
Tillage practices (T)	0.0277	<0.0001	0.0002	<0.0001	<0.0001
T*R	0.7313	0.8398	0.3332	0.1255	0.9693
Sampling depth (D)	0.5823	0.1588	0.0102	<0.0001	<0.0001
T*D	0.0304	0.0003	0.0006	<0.0001	<0.0001
Tillage practices	VMC (m^3 m^{-3}) Means				
ZT	0.450a	0.394a	0.354a	0.316a	0.185a
MT	0.440b	0.368b	0.342b	0.254c	0.144c
CT	0.450a	0.375b	0.342b	0.290b	0.164b
DT	0.445ab	0.373b	0.336b	0.223d	0.106d
LSD	0.006	0.008	0.007	0.006	0.006
Sampling depth(cm)					
0-10cm	0.452a	0.381a	0.347a	0.267b	0.146b
10-20cm	0.446ab	0.378ab	0.347a	0.278a	0.156a
20-30cm	0.451a	0.379ab	0.343ab	0.279a	0.159a
30-40cm	0.445ab	0.372b	0.336b	0.255c	0.137c
LSD	0.006	0.008	0.007	0.006	0.006

Mean comparisons were made only when *P* values for the main effects were ≤0.05. Means followed by same letters within the same column (for a given soil water pressure) are not significantly different from one another at 5% level of significance.

Figure 2 indicated that water content of a soil under different tillage practices can be different at all the soil water pressure levels. In terms of plant available water (FC-PWP), significant difference (P<0.05) was observed only at 5 and 35 cm average depth. However, at 5 cm depth the PAW was recorded in the order of ZT>CT>DT>MT with ZT having an increase of 31.5%, 42.1% and 47.3% over CT, DT and MT respectively. At the deeper/lower depth, the increase in PAW followed this order ZT>CT>MT>DT with 7.8% and 15.4% each of MT and DT. MT and DT had similar PAW at the lower depth (35 cm). The result disagrees with Alam et al. (2014) who found the maximum Available water content under DT, then followed by ZT, even though, it was after 2 cropping cycles.

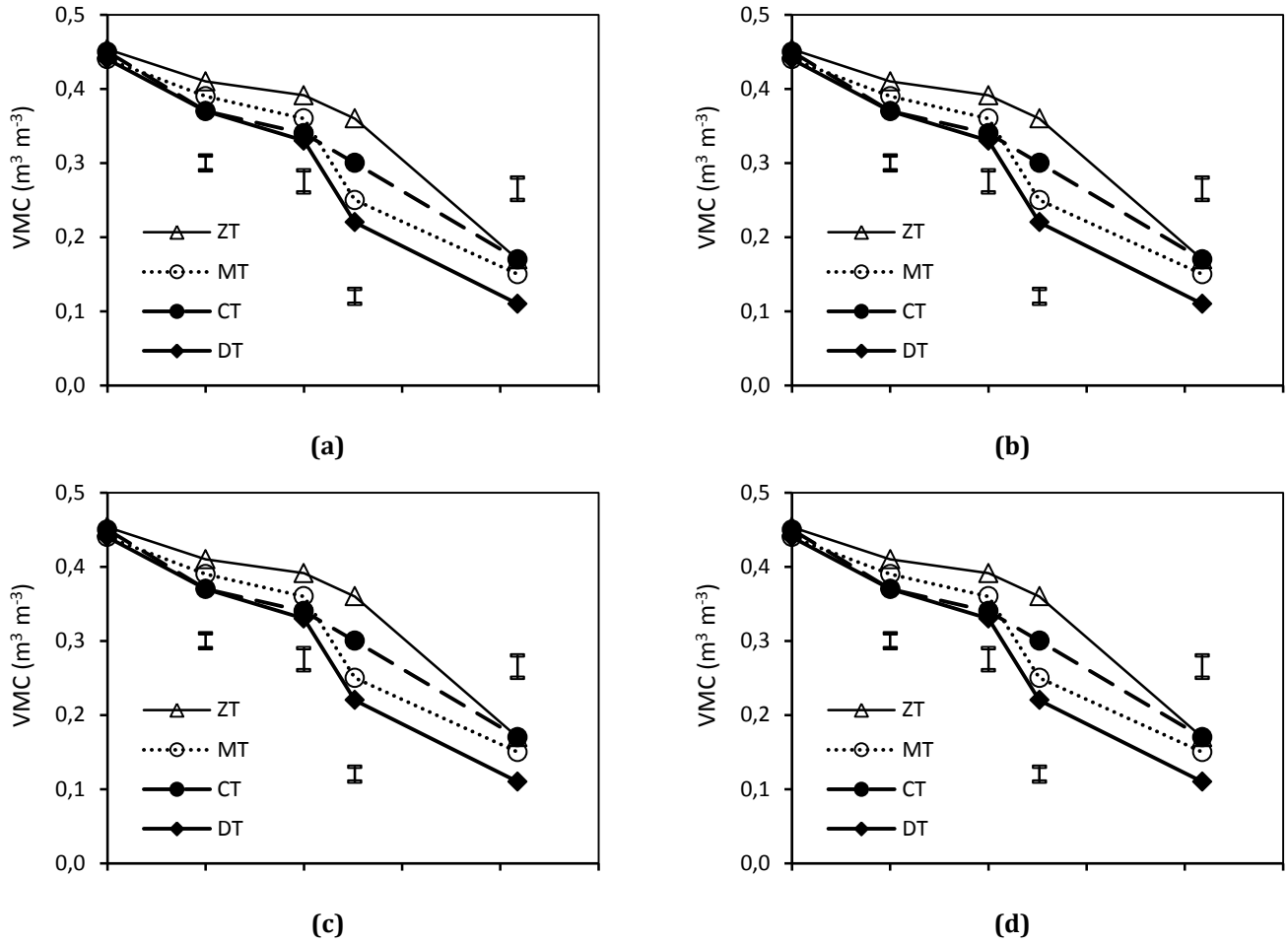


Figure 2. Effect of tillage practices on soil water retention by sampling depth: (a) 5 cm, (b) 15 cm, (c) 25 cm, (d) 35 cm. Bars indicate LSD values at a given soil water pressure when significant at 5% level.
ZT: Zero tillage, MT: Minimum tillage, CT: Conventional tillage, DT: Deep tillage.

