2022

Volume : 11
Issue : 4
Page : 275-362

e-ISSN : 2147-4249

Eurasian Journal of Soil Science

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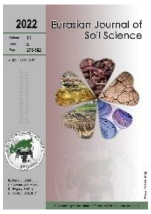
EURASIAN JOURNAL OF SOIL SCIENCE

(Peer Reviewed Open Access Journal)

Published by Federation of Eurasian Soil Science Societies



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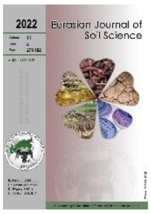
ABSTRACTING AND INDEXING: SCOPUS, CABI, FAO-Agris, EBSCOhost, ProQuest, DOAJ, OAJI, TR Dizin, TURKISH Journalpark, CrossRef, CiteFactor, CAS, etc.

Year: 2022, Volume: 11, Issue: 4, Page: 275 - 362 **e-ISSN 2147-4249**

EURASIAN JOURNAL OF SOIL SCIENCE

(Peer Reviewed Open Access Journal)

Published by Federation of Eurasian Soil Science Societies



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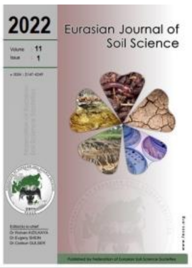
Journal homepage : http://ejss.fesss.org



Deforestation effects on soil properties and erosion: a case study in the central Rif, Morocco

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Article Info

Received : 18.04.2021
Accepted : 07.03.2022
Available online : 04.04.2022

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Abstract

In the Central Rif in the north of Morocco, forest ecosystems have suffered a very sharp decline in favor of crops. Deforestation followed by cultivation illustrates the important environmental, economic and social roles of forests. The objective of this work is to assess the impact of deforestation on soil properties and erosion in the southern Central Rif. The loss of fertility of cleared soils was assessed using physico-chemical analyses after 2, 8 and 20 years of cultivation. A manual rainfall simulation was used to assess the impact of cultivation on the hydrodynamic behavior of the soil. The results show that the conversion of forests into agricultural areas has multiple consequences on the natural system. The general trend of soil texture elements after cultivation shows a significant increase in sand content, and a decrease in clay and silt content. Soil erodibility measured by USLE-K factor increased 3.5 times in the cultivated soil for 20 yrs. compared to the forest soil. Subsequent tillage of cultivated land increases bulk density and fragments large aggregates into smaller ones. Cultivation for 8 and 20 yr decreased SOM by 41 and 82% respectively. Total Nitrogen decreased by 45%, acidity increased by 0.8 unit after 20 years of cultivation. Conversion of natural forest to agricultural land significantly increases soil erosion. The erosion rate becomes higher in the cultivated the 8 and 20 yr cultivation, with an average of 219.60 ± 19.3 and 989.17 ± 68.4 g m^{-2} h^{-1} respectively. This degradation hinders agricultural productivity, leading farmers to abandon the land and seek new plots at the expense of forests to meet their agricultural land needs.

Keywords: Land use change, cultivation, rainfall simulation, organic matter, erosion.

Introduction

Land use change characterized by deforestation for expansion of agricultural land increases the effects of climate change on land degradation (Kassa et al., 2017; Eekhout and de Vente, 2022). Erosion and soil fertility decline contribute to about 84% and 7% of soil degradation worldwide respectively (Oldeman, 1994). Soil erosion affects about 1100 Mha of cultivated land worldwide, and results in the transport of 20-25^{109} Mg of sediment to the oceans per year (Brown, 1984). Incompatible local human land use such as deforestation and soil fertility weakness, have led to changes in the physical and biological properties of the soil (Lu et al., 2002; Khormali et al., 2009). Deforestation and subsequent tillage practices increases the soil erosion, which can reduce soil quality and hinder soil productivity. Subsequent tillage of cultivated land can affect soil structural stability, nutrients and cause mineralization of organic carbon (Veldkamp et al., 2020), and therefore affect the amount of different organic nutrient reserves (Parton et al., 1987). For example, deforestation followed by cultivation decreases soil organic carbon (SOC) stocks, increases soil bulk density, and causes significant changes in soil pH (Assefa et al., 2017; Kassa et al., 2017; Veldkamp et al., 2020).

: https://doi.org/10.18393/ejss.1098600
: http://ejss.fesss.org/10.18393/ejss.1098600

Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

In Morocco, the rate of deforestation for the extension of living agriculture exceeds 31.000 ha/year (HCEFLC, 2004). This is remarkable in the Rif's mountains chain in northern Morocco. Indeed, the rapid demographic growth associated with the scarcity of land for agricultural purposes has led to strong pressure on forest environments. The expansion of cannabis cultivation has increased rapidly since 1980 (illegal cultivation at that time) and has largely contributed to the fixation of the population in the region (Chouvy, 2020), but also to increase the rate of deforestation over their agricultural needs. The rapidity of clearing operations is directly associated with the depletion of the soil's fertility potential due to the effect of erosion and cannabis cultivation, which consumes a lot of forest space and its humus (Grovel, 1996; Benabid, 2000). At present, more than two thirds of the old clearings are permanently abandoned, and the slopes are completely stripped (El Mazi et al., 2021). Land degradation caused by erosion increases the concentrations of suspended sediments in watercourses, which leads to silting of downstream dam reservoirs and damage to road infrastructure and even houses (Al Karkouri et al, 2000; Tribak, 2020).

The study area has been the subject of several studies on morphogenesis and water erosion over the last decades (Sabir et al., 2004; Tribak, 2020; Zaher et al., 2021; Arrebei et al., 2020; El Mazi et al., 2021). These studies showed that the deforestation and the successive tillage led to profound changes in soil properties affect directly the soil functional processes. Sabir et al. (2004) showed that the cultivation of cork oak forest land in northern Morocco reduces the organic matter content by 47% and greatly increases the risk of runoff and erosion. However, El Mazi et al. (2021) reported that this cultivation of forest soils on siliceous substrate in northern Morocco for 22 years decreased OM by 73% and made the soil instable and more susceptible to the erosion. Zaher et al. (2021) studied the effects of land use change on soil erosion and hydrological behavior in the Tlata watershed in northern Morocco, showed that detachability increased in the cultivated soil by 3 times compared to the forest soil. The objective of this work is, on one hand, to evaluate and quantify the impact of the conversion of forests into agricultural land on the physico-chemical, hydrodynamic properties of the soil in the Central Rif in northern Morocco, and on the other hand, is to determine the impact of cultivation and subsequent works on soil quality, structural stability, runoff and water erosion.

Material and Methods

Study site

The area concerned by this study is located in the Jbel Lerz and Outka forest massif, part of the central Rif in northern Morocco (Figure 1). It extends between longitudes 4°54' and 5°W, and between latitudes 34°40' and 34°54'N. It is characterized by a bioclimatic gradient ranging from subhumid in the valleys to perhumid in the high peaks. The Mediterranean rainfall is abundant with an average of between 800 mm/year and 1500 mm/year. More than 70% of the rainfall is concentrated between October and March. The number of rainy days exceeds 70 days/year. Autumn rainfall is marked by an often very high daily intensity of certain showers, with intensities of more than 150 mm/day, thus opening up runoff phenomena and water erosion. The most dominant substratum is the Ketma unit shale, the sandstone with albo-aptian shale and quartzite sandstones (Maurer, 1968).

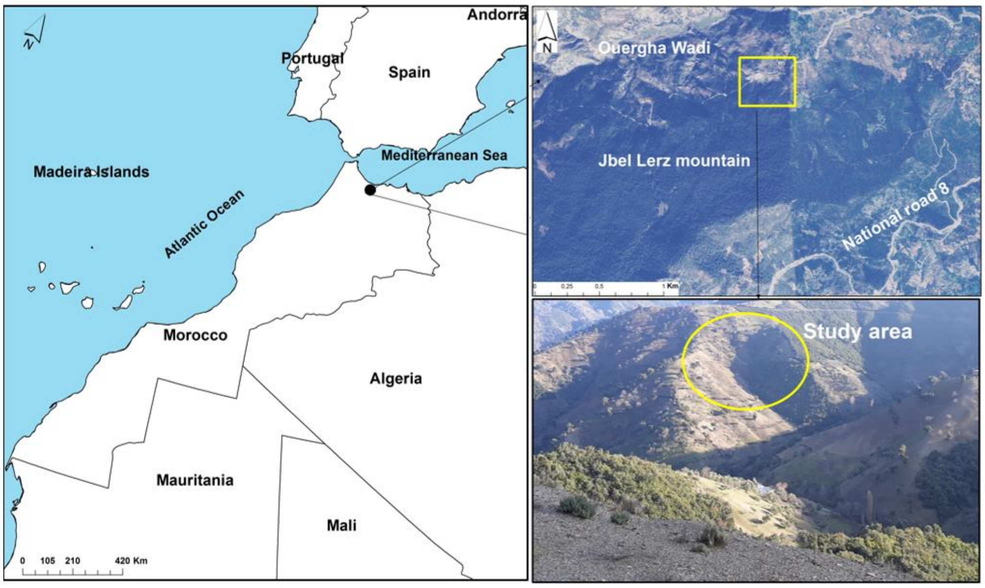

Figure 1. Study area

Characterization of the study plots and cultivation practices

In order to study the impact of the evolution of land use on the degradation of soil resources, 24 plots were selected so as to be as homogeneous as possible in terms of physiography (relief, lithology, soil, climate, etc.). All the sites belong to the burnished soil according to the CPCS classification of its 1967 edition. The sites are located between 1300 and 1400 m altitude, and the slope variations were minimised (<5%). Soil samples were taken from a control plot under the forest, whose soil characteristics are therefore well preserved, and from plots cleared and cultivated for 2, 8 and 20 yr. The forest is composed of *Cedrus atlanticae, Quercus rotundifolia, Arbutus unedo, Genista quadriflora, Daphne gnidium, Cistus laurifolius and Cistus ladaniferus*. The soil under the forest is moderately deep (40-60 cm) and the litter layer is thick (0-5 cm). The recently cultivated plot (2 yr) was under cultivation after a fire. The plots that have been cultivated for 8 and 20 yr were cultivated after traditional land clearing. In these plots the soil is shallow (25-40 cm), with almost no vegetation cover. The conventional tillage system adopted by farmers in the region consists at least two medium-depth soil turnings (10-20 cm) between February and April to prepare the seedbed. The soil is regularly fertilized with chemical fertilizers during the growing season (between April and July). This crop reaches maturity in August before the violent autumn rains of the Mediterranean regime compromise the harvest. The samples for the physico-chemical analyses were taken from the 20 cm depth of the soil, in December after a period of intense natural rainfall between October and November 2019.

Physico-chemical analysis protocols

The samples intended for physico-chemical analyses were air-dried and then sieved to 2 mm by taking 2mm/10 g as the standard measurement of the intended size of each sample for the granulometric analysis. The organic matter was destroyed by hydrogen peroxide. The samples are then dispersed by mechanical agitation after the addition of sodium hexametaphosphate. The suspension is sieved to recover the sands. The silt and clay contents are determined by using the "Robinson pipette" method. The texture is determined using the U.S.D.A. triangle, to assess the clay, silt and sand contents. The measurement of the water pH is done on a soil-water suspension in the ratio 1/2.5 in the laboratory. It was measured by the potentiometer method using a pH meter with electrodes. Organic matter was quantified by the Walkley and Black (1934) method. Exchangeable bases (Ca^{2+}, Mg^{2+}, Na, K) were saturated with a 1 N ammonium acetate solution. The reading and determination of cations were conducted by atomic absorption and flame photometer. Available phosphorus content is determined using Olsen method (Olsen et al., 1954). Total Nitrogen is quantified by the Kjeldahl method (Bremner, 1965).

The structural stability of the soil was evaluated by the procedure proposed by Le Bissonnais (1996), using a combination of three tests simulating the behavior of the soil under different climatic and hydric conditions. The results obtained are presented in mm in the form of mean weight diameter (MWD). The soil erodibility index was measured using the equation proposed by Wischmeier and Smith (1978). The bulk density was measured gravimetrically using a cylinder (0-10 cm), which were then weighed and oven dried (105°C) for 24 h and then reweighed to obtain the soil porosity and bulk density of the sample.

Runoff and erosion

A portable rainfall simulator based on the model produced by Roose and Smolikowski (1997) was used to simulate soil hydrological behaviour and solid transport. The rainfall simulator on a 1 m^2 plot consisted of a device capable of providing a homogeneous rainfall intensity of 75-80 mm h^{-1}. The choice of this intensity is based on the observations of daily rainfall of the hydrological station located 10 km from the study area. The rainfall simulations lasted 40 minutes. The tests were carried out in December after an intense period of natural rainfall. Runoff and sediment transport were collected and laboratory analyses were performed to determine the sediment concentration.

The following parameters could be derived:

- Runoff (LR in mm h^{-1});
- The infiltrated water level (Linf in mm h^{-1}) calculated as follows: (Linf= Rainfall-LR);
- The runoff coefficient (KR in %) with: KR= (LR/rain) × 100;
- The sediment concentration of the runoff water in g l^{-1};
- Soil erosion in g $m^{-2}h^{-1}$ is calculated with erosion= sediment concentration ×LR.

Statistical analysis

The effects of deforestation followed by cultivation on soil hydrological behavior and water erosion were tested using statistical treatments. These treatments consisted of simple regressions, analysis of variance (ANOVA) and comparisons of means using the least significant difference (LSD) method with $P<0.05$. A correlation matrix was calculated, including soil physico-chemical properties and soil erosion. All correlation coefficients were reported at 5% probability. The program used for this analysis is SPSS version 21.

Results and Discussion

Effects of deforestation on soil physical properties

The particle size distribution of the top soil layer (0-20) shows highly significant variations between the forest and the cleared cultivated soils ($P < 0.01$) (Table 1). In this layer, the forest has lower values for sand and higher values for silt and clay content compared to cultivated soils. The general trend of soil texture elements after cultivation shows a significant increase in sand content, and a decrease in clay and silt content. The clay content decreased from 22.62±1.94 of the soil under forest to 21.84±1.3% after 8 yr and to 17.71±0.7 after 20 yr of cultivation (Table 1). On the other hand, the sand content increased significantly in the cultivated soils, reaching 50.03 ± 36% for the soil cultivated 8 yr and 53.2±3.78% for the soil cultivated 20 yr, compared to 42.52±2.8% for the soil under forest. These changes in the physical properties of the soil observed in cultivated soils compared to forest soils are the result of deforestation and subsequent tillage, which favors the erosion of the finest soil particles. Several studies have shown dramatic changes in particle size distribution after deforestation followed by cultivation (Nunes et al, 2012; Assefa et al., 2020; Gülser et al., 2021).

Table 1. Comparison of average soil physical property between forest and cleared plots under cultivation

Land uses	Forest (control)			Cultivated 2 yr			Cultivated 8 yr			Cultivated 20 yr			ANOVA
Statistics	Mean ± STD			Mean ± STD			Mean ± STD			Mean ± STD			
Sand (%)	42,50	±	2,87	41,07	±	4,01	50,03	±	3,60	53,20	±	3,78	**
Silt (%)	33,28	±	2,37	34,98	±	2,98	29,72	±	2,91	29,11	±	3,80	*
Clay (%)	22,62	±	1,94	23,14	±	0,86	21,84	±	1,36	17,71	±	0,79	***
Bulk density (g cm^{-3})	0,95	±	0,09	1,04	±	0,07	1,24	±	0,07	1,49	±	0,06	***
Porosity (%)	65,06	±	3,20	65,24	±	5,20	53,23	±	2,60	53,04	±	3,80	*
K-USLE	0,11	±	0,02	0,09	±	0,02	0,22	±	0,04	0,35	±	0,07	***
MWD (mm)	2,51	±	0,20	1,96	±	0,09	0,87	±	0,16	0,39	±	0,12	***

Notes: * $p < 0.05$; ** $P < 0.01$; *** $p < 0.001$.

The hydrodynamic behavior of the soil depends on several physical characteristics such as porosity and bulk density which appear to be most strongly and rapidly affected by forest clearing followed by cultivation (Table.1). Bulk density was significantly higher in soils cultivated for 8 (1.24 ± 0.07 g cm^{-3}) and 20 yr (1.49 ± 0.06 g cm^{-3}) than in soils under forest (0.95 ± 0.09 g cm^{-3}). Bulk density did not differ between soils cultivated for 2 yr and forest soils. The table also shows that TP is higher in forest areas than in cultivated soils. Low values were found in soils cultivated for 8 and 20 yr. These changes are related to the decomposition of pre-existing roots and the loss of SOM due to the loss of forest cover to agricultural land (Roose, 1985; Sabir et al. 2004). Furthermore, the low Da value in the forest and cultivated soil for the last 2 yr is probably due to the high SOM content.

The USLE-K factor, used to assess soil erodibility, shows a significant difference between cultivated and forest soils ($P < 0.01$). USLE-K values were lower in forest soil (0.1 ± 0.02) and higher in soils cultivated for 20 yr (0.35 ± 0.07). There was no significant difference in the K-factor between the forest and the soil cultivated for 2 yr. However, the K-factor increased 2.2 times in the soil cultivated for 8 yr and 3.5 times for the soil cultivated for 20 yr compared to the forest soil. Indeed, land clearing, loss of SOM and increase of fine particles caused by successive tillage have increased soil erodibility (Celik, 2005; Khormali et al., 2009).

The mean weight diameter (MWD), an index of soil structural stability, shows significant differences between clearing stages. MWD was tested significantly higher in forest soils than in cultivated soils. Deforestation resulted in a significant decrease in the MWD of the topsoil ($P < 0.01$). With reference to the standards established by Le Bissonnais (1996), the structural stability in the soil under the forest tested high (MWD = 2.5 mm), and provides good protection against erosion, even during the devastating late summer and spring storms. However, this stability decreases significantly over time after cultivation. Indeed, the soil of the plots cleared for 2 and 8 yr becomes moderately unstable (Table 1). The soil of the plot cultivated 20 yr is very unstable (MWD = 0.37 mm). This result may be related to cultivation practices, which loss of organic matter and weaken the resistance of soil aggregates to aggressive rainfall (Celik, 2005; Laghrour et al., 2016).

The distribution of soil aggregates after disaggregation shows highly significant differences between forest and cultivated soils (Figure 2). The forest soil had a high percentage of large aggregates (> 2mm), however the cultivated soils had significantly more aggregate mass in the small diameter classes (<1mm) than the forest soil. This increase could be explained by the intensification of successive tillage practices (more than 3 times per year), which fragment large aggregates into smaller ones (Unger, 1997; Materechera and

Mkhabela, 2001). The increase of fine aggregates is an indicator of degradation of cultivated soils (Whalen and Chang, 2002). Smaller aggregates in cultivated land hamper soil productivity, as they reduce the water infiltration capacity, macroporosity and water storage potential of the soil, thus increasing the potential for water erosion (Whalen and Chang, 2002).

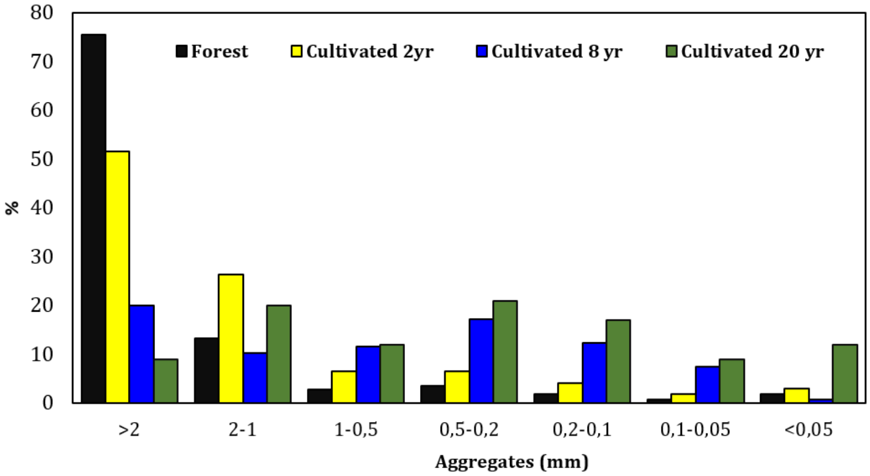

Figure 2. Effects of deforestation and cultivation on the size distribution of soil aggregates

Effects of deforestation on soil chemical properties

The ANOVA test showed that deforestation followed by cultivation resulted in significant changes in soil pH values. ($P<0.001$) (Table 2). There was a decrease in soil acidity in the short term (2 yr), but in the medium and long term there was a significant increase in acidity. Indeed, the pH$_{w}$ value decreases from 6.2 ± 0.26 for the soil under forest to 5.87 ± 0.12 for the soil cultivated 8 yr, and to 5.42 ± 0.24 for the soil cultivated 20; this indicates that the soil becomes strongly acidic. The pH$_{Kcl}$ has a similar evolution to that of the pH$_{w}$. It increased from 5.63 ± 0.21 for the soil under forest to 6.1 ± 0.12 for the soil cultivated for 8 yr and to 5.23 ± 0.16 for the soil cultivated for 20 yr. The weakly acidic soil under forest is probably due to the acidic nature of the lithological substrate, namely shale and sandstone. The increase in pH in the recently cultivated soil (2 yr) compared to the soil under forest is explained by the fact that the plot had suffered a fire and carried a large quantity of large branches that had burnt poorly in the first year and whose complete combustion in the second year provided a greater abundance of ash rich in mineral elements and exchangeable bases, notably Ca^{2+}, which can increase the soil pH (Francos et al., 2019). The decrease in acidity in cultivated soils in the medium and long term can be explained by the leaching of exchangeable bases under the effect of heavy rainfall, which consequently lowers the soil pH (Olorunfemi et al., 2018; Assefa et al., 2020). Thus, tillage mineralizes SOM and continuous application of acidifying ammonium phosphate fertilizers such as ammonia phosphate (Assefa et al., 2017). Serious problems are encountered when the soil pH is below 5.5 (Norton et al., 1999). Indeed, in acidic soils, Al^{3+} and H^{+} ions replace Ca^{2+} and Mg^{2+} in the absorbing complex, causing leaching and a reduction in the availability of some exchangeable bases essential for plants (Nunes et al., 2012). These results are similar to others found by other authors. Indeed, Assefa et al. (2020) and Khormali et al. (2009) found that the conversion of forests to agricultural land decreases the soil pH.

Table 2. The effect of deforestation on the chemical properties of the soil

Land uses	Forest (control)	Cultivated 2 yr	Cultivated 8 yr	Cultivated 20 yr	ANOVA
Statistics	Mean ± STD	Mean ± STD	Mean ± STD	Mean ± STD	
Soil pH$_{w}$	6.20 ± 0.26	6.61 ± 0.15	5.87 ± 0.12	5.42 ± 0.24	***
Soil pH$_{KCl}$	5.63 ± 0.21	6.10 ± 0.12	5.11 ± 0.30	5.23 ± 0.16	*
SOM (%)	5. 90 ± 0.67	6.83 ± 0.84	3.24 ± 0.78	1.01 ± 0.21	***
TN (%)	0.11 ± 0.03	0.12 ± 0.04	0.11 ± 0.02	0.08 ± 0.02	**
Available P (mg kg^{-1})	10,90 ± 1.59	53.15 ± 9.40	163.00 ± 37.47	206.30 ± 13.60	***
Available K (mg kg^{-1})	267.67 ± 15.80	511.70 ± 76.90	122.3 ± 10.4	195.00 ± 8.91	***
Ca^{2+} (mmol$_{c}$ kg^{-1})	7.41 ± 1.17	10.31 ± 3.40	5.90 ± 1.28	5.68 ± 2.46	***
Mg^{2+} (mmol$_{c}$ kg^{-1})	1.83 ± 0.20	1.77 ± 1.40	0.83 ± 0.71	0.81 ± 0.80	**
Na (mmol$_{c}$ kg^{-1})	4.50 ± 0.19	0.50 ± 0.02	1.01 ± 0.04	0.51 ± 0.02	**

Notes: * $p < 0.05$; ** $P < 0.01$; *** $p < 0.001$.

Organic matter (SOM) is a significant index of soil fertility and plays an important role in the sustainability of natural systems (Laouina, 2013). Deforestation resulted in a significant decrease in soil SOM ($P \leq 0.01$). Cultivation for 8 and 20 yr decreased SOM by 41 and 82% respectively compared to the soil under forest.

The loss of soil SOM may be related soil water erosion and mineralization (Sabir et al., 2004; Gülser et al., 2021). These results are similar to those found by several authors. Indeed, Sabir et al (2004) showed that conversion of cork oak forests to cannabis cultivation has decreased soil OM by 47% in the western Rif in northern Morocco. Furthermore, Gülser et al. (2021) found that cultivation of forest soils in Turkey for 50 years ago decreased OM by 57.14%.

The highest values of total nitrogen content were observed for forest and varied significantly from cultivated soils in the long term. There was no significant difference in total N between the forest and the soil cultivated for 2 yr and 8 yr (P > 0.05). However, about 45% of the total N was lost after the 20 yr cultivation. This decrease is completely associated with runoff, hypodermic flow and especially leaching, which is observed the most common loss of nitrogen nitrates (Bonneau and Souchier, 1979). It is also correlated with the acidification of the soil after cultivation. Indeed, some authors have shown that the increase in acidity results in a decrease of nitrogen fixing organisms in the soil (Demolon, 1960). Similarly, if the soil becomes more acidic, certain elements such as nitrogen may be blocked despite the intensive use of chemical fertilizers (FAO, 1989). Concerning available phosphorus content the highest values were found in cultivated soils than in soils under forest. The concentration of this element is higher in the soil cultivated for 8 yr (122 ± 11 mg kg^{-1}) and in the soil cultivated for 20 yr (195 ± 8.91 mg kg^{-1}). The increase in available P in cultivated soils can be explained by the fact that the cultivated plots were amended with a large quantity of complex chemical fertilizers, an amendment that stimulates, especially in acidic soils, the fixation of phosphorus with Al and Fe to form compounds that are not very soluble in the soil (Demolon, 1960). The evolution of available potassium is similar to that of available phosphorus. Higher values of available potassium were found in cultivated soils than in soils under forest. The concentration of this element appears to be very high in the cultivated soil for the last 2 yr with a concentration of 580.33 ± 31.58 mg kg^{-1}. This increase can be attributed to the use of chemical fertilizers by farmers at the time of cultivation to improve the productivity of their fields.

The distribution of calcium (Ca^{2+} in mmol$_c$ kg^{-1}) in the surface layer of the soil also shows significant differences between the forest and cultivated soils. All the plots revealed a very low Ca^{2+} content of less than 11 mmol$_c$ kg^{-1}.The highest levels were found in the soil cultivated for 2 yr (10.31 ± 3.4 mmol$_c$ kg^{-1}), due to the contribution of ash which is a good source of calcium, potassium, phosphorus and magnesium (Giovannini and Lucchesi, 1997). In soils cultivated for 8 and 20 yr the Ca^{2+} content starts to decrease significantly by -18% and -20% respectively compared to the control. Magnesium (Mg^{2+}) content, which is already low at the initial state (< 2 mmol$_c$ kg^{-1}), shows a slight increase in soils cultivated for 2 yr, and then shows a significant decrease in soils cultivated for 8 and 20 yr by about -56% compared with forest. Sodium (Na) losses are higher and exceed those for other exchangeable bases. After clearing, the content of this element starts to fall rapidly during the 2 yr of cultivation (-70.83% of the control), indicates severe degradation and considerable soil poverty. These results are similar to those of Assefa et al. (2020) and Olorunfemi et al. (2018) whose studies showed that deforestation leads to a significant decrease in exchangeable bases in acidic soil due to leaching.

Runoff and soil erosion

The results intended for the runoff coefficient on micro plots (1 m^{-2}) obtained by conducting the rainfall simulation tests with an intensity of 75-80 mm h^{-1}, show that the conversion of forests into agricultural land resulted in a significant increase in runoff rate as a function of time after cultivation (Table 3).

Table 3. The effect of deforestation on the runoff and soil erosion

Land uses	Forest (control)	Cultivated 2 yr	Cultivated 8 yr	Cultivated 20 yr	ANOVA
Statistics	Mean ± STD	Mean ± STD	Mean ± STD	Mean ± STD	
Linf (mm h-1)	78.00 ± 0.80	69.53 ± 1.15	45.20 ± 2.18	27.70 ± 2.70	***
Runoff (mm h-1)	2.00 ± 0.80	10.47 ± 1.15	34.80 ± 2.18	52.32 ± 2.70	***
Runoff coef (%)	2.50 ± 0.90	2.32 ± 1.43	48.50 ± 2.72	65.40 ± 3.37	***
Erosion (g m-2 h-1)	n.a	10.06 ± 3.04	219.60 ± 19.3	989.17 ± 68.40	***

Notes: * p < 0.05; ** P < 0.01; *** p < 0.001.

