2022

Volume : 11
Issue : 1
Page : 1 - 92

e-ISSN : 2147-4249

Federation of Eurasian Soil Science Societies

Eurasian Journal of Soil Science

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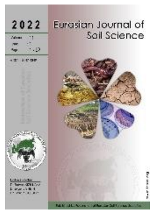

EURASIAN JOURNAL OF SOIL SCIENCE

(Peer Reviewed Open Access Journal)

Published by Federation of Eurasian Soil Science Societies

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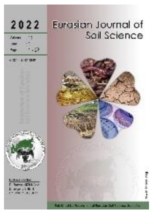
ABSTRACTING AND INDEXING: SCOPUS, CABI, FAO-Agris, EBSCOhost, ProQuest, DOAJ, OAJI, TR Dizin, TURKISH Journalpark, CrossRef, CiteFactor, CAS, etc.



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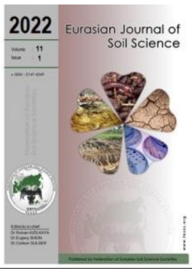
CONTENTS

Eurasian Journal of Soil Science 2022, 11(1), 1 - 9

Eurasian Journal of Soil Science

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



Estimation and spatial distribution of some soil erodibility parameters in soils of Ilgaz National Park

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Article Info

Received : 12.03.2021
Accepted : 19.07.2021
Available online : 25.07.2021

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Abstract

The aim of this research was to determine some erodibility factors, aggregate stability, structure stability and crust formation, in soils located at Ilgaz National Park and to generate their spatial distribution maps using fifteen different interpolation models in GIS medium. For this aim, total 151 soil samples were collected from surface (0-20 cm) soil depth. According to analysis results, it was determined that most part of the investigated soils has high erodibility value. In addition, correlation analysis was performed between erodibility factors and some soil physical and chemical properties. According to analysis results, it was found that a significantly positive relationship was found between AS and EC (0.460**) and OM (0.603**) at the 1% importance level whereas, a negative relationship was found between BD (-0.544**) at the 1% importance level. A positive relationship was also found between SSI values and EC (0.418**) and OM (0.565**) at a 1% significance level, and a negative relationship was found at a 1% significance level with BD (-0.542**). Moreover, a positive relationship was found between CF and EC (0.523**), OM (0.894**) and sand (0.345**) at a 1% importance level, and a negative relationship was found at a 1% importance level with clay (-0.376**) and BD (-0.811**).

Keywords: Aggregate stability, structure stability, crust formation, GIS, Ilgaz national park.

Introduction

Given its ecological differences, Turkey has favourable conditions for erosion, especially due to climate, topographic conditions, and land use pressures (Kanar and Dengiz, 2015). Erosion is generally defined as transport and accumulation of soil from where it is located by various factors such as water, wind and gravity. Although soil erosion is a natural event that occurs on the surface of the earth, it is accelerated as a result of human effects and can lead to serious environmental problems. As a result of erosion, problems such as decreased nutrient content in the soil, acidification of the soil, formation of poor drainage conditions, deterioration of water balance in the root zone, loss of soil productivity, accumulation of sediment in water channels, increased amounts of floods, contamination of water resources are caused (Singer and Warkentin, 1996; Li and Fang, 2016; Wang et al., 2017). From these negative effects of erosion, soil and water resources, which are our most important natural resources, need to be protected. Effective and sustainable use of these two important natural resources is very important both in terms of the continuity of the terrestrial ecosystem and in terms of food security, given the rapid increase in population (Saygın et al., 2019). Because of this, soil erosion studies are critical in creating successful land use and management planning and in developing appropriate conservation practices at different scales (Breetzke et al., 2013). It is well known that many factors influence the severity of soil erosion. These factors such as the characteristic and erosivity of rainfall, the degree and length of slope, vegetation cover and land management can be more effective than the natural properties of the soil. On the other hand, even if all these factors are the same, some soils are

: https://doi.org/10.18393/ejss.974219
: http://ejss.fesss.org/10.18393/ejss.974219
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

more easily eroded or sensitive for erosion. This difference, caused by the soil's own properties, is called soil erodibility (Wischmeier and Smith, 1978; Kanar and Dengiz, 2015). In this case, in order estimate soil loss and understand the functioning of soil erosion, it is necessary to evaluate soil erodibility. However, since soil erosion studies are often expensive and time-consuming, some soil characteristics that are closely related to erosion are identified and soil erodibility is estimated (Carlos and Odette, 2012; Stanchi et al., 2013).

Aggregate stability, one of the physical properties of the soil, is a measure of the ability of the soil to maintain its structure when the soil is under mechanical stress or subjected to destructive forces. Shi et al. (2010) noted that soil aggregate structure is an appropriate indicator of soil sensitivity to erosion, while Igwe and Obalum (2013) reported the importance of micro aggregate stability as an indicator of soil erodibility. After precipitation, a crust is formed on the surface following the breakdown of aggregates on the soil surface, and as a result, water infiltration into the soil decreases and surface flow occurs. Many studies have been carried out examining the relationship of crust formation with infiltration rate and erodibility of soils (Le Bissonnais, 1996; Issa et al., 2004; Darboux and Le Bissonnais, 2007). In addition, the crust formation is an indicator of the physical deterioration in the structure when the soil is wet, and a decrease in this ratio means an increase in resistance to erosion. In order to eliminate these adverse conditions, it is necessary to improve the physical properties of soils and increase their structural stability. For that reason, some studies are also conducted in which the relationship between the dispersion ratio and structural stability indexes of soils and their erodibility is evaluated (Mbagwu et al., 1999; Özdemir et al., 2005).

The aims of this study are to determine some erosion sensitivity parameters such as aggregate stability, structure stability index and crust formation of soils distributed within Ilgaz National Park area in Turkey and to map their spatial distributions using different interpolation methods using geographical information techniques.

Material and Methods

Ilgaz Mountain National Park is located in the Western Black Sea region of the Black Sea region of Turkey and within the borders of Kastamonu and Çankırı provinces. The study area is located between 558759 - 4548060 East longitudes and 563823-4544347 North latitudes (WGS84- Zone 36, UTM m) (Figure 1).

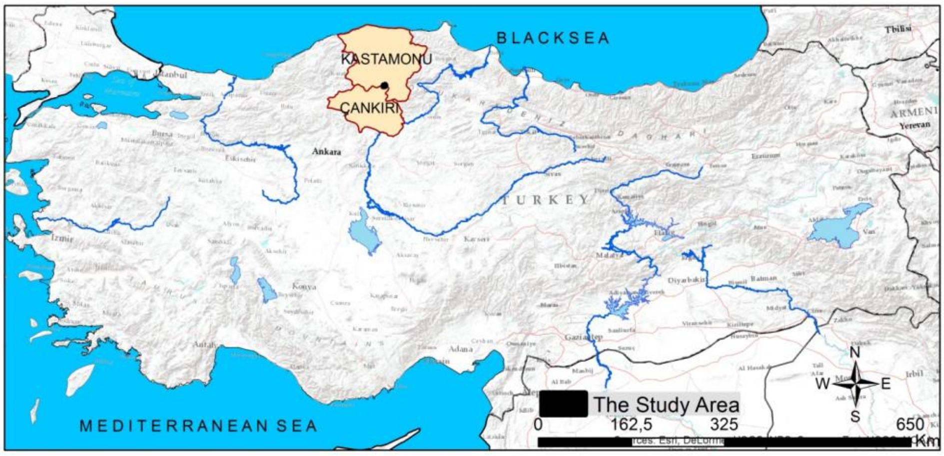

Figure 1. Location of the study area

The National park has an area of 1117.54 ha, 778.93 ha of the study area is within the borders of Kastamonu province, while 337.75 ha of the area is within the borders of Çankırı province. Kozançal Tepe (2070 m), Karakeçilik Tepe (1999 m), Hemdir Tepe (1931 m), Şadımın Tepe (1843 m), Haydarın Ridge and Arpasekisi Ridge are important hills and ridges within the borders of the National Park (Anonymous, 2009). The National Park has an undulating and mountainous topography and is located between 1519m and 2072m above sea level (Celilov and Dengiz, 2019) (Figure 2).

For long years (2009-2017) meteorological station located about 885m from sea level data was used in the Çankırı Ilgaz District in the research area. According to the Thorthwaite climate classification, it was coded as “B2C2sb2”; subhumid, microthermal climate, moderate water deficiency in summer, 2. decree shows marine characteristics. The average annual rainfall in the research area is 680.5 mm and the temperature is 5.1 ^{o}C. According to the Newhall simulation model (Van Wambeke, 2000), the soil moisture of the working area soils was classified as Udic and in the sub classification as Dry Tempudic. According to soil taxonomy (Soil Survey Staff, 1999), the majority of the study area soils are still at the beginning of pedological development and can be characterized as young because they do not have any sub-surface diagnostic horizons. Soils have formed on sloping land and have shallow depth. There are no diagnostic horizons except for a lithic contact within 50 cm depth under the surface of these soils. Soils are classified in the orthent suborder due to their location on hillside shallow depth and classified as Cryorthent great group due to soil temperature regime. In addition, they were classified as Lithic Cryorthent in subgroup level due to reaching bedrock at depths of 50 cm. The type of bedrock distributed within the study area is sandstone-mudstone, limestone in the north-eastern parts, while in the south-eastern parts there are mostly flints. In addition, in the cross-section located in the northeast-southwest direction of the study area, there are lime stones (Celilov and Dengiz, 2019).

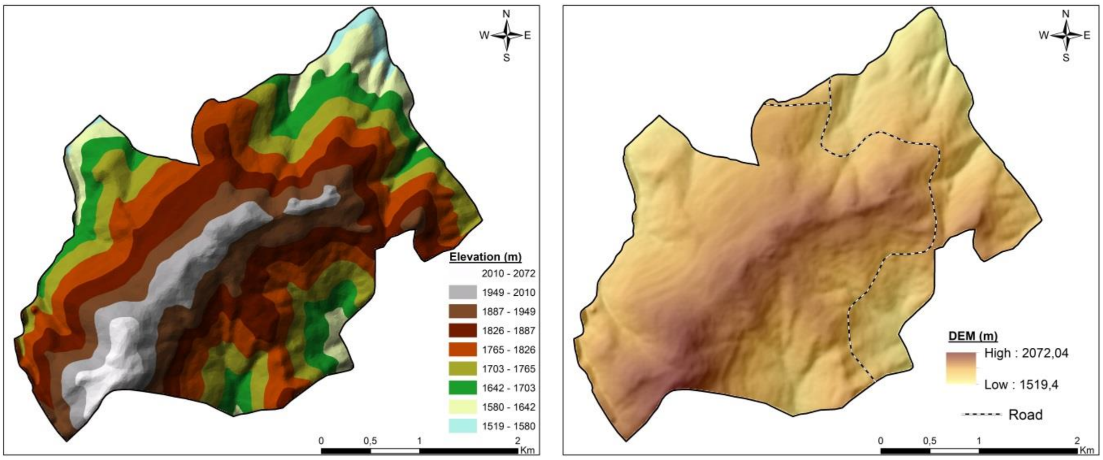

Figure 2. DEM and elevation maps of the study area

Soil sampling and analysis

A total of 151 soil samples were collected from a depth of 0-20 cm within the study area (Figure 3).

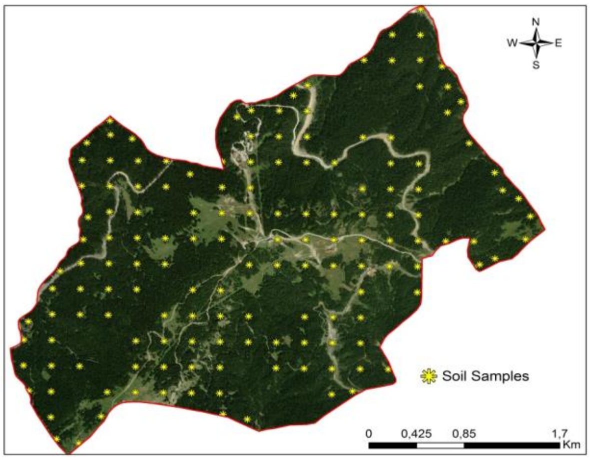

Figure 3. Soil samples patter of the study area

Collected soil samples brought from the field to the laboratory separated from stone and gravel after air dry and they were made ready for analysis by sieving with 2 mm sieve and some physical and chemical analyses were performed. Sand, silt and clay percentages of soils were determined by Bouyoucos hydrometer method (Bouyoucos, 1962), and bulk density was determined with the help of cylinders with a volume of 100 cm^3 in undisturbed soil samples. Hydraulic conductivity was measured using a hydraulic permeable set (Klute and Dirksen, 1986). The electrical conductivity (EC) and pH of soils were determined in 1:1 soil-water suspensions. Lime ($CaCO_3$) content was calculated with volumetric calcimeter method (Soil Survey Staff, 1992), and organic matter (OM) was calculated using the modified Walkley-Black method (Soil Survey Field and Laboratory Methods Manual, 2014).

Soil erodibility parameters

Aggregate Stability Index (ASI): Aggregate stability index was determined according to wet sieving method using Yoder type sieving set (Kemper and Rosenau, 1986).

$$\text{ASI}\ (\%) = \frac{(\text{Weight of soil} + \text{sand}) - (\text{Weight of sand})}{\text{weight of sample}} \times 100 \qquad (1)$$

Structural stability index (SSI): It was calculated by subtracting total silt + clay value measured in suspension without being dispersed from the total silt + clay value measured by mechanical analysis (Eq. 2). Soils with a SSI value less than 40% are considered susceptible to erosion. As the SSI values of soils increase, their erodibility decreases (Lal and Elliot, 1994).

$$SSI\ (\%) = \Sigma b - \Sigma a \quad (2)$$

Where; a: silt plus clay content measured in suspension with no calgon agent (%), b: silt plus clay content measured by mechanical analysis with calgon agent (%)

Crust Formation Index (CFI):

$$CFI = \text{Organic matter } (\%) \times 100 / \text{Clay } (\%) + \text{Silt } (\%) \quad (3)$$

Another important indicator of physical degradation of soils, the soil crust index, a state of sensitivity to crust formation, was determined by the following Formula (Eq. 3) (Pieri, 1989). It was given class of CFI in Table 1.

Table 1. Class of the Crust Formation Index

Class	Values	Description
1	CFI < 5	High physical degradation
2	5< CFI <7	Moderate physical degradation
3	7< CFI < 9	Low physical degradation
4	CFI > 9	No physical degradation

Interpolation analyses and descriptive statistic

In this study, different interpolation methods (Inverse Distance Weighing-IDW with the weights of 1, 2, 3 and radial basis function-RBF with thin plate spline (TPS), simple kriging (OK) with spherical, exponential and gaussian variograms, ordinary kriging (OK) with spherical, exponential and gaussian variograms, universal kriging (OK) with spherical, exponential and gaussian variograms) were applied for predicting the spatial distribution of soil quality index criteria with ArcGIS 10.2.2.

In the present study, root mean square error (RMSE) was used to assess and figure out the most suitable interpolation model. That's why, the lowest RMSE indicates the most accurate prediction. Estimates are determined by using the following formula (Eq. 4):

$$RMSE = \sqrt{\frac{\sum(z_{i^*} - z_i)^2}{n}} \quad (4)$$

where; RMSE: root mean square error, Z_i is the predicted value, Z_{i^*} is the observed value, and n is the number of observations. Descriptive statistics as minimum, maximum, mean, standard deviation, skewness, kurtosis coefficient and coefficients of variation of physico-chemical properties of surface soil samples were calculated.

Results and Discussion

Soil physico-chemical properties and correlation analysis

The sensitivity of soils to erosion is due to differences of their physical and chemical properties which affect soil erosion. In many studies conducted by some researchers, it has been reported that the texture, structure, hydraulic conductivity, organic matter content are the most important soil properties which affects soil erodibility (Imani et al., 2014; Yakupoğlu et al., 2017; Celilov and Dengiz, 2019). A total of 151 soil samples were taken at the research site. In these samples, twelve different physical and chemical properties were examined. The Normal distribution is a symmetric distribution. The degree of distortion of symmetry in the Normal distribution is called skewness. The distribution is called right (positive) skewness if it is long-tailed to the right and left (negative) skewness if it is long-tailed to the left. The degree of tapering or roundness of the normal distribution curve is called kurtosis (Yıldız et al., 1999; Saygın et al., 2019). Results of some descriptive statistics features of soils are given in Table 2. In Table 2, the skewness values of clay, silt, sand, BD, SSI and pH showed normal distribution, while other properties were determined to be far from normal distribution. ASI that is away from the Normal distribution has a negative (left) skewness, while other properties that showed non normal distribution are a positive (right) skewness.

Many researchers accept coefficient of variability as an important indicator to explain changes of soil properties and classify it as low (<15%), medium (15-35%) and high (>35%) according to the values it receives (Wilding, 1985; Mallants et al., 1996; Çelik and Dengiz, 2018; Aydın and Dengiz, 2019). In this case, clay, silt, sand, ASI, SSI and CFI have high variability in soil samples taken from the research area, OM and $CaCO_3$ have medium variability, and other soil characteristics have low variability. Similar results were obtained as a result of research conducted by Özyazici et al. (2016). According to the their study, it was reported that all physical and chemical soil properties except for (pH and silt content) have high variability, and the most variable soil property is $CaCO_3$. The amount of organic matter in soils varies between 0.37% and 21.42 %. According to the classification reported by Ülgen and Yurtsever (1995), it was determined that soils contain an amount of organic matter ranging from less to more. In addition, it was determined that the $CaCO_3$ content of soil samples taken from the research area had the highest coefficient of variability in chemical properties. It was determined that the lime content of soils ranged between 0.8% and 44.1 %. According to Ülgen and Yurtsever (1995) classification, soils are distributed between less calcareous and more calcareous in terms of their lime content. EC values of research soils vary between 0.047 and 0.118 dS m^{-1} and there is no any salinity problem in the study area while the pH values of soils range from 4.09 to 7.38 which can be called ranging from strong acid to slightly alkaline soil.

Table 2. Descriptive statistics of some erodibility factors and phsico-chemical properties of soil sample.

Criteria	Mean	SD	CV	Variance	Min.	Max.	Skewness	Kurtosis
Clay (%)	20,62	8,59	41,37	73,88	4,08	45,45	0,35	-0,09
Silt (%)	25,76	6,04	37,69	36,53	8,69	46,38	0,43	0,38
Sand (%)	53,33	12,28	64,81	150,82	14,59	79,40	-0,14	-0,34
pH	5,72	0,77	3,29	0,60	4,09	7,38	-0,07	-0,74
EC (dS/m)	0,27	0,18	1,14	0,03	0,05	1,19	1,60	3,71
$CaCO_3$ (%)	2,14	4,76	33,99	22,73	0,11	34,10	5,40	31,00
OM	6,04	3,35	21,05	11,26	0,37	21,42	1,06	2,32
BD	1,28	0,14	0,60	0,02	0,99	1,59	0,10	-1,00
HC	3,59	2,88	14,75	8,33	0,18	14,93	1,54	2,69
ASI	57,57	14,40	66,22	207,41	16,52	82,74	-0,61	-0,27
SSI	27,48	8,54	41,10	73,03	8,47	49,57	0,18	-0,34
CFI	13,55	7,95	35,50	63,30	1,48	36,98	1,01	0,79

OM: Organic matter, EC: Electrical Conductivity, HC: Hydraulic Conductivity, AS: Aggregate Stability, SSI: Structure stability index,, CF: Crust Formation, SD: standard deviation, CV: coefficient of variation, Min: Minimum, Max: Maximum

As for the changing in physical properties of soils, it was determined that sand, clay and silt content of soils of the study area varied between 14.59-79.40%, 4.08-45.45% and 8.69-46.38%, respectively. Texture classes of soil samples were generally determined as clay, clay loam, loam, loamy sand, sandy clay loam and sandy loam. Besides, bulk density values of soils range from 0.99-1.59 gr cm^{-3}. This high variation of bulk density resulted from textural changing and organic matter content. Finally, when looking at the changing of the soil erodibility factors which are ASI, SSI and CFI, it was found that values of ASI, SSI and CFI are 16.52-82.74%, 8.47-49.57% and 1.48-36.98%, respectively. Stanchi et al. (2015) stated that a relationship between soil erodibility and aggregation should therefore be expected. However, erosion may limit the development of soil structure; hence aggregates should not only be related to erodibility but also partially mirror soil erosion rates. Therefore, it can be said that the higher the aggregate ratio of soils, the more resistant the soil is to erosion.

Interpolation models and distribution maps of erosion sensitivity parameters

Determining the spatial changing pattern of any soil property using interpolation models allows to estimate the value of the studied soil property at any point in the study area with minimal errors. Thus, distribution maps obtained as a result of interpolation analysis of soil characteristics allow the most appropriate planning and management decisions related to land management to be taken and implemented for the study area (Arslan, 2014; Özyazıcı et al., 2015; Gülser et al., 2016; Alaboz et al., 2020). RMSE values of 15 interpolation models were obtained in order to create distribution maps of the selected soil erodibility parameters and their values have been given in Table 6. IDW-2 with the lowest RMSE value for SSI (7.9195) was determined as the most appropriate model in terms of distribution mapping, while IDW-1 model for ASI with a RMSE value (12.7548) was determined to be the most suitable model. In addition, the Gaussian model of simple Kriging with the lowest RMSE value (7.3754) for CFI has been determined to be the most appropriate model in terms of distribution map creation.

In planning for soil and water conservation, it is necessary to know the resistance of the soil to changing the structural continuity of the soil and its tendency to erosion. Many erosion sensitivity indices have been developed for this purpose. One of these erosion sensitivity indices is the aggregate stability index.

Aggregate distributions and stability measurements of soils are considered a quality indicator of soils (Six et al. 2000), as well as aggregate stability measurements are considered as an important indicator in determining the resistance of soil aggregates to environmental factors that cause degradation (Hillel, 1982). Aggregate stability values were found to be between 16.52% and 82.74% in soil samples taken from study area. The mean aggregate stability value of the research area was found 57.57 %. Furthermore, when the aggregate stability values of soil samples of the study area were examined, it was determined that almost half of soil samples had more than 60% aggregates stability value. Considering the frequency distribution and statistical information of values in the creation of distribution maps for aggregate and stock stability, it was evaluated in 10 (ASI) and 5 (SSI) classes using the Natural Breaks (Jenks) method by means of GIS. This methodologic approach is used in cases where data is not evenly distributed, there are large differences between values, and differences between classes should be given prominently. Aggregate stability distribution map was given in Figure 4. According to Figure 4, particularly centre and south west part of the study area has more than 56% aggregate stability values whereas aggregate stability values is increasing in south east part of the study area. The use of aggregate stability to estimate soil sensitivity to erosion has been proposed by various researchers (Le Bissonnais et al., 1989; Barthès and Roose, 2002; Stanchi et al. 2015). Kanar and Dengiz (2015) carried out a research to the determination of the relationship between land use/land cover and some erodibility indices in Madendere Watershed soils after taking from surface (0-20 cm) soil samples based on grit system. They reported that small part of the study area has less than 20% aggregate stability index value which was generally located on agriculture lands. On the other hand, the highest aggregate stability index value was determined under forest lands. Another of the erosion sensitivity parameters is the structure stability index of soils, and there is no limit value for this ratio. Structural stability index (SSI) by the sum of the difference between mechanical and aggregate analyses of silt plus clay fractions was introduced as a rapid technique for estimating structural stability of soils (Leo, 1963; Özdemir and Gülser, 2017). In general, as the SSI value decreases, the degree of erosion resistance of soils also decreases. When looking at the Figure 4, distribution map of the SSI pattern shows parallel trend with map of ASI. İmamoğlu and Dengiz (2020) performed a research to determination of relationship between situation of soil erosion sensitivity using SSI and land use/land cover in two adjacent micro catchments called Ilıcak and Kum Çay located in Gediz Basin soils. In this study, it was determined that the lowest SSI of the study area was found on agriculture lands whereas the highest SSI values located on the pasture and forest land in the Basin. As for crust formation, Öztürk and Özdemir (2006) stated that some practices to take under control the crusting, increase the seedling emergence, improve the aggregation, increase the resistance of soil aggregate, and control the erosion are these; soil organic matter management, use of soil surface covers, the application of amendments and improve the irrigation management. In addition, İmamoğlu et al. (2018) reported that crust layer formation is not only related to the structure, but also the dispersion rate and aggregate stability values of factors that accelerate erosion also affect crust formation. According to CFI class in Table 1, less than 5 and between 5 and 7 values of CFI mean highly and moderately physical degradation and this case was also found at the same areas which located on south-east part of the study area, when compared SSI and ASI maps. On the other hand, most part of the study area has low or no physical degradation.

Table 6. Cross validation according to different interpolation models

Interpolation Models	Semivariogram models		Soil erodibility parameters		
			SSI	ASI	CFI
Inverse Distance Weighing-IDW	IDW -1		7,9854	12,7548	7,4008
	IDW -2		7,9195	12,7648	7,5227
	IDW -3		8,0102	12,9599	7,7297
Radial Basis Function-RBF	TPS		9,1378	15,4610	9,2049
	CRS		7,9605	12,7989	7,5152
	SWT		7,9575	12,7752	7,4768
Kriging	Ordinary	Gaussian	7,9755	12,7915	7,3970
		Exponential	7,9305	12,8217	7,5114
		Spherical	8,0014	12,7920	7,4438
	Simple	Gaussian	8,0146	12,8227	7,3754
		Exponential	7,9853	12,8719	7,4911
		Spherical	7,9722	12,8299	7,4228
	Universal	Gaussian	7,9755	12,7915	7,3970
		Exponential	7,9305	12,8217	7,5114
		Spherical	8,0014	12,7920	7,4438

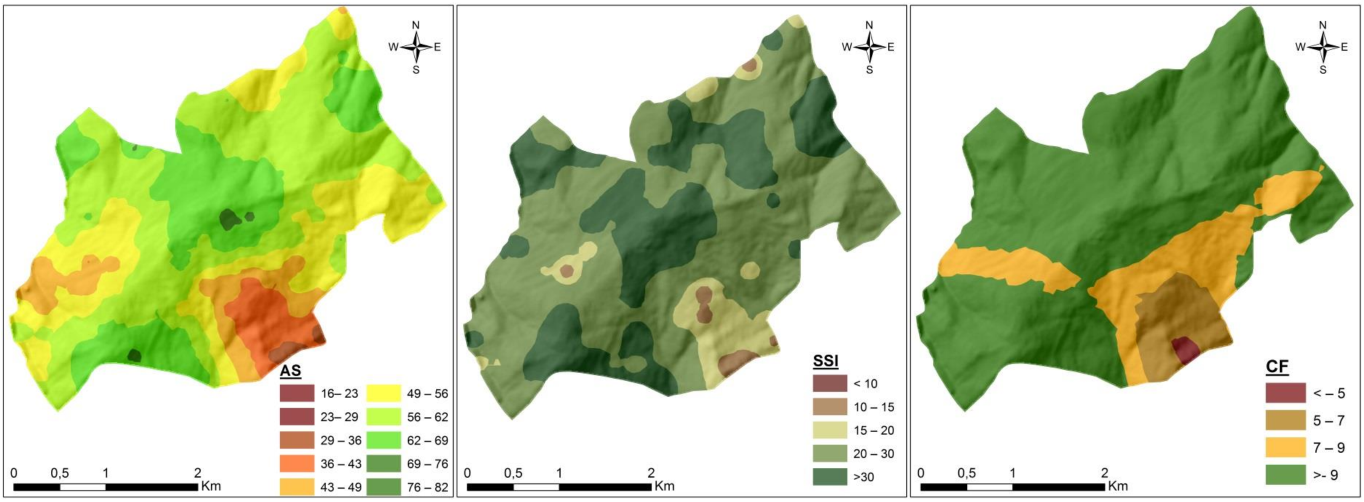

Figure 4. Distribution maps of the AS, SSI and CF in the study area

Correlation analysis between erosion sensitivity parameters and soil properties

Kolmogorov-Smirnov (K-S) test was applied to determine whether soil parameters showed normal distribution. As a result of the K-S test, it was found that not all parameters showed normal distribution. For this reason, Spearman correlation was applied to reveal the correlation relationship of the data. As a result of the study, 18 correlation pairs were found as statistically significant ($p<0.05$; $p<0.01$) and results were given in Table 7. A positive relationship was found between AS and EC (0.460**) and OM (0.603**) at the 1% importance level whereas, a negative relationship was found between BD (-0.544**) at the 1% importance level. There are many studies in the literature on this subject that show change in OM level due to change in land use (Chan, 2001; Neufeldt et al., 2002; Dengiz, 2007) and increasing soil erosion due to diminishing OM content (Celik, 2005; Cerda and Doerr, 2007; Yilmaz et al., 2008). On the other hand, effect of total OM on aggregation was defined in many studies, but in some cases, the origin of organic matter and dominant clay mineralogy rather than total quantity play a role in aggregation. A positive relationship was also found between SSI values and EC (0.418**) and OM (0.565**) at a 1% significance level, and a negative relationship was found at a 1% significance level with BD (-0.542**). Moreover, a positive relationship was found between CF and EC (0.523**), OM (0.894**) and sand (0.345**) at a 1% importance level, and a negative relationship was found at a 1% importance level with clay (-0.376**) and BD (-0.811**).

Table 7. Analysis results of correlation between erosion sensitivity parameters and some physical and chemical properties of soils

Soil parameters	Erodibility factors		
	ASI	SSI	CFI
pH	0,057	-0,015	-0,001
EC (dS/m)	0,460**	0,418**	0,523**
OM (%)	0,603**	0,565**	0,894**
$CaCO_3$ (%)	0,147	0,053	0,132
Clay (%)	0,144	0,121	-0,376**
Silt (%)	0,159	0,137	-0,146
Sand (%)	-0,139	-0,123	0,345**
BD (gr/cm^3)	-0,544**	-0,542**	-0,811**
HC mm/h	0,131	0,141	0,679**

*: $p<0,05$; **: $p<0,01$

Conclusion

In this present study, the determination of some erodability factors such as ASI, SSI and CFI of soils distributed within the Ilgaz National Park area and its relationship with some other soil properties were examined. In addition, the distribution maps of the sensitivity some factors were produced using different spatial distribution interpolation models. In this case, IDW-2, IDW-1 and Gaussian model of simple Kriging were determined the most semivariogram model for SSI, ASI and CFI, respectively. According to three erodobility factors, it was found that some south east part of the study area has sensitive for erosion risk and physical degradation. Therefore, this side of the study area should be taken some measurement to protect from soil erosion and physical degradation. In addition to being possible by taking measures to increase the scope of organic matter and hydraulic permeability of the soil and improve its structure, the vegetation on it is not destroyed.

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Eurasian Journal of Soil Science 2022, 11(1), 10 - 16

Eurasian Journal of Soil Science

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

Identification of humic substances on the transformation of an organic substrate

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Article Info

Received : 08.02.2021
Accepted : 20.07.2021
Available online : 25.07.2021

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Abstract

The organic matter of the soil and humus are heterogeneous in composition. That is why the nature of soil organic matter has not yet been fully understood and causes many discussions. The purpose of this work is to study the identification features of humus in the process of peat's transformation. The results of a five-year experiment studying the processes of organic matter transformation in organic-mineral substrate based on peat growing coniferous seedlings in greenhouses are presented. In the dynamics of the group and fractional composition of humus for several years the biochemical essence of the humification process is revealed. To extract specific humic substances from the organogenic substrate, we used the method of fractioning humus into groups and fractions, which is a stepwise sequential extraction using solutions of sodium hydroxide and sulfuric acid. It was found that the organic matter of the substrate passes through three stages of transformation: hydrolysis of organic products → initial transformation → humification of lignin structures. There is a significant change in the ratio of the three main groups of humus in favor of humic acids at the third stage. In general, the system is tends to its most stable state: aromatic structures are copolymerized, the core of humic substances is densified, and humus gradually "matures".

Keywords: Fulvic acid, humic acid, humus formation, peat.

Introduction

The organic matter of the soil and humus are heterogeneous in composition. That is predetermined by their chemical properties, the degree of organic residues transformation, the nature of their transformations, and also due to the connection of humic compounds with each other and with the mineral part of the soil. Humic acids (HA) are known to be the most specific part of humus. The amphiphilic nature of humic acids determines the formation of micelle-like structures. This property of HAs in neutral and acidic conditions determines the protective properties of soil organic matter, binding pollutants and preventing their entry into adjacent environments (Sannino and Piccolo, 2013). The composition of soil organic matter and its properties cause intense interest of scientists (Sbih et al., 2012; Hristov and Filcheva, 2017; Shahin and Khater, 2020). Its study is possible from different perspectives, so various approaches were developed at different times and in different countries to solve this very difficult task.

In particular, the method of Tyurin (1951) was widely used in the USSR. This method involved the fractionation of soil organic matter into groups by the relation to solvents and into fractions by the connection of organic molecules with the mineral part of the soil. The most famous adaptation of Tyurin's method became modification of Ponomareva and Plotnikova (1980).

doi : https://doi.org/10.18393/ejss.974224
: http://ejss.fesss.org/10.18393/ejss.974224
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

The principles underlying the method have been very productive, proof of which is the establishment with its help of the geographical patterns of humus formation. Studies (Orlov et al., 2004) have shown, that the degree and depth of organic matter humification in the upper humus horizon, established according to the number and ratio of the main humus groups in its composition, closely correlated with the duration of the period of biological activity (PBA). Modern studies using this method show that variations in the humus composition, ceteris paribus, can be caused not only by zonal differences in climate, but also by the relief features (Dubovik and Cherkasov, 2013). Even such a factor as location on slopes of different exposure affects the composition of humus. The authors showed that "humid climate of northern slopes leads to formation of brown type of humic acids (HA), while the conditions of eastern slopes assist to formation of gray HA". Moreover, for example: "Cambisols are characterized by dominance of fulvic acids (FA) above HA" regardless of the location: whether it is Jiguli Ridges in Samara Region of Russia (Abakumov et al., 2009) or Caucasus mountains of the Republic of Armenia (Kroyan, 2018).

The presence of such regularities disproves the postulate that humic substances are just a complex mixture of identifiable biopolymers (Lehmann et al., 2008; Lehmann and Kleber, 2015). The specificity of humic substances acts here as a sign of identification of the soil state, which an ordinary mixture of organic compounds just cannot do. More convincing is the concept that HSs have a supramolecular structure (Piccolo, 2001; 2002). According to Piccolo, this concept of HS structure gives a better insight into the key contribution of HSs to ensuring and maintaining the physical and chemical properties of soils and their reactivity to pesticides and other environmental pollutants. Later on Nebbioso and Piccolo (2011; 2012) with the help of the fractionation and chromatography of HSs, found that hydrophobic compounds are mainly distributed in the coarsest fraction, while hydrophilic components are eluted in the finest fraction. The proportions of these molecular components determine the hydrophobicity or hydrophilicity of HSs. This hypothesis is consistent with the hydrophobic properties of HA nucleus and the hydrophilicity of peripheral chains (Orlov, 1992). In fact, humus compounds are a humus matrix of complex multilevel organization (Fedotov and Dobrovolskiy, 2012). It was also found that the supramolecular organization of HAs in Chernozems and Kastanozems can be described as a spatial structure composed of 2–4 layers of condensed aromatic systems supplemented by a network of chain fragments with different degrees of regularity and lengths (Bezuglova, 2019). The purpose of this work is to study the identification features of humus in the process of long-term transformation through successive stages of the extraction of specific humic substances from a typical organogenic substrate (greenhouse soils) using alkaline and acid reagents.

Material and Methods

Materials

The object of research was an organic-mineral substrate based on peat, which is used in the system of the protected ground when growing plants in greenhouses. The main part of its composition is the peat mined by milling process. It has a favorable air-water regime for plants, and it is a good antiseptic. Peat was preliminarily neutralized by lime until a neutral medium reaction and was saturated with nutrients by adding mineral fertilizers according to the technology of preparing greenhouse soils (Lapin and Nollendorf, 1975). The transformation of the substrate used for growing coniferous seedlings is considered in this work. The initial characteristics of the substrate and its biochemical and agrochemical parameters were performed in accordance with State Standard 27753.0-88 (Greenhouse soils. General requirements for methods of analysis), are presented in Table 1.

Table 1 The five-year dynamics of organomineral substrates' agrochemical indicators in greenhouse conditions

Agrochemical indicators	The term of the substrate use, years			
	Initial	1	3	5
Ash content, %	2,2 ± 0,2	5,8 ± 0,6	10,2 ± 0,7	41,7 ± 0,7
C, %	44,2 ± 0,7	36,7 ± 0,5	29,6 ± 0,3	19,5 ± 0,3
N, %	1,01	0,95	0,93	0,90
P_2O_5, %	0,07	0,16	0,17	0,18
C/N	41,70	38,60	31,8	21,70
$N\text{-}NH_4$, mM(+)/100 g	17,5 ± 5,2	12,5 ± 2,3	8,7 ± 1,7	11,8 ± 2,7
$N\text{-}NO_3$, mM(+)/100 g	2,80	5,20	3,10	5,30
N mineral, mM(+)/100 g	20,30	17,70	11,80	17,10
P_2O_5, mM(+)/100 g	16,20	16,20	24,90	61,70
K_2O, mM(+)/100 g	29,00	298,00	111,00	30,00
The total amount of exchangeable bases, mM(+)/100 g	1,12	6,12	6,00	6,80
Hydrolytic acidity, mM(+)/100 g	9,33	4,26	3,91	2,34
Base saturation, %	10,70	58,90	60,50	74,40

± – the standard deviation (SD)

Fractionation of humic substances

To extract specific humic substances from the organogenic substrate, the method of fractionation of the group and fractional composition of humus was proposed by Tyurin and modified by Ponomareva and Plotnikova (1980). This method is a step-sequential extraction using dilute solutions of sodium hydroxide and sulfuric acid. The analysis scheme of the humus composition according to Ponomareva and Plotnikova (1980) provides for the determination of three groups of humus: humic acids (HA), fulvic acids (FA) and humins (Hm) – non-hydrolyzable residue. Humic acids are extracted from the soil with 0.1 N sodium hydroxide solution. The combination of soil treatment with an alkaline solution with other operations allows us to subdivide humic acids into three fractions according to their relationship with the mineral part of the soil. Free and associated with mobile sesquioxides humic compounds (HA-1) are extracted directly from the soil in its natural state. Humic acids associated with exchange calcium and magnesium (HA-2) are extracted with an alkaline solution from decalcified soil, previously destroying salts of divalent cations by treating the soil with a 0.1 N sulfuric acid solution. Humic acids associated with stable forms of iron and aluminum oxides and clay minerals (HA-3) are extracted from soil already devoid of calcium and magnesium humates using a 0.02N solution of NaOH, after being subjected to 6-hour alkaline hydrolysis in aqueous bath.Fulvic acids, in turn, are divided into 4 fractions. One fraction, represented by free fulvic acids (FA-1a), is extracted from the soil using 0.1N solution of H_2SO_4. Three fractions of fulvic acids, which are in polymer bonds with the humic acids of the corresponding fractions and the mineral components of these fractions, are extracted together with humic acids and then the HA is separated by its precipitating from an alkaline solution using sulfuric acid. Fulvic acids remain in solution. The non-hydrolyzable residue (humine) is found by the difference between the total content of organic carbon in the soil and the sum of the organic carbon of all fractions. It characterizes the strength of fixing humic substances by the mineral part of the soil. However, compounds having a weak degree of humification of organic matter, such as those that are in peat, forest litter, etc., also get in this fraction. The processing of the experimental results was carried out in accordance with the method of field experiment (Dospekhov, 1985) and with the help of Statgraphics Plus software for Windows (Matveeva and Valeeva, 2012).