Conclusion

Based on the given conditions under which the work was carried out, the study showed the importance of zero tillage among other soil conservation practices and based on the conditions under which this work was carried out the results of the research indicated that ZT attributes can be reaped beyond upper depth. Among the tillage practices, ZT had the lowest bulk density value (1.42 and 1.46 Mg m^{-3}) at the upper (5 cm) and lower (35 cm) depth while DT had the highest values at the corresponding depths, with 5.3 and 6.4% increment respectively. Ksat ranges in the order of DT>MT>CT>ZT. The highest Ksat value in DT was due to the higher proportion of sand particles in soil under the practice. However, the hydraulic conductivity classes of the area, showed that all the soils under the tillage practices fall under same category of moderately slow hydraulic conductivity with the exception of DT practice. The VMC retained at varying soil water pressure potential was significantly different at all depths. It followed the order of ZT>MT>CT>DT from highest to lowest. ZT also recorded the highest plant available water (PAW) with 47.3 and 15.4%, 31.5 and 7.4% and 42.1 and 15.4% increase over MT, CT and DT practices respectively at the average depth of 5 and 35 cm. There was no significant difference observed among the TP at the average depth of 15 and 25 cm.

Based on the findings of this study, the following recommendations were made:

1. The use of zero tillage (ZT) should be encouraged since it neither involves cost nor adverse effects on the soils.
2. The study should be extended to cover long time effects for tillage practices-TP (Zero tillage-ZT, Minimum tillage-MT, Conventional tillage-CT and Deep tillage-DT)
3. Farming practices in an upland soil that rely solely on rainfall should be based on conservation tillage practices as this study revealed their important contributions to some soil physical and hydraulic properties as well as to the plant.

References

Afzalinia, S., Zabihi, J., 2014. Soil compaction variation during corn growing season under conservation tillage. *Soil and Tillage Research* 137: 1–6.

Alam, M.K., Islam, M.M., Salahin, N., Hasanuzzaman, M., 2014. Effect of tillage practices on soil properties and crop productivity in wheat-mungbean-rice cropping system under subtropical climatic conditions. *The Scientific World Journal* Article ID 437283.

Bai, Z.G., Dent, D.L., Lennart, O., Schaepman, M.E., 2008. Global assessment of land degradation and improvement: 1. Identification by remote sensing, Report 2008/01 (GLADA Report 5). ISRIC-World Soil Information, Wageningen, The Netherland, 70p.

Blake, G.R., Hartge, K.H., 1986. Bulk density. In: Methods of Soil Analysis Part 1 Physical and Mineralogical Methods. 2nd Edition, Klute, A., (Ed). American Society of Agronomy,-Soil Science Society of America. Madison, Wisconsin, USA. pp. 363-375.

Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. *Geoderma* 124(1-2): 3–22.

Fuentes, J.P., Flury, M., Bezdicek, D.F., 2004. Hydraulic properties in a silt loam soil under natural prairie, conventional till, and no-till. *Soil Science Society of America Journal* 68(5): 1679–1688.

Gee, G.W., Bauder, J.W., 1986. Particle-size Analysis. In: Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, A.L. Page, R.H. Miller, D.R. Keeney (Eds.), 2nd Edition. Agronomy Monograph No. 9, American Society of Agronomy, Soil Science Society of America. Madison, Wisconsin, USA. pp. 383-411.

Iqbal, M., Hassan, A.U., Ali, A., Rizwanullah, M., 2005. Residual effect of tillage and farm manure on some soil physical properties and growth of wheat (Triticum aestivum L.). *International Journal of Agriculture and Biology* 1: 54–57.

Khan, F.U.H., Tahir, A.R., Yule, I.J., 2001. Intrinsic implication of different tillage practices on soil penetration resistance and crop growth. *International Journal of Agriculture and Biology* 3(1): 23–26.

Khurshid, K., Iqbal, M., Arif, M.S., Nawaz, A., 2006. Effect of tillage and mulch on soil physical properties and growth of maize. *International Journal of Agriculture and Biology* 8: 593–596.

Klute, A., Dirksen, C., 1986. Hydraulic conductivity and diffusivity: Laboratory methods. In: Methods of Soil Analysis Part 1 Physical and Mineralogical Methods. 2nd Edition, Klute, A., (Ed). American Society of Agronomy, Soil Science Society of America. Madison, Wisconsin, USA. pp. 687-734.

Lal, R., Stewart, B.A., 2013. Principles of sustainable soil management in agroecosystems. CRC Press. New York, USA. 552p.

Licht, M.A., Al-Kaisi, M., 2005. Strip-tillage effect on seedbed soil temperature and other soil physical properties. *Soil and Tillage Research* 80(1-2): 233–249.

Liebig, M.A., Johnson, H.A., Hanson, J.D., Frank, A.B., 2005. Soil carbon under switchgrass stands and cultivated cropland. *Biomass and Bioenergy* 28(4): 347–354.

López-Garrido, R., Deurer, M., Madejón, E., Murillo, J.M., Moreno, F., 2012. Tillage influence on biophysical soil properties: The example of a long-term tillage experiment under Mediterranean rainfed conditions in South Spain. *Soil and Tillage Research* 118: 52–60.

Mehari, A., Schultz, B., Depeweg, H., 2005. Where indigenous water management practices overcome failures of structures: The WadiLaba Spate Irrigation System in Eritrea. *Irrigation and Drainage* 54(1): 1–14.

Mudgal, A., Anderson, S.H., Baffaut, C., Kitchen, N.R., Sadler, E.J., 2010. Effects of long-term soil and crop management on soil hydraulic properties for claypan soils. *Journal of Soil and Water Conservation* 65(6): 393–403.

Muñoz, A., López-Piñeiro, A., Ramírez, M., 2007. Soil quality attributes of conservation management regimes in a semi-arid region of south western Spain. *Soil and Tillage Research* 95(1-2): 255–265.

O'Neal, A.M., 1949. Soil characteristics significant in evaluating permeability. *Soil Science* 67(5): 403-409.