The highest runoff rate values found in soils cultivated for 8 and 20 yr with an average of 34.8 ± 2.18 mm h^{-1} and 52.32 ± 2.7mm h^{-1} respectively. Soils cultivated 2 yr recorded as the lowest runoff rate values with an average of 10.47±1.15 mm h^{-1}. Figure 3 shows the behaviour of runoff rates as a function of time. The runoff rate increases rapidly in the cultivated for 8 and 20 yr, between 15 min and 27 min after the start of the rainfall simulation. Runoff became very high in these plots at the end of the simulation, reaching 55.2 mm h^{-1} and 59.7 mm h^{-1} respectively. The soil under the forest is clearly different from the other cultivated soils, with a low water runoff (2.0±0.8 mm h^{-1}), due to the dense vegetation cover, litter and root system that facilitate water infiltration. Thus, the emergence of cannabis cultivation has led to a loss of interest in livestock farming, which is significantly regressing. This regression has reduced both the compaction of the soil under the forest by the animals, and favors the installation of dense undergrowth that represents a barrier to raindrops, and favours the infiltration of water into the soil. These results are consistent with several studies in the Mediterranean region (Al Karkouri et al., 2000; Martinez-Mena et al., 2008 and Kavian et al., 2014) , which found that conversion of forests to agricultural land increases runoff and soil erosion.

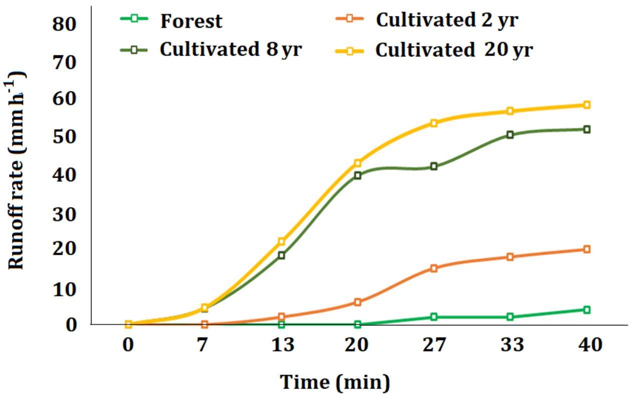

Figure 3. Evolution of runoff in the experimental sites as a function of time

Similarly, soil loss calculated at the microplot scale of the simulation shows that conversion of natural forest to agricultural land significantly increases soil erosion. The erosion rate becomes higher in the cultivated the 8 and 20 yr cultivation, with an average of 219.60 ± 19.3 and 989.17 ± 68.4 g m^{-2} h^{-1} respectively. Soil cultivated for 2 yr with an average of 10.6 g $m^{-2}h^{-1}$ recorded a low value of land loss compared to long-term cultivated soils, due to their high SOM content which facilitates water circulation within the soil and, consequently, reduces soil erosion (Moussadek et al., 2011). Several studies have found similar results. Indeed, (Bensalah et al., 2012) showed that cultivated soils in the Bouregrag watershed (Morocco) show very different hydrological behaviours compared to natural forest soil depending on their textural nature and changes in their surface states. Martinez-Mena et al. (2008) found that conversion of forest to agricultural land in a semi-arid area of southeastern Spain increased the average sediment concentration seven times more than forest. (Kavian et al., 2014) showed that soil erosion in cultivated land is 1587 times higher than the forest in northern Iran.

Correlation between physical-chemical properties, runoff and soil erosion

Table 4 shows the different correlations between the components of physical and chemical properties, runoff and soil erosion. The linear correlation analysis showed a significant correlation between 29 of the 55 pairs of soil attributes (P<0.05).

Table 4. Correlation matrix for physicochemical soil, runoff and soil erosion

	MWD	MO	K	BD	Sand	Silt	Clay	TN	P_2O_5	Ca^{2+}	K_2O	Na	Mg	pH w	KR	Erosion
MWD	1															
MO	$0,731^{**}$	1														
K	$-0,603^{**}$	$-0,637^{**}$	1													
BD	$-0,375^{*}$	$-0,570^{**}$	$0,401^{*}$	1												
Sand	-0,015	-0,060	$0,570^{*}$	0,214	1											
Silt	0,270	0,205	$-0,371^{*}$	-0,162	$-0,387^{*}$	1										
Clay	$0,660^{**}$	0,023	-0,418	-0,176	$-0,586^{*}$	0,213	1									
TN	0,340*	$0,447^{*}$	0,035	$0,596^{**}$	0,047	0,034	0,262	1								
P_2O_5	-0,055	$-0,645^{*}$	$0,511^{*}$	0,237	0,270	-0,120	$-0,548^{**}$	0,060	1							
Ca^{2+}	$0,457^{**}$	$0,490^{**}$	$-0,434^{**}$	-0,346	-0,160	0,040	0,317	$-0,580^{**}$	-0,039	1						
K_2O	-0,230	-0,310	0,268	0,122	0,155	-0,070	-0,041	-0,393	0,622	-0,191	1					
Na	$0,455^{**}$	$0,350^{*}$	-0,340	-0,171	-0,261	0,285	$0,376^{*}$	-0,263	$-0,644^{**}$	0,053	$-0,859^{**}$	1				
Mg	0,266	0,197	-0,556	$-0,379^{*}$	-0,255	0,265	0,131	0,312	$-0,454^{**}$	$0,427^{*}$	-0,241	0,313	1			
pH w	$0,344^{*}$	0,156	-0,343	$-0,378^{*}$	$-0,430^{**}$	0,172	0,317	$-0,590^{**}$	$-0,514^{*}$	$0,540^{**}$	-0,174	0,281	0,280	1		
KR	$-0,530^{**}$	$-0,760^{**}$	$0,619^{**}$	$0,287^{*}$	$0,710^{**}$	-0,259	$-0,648^{**}$	-0,230	0,115	$-0,462^{**}$	$0,360^{*}$	-0,394	-0,172	$-0,400^{*}$	1	
Erosion	$-0,780^{**}$	$-0,640^{**}$	$0,536^{**}$	0,194	$0,750^{**}$	$-0,305^{*}$	$-0,711^{**}$	$0,490^{*}$	$0,670^{*}$	$-0,414^{*}$	0,318	$-0,350^{*}$	-0,102	$-0,460^{**}$	$0,740^{**}$	1

Soil organic matter and MWD showed highly significant negative correlations with runoff (-0.530**) and soil erosion (0.780**). This result is compatible with good agreement of other authors (Sabir et al., 2004; Martinez-Mena et al. (2008). The influence of organic matter, which mainly acts on structural stability, is important for high contents that increase macroporosity and decrease soil erosion. Significant correlations were also found between SOM and soil physical and chemical properties (P<0.05) such as structural stability, bulk density, soil porosity, total nitrogen and exchangeable bases (Ca^{2+}, Na). Bulk density shows a significant positive correlation with runoff (0.287*). Increasing bulk density reduces soil porosity and water and air storage capacities and increases surface runoff. Runoff showed a highly significant positive correlation with water erosion rate (0.740**). Clay content shows a significant negative correlation with runoff (-0.648**) and erosion (-0.711**). Sand content was positively correlated with runoff (0,710**) and soil erosion (0.750**). Sand particles have larger sizes, so they are resistant to movement (Kavian et al., 2014). These results are similar to those found by several authors, among others Bensalah et al. (2012); Moussadek et al. (2011), who showed that the erosion of Moroccan soils is strongly related to soil texture.

Conclusion

The chronosequence study of the physico-chemical, hydrodynamic and erosion properties of the soil highlighted the transformations of soil after deforestation followed by cultivation. The intended results of the study show that the transition from natural forest to cultivation has a negative impact on the structural stability of the soil, on the physico-chemical properties and on the hydrodynamic behaviour of the soil. This study suggests that well-conserved forests provide good soil cover, enrich soil OM, improve soil stability, and provide excellent protection against erosion. In contrast, successive tillage is the main factor in modifying soil structure and depleting the fertility of cleared and cultivated land. Soils become extremely degraded by the eighth year after conversion of forests to agricultural land. Organic matter decreased by 82%, nitrogen and exchangeable bases were reduced alarmingly, soil acidity increased by 0.78 units, and bulk density increased by 0.54 g cm^{-3}; making runoff and erosion risks very high (989.17±68.4 g m^{-2} h^{-1}). In this context, a set of measures should be put in place to reduce the anthropic pressure on forests, in order to reduce the degradation of natural environments in the Central Rif. This leads to the search for viable solutions focused on the safeguard and rehabilitation of degraded forest ecosystems and socio-economic development.

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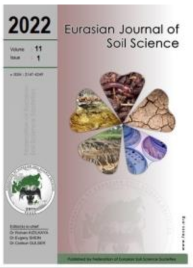

Isolation and characterization of salt tolerant bacteria from saline soils of Bangladesh

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Article Info

Received : 16.11.2021
Accepted : 19.04.2022
Available online : 25.04.2022

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Abstract

Salinity is an important abiotic stress that limits the productivity of crops growing on the salt affected areas because excess salt concentration in the soil has detrimental effect on growth and development of plants. Beneficial microorganisms having the inimitable characteristics like tolerance to soil salinity, synthesis of plant growth hormones, facilitating nutrient uptake, bio-control ability and beneficial interaction with plants could be vital to address the problem. An experiment was carried out with the objectives of isolating and characterizing saline tolerant bacteria for utilizing as a tool for bioremediation. Soil samples were collected from three saline affected districts of Bangladesh viz. Khulna, Satkhira and Bhola. The highest bacterial population was found in Satkhira followed by Khulna and the lowest was found in Bhola. Eighteen (18) bacterial isolates viz. BU B1, BU B2, BU B3, BU B4, BU B5, BU B6, BU B7, BU B8, BU B9, BU S1, BU S2, BU S3, BU S4, BU S5, BU S6, BU S7, BU K1 and BU K2 were identified according to the colony color and shape. All the isolated bacteria showed positive response to produce IAA. Isolates BU S4, BU B7 and BU S1 showed highest IAA production ability. Among the 18 isolates, 12 were Gram positive and showed negative reaction on KOH test and the rest 6 isolates were Gram negative and showed positive reaction on KOH test. The isolates BU B1, BU B4, BU B6, BU S6, BU K1 and BU K2 were slow growing bacteria and the rest were fast grower. Biochemical tests indicate that 13 isolates were positive for catalase and P solubilization test. Whereas, 11 isolates could degrade the cellulose. For screening of bacterial isolates against NaCl tolerance, the isolates were cultured on NA medium having different salt concentrations. Experimental results reveal that all the isolates could tolerate 4.0% NaCl concentration except BU B6. Ten isolates showed the ability to tolerate NaCl up to 8.0%. The isolates BU B7 and BU S4 showed highest salinity tolerance along with better response to different biochemical characteristics. Therefore, these isolates may become promising for the bioremediation of soil salinity in the saline affected areas of Bangladesh.

Keywords: Salinity, bacteria, salinity tolerance, bioremediation.

Introduction

Soil salinity is considered as a vital abiotic stress that negatively influence the agricultural production all over the world. Research report indicated that 20% of the cultivable land and 33% of the irrigated farming lands of the world are saline affected (Shrivastava and Kumar, 2015). In Bangladesh, the coastal region comprises of 19 districts that covers around 32% of the country and accommodates about 35 million people (Huq and Rabbani, 2011). The salt-affected area in Bangladesh is increasing gradually due to the influence of

: https://doi.org/10.18393/ejss.1108521
: http://ejss.fesss.org/10.18393/ejss.1108521
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

cyclonic storms, leading to salt water intrusion in the non-saline crop lands (SRDI, 2010). Higher soil salinity level affects the growth of most of the field crops and thus hampers crop productivity significantly.

To fulfill the excess food requirement of the increasing population, it is vital to find out the possible ways of utilizing saline soils for better agricultural production (Haque, 2006). Utilization of salt-tolerant bacteria to face the negative influence of soil salinity on crop production is getting popularity in many parts of the world. Research findings reveal that utilization of plant growth promoting bacteria (PGPB) is one of the important strategies to alleviate salinity induced plant stress (Yao et al., 2010). These beneficial microorganisms facilitate growth of the plants through a number of mechanisms (Nia et al., 2012; Solaiman et al., 2012; Ramadoss et al., 2013; Alam et al., 2015) including phytohormone production, nitrogen fixation, nutrient solubilization, disease suppression, siderophore production etc. Therefore, the halotolerant and halophilic organisms are important for maintenance of soil health and nutrition recycling in saline environment. It is hypothesized that the isolation and characterization of saline tolerant bacteria and their application would have immense potential for increased crop productivity in saline affected areas. Considering the above-mentioned facts, the present research work was undertaken with the objectives of isolating and characterizing saline resistant bacteria and to screen promising bacteria against soil salinity for better crop production in coastal saline areas.

Material and Methods

The research study was carried out at the Laboratory of Soil Microbiology under the Department of Soil Science of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh.

Collection of soil samples

Soil samples were collected from salt affected regions of Bangladesh viz. Bhola, Satkhira and Khulna. Randomly collected soil samples were carefully labeled and put in an ice box and transported to the research laboratory and preserved in a refrigerator at -4°C temperature for culturing the bacteria. A portion of each sample was transferred to the laboratory, air dried at room temperature and then sieved using a 2 mm sized sieve and used for subsequent physical and chemical analysis.

Analysis of collected soil samples

The mechanical analysis of soil was performed following hydrometer method (Bouyoucos, 1962) using standard hydrometer (ASTM No. 152H). Cation exchange capacity (CEC) of the collected samples were estimated following ammonium acetate (1N) extraction method (Jackson, 1973). Organic carbon of the soil samples was determined by Walkley and Black wet digestion method (Nelson and Sommers, 1982). Electrical conductivity of saturated paste extract (EC) was determined by the procedure as described by Rhoades et al. (1999). Exchangeable cations like K, Ca, Mg and Na were estimated by 1N ammonium acetate extraction method as prescribed by Soil Survey Staff (2011).

Isolation of bacteria

From each soil sample, 1g of soil was suspended in 9 ml of double distilled water and vortex for 3 minutes. The resulting suspensions were serially diluted to 10^{-10}. An amount of 0.1ml dilution fluid from each dilution tube was taken and transferred separately into freshly prepared Nutrient Agar (NA) media and then incubated at 37°C for bacterial growth. pH of the NA culture media was adjusted to 7. Bacterial growth was monitored on a daily basis and single colonies were picked up with sterile tooth pick and sub-cultured over the pre-solidified NA media. All the subsequent *in vitro* plate assay analyses were done in triplicate until the colonies were more purified. After bacterial growth on NA media, the pure cultures were sub-cultured in NA slants. The slants were incubated at 37°C to get vigorous bacterial growth and therefore preserved in 20.0% glycerol eppendorf tubes at -4°C (Saha and Santra, 2014).

Enumeration of bacteria from saline soil was done by using serial dilutions through drop plate count method. Nutrient agar (NA) (MacFaddin, 2000) media was used for the counting of bacterial populations. After 3 days of incubation, bacterial population was counted as CFU (colony forming unit) that developed on the particular agar plates.

CFU or viable cells / gm of dry soil was calculated by the following formula-

$$= \frac{\text{Mean plate count} \times \text{Dilution factor}}{\text{Amount of dilution}}$$

Morphological and biochemical tests

The morphology of the bacterial colony was observed on NA plates. Individual colonies of each of the isolates were examined based on morphological features including shape, margin, elevation, surface and color (Aneja, 2003). Gram staining tests were performed following standard protocols. Bacterial isolates were also grown in glucose peptone agar, NA media containing bromothymol blue (BTB), NA media containing Congo red.

Catalase test

Different isolates were flooded with 3.0% freshly prepared hydrogen peroxide (H_2O_2) to perform the catalase test. The effervescence indicated catalase activity that is formation of air bubbles within 10 seconds indicates positive result for catalase test. Negative catalase test was confirmed by the formation of no bubbles or few scattered bubbles.

Phosphate solubilizing assay

Assessment of P-solubilization was carried out following the method as described by Sharma et al. (2012). The cultures of bacterial isolates were spot inoculated on the plates containing Pikovaskya's medium. The plates were incubated in an incubator at 28°C for 4-5 days. Phosphate solubilization was ensured by the formation of clear zone around the bacterial colonies.

Determination of cellulose activity

Cellulose degradation test was done to confirm whether the isolates could degrade the cellulose or not. The test was performed on Jensen's agar plates containing 0.1% carboxymethyl cellulose (CMC). About 10 µl liquid culture of the bacterial isolates were spot inoculated. The plates were incubated for 24 hours and then the colonies were streaked and washed off with sterile water and discarded. Examination plates were then stained with 0.1% congo red for the period of 30 minutes and subsequently rinsed with 1M NaCl. Cellulose degradation activity of the isolates was confirmed by the formation of clear hallo zone around the colony.

Determination of indole acetic acid (IAA) production

Indole acetic acid production test was carried out by the inoculation of desired bacterial isolates in Jensen's broth media. The media containing bacterial isolates were incubated at 29 ±2^{0}C for 48h according to Bric et al. (1991). From the inoculated broth media, 1 ml was transferred into freshly prepared 50ml Jensen's broth culture having 2mg ml^{-1} of tryptophan. The culture was then incubated at 29±1^{0}C for 72h. About 2ml of culture was then centrifuged for 7 min at 7000 rpm. After completion of the centrifugation, 1 ml of the supernatant was mixed with 2ml of Salkowsky's reagent (2.0% of 0.5M $FeCl_3$ in 35.0% perchloric acid) according to Gordon and Weber (1951). Color absorbance was measured by a spectrophotometer at 530nm. Standard curve for Indole Acetic Acid was prepared from pure IAA as 0, 5, 10, 15, 20, 25, 30, 35, 40 and 45µg ml^{-1} of IAA. The concentration of IAA was obtained from the standard graph. Supernatants collected from un-inoculated test tubes were used as control, where no visible color was found.

Growth of the isolates at 37°C temperature

Bacterial isolates were cultured (nutrient agar media) in the growth chamber at 37^{0}C to find out whether the bacterial isolates could survive high temperature or not.

Screening of bacterial isolates against NaCl tolerance

The isolates were tested for their sodium chloride (NaCl) tolerance by growth in nutrient agar plates containing various concentrations of NaCl (0%, 2.0%, 4.0%, 6.0% and 8.0%).

Results and Discussion

Bio physico-chemical properties of the collected soil samples

The soil sample collected from Khulna had organic carbon 1.0%, electrical conductivity 7.09 ds/m and cation exchange capacity 15.50 cmol/kg (Table 1). The soil of Satkhira had organic carbon 0.73%, electrical conductivity 6.68 ds/m and cation exchange capacity 15.33 cmol/kg. On the other hand, soil sample collected from Bhola had organic carbon 0.95%, electrical conductivity 12.29 ds/m and cation exchange capacity 12.34 cmol/kg. Soil texture of the Khulna soil was silt loam, while the texture of Satkhira and Bhola soils were sandy loam. Exchangeable cations (Na, K, Ca and Mg) also vary from one location to another. Bhola soils demonstrate higher exchangeable cations as compared to Khulna and Satkhira soils. Results obtained from the present study (Table 1) reveal that bacterial populations also differ from one place to another. Higher bacterial population was enumerated in Satkhira soil (6.7× 10^5 cfu g^{-1} soil) which was very close to Khulna soil (6.5× 10^5 cfu g^{-1} soil). On the contrary, lowest bacterial population was counted in Bhola soil (1.31× 10^5 cfu g^{-1} soil). It seems that bacterial population in saline soil is related with the EC value. Increasing the EC value might decrease the bacterial population in saline soil. In the present study Bhola soil had the highest EC value and consequently showed lowest bacterial population. This finding implies that soil salinity adversely influences the bacterial population. Our findings are in harmony with many other findings (Moradi et al., 2011; Ma and Gong, 2013) that illustrated the negative influence of soil salinity on microbial abundance.

Table 1. Properties of the collected soil samples

Sample ID	EC (ds/m)	CEC (cmol/kg)	OC (%)	Exchangeable cations (cmol/kg)				Texture	Bacterial population (cfu g^{-1} soil)
				Na	K	Ca	Mg		
K (Khulna)	7.09	15.50	1.00	0.79	0.62	5.67	4.00	Silt Loam	6.5×10^5
S (Satkhira)	6.68	15.33	0.73	0.86	0.74	6.06	5.70	Sandy Loam	6.7×10^5
B (Bhola)	12.29	12.34	0.95	0.93	1.20	9.38	6.23	Sandy Loam	1.31×10^5

Isolation of bacteria from saline soils

A total of 18 bacterial isolates viz. BU B1, BU B2, BU B3, BU B4, BU B5, BU B6, BU B7, BU B8, BU B9, BU S1, BU S2, BU S3, BU S4, BU S5, BU S6, BU S7, BU K1 and BU K2 were isolated from 3 soil samples that were collected from salt affected areas of Bangladesh (Table 2). Out of the 18 bacterial isolates, 9 isolates were obtained from Bhola soil, 7 isolates from Satkhira soil and remaining 2 isolates were isolated from Khulna soil.

Table 2. Isolates with their codes and location of collections

Serial No.	Location of collected soil sample	Isolate code	Serial No.	Location of collected soil sample	Isolate code
1	Bhola	BU B1	10	Satkhira	BU S1
2	Bhola	BU B2	11	Satkhira	BU S2
3	Bhola	BU B3	12	Satkhira	BU S3
4	Bhola	BU B4	13	Satkhira	BU S4
5	Bhola	BU B5	14	Satkhira	BU S5
6	Bhola	BU B6	15	Satkhira	BU S6
7	Bhola	BU B7	16	Satkhira	BU S7
8	Bhola	BU B8	17	Khulna	BU K1
9	Bhola	BU B9	18	Khulna	BU K2

Characterization of the bacterial isolates

Morphological characteristics of the isolates

The isolates were characterized based on their morphological features such as shape, margin, elevation, surface, color. All the isolates have different morphological characters in the nutrient agar medium. All of the isolates were sticky in nature. On nutrient agar (NA) media, 3 isolates (BU B2, BU B4 and BU S1) out of 18 isolates produced round and flat colonies, having smooth margin with smooth shiny surface (Table 3). Colony color of the isolates vary from one isolate to another. In the present study, creamy colored (4 isolates), off-white (4 isolates), yolk yellowish colored (3 isolates), orange colored (2 isolates), greenish yellow colored (2 isolates), brownish colored (2 isolates) and whitish (1 isolate) colored colonies were observed. The results of the study indicate that all the isolates were able to grow in the nutrient agar medium with variable morphological features (Table 3 and Figure 1). Our findings are in harmony with the findings of Hossain et al. (2021) where they isolated bacteria from soil having different morphological features which indicates the diversified presence of bacterial community in soil.

Table 3. Morphological characteristics of the bacterial isolates

Isolates	Colony shape	Elevation	Surface	Margin	Color
BU B1	Round	Raised	Smooth shiny	Smooth	Yolk yellowish
BU B2	Round	Flat	Smooth shiny	Smooth	Creamy
BU B3	Round	Raised	Smooth shiny	Smooth	Off white
BU B4	Round	Flat	Smooth shiny	Smooth	Orange
BU B5	Round	Raised	Smooth shiny	Smooth	Creamy
BU B6	Round	Raised	Smooth shiny	Smooth	Creamy
BU B7	Round	Raised	Smooth shiny	Smooth	Greenish yellow
BU B8	Round	Raised	Smooth shiny	Smooth	Brownish
BU B9	Round	Raised	Smooth shiny	Smooth	Brownish
BU S1	Round	Flat	Smooth shiny	Smooth	Creamy
BU S2	Round	Raised	Smooth shiny	Smooth	Whitish
BU S3	Round	Raised	Smooth shiny	Smooth	Orange
BU S4	Round	Raised	Smooth shiny	Smooth	Greenish yellow
BU S5	Round	Raised	Smooth shiny	Smooth	Yolk yellowish
BU S6	Round	Raised	Smooth shiny	Smooth	Off white
BU S7	Round	Raised	Smooth shiny	Smooth	Yolk yellowish
BU K1	Round	Raised	Smooth shiny	Smooth	Off white
BU K2	Round	Raised	Smooth shiny	Smooth	Off white

Figure 1. Pure culture of different bacterial isolates

Biochemical characteristics of the isolates

Gram staining and KOH string test

Result presented in Table 4 reveal that 6 isolates were Gram negative in reaction, whereas remaining 12 isolates were Gram positive in reaction. Red colored cells under the microscope were considered as Gram negative and violet-colored cells were considered as Gram positive. The KOH test also confirmed that 6 isolates were Gram negative as the KOH test was positive while 12 isolates were Gram positive as the KOH test was negative (Table 4).

Table 4. Gram staining, KOH, Catalase activity, cellulose degradation and phosphate solubilization test of the bacterial isolates

Isolates	Gram reaction	KOH test	Catalase Test	Phosphate Solubilization Test	Cellulose Degradation Test
BU B1	+ve	-ve	+	+	+
BU B2	+ve	-ve	+	+	+
BU B3	+ve	-ve	-	+ +	-
BU B4	+ve	-ve	+	-	-
BU B5	-ve	+ve	+ +	+	-
BU B6	+ve	-ve	+ +	+	+ +
BU B7	+ve	-ve	+ +	+ +	+ +
BU B8	+ve	-ve	+	-	-
BU B9	-ve	+ve	-	+	+ +
BU S1	+ve	-ve	+ +	+	+ +
BU S2	-ve	+ve	+	+	+
BU S3	-ve	+ve	+ +	-	-
BU S4	-ve	+ve	+ +	+ +	+ +
BU S5	+ve	-ve	+	+	-
BU S6	+ve	-ve	-	-	-
BU S7	-ve	+ve	+ +	+ +	+
BU K1	+ve	-ve	-	-	+
BU K2	+ve	-ve	-	+	+

(-) ve = Negative test, (+) ve = Positive test, Note: (-) =No ability, (+) = Weak and (++) = High

Catalase test

Catalase activity test was carried out with 18 bacterial isolates to find out whether the isolates could decompose the added H_2O_2 or not. Results of the present study (Table 4 and Figure 2) demonstrate that among 18 isolates, 13 were catalase positive as implied by the formation of bubbles upon addition of hydrogen peroxide to the cultures and 5 isolates were catalase negative as they formed no bubbles (Reiner, 2010). Catalase test positive bacterial isolates are highly resistant to harsh environmental conditions as well as have the ability to resist chemical and mechanical stresses (Glick et al., 1999). Since most of the bacterial isolates under the present were found as catalase positive, therefore it implies the possibilities of utilizing these isolates to alleviate salt stresses for better crop production in coastal areas.

Figure 2. Catalase test of different bacterial isolates

Phosphate solubilizing assay

Experimental results confirm that out of 18 bacterial isolates, 13 bacterial isolates were positive for P-solubilization, while rest of the isolates were found negative for P-solubilization test (Table 4 and Figure 3). Phosphorus is an essential and primary plant nutrient. In both acidic and alkaline soils, phosphorus fixation is a common phenomenon which makes the soluble P into insoluble form, thus the efficiency of applied P become low (Mahdi et al., 2012). Therefore, bacterial isolates having P solubilizing ability would play vital role in enhancing the availability of this crucial nutrient for the plant uptake (Richardson, 2001). The most important microbiological way through which insoluble phosphorus compounds are solubilized is by the production of organic acids by the P solubilizing microorganisms.

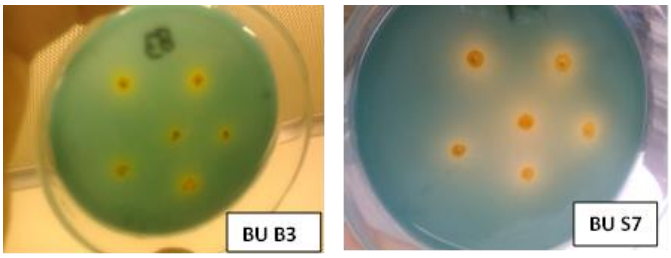


Figure 3. Phosphate solubilization assay by the bacterial isolates

Cellulose degradation activity test

Results presented in Table 4 and Figure 4 show that 11 bacterial isolates (BU B1, BU B2, BU B6, BU B7, BU B9, BU S1, BU S2, BU S4, BU S7, BU K1 and BU K2) out of 18 were positive for cellulose degradation activity. The remaining 7 isolates viz. BU B3, BU B4, BU B5, BU B8, BU S3, BU S5 and BU S6 were identified as negative for cellulose degradation activity. Biodegradation of various organic wastes through efficient microorganisms is an excellent approach (Rahman et al., 2020) which is getting popularity during the last few years. Under normal conditions, compost preparation takes longer period of time due to the slow decomposition of the organic residues (Bui et al., 2014). Thus, bacterial isolates having cellulose degrading activity might play important role in preparing agricultural compost in a useful and ecofriendly way.