Results

Change in soil-agrochemical indicators of soil

The dynamics of changes of the main agrochemical and biochemical parameters in the process of using the substrate are presented in Table 1. The content of basic nutrients in the process of using the substrate changed slightly and was at the optimum level to provide the cultivated plants.

The total amount of exchangeable bases increased from 6.12 (in the first year) to 6.80 mM(+)/100 g. (by the fifth year). The degree of saturation with bases also increased (from 58.9 to mM(+)/100 g.), in parallel, hydrolytic acidity significantly decreased – from 4.26 to 2.34 mM(+)/100 g. All this indicates significant improvement in soil quality by agrochemical indicators and characterizes the intensive transformation of organic matter from an organic substrate into organic-mineral soil.

This demonstrates that the transformation of the substrate in the system of estimated indicators over a period of 5 years has a continuous progressive character. Thus, two processes are observed: the process of mineralization of organic matter, which is accompanied by an increase in ash content from 2.2% to 41.7% (Figure 1), and a decrease in the total carbon content from 44.2% to 19.5% (Figure 2 (C, %)). The change in the ratio of carbon to nitrogen by the fifth year of the experiment is fixed at C / N = 21.7. This indicates directed optimization of the balance between carbon and nitrogen (Figure 2 (N, %)), which is not so much characteristic of a mixture of mineralized substrate as a self-regulating system, which is characteristic of highly productive soils. Thus, the transformation of the substrate is aimed at changing the ratio of the mineral and organic parts to the level of ratios close to the natural soil.

It is known (Gmurman, 2004) that the correlation coefficient (r) serves as a mathematical measure of the relationship between two random variables. Evaluating the statistically significant (P=95%) relationship between the main indicators that determine the transformation of the substrate (Ash content, total C, total N, one can observe a fairly strong relationship between the estimated indicators. For Ash content – total C and for Ash content – total N, there is a strong negative correlation (r = - 0.91 and r = - 0.80, respectively). At the same time, a very strong positive correlation is observed between total C and total N content (r = 0.96).

Summing up the analysis of the quality of the substrate according to the main agrochemical indicators, it can be noted that the nutritional qualities of the substrate during its use did not deteriorate but improved.

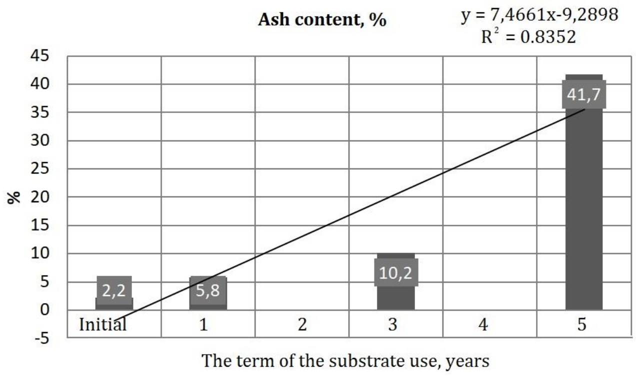

Figure 1. Dynamics of ash content over time, %

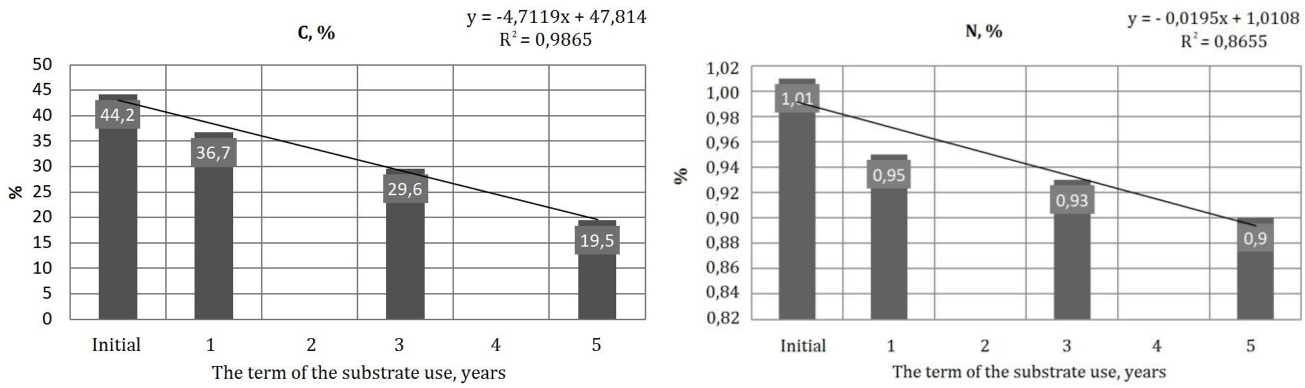

Figure 2. Dynamics of total carbon (C, %) and total nitrogen (N, %) content over time,%

Change in the quality state of humic substances

Agrochemical indicators cannot serve as a universal criterion for assessing the suitability of a substrate for plants growing. The criterion for assessing the suitability of peat grounds for their use in the form of a nutrient substrate in the conditions of protected ground can be an assessment of the specifics of the formation of humic substances that regulate plant growth and development. For this purpose, the dynamics of the group and fractional composition of humic substances formed during the transformation of organic-mineral substrate for several years was evaluated. We were interested in this criterion as evidence of the specificity of humus formation, during which a special class of organic substances that can fulfill the communication-regulatory role in the functioning of plants is gradually formed.

Table 2 presents the data on changes in the group and fractional composition of the humic substances of the organic-mineral substrate in the process of its use. They indicate a significant increase of all fractions of humic acids, especially the first fraction (brown HA), with an increase in the period of the substrate use. Statistical processing of the experimental results has shown, that the dynamics of various fractions of humic substances changes in the substrate can be described by a simple linear function. A high level of reliable approximation is noted for the total accumulation of humic acids (R^2 = 0.76) and especially the HA-2 fraction (R^2 = 0.94). Somewhat lower (R^2 = 0.60-0.61) was the approximation for HA-1 and HA-3 fractions. For the fractions of fulvic acids, an increasing linear function was noted for the FA-1a (R^2 = 0.74) and FA-1 (R^2 = 0.82) fractions. For the FA-2 (R^2 = 0.56) and FA-3 (R^2 = 0.78) fractions, a decrease in the accumulation was noted. Thus, the total accumulation of fulvic acid fractions had an unreliable level of approximation (R^2 = 0.43). The most significant statistical indicators (R^2 = 0.99) are noted for a decrease in the total carbon content and the proportion of non-hydrolyzable residue (R^2 = 0.71), which can be explained by the mineralization of the substrate.

It was revealed that in the process of transformation of the organic-mineral substrate, coupled processes of both mineralization and humification of the source material are observed. These processes involve not only labile, easily hydrolyzable components of organic matter, but also a non-hydrolyzable residue (represented mainly by lignin), as evidenced by a threefold decrease in its participation in the composition of organic matter in the fifth year of the study. This result is in good agreement with the concept of humification, that is unambiguously described as the progressive accumulation of hydrophobic molecular components (Piccolo et al,, 2019).

Table 2. Changes in the group and fractional composition of humic substances of the substrate in the process of its use (% of total carbon)

Years	Corg, %	% of the soil Corg.										$\frac{C_{HA}}{C_{FA}}$	$\frac{C_{HA}+C_{FA}}{C_{NHR}}$
		Humic Acids				Fulvic Acids					Hm		
		1	2	3	Total	1a	1	2	3	Total			
0	44.23	12.65	0.41	17.57	30.36	0.41	8.46	4.18	15.28	28.33	41.31	1.07	1.42
1	36.73	13.34	1.80	15.30	30.44	0.38	8.66	3.57	12.47	25.07	44.48	1.21	1.25
3	29.60	17.71	2.16	15.68	35.55	0.58	10.84	4.02	12.64	28.08	36.37	1.27	1.75
5	19.52	26.02	4.05	23.26	53.33	1.33	16.50	3.07	11.63	32.53	14.14	1.64	6.07
Formula	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9	Y10	Y11		
R^2	0.99	0.61	0,94	0,60	0,76	0,74	0,82	0,56	0,78	0,43	0,71		

Linear function: Y1=-7,9x+52.5 Y2=3,242x+10,32 Y3=1,128x-0,715 Y4=-0,945x+18,073 Y5=2,595x+26,927
Y6=0,296x-0,065 Y7=2,63x+4,54 Y8=-0,288x+4,43 Y9=- 1,078x+15,7 Y10= 1,561x=24,6 Y11=- 8,962x+56,48

Discussion

The dynamics of this process are specific. Modeling the dynamics of the process, we divided it into three stages. The initial stage of the substrate's transformation – the hydrolysis of organic products. At the initial stages of the transformation of the organic substrate (in the first year of the experiment), the non-hydrolyzable residue is not yet involved or is poorly involved in the mineralization process, but it takes an active part in the humification process. Moreover, due to the intensive mineralization of hydrolyzable products, there is a relative increase in the proportion of non-hydrolyzable products in the substrate – from 41.31 to 44.48 %. This first stage of the oxidative-hydrolytic transformation of the organic matter of the substrate we called the "hydrolytic stage" or "hydrolysis".

Hydrolysis attenuation and increase in transformation: beginning of phase the stabilization. At subsequent stages (second and third year of substrate mineralization), non-hydrolyzable products (lignin) are gradually involved in the general transformation process. The proportion of non-hydrolyzable residue at this stage of the transformation decreases from 44.48 % to 36.37 %. This stage, which we called "the beginning of the stabilization phase", is characterized by the attenuation of hydrolytic processes. Attenuation occurs due to a decrease in the proportion of hydrolyzable substrate products with a relative increase in transformation processes associated not only with the loss of mineralizable carbon, but with the redistribution and rearrangement of difficultly hydrolyzable and non-hydrolyzable components of the substrate.

This is confirmed by an increase in the proportion of specific humic substances formed during the transformation of the substrate as well as a change in the distribution of specific fractions of humic acids and fulvic acids. The changes in the content of humic substances in the first year (at the "hydrolysis" stage) were insignificant and associated mainly with a change in the fractional composition of less mature fulvic acids. Moreover, both their total share and the composition of various fractions decreased (except the first fraction).

At this stage of the oxidative-hydrolytic transformation of soil organic matter, a decrease in the proportion of non-hydrolyzable residue begins (by 18.2 %). This occurs due to the involvement of lignin structures in the transformation process. In this case, the proportion of humic substances increases – from 30.44 to 35.55 % of the total carbon (C) or by 14 % for humic acids, and from 25.07 to 28.08 % of the total C or by 11 % for fulvic acids. Moreover, a gradual increase in the share in all fractions of both humic and fulvic acids is noted.

These data confirm once again that the process of humification is a process of oxidative-hydrolytic transformation of organic matter with a phased transformation of lignin structures into specific substances of humus (Alexandrova, 1980).

Humification of lignin structures – stage of transformation. The process of transformation of the non-hydrolyzable residue was especially intense in the subsequent stage (from the third to fifth year of the experiment). This stage, called "transformation", provided a more complete involvement of lignin structures in the humification process. Thus, the proportion of non-hydrolyzable residue decreased from 36.37 to 14.14 % or by 61 %. The proportion of humic substances also increased significantly: for humic acids – from 35.5 to 53.33 % of total C or by 33 %, and for fulvic acids – from 28.08 to 32.53 % of total C or by 14 %.

The characteristic pattern of redistribution of the share of humic substances in fractions is also traced. Their relative increase was observed in all fractions of humic acids. For fulvic acid fractions, a relative increase was recorded only in 1A and 1st fractions, while a relative decrease was observed for the 2nd and 3rd fractions. This once again indicates the nature of the smooth and gradual transition of the stages of organic matter transformation into specific humic compounds, characterizing the "maturation" of humus.

It is known that the degree of humification is estimated as the proportion of humified material (humic acids) in the composition of organic matter. The calculation of the degree of humification, using the Orlov (1992) formula (1), reveals that humification reaches a high degree during the transformation of the substrate.

$$H = [(CHA : SOM) \times 100] \quad (1)$$

The relative change in indicators increases almost 4 times from the initial substrate, where it is 68.6, to the substrate after five years of transformation, where this indicator is already 273.2. According to Orlov (1992), this important indicator shows how fully organic residues are converted into humic substances.

The relative change in the ratio of CHA to CFA during the transformation of the substrate (Table 2) indicates an increase of humic structures in the humus composition. So, if in the initial substrate the CHA:CFA was 1.07, indicating almost equal participation of humic and fulvic acids, finally, by the fifth year, it increased to 1.64, clearly fixing the predominance of humic acids in the composition of humus. It should be noted that different authors proposed different gradations for typing humus according to this ratio (Table 3). The difference in the qualitative composition of organic matter at different stages of transformation is seen especially well if the assessment is carried out according to Alexandrova (1980).

Table 3. Classification of soil organic matter by the ratio of humus groups in its composition

Humus type	According to Orlov	According to Alexandrova	According to Marchik, Efremov
		C_{HA} / C_{FA}	
Fulvate	< 0,5	<0,6	< 0,5
Humate-fulvate	0,5—1,0	0,6—0,8	0,5—1,0
Fulvate-Humate	1,0—2,0	0,8—1,2	1,0—1,5
Humate	>2,0	>1,2	>1,5

The difference in the composition of organic matter is even clearer if the assessment according to Aliev (1978) takes into account the state of the non-hydrolyzable residue. The ratio (C_{HA} + C_{FA}) / C_{Hm} sharply increases from 1.75 to 6.07 at the third stage when lignin structures are actively involved in the transformation processes. If we consider the significant predominance of HA over FA, we can talk about an increase in the copolymerization of aromatic structures, densification of the core of humic substances and the gradual "maturation" of humus. It is known that organo-mineral substrates of greenhouses in the process of their transformation acquire toxic properties, forcing to replace the soil after 2–3 years of their use. The effect of humic compounds as regulators of the growth and development of plants and microorganisms is widely known (de Melo et al., 2016; Bezuglova et al., 2019). The presented data by a new way reveal the hypothesis of the manifestation of the humic substances physiological activity. The structural and physiological similarity of different fragments of humic substances and natural phytohormones (Pirog et al., 2018) explains the specifics of the change in the physiological reaction of humic substances formed from the original plant residues during their transformation into humus.

At the first stage, the initial humus-forming agents get the properties of humic acids analogues in the process of carboxylation and demethoxylation. The resulting intermediate ligno-humic acids (LHA) had structural and physiological similarities with natural gibberellins. At the second stage, nitrogen was included in the structure of LHA and formed compounds were close to natural auxins. The final stage of humus formation was characterized by saturation of the aromatic matrix with nitrogen, forming structures close to natural cytokinins (Komarov and Komarov, 2017). Specific humic substances formed during the transformation of organic matter in a closed system, such as a greenhouse, can cause a toxic effect on plants due to physiologically active compounds, such as phytohormones, when the concentration rises to a critical level.

Conclusion

Studying the specifics of the formation of humic substances formed during the long-term transformation of an organogenic substrate allows us to answer the questions of what the organic matter of soils and soil-like formations is, and how specific this substance is. In the dynamics of the group and fractional composition of humus for several years, during which the stages of transformation of the organic matter of peat were successively monitored, the biochemical essence of the humification process is revealed. It was found that the substrate organic matter passes through three stages of transformation: hydrolysis of organic products → initial transformation → humification of lignin structures. At the third stage, there is a sharp change in the ratio of the three main groups of humus in favor of humic acids due to a decrease in the amount of lignin structures that made up the bulk of the non-hydrolyzable residue and were not fully humified in the previous stages. In full accordance with the thermodynamic theory of humification (Orlov, 1992), the system tends to its most stable state: aromatic structures are copolymerized, the core of humic substances is densified, and humus gradually "matures".

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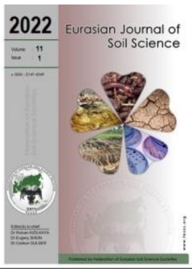
Eurasian Journal of Soil Science 2022, 11(1), 17 - 24

Eurasian Journal of Soil Science

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



Nitrogen and phosphorus leaching and vegetative growth of maize as affected by organic manure application

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Article Info

Received : 03.04.2021

Accepted : 01.08.2021

Available online : 03.08.2021

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Abstract

Maize production in Asia is rapidly increasing. For its sustainable production, the effects of raw and dry manure application on soil water dynamics, nutrient leaching, and plant growth were investigated. Nitrogen (N) and phosphorus (P) concentrations in the percolated water below a 110-cm depth of field-lysimeter columns were analyzed to quantify leaching. Soil water, soil temperature, and plant growth were routinely monitored. The manure application practices increased soil water content by 0.008–0.025 cm^3 cm^{-3} throughout the vegetative period by reducing bulk density and reduced the daytime temperature range by 0.4–1.2°C. The average leaching concentrations of total N increased from 2.6 to 4.7 mg N L^{-1} and available P decreased from 0.12 to 0.04 mg P L^{-1} between 63 and 93 DAS (day after sowing), respectively. The manure treatments did not increase nutrient leaching load at 63 DAS, but at 93 DAS the N load was increased by 219–324 g ha^{-1} and P load by 2.0–3.1 g ha^{-1} compared with the control treatment. The dry manure released a larger amount of N (30.7%) and P (3.2%) in the leachates than the raw manure. The dry and raw manure treatment produced 14.5 and 5 cm taller plants, respectively than the control treatment. Manure application with a slight modification in nutrient management can avoid the nutrient leaching problem.

Keywords: Dry manure, maize root growth, raw manure, soil temperature, soil water conservation.

Introduction

A major challenge for sustainable agriculture is to continue supplying the ever-increasing food demand without deteriorating environmental quality. Agriculture is a major non-point source of nitrogen (N) and phosphorus (P) loss to the environment (Amin et al., 2018). Even in the relatively well-managed Chesapeake Bay catchment in the USA, agricultural land releases 34% of total N and 50% of total P load of the catchment outlet, for instance (CBP, 2016). The nutrient that leached below root zone soils can join shallow aquifers and then pollute nearby surface water through surface water-groundwater interactions, and cause eutrophication of aquatic ecosystems (Norring and Jørgensen, 2009). Nutrients joining groundwater can appear in pumping wells that supply water to households or industries (Giacomoni et al., 2014). Appropriate incorporation of conservation practices can reduce nutrient losses from agricultural fields. Among major crops, maize cultivation exerts comparatively higher nutrient leaching (Amin et al., 2018).

To meet the national food and feed demand, maize production is rapidly increasing in many Asian countries (FAO, 2018). Maize is a dry season crop grown after monsoon season when soil water content starts to deplete steadily because of continuous rainless days. Therefore, the effective use of residual water in the root zone is crucial, especially in the water-scarce areas (Rahman et al., 2015; Huhmann et al., 2017). Organic manure application can help use the residual soil water effectively (Amin et al., 2014; Xia et al., 2017; Eze et al., 2020). A number of studies assessed the effects of these practices on soil water reserve and

: https://doi.org/10.18393/ejss.977955

: http://ejss.fesss.org/10.18393/ejss.977955

Publisher : Federation of Eurasian Soil Science Societies

e-ISSN : 2147-4249

plant growth; however, scientists are still working to explicate these issues (Du et al., 2020; Eze et al., 2020; Jjagwe et al., 2020). Dry or compost animal manure is usually applied to crop fields, but relatively fresh manure may end up in agricultural fields due to storage limitations. How the application of different types of animal manure affects N and P leaching in maize fields is still somewhat unclear (Sainju, 2017; Amin et al., 2018).

Animal manure application increases soil water retention in and around the manure-application slit (Amin et al., 2014; Xia et al., 2017) and improves soil properties (Admas et al., 2015). Land application is a profitable option to handle the huge amount of wastes generated from the expanding animal industries in developing countries (ILMM policy, 2015). However, N and P can slowly mineralize from organic manure and be available for leaching to shallow groundwater during and between the main crop seasons (Amin et al., 2014; Sainju, 2017; Xia et al., 2017; Amin et al., 2018). The overall effects of these practices on agroecology need to be assessed before large-scale implementation in a region. We hypothesized that a better soil water reserve under different types of organic manure application can enhance nutrient transport and leaching in the soil. This study was, therefore, conducted with three specific objectives: (i) to quantify the effectiveness of two manure types on soil water content and soil temperature; (ii) to investigate the impact of the organic manures on N and P leaching; and (iii) to evaluate the impacts of the organic manures on the vegetative growth of maize.

Material and Methods

Study site

A field lysimeter at the Field Irrigation Lab, Bangladesh Agricultural University, Mymensingh, Bangladesh (24°55´ to 25°55´ N and 90°10´ to 90°30´ E at 18 m above the mean sea level) was used for this experiment. The study location is situated in the Old Brahmaputra Alluvial Floodplain having non-calcareous dark gray floodplain soil. The local climate is sub-tropic with summer-dominant rainfall mostly concentrated over April to October, but November to March is dry (Ali et al., 2007). Daily weather data were collected from an on-site weather station. Rainfall started after a month of sowing and then had an increasing frequency in the later part of the experiment (Figure 1). The wind speed increased, whereas sunshine hour decreased due to frequent cloud formation in the later part. The daily mean temperature during the study period ranged from 20 to 32.4°C, and the mean relative humidity fluctuated between 57 and 95% during the growing period (Figure 1).

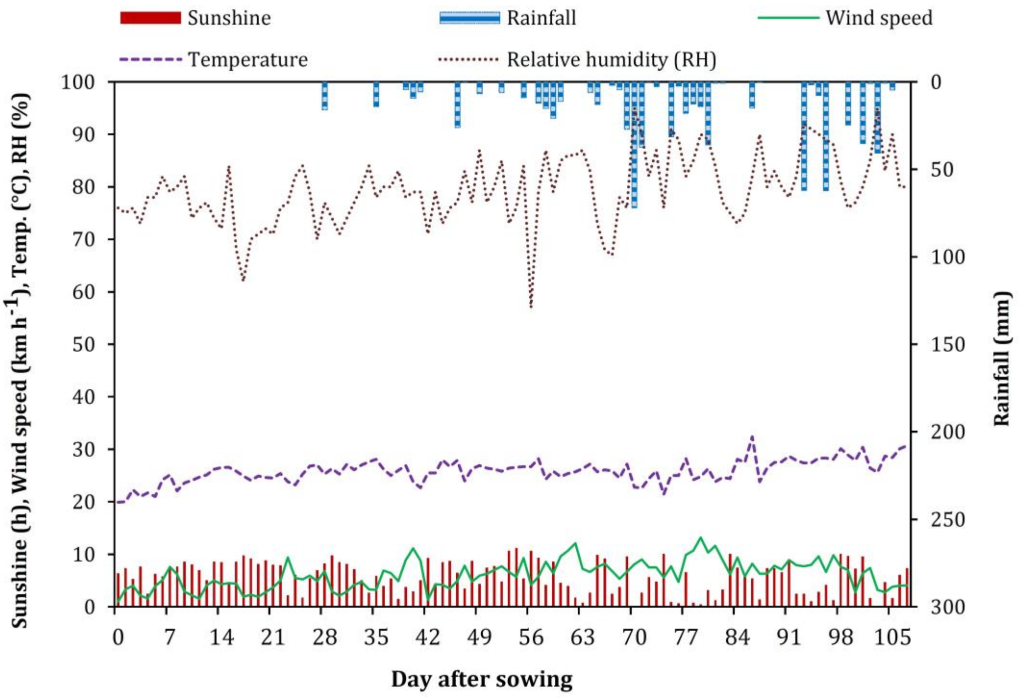

Figure 1. Daily mean temperature, rainfall, sunshine hours, wind speed, and relative humidity during the study period

Lysimeter

The dimension of each soil column of the lysimeter is 1.2 m × 1.2 m × 1.1 m (Figure 2). A 1-m buffer zone separated the columns from each other. A perforated pipe covered with envelope materials was built-in at the bottom of the soil column to collect percolated water and leachate samples. This lysimeter is suitable to measure evapotranspiration, crop-water requirement, water percolation, and chemical leaching in the soil profile (Xue et al., 2013). A test before the experiment confirmed that each of the soil columns was hydraulically isolated from the surrounding soil. Locally available perennial grass was grown in the previous three years so that the soil columns represent local field conditions. Soil samples collected from the lysimeter columns were analyzed for some selected physicochemical properties. The soil was silt loam (sand 42%, silt 49%, and clay 10%) with pH 6.49, electrical conductivity of 131.6 µS cm^{-1}, organic matter of 1.1%, bulk density of 1.33 g cm^{-3}, field capacity of 38.2%. The soil was low in nutrient content, i.e., TN (0.63 g kg^{-1}), P (0.15 ppm) and K (12.6 ppm).

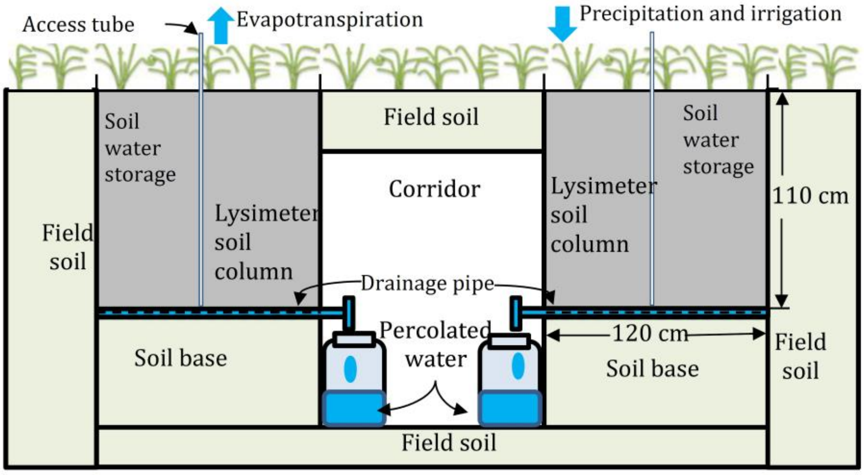

Figure 2. Cross-sectional view of a soil column in the lysimeter

Manure treatments

The effects of manure application on the soil water and temperature dynamics, nutrient leaching, and vegetative growth of maize were investigated. The following manure application treatments with three replications of each were established: (i) no-manure (manure not applied), (ii) raw manure (one-week-old cow dung applied at 110 t ha^{-1}), and (iii) dry manure (cow manure stored in an open pit for six months applied at 60 t ha^{-1}). The raw manure had 16.8% dry matter (3.0±0.3 kg N t^{-1}, 0.7±0.06 kg P t^{-1}, 2.5±0.22 kg K t^{-1}) and the dry manure had 30.8% dry matter (5.0±0.6 kg N t^{-1}, 1.5±0.16 kg P t^{-1}, 2.3±0.19 kg K t^{-1}); as a result, both treatments received an equal amount of manure dry matter and N. Land was prepared before the surface application of the manure and then the manure was mixed with the top 15-cm soil.

Agronomic management

Inorganic fertilizer was applied according to the recommended dose (FRG, 2012): phosphorus (110 kg P ha^{-1}) as triple superphosphate, potassium (120 kg K ha^{-1}) as muriate of potash, sulfur as gypsum (15 kg ha^{-1}), and zinc as zinc sulfate (5 kg ha^{-1}) only once before sowing. Nitrogen as urea was applied thrice (total 240 kg N ha^{-1}); one basal before sowing and two side-dresses at 35 and 60 days after sowing (DAS). On 18 February 2018, seeds of hybrid maize variety Kaveri-3696 were sown 20 cm apart with a row-to-row distance of 60 cm. The plots received no irrigation. Other agronomic requirements of the plants were provided equally.

Vegetative growth

The shoot length of randomly selected 50% plants from each plot was measured at 30, 60, and 107 DAS. Root length of maize plants was measured at 30 DAS. Surrounding soil (20 cm × 20 cm × 20 cm) having the plant roots was collected very carefully and the roots were gently rinsed in the laboratory to wash away the soil before measuring the length. The fresh weight and air-dried weight of the root and shoot of each plant were recorded. The full-matured maize plants were harvested on June 06, 2018 (107 DAS).

Soil and water sampling

Soil water content at 5, 15, and 30 cm below the ground surface was measured at 10–15-day interval. Soil thermometers set at 15-cm depth in the plots gave soil temperature record. Soil water content and soil temperature were measured during the first two months after the sowing. After that period, crop canopy was fully established and soil surface was fully covered, and thereby the differences in soil temperature under different treatments became insignificant. Plastic water containers were used to collect leachate from each soil column of the lysimeter (Figure 2). Rainfall events at 63 and 93 DAS produced adequate leachate samples to be chemically analyzed. The total leachate volume of each column was recorded. The leachate samples were immediately taken for the analysis of total N and available P concentration.

Sample analysis

The oven drying method (105°C for at least 24 hours to reach a constant weight) was used to determine soil water content. Soil organic carbon content was analyzed using the wet oxidation method and cation exchange capacity by the sodium saturation method (Black, 1965). Exchangeable K was extracted with 1.0 N NH_4OAc (pH 7) solution and then a flame photometer was used to determine the extractable K (Black, 1965). Total N (TN) was estimated by the Micro-Kjeldahl method (Bremner and Mulvaney, 1982) where samples were digested with 30% H_2O_2, conc. H_2SO_4 and catalyst mixture of K_2SO_4: $CuSO_4$:$5H_2O$: Se=100:10:1. Nitrogen in the digest was measured by distillation with 40% NaOH followed by titration of the distillate trapped water in H_3BO_3 with 0.01N H_2SO_4. The samples were shaken with 0.5M $NaHCO_3$ solution at pH 8.5 to extract available P, and then P was measured by developing a blue color using $SnCl_2$ reduction of phosphomolybdate complex solution. The absorbance of the complex was measured at 600 nm wavelength in a spectrophotometer and available P was calibrated with a standard P curve.

Data analysis

The experimental data were analyzed by one-way Analysis of Variance by using MS Excel 2016. The differences between the treatment-means were tested with the Least Significant Difference (LSD) value at a significance level of 0.05.

Results and Discussion

Soil water and soil temperature

The dry manure treatment had higher soil water contents than the no-manure treatment, and dry manure outperformed raw manure in increasing soil water availability (Figure 3a). Celik et al. (2004) also observed higher soil water availability due to higher porosity and hydraulic conductivity induced by dry manure application compared with raw manure application. The raw manure constituents were possibly redistributed better in the soil because of the higher water content in it, as suggested by Amin et al. (2014). Nahar et al. (2006) reported that fresh manure loosened soil aggregates by increasing microbial activities more than the composted dry manure. The loose aggregates can facilitate soil water transport through its pore spaces (Amin et al., 2016). Manure application increased soil organic matter from 1.1 to 1.4–1.9% and electrical conductivity from 132 to 156–206 µS cm^{-1} but reduced bulk density from 1.33 to 1.25 g cm^{-3} in the topsoil. The saturated soil water content (45.9 ± 2.4%) observed in the manure-treated soils was considerably higher than that in the un-amended soils (40.8 ± 1.8%). Wortmann and Shapiro (2008) reported that manure organic matter increased total porosity and infiltration capacity, thereby increasing water-holding capacity. The decreasing soil water content between 28 and 38 DAS indicates that total evapotranspiration during this vegetative growth period under this warm and dry weather was higher than the total rainfall (Figure 3a).

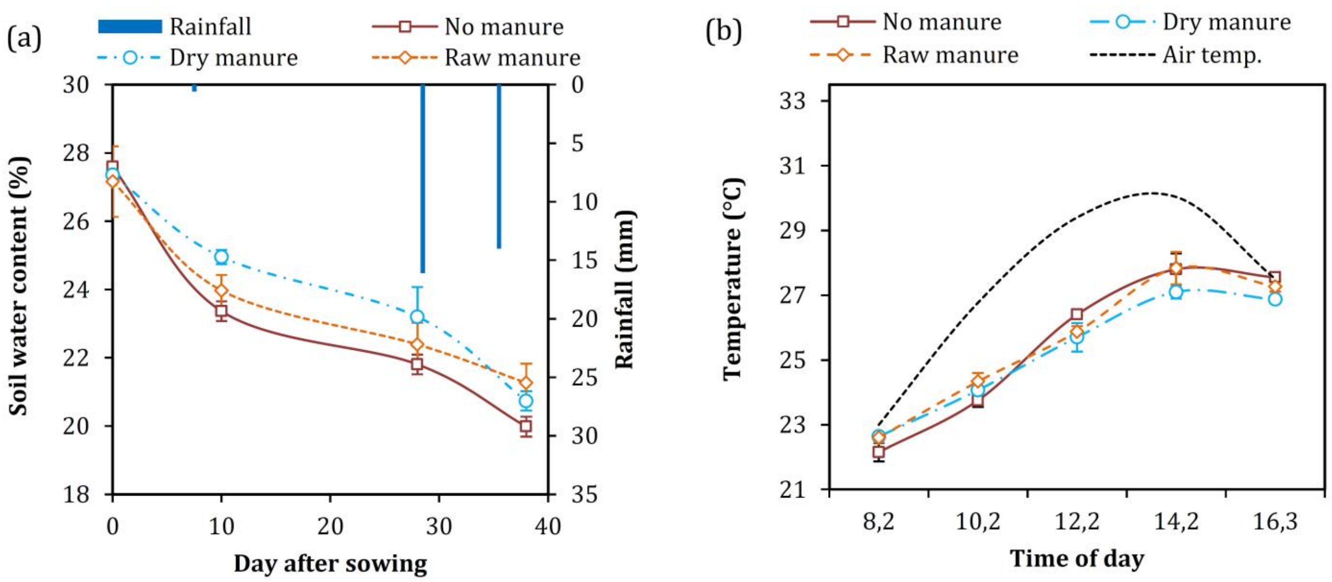

Figure 3. Temporal changes of soil water content at 15-cm depth (a) and daytime soil temperature fluctuation at 24 days after sowing (b) for different manure applications (error bars show standard errors)

The manure treatments had a slightly higher soil temperature than that of the no-manure in the morning but had a lower temperature in the afternoon at 24 DAS (Figure 3b). Organic matter in the applied-manure can reduce the thermal conductivity of the topsoil due to the increased soil porosity and reduced bulk density as suggested by Adekiya et al. (2016). Manure application reduced the daily temperature range by increasing the daily minimum temperature and reducing the daily maximum temperature (Figure 3b). Agbede et al. (2017) stated that manure application raised the minimum temperature and reduced the maximum temperature of a day. Reduction of daily temperature range can create a suitable soil environment for plant growth in places with a long diurnal temperature range.

Nitrogen leaching

At the first leaching event, the dry manure treatment released the highest amount of total N (191 g N ha^{-1}), but the difference was not significant (Figure 4a). At 93 DAS, the dry manure and raw manure treatments exerted a similar amount of total N leaching, and the amount was higher than that in the no-manure treatment (Figure 4b). The average leaching concentration of N under the manure treatments was 2.8 mg N L^{-1} at the first event and 5.8 mg N L^{-1} at the second event. At the latter event, the combined effect of rainfall and N mineralization from the land-applied manure released a higher amount of N. Asadu and Igboka (2014) obtained higher N leaching from the plots receiving manures compared with those receiving no-manure. The increased leaching of N in the manure treatments was attributed to the manure-borne N. Plants could not fully make use of the mineralized N, so the unused mineral N in soil subsequently started to leach.

The dry manure released a larger amount of N (30.7%) in the leachates than the raw manure. It is attributed to the steadier mineralization of N in soils amended with the compost-like dry manure. In contrast, straws present in the raw manure can increase N-immobilization (Lehrsch and Kincaid, 2007). The residual N content after maize harvesting was still higher in the manure-treated soils (0.77–0.97 g N kg^{-1}) than that in the non-manure treatment (0.63 g N kg^{-1}). Sanni (2016) observed an increase in available N in soils treated with the amendments that had slower nutrient release rates compared with the amendments with faster nutrient release rates.