Page, A.L., Miller, R.H., Kuny, D.R., 1986. Methods of Soil Analysis. Part 1 Chemical and Microbiological Properties. 2nd Edition, American Society of Agronomy, Soil Science Society of America. Madison, Wisconsin, USA.

Rachman, A., Anderson, S.H., Gantzer, C.J., Alberts, E.E., 2004. Soil hydraulic properties influenced by stiff-stemmed grass hedge systems. *Soil Science Society of America Journal* 68(4): 1386–1393.

Ramos, M.E., Robles, A.B., Sánchez-Navarro, A., González-Rebollar, J.L., 2011. Soil responses to different management practices in rainfed orchards in semiarid environments. *Soil and Tillage Research* 112 (1): 85–91.

Rashidi, M., Keshavarzpour, F., 2007. Effect of different tillage methods on grain yield and yield components of maize (Zea mays L.). *International Journal of Agriculture and Biology* 9(2): 274–277.

Richards, L.A., 1947. Pressure-membrane apparatus. Construction and use. *Agriculture and Engineering* 28: 451-454.

Salem, H.M., Valero, C., Muñoz, M.A., Rodríguez, M.G., Silver, L.L., 2015. Short-term effects of four tillage practices on soil physical properties, soil water potential, and maize yield. *Geoderma* 237-238: 60-70.

Seobi, T., Anderson, S.H., Udawatta, R.P., Gantzer, C.J., 2005. Influence of grass and agroforestry buffer strips on soil hydraulic properties for an Albaqualf. *Soil Science Society America Journal* 69(3): 893–901.

Teh, C.B.S., Jamal, T., 2006. Soil Physics Analyses. Vol. 1. University Putra Malaysia, Serdang. UPM press, Malaysia.

Zaibon, S., Anderson, S.H., Kitchen, N.R., Haruna, S.I., 2016. Hydraulic properties affected by top soil thickness in switchgrass and corn-soya-bean cropping systems. *Soil Science Society America Journal* 80(5): 1365-1376.

Eurasian J Soil Sci 2019, 8 (3) 275 - 281

Eurasian Journal of Soil Science

Journal homepage : http://ejss.fesss.org

The effect of NPK foliar fertilization on yield and macronutrient content of grain in wheat under Kostanai-Kazakhstan conditions

Coşkun Gülser [a,*], Zhenis Zharlygasov [b], Rıdvan Kızılkaya [a,c]

Niyazbek Kalimov [b], Izzet Akça [c,d], Zhaksylyk Zharlygasov [b]

[a] Ondokuz Mayıs University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Samsun, Turkey
[b] Ahmet Baytursynov Kostanai State University, Agrarian and Biological Faculty, Kostanai, Kazakhstan
[c] Agrobigen Research & Development Trade Ltd.Co, Samsun Technopark, Samsun, Turkey
[d] Ondokuz Mayıs University, Faculty of Agriculture, Department of Plant Protection, Samsun, Turkey

Abstract

The objective of this research were to determine the effects of foliar fertilization (20% N: 20% P_2O_5: 20% K_2O) at different growth stages on yield and nutrient contents of spring wheat (*Triticum aestivum* L.) and to reveal proper application time and frequency in Kostanai-Kazakhstan conditions. The field experiment was conducted according to randomized plot design with four replications with a seeding rate of 500 seed per m^2 on the plots having a size of 5.0 m length and 4.0 m width. Foliar applications of the fertilizer at 1%rate were done at tillering (T), stem elongation (S), heading (H) stages of wheat and the combinations of these stages (T+S, T+H, S+H and T+S+H). Wheat yield values varied between the lowest 1.32 t/ha in the control and the highest 2.14 t/ha in the foliar fertilization done at tillering and steam elongation stages (T+S). According to control treatment, increases in grain yields by the foliar fertilization done at the different growth stages were determined as follows; T+S (61.7%) > T+H (47.4%) > T+S+H (41.8%) > S+H (41.6%) > T (38.5%) > S (19.1%) > H (16.6%). There was no significant difference among the macronutrient contents in grain obtained by the foliar fertilization at the different growth stages. N, P and K contents in the grain were close the values cited in the literatures. Ca and Mg contents deficiency in grain were determined due to the acidic soil pH condition of Kostanai-Kazakhstan. Nitrogen, P, K and Ca uptakes by grain, except Mg, generally increased with the all application of foliar fertilization. When the foliar fertilization was done at T+S stages, the highest macro nutrient uptakes by grain in wheat were generally obtained. This research indicated that the first foliar fertilization should be applied at the tillering stage and the best foliar fertilization management for high grain yield and nutrient uptake should be done two times at the combination of tillering and stem elongation stages.

Keywords: Wheat, grain yield, foliar fertilization, nutrient uptake.

Article Info

Received : 10.10.2018
Accepted : 05.06.2019

Introduction

The nutrients in soil have an important role in the crop nutrition to achieve higher yield, better growth and plant development. Fertilization is one of the most important parts of the crop growing technology and has the highest dynamic effect on the grain wheat yield economically (Ivanova et al., 2007). It is generally assumed that if fertilizers are applied in autumn or spring then all crop nutrient requirements can be met from the soil. It is not always true, for example particularly late in the season when abundant nutrients may