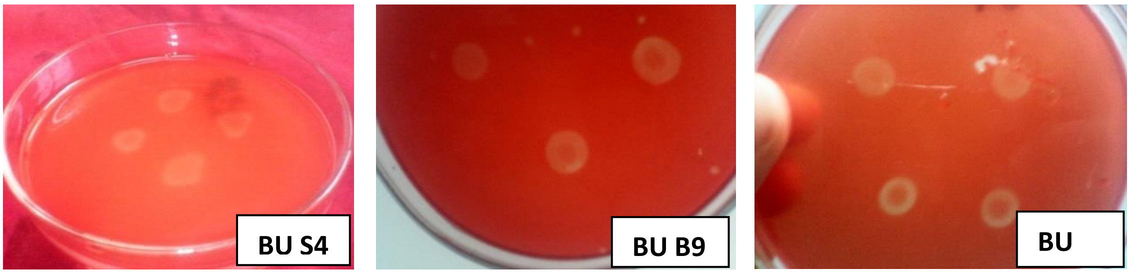
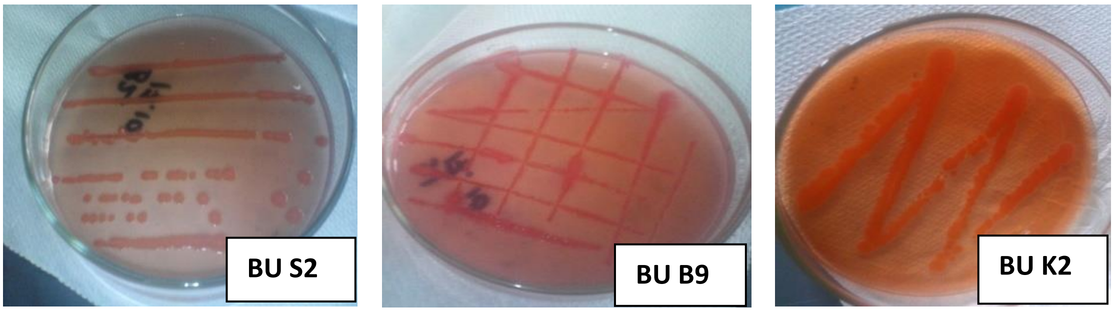


Figure 4 Cellulose degradation activity test of the bacterial isolates

Growth on Congo red dye

In general soil bacteria absorbs congo red dye highly as compared to *Rhizobium.* Results presented in Table 5 show that all the bacterial isolates absorbed the dye from Congo red NA agar media. Among 18 isolates, BU B2, BU B3, BU B5, BU B9, BU S1, BU S2, BU S3, BU S5, BU S6, BU K1 and BU K2 absorbed the dye strongly from Congo red NA agar media (Figure 5). In contrast, BU B1, BU B4, BU B7, BU B8, BU S4 and BU S7 absorbed the dye moderately and the isolate BU B6 show slight absorbance of the dye from Congo red NA agar media.



Figue 5 Growth of bacterial isolates on Congo red NA agar media

Table 5. Growth on Congo red NA agar media, growth on peptone glucose agar, Bromothymol blue test and Growth at 37^0C of the bacterial isolates

Isolates	Growth on Congo red NA agar (absorption of dye)	Growth on glucose peptone agar media	Bromothymol blue test			Growth at 37^0C
			Growth rate	Color	Result	
BU B1	Moderate	Moderate	Slow	Blue	Alkaline	+ve
BU B2	High	High	Fast	Yellow	Acidic	+ve
BU B3	High	High	Fast	Yellow	Acidic	+ve
BU B4	Moderate	Moderate	Slow	Blue	Alkaline	+ve
BU B5	High	High	Fast	Yellow	Acidic	+ve
BU B6	Slight	High	Slow	Blue	Alkaline	+ve
BU B7	Moderate	High	Fast	Yellow	Acidic	+ve
BU B8	Moderate	High	Fast	Yellow	Acidic	+ve
BU B9	High	High	Fast	Yellow	Acidic	+ve
BU S1	High	High	Fast	Yellow	Acidic	+ve
BU S2	High	High	Fast	Yellow	Acidic	+ve
BU S3	High	High	Fast	Yellow	Acidic	+ve
BU S4	Moderate	High	Fast	Yellow	Acidic	+ve
BU S5	High	High	Fast	Yellow	Acidic	+ve
BU S6	High	Moderate	Slow	Blue	Alkaline	+ve
BU S7	Moderate	High	Fast	Yellow	Acidic	+ve
BU K1	High	High	Slow	Blue	Alkaline	+ve
BU K2	High	High	Slow	Blue	Alkaline	+ve

Growth on glucose peptone agar media

Study results indicate that most of the bacterial isolates grow strongly on glucose peptone agar medium (Table 5 and Figure 6). Out of 18 bacterial isolates, 15 isolates showed high growth on this medium. Remaining 3 isolates BU B1, BU B4 and BU S6 showed moderate growth on glucose peptone agar medium (Table 5). The glucose peptone agar media is usually recommended for cultivation of wide variety of microorganisms. It has been reported that the growth of *Rhizobium* bacteria is normally poor whereas the other bacteria grow well on this media (Upadhayay et al., 2015). In the present study, as most of the bacterial isolates grew well on this media indicating the presence of diversified group of bacteria other than *Rhizobium*.

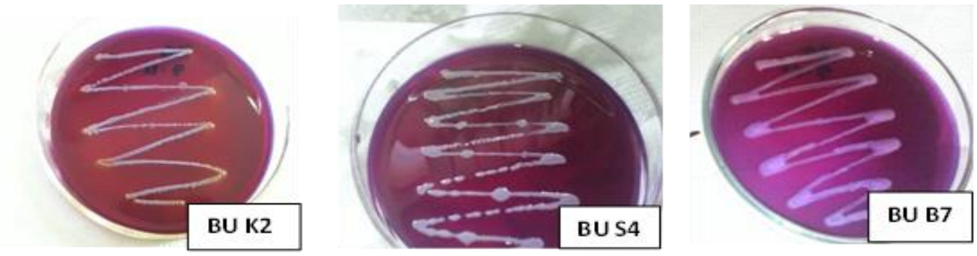


Figure 6. Growth of bacterial isolates on glucose peptone agar media

Acid/ alkali production in YEM agar medium containing Bromothymol blue

In the present study, bacterial isolates BU B2, BU B3, BU B5, BU B7, BU B8, BU B9 isolated from Bhola soil and BU S1, BU S2, BU S3, BU S4, BU S5, BU S7 isolated from Satkhira soil demonstrated acidic reaction during their one week of growth (Table 5). These isolates turned green color of the medium to yellow (Figure 7). On the other hand, the isolates BU B1, BU B4, BU B6, BU S6, BU K1 and BU K2 showed alkali reaction (Table 5). Alkali producing isolates turned green colored medium to blue. The isolates that produced acid indicating fast growing nature and the isolates that produced alkali indicating slow growing nature.

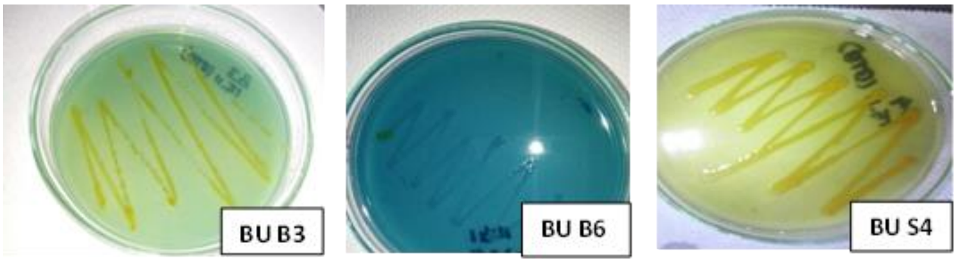


Figure 7. Growth of the bacterial isolates on NA agar medium containing BTB

Indole acetic acid production

Results presented in Figure 8 show that all the 18 isolates were capable of producing indole-acetic-acid (IAA) in variable quantity, indicating a substantial variability among the isolates in terms of IAA production. All isolates were positive for IAA production but among those, seven isolates BU B6, BU B7, BU S1, BU S2, BU S4, BU S6 and BU S7 were selected as potential IAA producers. Among the bacterial isolates, IAA production varied from 12.5 to 115µg/ml. The maximum IAA production was obtained from the isolate BU S4 (115µg/ml) which was followed by BU B7 (114µg/ml) and BU S1 (110µg/ml) (Figure 8).

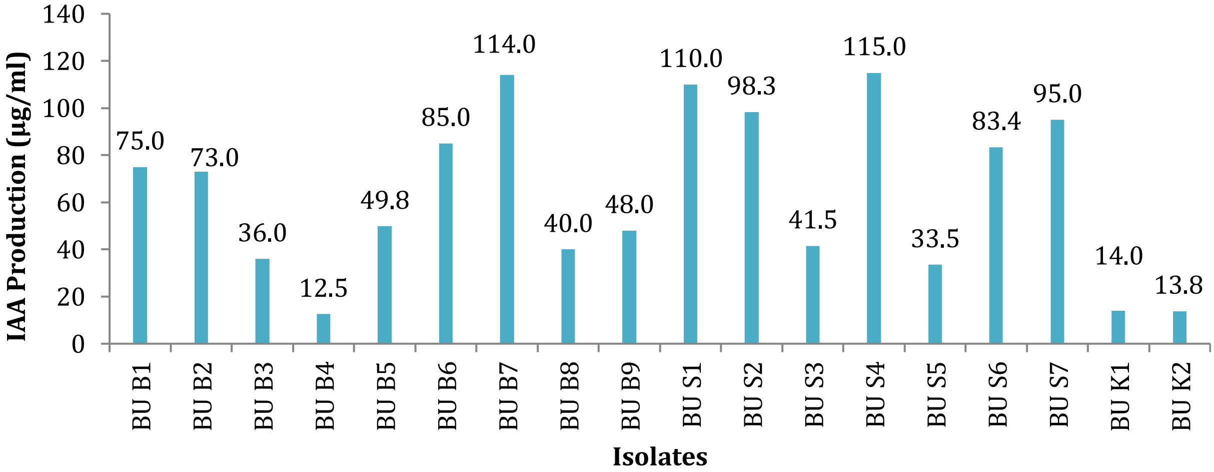


Figure 8. Indole acetic acid production (µg/ml) by different bacterial isolates

However, the lowest IAA production (12.5µg/ml) was found in case of isolate BU B4 (Figure 8). Joseph et al. (2007) showed that bacteria specially *Bacillus*, *Pseudomonas*, *Azotobacter* and *Rhizobium* could produce IAA. Due to the significant influence of IAA producing bacteria on plant growth promotion, production of IAA is considered as one of the vital tools for the screening of beneficial microbes (Wahyudi et al., 2011) The amount of IAA determined in the present study seems relatively higher as compared to the findings reported by Suliasih and Widawati (2020) but are in line with the findings of Hossain et al. (2021). Bacterial isolates that produced considerable amount of IAA might play effective role in plant growth enhancement under saline condition.

Growth of the isolates at 37^{0}C

In the growth chamber, all the bacterial isolates were able to grow in the NA growth medium at 37^{0}C (Table 5). Our findings indicate that all the isolates could tolerate high temperature. As the isolates were collected from harsh environment (saline soil), therefore they might tolerate other environmental stresses like high temperature.

Screening of bacterial isolates against NaCl tolerance

All the bacterial isolates were able to tolerate NaCl up to 2.0% (Table 6 and Figure 9). At 4.0% NaCl level, all the isolates were grown except the isolate BU B6 (Table 6). There was variation in the growth of the isolated bacteria at 6.0% and 8.0% NaCl level (Table 6). At 8.0% NaCl concentration, 9 isolates showed higher growth, 1 isolate demonstrate moderate growth and rest 8 isolates were failed to grow in the nutrient agar plate (Table 6 and Figure 10).

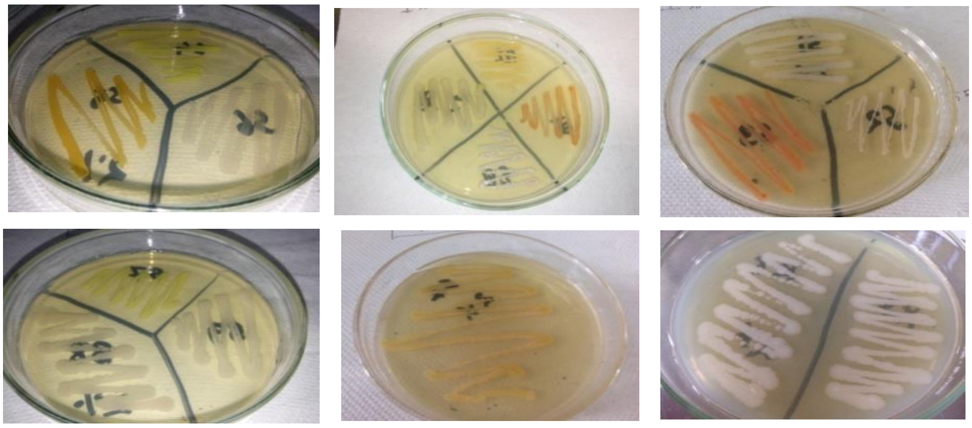


Figure 9. Growth of the bacterial isolates at 2.0% NaCl concentration

Table 6. Growth performance of the isolated bacteria in different NaCl concentration

Isolates	NaCl concentration				
	0%	2.0%	4.0%	6.0%	8.0%
BU B1	+ +	+ +	+ +	+ +	+ +
BU B2	+ +	+ +	+ +	+ +	+ +
BU B3	+ +	+ +	+	+	-
BU B4	+ +	+ +	+	-	-
BU B5	+ +	+ +	+ +	+ +	+ +
BU B6	+ +	+ +	-	-	-
BU B7	+ +	+ +	+ +	+ +	+ +
BU B8	+ +	+ +	+ +	+ +	+ +
BU B9	+ +	+ +	+ +	+ +	+ +
BU S1	+ +	+ +	+ +	+ +	-
BU S2	+ +	+ +	+ +	+	-
BU S3	+ +	+ +	+ +	+ +	+ +
BU S4	+ +	+ +	+ +	+ +	+ +
BU S5	+ +	+ +	+ +	+ +	+
BU S6	+ +	+ +	+	-	-
BU S7	+ +	+ +	+ +	+ +	+ +
BU K1	+ +	+ +	+ +	-	-
BU K2	+ +	+ +	+ +	-	-

Note: (-) =No growth, (+) = Moderate growth and (++) = High growth

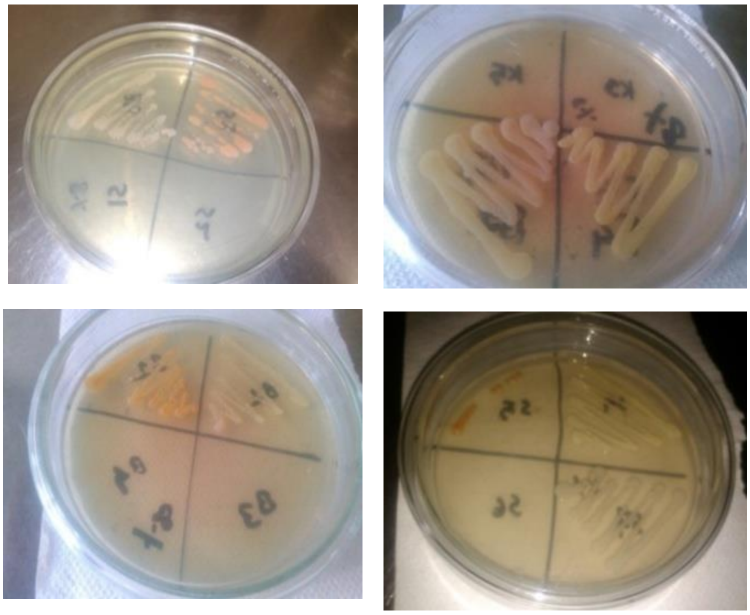


Figure 10. Growth of the bacterial isolates at 8.0% NaCl concentration

Results of the study reveal the possibility of using the high salt tolerant bacteria as effective biofertilizer to enhance the crop production in the saline areas.

Conclusion

A total of 18 isolates were isolated from saline soils of Bangladesh. All the isolated bacteria showed positive response to produce IAA but the Isolates BU S4, BU B7 and BU S1 showed highest IAA production ability. The isolates BU B7 and BU S4 showed better results in all biochemical tests including catalase test, cellulose degradation and P solubilization. All the bacterial isolates were found as salt tolerant against 4.0% NaCl concentration, except isolate BU B6. Ten (10) isolates showed tolerance up to 8.0% NaCl concentration of which BU B7 and BU S4 performed better for different biochemical characteristics. The isolates BU B7 and BU S4 might be recommended as effective bioremediation agent against soil salinity for better crop production in the coastal regions.

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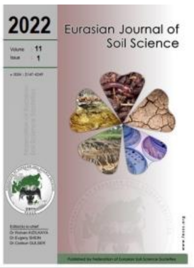
Eurasian Journal of Soil Science

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



Influence of inoculating microbes on municipal sewage sludge composting

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Article Info

Received : 21.11.2021
Accepted : 29.04.2022
Available online : 10.05.2022

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Abstract

The influence of Ilkompost and Micromix bacterial consortium inoculation during sewage sludge (SS) with wheat straw (WS) composting was assessed. The effect of inoculation on compost quality parameters such as pH, temperature, nutrient contents and C/N, bacterial and fungal population were determined. Compared to the control treatment, the temperature of piles and population of microorganism increased after inoculated bacterial consortiums at the beginning of compost. But, WS addition did not effect on compost quality parameters and microbial population. Fungal and bacterial population, the peak temperature, or heating rate, of Micromix bacterial consortium based on *Streptomyces pratensis, Bacillus mesentericus, Azotobacter chroococcum* inoculated treatments was clearly higher than that of Ilkompost bacterial consortium based on *Pediococcus pentosaceus, Streptomyces sindenensis, Bacillus megaterium* inoculated treatments.

Keywords: Sewage sludge, composting, inoculation, bacteria, fungi.

Introduction

Currently, with the development of industrialization and urbanization, the yield of sewage sludge (SS) in Kazakhstan sharply increases every year (Ospanov et al., 2022). SS is a by product of waste water treatment process, which contains lots of organic matter, plant nutrients (N, P, K, Ca, Mg, etc) and harmful components such as potential toxic heavy metals and pathogenic microorganisms (Delibacak et al., 2020; Kızılkaya et al., 2021). The use of SS in agriculture includes several operations that improve its efficiency in crop production, as compared to mineral fertilizers, which are related to the stabilising process before spreading (Shashoug et al., 2017; Jatav et al., 2021). These are principally aerobic digestion, anaerobic digestion, composting, liming, and pelletisation. SS has been globally applied as an effective and cost efficient process for its management and reuse (Wang et al., 2019a). However, SS cannot be composted alone owing to its high moisture content and poor air permeability (Zhou et al., 2014), and therefore must be mixed with bulking agents to reduce the moisture content and improve the free air space of composting materials (Ma et al., 2019a). Numerous studies have evaluated the effect of these bulking agents such as sawdust, straw, cotton waste, and matured compost to regulate the physicochemical properties of the matrix during composting (Fan et al., 2019; Nie et al., 2019; Ma et al., 2019b).

Numerous microbiological and physico-chemical techniques have been developed to characterize the agrochemical properties and the maturity of compost (Gülser et al., 2015). The previous studies indicated that the presence of more stable organic matter in the mature compost, which may be good indicators of SS composting with microbial inoculation. The results on the microbial inoculation of different composting processes can be found in the literature (Biey et al., 2000; Baheri and Meysami, 2002; Barrena et al., 2006; Kızılkaya et al., 2015). It seems clear that microbial inoculation can have a positive effect on composting (Tiquia et al., 1997; Bolta et al., 2003).

doi : https://doi.org/10.18393/ejss.1114772
: http://ejss.fesss.org/10.18393/ejss.1114772
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

In this study, inoculation microorganisms were used in SS composting in order to increase temperature in thermophilic phase and improve the composting process. The effect of two types of bacterial consortium (named Micromix and Ilcompost) inoculation on the microbial population and compost quality parameters during the composting was also determined.

Material and Methods

Preparation of bacterial consortium

To preparation a bacterial consortium, 65 different microorganisms were isolated from samples of sewage sludge, then 15 microorganisms were selected after assessing their agro-ecological properties: nitrogen-fixing ability, growth stimulation of seedlings; cellulose destruction, inhibitor possibilities of growth and reproduction of soil pathogens. Next, microorganisms that could interact without suppressing any strain in the consortium were united. As a result, 3 consortiums of microorganisms were created, one of them did not show good growth on a nutrient medium from sewage sludge, for the remaining two consortium, liquid nutrient media were tested for growth and development activities of microorganisms. As a result, two types of bacterial consortium (named Micromix and Ilcompost) were used in the study:

i. Micromix bacterial consortium based on *Streptomyces pratensis, Bacillus mesentericus, Azotobacter chroococcum.* The inoculated micromix bacterial consortium was comprised of the enriched ONB (Oatmeal based liquid nutrient medium) and the consortium was obtained from composting samples by ONB medium (28 g Oatmeal, 0.5 g NaCl, 0,01 g $FeSO_4$ in 1 L distilled water, pH 7,2).

ii. Ilcompost bacterial consortium based on *Pediococcus pentosaceus, Streptomyces sindenensis, Bacillus megaterium*. The inoculated Ilcompost bacterial consortium was comprised of the enriched WNB (wheat bran based liquid nutrient medium) and the consortium was obtained from composting samples by WNB medium (15 g weat bran, 18 g sucrose, and 0,01 g $FeSO_4$ in 1 L distilled water, pH 7,2).

The enrichment process of ONB and WNB were as follows. 5 ml fresh suspensions of microorganisms (concentration was 1×10^8 CFU/ml) was took out and injected into 250 ml ONB and WNB medium, then cultivated at the temperature (30 °C) and shaking speed (100 rpm/min). Culture time was set to 7 days. After 7 days of cultivation, 10 ml inoculant was transferred into fresh 250 ml enrichment medium and cultivated at the same condition. The inoculant of enriched ONB and WNB was centrifuged and suspended in sterile water, then sprayed on the composting mixture. The concentration of the inoculant was 1×10^8 CFU/ml.

Composting materials and experimental design

The dewatered municipal sewage sludge from waste water (SS) and wheat straw (WS) were used as the raw materials for composting. The dewatered SS was collected from a municipal wastewater treatment plant in Astana province, Kazakhstan. WS was collected on the field of the Research and Production Center for Grain Farming named after A.I. Baraev. The main characteristics of the raw materials were shown in Table 1.

Table 1. Physicochemical characteristics of the raw materials.

	Organic matter, %	Organic C, %	Total N, %	Total P, %	Total K, %	C/N
SS	48.0	27.8	5.2	1.1	0.2	5.4
WS	80.0	46.4	0.6	0.2	0.7	77.3

SS: dewatered municipal sewage sludge from waste water; WS: Wheat straw

The moisture content of every pile (width: 40 cm, height: 20 cm, length: 50 cm and total dry weight: 12-12.6 kg) was adjusted to 60% by addition of sterile water. Fresh air was supplied into piles by mixing for active aeration. The piles was regularly turned to provide sufficient aeration (every 3 days) and maintain the temperature. Temperature of the pin was monitored weekly by a digital thermometer during SS composting. A randomized complete plot design with three replicates per treatment was used. The experiment was performed with the following 8 treatment:

1 (control) - SS (12 kg) and no inoculation.
2- SS (12 kg) and only inoculation of Ilkompost bacterial consortium (1 ml/kg).
3- SS (12 kg) and only inoculation of Micromix bacterial consortium (1 ml/kg).
4- SS (12 kg) and only inoculation of Ilkompost bacterial consortium (2 ml/kg).
5- SS (12 kg) and only inoculation of Micromix bacterial consortium (2 ml/kg).
6- SS + WS (12 kg/0.6 kg, 1/0.05, w/w) and inoculation of Ilkompost bacterial consortium (2 ml/kg).
7- SS + WS (12 kg/0.6 kg, 1/0.05, w/w) and inoculation of Micromix bacterial consortium (2 ml/kg).
8- SS + WS (12 kg/0.6 kg, 1/0.05, w/w) and no inoculation

The whole time of experiment in this study was 52 days. Every week, Triplicate samples were collected from each pile at a depth of about 10 cm and stored at −4°C immediately until used for determination of microbial population, pile temperature and pH. And, at the end of the composting period, Triplicate samples were collected from each pile at a depth of about 10 cm and stored at −4°C immediately until used for determination of other compost quality parameters such as moisture content, organic matter, N, P, K contents and C/N ratio.

Determination of Microbial Population

Bacteria, fungi and actinomycetes play an important role in the composting. They were determined by using different medium throughout the serial dilution method (Germida, 1993). **MPA** (Meat-peptone agar media: 13 g meat peptone broth and 15 g agar, in 1 L distilled water, pH 7,6), **SAA** (Starch ammonia agar media: 10 g starch, 2 g $(NH_4)_2SO_4$, 1 g K_2HPO_4, 1 g $MgSO_4$, 3 g $CaCO_3$ and 20 g agar in 1 L distilled water, pH 7,2) and **ASM** (Ashby media: 20 g sucrose, 0.2 g K_2HP0_4, 0.2 g $MgS0_4.7H_20$, 0.2 g NaCl, 0.1 g $FeSO_4$, 5.0 g $CaCO_3$ and 20 g agar in 1 L distilled water, pH 7.3) were used for evaluating bacteria population. **CDA** (Chapek-Dox Agar media: 14 g glucose, 0.7 g $CaCO_3$, 0.7 g KNO_3, 0.35 g $MgSO_4$, 0.35 g NaCl, 0.35 g K_2HPO4, 0.01 g $FeSO_4$ and 20 g agar in 1 L distilled water, pH 6,0) **GAM** (Gause's No.1 media: 20 g soluble starch, 1 g KNO_3, 0.5 g KH_2PO_4, 0.5 g $MgSO_4$, 0.5 g NaCl, 0.01 g $FeSO_4$ and and 20 g agar in 1 L distilled water, pH 7,2) and **HUM** (Hutchinson's media: 2.5 g $NaNO_3$, 0.01 g $FeCl_3$, 1 g K_2HPO_4, 0.3 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.1 g $CaCl_2$ 20 g agar in 1 L distilled water, pH 7.2) were used for evaluating fungi and actinomycetes population (Germida and de Freitas, 2008). 1 g of compost sample was diluted in 10 ml sterile water and shaken at 150 rpm for 1 h, then the suspension was diluted with sterile water at 10^{-1}–10^{-6}. 0.1 ml diluted solution (10^{-1}–10^{-6}) was inoculated into 7 ml of MPA, SAA, ASM, CDA, GAM and HUM. Each treatment repeated 3 times. Mediums were incubated at 30 ℃for 7 days. Hutchinson media was incubated 30 ℃for 21 days.

Determination Compost Quality Parameters

The changes in temperature of the compost piles were monitored using mercury thermometer. The pH of the compost sample was determined by pH meter using 1:10 sample water suspension ratios. Compost samples were dried and sieved to less than 0.25 mm. The organic matter content was determined by measuring the loss of dry-solid mass in muffle at 550 ℃for 6 h. Total nitrogen content was determined using the Kjeldahl digestion method. Total phosphorus content was determined using the Vanadomolybdo phosphoric Acid colorimetric method and total potassium content was determined Flame photometric method after wet digestion (Jones et al., 1991; Jones, 2001). All the experiments were performed in triplicate.

Results and Discussion

Microbial Populations

The change of bacterial population in different media was shown in Figure 1. The MPA (Meat peptone agar media), SSA (Starch ammonia agar media) and ASM (Ashby media) media were used as measures of the number of bacterial community in the piles. The population of bacteria in MPA and ASM media all showed an upward trend over the maturation phases and reached the peak value on the day 50. On the other hand, bacterial population in SAA media showed an upward trend over the mesophilic phase after thermophilic phase and reached the peak value on the day 40. Vargas-Garcia et al (2010) presented the similar trend in population of ammonifying bacteria during the compost of sewage sludge, which was also reported by Meng et al. (2016). In SSA media, at the early mesophilic and thermophilic phases of composting, the population of bacteria had increased due to the presence of readily available organic substrates which was energy for microbial growth. Later, the decrease in the population of bacteria for all treatments from day 40 to the end of composting was probably because the energy sources such as proteins, fats and amino acids were exhausted. The bacterial population in treatments was a little higher than that in control treatments within the initial 10 days and all treatments showed the similar tendency on day 20 to day 50. Therefore, inoculation of enriched Micromix and Ilkompost bacterial consortium was not detrimental to the growth of indigenous bacteria.