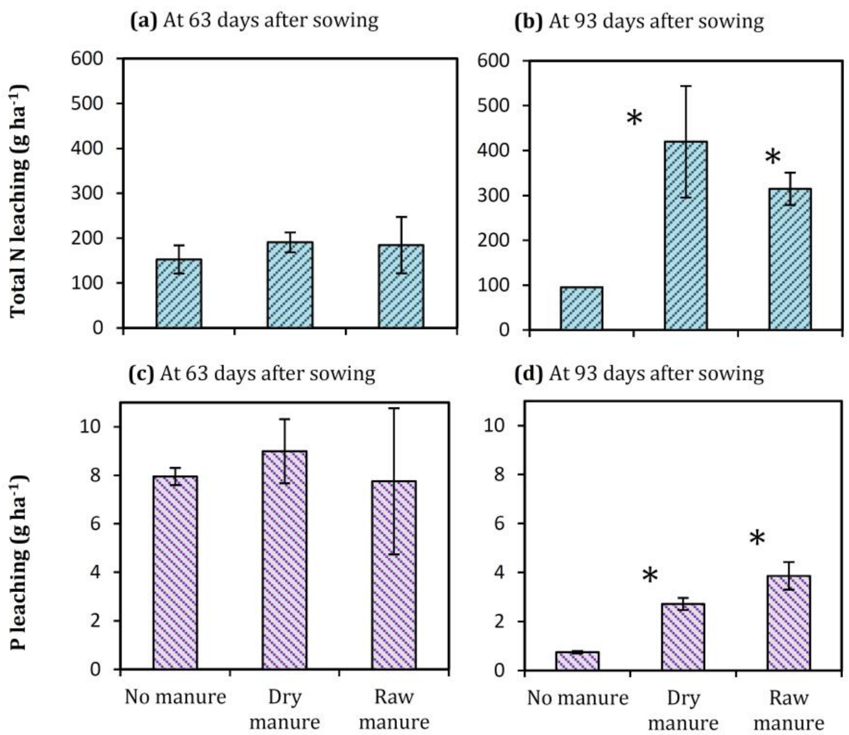

Figure 4. Leaching of total nitrogen (a and b) and available phosphorus (c and d) for rain events at 63 and 93 days after sowing for different manure application (* sign indicates significantly different compared to the control treatment at 5% significance level)

Phosphorus leaching

At 63 DAS, the difference in P leaching for no-manure and manure application was not significant (Figure 4c). At 93 DAS, the manure-treated plots released a higher amount of P compared with the control plots (Figure 4d). This finding agrees with the results of Chardon et al. (2007), who observed that land application of manure gradually increased P concentration in leachates. Phosphorus slowly mineralized from the land-applied manure, and then it started to leach, as stated by Eghball et al. (2002). The gradual increase in rainfall frequency and intensity augmented the vertical flux of water, which assisted the downward movement of P in the soil (Xue et al., 2013). The raw manure treatment released a higher amount of P than the dry manure treatment at 93 DAS. Ksheem et al. (2015) also found that raw manure released more water-soluble P in leachates than dry manure.

The N leaching rate was much higher than the P leaching because the NO_3-N form of N is more mobile in the soil. Moreover, the N application rate was more than double of P application rate. The average leaching concentration of total N increased from 2.6 mg N L^{-1} at 63 DAS to 4.7 mg N L^{-1} at 93 DAS, whereas available P leaching decreased from 0.12 mg P L^{-1} to 0.04 mg P L^{-1} between these two events. Leaching of N usually increases gradually up to a certain duration after field application because NO_3-N accumulates slowly through nitrification depending on the soil condition and subsequently starts to leach (Amin et al., 2014). In contrast, P is immobile in bulk soils and consequently often takes a preferential flow path to move (Williams et al., 2018). However, the effects of the manure treatments on the N and P leaching were almost the same, i.e., the treatments did not increase the leaching at the first event but had higher leaching at the second event.

Vegetative growth

Both the manure treatments enhanced plant growth, but the dry manure treatment outperformed the raw manure treatment (Table 1). This result agrees with De Boer (2008), who found an increased maize yield for dry manure application compared with raw manure application. Water content and crop-available nutrient in the topsoil were relatively higher for the dry manure treatment than that for the raw manure treatment. The end-of-season total N content in soil was 0.97 ± 0.02 g kg^{-1} for the dry manure treatment and 0.77 ± 0.01 g kg^{-1} for the raw manure treatment. Lehrsch and Kincaid (2007) found up to 17% more N uptake in compost-amended soils than that in manure-amended plots. The dry manure used in the study was more decomposed than the raw manure. Aziz et al. (2010) also observed that dry manure application improved plant growth and shoot weight compared with raw manure application. Saunders et al. (2012) found better forage production and N use efficiency for compost slurry application compared with the raw slurry application. However, Eghball et al. (2002) and Loria et al. (2007) found similar positive effects of compost and raw cattle or swine manure on maize yield and soil characteristics.

Table 1. Vegetative growth of maize for different manure types

Treatment	At 30 days after sowing (DAS)			Shoot length at 60 DAS (cm)	Shoot length at 107 DAS (cm)
	Shoot length (cm)	Root-shoot length ratio	Root-shoot mass ratio		
No-manure	50.3^{c}	0.22^{a}	0.13^{c}	161.6^{b}	190.7^{b}
Dry manure	69.7^{a}	0.19^{b}	0.20^{a}	187.3^{a}	205.2^{a}
Raw manure	59.9^{b}	0.22^{a}	0.15^{b}	164.5^{b}	195.7^{ab}

*Values with different letters (a, b, and c) are significantly different at 5% significance level.

The no-manure and raw manure treatments had a higher root-shoot length ratio than that of the dry manure treatment (Table 1). The raw manure treatment had a higher root length than the dry manure treatment probably because the nutrient in the raw manure redistributed more into the deeper soil. The raw manure had a larger liquid fraction than the dry manure, which could have facilitated the redistribution of manure-borne nutrients in the soil. Amin et al. (2014) found higher redistribution of manure-borne constituents in soil when the water content in manure was higher. Lynch (2013) reported that a better development of root foraging into deep soil strata occurred when N became available in the subsoil. The low soil water and nutrient availability in the topsoil under the no-manure treatment presumably increased the root length compared with that in the dry manure treatment (Table 1). Sharp et al. (2004) suggested that water stress condition exerts pressure on roots to spread more to search for water and nutrient into the soil to check crop failure. However, the dry manure treatment produced the highest root-shoot mass ratio followed by the raw manure treatment.

Conclusion

Manure application effectively conserved soil water throughout the vegetative stage of maize. Manure application raised the daily minimum temperature and reduced the daily maximum temperature. The average total N content in leachates was 2.6 mg N L^{-1} at 63 DAS and 4.7 mg N L^{-1} at 93 DAS, and the values for available P were 0.12 and 0.04 mg P L^{-1} at 63 and 93 DAS, respectively. These practices did not increase nutrient leaching at the first leaching event but released a higher amount of nutrient in the leachates at the second event than the control treatment. The manure treatments gave longer shoots due to the increased soil water availability and thus augmented crop uptake of nutrients. A nutrient management plan should be in place to avoid the leaching problem before any large-scale implementation of these practices.

Acknowledgements

The research work was supported by a research project # LS2017573 funded by the Ministry of Education, Government of the People's Republic of Bangladesh. We also acknowledge the research support from the BAURES of Bangladesh Agricultural University.

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Eurasian Journal of Soil Science 2022, 11(1), 25 - 32

Eurasian Journal of Soil Science

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

Yield of sugar beet and changes in phosphorus fractions in relation to long term P fertilization in chestnut soil of Kazakhstan

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Article Info

Received : 11.02.2021
Accepted : 02.08.2021
Available online : 08.08.2021

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Abstract

Excessive phosphorus (P) application can alter soil P availability and limit plant growth by P fixation into different organic and inorganic P forms. However, it remains uncertain whether these changes happen after limited fertilization or an excessive rate applied under the crop rotation. The current study aimed to investigate the yield of sugar beet in response to long term P fertilization, and to investigate long-term P fertilization effects on soil P fractions after long-term fertilizations in chestnut soil of Kazakhstan. A long-term study (56 years) was conducted to assess the changes in total P, available P and inorganic P (Pi) fractions in response to different P rates applied to sugar beet. Inorganic P fractions were determined using the Ginzburg and Lebedeva (1971) and Ginzburg (1981) methods. Our findings demonstrated that different P rates significantly increased the total P and available P in the inorganic P fractions compared to $N_0P_0K_0$ treatment (Absolute control). The $N_1P_2K_1$ (100% of recommended level of NK but 200% of P) treatment had a maximum yield and sugar content of sugar beet. Compared with $N_0P_0K_0$, the proportions of Ca-P_I, Ca-P_{II}, Fe-P and Al-P of total inorganic P fractions associated with under fertilizer treatments increased. The highest content of fractioned P was found in the form of Ca–P_{III}.

Keywords: Sugar beet, chestnut soil, phosphorus, P fertilization, inorganic P fractions.

Introduction

In modern agriculture, maximizing and sustaining crop yields are the main objectives. One of the major problems constraining the development of an economically successful agriculture is nutrient deficiency (Fageria and Baligar, 2005). Phosphorus (P) deficiency is a universal constraint to crop production and constitutes the second most important soil fertility problem throughout the World (Rashid et al., 2005). Phosphorus is an essential nutrient for both plants and animals. It is estimated that some 30 to 50% of the increase in world food production since the 1950s is attributable to fertilizer use, including P use (Higgs et al., 2000). Phosphorus deficiency in crop plants is a widespread problem in various parts of the world, especially in highly weathered acidic soils (Fageria and Baligar, 1997, 2001; Faye et al., 2006). Worldwide applications of phosphate fertilizers now exceed over 30 million metric tons annually (Epstein and Bloom, 2005). The deficiency of this element is related to several factors. These factors are low natural level in some soils, high immobile or fixation capacity of acidic soils, uptake of modern crop cultivars in large amount, loss by soil erosion, and use of low rate by farmers in developing countries. Biotic stresses such as crop infestation of insects, diseases, and weeds also reduce P use efficiency in crop plants (Fageria, 2009).

Soils contain organic and inorganic phosphorus compounds. Because organic compounds are largely derived from plant residues, microbial cells, and metabolic products, components of soil organic matter are often

doi : https://doi.org/10.18393/ejss.980372
: http://ejss.fesss.org/10.18393/ejss.980372
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

similar to these source materials. Approximately 1% of the organic phosphorus is in the phospholipid fraction; 5 to 10% is in nucleic acids or degradation products, and up to 60% is in an inositol polyphosphate fraction. Phospholipids and nucleic acids that enter the soil are degraded rapidly by soil microorganisms (Ko and Hora, 1970; Anderson, 1967). The more stable, and therefore more abundant, constituents of the organic phosphorus fraction are the inositol phosphates. Inositol polyphosphates are usually associated with high-molecular- weight molecules extracted from the soil, suggesting that they are an important component of humus (Omotoso and Wild, 1970).

Soils normally contain a wide range of microorganisms capable of releasing inorganic orthophosphate from organic phosphates of plant and microbial origin (Alexander, 1977). Conditions that favor the activities of these organisms, such as warm temperatures and near-neutral pH values also favor mineralization of organic phosphorus in soils (Alexander, 1977; Anderson, 1975). The enzymes involved in the cleavage of phosphate from organic substrates are collectively called phosphatases. Microorganisms produce a variety of phosphatases that mineralize organic phosphate (Feder, 1973). Humic acids and other organic acids often reduce phosphorus fixation through the formation of complexes (chelates) with Fe, Al, Ca, and other cations that react with phosphorus (Holford and Mattingly, 1975; Hedley et al., 1982; Wang et al., 2010). Studies have shown that organic phosphorus is much more mobile in soils than inorganic sources.

Inorganic phosphorus entering the soil solution, by mineralization or fertilizer additions, is rapidly converted into less available forms. Sorption and precipitation reactions are involved. The sorption of inorganic phosphorus from solution is closely related to the presence of amorphous iron and aluminum oxides and hydrous oxides and the amounts of calcium carbonate ($CaCO_3$) (Holford and Mattingly, 1975; Cogger and Duxbury, 1984; Solis and Torrent, 1989; Kızılkaya et al., 2007). Hydrous oxides and oxides of aluminum and iron often occur as coatings on clay mineral surfaces (Williams et al., 1958; Greenland et al., 1968, Shen and Rich, 1962), and these coatings may account for a large portion of the phosphorus sorption associated with the clay fraction of soils. Even in calcareous soils, hydrous oxides have been demonstrated as being important in phosphorus sorption, as was demonstrated by Shukla et al. (1971) for calcareous lake sediments, Holford and Mattingly (1975) for calcareous mineral soils, and Porter and Sanchez (1992) for calcareous Histosols. In calcareous soils, phosphorus (or phosphate) sorption to CaCO3 may be of equal or greater importance than sorption to aluminum and iron oxides (Porter and Sanchez, 1992). In a laboratory investigation with pure calcite, Cole et al (1953) concluded that the reaction of phosphorus with CaCO3 consisted of initial sorption reactions followed by precipitation with increasing concentrations of phosphorus. Phosphorus sorption may occur in part as a multilayer phenomenon on specific sites of the calcite surface (Holford and Mattingly, 1975, Griffin and Jurinak, 1973). As sorption proceeds, lateral interactions occur between sorbed phosphorus, eventually resulting in clusters. These clusters in turn serve as centers for the heterogeneous nucleation of calcium phosphate crystallites on the calcite surface.

Crop yields are often limited by low P availability in soils, owing mainly to adsorption and precipitation reactions of both indigenous soil P and applied fertilizer P with iron (Fe), aluminum (Al), or calcium (Ca) (Khiari and Parent, 2005). Low P uptake efficiency of plants is associated primarily with limited P availability in native soil. Consequently, large amounts of expensive inorganic P fertilizers need to be applied to many agricultural soils to attain reasonable crop yields (Ayaga et al., 2006).

Soil inorganic P (Pi) represents the dominant component in the soil P pool, accounting for about 75–85% of soil total P. Soil Pi is represented as various fractions such as Ca–P, Fe-P, Al–P and O–P (P occluded within Fe oxides) (Solis and Torrent, 1989; Kızılkaya et al., 2007). However, in calcareous soils, most Pi is present in various Ca-bound forms and there are great differences in P availability among these Ca–P fractions (Yang and Jacobsen, 1990). A few studies have assessed fractionated P and available P in soils on a regional or country scale in chestnut soil of Kazakhstan. Chestnut soils a soil type occurring in arid steppes. The soils cover large areas of Turkey, Mongolia, northern China, the United States, and Kazakhstan (Saparov, 2014; Yertayeva et al., 2018; 2019; Suleimenova et al., 2019). The climate in the chestnut soil zone is continental and arid. The genetic and zonal properties of chestnut soils include deficient drainage, a shortage of productive moisture, alkalinity, and soil heterogeneity. The parent material consists chiefly of calcareous deposits with a predominance of loess like loams, calcareous sandy loams, calcareous sands, sandy loams, and alluvium. Chestnut soils contain carbonates and, in most cases, gypsum in the lower part of the profile. The presence of readily soluble salts causes the alkalinity of chestnut soils.

The objectives of the present study were (i) to investigate the yield of sugar beet in response to long term P fertilization, and (ii) to investigate long-term P fertilization effects on soil P fractions after long-term fertilizations in chestnut soil of Kazakhstan.

Material and Methods

Site Description

The long-term field experiment was located on the experimental station of the Kazakh Research Institute of Agriculture and Plant Growing, Almaty, Kazakhstan (43°09'32.8"N 76°26'57.3"E). Sugar beet (*Beta vulgar*is L.) is the major crop in this region, which are generally planted in May and harvested in October. The experiment was established to study the effect of different fertilizer treatments and crop rotations on yield of sugar beet and soil phosphorus fractions. The year the experiment was established was 1962. The locations of the experimental site were characterized by the continental climate (large daily and annual fluctuations in air temperature, characterized by cold winters and long hot summers). The annual mean precipitation and mean temperature from the establishment of the experiment is shown in Figure 1.

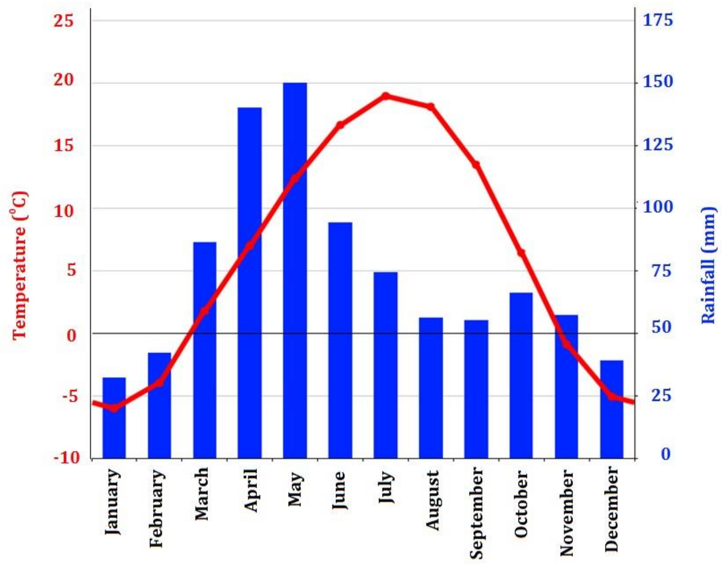

Figure 1. Monthly average temperature (°C) and distribution of precipitation (mm) of the experimental area.

The standard climatological long-term average (1961–2020) precipitation and temperature was 863 mm and 6.8 °C, respectively. The altitude of the trial site is 700 m. The soil belongs to the general soil type of dark chestnut. The pH was 8.61-8.62 (alkaline reaction), soil organic matter content was 2.27-2.30% (moderate). Total N was 0.171-0.182%, total phosphorus was 0.20-0.21% and total potassium was 1.62-1.75%. Available nitrogen, phosphorus and potassium contents were 23.1-24.8 mg/kg, 20.2-27.0 mg/kg and 424-455 mg/kg, respectively.

Experimental Design Description

A long term experiment was established at the experimental station of the Kazakh Research Institute of Agriculture and Plant Growing in May 2018. Twelve experimental plots of 216 m^2 area (11.2 m x 19.3 m) separated by 0.7m cement barriers were set in completely randomized block design with five treatments and four replications. The crop rotation in these fields was equal, consisting of alfalfa + winter wheat, alfalfa, and alfalfa, sugar beet, winter wheat, sugar beet, corn. The sources of fertilizers used were urea 46% N, double superphosphate 47% P_2O_5 and potassium chloride 60% K_2O. The doses of mineral N, P, and K are shown in Table 1.

Table 1. Doses of applied N, P, and K in the field experiment

Fertilizer treatments	In 2018			Totally (from 1962 to 2018)		
	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
$N_0P_0K_0$	0	0	0	0	0	0
$N_1P_0K_1$	100	0	60	5600	0	3360
$N_1P_1K_1$	100	90	60	5600	3360	3360
$N_1P_{1.5}K_1$	100	135	60	5600	5040	3360
$N_1P_2K_1$	100	180	60	5600	6720	3360

$N_0P_0K_0$ = Absolute control (no applied fertilizers)
$N_1P_0K_1$ = Phosphorus control, 100% of recommended level of NK
$N_1P_1K_1$ = 100% of recommended level of NPK
$N_1P_{1.5}K_1$ = 100% of recommended level of NK but 150% of P
$N_1P_2K_1$ = 100% of recommended level of NK but 200% of P

Sugar beets were grown after winter wheat, and their nourishment consisted exclusively of mineral fertilization according to the above variants. The row spacing was 45 cm x 17 cm. Theoretically, the plant density was 130 thousand plants per ha^{-1}. In the spring, prior to sowing beet seeds, Mineral P and K fertilizers were applied in autumn and were incorporated into the soil by moderate deep tillage (0.2 m). Mineral N was applied in the spring, before the beet planting, applied in a topdressing treatment during the sugar beet growth phase of 6 leaves, according to the experiment design. The surface area of the experimental plots was 28 m^2, of which 21.6 m^2 was harvested in October. At harvest (205 days after sowing), plants of each plot were harvest to determine roots yield (ton ha^{-1}). Sugar Content Analyses (expressed as %) was estimated in fresh samples of sugar beet root by using Saccharometer/Polarimeter with SU-4 model.

Soil Sampling: The soil samples were collected at two depths: 0-20 cm and 20-40 cm. Soil samples were processed in the laboratory by removing and visible plant residues and Stones larger than 2 mm immediately after sampling. Soil samplings were then air-dried.

Soil sample analyses: The air dried soil samples were ground to pass through a 2 mm sieve for laboratory analysis. Soil samples were digested in a tri-acid mixture (HNO_3, $HClO_4$, and H_2SO_4 at a 3:1:1 ratio) for determining total phosphorus (Total P). The P concentration in the digest was determined colorimetrically using the vanadomolybdate method. Soil organic phosphorus (Po) was determined by combustion at 550^{0}C and extraction with 4 M H_2SO_4. Machigan method was used to determine available P using the colorimetric method after the extraction with 1% $(NH_4)_2CO_3$ (GOST 26205-91)

Soil inorganic P fraction: Inorganic P (Pi) fractions were measured according to a fractionation scheme of Ginzburg and Lebedeva (1971) and Ginzburg (1981). Briefly, the fractionation involved a sequential extraction with (i) 1% $(NH_4)_2SO_4$ + 0.25% $(NH_4)_2MoO_4$ (pH=4.8) to extract Ca-P_I, (ii) CH_3COONH_4 + CH_3COOH + 0.25 % $(NH_4)_2MoO_4$ (pH= 4.3) to extract Ca-P_{II}, (iii) 0.5 N NH_4F+ 0.1 N NaOH + 0.5 N H_2SO_4 to obtain Al-P, (iv) 0.5 N NH_4F+ 0.1 N NaOH + 0.5 N H_2SO_4 to obtain Fe-P, (v) 0.5 N NH_4F+ 0.1 N NaOH + 0.5 N H_2SO_4 to extract Ca-P_{III}.

Results and Discussion

Yield and sugar content of sugar beet

Effect of long term P fertilization on yield and sugar content of sugar beet was evaluated (Figure 2). Application of all fertilization significantly influenced the sugar beet yield and sugar content compared to untreated (control) plants (Figure 2). On average, plants grown on the absolute control plot ($N_0P_0K_0$) yielded the lowest, but at the same level, as those fertilized 100% of recommended level of NK + 150% of P ($N_1P_{1.5}K_1$) and 100% of recommended level of NK but 200% of P ($N_1P_2K_1$). This is the indicator that P was the most limiting yield forming nutrient. Yield of sugar beet in-growth in chestnut soil showed high differences, related to P rates. The highest yield increase of 98.7% was noted for the treatment $N_1P_{1.5}K_1$. Similar results were obtained by Gunarto et al. (1985) and Yousaf et al. (1999) on several vegetable crops. Numerous studies have reported that inorganic NPK fertilizer increased growth in some species by enhancing nitrogen, phosphorus and potassium uptake (Gülser et al., 2019). In the light of above presented facts, the key problem concerns yield forming functions of P. Effect of tested fertilizing treatments were variable, depending on the P doses. This is in agreement with study other studies (Barlog et al., 2013). In 2018, beet yield increased along the increasing degree of P balancing. The highest yield produced crop grown in the treatment $N_1P_{1.5}K_1$ (Figure 2). Our results corroborate earlier studies about the positive response of sugar beet to NPK fertilizers to exploit its yielding potential (Barłóg et al., 2010, 2013).

Total P and available P

Long term P fertilization significantly increased total P and available P concentrations within 0-20 and 20-40 cm soil depth (Figure 3a,b). Compared to $N_0P_0K_0$ (Absolute control), total P concentration in the 0-20 cm soil depth was increased by 4.96%, 12.77%, 24.64% and 20.22% in $N_1P_0K_1$, $N_1P_1K_1$, $N_1P_{1.5}K_1$ and $N_1P_2K_1$, respectively, while available P concentration was increased by 14.49%, 136.71%, 148.33% and 185.02% in in $N_1P_0K_1$, $N_1P_1K_1$, $N_1P_{1.5}K_1$ and $N_1P_2K_1$, respectively. Phosphorus was accumulated in the 0-20 cm soil depth in all fertilizer treatments. The majority of P accumulated in the 0-20 cm soil depth under $N_1P_{1.5}K_1$ treatment. The highest increase in available P concentration in $N_1P_2K_1$ treatment observed in the 0-20 cm soil depth, with the increase of 145.92% in 20-40 cm soil depth over $N_1P_2K_1$ treatment.

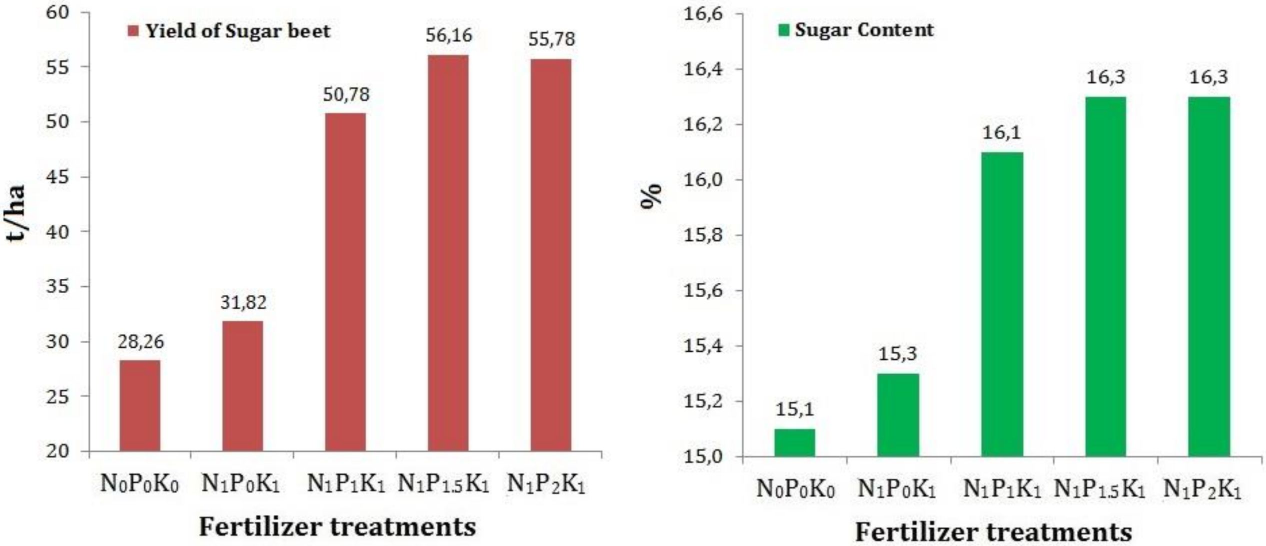

Figure 2. Effect of long term P fertilization on yield and sugar content of sugar beet

Continuous or long term P fertilization significantly increases P accumulation in soils (Ahmed et al., 2019, 2020). Long-term P fertilization causes a prominent increase in different inorganic P (Pi) forms, including available P fraction (Meason et al., 2009). The results of the present study revealed that long-term P fertilizer application effects the soil P directly in loess soils. Compared with the $N_0P_0K_0$ treatment, NPK treatments significantly increased the accumulation of total P in the soil (Figure 3a). Previous studies have examined the possibility of increasing the available P and total P after long-term P fertilization (Mao et al., 2015). In this study, the total and available P content was maximized in the $N_1P_2K_1$ treatment compared to the $N_0P_0K_0$ treatment. Zhang et al. (2003) found that the soil content of available Pi that ranged between 5 and 10 mg kg $^{-1}$ would be enough for plants, whereas a concentration <5 mg kg^{-1} would cause P-deficiency. In another study, Bravo et al. (2006) reported that the available concentration of P should be above the critical 6 or 7 mg kg^{-1} level for optimal cultivation growth. In our findings, NPK application treatments showed a significant increase in total P and available P content compared to the treatment of $N_0P_0K_0$, thus demonstrating interactive effects on the soil concentrations of available P under the application of NPK fertilizers. The long-term effects of the NPK application could be associated with the continuous addition of different rates of inorganic P in a balanced quantity, which induced the available P and avoided high fixation of different P forms (Hinsinger, 2001; Laboski et al., 2004).

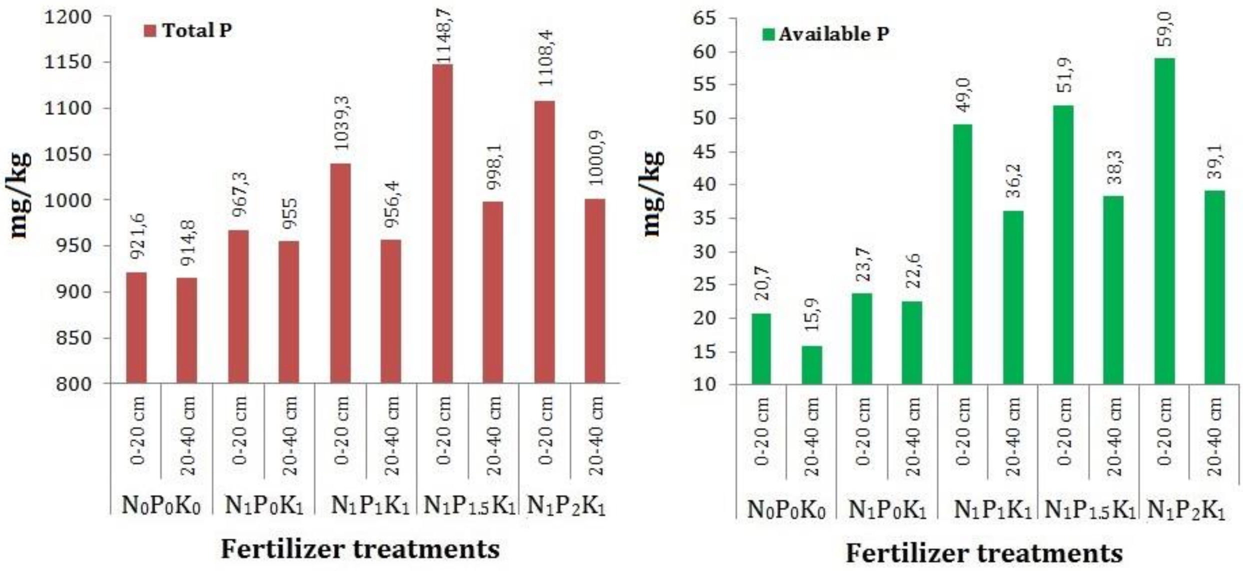

Figure 3. Effect of long term P fertilization on total P (a) and available P (b) concentrations

P fractions

Organic P proportion in total P decreased with the increase of P concentration of fertilizer regardless of treatments, while inorganic P proportion in total P increased under all fertilizer treatments in the 0-20 cm and 20-40 cm soil depth (Figure 4a). The concentrations of inorganic P fractions (Pi) increased significantly in the 0-20 and 20-40 cm soil depth (Figure 4b). Compared with $N_0P_0K_0$ (Absolute control), the proportions of Po fractions of the total P associated with $N_1P_0K_1$, $N_1P_1K_1$, $N_1P_{1.5}K_1$ and $N_1P_2K_1$ treatments decreased, while the of proportions of Ca-P_I, Ca-P_{II}, Fe-P and Al-P of total inorganic P fractions associated with under fertilizer treatments increased. Five fractions of P were quantified from different treatments: Ca-P_I, Ca-P_{II}, Al–P, Fe–P, and Ca-P_{III}. The highest content of fractioned P was found in the form of Ca–P_{III} (Figure 4b). Each P fraction was highest under the $N_1P_2K_1$ treatment and the lowest under the $N_0P_0K_0$ (Absolute control) treatment. Among the five long-term treatments, where NPK fertilizers were applied for 56 years along with treatments, Pi fractions followed the trend Ca-P_{III} > Ca-P_{II} > Fe–P > Al–P > Ca–P_I. These results are in line with the study findings of Dobermann et al. (2002), who found that the application of P fertilizer increases inorganic P fractions.

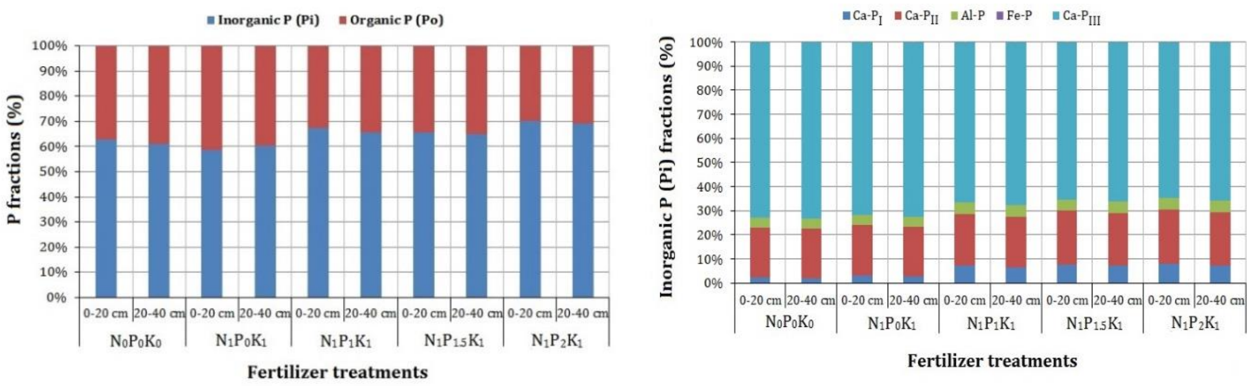

Figure 4. Effect of long term P fertilization on P fractions (a) and inorganic P fractions (b)

Conclusion

In this study, we compared the responses of soil P fractions and sugar beet yields to different rates of NPK fertilizer added to a sugar beet in a long-term field experiment. The total P and available P increased significantly due to the application of different rates of NPK compared with the $N_0P_0K_0$ (Absolute control) treatment. Long-term NPK fertilization with different level of P influenced the content of all forms of P as determined by a modified Ginzburg and Lebedeva (1971) and Ginzburg (1981) method. The 56 years long fertilization period did not affect the content of organic P in chesnut soils. On the contrary, the results obtained by the Ginzburg methods showed that the contents of Pi (Ca-P, Fe–P and Al–P) fractions were increased. Fertilization considerably increased the content of available P, especially of P bound to Ca. Application of higher amounts of P-fertilizer resulted in the dominance of the Ca–P fraction in the studied soil. The application of NPK fertilizer on the soil produced a significant increase in the available phosphorus.

Acknowledgements

The study was carried out with the financial support of the Ministry of Education and Science of the Republic of Kazakhstan (Project No: AP05133193)

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Eurasian Journal of Soil Science 2022, 11(1), 33 - 42

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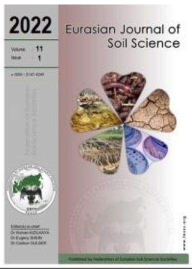

Splitting nitrogen fertilization improves growth, yield and profit of soybean (Glycine max) production in the semi-arid Afghanistan

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Article Info

Received : 19.02.2021
Accepted : 09.08.2021
Available online : 15.08.2021

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Abstract

The objectives of this study were to evaluate the effects of splitting application of 30 kg N ha^{-1} on the growth, yield and economics of soybean (*Glycine max* var. Dea-won) in the semi-arid and sub-tropical Afghanistan. Besides no-N-fertilization control, urea (30 kg N ha^{-1}) was applied to fields in four-splits: S_1, one time basal application at sowing; S_2, two-splits of 50% N at sowing and 10 DAS (days after sowing); S_3, three-splits of 33% N at sowing, 10 and 20 DAS; and S_4, four-splits of 25% N at sowing, 10, 20 and 30 DAS. Aboveground growth and yield parameters were compared at 30, 60, 90 and 127 DAS. Soybean's growth and yields increased in corresponding with the increased frequency of split fertilization. Three- or four-splits significantly increased plant height, leaf area index, aboveground biomass, crop growth rate, net assimilation rate, relative growth rate, pod and seed numbers, 1,000-seed weight, yield production and economics (gross and net returns and benefit cost ratio) than those at one or two-splits N-application at all these four harvests. Positive relationships were observed among growth parameters and yield traits and yield production. Three- or four-splits at tested N rate and growth stages can meet N requirement for soybean's growth and yield while improving N use efficiency in semi-arid Afghanistan.

Keywords: Biomass production, *Glycine max,* N demanding, N use efficiency, N split application, urea.

Introduction

Soybean is the most important oil crop for human beings, animals and the biodiesel industry due to its high contents of oil (19%) and protein (40%) (Mon et al., 2017). Physiologically, soybean's growth has a high nitrogen (N) demand that is required mainly for protein synthesis. For example, a maximal daily uptake of 4.6 kg ha^{-1} is required at the R4 (full pod) stage (Bender et al., 2015) and approximately 300 kg N is needed to produce 3 t ha^{-1} of soybean (Youn et al., 2009). In an environment that is ideal for crop's growth, especially for soybean, biological nitrogen fixation (BNF) can fulfil 50% of soybean's total N demand (Bender et al., 2015). However, high soil NO_3^-, low moisture, low pH, compaction, acidity or drought can inhibit soybean's BNF process, growth and yield production (Mourtzinis et al., 2018). The present 0.6 to 1.2 t ha^{-1} productivity in Afghanistan is substantially low compared to the global 2.77 t ha^{-1} productivity (NEI, 2017). It is most likely due to the lack of indigenous N fixing bacteria and soil residual N, without rhizobial inoculation, excessive or inappropriate N application and low soil fertility in Afghanistan. As a result,

: https://doi.org/10.18393/ejss.984060
: http://ejss.fesss.org/10.18393/ejss.984060
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

external N fertilizer has to be supplied to promote soybean growth and productivity. Furthermore, overuse of NO_3^- has always resulted in leaching or denitrification. Even if N was applied as a basal application, plant roots could not possibly absorb all of them at once. In such soil conditions, N fertilization should be carefully managed throughout the crop growth cycle. One of the effective methods of N fertilization management is split application by adjusting the timing of fertilizer application within the plant growth stage. The fertilizer split can also improve the efficiency of crop uptake, re-translocation, and nitrogen use efficiency (Khan et al., 2017). Positive response of different rates and time of N fertilization on soybean's growth, nutrient uptake and seed yield have been studied. For example, fertilization of 30 kg N ha^{-1} or 7.5 mM pot^{-1} as a basal application and at the beginning of flowering (R1) stage increased plant height, leaf area index (LAI), dry matter accumulation and seed yield (Singh and Singh 2013; Bhangu and Virk, 2019; Zhou et al., 2019). The supply of 100, 50, 40, 30 or 10 kg N ha^{-1} as basal, at 25 DAS (days after sowing), the R1, pod initiation (R3) and beginning of seed (R5) stages significantly increased the soybean's aboveground biomass and seed yield (Gan et al., 2002; Bhangu and Virk, 2019).