* Corresponding author.
Ondokuz Mayıs University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Samsun, Turkey
Tel.: +90 362 3121919 E-mail address: cgulser@omu.edu.tr
e-ISSN: 2147-4249 DOI: 10.18393/ejss.575026

be present in the soil but unavailable to a crop due to dry conditions. In this case, soil nutrient supply combined with internal re-translocation may not always be adequate for setting and filling the maximum number of grains, and crops might benefit from supplementary foliar applications of nutrients (Barraclough and Haynes, 1995). Many studies showed that foliar fertilization at different growing stages of wheat increased grain yield over the control (Bhutto et al., 2016; Jarecki et al., 2017; Mikos-Szymańska, 2018). Rusek et al. (2016) found that the average yields of wheat were significantly higher about 30% after urea-superphosphate and concentrated superphosphate with urea fertilizers over the non-fertilized plots. They determined that N and P fertilization increased the N, protein and gluten contents in grains and had the positive effect on wheat quality. Czuba (1993) reported that nitrogen use efficiency (up to 160%) through foliar application was greater than soil application of urea. Sarandón and Gianibelli (1990) determined that foliar application of urea at the end of tillering increased dry matter yield, grain yield, harvest index and total N uptake by wheat and maximum grain yield was obtained with the application of N at sowing plus a foliar application at the end of tillering. In a field experiment by Khan et al. (2009), foliar application of urea in different concentrations and at different stages increased yield and yield components of wheat. Potassium nitrate is readily available and widely used in horticulture (Weinbaum, 1988), and in view of the well-known synergisms between N and K during uptake and translocation in plants, it would seem to be an ideal source of macronutrients for late, supplemental foliar feeding. Munson (1982) reported that nutrient uptake by wheat was 125 kg/ha N, 22 kg/ha P, 92 kg/ha K, 16 kg/ha Ca, 14 kg/ha Mg, and 14 kg/ha S when 8 t/ha of dry matter was produced. In a greenhouse study, low concentrations of KNO_3 (20 mM) sprayed on wheat at anthesis considerably increased grain numbers (Prakash and Joshi, 1973). In addition to their benefits, foliar fertilization may also help reduce the denitrification, leaching and immobilization, frequently related to N fertilization to the soil system (Gooding, 2005) by improving N use efficiency to a greater extent (Czuba, 1993). It is also reported that the foliar application of urea, applied at and after anthesis, is beneficial over soil treatments in increasing grain N content of wheat (Zhigulev, 1992). Gülser et al. (2017) reported that the application of 1.0% rate of foliar fertilization (10% N, 5% P_2O_5, 5% K_2O) only at stem extension stage or together with tillering stage increased the yield components and raw protein contents of grains in wheat plant.

The objectives of this research were to determine the effects of foliar fertilization (20% N: 20% P_2O_5: 20% K_2O) at different growth stages on yield and nutrient contents of spring wheat (*Triticum aestivum* L.) and to reveal proper application time and frequency in Kostanai-Kazakhstan conditions.

Material and Methods

This research was carried out at the experimental field of Kostanai Agricultural Research Institute in Kazakhstan. Soil properties of the experimental field were determined on the soil samples taken from the field randomly before seeding. Soil samples taken from the field were air dried and sieved from a screen with 2 mm opening size to prepare for analysis. Sand, silt, clay contents of the soil samples were determined by Hydrometer method (Bouyoucos, 1962). Soil pH values were measured in soil suspension (1:1 w:v) by glass electrode pH meter and EC values were determined in the same soil suspension (1:1 w:v) by EC meter (Rowell, 1996). Soil organic matter (OM) contents were determined by modified Walkley-Black method, (Walkley and Black, 1934). Lime ($CaCO_3$) contents of soils were determined by Scheibler calcimeter according to Nelson (1982). Some properties of the soil used in this research are given in Table 1. The soil of experimental field had sandy clay loam (SCL) textural class, moderate in organic matter content, acidic in soil pH, non-saline and limeless (Soil Survey Staff, 1993).

Table 1. Some properties of the experimental soil.

Clay, %	25.87	pH(1:1)	5.45
Silt, %	11.04	EC, dS/m	1.272
Sand, %	63.09	Organic matter, %	3.18
Texture	SCL	Lime ($CaCO_3$), %	1.06

Average climatic data of temperature and rainfall for long term in Kostanai are given in Figure 1. According climatic data, the highest mean temperature is on July (27°C) while the lowest mean temperature is on January (-23°C). The total precipitation per year is about 347 mm and the region receives the highest mean precipitation on July (61 mm) and the lowest precipitation is on December (10 mm).

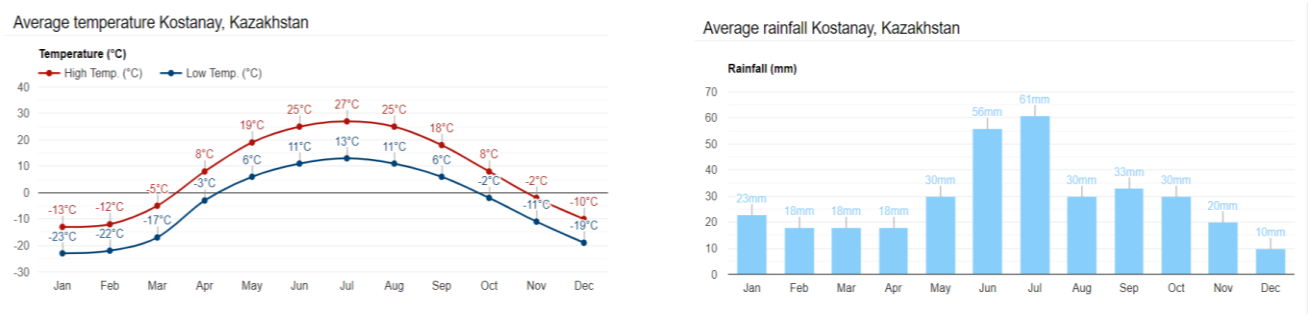


Figure 1. Climatic data for long term in Kostanai, Kazakhstan

A field experiment was conducted at the fields of Kostanai Agricultural Research Institute with randomized complete plot design having three replications. The plot size was kept 20 m^2 (5x4m) and 2 m spacing among the parcels was left to avoid edge effect. After ploughing and land leveling, seeds of spring wheat variety Omskaya-18 at a recommended rate of 500 seeds per m^2 were sown throughout the plots in 26.05.2016. In the control plots, only basic recommended NPK fertilization for wheat in Kostanai conditions was applied from soil and there was not foliar fertilization. Also, the basic NPK fertilization was applied for the experimental plots including foliar fertilization. The foliar fertilizations at 1% rate of totally water soluble fertilizer (20% N: 20% P_2O_5: 20% K_2O) produced by Agrobigen R&D using with nano-technological methods were applied with spraying 0.5 L fertilizer solution to plant leaves in each plot to wet all plants completely at tillering (T), stem elongation (S), heading (H) stages of wheat and at the four combination times of these stages (T+S, T+H, S+H and T+S+H). The foliar fertilizations were applied at tillering stage in 25.06.2016, stem elongation stage in 12.07.2016 and heading stage in 23.07.2016.