The change of fungal population throughout composting was shown in Figure 2. Fungal population in different media had been detected in the piles. The CDA (Chapek-Dox agar media), HUM (Hutchinson's media media) and GAM (Gause's No.1 media) were used as measures of the number of fungal community in the piles. The profile indicated that the fungal populations of inoculation of enriched Micromix and Ilkompost bacterial consortium were much larger than those of control treatment during the whole composting time, with the order as following: Micromix bacterial consortium > Ilkompost bacterial consortium > No inoculation > Control. In treatments, the fungal population increased quickly in the initial mesophilic phase, and later reached at thermophilic and second mesophilic phase. As a comparison, the corresponding peak level of fungal population in treatments was done at fourty days.

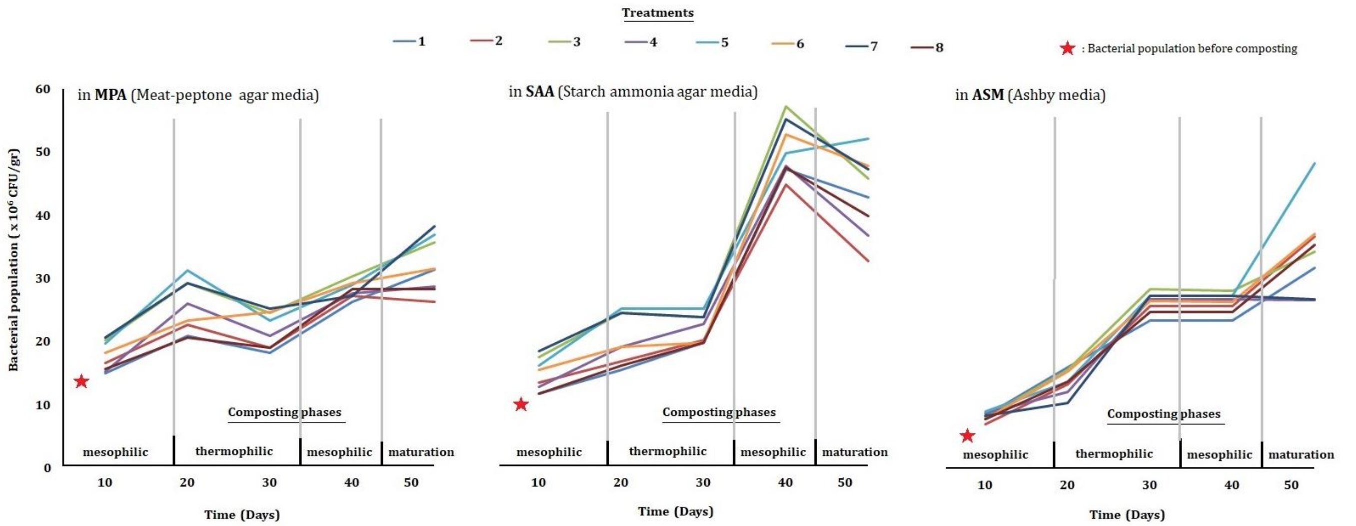


Figure 1. Changes of bacterial population in MPA-Meat peptone agar media (a), SSA-Starch ammonia agar media (b) and ASM-Ashby media (c) during composting

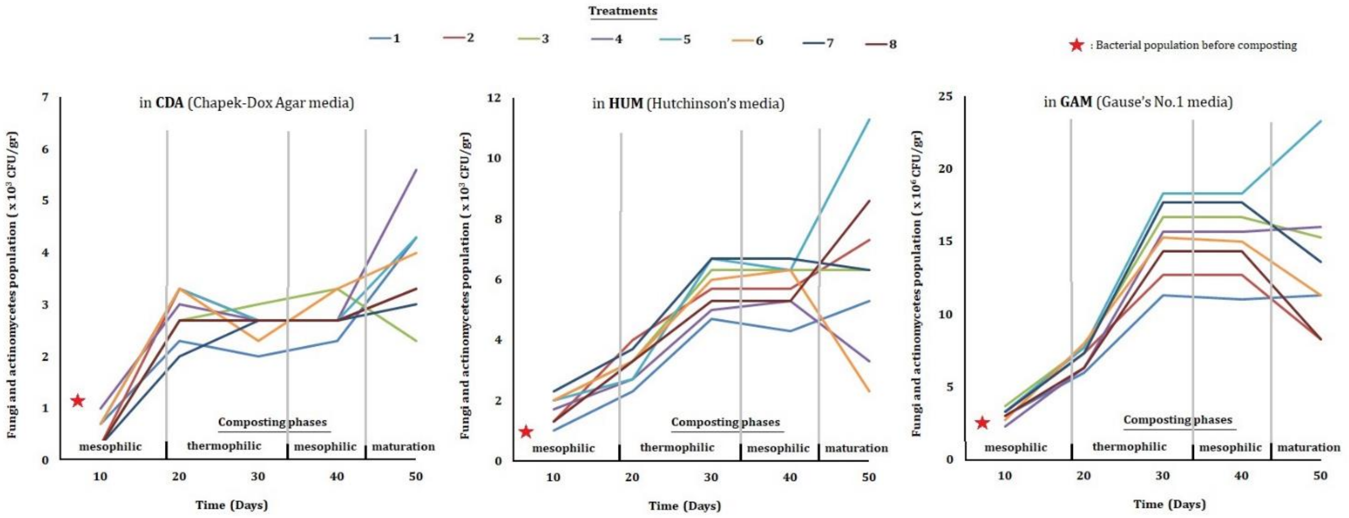
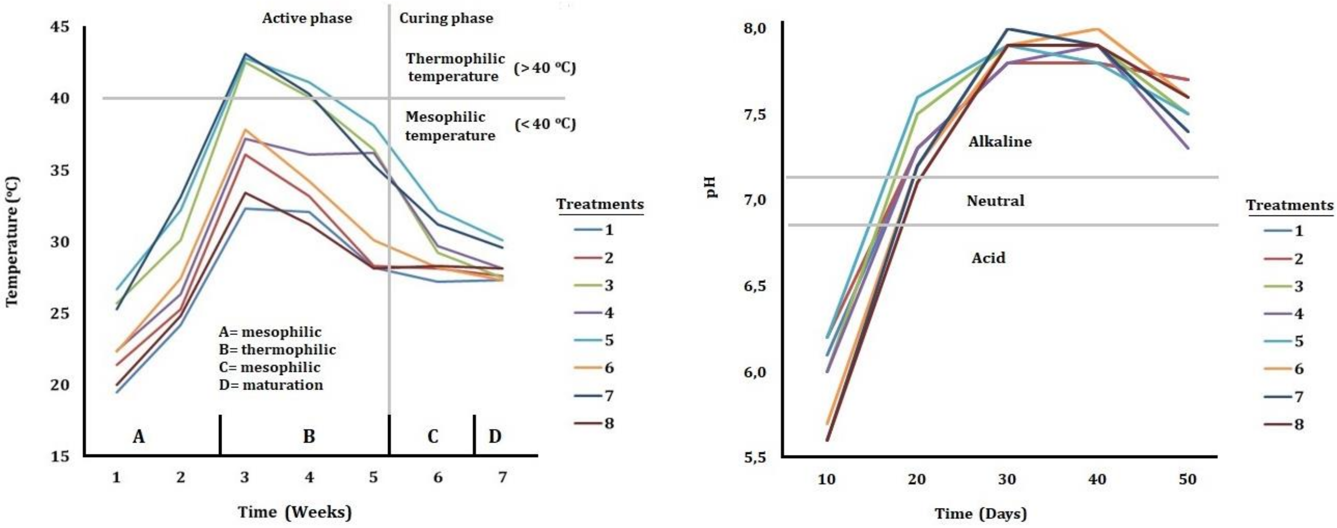


Figure 2. Changes of fungal population in CDA-Chapek-Dox agar media (a), HUM-Hutchinson's media media (b) and GAM-Gause's No.1 media (c) during composting

Compost Quality Parameters

Temperature has been widely recognized as one of the most important parameters in the composting process (Sullivan and Miller, 2001; Zhou, 2017), which can directly reflect the composting efficiency and microbial activity (Manu et al., 2017; Wang et al., 2019b). The variation of temperature during composting process was shown in Figure 3a. The temperature during composting went through three distinct phases, including mesophilic phase (0–2,5 weeks), thermophilic phase (2,5-5 week) and cooling phase (5-7 week), which were similarly found by Li et al. (2019) and Wang et al. (2019c). Compared to control (treatment 1), the temperature of treatments rapidly increased after inoculated Micromix and Ilkompost bacterial consortium at the beginning of compost. As shown in Figure 1a, Treatment 3, 5 and 7 reached the highest temperatures on 3 weeks at 42.5°C, 42.8°C and 43.1°C, respectively. The peak temperature, or heating rate, of Micromix bacterial consortium inoculated treatments (3, 5 and 7) was clearly higher than that of Ilkompost bacterial consortium inoculated treatments (2, 4 and 6). This may be due to the inoculation of the Micromix bacterial consortium in piles, which would result in a more affective microorganism in this consortium. In addition, Micromix bacterial consortium inoculated treatments had a long high thermophilic duration of 3 weeks and 5 weeks (>40°C), which could meet the hygienic index of composting, while effectively killing pathogenic microorganisms and weed seeds in the compost (Wang et al., 2019b), respectively. It could be the result of inoculation of enriched Micromix and Ilkompost bacterial consortium, which could accelerate the decomposition rate of organic matters and contribute more heat from microorganisms, thereby promoting a large temperature fluctuation. As the reaction progressed, when the temperature of composting decreased to 40 °C on 4 weeks for Micromix bacterial consortium, the process had mostly finished (Bertoldi et al., 1983). Inoculation with enriched Ilkompost bacterial consortium had no significant impact on shortening composting time in comparison with control ratment. However, inoculation with Ilkompost bacterial consortium could accelerated the rise of pile temperature and had a longer duration of high temperature, which could kill many kill many pathogenic microorganisms and weed seeds (Wan et al., 2020).



(a) (b)

Figure 3. Changes of temperature (a) and pH values (b) during composting

The pH also strongly affects microbial activity during composting (Bareither et al., 2013), and the degradation process can be enhanced by appropriate pH control (Gajalakshmi and Abbasi, 2008). The change of pH in treatments was shown in Figure 3b. As shown in Figure 1b, the trend of the eight piles was similar, with an overall trend of a gradual increase in the pH with ultimate stabilization at an alkaline pH, which is consistent with previous results (Chang et al., 2019). During the mesophilic phase, the pH in all piles increased slightly. The increase in pH might be attribute to consumption of short-chain organic acids and the formation of ammonium (NH_4^+) ions (Tong et al., 2019). Moreover, the addition of matured compost significantly reduced the pH in the mesophilic phase, and significantly increased the pH of thermophilic phase. However, there was no significant difference in the pH among the piles during the cooling phase of composting. Afterward, the pH of treatments slowly decreased and kept approximately 7.5 until the end of compost because of the ammonia emission and ammonium oxidation by nitrobacteria (Rihani et al., 2010). Wang et al. (2017) pointed out that the optimal pH value of final composting production ranged from 6.9 to 8.3. In this study, the final pH value of composting production of all treatments reached the standard (pH 7.29–7.67).

The change of some compost quality parameters in different media was shown in Table 2. The initial organic matter and organic carbon contents in SS and WS were higher than in all treatments. The total carbon and organic matter content of the compost showed a decreasing trend with the advancement of composting. The release of CO_2 leads to a reduction in C/N ratio. The composting is an aerobic process, in which diverse microbes are involved. By changing the microbial diversity, the composting process can be altered. Most of the organic material consists of macromolecules, which cannot be penetrated easily. Therefore, microorganisms secrete enzymes, which degrade the polymers to small organic materials. According to Golueke (1992) and Sivapalan et al. (1994), low colony forming unit's value must be taken as an indicator of matured compost. Death cells of microbes in turn, increase the nitrogen content.

Table 2. Changes in some compost quality parameters at the end of the composting period

Treatments	Organic matter,%	Organic C, %	Total N, %	Total P, %	Total K, %	C/N
1	34.25	19.87	1.77	1.87	0.21	11.22
2	34.10	19.78	1.94	1.62	0.31	10.19
3	35.29	20.47	1.97	1.87	0.23	10.39
4	36.74	21.31	2.04	1.89	0.41	10.44
5	35.52	20.60	2.07	1.75	0.24	9.95
6	42.52	24.66	4.37	1.91	0.30	5.64
7	43.86	25.44	4.02	1.80	0.27	6.32
8	40.52	23.50	3.94	1.72	0.26	5.96

Composting materials with a low C/N ratio results in greater N losses than waste with a high C/N ratio (Zhou, 2007). The C/N ratio is an important quality parameter in composting because it gives a characterization of the decomposition of organic matter in the compost. Microorganisms use about 30 parts of carbon for every part of N. Thus, an initial C/N ratio of 20–35 will be most favorable for quickly converting organic waste into compost. Sewage sludge typically has a C/N ratio of less than 15. Although decomposition will be rapid at this ratio, nitrogen can be lost in large amounts as ammonia (Jiang et al., 2011). In the present study, the C/N ratio was 5.4 in the treatment 1, 2, 3, 4 and 5 and 8.82 in the treatment 6, 7 and 8 with WS added at the beginning of composting. The C/N ratio of compost pile provides an indication of the kind of compost and how it can be managed while mixed to the soil. Initially the C/N ratio of SS and WS were 5.4 and 77.3, respectively. At the end of composting period, it was determined that the C/N ratios of the treatments varied between 5.64 and 11.22. Asija et al. (1984) also recorded a decrease in C/N ratio with the increase in the period of decomposition (Table 2).

In this study, results showed that the level of P in all piles was high compared to the initial contents of SS and WS. Similar results have been determined Wei et al. (2015) and Du et al (2018). On the contrary, there was also a significant decrease in the N concentration in the final product compared to the initial contents of SS and WS. Regarding the loss of nitrogen during composting, Wang et al. (2016) determined that nitrogen is reduced as a result of ammonia volatilization at the initial stage of composting. Similarly, Hua et al (2009) determined that the decrease in total N content in the early stages of composting was due to loss of N in the form of ammonia, which in turn depends on the type of material and its C / N ratio.

Conclusion

In this study, Ilkompost and Micromix bacterial consortium were inoculated in composting of SS. Compared to control, the temperature of piles rapidly increased after inoculated Micromix and Ilkompost bacterial consortium at the beginning of compost. Moreover, inoculation increased the population of bacteria and fungi in the composting phases. During aerobic composting of SS, addition of WS enhanced the population of bacteria and fungi during the composting phases, but reduced the peak temperature. WS addition in aerobic SS composting as a bulking agent is not necessary. But, the inoculation of Micromix is more active bacterial consortium than Ilkompost might be a useful strategy to improve compost quality parameters and increase microbial population in aerobic SS composting.

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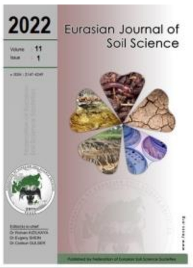
Eurasian Journal of Soil Science

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



A study about radiation dosimetry and heavy metal pollution in the Küçük Menderes Basin, Turkey (Radio-ecological and Heavy Metal Risks)

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Article Info

Received : 07.12.2021
Accepted : 10.05.2022
Available online : 24.05.2022

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Abstract

Agricultural researchers in many countries investigate radiological risks in soil and crops because it concerns human health. In addition, they also study heavy metal pollution in plants in cultivated soil for ecological safety. This study aims to analyze the activity concentrations of radionuclides and heavy metals in soil and corn crops in the Küçük Menderes Basin (Izmir, Turkey) – which is enriched with phosphatic fertilizers. We collected soil and corn samples from the area, and then separately measured concentrations of radionuclides (^{226}Ra, ^{232}Th and ^{40}K) and trace elements (Cd, Cr, Cu, Hg, Ni, Pb and Zn) they contain. Activity concentrations of the radionuclides were acquired by radiometric methods (gamma spectroscopy). Heavy metal amounts were calculated using ICP-MS (inductively coupled plasma-mass-spectrometry). The mean heavy metal concentrations in the soil (Cd, Cr, Cu, Zn, Ni, Pb, Hg) were 0.096, 40.26, 26.51, 72.43, 32.24, 7.05 mg kg^{-1}, 158.28 µg kg^{-1} and in the corn (Cd, Cr, Cu, Zn, Ni, Pb, Hg) were 0.01, 1.09, 2.05, 22.00, 0.54, 0.24 mg kg^{-1}, 12.15 µg kg^{-1}. The heavy metal concentrations in soil samples were as follows: Hg<Cd< Pb<Cu<Ni<Cr< Zn and in corn samples were as follows: Hg<Cd<Pb<Ni<Cr<Cu<Zn. Also, the mean activity concentrations in the soils (^{226}Ra, ^{232}Th, ^{40}K) were 36.2±2, 32±1, 615.44±7 Bq kg^{-1}. The ^{226}Ra and ^{232}Th concentrations in the corn samples are smaller than the Minimum Detectable Activity (MDA). However, the mean activity concentration of ^{40}K in the corn samples is 310.7±8 Bq kg^{-1}. These values considered are acceptable for human health according to UNSCEAR (2000). The heavy metal concentrations in the soil and corn samples are within acceptable limits for Turkish Government. The level of radionuclide activity and heavy metal concentrations, as well as both transfer and bio-concentration factors are comparable with those of a handful of other countries. Long-term research on radio-ecological risks is very important for agricultural control. In addition, the data set of radiation levels and pollutant elements do not have a fixed amount in related materials such as soil and plants. On the other hand, the quantity of pollutants soil (via plants) has risen due to activity from non-controlled industrial facilities. Researchers and governments alike therefore must monitor ecological pollution of terrestrial radionuclides and heavy elements on a routine basis.

Keywords: Radioecology, agronomy, heavy metals, soil, corn.

Introduction

In recent years, many radio-ecological studies have focused on the deposition of radionuclides in different ecosystems (Kuo et al., 1997; dos Santos Amaral et al., 2005; Abbady et al., 2005; Bolca et al., 2007; Yadav et al., 2017). It takes longer for soil in nature and semi-natural eco-systems to absorb radionuclides than it does cultivated soil. Many environmental factors affect the horizontal distribution of radionuclides in the soil: namely change in topography, falling, and wind. Furthermore, local plant flora alongside animal movement

: https://doi.org/10.18393/ejss.1120539
: http://ejss.fesss.org/10.18393/ejss.1120539
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

(surface or underground) also can biologically affect how radionuclides get distributed. Radionuclides reach the plant roots and enter the food chain via vertical distribution. Moreover, they can mix into ground- and drinking water (Epik, 2005). According to UNSCEAR (2000a,b), gamma radiation from ^{238}U and ^{232}Th series and from ^{40}K can exist inter-bodily; gamma irradiation – like beta and alpha irradiation – can occur in all organs like. This in turn leads people to develop inescapable health problems such as cancer (Kapdan et al., 2018). The world-mean values for ^{226}Ra, ^{232}Th, and ^{40}K activity concentrations (including their deviation intervals) in soil are 35 Bq kg^{-1} (17-60), 30 Bq kg^{-1} (11-64), and 400 Bq kg^{-1} (140-850), respectively (UNSCEAR, 1988). The now extensive use of phosphatic fertilizer by farmers has caused natural radionuclide concentrations to increase in soil, and thus agricultural products (Khalf and Mohammad, 2021; Sallam et al., 2021).

Terrestrial ^{238}U and its daughter products are at radioactive equilibrium in phosphate rocks. Its radioactive equilibrium breaks down during industrial processing and creates ^{238}U, ^{226}Ra, ^{210}Pb and ^{210}Po radionuclides in industrial by-products. Therefore, phosphatic fertilizer is an important of source of TENORM (Technologically Enhanced Naturally Occurring Radioactive Materials). One might deem this a radio-ecological risk (Camgöz and Yaprak, 2009). The annual effective dose equivalent (per person) for phosphate production is 0.04 µSv in industrial applications, 2 µSv in fertilizer, and 10 µSv in phosphate waste (UNSCEAR, 1993). Processing phosphate rocks and using them in various areas of industry creates TENORM. They also are an important source of energy (via coal and other fossil fuels) and radioactive minerals. The radiation dose from industrial activities is 100 µSv. This is very small in comparison with natural radiation (UNSCEAR, 2000a,b).

Heavy metals concentrations in cultivated soil depend on geologic construction. Heavy metal concentrations have been identified in the Earth's crust (Carnelo et al., 1997) – namely Cd, Cr, Cu, Ni, Pb and Zn at 0.5, 200, 100, 80, 16 and 50 mg kg^{-1}, respectively. However, fertilization, atmospheric deposition, agricultural chemicals, industrial, household (namely organic) waste, and other inorganic sources of pollution (ore bed and mine waste) cause soil to accumulate heavy metals (Taşkaya, 2004). Phosphatic fertilizers made from phosphate rocks incorporate several heavy metals (Co, Cu, Fe, Mn, Mo, Ni, Zn), Fluorine, and toxic metals (As, Al, Cd, Pb and Hg), alongside radioactive elements (Camgöz and Yaprak, 2009). Extensively using phosphatic fertilizers can increase how much Fluorine, heavy metals, and radioactive elements soil – and thus plants – absorb. Both organic and phosphatic fertilizers (can) cause soil to accumulate heavy metals such as Zn, Cu, and Cd.

Plants enhance radioactivity in soil by absorbing radionuclides via their roots or by means of surface deposition due to atmospheric precipitation. They absorb heavy metals and radionuclides through the soil from water, salt, and minerals. However, plants will recycle those radionuclides back into soil. The rate at which plants absorb radioelements and other chemicals by plants depends on how productive the soil is, how acidic/alkaline and reductive - oxidative agents in soil are, and its organic composition (Grytsyuk et al., 2006). This rate for radioactivity absorption is defined as the transfer factor (TF) or the rate at which radionuclide penetrates the crop via contaminated soil (Alharbi and El-Taher, 2013). TF is calculated the ratio of radionuclide concentrations in crops (Bq kg^{-1} dry mass) to concentration of radionuclides in soil (Bq kg^{-1} dry mass) (Vandenhove et al., 2009). TF is a prediction indicator for risk to human health risk because of how many radionuclides gets transferred into the food chain (Tome et al., 2003; Ali et al., 2020). The bio-concentration factor (BCF) is trace element concentration in crop tissues (mg g^{-1}) over the background concentration of metals in the soil (mg g^{-1}) (Tiwari et al., 2011). It quantifies the bioavailability of heavy metals in agricultural products. (Kim et al., 2012). Soil-to-plant transfer exposes humans to heavy metals. The health risks associated with heavy metal contaminations from soil to agricultural food has been widely studied (Cui et al., 2004).

This study investigates the possible pollution of radionuclides and heavy elements by assessing the terrestrial gamma doses rate in agricultural soil and corn samples taken from Küçük Menderes Basin. This study is local in nature. That noted, while local databases are important for environmental efforts, every local study area is nevertheless can serve research in neighboring countries – give or take variation in atmospheric activity and ground transfer rates. One can in turn use such data for comparison purposes and to track environmental relationships.

Why are environmental radiometric studies important? While they may not offer us improved techniques or new fundamental approaches, their data nonetheless contains important data – especially where agricultural product trade between countries is concerned, namely when it comes to government procedures and people requesting product information for health purposes. Environmental studies can supply this. One should consider case studies as a scientific database case.

Material and Methods

The Küçük Menderes Basin is an important agricultural area in western Turkey for corn farming. Farmers there moreover make extensive use of phosphatic fertilizers. Its geographical position of the basin that feeds into is 38º41´05" by 37º53´08" N (latitude) and 28º41´36" and 26º11´48" E (longitude). This basin forms Küçük Menderes's quaternary sediment filling graben (broken in the Menderes Massif). That filling is composed of crystalline rocks; the basin likewise covers a broad surface area (Dora et al., 1992). We collected raw soil samples (55) as well as those from principal crops (13) (where crop roots grow) from various points along the basin (Figure 1 and 2).

Figure 1. Soil sample points in Küçük Menderes Basin (maps.google.com)

Figure 2. Corn sample points in Küçük Menderes Basin (maps.google.com)

First, we marked every point of each soil sample (3 kg), sifted them, left them to dry under the sun for three days, and then baked them in an 105°C degree oven for between two and forty eight until they reached a constant weight. Next, the dried and homogenized soil samples were placed into Marinelli beakers (1 L). Corn is classic product grown around the basin. As such, we collected and air-dried samples (at 105°C for a few days) of corn grains until they reached a constant weight. We then ground the corn grain down and filled it into 100 cc plastic containers. Each sample was sealed and stored for four weeks in order to study their secular radioactive equilibriums between ^{226}Ra and ^{222}Rn.

We identified ^{226}Ra, ^{232}Th and ^{40}K in the samples using gamma spectroscopy. Analytical quality control of both gamma spectrometer systems done by using standards prepared from IAEA and Amersham-sourced reference materials whose matrices and geometries were similar to the samples. We used two types of detectors due to how much of each sample there was. We used one-liter Marinelli Beakers on the HPGe detector system (184 cc HPGe coaxial, efficiency: 25%, for 1.33 MeV ^{60}Co FWHM: 1.83 keV and peak/Compton; 57:1, Ortec Model-671 amplifier and Canberra PC base MCA (8K) Wilkonson ADC, 100 mm shielding). The HPGe detector has good resolution however, it does not have enough efficiency in some conditions, especially for low activities. The lower detection limits were 2 Bq/kg for ^{226}Ra, 1 Bq/kg for ^{232}Th and 4 Bq/kg for ^{40}K. As the plant samples were limited in terms of both quantity and volume, we thus needed high efficiency more than resolution. Therefore, we turned to a NaI (Tl) scintillation gamma spectrometer (Tennelec 3" X 3" NaI (Tl) detector (shielded with 50 mm lead) as well as a computer-based multi-channel analyzer) to examine the corn samples.

Due to the limited separation efficiency of NaI (Tl) scintillation detectors, the gamma energies (2.6 MeV, 1.76 MeV, 1.46 MeV) that we had selected for these primordial radionuclides could not directly be used to measure the concentrations by scintillation gamma spectroscopy. The lower detection limits were 1 Bq/kg for ^{238}U, 1 Bq/kg for ^{232}Th and 5 Bq/kg for ^{40}K (Canbaz Öztürk, 2015). One therefore must calculate how each radionuclide contributes to one another according to the appropriate factors. To resolve this, we used the following three equations (Akakçe, 2008):

$$^{232}\mathrm{Th}\ (\mathrm{Bq\ kg^{-1}}) = \frac{C(\mathrm{Th})}{K_1}\alpha \tag{1}$$

$$^{238}\mathrm{U}\ (\mathrm{Bq\ kg^{-1}}) = \frac{1}{K_2}[C(\mathrm{U})-\alpha C(\mathrm{Th})] \tag{2}$$

$$^{40}\mathrm{K}\ (\mathrm{Bq\ kg^{-1}}) = \frac{1}{K_3}[C(\mathrm{K})-\gamma[C(\mathrm{U})]-\alpha C(\mathrm{Th})-\beta C(\mathrm{Th})] \tag{3}$$

K_1, K_2, and K_3 constitute sensitivity factors – i.e. count rates per unit activity concentration (IAEA, 2003). The method for both how we determined the stripping rates (α, β and γ) that gave us these additive rates (depending on the geometry and various settings of the spectrometer), and how we found out the sensitivity factors that enable the transition from net counts to activity concentration in terms of K (%), U (mg kg^{-1}), Th (mg kg^{-1}) was as follows.

K_1= 6.2 counts/ 10000s per Bq/kg ^{232}Th
K_2= 7.3 counts/ 10000s per Bq/kg ^{238}U
K_3= 2.4 counts/ 10000s per Bq/kg ^{40}K

The stripping rates of the gamma spectroscopy system were $\alpha = 0.75$, $\beta = 0.81$, and $\gamma = 1.32$, respectively.

According to UNSCEAR (1993), the equation to determine the terrestrial gamma dose rate in soil is:

$$D\ (\mathrm{nGY\ h^{-1}}) = 0.461\ C_{Ra} + 0.623\ C_{Th} + 0.0414\ C_K \tag{4}$$

C_{Ra}, C_{Th} and C_K are concentrations of ^{226}Ra, ^{232}Th and ^{40}K, respectively.