Taking all above-mentioned information into consideration for rational splitting fertilization to harness maximum productivity, we hypothesized that (1) applying N fertilizer corresponding with plant growth stages could have significant effects on the growth and yield of soybean; and (2) a gradual supplement of the same amount of N fertilizer under split applications could be better than an one-time fertilizer supplement. These hypotheses are based on a fact that soybean can progressively establish its BNF capacity to meet its N-demanding while lessening the N-demand from soil mineralization, particularly in the earlier seedling or vegetative growth stages. A starter N application reportedly improved soybean's biomass accumulation and seed yield (Starling et al., 1998; Hellal et al., 2013). Even though chemical starter fertilizer N tends to lost within a few weeks after planting (Ohyama et al., 2017), subsequent supplementation of N fertilizer via the split application may replenish the soil N pool. Therefore, in this study, a locally practised N fertilization rate of 30 kg N ha^{-1} was employed as four splits application that corresponds to the growth and development stages of a local soybean cultivar to enhance the N use efficiency while increasing its primary productivity.

Therefore, the objectives of this present study were to determine (i) to eveluate the effects of splitting patterns of N fertilizer on growth and yield, and (ii) to eveluate the optimal splitting application of both timing and rates across four splitting times to ensure a higher N-use efficiency, productivity and profitability of soybean in Kandahar, Afghanistan. Findings from the study can provide a foundation for promoting crop production and resource use efficiency in the semi-arid and sub-tropical regions around the world.

Material and Methods

Site specification

A field experiment was conducted between May 13 and September 20, 2017 in the Afghanistan National Agricultural Sciences and Technology University Research Farm in Kandahar (31°26'N and 65°51'E, 1,010 m above mean sea level), Afghanistan. The region has a semi-arid to sub-tropical climate with a mean annual temperature of 5–6°C in winter and of 24.3–35.8°C in summer, and an annual precipitation of 199 mm (most between January and March) (Table 1). The soil is an Aridisol soil (according to the USDA Soil Taxonomy) that has 56.2% sand, 14.3% silt and 29.5% clay. The soil pH (1:2.5; H_2O) was 7.9 with cation exchange capacity of 80.58 meq 100 g^{-1}. Soil organic carbon, available N, P, and K, and total Fe, Zn, Cu and Mn were 9.40 g kg^{-1}, 700, 1.29 and 956.64, and 2.89, 1.35, 0.98 and 3.51 mg kg^{-1}, respectively.

Table 1. Monthly temperature and rainfall during the soybean growing season (2017)

Months (2017)		April	May	June	July	August	September
Temperature (°C)	Maximum	26.65	31.63	35.94	35.56	33.69	29.87
	Average	19.46	24.85	29.55	29.37	27.55	22.87
	Minimum	11.65	17.11	22.41	23.17	20.65	15.84
Rainfall (mm)		0.178	0.001	0.000	0.000	0.000	0.000

Experimental design and treatment

To examine the growth response of soybean to different timing of fertilization, conventional urea (46% N) was applied in four splits for a total rate of 30 kg N ha^{-1} based on the local fertilization rate. In addition, P_2O_5, K_2O and $ZnSO_4$ were applied at sowing to all plots at the rate of 60, 40, and 10 kg ha^{-1}, respectively. The field experiment was designed in a randomized complete block design with three replications. There were a total of 15 plots, each measured at 4.0 m x 3.0 m with 5 numbers of rows spaced at 0.4 m apart. The treatments were consisted of five fertilization rates applied at different days after sowing (DAS): (1) the no-fertilization control, (2) S1 = one-time basal application (100% N) at sowing, (3) S2 = two-times split at 50% N each at sowing and 10 DAS; (4) S3 = three-times split at 33% N each at sowing, 10 DAS and 20 DAS; and (5) S4=four-times split of 25% N each at sowing, 10 DAS, 20 DAS and 30 DAS. The timing of fertilizer application at 10 DAS corresponded to the cotyledon or unifoliate leaves (VC) stage, 20 DAS to the 3rd trifoliate (V3) stage and 30 DAS to the beginning of flower (R1). Soybean (Glycine max cv. Dea-won) was sown at 40 cm x 15 cm without inoculation with a bacterium culture. This cultivar, selected with its wide adaptability in the study region, shows resistance against pest and disease and a productivity of 1.1 to 2.8 t seed ha^{-1}. No information is available to the soil microbiota, to our knowledge. The experiment was conducted under irrigation with wheat as the previous crop and the field was Mouldboard–ploughed once before sowing. In next month, land preparation was started with cross–cultivator to obtain a good soil tilth, followed by two times of rotaries to break soil clods.

Measurements

Five plants were randomly selected as representative samples for a replicated treatment at 30, 60 and 90 DAS corresponding to R1, full pod (R4) and full seed (R6), respectively, to determine (i) plant height, measured from ground level to the tip of the plant, and (ii) leaf area per plant from three sizes of leaves (i.e., small, medium, large). The leaf area was determined using millimetre graph paper and then converted to cm^2 $plant^{-1}$. The leaf area index was calculated using the following equation:

$$LAI = \text{leaf area per plant (in cm}^{-2}\text{)/plant ground area (cm}^2\text{)}.$$

Plants were harvested at 127 DAS where the straws (leaf + stem + pod) were sun-dried for several days to a constant weight. The outcome was recorded as g $plant^{-1}$ and then converted to t ha^{-1}. The total pods per plant were determined by the number of pods (containing one or more seeds) from ten randomly selected plants. The average number of pods per plant was derived by dividing the total number of pods by ten. Total seed pod^{-1} was determined by the total number of seeds from ten pods randomly selected from each treatment divided by ten and then averaged to obtain the total seed per pod. 1,000 of clean dried seeds counted randomly from each sample were weighted by using digital electric balance and the results were expressed in grams. The seeds or grains obtained from each net plot were sun-dried for 4-5 days, weighed carefully and recorded in t ha^{-1}. The seed yield was recorded at 10% moisture content. Total aboveground biomass (straw + seed) was determined as the total weight of straw t ha^{-1} plus seed yield t ha^{-1} and harvest index as seed yield t ha^{-1} divided by aboveground biomass and multiply by 100.

Physiological parameters

Net assimilation rate (NAR; g cm^{-2} d^{-1}), crop growth rate (CGR; g $cm^{-2}d^{-1}$), relative growth rate (RGR; g g^{-1} d^{-1}), and agronomic nitrogen use efficiency (ANUE; kg seed kg N^{-1}) were determined using the following formulas (Delogu et al., 1998; Sun et al., 2019; Díaz-López et al., 2020).

$$NAR = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{Log_e L_2 - Log_e L_1}{L_2 - L_1}$$

Where W_1 and W_2 are the total weight of aboveground biomass of plant at time t_1 and time t_2, respectively and L_1 and L_2 are total leaf area of plant at time t_1 and t_2, respectively.

$$CGR = \frac{W_2 - W_1}{t_2 - t_1}$$

WhereW_1 and W_2 are total weight of aboveground biomass at time t_1 and time t_2 respectively.

$$RGR = = \frac{Log_e W_2 - Log_e W_1}{t_2 - t_1}$$

W_1 and W_2 are the total dry weight at times t_1 and t_2, respectively.

$$ANUE = \frac{\text{seed yield at N treatment-seed yield at zero N treatment}}{\text{applied N at N treatment}}$$

Economic evaluation

All costs and returns per hectare were initially calculated based on Afghan Afghani (AFN) and thereafter converted to US$ based on the exchange rate of 1 AFN=0.015 US$, as of September, 2017, though such conversions only reflected relative values. All the expenses included land lease, labour and farmer segment, land preparation, irrigation, fertilization, weeding, harvesting and threshing, seeds, insecticide and the cost of fertilizers were computed as the costs of cultivation based on individual treatment. The production prices for straw and fallow land wad (t ha^{-1}) were considered as per the average price of domestic market. The seed/grain was counted 395 US$ per tonne as per the FAO international price review for soybean observed in September, 2017. Gross return was calculated as the price of whole plant production (straw + seed t ha^{-1}) and the net return was the difference of gross return and the total cost of all variables. The ratio between gross return to cost of cultivation was computed as benefit/cost ratio (B/C ratio).

Statistical Analyses

Data were subjected to one-way ANOVA. Significant differences among fertilization treatments for the same harvest day or among harvest days for the same fertilization rate were compared by the Duncan's multiple range test at *P* < 0.05 using a SPSS 24 software (Chicago, USA). Microsoft Office Excel (version 2016, Microsoft, Redmond, USA) was used for graphs, correlation, and regression analysis.

Results

Effects of splitting nitrogen applications on growth attributes

Plant heights observed within the same days after sowing (DAS) at 30, 60 and 90 increased with the number of split fertilization compared to control (no-fertilization) (Table 2). At 90 DAS, soybean plants treated with four splits of N (S4, at a rate of 25% N) had the tallest height, which was significantly taller than plants treated with S0-S2 but not significantly different compared to plants treated with S3. In addition, significantly taller plant height for the same fertilization among different growing days patterned as 90 DAS > 60 DAS > 30 DAS (Table 2).

Table 2. Effects of splitting N fertilizations on plant height, leaf area index and aboveground biomass of soybean at 30, 60 and 90 days after sowing (DAS).

Treatment	Plant height (cm)			Leaf area index			Aboveground biomass (g plant^{-1})		
30 kg N ha^{-1}	**30 DAS**	**60 DAS**	**90 DAS**	**30 DAS**	**60 DAS**	**90 DAS**	**30 DAS**	**60 DAS**	**90 DAS**
Control	8.1 ± 0.3 (b, z)	29.2 ± 2.8 (b, y)	34.1 ± 2.9 (c, x)	0.24 ± 0.02 (a, z)	3.1 ± 0.22 (c, y)	3.5 ± 0.15 (c, x)	1.63 ± 0.03 (b, z)	12.6 ± 0.48 (c, y)	21.0 ± 2.2 (c, x)
N_{30}-S1	8.6 ± 0.5 (ab,z)	31.8 ± 3.1 (ab, y)	36.7 ± 1.4 (bc, x)	0.27 ± 0.01 (ab, y)	4.3 ± 0.21 (b, x)	4.6 ± 0.57 (b, x)	1.74 ± 0.11 (b,z)	17.6 ± 0.98 (b, y)	35.9 ± 2.7 (b, x)
N_{30}-S2	8.8 ± 0.7 (ab, z)	32.2 ± 3.5 (ab, y)	39.1 ± 1.8 (b, x)	0.27 ± 0.02 (ab, z)	4.3 ± 0.39 (b, y)	4.7 ± 0.52 (b, x)	1.74 ± 0.07 (ab, z)	18.4 ± 1.09 (b, y)	38.0 ± 3.1 (b, x)
N_{30}-S3	9.0 ± 0.7 (a, z)	34.5 ± 3.1 (a, y)	43.8 ± 4.1 (a, x)	0.30 ± 0.07 (a, z)	4.9 ± 0.85 (a, y)	5.6 ± 0.32 (a, x)	1.75 ± 0.04 (ab, z)	23.9 ± 0.95 (a, y)	59.2 ± 2.9 (a, x)
N_{30}-S4	9.0 ± 0.8 (a, z)	34.6 ± 2.8 (a, y)	45.1 ± 2.6 (a, x)	0.32 ± 0.09 (a, z)	4.9 ± 0.42 (a, y)	5.7 ± 0.61 (a, x)	1.77 ± 0.03 (a, z)	24.6 ± 0.83 (a, y)	61.6 ± 4.0 (a, x)

Data are means ± SD (*n*=3). S1 = One-time basal application at sowing; S2 = two-times split application at 50% N each at sowing and 10 DAS; S3 = three-times split at 33% N each at sowing, 10 DAS and 20 DAS; and S4 = four-times split of 25% N each at sowing, 10 DAS, 20 DAS and 30 DAS. N_{30} indicates 30 kg N ha^{-1} season^{-1}.
Significant differences between N fertilizations for the same day (a, b, c).
Significant differences between growing days for the same N fertilization (x, y, z) at *P* < 0.05.

There were no significant differences in leaf area index (LAI) observed at 30 DAS when the plants were treated with different rates of fertilization (Table 2). However, at 60 and 90 DAS, the LAI increased significantly until the plants were treated with three-times split at 33% N (S3). Increasing the number of split fertilizations to four times (S4) at 25% N did not affect the LAI significantly when observed at 60 and 90 DAS. Moreover, significant higher LAI was observed for the same fertilization among growing days when the plant was progressed toward the growing period between 30, 60 and 90 DAS and pattern as 90 DAS > 60 DAS > 30 DAS (Table 2).

Similar trends were also observed for the aboveground biomass (Table 2). Plants accumulated significantly more aboveground biomass with an increased split application of fertilizers until S3 at 60 and 90 DAS compared to control. However, there was no significant difference in aboveground biomass accumulation in plants treated with S4 of N fertilizer at 60 and 90 DAS compared to S3. Furthermore, significant difference was also observed for the same fertilization among growing days and rank as 90 DAS > 60 DAS > 30 DAS (Table 2)

Table 3 showed the soybean crop growth rate (CGR), net assimilation rate (NAR) and relative growth rate (RGR) in response to four different splits application of 30 kg N ha^{-1} compared to non-fertilized plants. The split N application significantly influenced the soybean CGR, NAR and RGR compared to the control. However, increasing the frequency of N supplies in split application to four times (S4) did not influence the CGR, NAR and RGR significantly compared to S3 for both observations at 30-60 DAS and 60-90 DAS. In addition, significant difference was observed when the soybean plants treated within the same fertilizer treatment (in S1, S2, S3 and S4) progressed towards the growing period between 60 and 90 DAS.

Yield attributes and agronomy N use efficiency (ANUE)

The yield attributes and ANUE were significantly affected by the increased frequency of split N applications (Table 3 and 4). In terms of ANUE, there were no significant differences between the fertilizer treatment of S1 and S2. However, ANUE increased significantly as the frequency of split application increased from S2 to

S3. A maximum ANUE was achieved in the S4 treatment but, it was not significantly different from S3 treatment (Table 3). The yield attributes including 1,000-seed weight, total pods plant^{-1}, seed weight plant^{-1} and seed pod^{-1} showed parallel trend toward different split fertilization treatments. However, significantly yield attributes were increased under the S3 and S4 than under the S1, S2 and control (Table 4).

Table 3. Effects of splitting N fertilizations on agronomy N use efficiency (ANUE), crop growth rate (CGR), net assimilation rate (NAR) and relative growth rate (RGR).

Treatment	ANUE	CGR (mg cm^2 d^{-1})		NAR (mg cm^2 d^{-1})		RGR (mg g^{-1} d^{-1})	
30 kg N ha^{-1}	(kg seeds kg N^{-1})	30-60 DAS	60-90 DAS	30-60 DAS	60-90 DAS	30-60 DAS	60-90 DAS
Control		318.6 ± 39 (c, ns)	329.5 ± 92 (c, ns)	0.42 ± 0.04 (c, ns)	0.16 ± 0.04 (c, ***)	64.00 ± 3.1 (c, ns)	21.11 ± 5.6 (b, ***)
N$_{30}$-S1	13.6 ± 8.4 b	530.4 ± 34 (b, ns)	608.8 ± 88 (b, *)	0.60 ± 0.05 (b, ns)	0.22 ± 0.03 (b, ***)	77.20 ± 3.4 (b, ns)	23.63 ± 2.6 (b, ***)
N$_{30}$-S2	17.3 ± 9.4 b	558.3 ± 35 (b, ns)	651.6 ± 97 (b, *)	0.63 ± 0.05 (b, ns)	0.24 ± 0.05 (b, ***)	78.69 ± 2.1 (b, ns)	23.99 ± 2.7 (b, ***)
N$_{30}$-S3	32.0 ± 10.6 a	738.2 ± 31 (a, ns)	1178.5 ± 99 (a, ***)	0.76 ± 0.06 (a, ns)	0.37 ± 0.04 (a, ***)	87.00 ± 1.5 (a, ns)	30.25 ± 2.0 (a, ***)
N$_{30}$-S4	38.8 ± 10.8 a	761.2 ± 27 (a, ns)	1234.5 ± 150 (a, ***)	0.75 ± 0.07 (a, ns)	0.38 ± 0.04 (a, ***)	87.66 ± 1.0 (a, ns)	30.55 ± 2.9 (a, ***)

Data are means ± SD (n=3). N$_{30}$ indicates 30 kg N ha^{-1} season^{-1}.
Significant differences between N fertilizations for the same growing period (a, b, c).
Significant differences between growing period for the same N fertilization (*P < 0.05; **P < 0.01; ***P < 0.001 and ns not significant).

Biomass and yield production

At harvest (127 DAS), the total aboveground biomass was significantly influenced by the split application of N (Table 4). Production of straw biomass, aboveground biomass (straw + seed) and seed yield were highest under S4, although they were not significantly different from S3 treatment. The harvest index varied significantly between the no-N fertilization control and N fertilizer treatments. There were no significant changes in the harvest index among the four split fertilization, but a significantly higher harvest index was observed under the no-N fertilization control (Table 4).

Table 4. The effects of splitting N fertilizations on yield attributes and total aboveground biomass of soybean at 127 DAS.

Treatment 30 kg N ha^{-1}	Yield attributes				Total aboveground biomass (straw + seed)			
	Total pod (plant^{-1})	Total seed (pod^{-1})	1,000-seed weight (g^{-1})	Seed weight (g plant^{-1})	Straw (t ha^{-1})	Seed yield (t ha^{-1})	Straw + seed (t ha^{-1})	Harvest index (%)
Control	29.1±0.3 c	1.80±0.05 c	97.4±5.03 c	6.01±0.7 c	1.27±0.04 c	1.42±0.08 c	2.6±0.09 c	52.8±1.8 a
N$_{30}$-S1	35.6±2.9 b	2.40±0.15 b	108.9±4.6 b	12.00± 0.1 b	1.65±0.08 b	1.64±0.15 b	3.3±0.21 b	49.7±1.9 b
N$_{30}$-S2	36.3±1.9 b	2.50±0.07 b	112.0±3.4 b	12.00± 0.1 b	1.69±0.04 b	1.71±0.10 b	3.4±0.11 b	50.2±1.5 b
N$_{30}$-S3	41.5±2.1 a	2.90±0.13 a	122.1±3.5 a	17.10±0.9 a	2.03±0.05 a	1.95±0.17 a	3.9±0.17 a	48.8±2.2 b
N$_{30}$-S4	42.2± 2.2 a	3.02±0.04 a	122.2±1.5 a	17.40±0.6 a	2.07±0.25 a	2.06±0.14 a	4.1±0.34 a	49.9±2.5 b

Data are means ± SD (n=3). N$_{30}$ indicates 30 kg N ha^{-1} season^{-1}.
Values followed by the same letter within the column are not significantly different at P < 0.05.

Economics

The statistical significance of the soybean productivity that was affected by different rates of fertilizer treatment demonstrated concomitant economic returns. That is, the increasing frequency of split N applications produced higher grain yield, gross and net returns with a higher benefit-cost ratio despite the higher cost of cultivation (Table 5). The maximum values were recorded under S4, although they were not significantly different compared to S3.

Table 5. Effects of splitting N fertilizations on grain yield price, cost of cultivation, gross return, net returns and benefit cost ratio of soybean. Data (means ± SD, n = 3). Abbreviations: N$_{30}$= 30 kg N ha^{-1} season^{-1}; S$_1$= One time basal application at sowing; S$_2$= an equal two times of 50% N split rate at sowing and 10 DAS; S$_3$= an equal three times of 33% N split rate at sowing, 10 DAS and 20 DAS; and S$_4$ = an equal four times of 25% N split rate at sowing, 10 DAS, 20 DAS and 30 DAS.

Nitrogen 30 kg ha^{-1}	Economics				
	Grain yield (US$ tonne^{-1} price)	Cost of cultivation (US$ ha^{-1})	Gross returns (US$ ha^{-1})	Net returns (US$ ha^{-1})	Benefit cost ratio
Control	1084.5 ± 23.2c	641.0 ± 3.1c	829.4 ± 18.5c	188.3 ± 15.4c	0.30 ± 0.03c
N$_{30}$-S$_1$	1254.7 ± 126.2b	665.1 ± 13.6b	973.6 ± 69.3b	308.4 ± 58.3b	0.46 ± 0.09b
N$_{30}$-S$_2$	1303.0 ± 112.2b	670.2 ± 16.1b	1004.6 ± 46.6b	334.4 ± 46.7b	0.50 ± 0.06b
N$_{30}$-S$_3$	1485.9 + 93.6a	694.6 ± 7.7a	1150.7 ± 46.1a	456.1 ± 38.4a	0.66 ± 0.05a
N$_{30}$-S$_4$	1623.0 ± 69.4a	708.1 ± 11.2a	1232.0 ± 67.1a	523.8 ± 55.9a	0.74 ± 0.07a

Significant differences between N fertilizations (a, b, c, d, e) at P < 0.05.

Relationships between plant growth parameters and biomass and/or yield production

The aboveground biomass (leaf + stem) showed positive relationships with plant height (r^2= 0.64, P = 0.001) and leaf area index (r^2= 0.74, P = 0.002) at 90 DAS (Figure 1a-b). Similar positive relationships were also observed with the total pod per plant (r^2= 0.66, P = 0.003) and CGR (r^2= 0.98, P = 0.002) (Figure 1 c-d). The seed yield at harvest 127 DAS had a strong positive relationship with aboveground biomass (straw + seed) (r^2 = 0.91, P = 0.002), but fairly positive relationship with total pod plant^{-1} (r^2 = 0.54, P = 0.002), 1,000-seed weight (r^2 = 0.55, P = 0.003) and CGR (r^2= 0.67, P = 0.002) (Figure 1 e-h).

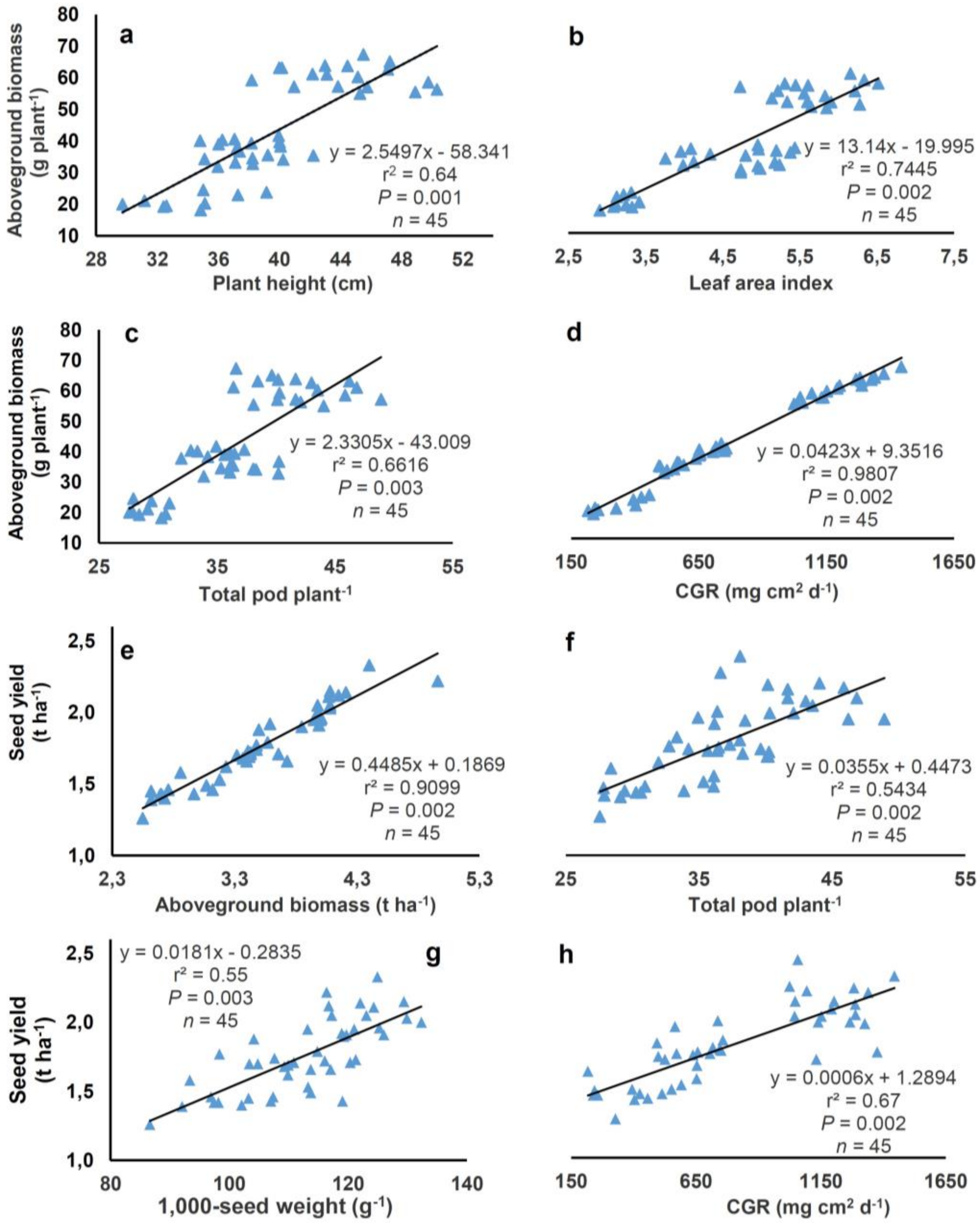

Figure 1. Relationship between aboveground biomass (leaf + stem) with plant height (A) or leaf area index (b) at 90 DAS and total pod plant^{-1} (c) at harvest 127 DAS or CGR (d), between seed yield with aboveground biomass (e) or total pod plant^{-1} (F) and 1,000-seed weight (g) or CGR (h) at harvest 127 DAS.

Positive relationships were also observed between leaf area index (r^2 = 0.69, P = 0.003) and NAR (r^2 = 0.93, P= 0.001) with CGR at 90 DAS (Figure 2 a-b), between seed yield (r^2= 0.88, P = 0.03) and aboveground biomass (straw + seed) (r^2= 0.84, P = 0.004) with agronomy N used efficiency (ANUE) at harvest 127 DAS (Figure 2 c-d), between leaf area index with plant height at 90 DAS (r^2= 0.52, P = 0.002, Figure 2e) and between NAR with RGR (r^2 = 0.83, P = 0.00; Figure 2 f) and seed yield (r^2 = 0.63, P = 0.001; Figure 2 g).

Discussion

In Afghanistan, the occasional cultivation of legume crops, without rhizobial inoculation coupled with certain environmental factors have considerably reduced the number and activities of N_2 fixing bacteria in soil. Hence, the crop growth and yield production mostly depend on the external application of N fertilizer and its management.

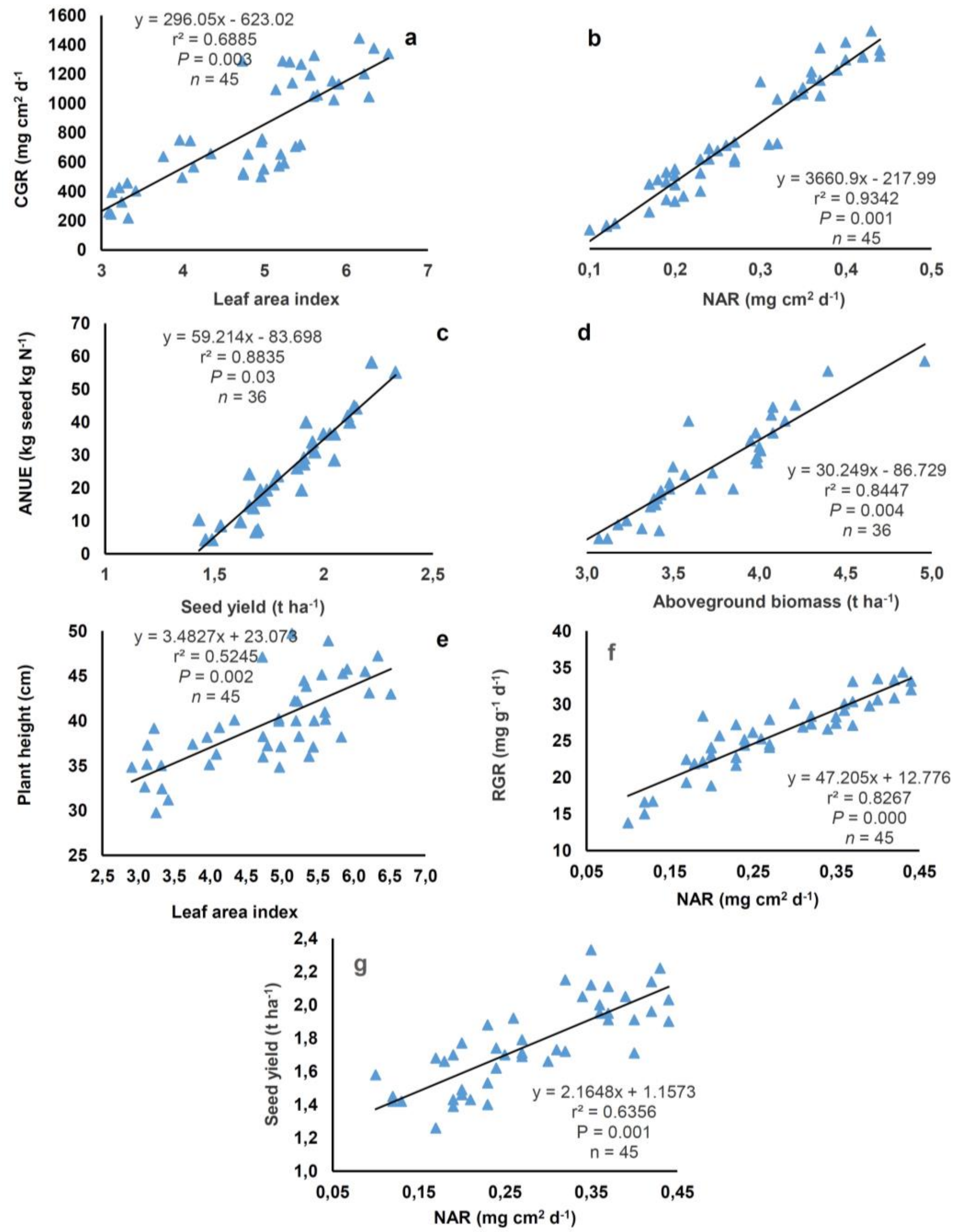

Figure 2. Relationships between CGR with leaf area index or NAR (a or b) at 90 DAS, between Agronomy N use efficiency (ANUE) with seed yield or aboveground biomass (straw + seed) at harvest 127 DAS (c and d), between plant height with leaf area index (e) or NAR with RGR at 90 DAS (f) and NAR with seed yield at harvest 127 DAS (g).

In our field experiment, splitting the 30 kg N ha^{-1} into three and four splits (at 33% and 25% N, respectively) and applied at sowing, 10, 20 and 30 DAS resulted in significantly higher physiological traits of soybean i.e., taller plant height, higher leaf area index and greater aboveground biomass (Table 2). Meanwhile, the application of N fertilizer in fewer splits notably two-times at 50% N each at sowing and 10 DAS as well as the one-time basal application at sowing resulted in significantly lower physiological traits; there were no significant differences compared to plants treated with no-N fertilization (Table 2).

The differences observed could be attributed to N management since the gradual application in splits from sowing to 30 DAS allows the soil to replenish its N pool, timely for plant uptake and thereby increased plant growth and yield. Furthermore, soybean requires less amount of N at its initial growth days since the plant root is not well developed to absorb more N from soil. If all N is applied at sowing, a portion will be taken up by the plant while the remaining will be lost through denitrification, volatilization, leaching and runoff (Li et al., 2018). Hence, the split application of N fertilizer during the crop growth reduces N losses while improving N use efficiency and fulfilling the crop N demand at the early growth and later in the reproductive stage. For example, a frequent rainfall at the early vegetative stage during the spring could often result in N losses due to run-off and/or leaching, while a split fertilization would decrease N leaching in the soil profile (Gan et al., 2002). Higher yield production and reduce N losses to the environment was positively related with the decrease of the basal N ratio while the increase of N split numbers during growth stages of rice (Li et al., 2018). Fertilization of external N at an earlier growth stage to sustain later growth/development and seed yield (Salvagiotti et al., 2009).

The results from our field study further indicate that soybean's growth increased in correspondingly increased with the increased split application of N fertilizers (Table 3). Both S3 and S4 significantly increased CGR, NAR and RGR. In addition to the abovementioned plant height, LAI and aboveground biomass. Gradual supplementation of N fertilizers from sowing to the initial flowering stage enhanced the soybean's growth. Notably, the beginning of the flowering stage is characterized as a rapid stage with the plant transitioned from vegetative growth to reproductive growth. A substantial amount of nutrients are required at this stage to maintain a better canopy for photosynthesis and accordingly, higher biomass production and grain yield.

These above-mentioned findings are in accordance with results from other studies. For example, Begum et al. (2015) applied 25 and 40 kg N ha^{-1} at sowing and 25 DAS respectively while Mohan and Angadi (2016) supplied 60 kg N ha^{-1} in equal split, each at basal and 40 DAS. Both group of researchers reported significantly higher plant height, LAI and biomass. Furthermore, the application of 20, 25, 10 and 66 kg N ha^{-1} as basal, at V3 stage, 25 DAS and R1 stage or 1.8 g N cm^2 at sowing and 4.2 g N cm^2 at R3 of soybean produced a significantly higher LAI (Zhang et al., 2014; Begum et al., 2015; Mon et al., 2017). Significantly higher biomass was also obtained when 30 N ha^{-1} was applied at sowing and 50 kg N ha^{-1} at the R1 stage (Gan et al., 2002) and higher biomass production was reported when 30 kg N ha^{-1} was applied at 42 DAS (Masaka et al., 2007). The application of 50% before sowing and 50% at a full flowering stage at a total rate of 80 kg N ha^{-1} resulted in higher LAI, biomass production and leaf photosynthesis (Caliskan et al., 2008).

RGR, CGR and NAR are the important plant growth parameters that indicate the net increase in dry matter per unit of dry matter, gain in dry matter production on a unit of land area, respectively. Results from our field study showed that these parameters in parallel with an increasing split number of N fertilization (Table 3). A similar increment was also observed in other studies with the application of 60 kg N ha^{-1} in four splits (basal ½ + V3 $^1/_8$ + R1 $^1/_8$ + R3 $^1/_8$, R5 $^1/_8$) (Mon et al., 2017) or the fertilization of 1.8 g N cm^2 at sowing and 4.2 g N cm^2 at the R3 stage (Zhang et al., 2014) and the application of 0.6 g N pot^{-1} as basal and at the R1 stage (Youn et al., 2009).

The accumulation of exogenous N in plant tissue at the vegetative stage is important for higher biomass and yield production. The plant organ including leaf, pod wall and stem at the vegetative period acquire N from BNF and the soil. At the onset of the seed filling stage, plants remobilized their stored N from vegetative tissue to reproductive organ and fulfil most of the N demand for seed. Ortez et al. (2019) demonstrated that 61% of N remobilization from vegetative tissue to seed at the R5 stage in soybean and 12% of more N uptake from the soil at the seed filling stage. It also indicates that split application of N at the vegetative stage remobilize more N compared to a single N application at sowing (Table 4). Salvagiotti et al. (2009) studied the influence of the application of 180 kg N ha^{-1} application in two equal splits on N remobilization i.e., 50% before planting and at the vegetative stage of V6. As a result 164 kg N ha^{-1} was remobilized from vegetative to reproductive components, which was 24% greater than a single application either before planting or at the R5 stage. Accordingly, this also translates to a close relationship between biomass, seed components and seed yield. Moreover, Fageria (2014) has indeed shown that there is a strong positive relationship between plant dry weight and seed yield for soybean and faba bean.

In this study, the effects of splitting the N fertilizer produced a consistent trend in aboveground biomass, yield components and seed yield at 30, 60, 90 and 127 DAS (Table 4). Positive relationships were observed between the aboveground biomass, yield components and seed yield (Figure 1 and 2). Seed yield tended to increase with increasing accumulation of aboveground biomass (r^2 = 0.90, P = 002). A linear regression produced a slope of 0.44 t ha^{-1} of seed yield from 1 t ha^{-1} aboveground biomass. Accordingly, the positive relationship between ANUE with seed yield was observed in this study. This is expected since soybean yield is heavily influenced by N uptake. The data from our field experiment further indicate that split application of 30 kg N ha^{-1} into S4 or S3 significantly increased pod $plant^{-1}$, total seed pod^{-1}, 1,000-seed weight, seed weight $plant^{-1}$ than with fewer splits application (S1 and S2) of the same N rate and the no-N control (Table 4). Our findings are in accordance with results from other studies where 60, 50, 20, 10 and 25 kg N ha^{-1} rates were applied before sowing, at V2, 25 DAS, R1 and R2 stages and significantly increased pod $plant^{-1}$, total seed pod^{-1}, 1,000-seed weight, seed weight (Gan et al., 2003; Singh and Singh, 2013; Begum et al., 2015; Bobrecka-Jamro et al., 2018).

At the harvest at 127 DAS, soybean aboveground biomass and yield production increased with increased frequency of split N fertilization (Table 4). Among the four split treatments, S_4 or S_3 significantly increased seed yield, straw, total aboveground biomass, compared to S_2, S_1 or control (Table 4). Seed yield treated in S4 or S3 significantly increased by 25% and 18% over S1 and 21% and 14%, respectively over S2 and 45 and 37% compared to the control. Similar findings were also reported in the previous studies. For instance, lowering the dose of N fertilization and the timing of application produced a significantly higher seed yield of soybean (Gan et al., 2002; Masaka et al., 2007) or splitting the rate from 60 or 90 to 30 kg N ha^{-1} as a basal, 30 DAS, and at the (R1) stages (Sawyer 2001; Bobrecka-Jamro et al., 2018). Splitting the application of 30, 60, 90 kg N ha^{-1} as basal and topdressing also resulted in higher straw, biological and seed yield (Singh et al., 2001).

Economically, increasing the frequency of split fertilizer treatments incurred a higher cost by 3.7, 4.6, 8.4, 10.5 % from S1 to S4 (Table 5). Compared to the control, the S3 and S4 treatment produced significantly higher gross returns of 38.7 and 48.5 %, respectively and net returns of 142.1 and 178.1 %, respectively. The net returns were mainly due to the grain yield associated with the market price. Despite incurring a higher cost of cultivation with the splitting frequency of fertilizer treatment, the benefits outweighed the cost because higher grain yield and gross net returns were obtained when the soybean plants were treated with an increased split application of N fertilizer (Table 5).