In order to determine the foliar fertilization effect, ripened plants were harvested in 27 August, 2016. Plants from all plots were sampled to measure yield and yield criteria parameters and to analyze plant nutrient contents. Plant samples were dried at 65°C with aeration until reaching a constant weight. Dried plant samples were grained using a stainless steel grinder to make samples ready for analysis. Grained seed samples were placed in the oven at 450±50°C for 8 hours. Seed ashes were treated with 10 N HNO_3 and filtrated using blue band filter paper according to Jones (2001). Total nutrient contents of seed samples were determined in filtrated solutions by the following methods at the laboratory of Soil Science and Plant Nutrition Department of Ondokuz Mayıs Uiversity, Samsun-Turkey; total nitrogen (N) contents by Kjeldahl method, total Phosphorus (P) contents with the vanado-molybdophosphoric acid color method spectrophotometrically; total potassium (K), calcium (Ca) and magnesium (Mg) contents using Perkin Elmer A400 Atomic Absorption Spectrophotometer (Jones, 2001). Variance analyses and correlations for the data were done using SPSS pogramme and significant differences between the treatment means were shown Duncan test.

Results and Discussion

Effect of NPK foliar fertilization on grain yield in wheat

Foliar fertilization with 1% rate of NPK (20:20:20) at different grown stages of wheat under Kostanai-Kazakhstan conditions significantly increased the grain yield over the control application (Figure 1A). While the lowest grain yield (1.32 t/ha) was obtained in the control, the highest grain yield (2.14 t/ha) was obtained with the foliar fertilizations at the combination of T+S stages. Although the increase in grain yield by foliar fertilization made only at S and H stages was not significant, the foliar fertilization in T stages significantly increased grain yield over the control. The foliar fertilization made more than one times at the combination of wheat grown stages significantly increased the grain yield compared with the control. The percentage increases in grain yield according to the control are given in Figure 1B. Percentage increment effect of foliar fertilization at different growth stages on grain yield over the control were determined as the following order; T+S (61.7%) > T+H (47.4%) > T+S+H (41.8%) > S+H (41.6%) > T (38.5%) > S (19.1%) > H (16.6%). Khan et al. (2009) found that the wheat grain yield was increased by 32% when 4% urea solution was applied as foliar spray at tillering, stem elongation and boot stage. Jarecki et al. (2017) reported that three times foliar fertilization increased wheat grain yield over the control. Mikos-Szymańska et al. (2018) determined that the standard NPK soil fertilization plus calcium micronized suspension foliar fertilization at tillering and stem elongation stages had the highest grain yield in spring wheat compare to control by 15.7%. In another study, Maitlo et al. (2006) found that the foliar application of urea done at tillering and ear head emergence stages by the integrated application of urea through broadcasting significantly increased the

wheat grain yield (4.11 t/ha) over soil application urea alone (3.47 t/ha). Gülser et al. (2017) determined that total biological wheat yield varied between the lowest 5.44 t/ha in control treatment and the highest 7.38 t/ha in 1.0% rate of NPK (10:5:5) foliar fertilization treatment at stem extension stage. In this study, similarly it was found that 1% foliar fertilizations of NPK (20:20:20) at different growing stages showed significant increments in wheat grain yield over the control. It was determined that if one or more foliar fertilization will be applied regarding the growing stages of wheat, the first one should be applied at the tillering stage and the best foliar fertilization management for high grain yield was the combination of tillering and stem elongation stages.

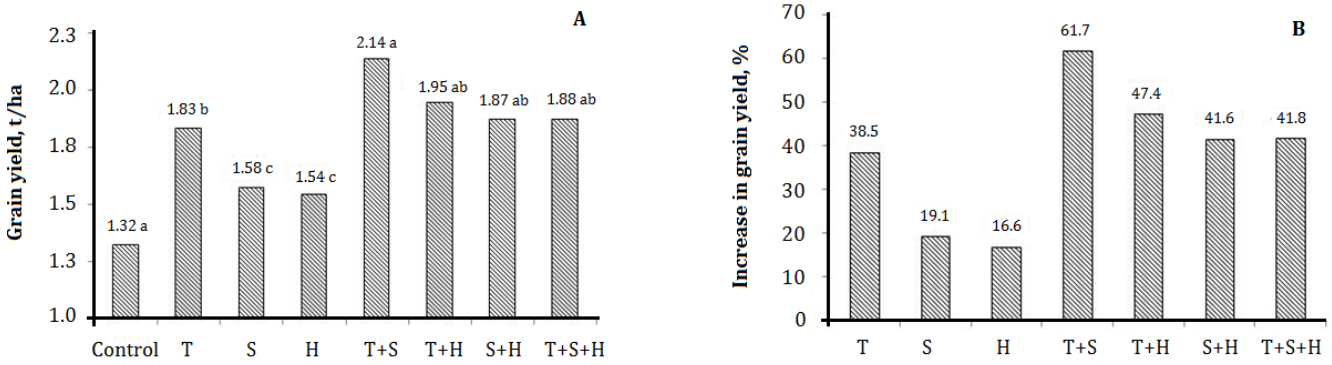


Figure 1. Effect of foliar fertilization at tillering (T), stem elongation (S) and heading (H) stages on wheat grain yield (A) and percentage increase in grain yield according to control (B).