We ground the dried corn and soil samples (5 g) at Dokuz Eylül University's Geology Engineering Lab (Retsch (RS 100)), and then measured all of the heavy metal levels in them at the ACME Analytic Lab. (ISO-9002) for analysing inductively coupled plasma-mass- spectrometer (ICP-MS). All analytical results of soil and corn in microwave extraction were obtained in mg/kg and µg/kg as well as for the laboratory's internal reference materials DS7 and NMKL186 inserted in parallel. Interference possibilities were evaluated by isotopic analysis. Heavy metal and radionuclide distribution were mapped on Surfer 8.0 (free demo version).

Results and Discussion

Radioelement and heavy metal data in soil and agricultural crops are largely based on how much background radiation there is in the soil, climatic factors, and present agricultural applications. We calculated concentrations of ^{226}Ra, ^{232}Th and ^{40}K radionuclides in 20 cm-deep cultivated soil samples as well. The mean of radionuclide activity concentrations in them were 36.2±2 Bq kg^{-1} (^{226}Ra), 32±1 Bq kg^{-1} (^{232}Th), and 615.44±7 Bq kg^{-1} (^{40}K). The mean terrestrial gamma dose rate was 62.1 nGy h^{-1}. We did not look at the activity concentrations of ^{226}Ra and ^{232}Th in any of the corn samples because they were below MDA (minimum detectable activity) and thus would not have been detected on a NaI (Tl) scintillation detector. However, there were nondedectable (ND) activities of U and Th in the corn. In contrast, the ^{40}K concentrations in the corn samples ranged between 136±8 and 712±7 Bq kg^{-1} (mean 310.7±8 Bq kg^{-1}).

We measured the concentrations of Cd, Cr, Cu, Hg, Ni, Pb, and Zn in both the soil and corn samples on an ICP-MS. What we discovered was that the Küçük Menderes Basin contains Cd 0.096 (0.01-0.21) mg kg^{-1}, Cr 40.26 (17.60-69.80) mg kg^{-1}, Cu 26.51 (9.45-58.01) mg kg^{-1}, Ni 32.24 (17.20-53.40) mg kg^{-1}, Pb 7.05 (3.53-12.57) mg kg^{-1}, Hg 158.28 (4.00-920.00) µg kg^{-1}, and Zn 72.43 (35.70-106.90) mg kg^{-1}. Also, mean heavy metal concentrations in its soil from highest to lowest were Zn>Cr>Ni>Cu>Pb>Cd>Hg. The heavy concentrations of Cd, Cr, Cu, Zn, Ni, Pb, and Hg in the corn samples were ~ 0.01 (<0.01-0.02), 1.09 (0.90-1.30), 2.05 (1.14-6.44), 22.0 (13.4-40.60), 0.54 (0.20-1.70), 0.24 (0.14-0.63) mg kg^{-1} and 12.15 (1.00-30.00) µg kg^{-1} (Zn>Cu>Cr>Ni> Pb>Cd>Hg), respectively.

The mean activity concentrations of ^{226}Ra and ^{232}Th did not exceed UNSCEAR (2000a,b) standards (Table 1). However, ^{40}K activity concentration exceeded global average in the basin's surface soil due to farmers intensive cultivation activities and because they extensively use fertilizers containing phosphate. Nevertheless, in 75% of the basin samples, activity concentrations of ^{40}K fell below the maximum of concentrations for natural soil in UNSCEAR (2000a,b).

Table1. Comparable radionuclide activity concentrations (Bq kg^{-1}) in soil

Country	^{226}Ra	^{232}Th	^{40}K	References
Australia	-	36 (1-342)	325 (2-1132)	(Kleinschmidt, 2017)
Nigeria	205.08	103.19	350.75	(Gbadamosi et al,. 2018)
Iraq	247	24.86	293.70	(Ridha et al., 2015)
India	41	32.3	544.7	(Yadav et al., 2017)
Serbia	40.6	48	743.2	(Gulan et al., 2013)
Greece	20-710	21 (1-193)	355 (12-1570)	(Anagnostakis et al., 1996)
Greece	21-80	16-85	337-1380	(Florou and Kritidis, 1992)
Greece	7-310	3-190	30-1440	(Probonas and Kritidis, 1993)
Greece	25 (1-238)	21 (1-193)	355 (12-1570)	(Anagnostakis et al., 1996)
Ireland	-	3-60	40-800	(McAulay and Morgan, 1988)
Italy	57-71	73-87	580-760	(Bella et al., 1997)
Norway	43.3 (12-137)	21.1 (4-52)	283 (31-564)	(Dowdall et al., 2003)
Serbia	21-29	25-43	348-441	(Djuric et al., 1996)
Spain	13-165	7-204	48-1586	(Baeza et al., 1992)
Spain	38.3 (36.2-40.59)	41(38.9-43.7)	653 (617-689)	(Baeza et al., 1992)
Spain	8-310	5-258	31-2040	(Quindos et al., 1994)
Turkey (Küçük Menderes Basin)	36.2±2 (13±2-58±2)	32±1 (12±1-74±1)	615.44±7 (72±7-1119±7)	This study
World	17-60	11-64	140-850	(UNSCEAR, 2000a,b)

The measured highest activity concentration of ^{226}Ra is 58±2 Bq kg^{-1} on the soil surface. As is seen in Figure 3, ^{226}Ra concentrations rise towards the east of basin. Also, the distribution map shows a spike in ^{232}Th radionuclides northeast (Kiraz district) and southwest (Selçuk district) of the basin at a maximum value worth 74±3 Bq kg^{-1} (Figure 4). In soil, ^{40}K distribution is homogeneous throughout the basin (Figure 5). The highest activity concentration of ^{40}K we found was 1119±11 Bq kg^{-1} in the Kiraz district.

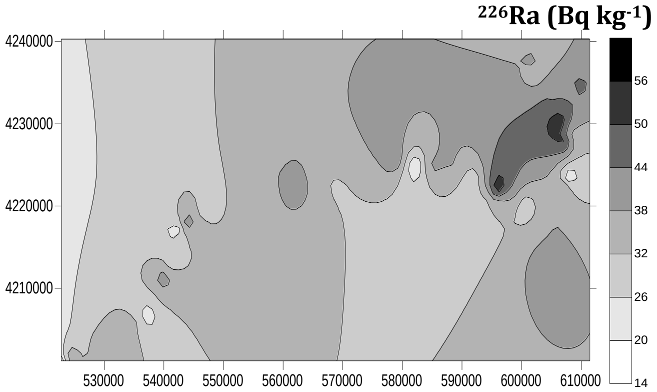

Figure 3. Distribution of ^{226}Ra concentration in soil (UTM)

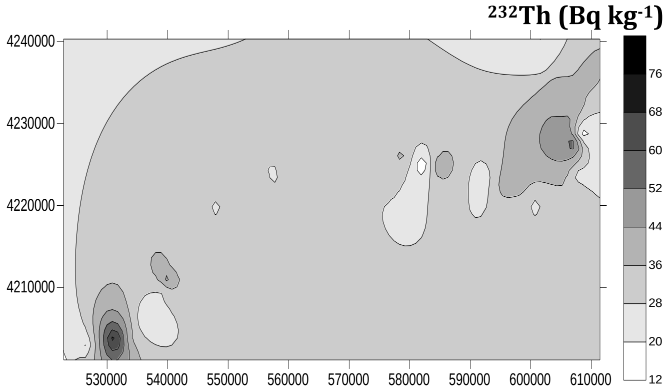

Figure 4. Distribution of ^{232}Th concentration in soil (UTM)

Figure 6 shows us that mean terrestrial gamma dose not exceeded UNSCEAR (2000a,b) standards [60 (20-200) nGy h^{-1}]. As one can see in Table 2, radionuclide concentrations in our corn samples are very low compared to grain crops. Potassium (K) is a "quality element" in crop production. A lack of K can disrupt enzyme system functions, photosynthesis, respiration, growth, and translocation. Potassium fertilization likewise affects corn grain quality (Usherwood, 1985). Generally speaking, radioelement concentrations (excluding ^{40}K) in grain crops are low at best. ^{40}K isotope is very for plant nutrition. The transfer factor of ^{40}K is very small (~10^{-4}) from soil to grain crops (Yaprak et al., 1998). ^{226}Ra, ^{232}Th and ^{40}K concentrations in grain crops varies from country to country (Table 2). ^{40}K activity concentration usually exceeds other radionuclides (^{226}Ra and ^{232}Th). We discovered that corn samples hailing from Izmir's Tire and Ödemiş districts contained the highest quantity of ^{40}K, whist those from the Torbalı district had the least (Figure 7).

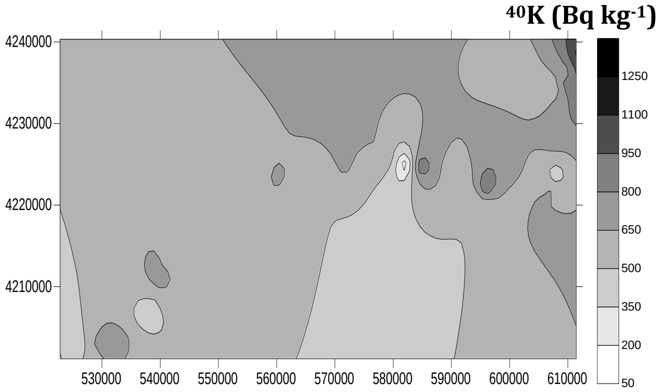

Figure 5. Distribution of ^{40}K concentration in soil (UTM)

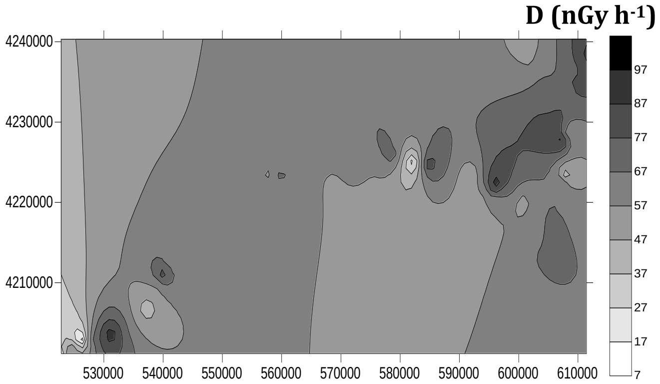

Figure 6. Terrestrial gamma dose rate (nGY h^{-1}) in soil (UTM)

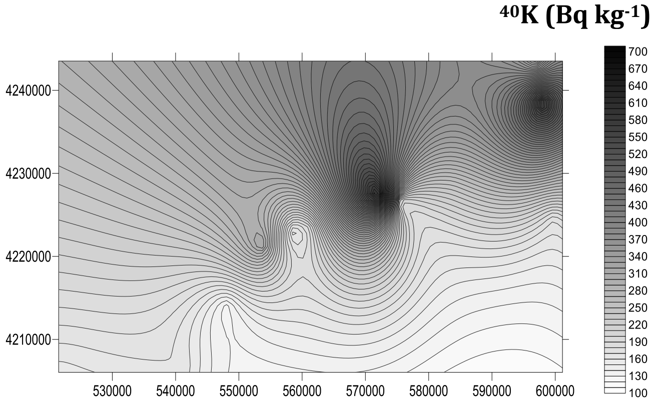

Figure 7. Distribution of ^{40}K concentration in corn samples (UTM)

Table 2. Radionuclide activity concentrations in grain crops in some countries

Plant	**Radionuclide Activity Concentrations (Bq kg^{-1})**			**Area**	**References**
	^{226}Ra	**^{232}Th**	**^{40}K**		
			Grain Crops		
Bean	0.748	-	-	Pernambuco, Brazil	(dos Santos Amaral et al., 2005)
	0.6	12.8	110.5	Upper Egypt	(Abbady et al., 2005)
Soy	8.3	ND	546.8	Jos Plateau, Nigeria	(Jibiri et al., 2007)
	≤4.3	-	745	Parana State, Brazil	(Scheibel and Appoloni, 2007)
Corn	0.13	-	-	Pernambuco, Brazil	(dos Santos Amaral et al., 2005)
	34.1	ND	243.2	Jos Plateau, Nigeria	(Jibiri et al., 2007)
	25.82	ND	491.62	Gediz Basin, Turkey	(Bolca et al., 2007)
	ND	ND	310.7±8 (136±8-712±7)	Küçük Menderes Basin, Turkey	This study
Sesame	2.5	11.5	125.5	Upper Egypt	(Abbady et al., 2005)
Wheat	3.4	9.7	104.8	Upper Egypt	(Abbady et al., 2005)
	0.04–0.37	0.015–0.11	111.3–245.7	India	(Yadav et al., 2017)
Rice	0.08	-	-	Taiwan	(Kuo et al., 1997)
Lentil	2.1	16.1	176.1	Upper Egypt	(Abbady et al., 2005)

When we compare radionuclide concentrations from other countries and regions of Turkey, we see that (Table 2), ^{40}K concentrations in the corn crops can reach as high as 243.20 Bq kg^{-1} (in Nigeria) and 491.62 Bq kg^{-1} (in the Gediz Basin, Turkey). In Izmir, the ^{40}K activity concentration value has been calculated as 310.7 Bq kg^{-1} (Table 2). ^{226}Ra concentrations in corn range from 0.13 Bq kg^{-1} in Brazil, to 25.82 Bq kg^{-1} in the Gediz Basin (Turkey) and 34.1 Bq kg^{-1} in Nigeria (Table 2). ^{232}Th concentrations in corn in Serbia is <0.2 Bq kg^{-1} – this is very low for a basin area. UNSCEAR (2000a,b) reference values in grain products are 80 Bq kg^{-1} (^{226}Ra), 20 Bq kg^{-1} (^{238}U), and 1 Bq kg^{-1} (^{232}Th). Our values (ND for ^{238}U and ^{232}Th) are below UNSCEAR (2000a,b)'s standards. ^{40}K activity concentration in this study (in Izmir) is lower than of other countries. However, the activity concentration found in the Gediz Basin is higher than literature data. Likewise, researchers have that ^{40}K in lentil, wheat, sesame, and bean crops are low, whilst in soy it is high. In India, ^{232}Th concentrations in wheat were found to be particularly low (Table 2).

In this study, we found that the TF of ^{232}Th and ^{226}Ra from soil to corn were almost zero. In contrast, the TF of radionuclides in most grain crops is ~10^{-4}, with the exception of ^{40}K (Yaprak et al., 1998). Our findings reveal that the mean TF of ^{40}K was 0.504 According to IAEA Report TRS 472 (2011), the transfer factor of K in grain cereals is 0.74. The TF of Ra, U and Th in grain maize are 2.4 10^{-3}, 1.5 10^{-2} and 6.4 10^{-5}, respectively. Alharbi and A. El-Taher (2013) discovered that of TF of ^{40}K from soil to alfalfa, wheat grains, and palm dates was 0.094, 0.16, and 0.22. They also also discovered that mean TF of ^{226}Ra from soil the same three items was 0.14, 0.12, and 0.12, respectively. Researchers in north-western Saudi Arabia obtained the soil-to-plant transfer factors of ^{226}Ra, ^{234}U and ^{238}U for crop plants in the range 0.07 ± 0.01 to 0.71 ± 0.15, 0.12 ± 0.02 to 0.44 ± 0.10, and 0.11 ± 0.02 to 0.40 ± 0.08 (Al-Hamarneh et al., 2016). Vandenhove et al., (2009) investigated TF of U, Th, ^{226}Ra, ^{210}Po, ^{210}Pb for maize respectively. Their findings: 0.121, 8.45 10^{-4}, 0.01, 1.68 10^{-3} and 2.42 10^{-4}. Spanish researchers found the transfer factors (TF) for ^{238}U, ^{234}U, ^{232}Th, ^{230}Th, ^{228}Th, and ^{226}Ra in grass samples taken from a region in south-western Spain were: 0.067, 0.072, 0.058, 0.056, 1.6, and 0.17 (Tome et al., 2003). Our TF values are similar to the literature.

In Table 3, one can see that heavy metal concentrations in our soil samples are very low relative to the Earth's crust. The same holds true when we compare them with EU Commission standards. We compared our values with those of other parts of the world (Table 3), namely: Kolkata (a disposal area), Vientiane (which receives > 300 tons of waste daily), Paramillo Massif (affected by mining areas upstream and inundated during seasonal floods), Peloponnese (that maintained uncontrolled application rates of fertilizers and pesticides–fungicides), South-west Nigeria (around a mega cement factory), Dhaka (around the Dhaka Export Processing Zone (DEPZ)), Gilgit (surrounded by volcanic rocks). All of the above sites exhibited higher levels of heavy metals than Küçük Menderes Basin (Table 3).

Table 3. Heavy metal concentration (mg kg^{-1}) in soil in some countries

Cd	Cr	Cu	Hg	Ni	Pb	Zn	Area	References
0.238	31.02	20.89	0.126	9.95	53.44	79.87	Jiedong District, China	(Jiang et al., 2020)
-	309	379	7	-	378	844	Kolkata, India	(Mukhopadhyay et al., 2020)
3.73	48.08	54.06	-	19.94	67.99	52.48	Vientiane, Laos	(Vongdala et al., 2019)
-	-	784.9	-	-	82.4	166.5	Kajaran, Armenia	(Tepanosyan et al., 2018)
0.008	-	118.1	0.028	14.1	0.012	107	Paramillo Massif, Colombia	Marrugo-Negrete et al., 2017)
0.09	21.72	13.01	0.004	18.36	15.3	-	EU countries	(Tóth et al., 2016)
1.48	-	17.18	-	88.7	28.9	34.94	Çanakkale, Turkey	(Sungur et al., 2014)
0.54	83.12	74.68	-	146.8	19.74	74.88	Peloponnese, Greece	(Kelepertzis, 2014)
547.9	156.6	613.4	-	-	666.1	188.5	Southwest Nigeria	(Ogunkunle and Fatoba, 2013)
0.0072	49.66	60.0	486.6	48.1	27.6	209	Dhaka, Bangladesh	(Rahman et al. 2012)
0.3-2.3	-	55-147	-	24-57	29-138	137-1194	Gilgit,Pakistan	(Khan et al., 2010)
1.5	-	100	-	70	100	200	EU Commission standard	(EU, 2000)
0.5	200	100	-	80	16	50	Earth crust	(Camelo et al., 1997)
0.096	40.26	26.51	158.28*	32.24	7.05	72.43	Küçük Menderes Basin, Turkey	This study

*µg kg^{-1}

As one can see in Figures 8, 9, and 10, we found Cd (0.21 mg kg^{-1}), Cr (69.80 mg kg^{-1}) and Cu (58.01 mg kg^{-1}) concentrations reaching maximum value in the districts of Kiraz and Tire. We also found Ni (53.40 mg kg^{-1}) and Pb (12.57 mg kg^{-1}) at the center of Tire (Figure 12-13). Figures 11 through 14 shows us the highest amounts Hg (920 µg kg^{-1}) and Zn (106.9 mg kg^{-1}) are concentrated in the districts of Belevi and Selçuk. Heavy metal concentrations commonly are observed in settlements and industrial areas, but minimum concentrations of heavy metals are determined from riverhead to Kiraz district.

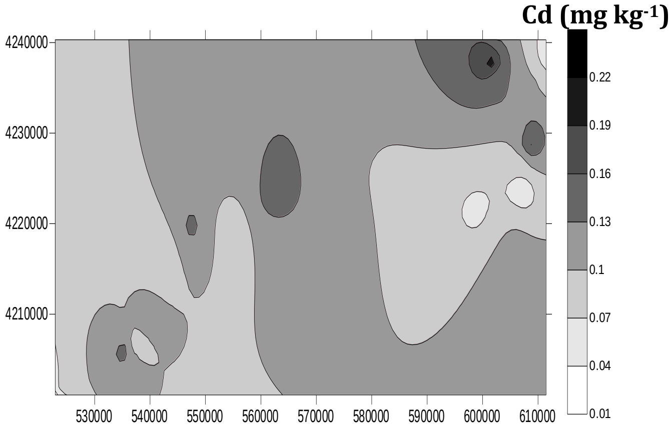

Figure 8. Concentration of Cd (mg kg^{-1}) in soil (UTM)

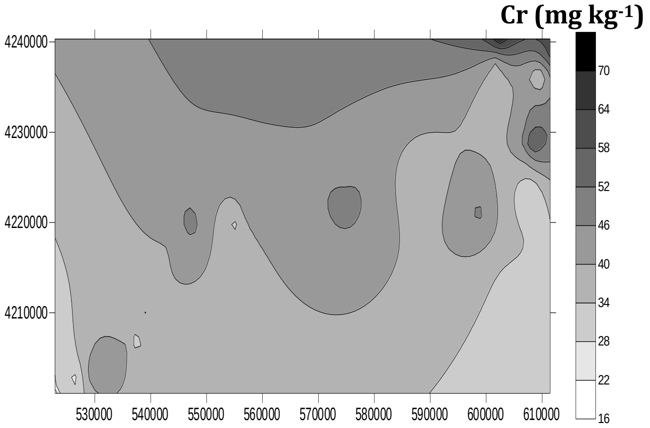

Figure 9. Concentration of Cr (mg kg^{-1}) in soil (UTM)

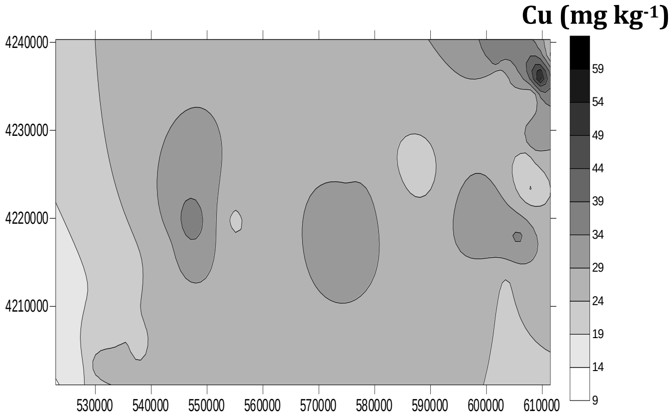

Figure 10. Concentration of Cu (mg kg^{-1}) in soil (UTM)

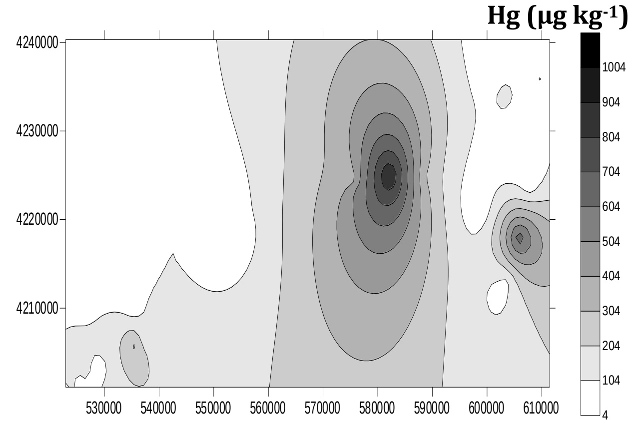

Figure 11. Concentration of Hg (µg kg^{-1}) in soil (UTM)

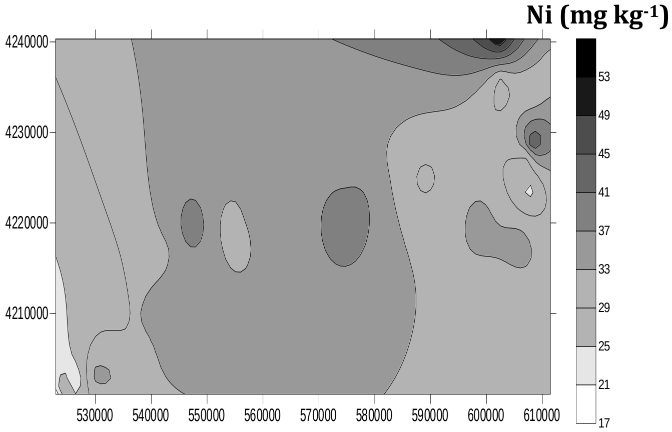

Figure 12. Concentration of Ni (mg kg^{-1}) in soil (UTM)

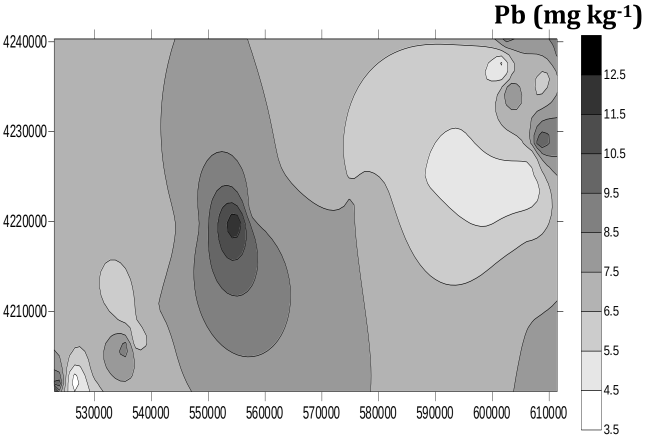

Figure 13. Concentration of Pb (mg kg^{-1}) in soil (UTM)

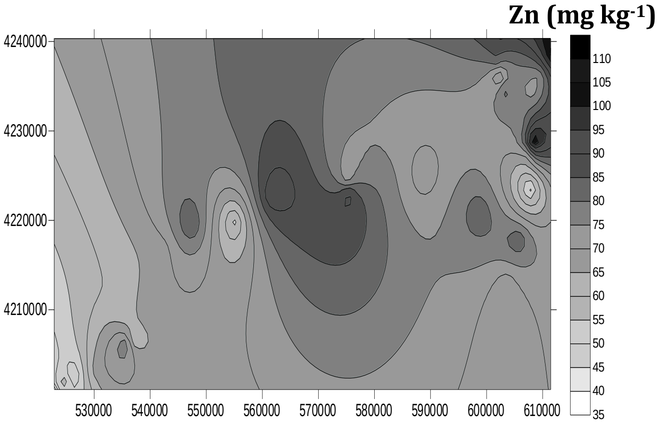

Figure 14. Concentration of Zn (mg kg^{-1}) in soil (UTM)

According to Table 4, we see that Cd, Cr, Cu, Pb and Zn concentrations in corn samples from Serbia are lower than ours, where as their Hg and Ni concentrations are higher than ours (Table 4). Cd, Cu, Pb and Zn concentrations in corn samples from China as well as Cu, Ni and Pb concentrations in corn samples from Argentina are also vary compared to what they are in our findings (Table 4). According to Turkish Ministry of Environment and Forestry, the limit of heavy metals (Pb, Cd, Cr, Cu, Ni, Zn, Hg) are respectively 50.00, 1.00, 100.00, 50.00, 30.00, 150.00, 1.00 mg kg^{-1} (Çevre ve Orman Bakanlığı, 2005). Our heavy metal values in the soil samples are below the limits of Turkish Ministry of Environment and Forestry.

Our findings demonstrate that the highest amount of Cd, Ni, and Zn in our corn samples hailed from the districts of Ödemiş and Kiraz – 0.02 mg kg^{-1}, 0.63 mg kg^{-1}, and 40.6 mg kg^{-1}, respectively (Figure 15, 19, 21). We observed that those corn samples from the districts of Selçuk and Torbalı likewise had the highest concentrations of Cr (1.3 mg kg^{-1}) and Cu (6.44 mg kg^{-1}) among the rest of the samples (Figure 16, 17). Hg concentrations reached 30 µg kg^{-1} in corn from Bayındır district (Figure 18). Pb concentrations reached 0.63 mg kg^{-1} in corn from Ödemiş district (Figure 20). According to Turkish Food Codex Regulation on Contaminations (Gıda, Tarım ve Hayvancılık Bakanlığı, 2012), the permitted limit of Pb level in corn (wet weight) is 0.10 mg kg^{-1} and, of Cd level in grain crops (except rice) is 0.10 mg kg^{-1}. Both Pb and Cd concentrations of corn samples are within the limits of Turkish Food Codex Regulation on Contaminations.