The benefit-cost (BC) ratio is an index of gross return and cost of cultivation (fertilizers, crop management costs, etc.) (Table 5). Similar benefits were also reported in other studies. For example, Ali et al. (2015) applied 90 kg N ha^{-1} in two equal split as ½ at sowing + ½ at 30 DAS in addition to 90 P_2O_5 + 60 K_2O kg ha^{-1} at sowing. As a result, the researchers obtained a higher BC ratio (1:6.05) as compared to the application of 120 kg N ha^{-1} in two equal split as ½ at sowing + ½ at 30 DAS + 90 P_2O_5 + 90 K_2O kg ha^{-1}. In another study, Chowdhury et al. (2014) conducted a field study on soybean and applied three doses of 17, 25, 28 N kg ha^{-1} along with other macro- and micro-nutrients. Each N dose was applied as in two equal splits at sowing and 22 DAS, and the results revealed that the application of 17 kg N ha^{-1} achieved significantly higher gross return (1347 US$ ha^{-1}), net returns (726 US$ ha^{-1}) and BC ratio (2.16) compared to other treatments in the study.

In the present study, however, it is important to highlight that the S3 treatment produced favourable outcomes compared to the S4 treatment since there were no significant differences in the agronomic performance between the two treatments. Besides, the S3 treatment is also more cost-effective and produced fairly decent economic returns compared to S4. This will bring relief to the farmers since a farmer who is taking care of a specific crop only received 1/6 of the total yield from her or his landlord at the end of a crop season in Afghanistan.

Conclusion

Both treatments in the three (33%) and four (25%) times of N split fertilization for a total rate of 30 kg N ha^{-1} have improved the N use efficiency and met the N requirement of soybean's growth and yield production. They also produced significantly positive effects on the growth and yield of soybean than those at one (100%) or two (50%) times of N split fertilization. Although splitting the N application four times would produce maximum agronomic performance – highest ANUE and seed yield, it was also costly with a disadvantage to the farmers. Results from this present study demonstrate that splitting the N fertilizer up to three times is both agronomically and economically optimum and therefore recommended for practice in Afghanistan.

Acknowledgements

This research was supported by the Higher Education Development Program, World Bank Kabul, Afghanistan under grant number (P146184) and the Afghanistan National Agricultural Sciences and Technology University (ANASTU), Kandahar, Afghanistan, and a PhD Scholarship from the China Scholar Council (2017GBJ009160).

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Eurasian Journal of Soil Science 2022, 11(1), 43 - 60

Eurasian Journal of Soil Science

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

A review on nanobioremediation approaches for restoration of contaminated soil

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Article Info

Received : 19.02.2021
Accepted : 29.08.2021
Available online : 03.09.2021

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Abstract

Nanotechnological approaches are emerging as one of the most contemporary restoration strategies that may be used to remove a variety of contaminants from the environment, including heavy metals, organic and inorganic pollutants. The application of nanoparticles (NPs) is entrenched with biological processes to boost up the removal of toxic compounds from contaminated soils. Many efforts have been taken to increase the effectiveness of phytoremediation such as the addition of chemical additives, application of rhizobacteria, and genetic engineering, etc. In this context, the integration of nanotechnology with bioremediation has introduced new dimensions to the reclamation methods. Thus, advanced remediation methods that combine nanotechnology with phytoremediation and bioremediation, where nano-scale process regulation aids in the absorption and breakdown of pollutants. NPs absorb/adsorb a variety of contaminants and also catalyze reactions by lowering the energy required for their breakdown due to unique surface properties. As a result, these nanobioremediation procedures decrease the accumulation of contaminants while simultaneously limiting their dispersal from one medium to another. Therefore, the present review is dealing with all the possibilities of the application of NPs for restoration of contaminated soils.

Keywords: Phytoremediation potential, phytorestoration strategy, NPs, contaminated soils, plants, microorganisms.

Introduction

Soil is the essence of agriculture and it is enriched with vital macro and micronutrients that promote healthy growth of the crops that ultimately impart health benefits to humans (Joshi et al., 2020). Several anthropogenic activities contaminate the soil with a load of synthetic organic compounds, heavy metals, agrochemicals, and an excess of nutrients as well (Minkina et al., 2019; Ghazaryan et al., 2020). Similarly,

: https://doi.org/10.18393/ejss.990605
: http://ejss.fesss.org/10.18393/ejss.990605
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

industrialization/urbanization is adding solid wastes, chemicals and solvents, and other persistent organic and inorganic materials to different environmental matrices (Midhat et al., 2019).

Advancement in nanotechnology and nanoscience provide new directions to research and development in almost every field of science. It is an expanding research field that involves structures, devices, and systems with unique properties owing to the arrangement of their atoms at the nanoscale (1–100 nm) (Bayda et al., 2019; Rajput et al., 2020b; 2021b). In recent decades, nanotechnology has been used in a range of contexts, notably medicine, textiles, pharmaceutics, electronics, optics, cosmetics, sports, and many others. The application of NPs in agriculture was accepted at the beginning of the twenty-first century (Fraceto et al., 2016), and more than 232 products are available for various agricultural uses (Rajput et al., 2021a). Also, it has not remained static in the field of environmental restoration (Guerra et al., 2018; Singh et al., 2020).

Recently, nanobioremediation (NBR) is declared as a technology for cleaning up environmental contamination by accelerating natural biodegradation processes using NPs. NBR is defined as a process that uses NPs with microorganisms, or plants to eradicate hazardous contaminants from the soils (Cecchin et al., 2017). Following that, distinct NBR procedures are defined based on the type of organism used for contaminants remediation (i.e., nanophytoremediation, and microbial nanoremediation (Burachevskaya et al., 2020; Rajput et al., 2020a,c; Singh et al., 2020; Kumari et al., 2021). The intensification in the expenses of chemical as well as physical processes, microbes- and plant-mediated NBR technologies are receiving more attention.

Coming to the benefits of NBR, there is a multitude of reasons why nanotechnology is integrated with bioremediation. For example, NPs have a large surface area per unit mass, which means that a greater number of particles can come into contact with the environment, boosting the remediation process (Fernández-Luqueño et al., 2018; Kaur et al., 2018). Thus, NBR efforts to minimize pollutant concentrations to risk-based thresholds while also decreasing secondary environmental impacts. Furthermore, this method of reclamation also combines the advantages of nanotechnology and bioremediation to create a remediation process that is more efficient, faster, and environmentally benign than the individual methods (Patil et al., 2016; Kumar et al., 2021).

However, every advance of the process of remediation has particular explicit merits as well as demerits that need to be taken into consideration for each location. In a nutshell, after the extensive literature survey, it can be concluded that integration of bioremediation with nanotechnology appears to be a feasible alternative to conventional remediation technologies either in sequence or in parallel to them. However, there are still more studies and development measures necessary to bring these types of sustainable technology to the market for full implementation.

Recent advances in bioremediation of polluted soil

Chemical and physical remediation, incineration, and bioremediation are some of the NBR technologies that are currently in use. With recent advances, NBR provides an environmentally friendly and economically viable option for removing contaminants (Patra Shahi et al., 2021). The fundamental principle behind the NBR is depicted as the degradation of organic wastes employing nano-catalysts as a medium that allows them to enter deep within contaminants, thereby executes the whole process safely without modulating the environment (Rizwan et al., 2014; Cecchin et al., 2017; Chauhan et al., 2020). The overview of NBR is presented in figure 1.

As bioremediation relies on live species to clean up contaminated environments, thereby a good relationship between NPs and living organisms is critical for the efficacy of this phenomenal technique (Sangwan and Dukare, 2018; Paterlini et al., 2021). In this context, it is documented that the physical and chemical interactions between NPs, biota, and contaminants are influenced by numerous factors viz., NPs' size and shape, surface coating, and chemical nature. Plus, the nature of contaminants, the type of organism used, the media, pH, and temperature are also recorded to impact the process considerably (Ibrahim et al., 2016; Tan et al., 2018).

These events grow complicated due to the large number of potential parameters that have a direct or indirect influence on such interactions. For example, temperature and pH of media are reported as important factors for the optimal development of biological organisms (Patra and Baek, 2014). Now pinpointing the different actions, such as dissolution, absorption, and biotransformation may occur when NPs and biota interact (Kranjc and Drobne, 2019; Vázquez-Núñez et al., 2020). On the other hand, interactions of NPs and biota can be toxic or stimulating which results in a biocidal or bio-stimulant effect, thus the performance of organisms involved in the NBR process could be impacted (Juárez-Maldonado et al., 2019).

Some of the most important NPs used in NBR are nano-iron, nanosized dendrimers, carbon nanotubes, single enzyme NPs, engineered NPs, etc. (Kaur et al., 2018; Patra Shahi et al., 2021). In the NBR technique, the contaminants are first broken down by NPs to a level that is conducive to biodegradation, and then the contaminants are biodegraded. The main advantages of bioremediation over conventional strategies are high competency, reduced generation of chemical and biological wastes, selectivity, no additional nutrient requirements, bio-sorbent regeneration, the probability of metal recovery, etc. (Juwarkar et al., 2010; Rizwan et al., 2014; Chauhan et al., 2020).

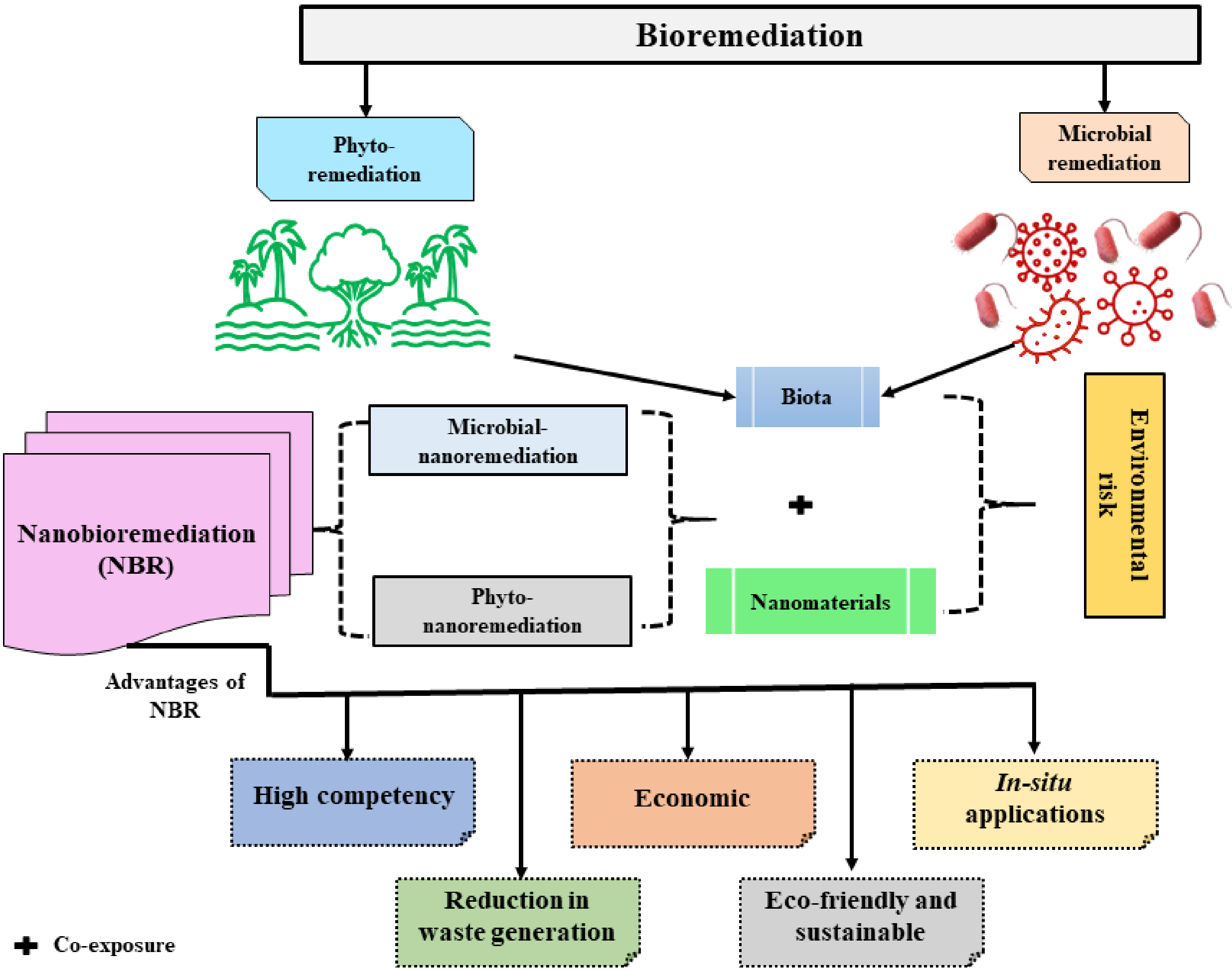

Figure 1. The pictorial representation of nanobioremediation and its types along with environmental risks

On top of it, most currently available conventional remediation procedures are based on the classic *ex-situ* strategy, which entails excavating contaminated material and then treating them with conventional means. Plus, some of these processes are energy-intensive, which makes them expensive, and they may also leave concentrated hazardous waste residues that require additional treatment and disposal (Wuana and Okieimen, 2011; Chauhan et al., 2020). On the flip side, *in-situ* remediation methods benefit greatly from the peculiar characteristics of NPs (Kumari et al., 2021; Rajput et al., 2021b). Thus, in-situ NBR can annihilate the need for draining out of groundwater and transportation of contaminated soils to treatment and disposal sites.

Nanophytoremediation of polluted soils

Nano-encapsulated enzymes also have greater potential in treating some complex organic pollutants, for example, persistent pesticides (organochlorines) and long-chain hydrocarbons are hard to degrade by microbial or plant remediation process (Chauhan et al., 2020). Few successful field applications of NPs have been done in past for the bioremediation of soils.

Heavy metals

Heavy metal pollution of arable soils is an increasing problem, as it poses a serious threat to food safety, public health, and the food chain and ecosystem. For *in-situ* treatment of polluted soils, phytoremediation is documented as a favored and cost-effective method by researchers (Liang et al., 2017). Phytoremediation of soils polluted with cadmium, chromium, lead, nickel, and zinc was improved with the introduction of NPs, according to several studies (Wani et al., 2017; Ekta and Modi, 2018; Kanwar et al., 2020). It is a well-established that exposure to heavy metal pollutants has major health risks to the well-being of humans (Rajput et al., 2020d; Zamani et al., 2020).

Phytoextraction is the most familiar method adopted to eliminate heavy metals from polluted soil (Ali et al., 2013). The application of NPs to enhance the phytoextraction efficiency has been a successful strategy towards nanophytoremediation (Ebbs and Kochian, 1998; Ghazaryan et al., 2018; Ghazaryan et al., 2019). Iron NPs are used as a strong reductant for those pollutants that require a reduction process for degradation (Sun et al., 2006), whereas, zerovalent iron (nZVI) has great potential in phytoremediation of a range of pollutants as it is a highly reactive reducing agent. Plants treated with the lower concentrations (100- 500 mg/kg) of nZVI have exhibited the maximum accumulation (1175.40 g per pot with 100 mg/kg of nZVI). Whereas, a higher dose of nZVI NPs (500-1000 mg/kg) caused oxidative stress in *Lolium perenne* thereby reducing the uptake of Pb (Huang et al., 2018). Another study reports the similar characteristics of nZVI of concentration 100-500 mg/kg that improves Pb uptake up to 857.18 µg per pot (at 500 mg/kg) in the ragweed *(Kochia scoparia* also known as *Bassia scoparia*).

The TiO_2 NPs of 100, 200, and 300 mg/kg spiked in soil have shown Cd accumulation in *Glycine max* by 1.9, 2.1, and 2.6 folds in the shoots and 2.5, 2.6, and 3.3 folds in roots, respectively. However, 1534.7 mg/kg per pot of Cd was reported to be the maximum accumulation (Singh and Lee, 2016). The inoculation of *Acaulospora mellea* considerably enabled the immobilization of heavy metals. The acceptable concentration of nZVI was 50 mg/kg to 1000 mg/kg (Cheng et al., 2021).

The concentration of nZVI at 100, 500, and 1000 of mg/kg showed effective uptake of Cd in the *Boehmeria nivea* L. root, stem, and leaves by 16–50%, 29–52%, and 31–73%, respectively (Gong et al., 2017). Arbuscular mycorrhizal (AM) fungi, *A. mellea* along with nZVI have shown uptake of Cd, Pb, and Zn from the acidic soil by *Sorghum bicolor* L. A table has been appended below that exhibits the role of NPs in the phytoremediation of heavy metals (Table 1).

Organic pollutants

Organic pollutants are a major threat to agricultural soil, food chain, ecosystem, and human health. They are majorly released from industrial operations and agricultural applications (Alharbi et al., 2018). Phenols, polycyclic aromatic hydrocarbons (PAHs), organochlorine insecticides, and polychlorinated biphenyls (PCBs) are all examples of cyclic organic compounds that are documented as persistent organic pollutants (Sushkova et al., 2016, 2018). Many of them are lipophilic, thereby they tend to get bioaccumulated and biomagnified in adipose tissues of several organisms in the food chains of aquatic and terrestrial ecosystems (Penell et al., 2014).

Hence, phytoremediation is always considered as a cost-effective and sustainable approach to remediate these organic pollutants (Kang et al., 2018). Application of NPs in phytoremediation of organic pollutants like trichloroethylene, endosulfan, and trinitrotoluene have been reported in the past (Pillai and Kottekottil, 2016). Fullerene NPs have been reported to enhance the uptake of trichloroethylene using *Populus deltoides*, 2 and 15 mg/L of fullerene NPs have enhanced the uptake by 26% and 82%. *Plantago major* with the appropriate adsorbent (activated charcoal) and solubilizing agent, SiO_2 as green synthesized NPs of Fe and Ag namely *Ficus*-FeNPs (F–Fe0) (size 2.46 nm–11.49 nm), *Ipomoea*–Ag (Ip–Ag0) (size 6.27 to 21.23 nm) and *Brassica*–AgNPs (Br–Ag0) (size 6.05 to 15.02 nm) were able to remove 93.7%, 91.30%, and 92.92%, respectively of chlorfenapyr (Romeh and Saber, 2020). Studies have also reported that pollutants like chlorpyrifos, molinate and, atrazine could be removed and broken by nanosized zerovalent iron.

Plants that absorb contaminants in their tissues to breakdown and detoxify from the environment are used in nanophytoremediation. Plants that are favored for phytoremediation purposes should have characteristics such as:

- Fast grower with higher biomass producer
- The highly branched and well-developed root system
- Potential to tolerate and accumulate pollutants
- Higher sink potential that allows hyperaccumulation
- Easy harvesting of plant's sink organs
- Genetic manipulation should be easier, and
- It should be non-consumable by humans

Table 1. Role of engineered NPs in the phytoremediation of Pb and Cd from the soil

Pollution	Applied NPs	Mode of action of NPs	Plant name	Remarks	Reference
Pb	nZVI	Lower concentration of nZVI promoted plant growth	*Kochia scoparia*	100-500 mg/kg of nZVI enabled 857.18 µg per pot of Pb accumulation in the plant	Zand and Tabrizi, 2020
	Nanohydroxyapatite	Promoted plant growth through phosphate mobilization in the soil	*L. perenne*	With nano-hydroxyapatite the concentration of Pb in the root was reduced by 2.86- 21.1% and in the shoots 13.19-20.3% reduction of Pb was observed	Ding et al., 2017
	nZVI	Lower concentration of nZVI promoted plant growth	*L. perenne*	Accumulated maximum concentrations of Pb in the root and shoot of the plants	Huang et al., 2018
	Nanohydroxyapatite	Reduced phytotoxicity, and enhance plant growth	*L. perenne*	The 21.97% remediation efficiency was observed within 6 weeks	Jin et al., 2016
Cd	TiO_2	Improved germination and photosynthetic capacity of the plant	*Glycine max*	Concentration (100 to 300 mg/kg) dependent increase in the uptake of Cd was observed (128.5 µg -507.6 µg of Cd per plant)	Singh and Lee, 2016
	nZVI	Promoted plant growth	*Boehmeria nivea*	Increase in the accumulation of Cd in the leaves by 31-73%, stems by 29-52%, and roots by 16-50% were recorded	Gong et al., 2017
Pb, Cd	nZVI	Promoted plant growth	*Sorghum bicolor*	Enhanced uptake of Pb and Cd of the concentration 50 mg/kg to 1000 mg/kg	Cheng et al., 2021
Pb, As	CNT with biochar	Reduced seed germination; however, toxicity was modulated by biochar	*Brassica rapa*	Pb was reduced by 1.2–3.8-folds and significantly reduced As accumulation in the soil	Awad et al., 2019
As, Cd, Pb, Zn	nZVI	Stabilized HMs	*Helianthus annuus*, *L. perenne*	Reduced up to 60% uptake of As, Cd, Pb, and Zn in roots and shoots compared to the control plants	Vítková et al., 2018
Cd, Pb	Nano-silica	Improved the availability of Pb and Cd to the plants, and also promoted the growth of the plant	*Secale montanum*	Accumulation of Pb in the roots was achieved up to 533.6 mg/kg DW and Cd up to 208.6 mg/ kg DW.	Moameri and Khalaki, 2019

Microbial nanoremediation

Microbes-mediated nanoremediation, a novel and efficient approach, involved the cellular enzymes secreted by microorganisms that successfully degraded and cleaned up the broad variety of organic pollutants in the contaminated ecosystem (Sangwan and Dukare, 2018; Torimiro et al., 2021). Numerous environmental conditions limit and influence the efficiency with which pollutants are degraded by microbes in contaminated soils. Within a microbial association, the biological response to environmental pollutants is differed, and the presence of co-contaminants may bring out changeable reactions to the bioremediation process (Sangwan and Dukare, 2018; Rajput et al., 2021c; Shende et al., 2021). Despite this, NBR offers a proficient and lucrative approach for contaminated soil and waste or groundwater treatment.

Microbes-mediated nanoremediation of xenobiotics is a fundamental environment-friendly approach to eradicate persistent toxic compounds gathered in the surroundings. The capacity of microbes to metabolize, transform, as well as degrade, xenobiotic compounds has been documented as a competent approach to remove dangerous and toxic wastes (Agarry and Solomon, 2008). Microorganisms are preferably appropriate to remove pollutants due to the enzyme system present that allocates them to utilize ecologically noxious pollutants as their energy and food source. The progressions in bioremediation science have been accredited to the individual as well as interdisciplinary contribution afforded by scientific areas of analytical chemistry, microbiology, biochemistry, molecular biology, environmental engineering, and very recently, nanobiotechnology (Hu et al., 2014; Sangwan and Dukare, 2018; Singh et al., 2020). The process of bioremediation includes mineralization and detoxification, in which the transformation of waste into inorganic compounds, like water, methane, and carbon dioxide has been carried out (Liu et al., 2018a;

Vázquez-Núñez et al., 2020; Paterlini et al., 2021). Microbes can alter almost all organic materials, with catalytic mechanisms and wider diversity (Paul et al., 2005). They can function still in anaerobic plus extreme environmental conditions, which constructs them a smart candidate for the process of bioremediation.

Additionally, microorganisms play a significant function in biogeochemical cycles as well as the ecosystems' sustainability. The conversion of xenobiotic contaminants by microbes may occur either in an anoxygenic or oxygenic environment. Nevertheless, molecular oxygen contributes to aliphatic as well as aromatic xenobiotic compounds (Cao et al., 2009; Sinha et al., 2009). Amid the different microorganisms, bacteria have been established as the most competent and prevailing in the natural bioremediation processes. In both the conditions, i.e., aerobic as well as anaerobic, bacteria have developed an approach for acquiring energy from nearly every compound by electron acceptors like ferric ions, sulfate, nitrate, etc. Several genera of bacteria, e.g., *Alcaligenes*, *Acinetobacter*, *Bacillus*, *Escherichia*, *Gordonia*, *Moraxella*, *Micrococcus*, *Pseudomonas*, *Pandoraea*, *Rhodococcus*, *Streptomyces*, and *Sphingobium* either independently or in amalgamation are implicated in oxygenic breakdown. In contrast, bacterial genera concerned with the anaerobic degradation of xenobiotics include *Azoarcus*, *Clostridium*, *Desulfotomaculum*, *Desulfovibrio*, *Geobacter*, *Methanococcus*, *Methanosaeta*, *Pelotomaculum*, *Syntrophobacter*, *Syntrophus*, and *Thauera* (Jindrova et al., 2002; Van Hamme Jonathan et al., 2003; Kulkarni and Chaudhari, 2007; Weelink et al., 2010; Sangwan and Dukare, 2018)

The remediation of extremely persistent and xenobiotic water and soil contaminants, such as hydrocarbons, heavy metals, dye in textile (acid dyes, cationic dyes, azo dyes), pharmaceutical constituents (antibiotics and antiseptics), and other such contaminants are critical for wastewater and soil treatment and its future application. These contaminants increase pollution and pessimistically affect the environment (Koul et al., 2021; Sushkova et al., 2016).

Since NPs have a larger surface area and are smaller, they can act as catalysts or adsorb contaminants above a larger surface area. Numerous reports documented the catalytic properties of various NPs together with the biological components have been assessed to reduce harmful pollutants (Zhao et al., 1998; Kharissova et al., 2013). Many microorganisms have been utilized to hone NPs exploitation for the NBR process as several researchers reported encouraging outputs in the application of microbe-mediated NPs in the process of remediation.

An extensive recognition of microbes for this scientific approach was recognized owing to their exceptional chemical, physical, biological, as well as optical properties like super-hydrophobic and filtering nature, sensitive affinity membranes, modifiable functionality, as well as a higher surface-to-volume ratio (Sarwar et al., 2017; Wang et al., 2015; Sangwan and Dukare, 2018).

A detailed description of microbes-mediated nanoremediation has been given in the forthcoming sections.

Hydrocarbons

Many researchers have been reported that the microbes-mediated nanoremediation of persistent organic pollutants; i.e., hydrocarbon. It was reported the electrostatic interaction of magnetic NPs functionalized by *Rhodococcus erythropolis* harnessing system that substantively bio-desulfurize hydrocarbon component dibenzothiopene (DBT) by 56%. Thus, validating the advantage of magnetic NPs functionalized by *R. erythropolis* above the solitary exploitation of every component for bioremediation (Ansari et al., 2009). The efficient synergistic effect of the nZVI with *Sphingomonas* sp. as an effectual twosome towards the de-bromination and gradual polybrominated diphenyl ethers (PBDEs) degradation in aqueous solution (Kim et al., 2012). Alternatively, the feasibility of the combined employ of bimetallic (Pd/nFe) NPs and *Sphingomonas wittichii* for the NBR 2,3,7,8-tetrachlorodibenzo-p-dioxin hydrocarbon was also recognized (Bokare et al., 2012). The active dechlorination facilitated by integrated hybrid (nano-bioredox) resulted to form dibenzo-p-dioxin.

A study has revealed the applications of Sphingomonas sp. as a bio-functionalized tool for carboxymethyl cellulose (CMC) stabilized bimetallic (Pd/Fe) NPs (Singh et al., 2013). The nano-composite was found to be triumphant for the deprivation of gamma-hexachlorocyclohexane (γ-HCH), generally identified as lindane and the main component in cosmetics (Singh et al., 2013). The study was performed to remove Aroclor 1248- a congener of PCBs, where the noteworthy de-chlorination, as well as conversion of the contaminant, was observed by the treatment of bimetallic (Pd/Fe) NPs under anoxic surrounding resulted in the formation of biphenyls (Le et al., 2015). Progressive bioremediation of the resulting biphenyls further catalytically decreased the persistent Aroclor 1248 from 33.8×10^{-5}µg/g to 9.5×10^{-5}µg/g with *Burkholderia* *xenovorans* (Le et al., 2015). The silica NPs biofunctionalized with lipid bilayers of *Pseudomonas aeruginosa* was investigated to clean up PAH (benzo[a]pyrene) (Wang et al., 2015). The 1,2-dimyristoylsn-glycero-3-phosphocholine, lipid molecule playing a dynamic role, to improve the sequestration or adsorption of the PAHs, when conjugated by silica NPs. The biofunctionalized graphene oxide NPs with laccase enzyme developed by *Trametes versicolor* were studied for their potential as well as combine enhance for the biodegradation of PAH (anthracene) (Patil et al., 2016). The amalgamation of laccase enzyme from fungi as conjugant was reported to have the enhanced ability of degradation than their single application and also extended their stability. The polymer (polyallylamine hydrochloride)-layered magnetic NPs functionalized by *Alcanivorax borkumensis* established an opportunity for vigorous hydrocarbon degradation (Konnova et al., 2016). Exceptional features like forming the neutral lipid inclusions in biofilms of *A. borkumensis*, the biosurfactant micelle ascertain the opportunity of hydrocarbon decomposition.

Bacillus licheniformis-mediated nanoremediation process was evaluated bio-functionalization of $Zn_5OH_8Cl_2$ modified Fe_2O_3 NPs with *B. licheniformis* to break crude oil into naturally degradable compounds. Additionally, demonstrate some prospects on the promising improvement of microbial bio-surfactants for efficient NBR of widespread oil pollution (El-Sheshtawy and Ahmed, 2017). The synergistic effect concerning iron oxide NPs and *Alkaligenes faecalis* improved the crude oil biodegradation in the contaminated environment (Oyewole et al., 2019). The authors observed that assessing variable deliberations of *A. faecalis* with iron oxide NPs, at 200 mg efficiently cleans up crude oil pollution.

Heavy metals

Microbes-mediated nanoremediation of heavy metals corroborates the potential of microorganisms in cleaning up the environment. NPs' effectiveness in bioremediation was accomplished during the *in-situ* fabrication of palladium (Pd) NPs from Pd (II) ions intervened by *Clostridium pasteurianum* acquired from sandy aquifer matter. The biosynthesized Pd NPs evidenced positive remediation in the alteration of hexavalent chromium; i.e., Cr (VI) into insoluble Cr (III) and, therefore, leading to the production of hydrogen gas (Chidambaram et al., 2010). In this study, the removal rate of Cr (VI) was considerably improved, reaching 7.2 g, indicating the importance of nano-catalysts over traditional in situ bio-simulation techniques. A comparable strategy accomplished was channeled towards reduction of Cr (VI) by sodium alginate, polyvinyl alcohol (PVA), as well as a matrix of carbon nanotubes (CNTS) immobilized upon *Pseudomonas aeruginosa* cells (Pang et al., 2011). The biogenic Cr (VI) reduction to soluble Cr (III) was shown in wastewater by the immobilized bacterial cells (Nancharaiah et al., 2010).

In the NBR process, algae also have revealed their significance. Iron NPs fabrication by *Chlorococcum sp*. demonstrated a noticeable elimination of Cr (VI) to Cr (III) about 92% of 4 mg/L (Subramaniyam et al., 2015). Iron NPs synthesized from algae was mediated with the biomolecules from algal cell illustrated more excellent stability, high reactivity, and proficient toxic pollutants reduction in the environment. On the other hand, the biogenic role of *Lysinibacillus sphaericus* in the production of magnetic oxide NPs intended to remove Cr (VI) contamination from the surroundings (Kumar et al., 2019). The authors reported the employ of exopolysaccharides (EPS) matrix of biofilm derived from *L. sphaericus* as a superior reducing, capping, and stabilizing agent, acquiring several binding sites for different metal ions. Magnetic oxide NPs functionalized with EPS illustrated the improved potential to adsorb Cr (VI). In another study, it was reported the integration of *Chlorella vulgaris* in ultrafine bi-metallic i.e., TiO_2/Ag chitosan nanofiber mats, as a functionalized agent, elucidated the significance of algae in the photo-removal strategy of Cr (VI) under UV light irradiation (Wang et al., 2017a). The discharge of organic substances such as chlorophylls, carboxylic acids, etc., through *C. vulgaris*, was documented to have an improved photocatalytic reduction of Cr (VI) on the TiO_2/Ag chitosan nanofiber mats, confirming the synergistic way of hybrid NPs by algae and TiO_2/Ag.

The fabrication of lead sulfide i.e., PbS NPs from *Rhodosporidium diobovatum* demonstrating the prospect of a straightforward breaking down of Pb(II) ions into less toxic and helpful forms by fungi (Seshadri et al., 2011). The triumphant elimination of Cd in Cd-polluted water illustrated the competence of *Pseudomonas aeruginosa* improved Cd bioreduction which in turn hasten the cadmium sulfide (CdS) NPs biosynthesis (Raj et al., 2016). Likewise, the removal and bioremediation of Cd from Cd-polluted soils also evaluated (Liu et al., 2018b). The authors demonstrated that the co-treatment of *Bacillus subtilis* and nano-hydroxyapetite (NHAP) efficiently eliminated the Cd contamination, encouraging the propagation of microbial community of rhizosphere along with the diversity of bacteria in the remediated soil (Liu et al., 2018b).

The evaluation of somewhat variable biofunctionalized approach including polyvinylpyrrolidone (PVP)-coated iron oxide NPs intermingled with *Halomonas* sp. isolated from the oil-contaminated soil, has been reported (Alabresm et al., 2018). Selenium NPs were found efficient in NBR of mercury polluted soil; those

NPs were formed by the occurrence of *Citrobacter freudii* (Wang et al., 2017c). The alteration of elemental form of (Hg0) to the insoluble form mercuric seleniide (HgSe) with biogenic selenium NPs evaluated under aerobic as well as anaerobic conditions accounted for a bioremediation value 39.1-48.6% and 45.8-57.1%, respectively. The nickel compound was removed in the effluent by introducing *Microbacterium* sp. resulting in the production and recovery of nickel oxide NPs (Sathyavathi et al., 2014). In another study, the potential of *Hypocrea lixii* was discovered to reduce noxious metals, specifically nickel, in contaminants and devising the nickel oxide NPs biosynthesis from the waste for further applications (Salvadori et al., 2015).

Recently, it was demonstrated that the silver (Ag) NPs synthesized through greener way assisted by *Bacillus cereus* was supported with alumina, found efficient in NBR of pharmaceutical effluents restraining heavy metals, mostly chromium (Cr) and lead (Pb) (Kumari and Tripathi, 2020). The bacterial cell-mediated nano-adsorbent method certified to remove about 98.13% (Cr) and 98.76% (Pb) that were discharged from pharmaceutical industries as waste effluents. The possibility of nanobioremediation of cadmium (Cd) and lead (Pb) in the soil by the mutual exploitation of *Escherichia coli* along with metal NPs towards the elimination of these heavy metals (Zhu et al., 2020).

Pharmaceutical ingredients

The recurrent emancipation of pharmaceutical ingredients (antiseptics and antibiotics) in wastewater is considered a serious concern. These mainly originate from domestic and industrial effluents, which have polluted not only the environment but also enhanced the appearance of antibiotic-resistant microbes in wastewater (Adesoji et al., 2020). Nevertheless, the prospect of eliminating these pharmaceutical ingredients by the NBR strategy was evaluated as per the many research studies. The biosynthesis of both Au and Ag NPs using *Turbinaria conoides*, an alga which was found useful as an antimicrofouling agent (Vijayan et al., 2014). Hydrogen peroxide, a common pharmaceutical ingredient, yet a pollutant of the environment, was proficiently removed from waste effluents from industries by the electrocatalytic reduction of the compound aided with Pd NPs synthesized *Sargassum bovinum* (Momeni and Nabipour, 2015).

Micro-accumulation of triclosan, which has been found to be linked with cancer, has frequently been used as an antibacterial and antiseptic agent. Nevertheless, the significance of fungi (*Trametes versicolor*) as an essential biofunctionalized agent for bimetallic (Pd/Fe) NPs to remove triclosan in liquid effluents was established (Bokare et al., 2010). In this work, *T. versicolor* was observed to secrete laccase enzyme that was found to play a vital role in the two-step redox strategy, which involved the anaerobic dechlorination as well as sequential oxidation of 2-phenoxy phenol. Similarly, (Adikesavan and Nilanjana, 2016) described the magnesium oxide (MgO) NPs biofunctionalization by yeast (*Candida* sp.). The myco-nano approach was found to have hastened the process of Cefdinir degradation and treatment in an aqueous environment. A group of bacteria conquered by *Bacillus* and *Pseudomonas* spp. accountable for the biosynthesis of manganese oxide (MnO) NPs was found to efficiently eradicate 1,2,4-triazole from wastewater (Wu et al., 2017). This study established the prospective of biogenic manganese oxide NPs to remove a variety of recalcitrant pollutants from bio-treated chemical industrial wastewater.

The efficiency of Pt and Pd NPs biosynthesized from *Desulfovibrio vulgaris* to remove effluents containing pharmaceutical compounds was reported. The numerous chemical compounds contribute greatly to the pharmaceutical industry. Likewise, 1,2,4-triazole used in different clinical applications because of a large number of compounds of the ring system. Besides, 1,2,4-triazole is also applied in the production of pesticides that often contributes to groundwater pollution during leaching (Martins et al., 2017). Similarly, picric acid (2,4,6-trinitrophenol (TNP), is a valuable constituent in the production of antiseptic, posturing hazard to the environment as a pollutant in an aqueous solution. The study established the progressive application of *Pseudomonas aeruginosa* mediated Fe_3O_4 NPs as a portion of multiwalled carbon nanotubes (MWCNT) to produce nanocomposite moderately employed for NBR of picric acid (Yousefi et al., 2020).

Dyes in textile

Dyes have been widely recognized as an essential component in a multitude of sectors, including cosmetics and textiles. Nevertheless, it is disposed-off mainly as liquid waste matter into the surroundings, which is poisonous to living beings (Asaduzzaman et al., 2016). A study ascertained the coalesce effect of biofunctionalized Ag NPs by *Chromobacterium violaceum* as a biosorption strategy to remediate washing water employed to process cotton fabrics (Durán et al., 2010; Duran et al., 2017). This process demonstrated the successful removal of organic compounds as well as dyes used in the production of fabrics. This treatment further illustrated its effectiveness for eliminating used Ag NPs and the revival of bacteria, posing lesser harm to the environment. The application of Ag NPs synthesized from *Bacillus pumilis* have been used to remediate the Congo red dye from wastewater, which was applied on cotton fabrics (Modi et al., 2015). The goal was to develop and implement an efficient method for removing Congo red dye because it is less resistant to light and washing. The highest revival of Ag NPs leached in the effluents to evade harm to the environment.