Effect of foliar fertilization macronutrient content and uptake by grain in wheat

The foliar fertilization at 1% rate of NPK (20:20:20) had no significant effect on macronutrient content of grain (Table 2). Smith et al. (2018) reported that increased grain yield by the N and P fertilization generally diluted grain nutrient concentration for P, Zn, Ca, K, Mg, and Mn. In this study, similarly N and Mg concentrations in grain generally decreased by the foliar fertilization and the concentrations of the other elements almost stayed stable due to dilution effect of increasing yield. While the highest N content (1.938%) was obtained in the control treatment, N content of grain generally decreased by the foliar fertilization. The highest P (0.404%) and K (0.563%) content were determined by the foliar fetilization applied at H stage. On the other hand, the highest Ca (0.017%) and Mg (0.089%) content were found by the foliar fetilization applied at T+S+H and S stage, respectively. Tiryakioğlu et al. (2014) found that there were high genotype effects for all macronutrients and plant organs and N, P, K, and Mg decreased during grain filling in all plant parts except the grain. They reported that the rate of decrease varied depending on plant organs and nutrients and grain nutrient concentration, except nitrogen content, increased up to physiological maturity, in contrast to the other nutrients, Ca content increased or remained stable depending on the plant organs. In this study gener ally, N and Mg concentrations in grain reduced with the application of foliar fertilization over the control when comparing with the other nutrients (Table 2). Ostrowska and Porebska (2017) indicated that N, Ca, Mg contents and Ca:Mg ratio of grain in wheat were 2.07%, 0.05%, 0.08% and 0.6, respectively. They reported that the Mg content higher than that of Ca in cereal grain and consequently the Ca:Mg ratios were lower than 1. The ratio of Ca:Mg is an indicator of Mg deficiency in plant and an imbalanced Ca:Mg ratio often negatively influences product quality (Gerendás and Führs, 2013). The ratio of Ca:Mg in cereals is an important parameter for human and animal health and the Mg content higher than that of Ca in cereal grain and consequently the Ca:Mg ratios are lower than 1. The low Ca content in plants is caused by intensive N fertilization in acidic soils (Ostrowska and Porebska, 2017). In this study, Ca content in grain was similarly low under the acidic soil condition.

Table 2. Effect of foliar fertilization on macronutrient content of wheat grain

Foliar fertilizations at grown stages	N, %	P, %	K, %	Ca, %	Mg, %	Ca:Mg
Control	1.938	0.356	0.532	0.013	0.078	0.169
Tillering (T)	1.803	0.377	0.529	0.013	0.058	0.229
Stem elongation (S)	1.858	0.393	0.504	0.014	0.089	0.156
Heading (H)	1.878	0.404	0.563	0.015	0.055	0.272
T+S	1.899	0.351	0.544	0.014	0.063	0.227
T+H	1.849	0.334	0.534	0.015	0.044	0.345
S+H	1.870	0.342	0.456	0.015	0.061	0.249
T+S+H	1.897	0.358	0.543	0.017	0.057	0.292

The foliar fertilization at 1% rate of NPK (20:20:20) significantly increased N, P, K and Ca uptakes by grain in wheat, except Mg uptake. The N uptake by grain varied between the lowest (25.6 kg/ha) in the control and the highest (40.6 kg/ha) in the foliar fertilization at T+S stages (Figure 2). Increments in N uptake over the control were significant in the foliar fertilization done at T, T+S, T+H, S+H and T+S+H stages (p>0.01). Increasing total N uptake also reflects the increase total protein content in grain of wheat plant. Siuliauskas et al. (2001) found that 30 kg N/ha, applied as foliar spray during heading and at the beginning of milky ripeness increased wheat yield upto 8.54 t/ha and protein content up to 15.29%. Khan et al. (2009) reported that integrated application of N through soil and foliage facilitated the higher N uptake in plants. They determined that the highest mean N uptake of 149.9 kg/ha was in the treatment where 6% urea solution sprayed at tillering, stem elongation and boot stage, which was significantly higher than all other treatments. In another study, Maitlo et al. (2006) found that the 2.5% foliar application of urea done at tillering and ear head emergence stages by the integrated application of urea through broadcasting significantly increased the N uptake by grain (59.36 kg/ha) over soil application urea alone (25.96 kg/ha). Gülser et al. (2017) reported that the highest raw protein content (18.5%) and the yield components in wheat was determined over the control with the application of 0.5% rate of foliar fertilization (10% N, 5% P_2O_5, 5% K_2O) at tillering plus stem extension stages. Similarly in this study, the highest protein amount or N uptake by grain in wheat was determined with the foliar fertilization done at the T+S stages.

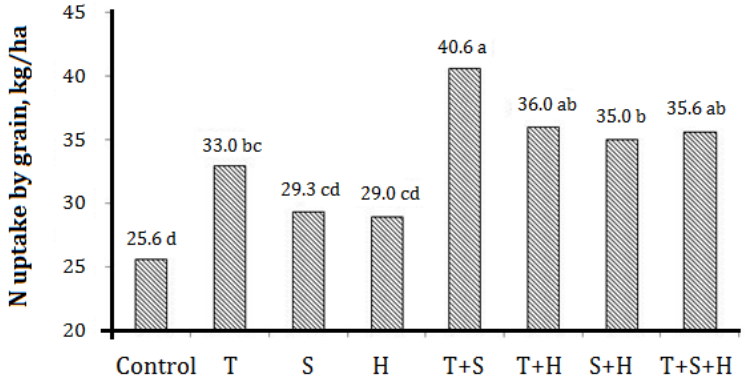


Figure 2. Effect of NPK foliar fertilization at tillering (T), stem elongation (S) and heading (H) stages on N uptake by grain in wheat (p>0.01).

The P uptake by grain varied between the lowest (4.65 kg/ha) in the control and the highest (7.45 kg/ha) in the foliar fertilization at T+S stages (Figure 3). Increments in P uptake over the control were significant in the all foliar fertilization treatments (p>0.05). The K uptake by grain also varied between the lowest (7.05 kg/ha) in the control and the highest (11.58 kg/ha) in the foliar fertilization at T+S stages (Figure 3). Increments in K uptake over the control were significant in the foliar fertilization done at the T, T+S, T+H, T+S+H stages (p>0.01). Maitlo et al. (2006) detrmined that the 2.5% foliar application of urea done at tillering and ear head emergence stages by the integrated application of urea through broadcasting significantly increased the P and K uptakes by grain (20.00 kg/ha and 24.65 kg/ha) over soil application urea alone (16.89 kg/ha and 20.89 kg/ha), respectively.