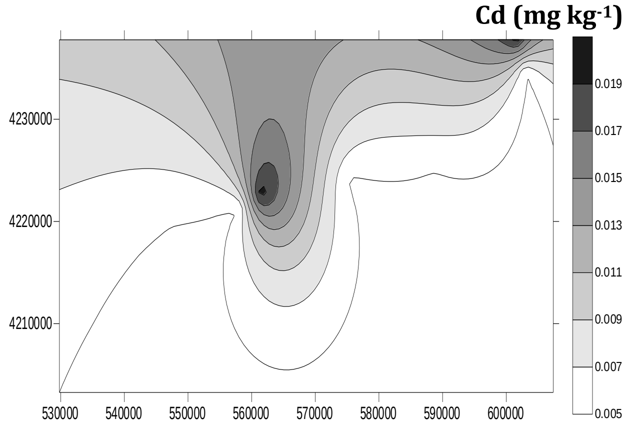

Figure 15. Concentration of Cd (mg kg^{-1}) in corn (UTM)

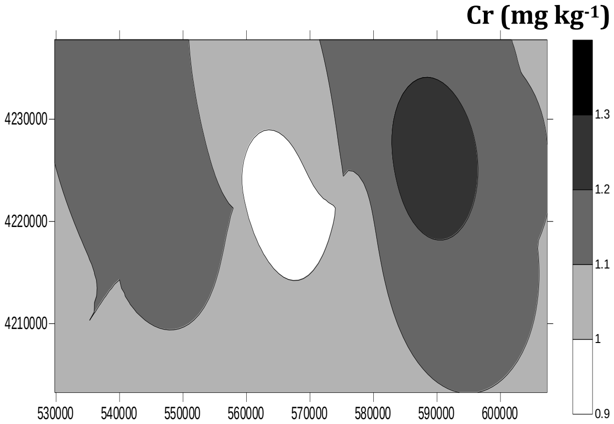

Figure 16. Concentration of Cr (mg kg^{-1}) in corn (UTM)

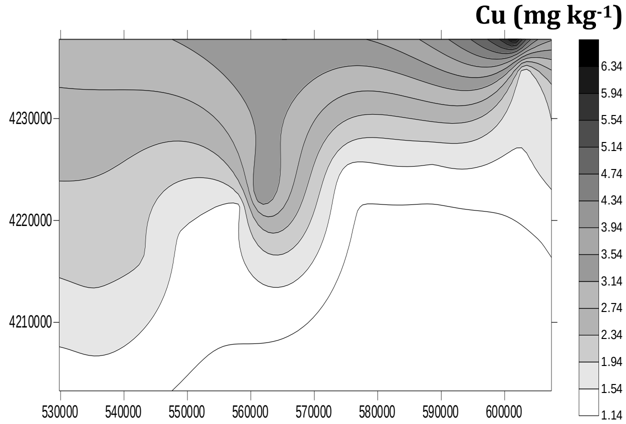

Figure 17. Concentration of Cu (mg kg-1) in corn (UTM)

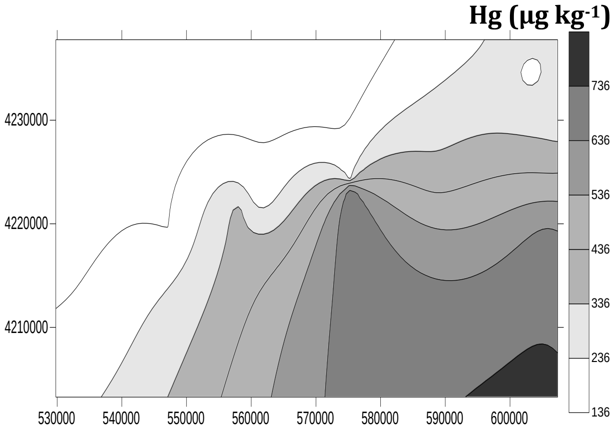

Figure 18. Concentration of Hg (μg kg^{-1}) in corn (UTM)

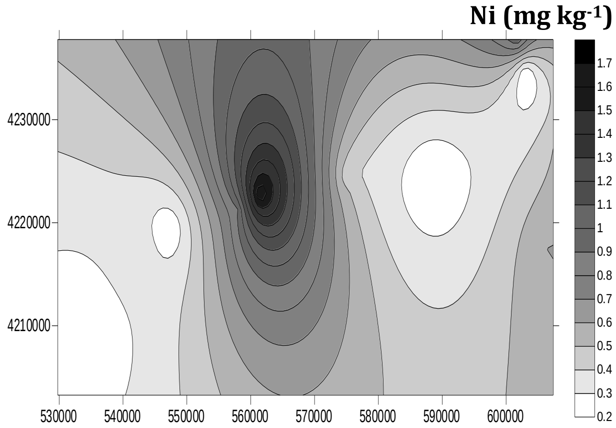

Figure 19. Concentration of Ni (mg kg^{-1}) in corn (UTM)

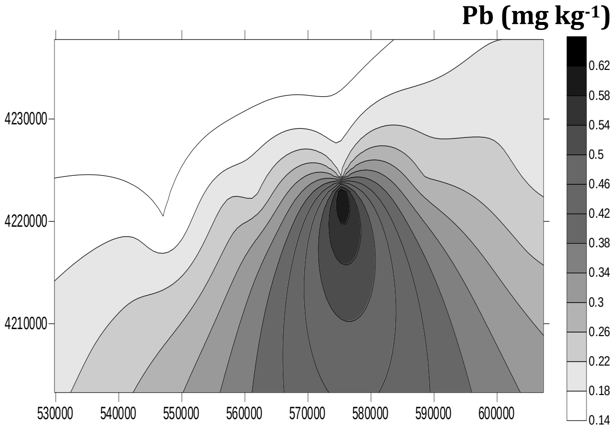

Figure 20. Concentration of Pb (mg kg-1) in corn (UTM)

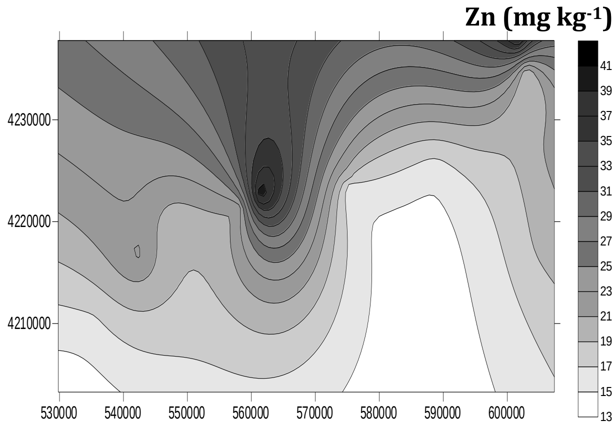

Figure 21. Concentration of Zn (mg kg^{-1}) in corn (UTM)

Table 4. Heavy metal concentration in corn crops in some countries

Heavy Metal Concentrations (mg kg^{-1})								
Cd	**Cr**	**Cu**	**Hg**	**Ni**	**Pb**	**Zn**	**Area**	**References**
<0.05	1.23	10.30	-	0.87	0.80	19.09	Pampas, Argentina	(Lavado et al., 2001)
0.03	-	6.71	-	-	0.29	51.57	Hunan,China	(Liu et al., 2005)
<0.10	0.60	1.80	0.02	0.80	1.60	20.00	Serbia	(Jakovljevic et al., 1997)
0.01	1.09	2.05	12.15*	0.54	0.24	22.00	Küçük Menderes Basin, Turkey	This study

*µg kg^{-1}

BCF in Pb, Cd, Cr, Cu, Ni, Zn and Hg was found in corn samples at the following means, worth: 0.035, 0.105, 0.026, 0.077, 0.016, 0.296 and 0.086, respectively. The IAEA Report TRS 472 (2011) indicates that the transfer factor in grain cereals for Ni and Cr are 2.7 10^{-2} and 2.0 10^{-4}. It also indicates that transfer factor of grain maize for Cd, Pb and Zn are 0.05, 1.2 10^{-3} and 0.58. Researchers found TF values in Cd, Zn, Pb and Cu for vegetables from Nanning, Southern China to be (0.001-1.83), (0.021-0.507), (0-0.031), (0.017-0.35) (Cui et al., 2004). South Korean has researchers investigated soil to corn BCF discovered figures worth 0.51 (As), 0.11 (Cd), and 2.54 (Pb) (Kim et al., 2012). Tome et al. (2003) found BCF values for Al, Cr, Cu, Fe, K, Mn, and Zn in plants worth 0.055, 0.03, 0.68, 0.088, 0.42, 1.4, and 1.1. BCF in various plants. Our BCF data is similar to Cui et al. (2004)'s and lower than Tome et al. (2003)'s findings.

Conclusion

In this study concentrations of radioactivity and heavy metal were calculated for soil and corn samples collected from the Küçük Menderes Basin, Izmir, Turkey. Results have shown that our corn samples contain low radionuclide concentrations compared with literature and national limits. It seems that radionuclide especially ^{40}K, and heavy metal content of the soil and corn samples stems from phosphatic fertilizers used by farmers around the basin, as most of it is agricultural land (there is little industrial or household waste). Despite this, corn cultivation poses very little radionuclide or heavy metal risk to the basin. Such levels may offer reliable agro-businesses a point of reference. This study can tell us agricultural products of the basin are reliable for people.

Radioactivity research on soil-plant interactions also carries remarkable importance. Researchers need to conduct radiological monitoring alongside chemical, biological, and ecological soil analysis. The same goes for plant nutrition and health, as well as for fertilizer application. Researchers should also study and monitor more than one agricultural area for radioactivity levels by observing terrestrial radionuclides and analyzing TENORM data. Globally speaking, natural radionuclide content in the soil does not receive any external contributions. Then, regionally speaking, industrial activity does enrich local values. Uncontrolled industrial facilities and activates in Turkey are causing a rise in soil – and thus plant – pollution. Ecological pollution caused by terrestrial radionuclides and heavy elements must be monitored on a regular basis. In short, the findings in this case study could serve researchers and agriculturalists alike as a potential database.

Acknowledgements

This work was partially funded by the Ege University Scientific Research Projects Committee (Project No: 07-NBE-007).

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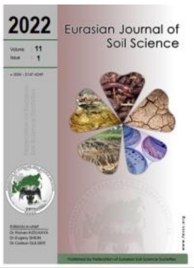
Eurasian Journal of Soil Science 2022, 11(4), 316 - 321

Eurasian Journal of Soil Science

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



Bioremediation of HCB-contaminated soil using *Comamonas testosteroni* and *Zea mays* L.

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Article Info

Received : 22.11.2021
Accepted : 13.05.2022
Available online : 01.06.2022

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Abstract

Bioremediation measures to restore soil ecosystems are environmentally safe, promising and relevant. Soil ecosystems contaminated with hexachlorobenzene require remediation measures. Studying the effectiveness of applying the microbial remediator *Comamonas testosteroni* UCM B-400, phytoremediator *Zea mays* L. cultivar Olena and microbial and phytoremediation complex to remove hexachlorobenzene contamination was carried out. The HCB content was determined by chromatographic method, the microbial groups reactions to application of various remediators in the soil were studied by classical microbiological methods. The results showed that the most effective is the complex using remediators *Comamonas testosteroni* UCM B-400 and *Zea mays* L. cultivar Olena, where HCB content was reduced to 82%.

Keywords: Bioremediation, phytoremediator, bacterial strain-destructor, hexachlorobenzene.

Introduction

Natural soil ecosystems and agrocenoses are constantly exposed to high pesticides loading, and the pesticide amount that has already accumulated requires measures to remove them from the soil. Hexachlorobenzene (HCB) is pesticide as to be removed, that is included in the list of POPs (persistent organic pollutants) prohibited for use by the Stockholm Convention (2019). The qualitative and quantitative composition of microbial representatives of ecologicy-functional groups are changes under pesticide loading conditions in soil microbiocenoses. Stressful conditions stimulate the activation of biochemical potential in microorganisms to metabolize toxic substances. Therefore, the introducing microorganisms into agrocenoses is one of the important feature of agricultural pesticides biodegradation (Jaiswal et al., 2017).

Bioremediation is a complex of processes for the recovering contaminated ecosystems, included the removal of pollutants from ecotopes, based on the physiological and biochemical activity of living organisms such as plants and microorganisms to be applicate as remediators (Jaiswal et al., 2017; Gupta et al., 2020). Bioremediation methods of soil restoration are environmentally friendly, cost-effective and efficient. Therefore, more and more research is devoted to the biological restoration of soil ecosystems. The most common bioremediation methods are microbial remediation, namely, bioaugmentation (introducing potentially capable microorganisms to destroy the target xenobiotics) (Ghosal et al., 2016), as well as phytoremediation, including rhizoremediation - the process of removing toxic substances through the microbial metabolic activity in the plant rhizosphere (Oberai and Khanna, 2018; Dubchak Bondar, 2019).

Using the potentially capable plants to remediate soils by accumulating toxic products in the phytomass is also a promising bioremediation way, but in the presence of large amounts of pesticides and other toxic substances, plant development is limited, which may reduce the phytoremediation effectiveness (Arslan et al., 2017). The same phytoremediant is noted can be absorb different amounts of toxic substances, depending on their

: https://doi.org/10.18393/ejss.1125125
: http://ejss.fesss.org/10.18393/ejss.1125125
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

composition and concentration in the soil (Chigbo and Batty, 2013). It is known that plants can absorb only a certain toxicant amount (Khoudi et al., 2013). However, the plant provides the rhizosphere microbiota with useful substances, as helping to survive and increase the tolerant microorganism number that have mechanisms to reduce the content of toxic substances (Hong et al., 2015).

Given the benefits of microbial remediation and phytoremediation, researchers' attention is focused on studying the combined using plants and bacteria to improve the remediation of soils contaminated with organic pollutants, including POPs (Becerra-Castro et al., 2013).

Consequently, developing effective bioremediation methods is important to remediate HCB contaminated areas. Therefore, the aim of this work was to study the applicating effectiveness of phytoremediator (*Zea mays* L. cultivar Olena) and the HCB destructor strain *Comamonas testosteroni* UCM B-400, as well as their integrated use to recovery the soil from HCB contamination.

Material and Methods

Field experiment design

The experiment was performed in the field on gray podzolic soil contaminated with substandard pesticides residues. Characteristics of gray podzolic soil were as follows: pH = 7.3 ± 0.20, alkaline nitrogen – 206.3 ± 20.6 mg / kg , mobile phosphorus – 99.3 ± 14.9 mg / kg, exchangeable potassium – 136.3 ± 13.6 mg / kg. The HCB content in the soil was determined at the beginning and after remediation measures. The experiment (plot size 1 m^2 , three repetition) included the following variants: control without remediators (a), growing *Zea mays L.* cultivar Olena as phytoremediator (b), application of liquid culture of *Comamonas testosteroni* UCM B-400, capable to HCB-destructing (c), complex liquid culture of *Comamonas testosteroni* UCM B-400 with phytoremediator (d). We isolated the *Comamonas testosteroni* UCM B-400 strain from the organochlorine pesticides (OCP) landfill (Kalush, Ivano-Frankivsk region, Ukraine) (Dimova et al., 2022). The culture liquid of the microorganism-destructor *Comamonas testosteroni* UCM B-400 was obtained by culturing in Menkina's liquid medium for 48-72 hours before the exponential growth phase. Menkina's liquid medium consisted following composition in g per l: $NaNO_3$ – 2, KCl – 0.6, sodium succinate – 4 (instead glucose -5), $MgSO_4$ – 0.5, K_2HPO_4 – 3. Microbial biomass after cultivating was determined colorimetrically (λ = 490 nm, cuvette thickness 3 mm. The concentration corresponded to 0.6 g per l in terms of dry biomass. The culture liquid *Comamonas testosteroni* UCM B-400 was applied to the soil immediately before sowing the seeds of phytoremediators, at the rate of 1 liter per 1 m^2. Biometric parameters of remediator *Zea mays* L. were determined at the seventh leaf stage and after full ripening.

Chemical analysis

The HCB content in the soil determaned used gas chromatograph Agilent 6890 N (Agilent, USA) in combination with software HP Chemstation (Agilent, USA), two microelectron capture detectors, two injectors with distribution and without flow distribution (Split / Splitless) autosampler on 100 samples, with synchronous in enter samples without flow distribution simultaneously (EPA, 1993; Levchuk et al., 2008). HCB analysis was performed using an HP-5 column (length 30 mm, inner diameter 0.32 mm, phase thickness 0.25 μm (HP cat. № 19091J-413). To analyze the component composition of the samples, a mass-selective gas chromatograph detector was used, which makes it possible to determine the mass spectra of the components in the pollutant mixtures. For identification, the obtained spectra were compared with the position in the NIST and AMDIS data libraries [http://www.sisweb.com/software/ms/nist.htm]. The using mass spectrometry to confirm and identify substances is highly effective and is accepted in modern analytical studies as the main method (Levchuk et al., 2008).

Microbiological analysis

Rhizosphere soil samples were taken from experimental sites where different bioremediation activities were applied, as well as from the control site without remediation. The microbial quantity of the main ecology - functional groups was determined by sowing methods of soil suspension on agar nutrient media and expressed by the counting of colony-forming units (CFU) per 1 g of dry soil. For the cultivating amylolytic microorganisms starch-ammonium agar was used; ammonifying - meat-peptone agar (MPA); pedotrophic-soil agar (contains soil extract); oligonitrophilic and nitrogen-fixing bacteria – Ashby medium; phosphate-mobilizing – Menkina's agar medium with sodium phenolphthalein phosphate (Tepper et al., 2004). To characterize the direction of microbiological processes, the coefficients of pedotrophicity were calculated as the ratio of the pedotrophic microorganism number to ammonifying bacteria, and the coefficient of nitrogen immobilization and mineralization as the ratio of the amylolytic microorganism number to ammonificators.

Statistical analysis of the data was performed by GraphPad Prism 8.0.1 software using Student's t-test. All values showed as mean ± SD.

Results

As a result of the study, data were obtained on the effectiveness of the using remediators on pesticide-contaminated soils. The effectiveness of bioremediation measures was assessed by the following indicators: the difference in concentrations of HCB in soil samples at the beginning and end of the experiment, the number and ratio of ecological-functional groups of soil microbiocenosis, assessment of biochemical processes in soil by ecological-trophic coefficients. The HCB content in the soil after remediation measures in all variants with remediators decreased compared to the initial content. Thus, due to the using bioremediation complex based on *Zea mays* L. cultivar Olena remediator and liquid culture of *C. testosteroni* UCM B-400, the greatest reduction in HCB content was 82%. When liquid monoculture of *C. testosteroni* UCM B-400 was introduced the HCB content decreased by 70%. The smallest effect was obtained with the use of the phytoremediator, which resulted in a decrease of 27.3%. The HCB content in the soil without remediators changed at the level of statistical error (Table 1).

Table 1. The HCB contain in soil under different bioremediation methods application

Bioremediation methods	HCB contain, µg per kg of dry soil		% decomposition from the initial level
	Initial level	Final level	
Without remediators	$1854.0 \pm 7.66^{****}$	$1804.0 \pm 10.33^{****}$	3
C. testosteroni UCM B-400 and Zea mays cultivar Olena	$1854.0 \pm 7.66^{****}$	$516.4 \pm 1.03^{****}$	82
Zea mays cultivar Olena	$1854.0 \pm 7.66^{****}$	$1237.4 \pm 0{,}77^{****}$	27,3
C. testosteroni UCM B-400	$1854.0 \pm 7.66^{****}$	$555.2 \pm 0.65^{****}$	70

****Correlation is significant at the 0.0001 level; ± the standard deviation (SD)

HCB presence in the plant mass confirmed the effectiveness of using complex "phytoremediator – microbial culture" and phytoremediator. In the phytoremediator sample from complex remediation with *C. testosteroni* UCM B-400 HCB (0.03 µg/kg) was detected, and in the sample from phytoremediator – 0.02 µg / kg. Thus, the plant is able to fully develop and accumulate more toxicant, probably due to the phytostimulant activity of *C. testosteroni* UCM B-400. However, HCB was not detected in grain samples. Thus, our results showed that bacteria play a key role in the metabolising toxic substances, but a certain contribution to this process are made by plant-remediators. Promising for the using complex remediation are on the one hand the bacterial culture is able to use HCB as a source of energy and carbon, and on the other hand – phytoremediator provides stability of biophysical processes during the period of detoxicating target substances, as well as root secretions stimulate the development of rhizosphere microbiota. Microorganisms that perform various functions to maintain the stability of soil ecosystems is played the leading role in ensuring high soil quality. We defined the development of the main ecological-trophic and taxonomic groups of soil microbiocenoses (Table 2).

Table 2. The microbial quantity in the soil under different bioremediation methods, CFU per 1 g of dry soil

Microbial ecology-functional and taxonomic group	Bioremediation methods			
	Without remediators (control)	*C. testosteroni* UCM B-400 and *Zea mays* cultivar Olena	*Zea mays* cultivar Olena	*C. testosteroni* UCM B-400
Pedotrophics	$1.59 \pm 0.11 \times 10^{8}$	$2.52 \pm 0.09 \times 10^{8***}$	$2.32 \pm 0.03 \times 10^{8***}$	$2.45 \pm 0.01 \times 10^{8***}$
Nitrogen-fixing and oligonitrophilic bacteria	$2.12 \pm 0.09 \times 10^{7}$	$3.20 \pm 0.21 \times 10^{7**}$	$3.07 \pm 0.23 \times 10^{7**}$	$2.45 \pm 0.14 \times 10^{7*}$
Amylolytics	$1.51 \pm 0.21 \times 10^{8}$	$1.94 \pm 0.40 \times 10^{8***}$	$1.56 \pm 0.01 \times 10^{8*}$	$1.90 \pm 0.08 \times 10^{7**}$
Ammonifying	$8.88 \pm 0.16 \times 10^{7}$	$1.03 \pm 0.48 \times 10^{8**}$	$1.03 \pm 0.15 \times 10^{8***}$	$1.05 \pm 0.15 \times 10^{8***}$
Phosphate - mobilizing	$7.12 \pm 0.18 \times 10^{6}$	$8.56 \pm 0.54 \times 10^{6*}$	$9.12 \pm 0.30 \times 10^{6***}$	$8.85 \pm 0.42 \times 10^{6**}$
Streptomycetes	$6.08 \pm 0.30 \times 10^{6}$	$7.56 \pm 0.48 \times 10^{6*}$	$8.10 \pm 0.36 \times 10^{6**}$	$9.76 \pm 0.54 \times 10^{6***}$
Micromycetes	$1.52 \pm 0.18 \times 10^{4}$	$2.12 \pm 0.30 \times 10^{4*}$	$2.18 \pm 0.32 \times 10^{4***}$	$1.90 \pm 0.12 \times 10^{4*}$

***Correlation is significant at the 0.001 level; ** Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level; ± the standard deviation (SD)

The number of pedotrophic bacteria, as played an important role in the formation of soil fertility, increased in all experimental variants compared to the variant without remediation. It should be noted that in variants with introduced liquid culture of strain *C. testosteroni* UCM B-400, or using it in combination with phytoremediator, the increase of the number of pedotrophic bacteria reached 30%, which indirectly suggests that the bacterial metabolic activity was associated with decreasing the concentration of toxic substances in the soil (Table 2). The quantity of amylolytic bacteria that perform the function of plant residues transformation was higher in all remediation variants, compared to the control without remediators. Thus, in the variant with mays Olena the number of this group increased to 15%, with introducing *C. testosteroni* UCM B-400 increased to 21%. The largest increase (49.7%) in the amylolytic bacteria amount was observed under complex remediation *C. testosteroni* UCM B-400 and mays Olena. The largest increasing the number of nitrogen-fixing and oligonitrophilic bacteria was under integrated using phytoremediant mays Olena and the culture liquid of *C. testosteroni* UCM B-400, almost 50% compared to the control without remediators. By separately application mays Olena and bacterial liquid of *C. testosteroni* UCM B-400, the number increased by 20 and 16%, respectively. Number of ammonifying bacteria as transformating organic nitrogen-containing compounds under applicating remediators increased significantly. Streptomycetes, which play an important role in the formation of productive microbial-plant systems and increase soil suppression to phytopathogens, have also increased in quantity. Under the phytoremediator applicating its number increased to 62%, under augmentation of *C. testosteroni* UCM B-400 and in the case of complex "phytoremediant and microbial culture of *C. testosteroni* B-400" – to 34 and 27.5%, respectively. Micromycetes play an important role in the soil fertility formation and prohumus compounds synthesis. According to the results of the study, micromycetes also showed an increase in the number from 16 to 24%. Thus, the quantitative and qualitative composition of the soil microbiocenosis changed as a resulted bioremediation measures. In all remediated variants, the number of studied groups of microorganisms was increased. Therefore, the practical applicating bioremediators will eventually lead to the restoration of soil fertility in polluted soil agrocenoses. Coefficients of nitrogen mineralization and pedotrophicity are indicators of soil quality and fertility, which are determined by the ratio of the specific microbial group number. It reflect to some extent the microbiological processes direction. Changes in the pedotrophic and mineralization of nitrogen indices under the HCB action were revealed compared to the control (Table 3).

Table 3. Coefficients of pedotrophic and nitrogen immobilization-mineralization in different remediation variants

Variant	Coefficient of pedotrophic	Nitrogen mineralization-immobilization coefficient
Without remediators	1.80 ± 0.15	1.70 ± 0.02
C. testosteroni UCM B-400 and mays cultivar Olena	2.45 ± 0.03**	1.89 ± 0.07*
C. testosteroni UCM B-400	2.33 ± 0.02**	1.80 ± 0.05*
Zea mays cultivar Olena	2.25 ± 0.06**	1.51 ± 0.02***

***Correlation is significant at the 0.001 level; ** Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level; ± the standard deviation (SD)

Calculated coefficients showed that the soil organic matter transformation processes after remediation were stabilized. In the control soil without remediators, the lowest pedotrophic coefficient (1.73) was noted compared to the experimental variants. It's indicated the inhibiting the processes of water-soluble humus fractions transformation. Mineralization indices after remediation measures in all variants did not exceed 2,0, as confirmed the balance of immobilization-mineralization processes. Only in the variant without remediation, this was slightly increased (2.13), that's emphasizes the imbalance in the nitrogen regime, due to the pesticides loading. Biometric parameters of plant-remediators demonstrated effectiveness of bioremediation measures. At the beginning growing season in the 6-7 leaves stage the height of the *Zea mays L.* plants growing under complex remediation with the *Comamonas testosteroni* UCM B-400 culture liquid was higher by 5% compared to the plant from phytoremediator. However, the plant mass with the root and the root system mass were greater to 46.5 and 33.6%, respectively, compared to the plants from phytoremediation variant (Table 4). The obtained results indicate the phytostimulating effect of the culture liquid *Comamonas testosteroni* UCM B-400. After full maturation, the plant-remediators were again selected, biometric indicators confirmed the presence of a stimulating effect on the growth and development of plant-remediators. The plant height under applicating *Comamonas testosteroni* UCM B-400 culture liquid was statistically significantly superior to the plant without treatment. The weight of plant cobs per 1 m^2 without treatment was lower by 9% compared to the variant applicated the *Comamonas testosteroni* UCM B-400 culture liquid. Thus, with the microbial inoculum applicating, a phytostimulating effect on plant-remediators was observed.

Table 4. Biometric indicators of *Zea mays L.* plants after full maturation

Characteristic	Phytoremediator *Zea mays L.* cultivar Olena	*C. testosteroni* UCM B-400 and *Z. mays L.* cultivar Olena
Plant heigth, cm	218.5 ± 2.65***	226.0 ± 1.58***
Mass of cobs per 1 m^2, kg	7,59 ± 0,36**	8,26 ± 0,24**

***Correlation is significant at the 0.001 level; ** Correlation is significant at the 0.01 level; ± the standard deviation (SD)

Discussion

Comamonas bacteria are known to be destructors of toxic polycyclic aromatic compounds. The effectiveness of the using *Comamonas sp.* CNB-1 isolated from activated sludge polluted with from 4-chloronitrobenzene (4-CNB) was studied. This strain was introduced into the system "plant - microbial strain-destructor 4-CNB" for bioremediating the contaminated environment. In the 8-day experiment the results of the 4-CNB degradation (100 µg per g of soil at the beginning) were compared in three variants: inoculating with *Comamonas* sp. CNB-1 strain and alfalfa, inoculation without alfalfa and alfalfa without inoculation with strain. The highest efficiency of 4-HNB degradation was in the system "alfalfa - *Comamonas* sp. CNB-1 ", where the toxicant was completely removed within 2 days. In the variant only with the strain inoculating 4-CNB degradation was lasted more than 6 days, and in the third variant (alfalfa only) removal of the pollutant after 8 days exposure was not observed. These results revealed that the *Comamonas* sp. CNB-1 played a key role in the 4-CNB degradation, and alfalfa was stimulated by bacteria (Liu et al., 2007). Similarly, our study showed that under the complex remediation the percentage of HCB degradation was the highest. It can be stated that it was due to the studied strain as the toxicant content in the soil was decreased. Since HCB and 4-CNB are derivatives of benzene compounds, the ability of our studied *C. testosteroni* UCM B-400) to destroy HCB is quite expected, as was confirmed by the our study results.

Studies concerning restoration HCB contaminated soil using a tandem of microorganisms with Reygrass, it was found which microorganisms were HCB destructors. Plant root exudates are known to affect the microbial community by providing available carbon sources. An increase in the numbers of representatives of genera *Comamonadaceae*, *Azohydromonas* and *Pseudomonas* were found, indicating as root exudates stimulated the growth of these bacteria (Yan et al., 2014; Zhang et al., 2017).