In another study, it was observed that Ag NPs competently decolorize the organic dyes during the catalytic activity and confirmed that NPs might be employed as catalysts in industries to degrade organic dyes with higher competence (Sharma et al., 2015). It has been reported that Ag and Au NPs demonstrated good catalytic activity in the removal of organic dyes. These NPs reduced the time requisite for eliminating dye while also competently improved the rate of reaction (Suvith and Philip, 2014). The Au NPs could also be employed as adsorbents for organic dyes. As Au NPs, comprising surface proteins produced from fungus *Cladosporium oxysporum* AJP03, efficiently enhanced the rhodamine-B organic dye adsorption (Bhargava et al., 2016). The roles of different NPs and nanocomposites such as TiO_2 NPs, FeNPs, magnetic NPs, bimetallic NPs, nanotubes, nanoclays, and nanosponges in the NBR of soil are also revealed (Koul and Taak, 2018). The authors accentuated that the synthesis of NPs by green methods might be an efficient approach for treating water and soil pollution. The efficient catalysis of Congo red dye by Ag NPs synthesized from green alga *Caulerpa serrulate* was reported (Aboelfetoh et al., 2017).

Even though methyl orange dye is infrequently employed in textile because of its susceptibility to acids, they still find expediency as a dye for wool fabrics, a type of contaminant in wastewater. Mechanism of NBR evaluated the consortium of *Cellulosimicrobium* sp., *Micrococcus lylae*, and *Micrococcus aloeverae* to produce TiO_2 NPs (Fulekar et al., 2018). The active degradation of methyl orange dye was achieved in a reactor by the influence of UV light. These rhizospheric root-associated microorganisms demonstrated the opportunity and efficiency of normal sources for the biosynthesis of NPs and around ~99 % of methyl orange dye photocatalytic degradation, a signal for the significance of photocatalytic process for a safe environmental and passable nanobioremediation system. A comparable discovery was recognized for algae *Hypnea musciformis* [wulfen] J.V Lamouroux-mediated synthesis of Ag NPs and their dynamic efficacy in humiliating methyl orange dye solution under visible light (Ganapathy Selvam and Sivakumar, 2015). An effort on the Azo dyes bio-reduction, which are imperative synthetic colorants are generally used in textile, paper manufacturing, printing, etc. was conceded out by Pd NPs fabricated from *Klebsiella oxytoca* (Wang et al., 2018). The synthetic organic colorants were effectively bio-reduced with recovery from the effluent liquids. The biosynthesized polysulfone nanofibrous web and *Chlamydomonas reinhardtii* were originated a synergistic effect that removes reactive dyes from wastewater (San Keskin et al., 2015).

The Ag NPs synthesized from microalgae *Caulerpa racemosa* and *Chlorella pyrenoidosa* were reported for the photo-catalytic degradation of methylene blue and the treatment of liquid effluent containing hazardous dye produced significant results i.e., dropping the level of the contaminants under controlled experimental conditions (Aziz et al., 2015; Edison et al., 2016). In recent work, the descriptive information on various approaches for the NPs synthesis using microbial cells; their applications in agriculture, bioremediation, diagnostics, and medicine; and their prospects are provided (Koul et al., 2021).

Other toxic chemicals

Besides these major groups of pollutants, there are found some other toxic chemicals in the environment. The biogenic synthesis of manganese oxide NPs by *Pseudomonas putida* documented the bacteria potential for sufficient removal of organic micropollutants (Furgal et al., 2015). Bisphenol A (BPA), generally known as an essential chemical substance exploited in the industries for developing resins and plastics, requisite for storage of food and beverages, has become an aggravation to the ecology. The elimination of bisphenol A by a route focused on applying MnO NPs biosynthesized from algae (*Desmodesmus* sp.) (Wang et al., 2017b). Commercially produced nitro compounds for solvents or chemical intermediates create a relatively extensive volume in effluents from industries (Torimiro et al., 2021).

The application of *Chlorella vulgaris* on nitrate removal from liquid effluents, in which algae played a dual role in biogenic production of Pd NPs and its immobilization on nanofibre mats prepared by an electrospun method that improves the catalytic activity of the complex to remove nitrate from liquid effluents was demonstrated (Eroglu et al., 2013). NBR mechanism evaluated in *Sargassum tenerrimum* and *Tubinaria conoides* for the biological production of Au NPs applied to reduce the nitro compounds in wastewater (Ramakrishna et al., 2016).

Environmental concerns and future perspective

Environmental contamination is a serious issue that humanity is currently struggling with (Litvinov et al., 2017; Sushkova et al., 2017; Rajput et al., 2017b,2018). Numbers of techniques are being used and some others are under trial for the remediation of contaminants of the environment (Song et al., 2019; Baig et al., 2021; Kumar et al., 2021; Kumari et al., 2021; Paterlini et al., 2021). There are several examples which are come under the category of contaminants, such as pesticides, herbicides, sewage and organic compounds, toxic gases, fertilizers, trace metals etc. (Vaseashta et al., 2007; Khan and Pathak, 2020). Therefore, to deal with these challenges, the engagement of NPs in the expansion of emerging green remediation technologies has been the subject of recent investigations (Tratnyek and Johnson, 2006). NBR is a unique technology employed in transforming the adverse effects of pollutants into safer molecules through NPs.

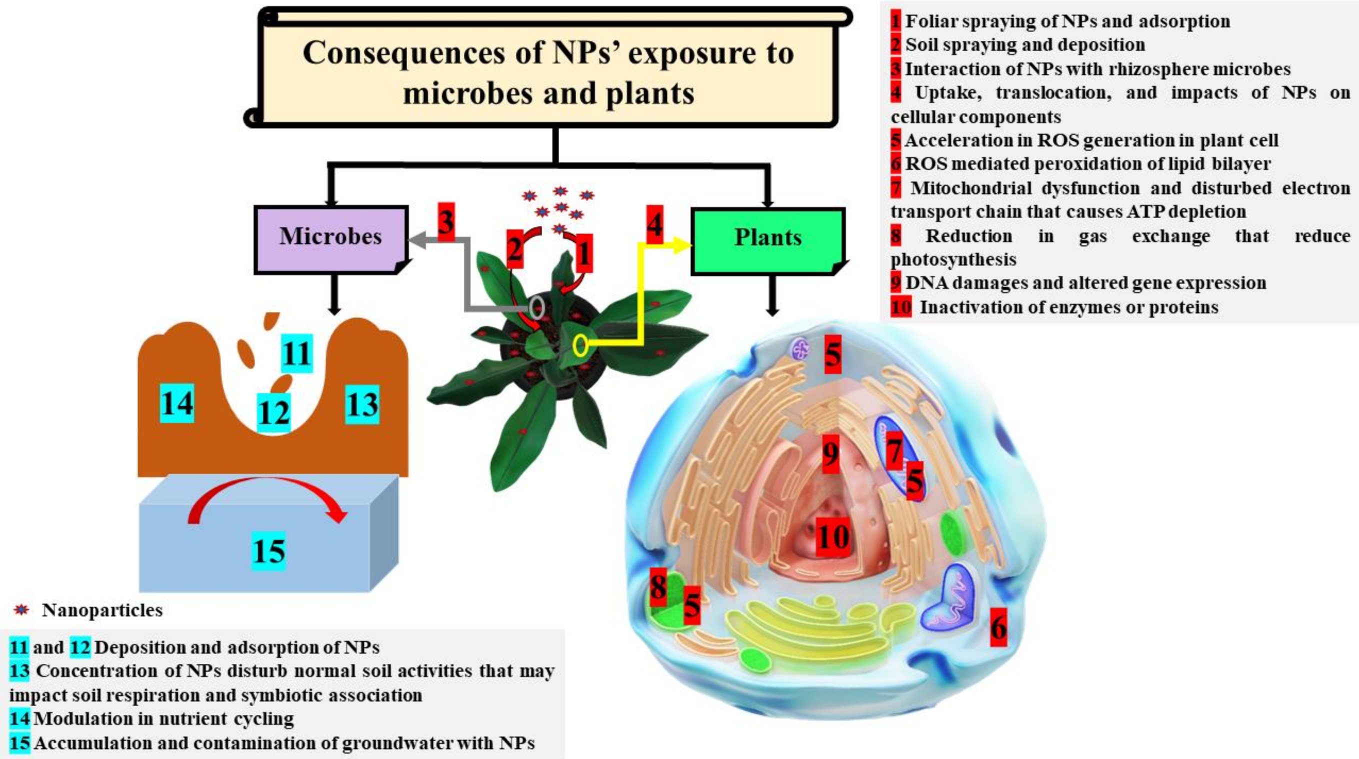

Figure 2. The exposure of NPs and associated impacts on microbes and plants

However, nanotechnology has gained very much importance in recent years because of its extraordinary properties. It has been accounted to play a major function in tackling diverse efficient and inventive resolutions to several ecological confront (Yan et al., 2013; Reddy et al., 2014). But pioneering thoughts for progress are similar a twice impacts. Every unique approach has been connected to pros and cons. It depends on researchers how they tackle and apply the new approach. In the turf of NBR, the negative aspects related to NPs are very significant and crucial which cannot be disregarded (Jiang et al., 2018; Rajput et al., 2021b).

Besides their positive effects, some negative aspects of NPs are also being seen in the environment. It is documented that NPs do not supply any profit in the situation of bioaugmentation, since they stop the microbial inhabitants in contaminated surroundings (Nzila et al., 2016; Amoatey and Baawain, 2019). The appliance of NPs for ecological action intentionally injects NPs into the soil or water body. This has finally involved rising anxiety from all stakeholders. The compensation of NPs such as their minute size, elevated activity, and immense capacity, could develop into a possible deadly feature by inducing unfavorable cellular toxic and damaging properties, abnormal in small-sized counterparts (Figure 2).

In stiff water and seawater, NPs tend to aggregate and are greatly influenced by the type of natural material or other natural colloids present in freshwater. The situation of dispersal will change the ecotoxicity, but several abiotic factors that influence this, such as pH, salinity, and the attendance of organic matters stay to be methodically investigated (Handy et al., 2008). It was demonstrated that the hindering effect of the nZVI in soils happens when the NPs begin to be putting on the facade of the soil particles, accumulating in such a method that they attract additional constituent parts in suspension, jamming the way of fluids (Reddy et al., 2014). Since the strainer result occurs as the concluding phase of deposition, which ends up providing a "clogging" of the soil hole, not allowing for the channel of the element. It has been confirmed that carbon nanotubes (CNT) abridged the biodegradation pace by hindering bacterial expansion and microbial action (Zhang et al., 2015).

Nanosorbent have a significant impact in explaining ecological subjects like the filtration of water that established immense interests because of their unique physicochemical properties. However, the use of nanosorbent material in water bodies can also have certain negative consequences (Yaqoob et al., 2020). Major drawbacks are the probable negativity of the remaining NPs in the water and their large size which causes that few probable functioning is not used (Zhu et al., 2019). The exercise of silver NPs in many products direct them to their discharge to the water body and befall a source of suspended silver and thus produce negative impacts on marine organisms (Navarro et al., 2008).

Several microbes are present in the water body; therefore, it is a very natural process for NPs to encounter microbes after they are released into the water body. When nZVI is in straight connection with bacterial cells, it results in oxidative stress and membrane demolition (Figure 2). The current study represents the thrashing of intracellular components and the disturbance of communication between the outside and inside environment of the bacteria (Lv et al., 2017).

Carbon nanotubes change the oxidation nature of enzymes in water molecules, which causes adverse impacts on microbes (Chen et al., 2016). Graphene oxide enhances the active oxygen application, but it does not harm cells. However, a higher concentration of silver NPs are used, the enzymatic action was retarded, but the genes for resistance were augmented (Li et al., 2019; Kolesnikov et al., 2021). The application of silver NPs and zinc oxide NPs on the activity of bacterial is reported to depend on the dimension of particles, and the microorganism concentration (Mboyi et al., 2017). Treatment of zinc oxide NPs to anaerobic fermentation, zinc ions are engrossed in the mud, however, bacterial quantity, cell activity, enzymatic activity, and zinc ion concentration significantly decreased (Figure 2).

Generally, the negative impacts of NPs on microbial activity largely engage membrane devastation and oxidative stress (Rajput et al., 2017a; Chen et al., 2019). Conclusively, microorganisms and planktons are highly vulnerable to the toxicity of NPs. Furthermore, these water-loving organisms are pretentious by the adverse effects of NPs, and occasionally it is quite hard to recover due to those NPs not simply root of cell injury, but also harm genes and influence reproduction.

Challenges associated with nanobioremediation

- Nanophytoremediation studies are yet to be adopted widely and need to explore rigorously.
- Most studies using nanophytoremediation approaches are microcosm therefore *in-situ* and realistic studies in future research could bring a new direction in this scope
- Time series and long-term research using NPs are also necessary, that can enable us to observe the actual effects of NPs in phytoremediation progression and also their effect on soil characteristics, microbiome, and nutrients
- NPs may get aggregate, dissolved, undergo dissociation in different soil pH, or it can also undergo photodegradation. These processes certainly affect their mobility. Application of doping, composite, or polymeric structure for nanophytoremediation must be explored in this regard.
- Assessment of effects and safety of NPs application in agriculture or polluted soil should be mandatory. Sustainable nanophytoremediation largely relies on climatological conditions hence our exploration should also include the identification of a naturally stable NPs

Conclusion

The advent in nanobiotechnology as a research field brings up possibilities for developing nanoremediation methods for the restoration of contaminated soils. Several investigations' experimental findings revealed the potential of nanobioremediation for the removal of various inorganic and organic pollutants from terrestrial ecosystems. Also, these techniques could be applied to decontaminating air, or water, in cost-effective ways; however, significant environmental concern regarding the application of NPs should be in the regulatory framework, and eco-friendly. Thus, the understanding of NPs interaction with plants, microbes, pollutants, and human health is of utmost importance as these effects might be negative or positive. Thus, nanobioremediation will undoubtedly be a promising tool for achieving environmental sustainability once these research gaps regarding its environmental concerns will have been revealed.

Acknowledgements

The study was supported by a grant from the Russian Science Foundation 19-74-10046.

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Eurasian Journal of Soil Science 2022, 11(1), 61 - 65

Eurasian Journal of Soil Science

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

Productivity of indigenous alfalfa (Medicago sativa) cultivar depending on agricultural practices on sierozem soils in South Kazakhstan

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Article Info

Received : 25.03.2021
Accepted : 01.09.2021
Available online : 10.09.2021

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Abstract

In Turkestan region of South Kazakhstan has large areas of fertile sierozem soils that are important for crop production and its accompanying economic development. And also, alfalfa (Medicago sativa) is an important forage crop grown for seed production. The soils are fertile loams, but because of the regions dry environment, they need to be irrigated. Field experiments were conducted from 2017 to 2020 on one-year or older alfalfa stands grown for seed production at various plots on sierozem soils in rainfed areas of Turkestan region to determine the influence of agricultural practices such as fertilizer use, pesticide use and conventional tillage on seed yield and dry hay of alfalfa stands. The findings of field research experiments indicated that agricultural practices of fertilizer use, pesticide use and conventional tillage was essential to obtain maximum seed yield and dry hay of alfalfa. But, it was determined that the best outputs tended to be obtained with Fertilizer use + Pesticide use + Conventional tillage.

Keywords: Sierozem, Alfalfa, fertilizer use, pesticide use, conventional tillage, agricultural practices.

Introduction

Agriculture is an important part of the economy in Kazakhstan and contributes significantly to the food security of the majority of population. Sustainable development of agriculture, increase of production and improvement of population welfare largely depend on soil condition and its fertility. Over the past decade, the agricultural lands, however, have suffered from progressive degradation leading steadily to the loss of their fertility and eventually to a low yield and inefficiency of production as a whole (Nurbekov et al., 2016).

In South Kazakhstan, alfalfa (*Medicago sativa*) is one of the important forage crops, providing high-protein fodder as herbage, haylage, hay and vitamin-enriched flour. Alfalfa stimulates recovery of soil fertility and structure, protects sloping land from water erosion and prevents soil salinization on irrigated areas (Massaliyev et al., 2015) Yield of alfalfa under rainfed production depends mainly on the soil moisture available from rainfall. Alfalfa is one of the best preceding cover crop in a crop sequence for all crops and is included in all types of crop rotations recommended for rainfed areas in South Kazakhstan Province (Toktarbekova et al., 2020). Alfalfa gives rich harvest of hay and seeds for four years of its life-cycle, followed by a decrease in yields caused by strong thinning of herbage and infestation from ephemeral weeds.

: https://doi.org/10.18393/ejss.993599
: http://ejss.fesss.org/10.18393/ejss.993599
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

Therefore, standing of alfalfa in cereal-fodder crop rotations should be for 3-4 years but sometimes it can be up to 5-6 years. Expansion of seeding areas under alfalfa on rainfed lands through outscaling Conservation Agriculture technologies is instrumental for building solid fodder reserves for livestock production.

South Kazakhstan is mostly an arid and semi-arid, strongly continental climate, with hot summers and cold winters. Sierozems are brown desert soils that are located in Turkestan region of South Kazakhstan and are extent of the alfalfa growing region (Shokparova and Issanova, 2013; Beketova et al., 2017; Yertayeva et al., 2018). Sierozem soils are a valuable resource because of their extent and because they are fertile. Sierozems must be properly managed and protected for efficient and sustainable productivity. They have been researched in the past but mainly as a soil-geographic resource. Further study is needed to quantify and expand their value in production and assure environmental sustainability (Jalankuzov et al., 2013; Saparov, 2014). Many factors are involved in producing a high-quality alfalfa crop. Although some factors (like rainfall and temperature) cannot be controlled, many other critical components of the production system can be carefully managed. High yields require maintenance of agricultural practices to meet the needs of the rapidly growing crop. As the demand for high-quality and high-yielding hay increases, closer examinations of the role of proper agricultural practices are needed.

Our objective was to study agricultural practices such as fertilizer use, pesticide use and conventional tillage, that would enhance alfalfa (*Medicago sativa*) on the sierozem soils of South Kazakhstan.

Material and Methods

Study Area

The experiment was performed at Kazygurt district of the Turkestan region, South Kazakhstan (Figure 1). The experimental fields had been in alfalfa growing regions of South Kazakhstan for at least 10 years. Alfalfa (*Medicago sativa*) is the major crop in this region, which are generally planted in March and harvested in October. This region is characterized by a semi-arid climate. Most of the precipitation occurred in June to September. The annual mean precipitation and mean temperature from the establishment of the experiment is shown in Figure 2.

Figure 1. Study area

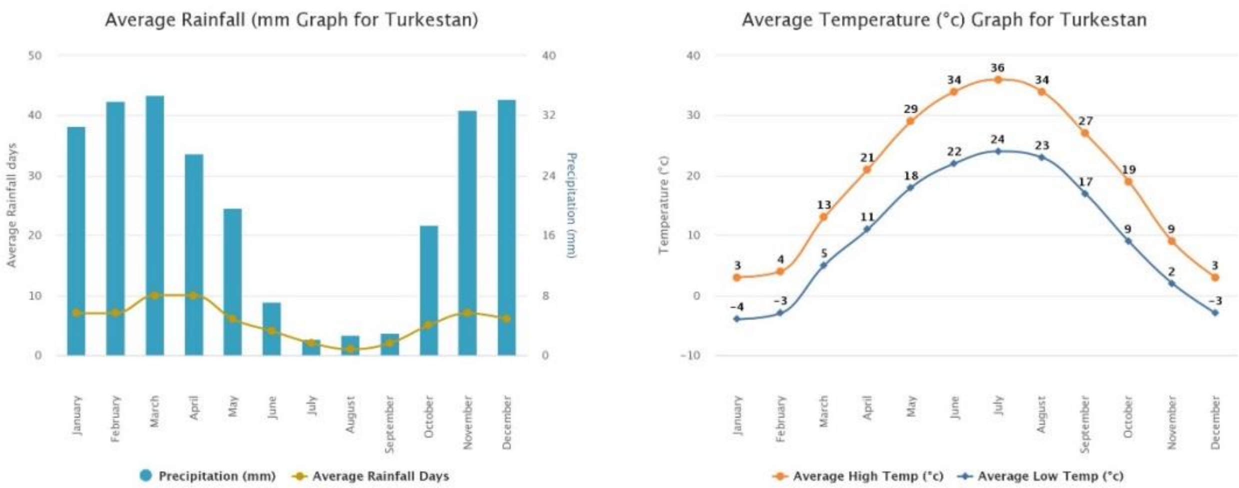

Figure 2. Monthly average temperature (0C) and distribution of precipitation (mm) of the experimental area.

Soil

The main soil type, which is typical for the region, is sierozem soils. The sierozem soils are found in arid regions which characterized by a brownish-gray surface on a lighter layer based on a carbonate or hard-pan layer (USDA, 1999). Ordinary sierozems develop on loess-like loams and have fully developed profile with a rather noticeable division into genetic horizons. Sierozems are marked by good water-physiological properties, high biological activity, and adequate fertility; they produce high yields when irrigated. There are various subtypes: light, conventional (standard), dark, and northern (Saparov, 2014). The soil belongs to the general soil type of dark sierozem. The soil had a mean soil bulk density of 1.30-1.50 g/cm^3, pH was 7,6-7,7 and calcium carbonate ($CaCO_3$) concentration was 6,6-7,4%, soil organic matter was 1.02-1.38%, total N was 0.058-0.126%, available phosphorus was 8,5-19,5 mg/kg and exchangeable potassium was 360-388 mg/kg.

Alfalfa

The objects of the research were indigenous alfalfa (Medicago sativa) cultivar, Krasnovodopadskaya 8, allowed for use. Krasnovodopadskaya 8 is a early ripening indigenous from Kazakhstan alfalfa (Medicago sativa) cultivar. Proper variety selection can have a dramatic impact on yield, quality, and stand longevity. Select adapted, high yielding, and pest-resistant varieties. Consider using more than one variety if the planting involves a large acreage. Most states in the South Kazakhstan publish recommended indigenous alfalfa (Medicago sativa) cultivar, Krasnovodopadskaya 8.

Treatments and Experimental design

The field experiment was performed using a completely randomized block design with four replications during the 2017-2020. In this experiment four different years old indigenous alfalfa used. These alfalfas are i) 1st years old, ii) 2nd years of life, iii) 3rd years of life, and iv) 4th years of life. The experimental unit was 200 m^2 (20 m x 10m). The experiment was performed with the following five treatments of agricultural practices given in Table 1. During the field experiment (2017-2020), every year same agricultural practices used in same plot of field experiment.

Table 1. Treatment description and practice of conservation agriculture used in the field experiment during the 2017 – 2020.

Treatments	Fertilizer Use	Pesticide Use	Conventional Tillage
T1 (Absolute Control)	-	-	-
T2	-	+	-
T3	-	-	+
T4	+	-	+
T5	+	+	+

The sources of fertilizers used were urea 46% N and triple superphosphate 44% P_2O_5. The dose of N was 12 kg/ha, P was 40 kg/da at the beginning of March. The source of pesticide used was Karate 5 EC (active ingredient is: Lambda-Cyhalothrin) and its dose was 0.2 lt/ha. A tillage method is 12-14 cm soil depth using with chisel cultivator at second decade of March. Data on seed yield and dry hay of alfalfa were measured from all plots during the 2018-2020. The economic efficiency of the factors under study is determined by calculating the actual costs of labor and funds for all types of work, the prevailing norms and market prices in the region of the Turkestan region, Kazakhstan.

Results and Discussion

Effect of different agricultural practices on seed yield and dry hay of alfalfa was evaluated (Figure 3). There were no significant differences among treatments for seed yield and dry hay of alfalfa between 2018 and 2020. According to the Figure 3, there was a significant difference between control treatments (T1) on seed yield and dry hay of alfalfa and agricultural practices (T2, T3, T4 and T5). All agricultural practices significantly influenced seed yield and dry hay of alfalfa compared to control treatment. Chemical inputs (Fertilizer and pesticide use) and conventional tillage increased the seed yield and dry hay of alfalfa. However, Fertilizer use + Pesticide use + Conventional tillage (T5) has shown a great influence on the seed yield and dry hay of alfalfa followed by Fertilizer use + Conventional tillage (T4). The lowest seed yield and dry hay recorded by the control experiment (T1) might be the reason why the yield parameters were very low compared with other treatments. Pesticide use (T2) and Conventional tillage (T3) were similar in their effect on seed yield and dry hay of alfalfa.

In South Kazakhstan, alfalafa is an important forage crop in sierozem soils. Alfalfa is a high-yielding, high-quality perennial forage that removes plant nutrients from soil in large quantities. For optimal production, the nutrients must be available at the appropriate level and time. It is common practice to not to apply fertilizer in the first year of alfalfa, which results in relatively low yields but newly planted alfalfa needs a readily available supply of phosphorus, potassium and other plant nutrients immediately after emergence. A well-planned fertilizer program is necessary for alfalfa forage production. Berg et al. (2003) reported that to meet the total seasonal nutritional requirements, an adequate nutrient supply must be available for uptake by the crop to meet periods of peak demand. In South Kazakhstan, this peak demand time for nutrients will be the late-bud stage when the crop is fully covering the ground and when intensive plant growth is going on. Applying 12 kg N/ha and 40 kg P/ha^{-1} (T4 and T5) during seeding has been shown to increase seedling size by four times compared to no fertilizer (control) treatment (T1). Between 2017 and 2020, the difference in seed yield and dry hay of alfalfa between unfertilized plots (T1) and fertilized plots (T4, T5) further increased, and a significant difference was observed between the T1 and T4 treatments (Figure 3). These findings corroborated the results of other studies that reported a positive influence of P and N on alfalfa yield even in soils (Markus and Battle, 1965; Kafkafi et al., 1977; Barbarick, 1985; Macolino et al., 2013; Yertayeva et al., 2019).

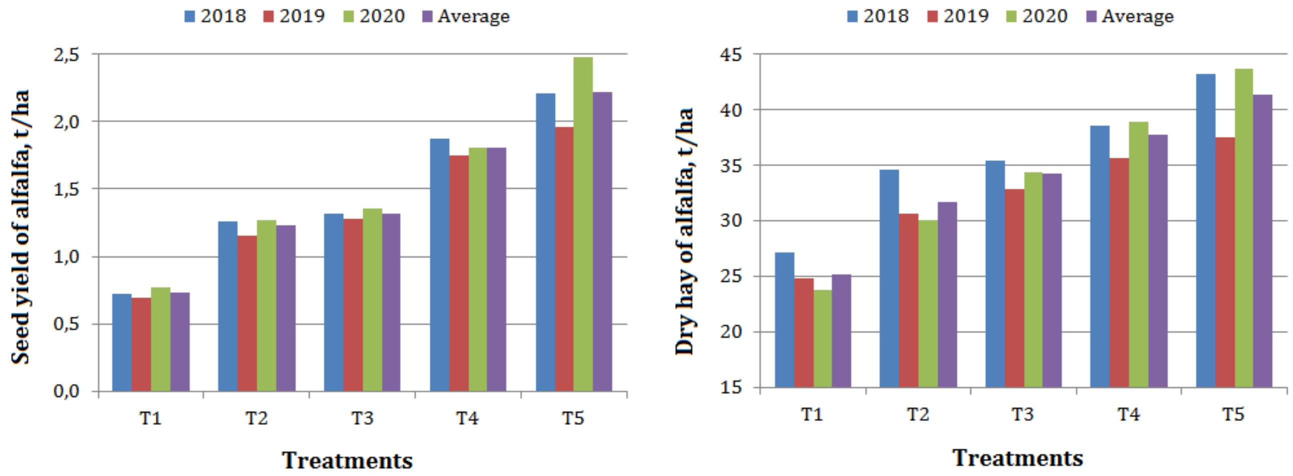

Figure 3. Effect of agricultural practices on seed yield and dry hay of alfalfa

Alfalfa weevil control is often necessary for high yields, high quality, and long-lived stands. Other insects may, at times, attack alfalfa. These include meadow spittlebugs, aphids, clover-root curculios, three-cornered alfalfa hoppers, and grasshoppers. In the South Kazakhstan, blister beetles will occasionally infest alfalfa, but are rarely problematic. Use of resistant varieties, proper harvest and fertility management, routine scouting, biological control, and selective use of insecticides are important factors in insect control. In this experiment, pesticide use (T2 and T5) increased the seed yield and dry hay of alfalfa compared to control treatment (T1). Similar results were obtained by Pellissier et al. (2017) and Harrington et al (2021) on alfalfa and the other crops. Numerous studies have reported that if plants have an insect pest problem, pesticide usage increased plant yield and yield parameters (Sun et al., 2014; Moyer et al., 2014).

In the South Kazakhstan, in rainfed areas under conventional tillage, the biggest problem is with open fallow when multiple tillage operations are conducted to control weeds, causing substantial soil erosion and degradation. It is established that through application of No-Till practices soil moisture can be increased and conserved compared with conventional tillage. However, it was determined that, seed yield and dry hay of alfalfa affected by conventional tillage practices (T3, T4 and T5) compared to absolute control plot (T1). Conventional tillage practices increased (T3, T4, T5) increased the seed yield and dry hay of alfalfa compared to control treatment (T1). In this research, we used Chisel cultivator to conventional tillage. Chisel plow has some adventages and disadvantages. Major advantages are : less erosion than from cleanly tilled systems and less wind erosion than fall plow or fall disk because of rough surface; Well adapted to poorly drained soils; Good to excellent incorporation. Major disadvantages are: Little erosion control; High soil moisture loss; Medium to high labor and fuel requirements (Amini and Asoodar, 2015). Similar results were obtained by Małecka et al. (2012) and Suleimenova et al. (2019) on alfalfa and the other crops.

Conclusion

Agriculture is an important part of the economy in Kazakhstan and contributes significantly to the food security of the majority of population. Sustainable development of agriculture, increase of production and improvement of population welfare largely depend on soil condition and its fertility. Traditionally, agriculture in South Kazakhstan province is dominated by medium-size and small farms. Water deficiency has remained one of the most important issues in the irrigated crop sector of South Kazakhstan province. In order to increase plant production in sierozems, which are widely distributed in Southern Kazakhstan, the effect of various agricultural practices other than irrigation has been one of the important research topics. In this study, the effects of fertilizer use, pesticide use and conventional tillage on alfalfa yields grown on sierozems were investigated. According to the results, it was determined that each of the agricultural practices had a significant effect on increasing alfalfa yield. However, the best outputs tended to be obtained with Fertilizer use + Pesticide use + Conventional tillage. However, it is necessary to detail the results obtained from this study with future studies.

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Eurasian Journal of Soil Science 2022, 11(1), 66 - 76

Eurasian Journal of Soil Science

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

Stubble burning and wildfires in Turkey considering the Sustainable Development Goals of the United Nations

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Article Info

Received : 19.02.2021
Accepted : 15.08.2021
Available online : 10.09.2021

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Abstract

There is a worldwide risk of fire spread due to the mismanagement of stubble in agricultural land. In 2019, 3.6% of the forest area was damaged by fires in Turkey due to stubble burning. The burning of agriculture residues negatively affects soil properties, air quality and water resources. This finally threatens humankind sustainability. However, there is a lack of information analyzing this problem from the current policies in Turkey. Therefore, this paper reviews the legislation currently applied to understand the specific competencies to achieve sustainable fire and forest management. A holistic analysis of the legal regulations and practices to prevent stubble burning in Turkey shown here the farmer´s perception of the use of fire as an agriculture tool and the relevance of stubble burning to explain the location and recurrence of wildfires. Then, we discussed the relevance to shed light on how effective are the laws to avoid stubble burning and its impact on the environment. We claim for an update of the legislation to allow the farmers to manage the stubble and encourage the policymakers to develop new strategies to compost the stubble and achieve sustainable management within the Sustainable Development Goals of the United Nations that will contribute to achieving the Land Degradation Neutrality Challenge.

Keywords: Legal measures, people awareness, stubble burning, stubble management, wildfires

Introduction

In 2019, approximately 15.4 million hectares of land, excluding fallow, are cultivated in Turkey. Among them, 6.8 million hectares of wheat, 2.9 million hectares of barley, and 0.6 million hectares of corn (TUIK, 2020) are cultivated. Rooted stems that remain in the field after the crops are harvested and defined as stubble. In Turkey, it is very common that farmers burn stubble considering a correct habit (Gursoy, 2012). Approximately 40% of the grain field in Turkey are exposed to stubble fire every year, and this fire results in the extinction of 10 million tons of stalks and straw (Temiz and Olgar, 2017). This is because farmers assume that fire provides convenience in tillage results and reduce diseases and pests, which allow obtaining higher crop yields, and reduce the economic costs to be transported (Korucu et al., 2007). There is a lack of suitable tools, equipment and financial support to process the stubble and the clogging of the sowing machines during planting which leave fire as a unique solution to the stubble burning by the Turkish farmers (Yilmaz et al., 2014). Another reason for burning stubble is that the stubble remaining on the soil surface causes

: https://doi.org/10.18393/ejss.993611
: http://ejss.fesss.org/10.18393/ejss.993611
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

Zabrus spp. beetle and plant root rot (Budak and Gunal, 2018). The perception of the farmers is that fire is positive to enhance their fields because the decomposition of the stubble will last for years and they need to plough the soil for the next harvest. Burning of agricultural residues is a harmful process in several ways but it is common in paddy, cotton, and cornfields, mainly cultivated with wheat in the United States (McCarty et al., 2009), New Zealand (Williams et al., 2020), Australia (Smith et al., 2007) or some countries of Asia (Mittal et al., 2009).

Stubble burning harms the soil in many aspects such as water-repellent conditions (Flow, 2016), changes in the water cycle by enhancing the runoff generation and reducing the infiltration capacity of the soils (Vermeire et al., 2004; McCarty et al., 2009), increasing the risk of soil erosion (De Bano et al., 1998), a greenhouse gas emissions rise (Venkataraman et al., 2006; Hemwong et al., 2008; Huang et al., 2012; Ravindra et al., 2019; Beig et al., 2020), and duplicating the nutrient losses (Fynn et al., 2003; Heard et al., 2006). Also, biodiversity is highly affected. For instance, many small reptiles and mammals, such as lizards or vole, whose value for the soil or life cycle is not fully appreciated by farmers, are also adversely affected by stubble fires (Akman, 2019). However, there are some contradictory results in this respect. For example, Christian et al. (1999) concluded that the burnt field's yield is higher than the yield from the unburned field.

Researchers explained this contradiction by the fact that yields are strongly affected not only by fire but also by soil, climate, and other environmental factors. In a study investigating the effects of forest fires on the biochemical properties of the soil (Kaptanoglu and Namli, 2019), it was concluded that low and moderate forest fires positively affected some chemical (pH, $CaCO_3$, Ca and P_2O_5) properties of soils, and negatively affected some biological properties (β-D glucosidase, urease enzyme activities). Burning the stubble left at the end of the harvest causes many risks besides its damage to the soil. These risks involve the emergence of acute respiratory diseases caused by smoke during and after stubble burning (Batra, 2017). Also, telephone and energy transmission lines can be damaged, traffic accidents due to fog formation can occur, fire splashes to neighbouring unharvested fields are registered (Korucu et al., 2005).

Wildfires also closely affect sociocultural life. Using data from the fires that occurred in Turkey between 1995 and 2004, Koulelis and Mitsopoulos (2007) found significant relationships among the population density of these years, the number of fires, the gross domestic product, and the width of the burning area. A large portion of forest fires in Turkey (93%) is removed by people, among them, negligence and carelessness forest fires are an important place (Inanc and Aydin, 2019). In Turkey, the primary starting point for forest fires is the agricultural area near or in the forests (Baltaci and Yildirim, 2021). According to the data of 2019, a total of 2698 forest fires broke out in Turkey, and 184 of these fires were reported to have occurred due to stubble burning. According to provinces, forest fires caused by burning stubble were located in Trabzon (28), Sakarya (27), Giresun (20), and Eskişehir (18). In 2019, the total size of the area damaged by forest fires was determined as 11332.44 ha. It was summarized that 406.45 ha was estimated to be burnt due to stubble burning. Forest areas damaged by stubble burning are registered in Trabzon (76.78 ha), Kahramanmaraş (67.45 ha), and Mersin (53.87 ha) (Anonymous, 2020a). Figure 1 shows different visuals from the stubble burning processes in Central Anatolia in October and November 2020.

Figure 1. Different visuals from the stubble burning processes in Central Anatolia in October and November 2020

Efficient and widespread training activities are required to prevent stubble burning. To date, various legal regulations have been developed in different countries to accomplish results in the short-term, in which policymakers are conscious of the enormity of the situation (Agudo-Gonzalez, 2010; Galiana-Martin et al., 2011; Montiel-Molina, 2013). In the European Union, the cross-compliance rules of the Common Agriculture Policy (CAP) regulates the management of agricultural residues according to the Council of the EU regulation no 73/2009, 6 (1) and it has prohibited the burning of stubble in EU member countries (Searle and Bitnere, 2017). Although there are many legal regulations and measures in the European Union countries, paradoxically, there have been severe wildfires in Europe (Fernandez-Anez et al., 2021), possibly, due to this fact (Xanthopoulos et al., 2006; San-Miguel-Ayanz and Camia, 2010; Mierauskas, 2012). However, a holistic analysis of the legal regulations and practices to prevent stubble burning in Turkey is scarce. Therefore, the main goals of this review are to describe: i) farmer´s perception; ii) wildfires in Turkey due to stubble burning; iii) and what can be considered to make the current laws effective to face this issue.

Legal measures in Turkey

Developing legal measures to face wildfires is essential to focus on fire prevention measures (Mavsar et al. 2013). In this sense, high-risk seasons and practices carrying a significant wildfire risk should be regarded when preparing laws and land management plans (Pausas, 2004; Pausas and Keeley, 2014). The burning of stubble and agricultural residues, particularly after harvest, coincides with the months when the air temperature is high (Erdal et al., 2016). During the next growing period coinciding with soil preparation, stubble burning can cause severe forest fires (Velez, 2010; Montiel-Molina, 2013).