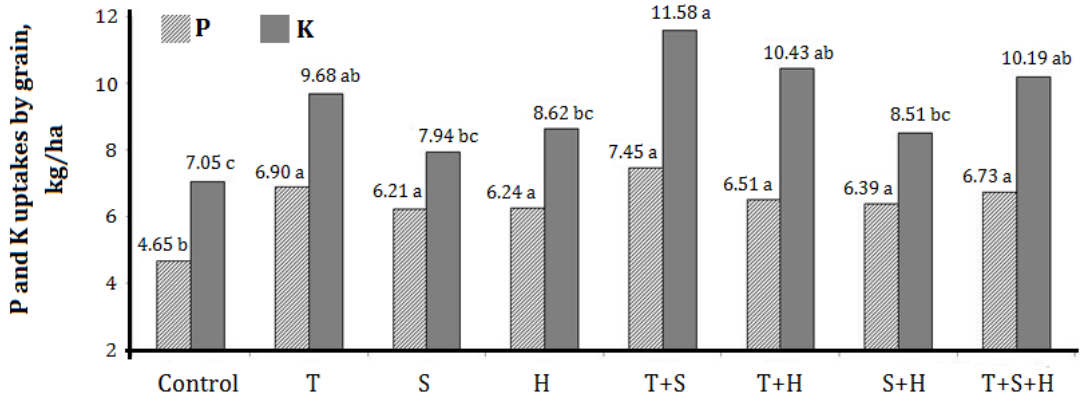


Figure 3. Effect of NPK foliar fertilization at tillering (T), stem elongation (S) and heading (H) stages on P (p>0.05) and K (p>0.01) uptakes by grain in wheat.

While the Ca uptake significantly increased by the foliar fertilization (p>0.01), there was no significant effect of foliar fetilization on Mg uptake over the control (Figure 4). It was found that the highest Ca uptake (0.31 kg/ha) was obtained by the foliar fetilizations done at the T+S and T+S+H stages, the lowest Ca uptake (0.17 kg/ha) was determined in the control. Except the foliar fertilization done at H and T+H stages, Mg uptake by grain generally increased by the foliar fertilization compared with the control. The highest Mg content (1.40 kg/ha) was determined with the foliar fertilization done at S stage. Klikocka (2018) reported that the uptake of K, Mg and Ca by grain significantly increased in direct proportion to the increase of the N fertilization dose and was the highest after application of 120 kg N/ha (K–22.55, Mg–8.36 and Ca–2.28 kg/ha).

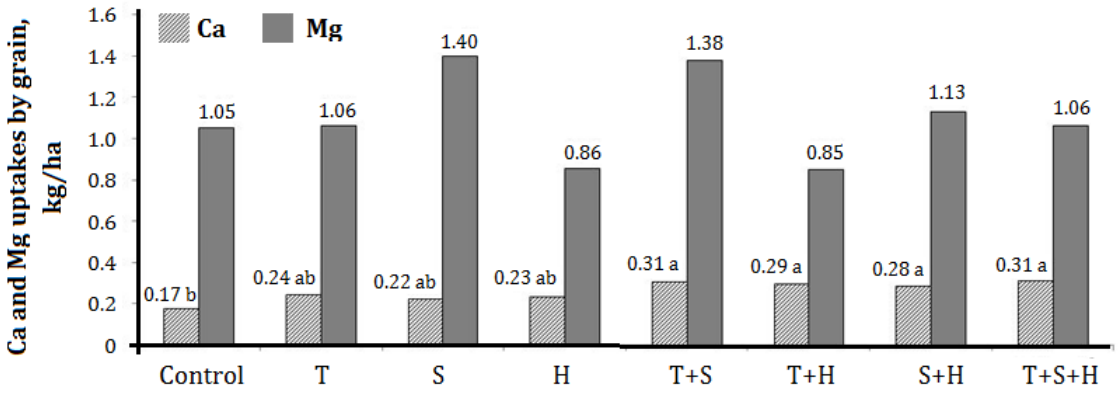


Figure 4. Effect of NPK foliar fertilization at tillering (T), stem elongation (S) and heading (H) stages on Ca (p>0.01) and Mg uptakes by grain in wheat.

The grain yield showed unsignificant negative correlations with N (-0.218), P (-0.395), K(-0.163), Mg(-0.264) and unsignificant positive correlation with Ca (0.042) contents. However, the significant correlation coefficients between the grain yield and uptake of macroelements by grain showed the following order; N (0.966**) > K (0.752**) > Ca (0.651**) > P (0.631**). Klikocka et al. (2018) determined that the correlation coefficients between grain yield and the content of macroelements decreased in the order Mg > Ca > K > P and the strength of the relationship between grain yield and uptake of macroelements by grain had the following order P > Mg > K > Ca.

Conclusion

The 1% rate of foliar fertilization (20% N: 20% P_2O_5: 20% K_2O) applied at the different growth stages increased the grain yield of wheat. The highest grain yield (2.14 t/ha) was determined with the foliar fertilization applied at the tillering plus stem elongation (T+S) stages compared with the control (1.32 t/ha). Increased grain yield by the foliar fertilization generally diluted macro nutrient content in grain. Therefore, there was no significant difference in macronutrient content in grain among the foliar fertilization done at the different growth stages and their combinations. Although N, P and K contents in the grain were close the values cited in the literatures, Ca and Mg contents were lower than the values indicated in the different studies. It shows that there are Ca and Mg deficiency in the gain grown under the acidic soil pH condition of Kostanai-Kazakhstan. The Ca:Mg ratio in grain for all treatments was lower than 1. Besides the basic N, P, K fertilizers, foliar or soil fertilizers including Ca and Mg should be used for wheat growth in this region. Macro nutrient uptakes by grain, except Mg, generally increased with the all foliar fertilization. The highest macro nutrient uptakes by grain in wheat were generally obtained with the foliar fertilization done at the T+S stages due to the highest grain yield. It can be suggested that if one or more foliar fertilization will be applied during the growing stages of wheat, the first one should be applied at the tillering stage and the best foliar fertilization management for high grain yield and nutrient uptake was the combination of tillering and stem elongation stages.

Acknowledgements

The authors would like to thank the Agrobigen R&D Ltd.Co., Turkey (Project No. AGROBIGEN.2016.01.01) for financial support.