The advantage of the microbial and plant-associated bioremediation methods is that bacteria are able to metabolize HCB or other toxic substances, and plants provide bacteria with high physiological activity through the supplying nutrients contained in root exudates, as well as provide oxygen needed for bacterial metabolism, namely the biophysical processes stability depends on the plant (Singh et al., 2011). High efficiency of removing hexachlorocyclohexane isomers from the soil by means of complex bioremediation based on the association of microbial culture with leguminous *Cytisus striatus* has been reported (Becerra-Castro et al., 2013).

Plants used as phytoremediators can also grow under toxic loads, although xenobiotics can be transported to the rhizosphere through transpiration flow and inhibit root development. Therefore, a certain amount of toxicant can accumulate in the plant, which was also shown in our study. However, an important condition for plants is the colonization of the root system by bacterial cells, as a result bacteria form biofilm on the surface, which can function as a protective barrier, filtering and destroying contaminants (Liu et al., 2007). On the other hand, plant root exudates stimulate an increase in the resident microbiota and also affect their activity, which leads to a change in the ratio of functional and systematic groups in soil microbiocenoses (Jha et al., 2015). Almost 20% of all photosynthetically formed carbon as transferred to the rhizosphere through root exudates is reported to use as a carbon and energy source (Dennis et al., 2010). According to the results of our study, there was also a positive dynamics in the development of microbial functional groups in the phytoremediator rhizosphere.

Plants are able to absorb a limited amount of toxic substances. For example, this has been demonstrated in a study of the effectiveness of soil phytoremediation from heavy metals using *Arabidopsis thaliana* (Khoudi et al., 2013). The results of our studies also confirmed the above, as *Zea mays L.* plants accumulated a small HCB quantity in the variants with phytoremediator and it complex with *C. testosteroni* UCM B-400 compared to the HCB amount that was removed from the experimental plots of these variants, relative to the remediation-free. It should be noted that in the variant of complex bioremediation, the plant accumulated 50% more HCB than with phytoremediator, due to the phytostimulanting activity of *C. testosteroni* UCM B-400, as also is reflected in the biometric indicators of plant- remediators. Liquid culture of *C. testosteroni* UCM B-400 recommended to be used in soil remediation measures from hexachlorobenzene pollution both in complex with phytoremediators and in the monoculture.

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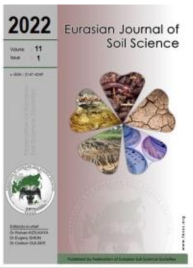
Eurasian Journal of Soil Science 2022, 11(4), 322 - 328

Eurasian Journal of Soil Science

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



Effect of foliar mineral fertilizer and plant growth regulator application on seed yield and yield components of soybean (*Glycine max*) cultivars

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Article Info

Received : 11.11.2021
Accepted : 21.06.2022
Available online : 24.06.2022

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Abstract

Soybean is known for its high protein content, which is the reason why it is widely used as one of the main food sources for humans and animals. In order to increase soybean yield, farmers tend to use foliar mineral fertilizer and plant growth regulator to this crop. Furthermore, a starter fertilizer application into the soil without foliar application may cause low yield contents of soybean. The aim of this investigation was to estimate the effects of different foliar mineral fertilizers (MF) and plant growth regulator (RGR) application on quantitative traits (plant height (PH), lower pods attachment height (LPH), number of seed pods per plant (NSPP), number of seeds per plant (NSP), weight of seeds per plant (WSP) and 1000-seed weight (TSW) and soybean grain yield (SGY)) in three soybean cultivars (Lastochka, Akku and Galina) in Shymkent of the Turkestan region, Kazakhstan. Four treatments of fertilization were tested: control (starter fertilizer, $P_{60}K_{45}$), $P_{60}K_{45}$ + Mo+B, $P_{60}K_{45}$ + Epin and $P_{60}K_{45}$ +Vuksal. Mo+ B, Epin and Vuksal were foliar applied one-two times at growth stage. The field experiments were carried out in South-Western Research Institute of Animal Husbandry and Plant Growing, during the years 2019, 2020 and 2021. In both research years, Akku had higher values for all investigated traits than Lastochka and Galina. Results showed that foliar MF and PGR application significantly increased the values for PH, LPH, NSPP, NSP, WSP, TSW and SGY. Vuksal is more effective than Epin and Mo,B in soybean cultivars because Vuksal is a liquid fertilizer that contains has higher concentration of macronutrients (16%N, 16%P_2O_5, 12%K_2O+me, w/v). Generally, cultivar Akku and treatment starter fertilizer ($P_{60}K_{45}$) + Vuksal (2,5 L/ha) may be recommended in soybean production in localities with similar agro-ecological conditions.

Keywords: Soybean cultivars, foliar application, seed yield, fertilizer.

Introduction

Soybean (Glycine max) is one of the agricultural commodities with important value to humans. Soybean is known for its high protein content, making this commodity widely utilized as a main food source for humans and husbandry as well as oil producer (Capriotti et al., 2014; Pagano and Miransari, 2016; Sandrakirana and Arifin, 2021). The lack of vegetable protein in human nutrition and livestock rations is one of the main problems of the agro-industrial complex of Kazakhstan (Beketova et al., 2017; Suleimenova et al., 2019). This problem can be solved by increasing the production of leguminous crops seeds and, first of all, soybean, which is considered as the most important protein and oil crop. Masuda and Goldsmith (2009) suggested that food

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: https://doi.org/10.18393/ejss.1135498
: http://ejss.fesss.org/10.18393/ejss.1135498

Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

production and security will be a problem in the future because of limited acreage for growing edible crops such as soybean, therefore, it is important to increase yields to meet national and global demands.

A balanced supply of macro-and micronutrients to the soybean crop is essential for achieving higher productivity, quality, and profitability. Starter fertilizers are used to increase initial soybean growth in terms of total plant biomass which will be partitioned into increased seed and oil yields in soybean at harvest (Osborne and Riedell, 2006). It requires nutrients, often the limiting factors are nutrients and in some cases they are found in the soil at a low level of what the crop needs, so foliar fertilization with nutrients is a fundamental practice to fulfill with the nutritional needs of the crop. The foliar fertilizers are used for the growth and development of the plants, the Molybdenum (Mo) is part of the nitrogenase enzyme, synthesized by bacteria during the process of biological nitrogen fixation by symbiosis, these elements increase in yield (Carlim et al., 2019) meanwhile, the Boron (B) acts in the formation of the pollen tube and it is also a nutrient that stimulates cell development, and due to inadequate fertilization and little use of foliar fertilizers the cultivation of soybean has lowered its yield. With the foliar application of B, Will et al. (2012) found an increase in yield, especially in a soil of low fertility. Indolebutryic acid, cytokinin, and gibberellic acid are classic plant hormones or growth regulators. They are chemicals that drive plant cell division, flowering, fruiting, and elongation (Zhang et al., 1997). But, Epin (24-epibrassinolide) is one of the most bioactive forms of brassinosteroids; it is extracted from plants and is biodegradable (Azhar et al., 2017). This steroid presents a broad spectrum of systemic action on plant metabolism, photochemical efficiency, antioxidant metabolism and growth rate (Abdullahi et al., 2002; Xia et al., 2009; dos Santos et al., 2020).

In order to optimize soybean growth and its yield, foliar mineral fertilizer and plant growth regulator application plays an important role to improve soybean productivity. The objective of this work is to evaluate the yield and yield components such as, plant height, lower pods attachment height, number of seed pods per plant, number of seeds per plant, weight of seeds per plant and 1000-seed weight of different soybean cultivars (Lastochka, Akku and Galina) with the foliar application of mineral fertilizer (Mo+B and Vuksal-NPK+me liquid fertilizer) and plant growth regulator (Epin, 24-epibrassinolide) in Shymkent of the Turkestan region, Southern Kazakhstan climatic condition.

Material and Methods

Soybean cultivars

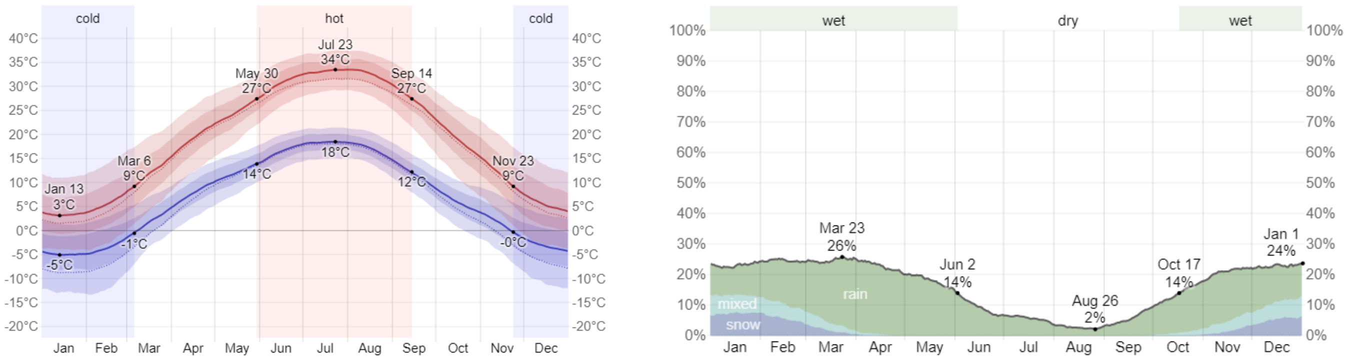
The soybean cultivars used were Lastochka, Akku and Galina. The Lastochka, Akku were artificial selected by Kazakh Research Institute of Agriculture and Plant Growing released and cultivated widely in Southern Kazakhstan, and the Galina was artificial selected by Institute of Vegetable and Field Farming, Novi Sad, Serbia and and gradually spread around this region.

Site Description

The field experiment was located on the experimental station of South-Western Research Institute of Animal Husbandry and Plant Growing, Shymkent of the Turkestan region, Southern Kazakhstan. The standard climatological long-term average precipitation and temperature was 283.3 mm and 15.3^{o}C, respectively (Figure 1). The altitude of the trial site is 650-800 m.

The hot season lasts for 3.5 months, from May 30 to September 14, with an average daily high temperature above 27°C. The hottest month of the year in Shymkent is July, with an average high of 33°C and low of 18°C. The cold season lasts for 3.5 months, from November 23 to March 6, with an average daily high temperature below 9°C. The coldest month of the year in Shymkent is January, with an average low of -5°C and high of 3°C. A wet day is one with at least 1.00 millimeters of liquid or liquid-equivalent precipitation. The chance of wet days in Shymkent varies throughout the year. The wetter season lasts 7.5 months, from October 17 to June 2, with a greater than 14% chance of a given day being a wet day. The month with the most wet days in Shymkent is March, with an average of 7.6 days with at least 1.00 millimeters of precipitation. The drier season lasts 4.5 months, from June 2 to October 17. The month with the fewest wet days in Shymkent is August, with an average of 0.8 days with at least 1.00 millimeters of precipitation. Among wet days, we distinguish between those that experience rain alone, snow alone, or a mixture of the two. The month with the most days of rain alone in Shymkent is April, with an average of 6.6 days. Based on this categorization, the most common form of precipitation throughout the year is rain alone, with a peak probability of 24% on April 6.

The soil belongs to the general soil type of dark gray soil. The pH was 7.47 (slightly alkaline reaction), soil organic matter content was 1.77% (low). NO_3-N was 50.8 mg kg^{-1}, available phosphorus was 11.4 mg kg^{-1} and exchangeable potassium was 162.1 mg kg^{-1}.



(A) (B)

Figure 1. Monthly average temperature (A) and distribution of precipitation (B) of the experimental area.

Experimental Design

The experiments were carried out in 2019, 2020 and 2021 at the experimental station of the South-Western Research Institute of Animal Husbandry and Plant Growing in 8 May 2019, 26 April 2020 and 23 April 2021. Soybeans were sown in a grain-grass rotation, the predecessor was winter wheat. Soybeans were sown in a grain-grass rotation, the predecessor was winter wheat. Soybean cultivars (Lastochka, Akku and Galina) were sown in a dotted way with a row spacing of 70 cm. Variants arrangement method - split plots, quadruple replication - 25 m^2 (each plot is 5 m long and 5 m wide, total 100 m^2 per cultivar or 400 m^2 per cultivars). To a seeding depth of 5-6 cm, with the placement of 30 seeds per 1 m^2.

The experimental designs used were completely randomized block design with five treatments and four replications. The land was disk ploughed, harrowed, and leveled with a tractor. Then ridging was done by hand. Fertilizer was applied using grain drill. The sources of fertilizers for soil application (starter fertilizer) used were single superphosphate ($Ca(H_2PO_4)_2.2H_2O$, 16%P_2O_5) and potassium sulphate (K_2SO_4, 50% K_2O). The source of mineral fertilizer and plant growth steroid for foliar application used were diammonium molybdate ($(NH_4)_2MoO_4$, 35%Mo), boric acid (H_3BO_3, 17%B), Epin (24-epibrassinolide) and Vuksal Universal (16%N, 16%P_2O_5, 12%K_2O, 1%B, 1%Zn, 8 mg Co kg^{-1}, 212 mg Cu kg^{-1}, 437 mg Fe kg^{-1}, 367 mg Mn kg^{-1}, 28 mg Mo kg^{-1}, w/v). The foliar application was applied one time at beginning of the flowering of the crop using a knapsack sprayer. 50 ml Epin/ha, 2,5 L Vuksal/ha, 20 g $(NH4)_2MoO_4$ + 25 g H_3BO_3/ha. No additional fertilizer was applied in to the soil. A combination of insecticide, herbicide and manual weed control was used to maximize the yield. Irrigation in this study was scheduled based on soil water content which was determined using a gravimetric method. All plots received the same irrigation water during the whole growth period of soybean. The entire field was irrigated by the pivot, with total irrigation amounts of 500-600 m^3/ha.

Plant measurements and sampling

The plants were harvested after 95% of the mature pods, that is, at the R8 stage. After the manual harvest, the plants were trod and evaluated individually to determine the Soybean grain yield (SGY) and yield components such as, plant height (PH), lower pods attachment height (LPH), number of seed pods per plant (NSPP), number of seeds per plant (NSP), weight of seeds per plant (WSP) and 1000-seed weight (TSW). After being harvested, the grain was dried and milled and the chemical analyses were carried out: oil and protein content and finally their respective yields.

Oil extraction was performed by the Soxhlet extractor using three samples per cultivar in each of the tests, and each sample weighing 2.5 grams of the dried and milled material. The oil yield was obtained by the product between the oil content and the grain yield (GOST 10857-64).

For the protein content, the methodology proposed by Kjeldahl was used, finding the value of the total nitrogen (N) of the sample and later, converting to crude protein by the factor 6.25 - using three samples to cultivate in each of the tests, and each sample weighing 0.5 grams of dry and ground material. The protein yield obtained through the product between the protein content and grain yield (GOST 10846-91).

Results and Discussion

Fertilizer management and application method can substantially affect yield response and producer's profitability. Starter fertilizer is a common practice to increase crop growth, stand uniformity and enhance yield potential. Some studies evaluated the effects of placement and fertilization of P and K on different crops and have shown that starter fertilizer often increase corn yield compared to a control treatment with no fertilization (Bundy et al., 2005; Randall and Hoeft, 1988). In this study, $P_{60}K_{45}$ was applied as starter fertilization in all applications and this parcel was accepted as the control parcel. No nitrogenous fertilization was made from the soil in starter fertilization. In the present research, it was determined that the three year average soybean seed yield was average, and it amounted 3.47 t/ha, whereas the highest yield was recorded for Akku cultivar (3.71 t/ha) and the lowest for Lastochka cultivar (3.16 t/ha) in the control application with only starter fertilization. The results are in agreement with findings Antony et al. (2012) and Borowska and Prusiński (2021) in soybean. Average soybean seed production is about 2.5 t/ha in all of the world (Basuchaudhuri, 2016).

The growth of soybean was influenced by its genetic factors and environment. Different foliar mineral fertilizers (MF) and plant growth regulator (PGR) application has a significant result on the Soybean grain yield (SGY) and yield components such as, plant height (PH), lower pods attachment height (LPH), number of seed pods per plant (NSPP), number of seeds per plant (NSP), weight of seeds per plant (WSP) and 1000-seed weight (TSW). Soybean yield components showed a noticeable difference according to the foliar application of MF and PGR at harvest where the role of MF and PGR application was of great importance (Table 1). Results of this study demonstrated that the foliar application of MF and PGR showed higher yield in soybean compared to treatment using only soil application (control treatment).

Table 1. Effect of MF and PGR application on plant height (PH), lower pods attachment height (LPH), number of seed pods per plant (NSPP), number of seeds per plant (NSP), weight of seeds per plant (WSP), protein and oil content of different soybean cultivars as influenced by different treatments

Cultivars	PH, cm	LPH, cm	NSPP, pics	NSP, pics	WSP, g	Protein, %	Oil,%
Control $P_{60}K_{45}$							
Lastochka	80,3	8,7	63,8	146,1	26,3	25,56	18,71
Akku	89,1	9,4	77,6	171,9	30,9	25,77	19,44
Galina	86,2	9,4	74,2	163,5	29,4	26,88	20,03
$P_{60}K_{45}$ + Mo,B							
Lastochka	82,5	9,2	64,8	148,5	26,7	32,02	23,14
Akku	94,6	9,3	77,3	177,7	32,0	32,12	21,38
Galina	88,0	9,3	73,0	167,6	30,2	37,12	21,82
$P_{60}K_{45}$ + Epin							
Lastochka	85,6	9,3	66,4	152,0	27,3	40,76	21,33
Akku	95,4	9,4	78,2	179,4	32,3	41,89	21,96
Galina	90,3	9,3	76,8	176,1	31,7	46,03	22,34
P_{60} K_{45} + Vuksal							
Lastochka	86,6	9,6	68,0	155,8	28,1	50,97	25,74
Akku	97,2	9,8	79,1	181,4	32,7	51,77	26,33
Galina	92,1	9,8	77,0	176,9	31,9	51,76	26,08

Different foliar application of MF and PGR exposed significant variation in plant height (PH) at harvest. At harvest, the tallest plant (86.6, 97.2, and 92.1 cm, respectively) was recorded from Vuksal sprayed at vegetative stage, the shortest plant (80.3, 89.1 and 86.2 cm) was observed from control treatments. In our experiment, PH also increased by the foliar application of Epin and Mo,B compared to that of Vuksal but little bit increased compared to control. Lower pods attachment height (LPH) varied significantly due to different foliar application of MF and PGR at harvest. At harvest, the highest LPH (9.8) was recorded from application of Vuksal whereas, the lowest (8.7) was recorded from control treatment. The NSPP, NSP and WSP of soybean had significant effect due to different foliar application of MF and PGR at harvest. At harvest, the maximum NSPP, NSP and WSP were recorded from Vuksal application and the minimum was obtained from control application. Similarly, different MF and PGR application showed significant variation in case of protein and oil content of soybean leaf at harvest. At harvest, the highest protein and oil content were recorded from Vuksal when applied whereas, the lowest was found from control application.

For all soybean cultivars (Lastochka, Akku and Galina), the highest PH, LPH, NSPP, NSP, WSP, protein content and oil content were determined Vuksal application whereas, cultivar Akku and Galina can promise better yield components after being treated with different foliar application of MF and PGR, but not with Lastochka at harvest. Soybean protein concentration has also been shown to be inversely correlated with oil and protein concentration and yield (Helms and Orf, 1998; Wilcox, 1998). The degree to which these correlations are related to genotypic or environmental influence is not entirely known (Wilson, 2004), but they are often associated with genotypic variation (Kravchenko and Bullock, 2002; Wilcox and Shibles, 2001). While genotype selection is an important component of crop management, considerable variation in soybean quality also exists within fields planted to single genotypes (Kravchenko and Bullock, 2002). A limited number of studies have reported effects of fertilization on soybean composition (Yin and Vyn, 2003). Reports of the relationship between within-field variation in soybean protein and oil concentration and soil chemical properties are also limited (Bellaloui et al., 2009; Anthony et al., 2012).

Yield obviously is the ultimate goal in growing any crop. Thus, the economic importance of foliar application of MF and PGR is largely depends on their ability to increase the crop yields. Different foliar application of MF and PGR had significant influence on SGY and TSW (Figure 2). The highest SGY (3.92 t ha^{-1}) was obtained from application of Vuksal the lowest (3.16 t ha^{-1}) was recorded from control treatment. At harvest, the maximum TSW (152.2 g) was obtained from Vuksal application and the minimum (143.3 g) was recorded from control application. These findings also correlate the above mentioned findings. It might be due to increased uptake of plant nutrients through effective translocation from sink to reproductive area of soybean enhanced yield attributes like PH, LPH, NSPP, NSP and WSP which finally increased the SGY and TSW. Figure 2 shows about the SGY and TSW based on the treatments. Application of Vuksal gave the SGY and TSW on Akku soybean cultivar at harvest as much as 3.92 t ha^{-1} and 152.2 g respectively. Lastochka cultivar showed lowest SGY and TSW result on control treatment as much as 3.16 t ha^{-1} and 143.3 g respectively. For cultivar Akku, the highest oil and protein yield were determined as much as 1032.1 kg ha^{-1} and 2029.4 kg ha^{-1} respectively on Vuksal application where control application of these cultivars showed the lowest oil and protein yield (Figure 3).

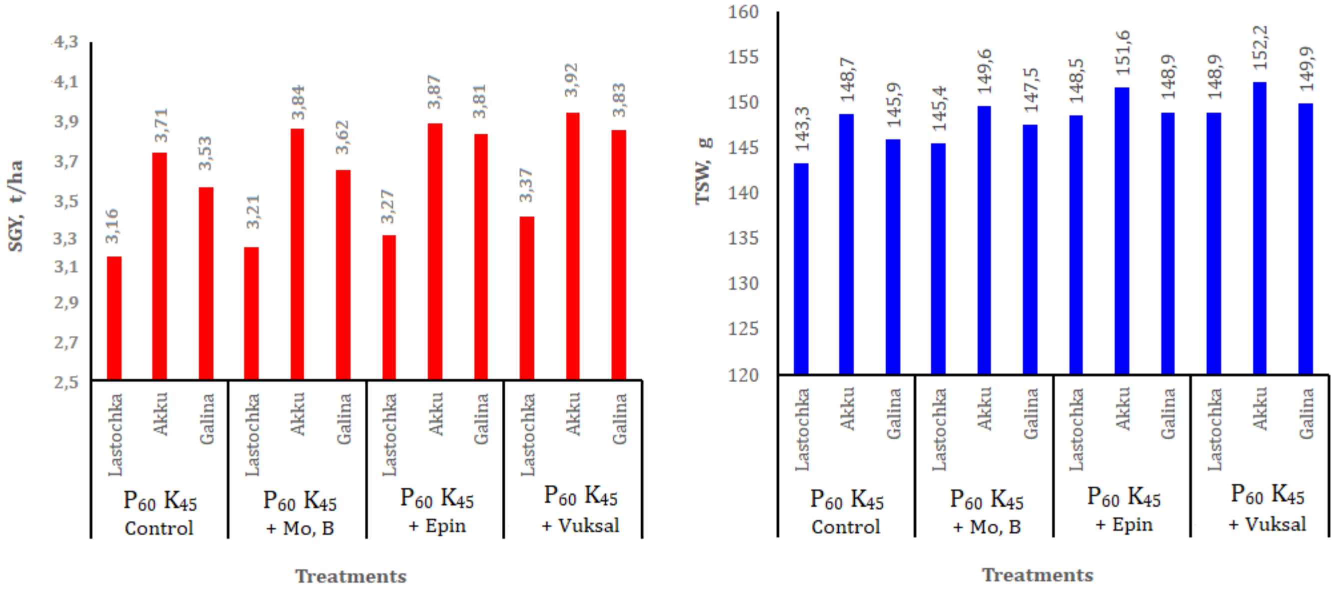


Figure 2. Effect of MF and PGR application on soybean grain yield (SGY) and 1000-seed weight (TSW) of different soybean cultivars as influenced by different treatments

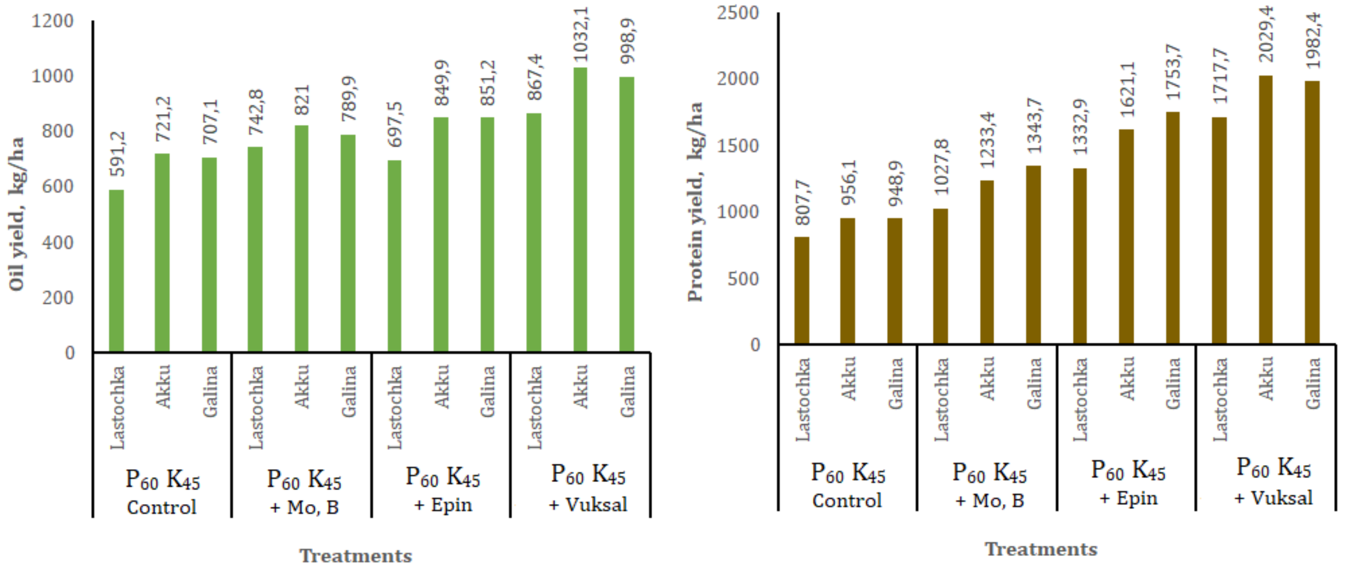


Figure 3. Effect of MF and PGR application on oil yield and protein yield of different soybean cultivars as influenced by different treatments

In both research years, cultivar Akku had higher values for all investigated traits compared to cultivar Lastochka and Galina. In addition, among the applications in the experiment, it was determined that the foliar application, which was effective on soybean seed yield and yield component was Vuksal. Vuksal universal is a liquid fertilizer that contains macronutrients (16%N, 16%P_2O_5, 12%K_2O, w/v) and micronutrients (1%B, 1%Zn, 8 mg Co kg^{-1}, 212 mg Cu kg^{-1}, 437 mg Fe kg^{-1}, 367 mg Mn kg^{-1}, 28 mg Mo kg^{-1}, w/v). Many researchers (Schon and Blevins, 1990; Reinbott and Blevins, 1995; Mandić et al., 2015) have reported that foliar fertilization treatments significantly increase soybean seed yield and yield components.

Conclusion

Cultivar Akku, with longer vegetation period (127-133 days), produced higher plant height (110-120 cm), grain yield, lower pods attachment height, number of seed pods per plant, number of seeds per plant, weight of seeds per plant and 1000-seed weight (TSW) then Lastochka and Galina. From this study it may be concluded that different foliar mineral fertilizers and plant growth regulator treatments effected the increasing of studied quantitative traits in both soybean cultivars. Method of foliar feeding has been proved as an effective tool for increasing of grain yield in both cultivars. However, Vuksal treatment is more effective than Epin and Mo+B in soybean. This follows from the fact that Epin including plant growth regulator (0.025 g/l 24-epibrassinolide). Generally, cultivar Akku and treatment starter fertilizer ($P_{60}K_{45}$) + Vuksal (2,5 L/ha) may be recommended in soybean production in localities with similar agro-ecological conditions in Turkestan region, Southern Kazakhstan.

Acknowledgment

This study (In 2018-2020, Program of Targeted Funding Creation and introduction of new highly productive, stress-resistant, high-quality soybean varieties for various regions of the country using world diversity, State Registration Number: 0118RK01208) was funded by the Ministry of Agriculture of the Republic of Kazakhstan.