According to official statistics, 3.6% of the forest area affected by fires in 2019 in Turkey are due to stubble burning (Anonymous, 2020a). While determining legal measures, deliberate forest burning and carelessness, such as stubble burning and forest burning by negligence, are separated. According to Forest Law No. 6831 of the Turkish Government, Article 76 "d). It is forbidden to burn stubble or similar vegetation within the villages' boundaries within the scope of articles 31 and 32 of this law or at a distance of four kilometers to forests". According to Article 110 of the same law, "Those who cause forest fires contrary to the obligation of attention and care are punished with imprisonment from two to seven years". Still, suppose the intention of the act of burning is determined; in that case, it is said that" the person who deliberately burns the forest is punished with imprisonment not less than ten years and a judicial fine up to ten thousand days". It is stated in the same law that "a person who burns the state forests within the framework of the activities of an organization established to commit crimes against the security of the state is punished with life imprisonment and a judicial fine of up to twenty thousand days". According to the 18th Article of the Forestry Law No. 6831, no activities other than forestry activities are allowed in forests exposed to fire (Anonymous 2020b).

In general, wheat straw in Turkey uses to be incinerated with maize, cotton, and sunflower residues to be favoured (Yilmaz et al., 2014). Barley lot and residues are nutritious and easy to digest for livestock and have a short growth cycle in the field against wheat growth; mainly used for livestock. In addition, since the amount of stubble on the field surface after the harvest of barley is low, the rate of burning is significantly lower (Gursoy et al., 2013). Depending on the harvesting height of the grain crop (i.e., barley and wheat), 3500 kg ha-1 for incineration of all crop residues and 1000 kg ha^{-1} for residues other than herds disappear without being added to the soil (Temel, 2012).

The preservation of agricultural lands and pastures in Turkey is also guaranteed by Article 44 and Article 45 of the Republic of Turkey's Constitution in 1982. Nowadays, Turkish policymakers continue to work on a new Constitution. In this new document to be prepared, the laws for protecting natural assets will undoubtedly be included. Currently, efforts are made to find a solution against stubble fires by applying penal sanctions (Dilber and Guler, 2015) by the Environmental Law No. 2872 (Anonymous, 1983), Forest Law No. 6831 (Anonymous, 1956), the Provincial Administration Law no 5442 (Anonymous, 1949) and the relevant articles of Turkish Penal Code No.5237 (Anonymous, 2004) and the circulars issued on this subject, through the Ministry of Agriculture and Forestry, the Ministry of Environment and Urbanization, the Ministry of Internal Affairs, Governorships, Municipalities, and related security units.

According to Article 76 of the Forest Law, it is forbidden to burn stubble and similar vegetation at a distance of 4km to the forest or within boundaries of forest villages, whose description is determined by law. This act is considered a forest crime. According to Article 110 of the Forest Law, people who commit the act mentioned above are sentenced to imprisonment for one to three years, and a fine is imposed. In the calculation of fire damage, the value of entirely burned trees, the decrease in the value of partially burnt trees, the damage, and efficiency losses in the substrate are taken into account (Anonymous, 1956).

Governorships in Turkey also publish notifications on this subject. The purposes of these rescripts are to take the necessary measures for the sustainability of the soil fertility in natural balance by protecting the soil's biological, chemical, and physical structure and the environment. This is the primary source of agricultural production to determine alternative measures to prevent stubble burning after the harvest in cereal agriculture. It considers the damage to the soil structure and the environment to train technical staff and farmers, and ensure that the public is sensitive to stubble fires.

Under subparagraph (I) of the Ministry of Environment and Urbanization's Communiqué numbered 2019/1 published in the Official Gazette dated 31 December 2018 and numbered 30642, subparagraph (I) of the 20th article of the Environment Law numbered 2872, those who burn stubble will be fined 60.11 TL (Approx. € 6.83) administrative fine for each decade. If the stubble burning is acted in areas adjacent to forests and wetlands and residential areas, the penalty is raised five times. On the other hand, in the regions where the second crop is planted, controlled stubble burning is permitted under the responsibility of the Governorships within the framework of the action plan prepared by the Governorships. Still, almost all governorships do not apply this provision in their provinces and do not allow stubble burning, including controlled stubble burning. Besides, a fine of 320 TL (Approximately € 35.00) is fined for 2019, according to Article 32 of the Misdemeanor Law No. 5326, along with the penalties accrued for those who act against this notification published by the Governorship (Anonymous, 2005). Provincial Agriculture and Forestry Directorates carry out the execution. Besides, the relevant institutions' responsibilities for stubble burning are also plainly declared in the rescripts mentioned above. The order not to cause any malfunctions in practice by closely monitoring the issues specified in the rescript by the relevant Public Institutions and Organizations (Anonymous, 2020d).

Responsibilities of institutions and organizations for the prevention of burning stubble

Public institution's and organizations' responsibilities in Turkey concerning the prevention of stubble burning are also clearly stated in the relevant rescripts. According to one of these rescripts (Anonymous, 2020c), the responsibilities of the relevant public institutions and organizations are given in articles:

The responsibilities of the Provincial Directorate of Agriculture and Forestry and the Presidencies of the Chamber of Agriculture confirm that: i) stubble fires will be included in farmer training programs extensively, and their damages will be explained in detail; ii) environmental awareness by providing the contributions of local government and non-governmental organizations in the training-publication activities; iii) public awareness will be raised with posters and brochures to be prepared; and, iv) with other public institutions and organizations to make more effective measures against stubble burning. Training requests from the Agricultural Chamber Presidencies will be fulfilled immediately by the Provincial Directorate of Agriculture and Forestry.

The Highways Branch Directorate's responsibilities clarify that stubble fires that pollute the air occasionally diminish visibility on highways, which causes traffic accidents and loss of life and property. Thus, weeds on the road slopes will be removed and flammable material too. Moreover, warning signs will be fixed on the roadside concerning the damages of stubble fires.

The responsibilities of the Provincial Gendarmerie Command and Provincial Security Directorate affirms that: i) control and patrol duty will be increased before, during, and after harvest; ii) vehicle and personnel will support in the transportation of living and non-living things affected by fire in case of fire and if desired; and, iii) despite the measures and announcements taken, the official reports and all evidence regarding the fire will be sent to the Provincial Directorate of Environment and Urbanization. This institution will take legal actions against those who begin stubble fire. In addition, the responsibilities of the Directorate of National Education claim for children and young people to better understand the harmful effects of stubble fires, education on stubble fires and their damages will be included in the education curriculum.

Also, the responsibilities of the Regional Directorate of Forestry/Forestry Operation Directorates and Chief Offices are planned. Firstly, farmers in the settlement where burning stubble is prohibited under the Forest Law's relevant articles will be informed about forest fires that may occur due to stubble burning. The technical staff of the Provincial/District Agriculture and Forestry Directorates will also participate in this information to be made in the form of neighbourhood meetings. Before, during and after harvest, inspections will be increased by making fire announcements and legal actions will be taken against those who cause fires. Also, between the agricultural areas and forest areas in fire-sensitive areas, lanes will be opened with a grader in a way to prevent fire. However, despite the measures and announcements taken, if the stubble fire is closer than 4 km to the forest area, criminal action will be applied to those who cause the stubble fire under the relevant provisions of Forest Law No. 6831. In this case, the official reports taken for legal action and all evidence regarding the fire will be sent to the Provincial Directorate of Environment and Urbanization.

Responsibilities of the Municipalities: i) announce the notification issued by the Provincial Governorship to inform the public and the concerned parties, regarding the prevention of stubble burning, ii) provide the intervention of the fire department units to extinguish the stubble fires within the boundaries of the municipality adjacent areas.

Responsibilities of the Neighborhood Mukhtars i): they will announce this notification published by our Provincial Governorship to inform the public and the concerned parties to prevent burning of stubble, ii) by determining those who cause stubble fires as soon as possible, informing relevant authorities immediately, iii) the time and place of training and activities related to stubble fires will be announced to the public.

The responsibilities of the Provincial Directorate of Environment and Urbanization: i) by making fire announcements before, during, and after harvest, inspections will be increased, legal action will be taken against those that cause fires, ii) studies will be carried out in coordination with the relevant institutions to prevent stubble fires, iii) will duly apply fines under the relevant laws.

Responsibilities of Provincial and District Mufti Offices: i) sermons on the prevention of stubble fires in mosques will be shared by mufti offices.

Responsibilities of combine harvester owners/operators in related rescripts on preventing stubble burning

Worldwide, various methods have been developed for the management of stubble left after wheat harvest. These are i) the stubble shredders separate from the combine harvester or the stubble shredder and spreader systems attached to the back of the harvester during harvesting, ii) collecting the stubble in the field during or after the harvesting process with the appropriate equipment, and making hay, iii) collecting bales iv) burying the stubble in the soil with tools such as a plough that tills the soil, v) performing stubble sowing directly with sowing machines (Erdal et al., 2016). One of the essential points in the correct control of the stubble is that the combine harvester operators performing the appropriate application. The responsibilities of the combined harvester owners/operators in the related communiqués regarding the prevention of stubble burning are listed in items (Anonymous, 2020d,e): i) those who do not have a G-class Driving License or Combine Harvester Operator's License will not be able to work as a harvester operator and cannot be operated by combine harvester owners Authorized operators will use combine harvesters. ii) harvester owners and operators will not exceed a 1.5% crop loss rate during harvesting within the provincial property borders for 2020. Product loss index values will be taken into consideration according to the land situation. iii) combine harvester owners shall not operate combine harvesters without two 6-kg fire extinguishers. iv) combine harvester owners and combine harvester operators are obliged to present their identification if requested by the authorized controllers. v) the combined header must be dismantled when they are travelling on highways. vi) in cases where the stalks left in the form of a barrel on the field during harvesting with a combine harvester are not counted as bales of straw, the straw chopper (harvester), which is attached to the straw hopper of the combine harvester and shreds the straw into the field by using a handle or chops the straw into a trailer attached to its back (combine harvester) schemes need to be installed and used on combines vii) the existing combine harvester owners, and the ones who come to the province from outside shall inform the Provincial or District Agriculture and Forestry Directorates about the combine harvester and the operator to be operated before starting the harvest. viii) harvester owners or operators are obliged to attend the training to be organized. ix) the combined harvester operator and owners will pay attention to the inspectors and law enforcement officers' warnings. x) all kinds of safety precautions will be taken in place and on time against fire hazards by those concerned not to damage the harvested product and the environment. xi) drivers with operator certificates will reap the crops from the most suitable height that will not allow the stubble's burning, taking into account the field and product condition and the land structure.

Responsibilities of field owners and villagers in the related communication on preventing stubble burning

If an effective struggle against stubble burning is desired, it is impossible to achieve success without the field owners and villagers involved in this struggle. Figure 2 shows some photos of the stubble burning in Ethiopia (Bahir Dar area, Nile) in January 2018. The responsibilities of the field owners and the local people in the related communiqués concerning the prevention of stubble burning are listed in items (Anonymous, 2020d): i) crop owners and mukhtars will ensure the formation of an 8-10 meters wide ploughed line, cleared from combustible materials (stubble) after harvest, in the grain fields adjacent to the forest, they will prevent the burning of stubble, grass, and bushes in the fields in and around the forest, and the mukhtars will notify the Gendarmerie Command or the Forest Management Directorate of those who act contrary. ii) under Forestry Law No. 6831, it is prohibited to spend the night in the state forests other than the accommodation places determined, burning all fires other than the quarries and burning stubble or similar vegetation. This issue will be followed by the Neighbourhood Mukhtar, Forest Management Directorate, the relevant Municipality, Gendarmerie Command, and the Police Department. iii) the control officers will make the necessary warnings to notify the public and the Neighbourhood Mukhtar, the prohibition of burning stubble and cleaning the field, starting fires, accommodation and overnight stays outside the forest picnic-promenade and being cautious in the forest areas.

Figure 2. View of the stubble burning in Ethiopia (Bahir Dar area, Nile) in January 2018.

Discussion

Stubble burning is not a Turkey issue as it is widespread around the world. Turkey can be considered a laboratory to understand the use of stubble burning as has a long tradition of farming and the use of stubble. Some of the problems of burning stubble go further than the risc to cause a wildfire. Stubble burning damage the quality of the air such as Mittal et al. (2009) found in Patiala due to the burning of wheat and rice crops. Dhammapala et al. (2006) measured high particulate emissions from wheat and Kentucky bluegrass stubble in eastern Washington and northern Idaho. Another impact of stubble burning is the changes in soil hydraulic and physical properties (Valzano et al., 1997) and the changes in soil organic matter, aggregation and earthworm population (Virto et al., 2007). There are other impacts such as the ones to the human health that Saggu et al. (2018) shown in the Malwa region of India, where the respiratory health of the school children was affected by stubble burning. All those problems request that stubble burning must be legislated and Turkey is an example of how the laws and their application will contribute to improving the management of stubble.

As can be assumed from the data given in this review article, it is forbidden to burn the country's stubble as in the European Union countries, and legal measures have been taken to prevent the stubble's burning (Mourao and Martinho, 2019). Turkish legislators, like their European counterparts, are aware of the harmful aspects of burning stubble. Despite this, it is impossible to prevent massive fires caused by burning stubble neither in Europe nor in Turkey, a candidate country for the European Union (Karmilowicz et al., 2018). This is a similar situation in the USA (Stephens and Ruth, 2005; Stephens et al., 2016), which is a worldwide trend along with the world (Herawati and Santoso, 2011; Stupak et al., 2007; Turyahabwe et al., 2008).

The proportion of stubble burning due to forest fires in Turkey in 2019 is 6.81%, while the ratio of the area of burning forests was determined as 3.58 % (406.45 ha). It is believed that the principal reason why stubble burning could not be prevented despite the laws is that the farmers and villagers, in particular, have not received sufficient training for not burning the stubble. Although there is an emphasis on education in the circulars issued by the governorships based on the laws, this training are either insufficient or cursory. The way to fight stubble should be not only with in-depth and ordinary training in the post-harvest seasons but also with methods embedded in formal education and training in village, town, and mukhtar meetings. The related units of institutions and organizations such as the Ministry of Agriculture and Forestry, Universities, Ministry of National Education, municipalities, fire brigade, gendarmerie, police, and non-governmental organizations should be involved in these training plans. Thus, lawmakers, advisors to lawmakers, law enforcement, and those who have to obey the law gather on the same platform. It is the only way for a society to become conscious of all age groups. The departments of universities such as forestry, environmental engineering, law, and medicine, primarily the faculties of agriculture, should prevent stubble fires with the outcomes of their studies. For instance, the availability of worms in stubble management can be studied by agricultural faculties.

On the other hand, settling a policy with target groups helps create more enforceable laws, and leads to less opposition and veto. While preparing laws for preventing stubble burning, getting opinions from all addressees, including those who burn stubble, will provide it possible to make more applicable, realistic, and rational laws. Effective laws are always more challenging to break. Perhaps the most crucial point in preventing stubble fires is that lawmakers and stubble burners understand each other. Sociologists who are quite familiarized with village sociology should build a bridge between lawmakers/enforcers and rural farmers. Thus, sociologists must be involved in both educational activities and legislative commissions.

The effectiveness of the legal regulations concerning the burning of stubble also depends on the deterrence of penalties. Although the penalties determined by the Forest Law in Turkey are very deterrent, the penalties determined by other laws are low. Of course, it is not the precise approach to impose strict punishments on a community that is not adequately aware of burning stubble. Nevertheless, when an adequate level of education is reached about the burning of stubble in rural areas, the fine for burning the stubble must be increased to a higher value than the current one. When the subject is viewed from another perspective, it occurs that reward may be more effective than punishment in obtaining success. Providing a state subsidy to the farmer who will manage accurately instead of burning the stubble can effectively fight stubble burning. The Turkish State provides farmers' support on many issues, and many successes have been achieved with these supports. For instance, a certain amount of money support for each declared stubble that is not burned; in other words, properly managed, can be an effective application. In addition to this financial support, farmers can be motivated by the projects to be developed. For example, in one of these projects, state support can be provided for the exemplary farmers who manage stubble most properly for a certain period, for instance, two or three years to pay a short-term visit with their families to a model agricultural enterprise in one of the European Union countries, as far as the allowances permit. On the one hand, it will contribute to the intellectual development and mental and spiritual relaxation of the rural people who visit abroad. Again, it will motivate other farmers in the region. This practice can be started by choosing one of the villages as a pilot area in Central Anatolia, where stubble burning is common. Consequently, the way to fight stubble burning can be possible by an educated and conscious society.

Stubble burning uses the roots and stems of the crops harvested during agricultural production. In Turkey, it is very common that farmers use to burn stubbles for various purposes; however, it also hurts soil properties deteriorating soil quality and soil human health. In addition to the serious environmental pollution it creates, stubble fires can damage energy and communication lines within agricultural lands or even generate wildfires. Therefore, the main aim of this review was to integrate information about the harmful effects of stubble burning, laws applicable in Turkey against stubble burning and some methods that can be applied instead of burning stubble. We summarize 6.8% of the forest fires in Turkey occurred in 2019 due to stubble burning. The most two relevant wildfires were located in Mersin-Gülnar (07 July 2008 and 5037 hectares of forest burned) and in İzmir-Gaziemir/Gümüldür (20 July 2008; 1228 hectares). In Turkey, stubble burning TV programs prepared for the farmers and broadcasting national channels in the 1980s had encouraged farmers who were still unconscious towards crop rests´ destruction by burning after harvesting (Coskan et al., 2006). However, with the entrance of Turkey as in the EU integration process, it has begun to be a series of measures to prevent these malpractices. The use of a straw to control soil degradation is very positive as previous researchers demonstrated in agriculture and forest land. Novara et al. (2018) demonstrated the positive use of a straw to increase the soil organic matter which confirms the positive effect of the cover management (Novara et al., 2019). The use of a straw to control the soil losses were demonstrated by Rodrigo-Comino et al. (2020) in olive orchards; Keesstra et al. (2019) in citrus plantations, Cerdà et al. (2016) in persimmon plantations and Cerdà et al. (2017) in the El Teularet soil erosion and degradation experimental station. The use of stubble will contribute then to more stable soils and accomplish the Sustainable Development Goals of the United Nations and the Land Degradation Neutrality objective (Keesstra et al., 2018; 2019; Visser et al., 2019). This is found in other recent publications such as Rodrigo-Comino et al. (2020); Novara et al. (2021), Barrena-González et al. (2020), López-Vicente et al. (2020) and Cerdà et al. (2021).

Limon-Ortega et al. (2002) investigated the change in soil structure, compaction and microbial biomass with the change in the next crop yield when wheat and corn stubble are burned or mixed into the soil without burning. The results obtained showed that if the wheat and corn stubble were burned or left to the soil as they were, there was no significant difference between the applications in terms of yield values in the first 2-3 years of the trial, and at the end of the 5-6 years, there was a higher grain yield in the stubble applications. Adding organic soil conditioners to stubble burned agricultural areas is a rational way to follow (Coskan et al., 2006).

Conclusion

We conclude that the current legislation in Turkey promote sustainable management of the soil resources as the maintenance of the stubble in the field will promote soil recovery and the soil functions and ecosystem services induced by the straw cover. This is similar to other legislation in other countries which has the target to achieve the Sustainable Development Goals of the United Nations and the Land Degradation Neutrality Challenge. The lack of information and subsidies to the farmers is the reason why the stubble is burnt now, and this should be changed with new policies based on the education of farmers and other users to promote the use of stubble as mulches and avoid their burning.

Acknowledgements

This paper was planned to be presented primarily at the GEA International Conference (Geo Eco-Eco Agro) in Podgorica, Montenegro on 28-29 May 2020. However, since the mentioned activity could not be carried out face to face due to the Covid-19 pandemic, only its abstract section was published in the abstract book of the conference. In addition, this paper has been prepared to support activities of EU COST action FIRElinks numbered CA18135. We thank the mentioned institutions and organizations.

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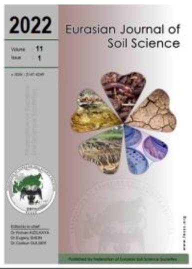
Eurasian Journal of Soil Science 2022, 11(1), 77 - 85

Eurasian Journal of Soil Science

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



Assessment of biological activity in mountain chernozems and mountain-meadow chernozemic soils of natural biogeocenoses in the Central Caucasus, Russia

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Article Info

Received : 05.02.2021
Accepted : 12.09.2021
Available online : 16.09.2021

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Abstract

Indicators reflecting various aspects of biological properties (genetic, microbiological, biochemical) were estimated for the upper horizons (0-20 cm) of mountain chernozems (Mollic Chernozems, WRB, 2015) and mountain meadow chernozemic soils (Mollic Leptosols Eutric, WRB, 2015), that were formed in the conditions of natural biogeocenoses of the Central Caucasus (Elbrus variant of zonality within Kabardino-Balkaria). A comparative assessment was performed for the biological activity parameters (humic content and stock, microbial biomass carbon (Cmic) content and stock, the enzyme activity of hydrolases (invertase, phosphatase, urease) and oxidoreductases (catalase, dehydrogenase)) in combination with indicators of the soil density and acid-base properties of various subtypes of the studied soils (typical and leached). The obtained results showed that the studied types of mountain soils in the upper horizons are characterized by a porous loose composition (0.75-1.07 g/cm^3), neutral (pH_{H2O} = 7.0-7.4) and slightly alkaline (pH_{H2O} = 7.9-8.0) by the reaction of the soil solution, high and very high content (9.5-19.1%) and stock of organic matter (173-276 t/ha). The maximal biological activity was noted in mountain-meadow chernozemic soils, which surpass mountain chernozems in humic content (by 42%) and stock (24%), Cmic content (38%) and stock (17%), relative total activity of hydrolases (36%), but inferior in activity of oxidoreductases (32%). Based on the data obtained, the integrative index of ecological and biological condition (IIEBC) was calculated, which reflects the general level of biological activity of the studied soils. Higher IIEBC values of mountain meadow chernozemic soils (80-100%) in comparison with mountain chernozems (70-74%) are due to the unique complex of soil-forming conditions in which these soils function. The established biological parameters of mountain soils of natural landscapes are necessary for use as reference in environmental studies of anthropogenically disturbed biogeocenoses.

Keywords: Carbon of microbial biomass, Central Caucasus, humus, soil enzymes.

Introduction

Kabardino-Balkaria Republic is located on the northern slopes of the Central Caucasus, mainly in the basin of the left tributaries of the Terek River and part of the Ciscaucasian plain. The Republic occupies an area of 12470 km^2, a height difference of the terrain varies from 150 to 5642 m above sea level. This territory is characterized by a significant variety of natural landscapes associated with a complex relief and various natural and climatic conditions (Fiapshev, 1996). The specificity of the ecological and geographical conditions of this relatively small area leads to the formation of various types of soils, the number of which

: https://doi.org/10.18393/ejss.996603
: http://ejss.fesss.org/10.18393/ejss.996603
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

reaches 29 (Molchanov, 1990). The soil cover of the foothills and uplands of the Republic is mostly formed by various subtypes of mountain chernozems (ordinary, typical, leached) and mountain meadow-chernozemic soils (typical, leached, calcareous). The soil formation conditions, genetic, morphological, and physicochemical features of these soils are described in detail in literature (Fiapshev, 1996; Kumakhov, 2007; Molchanov, 2008). However, studies of the biological state of mountain soils were started recently by the authors of this article (Khakunova et al., 2018).

An increase in anthropogenic load under conditions of mountainous landscapes leads to a rapid and sometimes irreversible change in soil properties (Molchanov, 2008; Egli and Poulenard, 2016). That's why it is relevant to study the biological properties, reflecting the most important aspects of the functioning of soils on the northern macroslope of the Central Caucasus (within Kabardino-Balkaria). It is necessary to establish the parameters and the general level of biological activity of undisturbed natural mountain chernozems (Mollic Chernozems, WRB, 2015) and mountain meadow chernozemic soils (Mollic Leptosols Eutric, WRB, 2015) in order to monitor the degree of their change under the influence of certain exogenous factors.

Of the whole variety of parameters characterizing the biological state of soils, the researchers (Murugan et al., 2014; Merino et al., 2016; dos Santos Soares et al., 2019; Ananyeva et al., 2020; Kazeev et al., 2021; Kolesnikov et al., 2021) propose to use indicators of biological activity, which are highly sensitive to negative processes occurring in soil. It is known that these indicators change earlier than other soil characteristics (e.g. agrochemical), when a stressful situation occurs in soil (Burns et al., 2013; Kazeev et al., 2016; Martinez-Mera et al., 2017).

An approach that provides the comprehensive determination of several informative indicators of biological activity (the humic content, the intensity of soil microbial respiration, the activity of soil enzymes of two classes: hydrolases (invertase, phosphatase, urease) and oxidoreductases (dehydrogenase, catalase)) proved to be effective for the assessment of a biological state of a soil cover (Kazeev et al., 2004; Gedgafova et al., 2015; Gorobtsova et al., 2016; Khakunova et al., 2018). These parameters reflect various aspects of the soil biological properties (genetic, microbiological, biochemical), and their combination makes it possible to establish the general level of biological activity and express it through the integrative index of ecological and biological condition (IIEBC) (Kazeev et al., 2004; Kolesnikov et al., 2021). The aim of this study is to establish the parameters and the general level of biological activity of the upper horizons (0-20 cm), undisturbed mountain chernozems and mountain meadow chernozemic soils of the northern macroslope of the Central Caucasus (Elbrus altitudinal zonality in Kabardino-Balkaria).

Material and Methods

The studied area

The objects of research are various subtypes of mountain chernozems (typical and leached) and mountain meadow chernozemic soils (typical and leached) under natural biogeocenoses. The study area covers the belts of meadow steppes and steppe meadows of the Elbrus altitudinal zonality variant of the Kabardino-Balkaria belt as typified by A.K. Tembotov, 1989 (Sokolov and Tembotov, 1989). The main massifs of mountain chernozems are located on elevated relief elements and smooth slopes with northern exposure of the Chalk and on the southern slopes of the Rocky ridge. The area covered by the characterized soils is about 640 km^2. Mountain meadow chernozemic soils are distributed on the acclivous, steep and less often smooth northern slopes of the Cretaceous and Dzhinalsky ridges and occupy about 523 km^2 (Soils of Kabardino-Balkar ASSR and recommendations on exploitation, 1984). Parent rocks for the studied soils are the products of carbonate rocks weathering: eluvio-diluvium of limestones and calcareous sandstones (Fiapshev et al., 1996).

The studied soils belong to the South European facies and are formed under the conditions of the shadow effect of the mountains of the North Caucasus, which determines the increased moisture content of the territory of its distribution (Valkov et al., 2002). Typical mountain chernozems occur at an altitude of 700-800 m above sea level, on the border of low mountains and foothills and develop at higher temperatures and lower precipitation (Table 1). Leached mountain chernozems are formed under conditions of periodically flushed water regime, at an altitude of 700 to 1000 m above sea level, in contact with mountain meadow chernozemic soils located in the altitude range of 1100-2000 m above sea level (Kumakhov, 2007).

Climatic conditions

The complex topography of the territory of Kabardino-Balkaria has led to a variety of climatic conditions of high-altitude zones within the boundaries of the distribution of the characterized mountain soils (Kumakhov, 2007; Molchanov, 2008), which are reflected in the table.

Table 1. Main climatic characteristics of the study areas (Elbrus variant of zonality, within the boundaries of Kabardino-Balkaria)

Altitudinal belts	Meadow steppe belt	Steppe meadow belt
Altitude, ma.s.l.	from 400-500 to 700-800	from 600-700 to 1500
Precipitation, mm/annual	580	706
Average annual temperature, ^oC	+9.9	+7.9
Hydrothermic coefficient (HTC)	0.67	1.5

Plant cover

More than 50% of the foothill areas are involved in agricultural production. About 34% of the area of mountain chernozems is used for arable land; hayfields and pastures are widespread (Fiapshev, 1996; Tsepkova and Fisun, 2005). However, in some areas, natural biogeocenoses have also been preserved, their phytomass stock in the air-dry mass is 20 c/ha (Tsepkova and Fisun, 2005). The vegetation cover is represented by herb-grasses and meadow-steppe vegetation. The most common forbs are *Salvia verticillata L.*, *Scabiosa ochroleuca L.*, *Achillea millefolium L.*, and *Agrimonia eupatoria L.* Such graminoids as *Elytrigia repens (L.) Nevski*, *Brachypodium rupestre (Host) Roem. and Schult.*, and *Phleum pretense L.* are most registered. Legumes are most presented by *Coronilla varia (L.) Lassen*, *Medicago falcata L.*, *Lotus corniculatus L.*, and *Trifolium pratense L.*

Mountain-meadow chernozemic soils are characterized by high potential fertility; however, due to the complexity of the relief, they are mostly used for hayfields and pastures (Fiapshev, 1996). The natural herbaceous cover is represented by forbs and bromegrasses, fescue grasses. The stock of phytomass of plant communities in the air-dry mass is about 40 c/ha (Tsepkova and Fisun, 2005). The most common forbs are *Achillea millefolium* L., *Scabiosa bipinnata* K. Koch, *Ranunculus oreophilus* M. Bieb, *Betonica officinalis* L., *Origanum vulgare* L., *Thymus pastoralis* Iljin ex Klokov, *Salvia verticillata* L., *Centaurea iberica* Trevir ex Spreng, and *Plantago media* L. are mostly widespread. Such graminoids as *Festuca pratensis* Huds, *Festuca rubra* L., *Poa pratensis* L. and *Phleum pretense L.,* are dominant. Tall grasses are most presented by *Cephalaria gigantean* (Ledeb) Bobrov, *Aconitum nasutum* Fisch ex Rchb, *Delphinium bracteosum* Somm. et Levier and some others. The species nomenclature is given in accordance with The Plant List (2021).

Soil sampling

The collection and analysis of soil samples to determine the physical and biological properties was carried out according to the methods generally accepted in ecology and soil science (Dobrovolskiy, 2001; Ananyeva, 2003; Kazeev et al., 2016). Soils were sampled by the "envelope" method from the 0-20 cm depth in natural biogeocenoses in the first decade of July 2016-2018. 13-15 mixed samples was picked to characterize each subtype of the studied soils. Cartographic materials were used to determine the sampling point's locations (Molchanov, 1990). The altitude above sea level and geographical coordinates were determined using a GPSMAP 60 CEX system navigator: the altitude limits of the sampling points for mountain chernozems were 515-1082 m above sea level, for mountain-meadow chernozemic soils - 990-1920 m above sea level, coordinates 48.23252 - 48.61473 N, 31.5684 - 37.4681 E (Figure. 1). Classification and diagnostics were carried out according to the genetic classification of soils (Valkov et al., 2002; Classification and diagnostics of soils of the USSR, 1977; IUSS Working Group, 2015).

Physical, physic-chemical and biological analyses

Laboratory and analytical studies were performed in 3-6 replicates. The humic content (%) in the soil was determined by the method of Tyurin modified by Nikitin, the pH of the water extract of the soil was estimated potentiometrically, the field moisture and soil density was measured using the gravimetric method (Dobrovolskiy, 2001). The humus stock in the 0-20 cm layer was calculated using the soil density indices.

The rates of basal and substrate-induced respiration (BR and SIR) that characterize background and potential respiration activity of soil microbial biomass were determined in accordance with the methodological developments of Ananyeva (2003). The carbon content of microbial biomass (C_{mic}) was calculated using the formula: Cmic (µg C / g soil) = SIR (µl CO_2 / g soil / hour) × 40.04 + 0.37 (Anderson and Domsch, 1978). C_{mic} stock in 0-20 cm layer was calculated using soil density indicators. The ratio of microbial biomass carbon in the total soil organic carbon (%) was calculated as $C_{mic}/_{Corg}$.

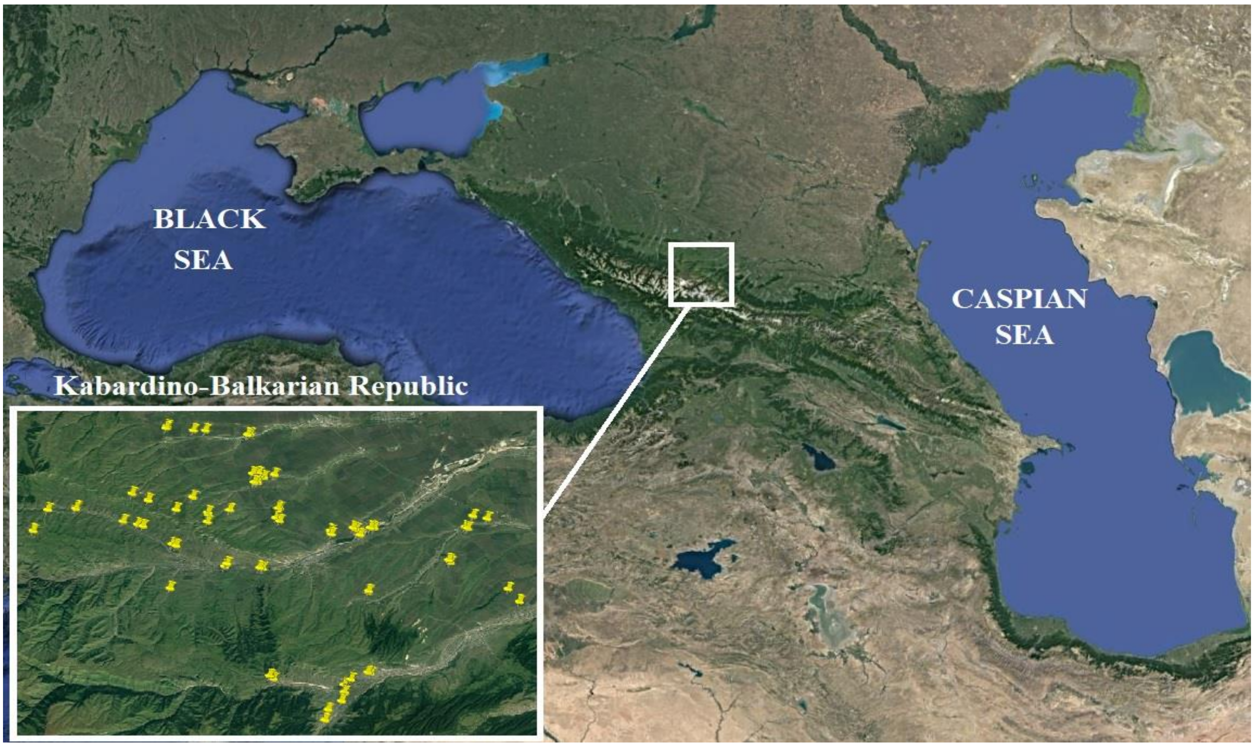

Figure 1. Map of the study area and sampling points of mountain soils of the Central Caucasus (Kabardino-Balkaria)

Activities of urease, phosphatase, invertase, dehydrogenase were measured colorimetrically; catalase activity was estimated gasometrically according to Galstyan's methods as modified by Khaziev (Kazeev et al., 2016). Sterilized soils (180°C, 3 hours) served as a control for determining the enzyme activity. The obtained biological parameters were assessed using the scale of Gaponyuk and Malakhova (1985). The calculation of the total relative enzyme activity was carried out according to the method proposed (Kazeev et al., 2016).

For a comparative assessment of the general level of biological activity of mountain chernozems and mountain meadow chernozemic soils, we used the methodology for calculating the integrative index of ecological and biological condition (IIEBC), which allows integrating the relative values of the studied parameters (Kazeev et al., 2004; Kolesnikov et al., 2021).

Statistical analysis

The obtained data was treated using STATISTICA 10 program. The significance of differences in the studied soil characteristics of the compared plots was assessed using the Student's t-test at a significance level of ≤0.05.

Results and Discussion

Physical and physicochemical soil properties

Soil biological activity to a significant extent depends on genetically-based physical and chemical parameters (soil density, acid-base conditions) and soil organic matter content (Breza-Boruta et al., 2016; Martinez-Mera et al., 2017; Ananyeva et al., 2020; Kolesnikov et al., 2021). The results of the estimation of physical and chemical properties of humus-accumulative horizons (0-20 cm) of the studied subtypes of mountain chernozems and mountain-meadow chernozemic soils are presented in Table 2.

The soil density affects the intensity of microbiological and biochemical processes (Mangalassery et al., 2019; Kazeev et al., 2004). The best conditions for the development of soil microbial biomass and catalytic activity of enzymes are formed at density values close to 1.0 g/cm^3, since the porous composition of the soil determines the hydrothermal and air regimes that are optimal for the soil biota (Kazeev et al., 2004). As the presented data show, the studied subtypes of mountain soils in the upper horizons (0-20 cm) have a porous loose composition, which is due to the activity of the root system of herbaceous plants, soil mesofauna and high organic matter content. The established values of the bulk density (Table 2) can be considered characteristic of the studied mountain soils.

Table 2. Average values ($\overline{X}$ ± m) of the indicators of the upper horizon (0-20 cm) of mountain soils of the Elbrus variant of zonality within Kabardino-Balkaria

Soils	Density, g/cm³	pH(H_2O)	Humus	
			Content, %	stocks, t/ha
Mountain chernozems typical	1.07±0.04	8.0±0.11	9.7±0.6	194±11
Mountain chernozems leached	1.01±0.03	7.9±0.12	9.5±0.6	173±12
Mountain meadow chernozem-likes typical	0.75±0.03	7.4±0.08	19.1±0.47	276±18
Mountain meadow chernozem-likes leached	0.99±0.06	7.0±0.11	13.8±1.1	209±23

Note: $\overline{X}$ ±m is mean and error of mean. The same is in Tables 3,4.

One of the most important factors regulating the level of biological activity is the acid-base conditions of the soil solution (Margalef et al., 2017; Kolesnikov et al., 2021). A favorable environment for microbial and enzymatic activity is created when the reaction of soil solution is neutral or close to it (Kazeev et al., 2004). Analysis of the results obtained (Table 2) showed that mountain chernozems in the upper humus-accumulative horizons are characterized by a weakly alkaline reaction. The pH_{H2O} level of mountain meadow chernozemic soils is in the range of neutral values. For this parameter, statistically significant differences were established between the considered soil types (t = 6.98; P = 0.00), which is due to the peculiarities of the genesis of the considered soils. The obtained average pH_{H2O} values characterize the acid-base conditions of the studied soils as favorable for most biological processes and promoting the activity of bacterial microbial biomass and most soil enzymes.