References

Barraclough, P.B., Haynes, J., 1995. The effect of foliar supplements of potassium nitrate and urea on the yield of winter wheat. *Fertilizer Research* 44(3): 217–223.

Bhutto, M., Shah, A.N., Leghari, U.A., Jatoi, G.H., Khaskheli, M.A., Khanzada, A., 2016. Growth and yield response of wheat (*Triticum Aestivum* L.) as affected by foliar fertilization of zinc. *Science International (Lahore)* 28(4): 4189-4192.

Bouyoucos, G.J., 1962. Hydrometer method improved for making particle size analyses of soils. *Agronomy Journal* 54(5): 464-465.

Czuba, R., 1993. The results of leaf nutrition of field crops. Part II. Response of plants to foliar application of micronutrients or nitrogen combined with micronutrients. *Roczniki Gleboznawcze - Soil Science Annual* 44(3/4):79-87 [in Polish].

Gerendás J., Führs, H., 2013. The significance of magnesium for crop quality. *Plant and Soil* 368(1-2): 101-128.

Gooding, M.J., 2005. Foliar urea fertilisation and the management of yield and quality in wheat. Proceedings of the International Fertiliser Society No. 573. Available at [Access date: 10.10.2018]: http://www.fertiliser-society.org/Proceedings/US/Prc573.HTM

Gülser, C., Kızılkaya, R., Küren, N., Oy, G., Yalçın, U., 2017. The effect of foliar fertilizer produced from biogas waste on yield and protein content of wheat. ICAFOF, International Conference on Agriculture, Forest, Food Sciences and Technologies, 15-17 May 2017., Cappadocia, Turkey, Book of Proceedings. p 260.

Ivanova, A., Nankova, M., Tsenov, N., 2007. Effect of previous crop, mineral fertilization and enviroment on the characters of new wheat varieties. *Bulgarian Journal of Agricultural Science* 13 (1): 55–62.

Jarecki, W., Buczek, J., Bobrecka-Jamro, D., 2017. Response of spring wheat to different soil and foliar fertilization. *Journal of Central European Agriculture* 18(2): 460–476.

Jones, Jr.J.B., 2001. Laboratory guide for conducting soil tests and plant analysis. CRC Press, New York, USA. 363p.

Khan, P., Memon, M.Y., Imtiaz, M., Aslam, M., 2009. Response of wheat to foliar and soil application of urea at different growth stages. *Pakistan Journal of Botany* 41(3): 1197-1204

Klikocka, H., Marks, M., Barczak, B., Szostak, B., Podleśna, A., Podleśny, J., 2018. Response of spring wheat to NPK and S fertilization. The content and uptake of macronutrients and the value of ionic ratios. *Open Chemistry* 16(1): 1059–1065.

Maitlo, A., Hassan, Z.U., Shah, A.N., Khan, H., 2006. Growth, Yield and Nutrient Uptake of Wheat (*Triticum aestivum* L.) in relation to Foliar and Soil Application of Urea. *International Journal of Agriculture and Biology* 8 (4):477-481.

Mikos-Szymańska, M., Borowik, M., Wyzińska, M., Rusek, P., 2018. Effects of different fertilizer treatments on grain yield and yield components of spring wheat. *Research For Rural Development* 2: 100-106.

Munson, R.D., 1982. Potassium, calcium, and magnesium in the tropics and subtropics. International Fertilizer Development Center. Technical Bulletin Series T-23. Muscle Shoals, TN, USA. 62p.

Nelson, R.E., 1982. Carbonate and gypsum. In: Methods of Soil Analysis, Part 2, Chemical and microbiological properties, Second Edition. Number 9, Page, A.L., Keeney, D. R., Baker, D.E., Miller, R.H., Ellis, R. Jr., Rhoades, J.D. (Eds.). ASA-SSSA, Madison, Wisconsin, USA. pp. 181-198.

Ostrowska, A., Porębska, G., 2017. The content of calcium and magnesium and the Ca:Mg ratio in cultivated plants in the context of human and animal demand for nutrients. *Journal of Elementology* 22(3): 995-1004.

Parkash, V., Joshi, Y.C., 1973. Influence of foliar feeding of kinetin and potassium nitrate on the grain setting of wheat. *Agrochemica* 17: 238–242.

Rowell, D.L. 1996. Soil Science: Methods and Applications. Longman, London, UK. 368p.

Rusek, P., Mikos-Szymańska, M., Karsznia, M., Sienkiewicz-Cholewa, U., Igras, J., 2016. The effectiveness of nitrogen-phosphorus fertilization in winter wheat (*Triticum aestivum* L.) cultivation. *Bulgarian Journal of Agricultural Science* 22(5): 752–755

Sarandón, S.J., Gianibelli, M.C., 1990. Effect of foliar urea spraying and nitrogen application at sowing upon dry matter and nitrogen distribution in wheat (Triticum aestivum L.). *Agronomie* 10(3):183-189.

Siuliauskas, A., Vagusevicience, I., Liakas, V., 2001. Additional fertilization of winter wheat through leaves. *International Conference on Sustainable Agriculture in Baltic States.* 28-30 June 2001, Tartu, Estonia.

Smith, E.G., Janzen, H.H., Ellert, B.H., 2018. Effect of fertilizer and cropping system on grain nutrient concentrations in spring wheat. *Canadian Journal of Plant Science* 98(1): 125-131.

Soil Survey Staff, 1993. Soil Survey Manuel. USDA Handbook No:18 Washington, USA.

Tiryakioğlu, M., Yildirim, M., Karanlik, S., 2014. Macronutrient concentration and remobilization in spring wheat organs during grain filling. *Turkish Journal of Agriculture and Forestry* 38: 488-494.

Walkley, A., Black, I.A., 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science* 37(1): 29-37.

Weinbaum, S. 1988. Foliar nutrition in fruit trees. In: Plant growth and leaf applied chemicals. Neumann, P.M. (Ed.). CRC Press, Boca Raton. USA. pp. 81-100.

Zhigulev, A.K., 1992. Effect of foliar application of nitrogen fertilizers on yield and quality of winter wheat grain. *Agrokhimiya* 3(3): 3-9.