Numerous soil characteristics are associated with the humic content that provide a certain level of biological activity (Sinsabaugh et al., 2010; Liang et al., 2017; Kolesnikov et al., 2021). The described types of mountain soils are characterized by the accumulation of a large amount of organic matter, which is due to climatic conditions and the development of rich meadow vegetation in the studied mountain landscapes (Tsepkova and Fisun, 2005). A feature of mountainous regions is a shorter warm period, where the processes of plant residues decomposition and soil organic matter mineralization are slowed down (Molchanov, 2008). As follows from the literature (Fiapshev, 1996; Egli and Poulenard, 2016; Kostenko, 2017), the general pattern for mountain soils is an increase in the humus stock as the amount of precipitation increases and the temperature decreases with the height of the terrain, which is confirmed by the presented data (Table 2).

According to the system of the humus state indicators for mountain soils (Kazeev et al., 2004), the studied mountain chernozems have high humic content, and in mountain meadow chernozemic soils it is very high. Statistically significant differences in the average content of soil organic matter were revealed between the studied soil types (t = 6.79; P = 0.00). So, for mountain meadow chernozemic typical soils that characterized by maximal humus content, the analyzed parameter is almost 2 times higher than the indicator for both subtypes of mountain chernozems (t ≥ 10.91; P = 0.00). In accordance with the rating scale (Valkov et al., 2004), humus stock in the upper horizons (0-20 cm) of mountain chernozems should be assessed as high, and in mountain meadow chernozemic soils - as very high. The difference in this parameter at the type level is 24% (t = 3.38; P = 0.00). Thus, the obtained average indicators, quantitatively characterizing the humus state of mountain chernozems and mountain meadow chernozemic soils, confirm their difference at the type level and can serve as reference characteristics in further monitoring studies.

Microbiological soil properties

One of the most important indicators of the biological activity of soils associated with the balance of organic carbon in the ecosystem are the rates of basal and substrate-induced respiration (BR and SIR) (Mangalassery et al., 2019; Zhao et al., 2019; Ananyeva et al., 2020). The studies carried out show that the BR rate (Table 3), which to a certain extent characterizes the amount of carbon available to support the vital activity of microorganisms, is higher in mountain meadow chernozemic soils in comparison with mountain chernozems, on average by 19% (t = 1.61; P = 0.11).

Higher (by 38%) values of the SIR rate, characterizing the potential activity of the soil microbial community, were also noted for mountain meadow chernozemic soils (Table 3). The differences found between the studied soil types are statistically significant (t = 5.85; P = 0.00), which indicates a higher potential of the microbial pool in mountain meadow chernozemic soils. In a number of works (Calderon et al., 2016; Liang et al., 2017; Sushko et al., 2019) it is shown that the amount of real and potential respiratory activity is influenced by the content of organic matter in soils, which is directly proportional to the formation of carbon dioxide. According to the results of the correlation analysis in the studied mountain soils, a positive relationship was revealed between the BR rate and the humic content (r = 0.33). The high correlation coefficient (r = 0.71) between the SIR rate and the amount of organic matter indicates a close relationship between these indicators.

Table 3. Average values of microbiological indicators of the upper horizon (0-20 cm) of mountain soils of the studied territories

Soil	BR	SIR	C_{mic}		C_{mic}/C_{org}, %
	mkg CO_2/g/h		Content, mkg C/g	Stock sg/m²	
Mountain chernozems typical	18.8±2.1	91.2±7.6	2018±168	446±45	3.9±0.35
Mountain chernozems leached	15.1±2.2	88.7±8.2	1964±181	458±58	4.1±0.37
Mountain meadow chernozem-likes typical	24.3±3.6	157.4±9.1	3483±200	522±38	3.1±0,.1
Mountain meadow chernozem-likes leached	18.4±2.7	130.2±13.1	2881±289	571±66	4.9±0.53

Note: Assessment scale of microbial biomass carbon content (mkg C/g) in the soils: < 200 is very low; 201-500 is low; 501-1000 is average; > 1000 is high (Ananyeva, 2003).

Determination of the SIR rate also makes it possible to establish the content of microbial biomass carbon (C_{mic}), an important quantitative characteristic of the state of the microbial community of the studied soils (Xu et al., 2019; Sushko et al., 2019). Analysis of the data obtained (Table 3) shows that all the studied subtypes of mountain soils, according to the assessment scale (Ananyeva, 2003), have a very high content of C_{mic}. It is noteworthy that the average values of the Cmic content of mountain meadow chernozemic soils are significantly higher (by 38%, t = 5.85; P = 0.00) than in mountain chernozems. The difference in the indices of the Cmic stock in the compared soil types is less pronounced (17%, t = 2.47; P = 0.02), which is explained by the higher indices of the soil bulk density in the sample characterizing the upper horizons of mountain chernozems (Table 2).

The ratio of microbial carbon (C_{mic}/C_{org},%) characterizes the content of the living and active part of soil organic carbon, which is involved in the subsequent processing of all soil organic matter (Ananyeva, 2003; Purtova et al., 2017). An increase or decrease in this indicator shows a change in the nutrients availability, since the microbial biomass reacts very sensitively to any factors of disturbance, while the total soil carbon content, on the contrary, is a stable indicator. The C_{mic}/C_{org} values presented (Table 3) are close to the data previously established for chernozems in the plain part of Kabardino-Balkaria (2.8-4.7%) (Gorobtsova et al., 2016). The differences found at the level of the type of the studied soils are not statistically significant (t = 0.065; P = 0.95), and the characteristic C_{mic}/C_{org} indicator can be taken to be close to 3-4%.

Biochemical soil properties

Enzyme activity is a relatively stable and informative indicator for studying and comparing various soil types in order to characterize their biological properties (Rao et al., 2014; Merino et al., 2016; Sudina et al., 2021). To estimate the biological activity of mountain soils, the catalytic activity of hydrolytic (invertase, phosphatase, urease) and redox (dehydrogenase, catalase) enzymes was measured. The activity of oxidoreductases characterizes the redox reactions of the transformation of organic substances, the activity of hydrolases characterises the intensity of the processes of organic substances mineralization, which include the most important nutrients - nitrogen, phosphorus, etc. (Utobo and Tewari, 2015; Kazeev et al., 2016; Kashirskaya et al., 2020).

In accordance with the data of the rating scale (Gaponyuk and Malakhov, 1985), in all studied subtypes of mountain soils the activity of catalase is average, dehydrogenase activity is medium in mountain chernozems and weak in mountain meadow chernozemic soils (Table 4). In the group of hydrolases, invertase exhibits weak activity in mountain chernozems and moderate in mountain meadow chernozemic soils. For all subtypes of mountain soils, an average level of phosphatase activity was noted and a high level of urease activity. The established differences in absolute values between soil types are statistically significant for all enzymes (t ≥ 2.72; p ≥ 0.00), except for urease (t = 1.41; p = 0.16).

Table 4. Mean values of enzymatic activity parameters for the upper horizon (0-20 cm) of mountain soils in the studied areas

Soil	Hydrolase activity			Oxidoreductase activity	
	Invertase	Phosphatase	Urease	Dehydrogenase	Catalase
Mountain chernozems typical	11.6±1.1	23.9±0.7	42.9±6.1	8.4±1.1	7.6±0.6
Mountain chernozems leached	14.1±1.5	25.3±1.7	57.3±10.7	9.2±1.3	6.8±0.3
Mountain meadow chernozem-likes typical	32.4±1.6	37.7±3.8	68.2±9.6	6.2±0.5	6.8±0.6
Mountain meadow chernozem-likes leached	22.8±2.2	34.8±3.4	68.6±6.1	4.2±0.3	4.5±0.4

Invertase, mg glucose/1 g/24h; Phosphatase, mg P_2O_5/100 g/1h; Urease, mg NH_3/10 g/24h; Dehydrogenase, mg TPF/10 g/24h; Catalase, mg O_2/1 g/1 min.

According to the literature data (Sinsabaugh et al., 2010; Oliveira Silva et al., 2018; Kazeev et al., 2021) and the results of previous studies of the lowland chernozems of Kabardino-Balkaria (Gedgafova et al., 2015; Gorobtsova et al., 2016) the activity of hydrolytic enzymes is closely related to the organic matter content. That was confirmed in this study of the biological properties of mountain soils. According to the data of correlation analysis, in the considered series of soils mainly an average positive relationship between the activity of urease (r=0.57) and a strong one - invertase (r=0.72) and phosphatase (r=0.74) with humic content were established. The catalytic activity of oxidoreductases, according to the authors (Kazeev et al., 2004; Kolesnikov et al., 2021), is more associated with the pH of the soil solution than with the humic content, which was confirmed in the present studies. Dehydrogenase and catalase are most active in mountain chernozems with a slightly alkaline reaction of the soil solution and the least in soils with neutral pH values (mountain meadow chernozemic soils). The studied subtypes of mountain soils in terms of the total relative activity of hydrolytic enzymes are arranged in the following row in descending order: typical mountain meadow chernozemic soils (100%) > leached mountain-meadow chernozemic soils (88%) > leached mountain chernozems (65%) > typical mountain chernozems (54%). The indicators of the total relative activity of oxidoreductases form a series - typical mountain chernozems (100%) > leached mountain chernozems (99%) > typical mountain meadow chernozemic soils (82%) > leached mountain-meadow chernozemic soils (55%). The higher activity of hydrolases in mountain meadow chernozemic soils is due to the optimal soil pH_{H2O} values for the action of hydrolytic enzymes and a relatively high humic content (Table 2), which regulate the level of activity of the corresponding enzymes of nitrogen and phosphorus metabolism. The total activity of oxidoreductases is higher in mountain chernozems, which is obviously associated with the slightly alkaline conditions of the soil solution. Indicators of the total relative enzymatic activity confirm that the intensity and direction of biochemical processes to a certain extent is associated with the genetic characteristics of the studied types of mountain soils.

The best results for diagnosing the biological activity of soils are obtained by a comprehensive assessment of biological parameters using the method for determining the integrative index of ecological and biological condition (IIEBC) (Kazeev et al., 2004; Kolesnikov et al., 2021). The given values of IIEBC (%), characterizing the general level of biological activity, summarize the following biological parameters: the humic content, carbon of microbial biomass (C_{mic}) content, the activity of hydrolytic (invertase, phosphatase, urease) and redox (dehydrogenase, catalase) enzymes (Figure 2).

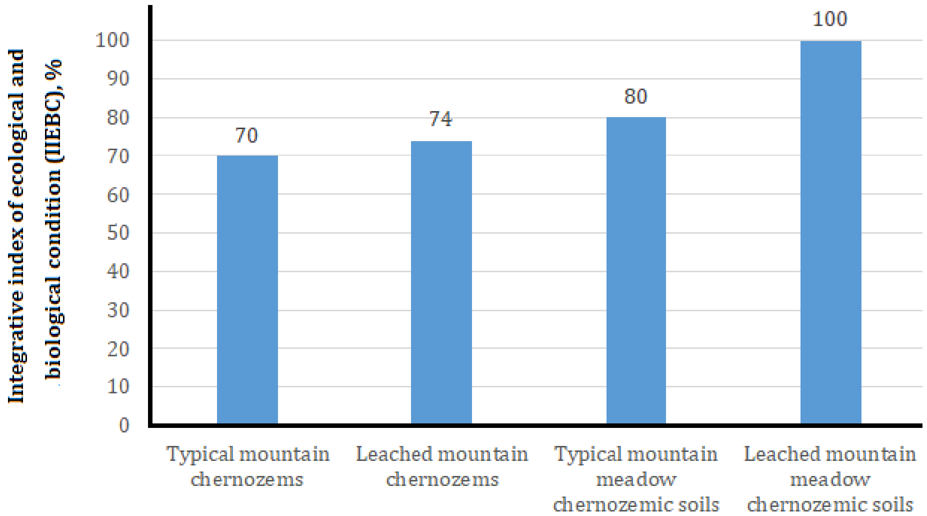

Figure. 2. Values of IIEBC (%) of the upper horizons (0-20 cm) of mountain soils of the Elbrus variant of the Kabardino-Balkaria zonality

The values of IIEBC calculated relative to this indicator for mountain meadow chernozemic typical soils are presented on Figure 2. Mountain meadow chernozemic typical soils are characterized by maximal total biological activity of the upper horizons (100%). The higher biological activity of these soils is due to the unique complex of soil-forming conditions in which these soils function (Molchanov, 2008).

Conclusion

According to the estimations, the average indicators characterizing the humus state of mountain soils make it possible to classify them as soils with high and very high humus content and reserves. It was shown that the general level of biological activity in soil types with different genesis, along with the conditions of soil formation, is regulated by the organic matter content and the acid-base reaction of the soil solution. The maximal biological activity and biogenecity were noted in mountain-meadow chernozemic soils, which surpass mountain chernozems in such parameters as humic content by 42%, humus stock - 24%, BR rate - 19%, SIR rate - 37%, Cmic content - 37% and stock - 17%, the relative total activity of enzymes of the class of hydrolases - 36% and are inferior in activity of oxidoreductases by 32%. According to the degree of decrease in IIEBC (%), the studied natural soils form the following row: typical mountain meadow chernozemic soils (100%) > leached mountain meadow chernozemic soils (80%) > leached mountain chernozems (74%) > typical mountain chernozems (70%). Higher indicators of biological activity of mountain meadow chernozemic soils are a consequence of the influence of better moisture conditions and richness of mountain meadow plant communities under which these soils were formed. The most characteristic biological indicators of mountain soils of natural landscapes have been established. These indicators can be used as reference in various environmental studies: while assessing the degree of soil cover degradation as a result of economic activities, for the needs of environmental regulation of agrogenic, pasture, or recreational load.

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Eurasian Journal of Soil Science 2022, 11(1), 86 - 92

Eurasian Journal of Soil Science

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

Morphophysiological response of young Frantoio olive tree under different fertilizer types in sierozem with surface drip irrigation

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Article Info

Received : 21.05.2021
Accepted : 15.09.2021
Available online : 17.09.2021

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Abstract

The amount of yield and adaptation of a cultivar to a new environment is strongly related to nutrient uptake ability. This study was carried out during 2019, 2020 and 2021 seasons to investigate the effect of different forms of chemical compose 20.20.20 fertilization alone or incombination with other fertilizers on morphological plant parameters (number of leaves per shoot, stem length and stem thickness) and leaf nutrient contents (N, P, K and Mg) of young Frantoio olive tree grown in Turkestan region, South Kazakhstan. The study was conducted on 1 years old olive trees of Frantoio in sierozem, under surface drip irrigation, system and uniform in shape and received the common horticultural practices. It was determined that fertilizer treatments significantly influenced number of morphological plant parameters and leaf plant nutrients compared to control treatments at all seasons. Results revealed that all fertilizers as well as the combination between 20.20.20 and Biohumus treatment and/or Nitroammophos treatment significantly increased morphological plant parameters and nutrient contents of young Frantoio olive trees. It can be concluded that the variation in the nutrient uptake ability may be used as a criterion for adaptation of a variety to a new ecological environment.

Keywords: Olive tree, fertilizer, drip irrigation, soil, sierozem.

Introduction

The olive tree (Olea europaea L.) is an ancient, traditional crop in the Mediterranean Basin (Langgut et al., 2019; Arenas-Castro et al., 2020). It is believed that the olive tree originated in the Mediterranean region and has been cultivated since 4800 BC (Fraga et al., 2021). It originated in the eastern Mediterranean and was spread widely around southern Europe, northern Africa, and the Iberian Peninsula. More recently, it has been introduced to other continents including the Americas, South Africa, Australia and Asia (Connor, 2005). The world area of olive is around 9 Mha, with major production (95% of 2.5 Mt oil) in 5 Mediterranean countries, Greece, Italy, Spain, Tunisia, and Turkey. Other continents, South America, South Africa, and Australia, are now becoming significant producers. The initial expansion around the Mediterranean moved the crop into comparable, although drier (southern) and colder (northern) environments. The present 'New World' expansion is taking olive into non-Mediterranean climates, e.g. subtropics in Australia and Argentina, where the response of the crop is yet to be studied in detail (Connor, 2005).

Olive is a drought-resistant plant. In general, the traditional olive orchards in Mediterranean areas are under rain-fed conditions without any form of irrigation (Sofo et al., 2008). However, in modern intensive

: https://doi.org/10.18393/ejss.996621
: http://ejss.fesss.org/10.18393/ejss.996621
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

orchards, more trees are planted per hectare than are in traditional orchards, which leads to decreased water availability for individual olive trees at a specific area with a relatively stable amount of rainfall (Fernández and Moreno, 1999). As a consequence, olive growth, including vegetative growth (the basis for flowering and cropping in the next year), flower-bud formation, and fruit development, could be limited by water shortage (Masmoudi-Charfi and Mechlia, 2008; Gucci et al., 2009). Studies have shown that irrigation during summer and autumn in a Mediterranean climate is an effective way to increase olive productivity (Proietti and Antognozzi, 1996; Sanz-Cortes et al., 2015; Liu et al., 2019).

South Kazakhstan is mostly an arid and semi-arid, strongly continental climate, with hot summers and cold winters. Sierozems are brown desert soils that are located in Turkestan region of South Kazakhstan (Shokparova and Issanova, 2013; Beketova et al., 2017; Yertayeva et al., 2018). Sierozem soils are a valuable resource because of their extent and because they are fertile, but, sierozems have low soil organic matter content. Sierozems must be properly managed and protected for efficient and sustainable productivity. They have been researched in the past but mainly as a soil-geographic resource. Further study is needed to quantify and expand their value in production and assure environmental sustainability (Jalankuzov et al., 2013; Saparov, 2014). Some fertilizers including humus and/or humic acids are a ready-to-use live formulation of such beneficial microorganisms which on application to seed, root or soil, mobilize the availability of nutrients by their biological activity. They help build up the soil micro-flora and there by the soil health. Use of fertilizer including humus and/or humic acids are recommended for improving the soil fertility in sustainable farming.

In the main growing area in the Turkestan region, South Kazakhstan, there is almost no rainfall during summer. In recent years, most of the olive trees in Turkestan region have been planted, and drip irrigation has been applied in the modern intensive olive orchards to replace traditional flood irrigation. However, little is known about the effects on olive productivity of fertilization regimes with surface drip irrigation in South Kazakhstan. Therefore, a 3-year field study was conducted to gain a better understanding the effect of chemical fertilizers alone or in combination with other fertilizers including humus and/or humic acids on vegetative growth, leaf mineral contents of Frantoio olive trees grown in sierozem with surface drip irrigation in Turkestan region, South Kazakhstan.

Material and Methods

Study Area

The experiment was performed at Ordabasy district of the Turkestan region, South Kazakhstan (Figure 1). The experimental fields had been in new olive growing area of Turkestan region at 3 years (2019-2021). In this area, efforts are being made to create new olive plantations. This region is characterized by a semi-arid climate. Most of the precipitation occurred in June to September. The annual mean precipitation and mean temperature from the establishment of the experiment is shown in Figure 2.

Figure 1. Study area

Soil

The main soil type, which is typical for the region, is sierozem soils. The sierozem soils are found in arid regions which characterized by a brownish-gray surface on a lighter layer based on a carbonate or hard-pan layer (USDA, 1999). Ordinary sierozems develop on loess-like loams and have fully developed profile with a rather noticeable division into genetic horizons. Sierozems are marked by good water-physiological properties, high biological activity, and adequate fertility; they produce high yields when irrigated. There are various subtypes: light, conventional (standard), dark, and northern (Saparov, 2014). The soil belongs to the general soil type of dark sierozem. The soil pH was 7,7 and organic matter content was 0.96%, NO_3-N was 5.4 mg kg^{-1}, available phosphorus was 6.04 mg kg^{-1} and exchangeable potassium was 380 mg kg^{-1}.

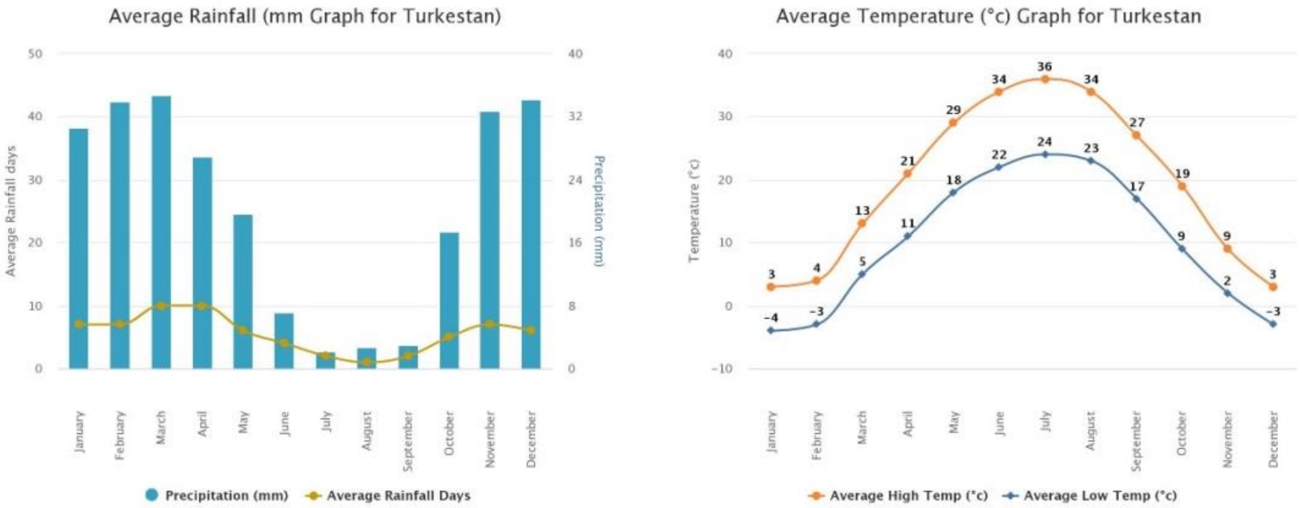

Figure 2. Monthly average temperature (^0C) and distribution of precipitation (mm) of the experimental area.

Olive (Olea europaea)

Frantoio olive variety were sampled and which was introduced from Turkey as 1-year-old cuttings in 2019. Some general informations and agronomic properties of Frantoio olive variety is given below and Table 1.

Table 1. Some agronomic properties of Frantoio olive tree

Origin	Italy
Cold tolerance	Low
Self sterile Productivity	High
Start of bearing	Early
Ripening	Late
Oil content	High

Frantoio is a moderately vigorous tree with a spreading-drooping growth habit and medium-dense canopy. It is one of the main varieties in the classic Tuscan blend. Frantoio is highly and consistently productive, and very adaptable. It is quite cold sensitive, however, and can suffer frost damage while other varieties in the same orchard are unscathed. Frantoio is self fertile, but the yields increase with the presence of pollinizers. Pendolino is the most popular choice for a pollinizer, but Leccino and Maurino are suitable as well. Frantoio is popular world-wide, with significant acreage in Australia, Argentina and Chile.

Fertilizers

Five different fertilizer were used as treatments. Nutrient contents of fertilizers are given in Table 2 and all fertilizers used were in the form of wholly soluble.

Table 2. Nutrient content of the fertilizers used in the experiment

Fertilizers	Humus,%	Humic acid, %	N,%	P_2O_5,%	K_2O,%	Ca,%	Mg,%
Compose fertilizer	-	-	20	20	20	0,2	0,3
Nitroammophos	-	-	16	16	16	0,1	0,3
Biohumus	10	-	0.9	1.3	1.5	4.5	0.5
Calcium Humate	-	80	0.8	0.9	0.7	0.2	0.1
Potassium humate	-	80	0.5	0.8	0.6	0.2	0.5

Treatments and Experimental design

A long term experiment was established at Ordabasy district of the Turkestan region, South Kazakhstan in April 2019 and the field trial was conducted during three consecutive seasons (2019, 2020 and 2021). The trees were evenly planted at three different planting density. These are i) 4.0 m x 3.0 m, ii) 4.0 m x 2.0 m, and iii) 4.0 m x 1.5 m. Two water-dripping lines were placed on the south and north sides along the trees, 30 cm away from trees. The drip lines ran east west, with a pipe diameter of 14 mm and emitter spacing of 30 cm. The drip rate was 2 L h^{-1}. Photographs from the experiment are given in Figure 3.

Figure 3. Photographs from the experiment

The experiment adopted a randomized block design with three different planting density and different fertilizer type and was performed with the following 15 treatments.

CO1 : Control ; + 20.20.20 (3 kg/ton water in week) with Drip irrigation during April to September ; Planting density is 4 m x 3 m

CO2 : Control ; + 20.20.20 (3 kg/ton water in week) with Drip irrigation during April to September ; Planting density is 4 m x 2 m

CO3 : Control ; + 20.20.20 (3 kg/ton water in week) with Drip irrigation during April to September ; Planting density is 4 m x 1,5 m

BH1 : Biohumus treatment (3kg /ton water in week) + 20.20.20 (3 kg/ton water in week) with Drip irrigation during April to September ; Planting density is 4 m x 3 m

BH2 : Biohumus treatment (3kg /ton water in week) + 20.20.20 (3 kg/ton water in week) with Drip irrigation during April to September ; Planting density is 4 m x 2 m

BH3 : Biohumus treatment (3kg /ton water in week) + 20.20.20 (3 kg/ton water in week) with Drip irrigation during April to September ; Planting density is 4 m x 1,5 m

CH1 : Calcium humate treatment (0,5 lt /ton water in week) + 20.20.20 (3 kg/ton water in week) with Drip irrigation during April to September ; Planting density is 4 m x 3 m

CH2 : Calcium humate treatment (0,5 lt /ton water in week) + 20.20.20 (3 kg/ton water in week) with Drip irrigation during April to September ; Planting density is 4 m x 2 m

CH3 : Calcium humate treatment (0,5 lt /ton water in week) + 20.20.20 (3 kg/ton water in week) with Drip irrigation during April to September ; Planting density is 4 m x 1,5 m

PH1 : Potassium humate treatment (0,5 lt /ton water in week) + 20.20.20 (3 kg/ton water in week) with Drip irrigation during April to September ; Planting density is 4 m x 3 m

PH2 : Potassium humate treatment (0,5 lt /ton water in week) + 20.20.20 (3 kg/ton water in week) with Drip irrigation during April to September ; Planting density is 4 m x 2 m

PH3 : Potassium humate treatment (0,5 lt /ton water in week) + 20.20.20 (3 kg/ton water in week) with Drip irrigation during April to September ; Planting density is 4 m x 1,5 m

NA1 : Nitroammophos treatment (2 lt /ton water in week) + 20.20.20 (3 kg/ton water in week) with Drip irrigation during April to September ; Planting density is 4 m x 3 m

NA2 : Nitroammophos treatment (2 lt /ton water in week) + 20.20.20 (3 kg/ton water in week) with Drip irrigation during April to September ; Planting density is 4 m x 2 m

NA3 : Nitroammophos treatment (2 lt /ton water in week) + 20.20.20 (3 kg/ton water in week) with Drip irrigation during April to September ; Planting density is 4 m x 1.5 m

Observed parameters

Four branches with flower buds and new apical shoots from the four directions (east, south, west, and north) in each sampling tree were labeled at the end of April for field survey, and counted in August. Observed morphological parameters in plants were number of leaves, stem length, stem thickness and leaf plate size. Nutrient concentration (N, P, K, Mg) in leaf. All parameters were measured according to Jones (2001).

Results and Discussion

Morphological plant parameters

Morphological plant parameters such as the number of leaves per shoot, stem length and stem thickness of young Frantoio olive tree differed significantly due to different fertilization all season (Table 3). At all seasons, fertilizer treatments significantly influenced number of leaves per shoot, stem length and stem thickness compared to control treatments. On the contrary, it was determined that planting density did not affected morphological plant parameters of young Frantoio olive tree at all season and fertilization compared to control treatments.

Table 3. Effect of different types of fertilizers on Morphological plant parameters of young Frantoio olive tree

Treatments	Number of leaves per shoot				Stem length, cm				Stem thickness, cm				Leaf plate size, cm			
	2019	2020	2021	mean	2019	2020	2021	mean	2019	2020	2021	mean	2019	2020	2021	mean
CO1	2,5	3,8	32,0	12,8	30,2	45,0	51,4	42,2	0,5	1,25	2,1	1,3	0,5	1,2	2,4	1,4
CO2	2,7	3,9	33,2	13,3	36,2	45,8	51,9	44,6	0,6	1,2	2,2	1,3	0,7	1,4	2,6	1,6
CO3	2,6	3,8	34,1	13,5	36,0	45,5	51,7	44,4	0,7	1,0	2,4	1,4	0,8	1,3	2,5	1,5
BH1	4,7	8,4	36,8	16,6	36,4	48,2	54,6	46,4	0,9	1,6	2,7	1,7	1,8	1,7	4,2	2,6
BH2	6,2	9,2	37,2	17,5	35,8	48,7	54,8	46,4	1,2	1,7	3,2	2,0	1,9	1,9	5,2	3,0
BH3	6,1	9,0	37,0	17,4	35,6	48,5	54,7	46,3	1,5	1,5	3,0	2,0	1,6	1,6	5,0	2,7
CH1	4,2	8,0	41,0	17,7	35,0	48,3	54,2	45,8	0,6	1,4	2,6	1,5	1,4	1,4	4,9	2,6
CH2	4,8	8,4	42,3	18,5	35,6	48,6	53,7	46,0	0,7	1,6	2,8	1,7	1,6	1,6	5,1	2,8
CH3	4,5	8,2	42,1	18,3	35,4	48,4	53,5	45,8	0,6	1,5	2,7	1,6	1,5	1,4	5,0	2,6
PH1	4,0	10,2	39,2	17,8	34,4	48,0	52,3	44,9	0,5	1,5	2,8	1,6	1,2	1,5	2,9	1,9
PH2	4,9	10,5	39,8	18,4	34,7	48,4	52,7	45,3	0,6	1,7	2,6	1,6	1,3	1,6	3,1	2,0
PH3	4,6	10,2	39,6	18,1	34,5	48,2	52,5	45,1	0,5	1,6	2,7	1,6	1,0	1,4	3,0	1,8
NA1	5,0	7,5	35,6	16,0	35,6	49,5	54,5	46,5	0,9	1,7	3,0	1,9	1,7	1,9	4,7	2,8
NA2	5,8	7,8	35,9	16,5	35,7	49,7	54,8	46,7	1,2	1,9	3,1	2,1	1,9	2,0	5,2	3,0
NA3	5,5	7,4	35,6	16,2	35,5	49,6	54,4	46,5	1,1	1,8	3,0	2,0	1,8	1,8	5,0	2,9
Mean	4,5	7,8	37,4	16,6	35,1	48,0	53,4	45,5	0,8	1,5	2,7	1,7	1,4	1,6	4,1	2,3

Data presented in Table 3 indicate that, number of leaves per shoot, leaf plate size, was affected by conducted treatments in the three seasons. BH (Biohumus treatment) resulted in highest significantly number of leaves per shoot in first, PH (Potassium humate treatment) was in second and CH (Calcium humate treatment) was in third seasons respectivitly. On the other side, the lowest number of leaves per shoot was obtained from CO (Control) treatment since it was in the first, second and third seasons. In addition, leaf plate size was affected by different treatments in first, second and third seasons. BH and NA treatments resulted in the largest leaf plate size in the first, second and third seasons. On the other contrary the lowest leaf plate size was found in CO treatments all seasons. Research results showed that stem length and stem thickness was significantly affected with different fertilizer treatments (Table 3). BH treatments and NA treatments recorded the highest value in the all seasons. On the contrary, the lowest stem length and stem thickness was recorded under the CO treatments in all season. Similar results were obtained by Bilalis et al. (2015), Wang and Xing (2017) and Alimkhanov et al. (2021) on several vegetable crops. Osman et al. (2010) found that bio-NPK fertilizer treatments soil applied significantly increased all leaf amino acid content and mineral composition, shoot nitrogen and total carbohydrates of Manzanillo young olive trees during the two seasons.

Leaf nutrient contents

Leaf nutrient contents such as N, P, K and Mg of young Frantoio olive tree differed significantly due to different fertilization all season (Table 4). At all seasons, fertilizer treatments significantly influenced leaf nitrogen, phosphorus, potassium and magnesium content of young Frantoio olive tree compared to control treatments. In addition, it was determined that planting density affected leaf nutrient contents of olive tree at all season and fertilization types compared to control treatments, and also plant density 2 (4 m x 2 m) treatments resulted in the highest leaf nutrient contents in all seasons and fertilization types. Numerous studies have reported that inorganic NPK fertilizer increased growth in some species by enhancing nitrogen, phosphorus, potassium and magnesium uptake (Shehu, 2014; Gülser et al., 2019; Alimkhanov et al., 2021).

Leaf N concentration was significantly affected by fertilization. Maximum N concentration in the leaves was observed for NA followed by BH at all season (Table 4). Only BH and NA treatments, The leaf N concentration of the cultivars was well above the critical N level (1.5%) in all seasons. Studies in Greece and Portugal showed that leaf N concentrations of various olive varieties were above the critical level (i.e., 1.84–2.15 %) (Dimassi et al., 1999; Jordao et al., 1999). However, Loupassaki et al. (2002) have reported comparatively higher values for leaf N concentration with a range of 1.68–2.89%. The phosphorus levels of the leaves in second and third years were above the optimum level (1.0%), but in control treatment in first season (2019) was close to the deficiency threshold level of leaf P concentration (Table 4). Leaf P concentration of Greek olives was in the range of 1.3–1.6% (Dimassi et al., 1999) while it was 1.2–1.9% in

Portugal (Jordao et al., 1999). In contrast, Loupassaki et al. (2002) have reported a much higher range of P concentrations (1.7–2.7%) for mature leaves. The potassium concentration of the leaves significantly changed as a fertilization and and years (Table 4). Maximum K content in the leaves was observed for NA followed by BH at all season (Table 4). Leaf content of K was increased in the second and third seasons than in the first season. 0.5–0.9 and 0.54–0.83% leaf K concentration were reported for Greece (Dimassi et al., 1999) and Portugual (Jordao et al., 1999), respectively. In this study, the K concentrations measured were comparatively higher due to the high exchangeable K content of the experimental soil (380 mg kg^{-1}). Magnesium concentration of the leaves was significantly influenced by fertilizer type and season (Table 4). Minimum values were observed for control treatments (CO) at all seasons. Loupassaki et al. (2002), Dimassi et al. (1999) and Jordao et al. (1999) have all reported that leaf Mg concentration of Greek and Portuguese olives were above 0.1%, which are highly similar to our data. Seasonal change of Mg concentration of olive leaves was reported to be in the range of 0.1–0.2% (Christos et al., 2005).

Table 4. Effect of different types of fertilizers on leaf nutrient contents of young Frantoio olive tree

Treatments	N, %				P, %				K, %				Mg, %			
	2019	2020	2021	mean	2019	2020	2021	mean	2019	2020	2021	mean	2019	2020	2021	mean
CO1	0,7	0,8	1,2	0,9	0,5	1,4	2,0	1,3	0,7	0,9	1,6	1,1	0,4	0,6	0,9	0,6
CO2	0,9	1,4	1,8	1,4	0,8	1,7	2,5	1,7	0,8	1,1	1,8	1,2	0,5	0,7	1,0	0,7
CO3	0,8	1,2	1,7	1,2	0,6	1,5	2,1	1,4	0,6	1,0	1,7	1,1	0,4	0,5	1,0	0,6
BH1	1,4	1,8	3,0	2,1	1,6	2,4	3,2	2,4	2,2	2,6	3,0	2,6	2,3	2,6	3,1	2,7
BH2	1,8	2,4	3,3	2,5	1,9	2,8	3,6	2,8	2,3	2,8	3,2	2,8	2,5	2,9	3,5	3,0
BH3	1,6	2,2	3,2	2,3	1,7	2,7	3,5	2,6	2,1	2,7	3,0	2,6	2,4	2,6	3,2	2,7
CH1	0,8	0,9	2,4	1,4	0,9	2,0	2,5	1,8	1,2	1,8	2,5	1,8	0,9	1,4	2,4	1,6
CH2	1,2	1,5	2,6	1,8	1,4	2,1	2,7	2,1	1,5	1,9	2,7	2,0	1,2	1,6	2,6	1,8
CH3	1,0	1,2	2,5	1,6	1,2	2,0	2,6	1,9	1,4	1,8	2,6	1,9	1,1	1,3	2,5	1,6
PH1	0,9	1,4	2,0	1,4	0,8	1,7	2,4	1,6	1,1	1,7	2,4	1,7	0,8	1,1	2,0	1,3
PH2	1,1	1,6	2,3	1,7	0,9	1,9	2,6	1,8	1,3	1,8	2,5	1,9	0,9	1,5	2,2	1,5
PH3	1,0	1,3	2,1	1,5	0,7	1,8	2,5	1,7	1,2	1,7	2,3	1,7	0,8	1,2	2,1	1,4
NA1	1,5	1,9	3,2	2,2	1,5	2,3	3,0	2,3	2,4	2,8	3,3	2,8	1,9	2,0	2,3	2,1
NA2	1,6	2,5	3,6	2,6	1,8	2,4	3,2	2,5	2,8	2,9	3,5	3,1	2,3	2,2	2,5	2,3
NA3	1,7	2,7	3,5	2,6	1,7	2,2	3,1	2,3	2,6	2,7	3,2	2,8	2,2	2,1	2,3	2,2
Mean	1,2	1,7	2,6	1,8	1,2	2,1	2,8	2,0	1,6	2,0	2,6	2,1	1,4	1,6	2,2	1,7

It can be concluded that nutrient uptake ability and usage efficiency of young Frantoio olive trees are different fertilization. Better yield performance, along with the nutrient uptake, could be an indication of adaptation of young Frantoio olive tree to a specific ecological environment and growing conditions. Leaf nutrient contents of young Frantoio olive trees in "on" years is usually increased significantly. In conclusion, the obtained data revealed that, all fertilizers as well as the combination between 20.20.20 (3 kg/ton water in week) and BH treatment (3kg /ton water in week) or NA treatment (2 lt /ton water in week) significantly increased morphological plant parameters (the number of leaves per shoot, stem length and stem thickness) and nutrient contents (N, P, K and Mg) of young Frantoio olive trees.

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