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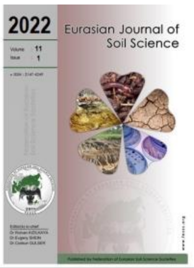
Eurasian Journal of Soil Science

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



Biomass yield, soil cover and minerals accumulation by two green manures species grown in soils of Chiapas Mexico

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Article Info

Received : 16.11.2021
Accepted : 17.06.2022
Available online : 24.06.2022

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Abstract

The aim of the current study was to assess the performance of *Canavalia ensiformis* and *Mucuna deeringiana* (Leguminosae) as a green manure in the agricultural soil of the Frailesca region of Chiapas, México, in terms of aboveground biomass accumulation, plant height, number of leaves, canopy coverage, and the accumulation of nitrogen (N), phosphorus (P), and potassium (K). Each species was sowed at two population densities under a randomized complete block design with three replications. Every 30 days after sowing (DAS), the following variables were quantified: plant length, number of leaves, canopy coverage, biomass yield, and N, P, and K content. A variance analysis and mean comparison test (Tukey 0.05) were performed for each variable. The biomass yield in *M. deeringiana* fluctuated from 9150 to 33,160 kg ha^{-1} on a fresh basis and from 4490 to 15,890 kg ha^{-1} on a dry basis, whereas the yield in *C. ensiformis* varied from 9343 to 26,390 kg ha^{-1} and from 4513 to 13,150 kg ha^{-1}, respectively. The longest recorded plant length was 513.00 cm in *M. deeringiana* and 155 cm in *C. ensiformis*, with a total of 353 and 322 leaves, respectively. The accumulation of N, P, and K was 463.99 kg ha^{-1}, 84.22 kg ha^{-1}, and 49.26 kg ha^{-1} in *M. deeringiana* and 341.90 kg ha^{-1}, 43.40 kg ha^{-1}, and 36.82 kg ha^{-1} in *C. ensiformis*, respectively. Both *C. ensiformis* and *M. deeringiana* have potential as green manure for the Frailesca region of Chiapas in terms of biomass production and N accumulation.

Keywords: *Canavalia ensiformis*, Canopy coverage, dry matter, ecotechnologies, legumes, *Mucuna deeringiana*.

Introduction

Incorrect management and overexploitation of soil resources has caused continuous degradation (Renté-Martí et al., 2018), as reflected in environmental pollution, erosion, and low fertility, which is mainly due to not considering agroecological practices for soil conservation (Serrano and Cano, 2007). Soil degradation in agriculture is appreciable in the long-term, as the excessive use of agrochemicals and other external technologies maintains crop yield levels; however, the cost of production increases each time and the negative effects to the soil increase (Amézquita et al., 2013). Therefore, one of the alternatives for soil improvement is the incorporation of green manures, mainly species of the Leguminosae family, due to their ability to fix atmospheric N (N_2) in association with bacteria of the genus *Rhizobium* (Mangaravite et al., 2014) and their different benefits to the physical, chemical and biological properties of the soil. Among these benefits is an increase in organic matter (Cruz et al., 2014) and the release of nutrients, mainly N, which is the most limiting nutrient for many of the basic crops with annual growth (Pereira et al., 2016). *C. ensiformis* and *M. deeringiana* are the most important species as green manures and cover crops in the tropics and subtropics, adapting to tropical and subtropical climates (Buckles and Triomphe, 1999), where they present optimal development in

: https://doi.org/10.18393/ejss.1135515
: http://ejss.fesss.org/10.18393/ejss.1135515
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

both clay and sandy soils under conditions of low fertility (Calegari et al., 1993). In some studies, it has been reported that *C. ensiformis* and *M. deeringiana* obtains large amounts of N through biological fixation (Bunch 2016; Sant´Anna et al., 2018). On the other hand in Cuba Renté-Martí et al. (2018) reported high biomass yields and a positive effect on physical properties of soil when the green manure was incorporate into the soil.

This ability to accumulate fresh or dry matter in the organs of a plant is known as crop yield (Barrientos-Llanos et al., 2015), which can be expressed as fresh or dry biomass, height, number of leaves, and stem diameter, among others, through the quantitative process of growth (Werner and Leihner, 2005). Therefore, knowledge of biomass yield and nutrient content throughout the growth cycle of plants is fundamental to establishing the potential for utilization in soil rehabilitation programs (Zapata et al., 2019). However, *C. ensiformis* and *M. deeringiana* have been little used to improve or preserve the fertility of agricultural soils in the tropical and subtropical regions of Mexico due to the competition that it can exert with the main crop, in addition to little knowledge of its use as forage in animal feeding (Eilittä and Carsky, 2003). Knowledge about the development and biomass production of *C. ensiformis* and *M. deeringiana*, as well as their roles in the improvement and rehabilitation processes of agricultural soils, is scarce in tropical regions of south-southeast Mexico. On the other hand, no investigations have indicated the best population densities for cultivation and dates of incorporation into the soil as green manures. In this context, it is important to for studies to focus on the vegetative characterization and biomass production in species that represent sustainable alternatives for improving and restoring soils in tropical and subtropical regions. Therefore, the objective of the present study was to characterize the vegetative development of *C. ensiformis* and *M. deeringiana* at different population densities in terms of biomass accumulation, plant height, number of leaves, canopy coverage, and the accumulation of N, P, and K for their potential use as green manures in agricultural soils of the Frailesca region of Chiapas, Mexico.

Material and Methods

Description of the experimental site

The evaluated species (*C. ensiformis* and *M. deeringiana* were cultivated at the University Center for Technology Transfer, CUTT San Ramón, of the Faculty of Agronomic Sciences, Campus V, of the Autonomous University of Chiapas, located in the municipality of Villaflores, Chiapas, Mexico. The geographical coordinates are 16°15'N and 93°14'W, with an altitude of 610 m.a.s.l., average annual temperature of 22°C, and annual precipitation of 1200 mm (Aguilar et al., 2019). Figure 1 shows the average temperature and precipitation during the experiment.

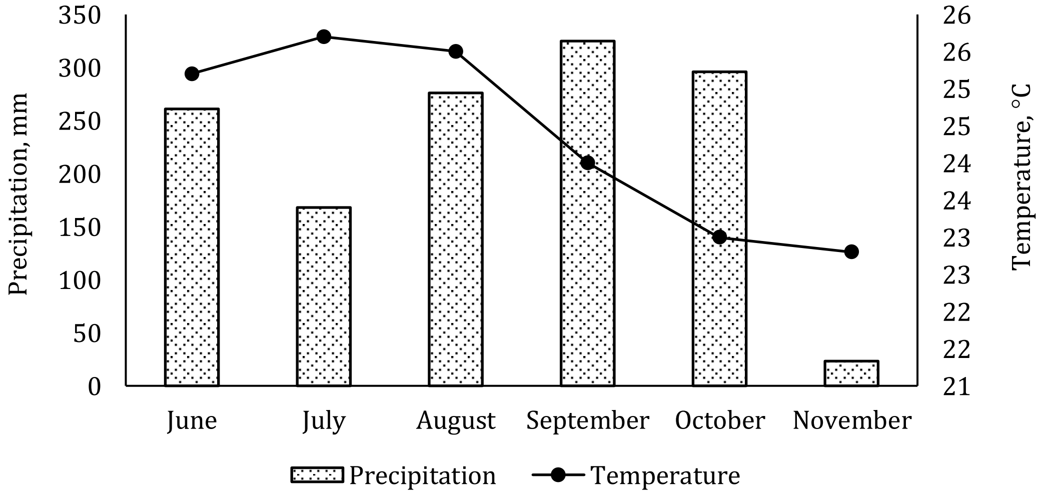

Figure 1. Average precipitation and temperature of the study area during the experiment.

Plant material and experimental design

The *C. ensiformis* and *M. deeringiana* seeds used in this experiment were obtained from the harvest of the previous agricultural cycle, spring/summer 2018, in the same experimental field. The seeds were sowed by hand June 1, 2019, at two different population densities using a randomized block design with four treatments and three replications (Table 1).

Table 1. Treatments, planting density, and population density of *Canavalia ensiformis* and *Mucuna deeringiana.*

Treatment	Planting distance between rows and plants (m)	Planting density (kg ha^{-1})	Population density (plants ha^{-1})
T1: *C. ensiformis*	0.5 × 0.5	95.2	80,000
T2: *C. ensiformis*	1 × 0.5	47.6	40,000
T3: *M. deeringiana*	1 × 1.5	10.66	13,333
T4: *M. deeringiana*	1 × 1	16	20,000

Each experimental unit consisted of 10×8 m plots, with a separation of 1 m between experimental plots. No scarification or inoculation treatment was applied to the seeds. In all four treatments, two seeds were placed per sowing point. The soil type used was a *Chromic Luvisol* (WRB, 2014), soil samples were collected and analysed according to methods described by Carter and Gregorich, (2007) and its initial chemical and physical characteristics are provided in Table 2. No agrochemicals were applied during the evaluation and weed control was carried out by hand.

Table 2. Physical and chemical characteristics of the soil used.

Characteristic	Method	Result
pH	$CaCO_2$	4.02
Organic matter (%)	Walkley and Black	1.41
N total (%)	Kjeldahl	0.07
P (mg kg^{-1})	Olsen	11
K (meq$_c$ 100 g)	Atomic emission	0.3
Ca (meq$_c$ 100 g)	Atomic absorption	2.1
Mg (meq$_c$ 100 g)	Atomic absorption	0.5
Na (meq$_c$ 100 g)	Atomic emission	0.1
Fe (mg kg^{-1})	Atomic absorption	104
Zn (mg kg^{-1})	Atomic absorption	1.0
Al (meq$_c$ 100 g)	Exchangeable acidity	0.3
CEC (meq$_c$ 100 g)	Atomic absorption	8.3
NO_3 (mg kg^{-1})	Steam entrainment	28
NH_4 (mg kg^{-1})	Steam entrainment	20
Sand (%)	Bouyoucos	55
Silt (%)	Bouyoucos	19
Clay (%)	Bouyoucos	26
Textural classification		Sandy-clay loam

Sample collection

The plants were harvested 30, 61, 92, 123, and 155 DAS and the following quantified in each sample: green and dry biomass yield, number of leaves, plant height, and canopy coverage. Both fresh and dry biomass yield were determined by the square method (1 m^2); the fresh biomass was quantified after cutting and extracting all plants located on the indicated surface, whereas for dry biomass the samples were taken to the Animal Nutrition laboratory of the Faculty of Agronomic Sciences of the UNACH, where they were cleaned of impurities and placed in an air circulation oven at 70°C for 48 hours. After obtaining the dry weight of the biomass, the samples were ground for further analysis of N, P, and K. In the last sampling (155 DAS), the root system was extracted in order to quantify the nodules. Ten plants located in the central furrows of the experimental plots were measured by a flexometer to obtain the plant length and the total number of leaves per plant, whereas the canopy coverage was obtained by the square method (1 m^2) subdivided into 100 squares of 10 cm each.

Nutrient analysis

The Kjedahl method was used (Bremner, 1996) to determine the N in whole plants of *C. ensiformis* and *M. deeringiana*, whereas P and K were obtained by colorimetric (Chapman et al., 1984) and atomic absorption spectrophotometry (Fishman and Downs, 1966), respectively. The accumulation of N, P, and K in dry biomass was calculated as follows:

$$\text{Accumulation x (g plant)} = \frac{(\text{g dry matter})(\text{x \% NPK total in the plant})}{100}$$

Statistical analysis

All data were subjected to an analysis of variance according to the selected experimental design. Subsequently, a comparison of means was carried out (Tukey test 0.05) using the statistical program Statgraphics Centurión XVII (Statgraphics, 2014).

Results and Discussion

The variance analysis for the fresh and dry biomass yield variables only showed significant differences ($p<0.05$) between treatments in the last two samplings (123 and 155 DAS). At the evaluated densities (T1 and T2), the *C. ensiformis* species registered the highest accumulation of biomass at 92 DAS with 22,320 and 26,390 kg ha^{-1} in fresh biomass and 11,080 and 13,150 kg ha^{-1} in dry biomass, respectively, but a decrease in biomass was observed at 155 DAS (Table 3). These biomass yields are within the values reported by Precoppe (2005), who recorded *C. ensiformis* yields of 20.00 to 40.00 t ha^{-1} for fresh matter and 3.00 to 6.00 t ha^{-1} for dry matter. In treatments T3 and T4, the biomass yields gradually increased from 123 and 155 DAS, with maximum values of 20,833 and 33,160 kg ha^{-1} for fresh biomass and 10,323 and 15,890 kg ha^{-1} for dry biomass, respectively (Table 3). The fresh biomass production recorded at T3 and T4 is within the yields reported by Costa-Mello et al. (2018) in *M. deeringiana* at 75 DAS; they recorded yields from 20.29 to 44.79 t ha^{-1}. However, the dry biomass yield found in this study was higher than that recorded by those authors (9.47 t ha^{-1}). The dry biomass yields obtained in this study are above the values recommended as cover/green manure.

The high biomass yields in both legume species sowed at different population densities confirm its potential as green manure for the improvement of degraded soils in the tropical and subtropical regions of south-southeast Mexico. In this regard, Aguilar (2014) reported that one of the desirable characteristics of the species used as green manures is that they can grow in poor soils with little or no management.

Table 3. Accumulation of fresh and dry biomass, plant length plant, and number of leaves in *C. ensiformis* and *M. deeringiana* at different growth periods.

Treatments	Days After Sowing				
	30	61	92	123	155
	Fresh biomass (kg ha^{-1})				
T1	13373 a	20950 a	22320 a	21280 b	9867 c
T2	9343 a	25946 a	26390 a	21560 b	13667 bc
T3	9150 a	18463 a	19723 a	20250 b	20833 b
T4	12270 a	23080 a	25103 a	26756 a	33160 a
CV %	25.04	19.48	19.33	19.65	23.38
	Dry biomass (kg ha^{-1})				
T1	7237 a	10337 a	11080 a	10447 b	4873 c
T2	4513 a	12647 a	13150 a	10623 b	6957 bc
T3	4490 a	8980 a	9843 a	10323 b	10270 b
T4	6170 a	11613 a	12547 a	13540 a	15890 a
CV %	27.63	18.67	19.53	18.55	21.69
	Plant length (cm)				
T1	60.67 b	113.33 b	147.67 b	149.00 b	146.67 b
T2	63.00 b	134.33 b	155.00 b	152.67 b	152.33 b
T3	126.00 a	320.67 a	407.33 a	470.00 a	512.33 a
T4	134.67 a	325.00 a	409.67 a	474.33 a	513.00 a
CV %	6.72	4.03	2.16	1.67	1.31
	Number of leaves				
T1	42 b	133 b	309 ab	265 c	94 b
T2	43 ab	147 a	322 a	277 b	97 b
T3	46 a	153 a	282 b	292 a	350 a
T4	47 a	155 a	286 b	299 a	353 a
CV %	4.62	3.64	3.97	1.27	2.84

Values in the same column with a different letter indicate significant ($p < 0.05$) differences between the means. CV=coefficient of variation.

Although the soil presented low fertility levels (Table 2), its physical and chemical characteristics and climatic conditions (Figure 1) were favourable for the production of biomass in the four treatments evaluated. This is probably due to the fact that these species adapted in tropical and subtropical zones, where agroclimatic conditions are favourable for their cultivation. A study under conditions of Kenia showed that the most outstanding legumes as green manure were *C. ensiformis* and *M pruriens*, based mainly on biomass accumulation (Murehiti et al., 2003). The length of the plant and the number of leaves showed a relationship with the accumulation of biomass; in the four treatments, the length of the plant gradually increased as the vegetative development of each species progressed. At 123 DAS, the maximum plant length was 149.00 cm and 155.00 cm for T1 and T2 at 92 DAS, whereas the longest plant length for T3 and T4 was 512.33 cm and 513.00 cm at 155 DAS (Table 3). The highest number of leaves accumulated for T1 and T2 was 309 and 322 at

92 DAS, respectively, whereas the maximum number of leaves for T3 and T4 was 350 and 353 up to 155 DAS (Table 3). These results show that the species evaluated in this study as green manures have suitable capacities to accumulate fresh matter and dry matter in their different organs, such as the leaves and stems, ensuring a proportional increase in biomass yield, which is essential in crop production (Barrientos-Llanos et al., 2015). The growth of these organs implies a physiological process, which depends on several factors, such as photosynthesis, respiration, elongation, and cell division (Gamage et al., 2018). In addition, other components that influence growth are the sowing date, planting density, fertilization, irrigation, pest control, and the duration of the crop (Vanek, 2009). The higher production of fresh and dry matter, higher plant height, and greater number of leaves with *M. deeringiana* during its growth (T3 and T4) was probably due to the fact that the flowering stage started earlier in *C. ensiformis* than in *M. deeringiana* (90 vs. 120 DAS). Thus, both densities of *M. deeringiana* showed a tendency to continue vegetative growth after 90 days (its life cycle was longer than that of *C. ensiformis*).

According to Rajwade et al. (2000), this behaviour is part of the typical characteristics of each species and the interaction with the environment. The canopy coverage presented a similar pattern (Figure 2), as *C. ensiformis* at the two densities (T1 and T2) showed a gradual increase of 30% and 27% at 30 DAS, respectively, 79% at 61 DAS, and 100% canopy coverage at 92 and 123 DAS, respectively. At 155 DAS, a small decrease of 92% was observed for T1 and 97% for T2. In contrast, *M. deeringiana* (T3 and T4) increased its canopy coverage from 30 DAS, exceeding 80%, and then remained constant at 100% until 155 DAS. This shows the efficiency of plants to accumulate biomass and translocate nutrients at certain times during growth to the organs with the highest demand. The decrease in biomass yield for T1 and T2 can be explained as a function of plant maturity, in which biomass production begins to decline due to senescence, which occurs mainly in mature leaves and with translocation of nutrients to the fruits (Zapata et al., 2019). Canopy coverage represents one of the most important characteristics of the species used as green manures in the tropical regions of Mexico. The orography in these regions makes it necessary to practice agriculture in hillside conditions, favouring erodibility, which requires maintenance of the surface area of agricultural soils due to the erosivity typical of these regions. The soil is covered to reduce hydric erosion (García-Hernández et al., 2010) and represents a fundamental principle of the ecological management of agricultural soils.

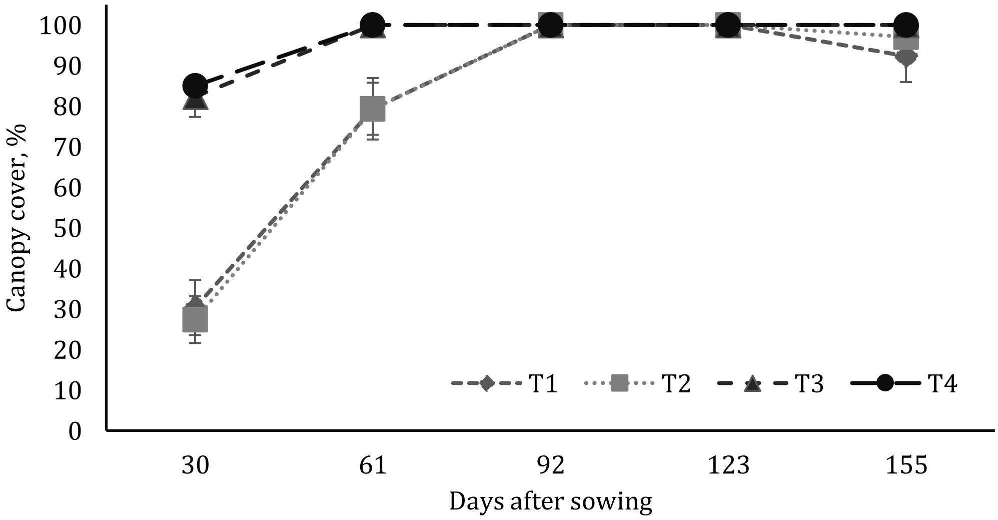

Figure 2. Percentage of canopy coverage during the growing season of *C. ensiformis* and *M. deeringiana* at different growth periods.

In practically all samples, the N, P, and K that accumulated in the biomass of the species under study had significant differences between treatments ($p<0.05$). The total N accumulated in *C. ensiformis* at the lowest density (T1) varied from 188 to 288 kg ha^{-1} from 30 to 90 DAS, whereas with the highest density (T2) the values ranged from 117.35 to 341.90 kg ha^{-1} during the same period. Subsequently, the N accumulation tended to decrease significantly for both treatments (Table 4). In *M. deeringiana*, a different behaviour was observed; the total N accumulated in the biomass of both densities (T3 and T4) increased until the last sampling period (150 DAS), but with a tendency to be significantly higher for T4 than T3 (463.99 vs. 301.44 ha^{-1}). With respect to accumulated P, a trend very similar to that recorded for N was observed, but in smaller amounts. For example, with *C. ensiformis* at the lowest density (T1), the total accumulated P varied from 23.8 to 36. 56 kg ha^{-1} from 30 to 90 DAS, whereas at the highest density (T2) the values ranged from 14.8 to 43.4 kg ha^{-1} during the same period. After 90 DAS, a tendency was also observed in both treatments for the accumulation to decrease significantly to 16.8 and 22.9 kg ha^{-1}, respectively. The P in the biomass of *M. deeringiana*, regardless of density (T3 and T4), also increased as the growth period progressed (from 30 to 150 DAS), with values from 23.80 to 54.43 kg ha^{-1} and 32.70 to 84.32 kg ha^{-1}, respectively, but with a tendency to be significantly higher for T4 than T3 in each sampling carried out (Table 4). K was the mineral that accumulated the least in the biomass of both legume species, with values from 20.26 to 31.04 kg ha^{-1} (T1), 12.64 to 36.82 kg ha^{-1} (T2), 13.92 to 32.00 kg ha^{-1} (T3), and 19.13 to 49.26 kg ha^{-1} (T4), but only the latter treatment gradually accumulated K until the end of the evaluation period (from 30 to 155 DAS).

Table 4. Nitrogen, phosphorus, and potassium accumulated in the dry biomass of *Canavalia ensiformis* and *Mucuna deeringiana* at different growth periods.

Treatments	Days After Sowing				
	30	61	92	123	155
	Accumulated N (kg ha^{-1})				
T1	188.15 a	268.75 b	288.08 b	271.61 b	126.71 c
T2	117.35 a	328.81 a	341.90 a	276.21 b	180.87 bc
T3	131.11 a	262.22 b	287.43 b	301.44 ab	299.88 b
T4	180.16 a	339.11 a	366.36 a	395.37 a	463.99 a
CV %	27.54	19.22	20.12	19.02	21.04
	Accumulated P (kg ha^{-1})				
T1	23.88 ab	34.11 b	36.56 b	34.47 b	16.08 c
T2	14.89 b	41.73 ab	43.40 ab	35.06 b	22.96 c
T3	23.80 ab	47.59 ab	52.17 ab	54.71 ab	54.43 b
T4	32.70 a	61.55 a	66.50 a	71.76 a	84.22 a
CV %	27.83	21.18	22.20	20.44	20.99
	Accumulated K (kg ha^{-1})				
T1	20.26 a	28.94 a	31.04 b	29.25 b	13.65 c
T2	12.64 b	35.41 a	36.82 a	29.75 b	19.48 bc
T3	13.92 b	27.84 a	30.51 b	32.00 b	31.84 b
T4	19.13 a	36.00 a	38.89 a	41.97 a	49.26 a
CV %	27.54	19.15	20.03	18.91	21.44

Values in the same column with different letters indicate significant ($p < 0.05$) differences between the means. CV=coefficient of variation.

In the edaphoclimatic conditions of this study, the amount of N accumulated in the aerial biomass of the legumes was higher than the amount of P and K, which is similar to previous reports (Mangaravite et al., 2014). This can be explained in terms of an adequate symbiosis between the roots of both *M. deeringiana* and *C. ensiformis* with bacteria of the genus *Rhizobium* or *Bradyrhizobium* to fix N_2 (Starovoytov et al., 2010; Saldaña-Acosta, 2017). According to Gerónimo et al. (2002), *M. deeringiana* can accumulate up to 260 kg ha^{-1} from fixation, whereas *C. ensiformis* can fix from 240 to 318 kg ha^{-1} (Vera-Núñez et al., 2008; Renté-Martí et al., 2018). Furthermore, approximately 80-85 % of the total N accumulated in biomass is derived from biological N fixation (Partelli et al., 2011). Although biological N fixation was not quantified in this study, it was possible to observe and quantify nodules on plant roots at 155 DAS in all treatments (Figure 3), with a greater tendency to find effective nodules (73 to 83) than nodules with no activity (36 to 39).

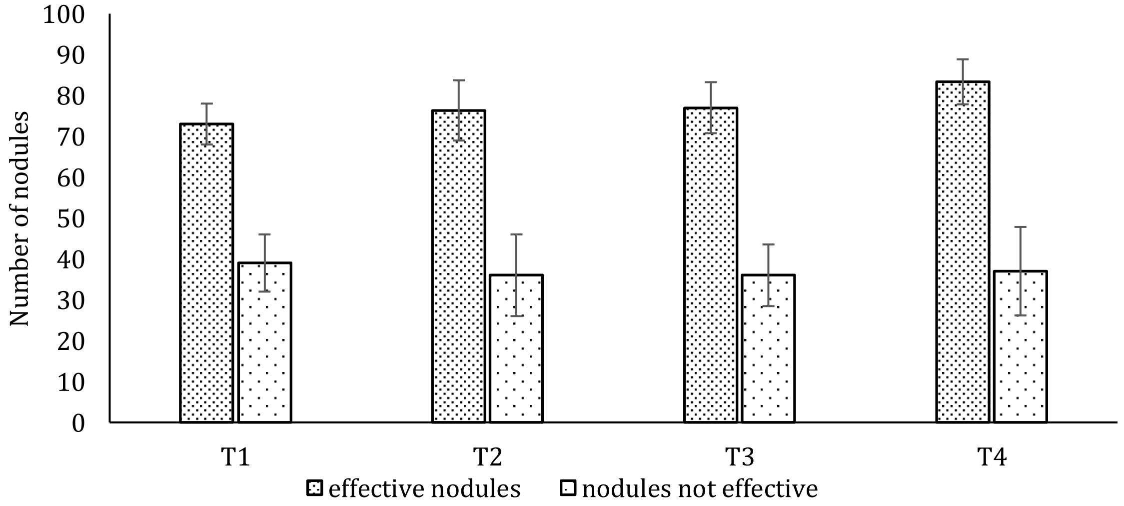

Figure 3. Number of effective and ineffective nodules in *C. ensiformis* and *M. deeringiana* at 155 DAS.

Fewer effective nodules were reported by Córdova-Sánchez et al. (2011), who recorded an average of 59 nodules in *C. ensiformis* and 61 nodules in *M. deeringiana*. Nodulation depends on other factors than the presence of effective rhizobia in the Fabaceae rhizosphere for an optimal interaction between the plant and bacteria (Bianco, 2020). Although P and K did not accumulate in large amounts in the present study, the values were generally higher than those reported by other authors for the same species, but with lower biomass yields, which may be related to differences in edaphoclimatic and management conditions during cultivation. For example, under the conditions in Brazil, Mangaravite et al. (2014) reported P and K values of 16 kg and 192 kg ha^{-1} for *C. ensiformis* biomass harvested at 74 DAS, whereas biomass from *M. deeringiana* cut at 104 DAS had values of 11 and 86 kg ha^{-1}, respectively. On the other hand, P and K availability is mainly related to the quantity and quality of effective nodules (Weisany et al., 2013; Sulieman et al., 2013; Divito and Sadras, 2014). The low accumulation of P and K in the biomass of the studied legumes indicates that the amount that will return to the soil after its incorporation as green manure is low; however, after decomposition and mineralization, these nutrients will be found in an easily accessible form for the next crop.

Conclusion

Overall, our results indicate that both *C. ensiformis* and *M. deeringiana* successfully established in tropical soils of Chiapas, Mexico; however in this study *M deeringiana* showed the greatest potential as a green manure in terms of biomass yield and minerals accumulation.

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Eurasian Journal of Soil Science 2022, 11(4), 337 - 344

Eurasian Journal of Soil Science

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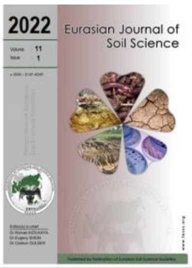

The effects of NPK fertilization on hay production and some yield components of crested wheatgrass (*Agropyron cristatum*) in the dry steppe zone of Eastern Kazakhstan

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Article Info

Received : 11.11.2021
Accepted : 07.08.2022
Available online : 15.08.2022

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Abstract

A three-year-long field experiment was conducted in a continuous grazing system with a variable stocking rate to evaluate effects of increasing NPK fertilization rates (Control- $N_0P_0K_0$, $N_{60}P_{40}K_{30}$, and $N_{80}P_{50}K_{40}$) in crested wheatgrass (*Agropyron cristatum*) on hay production, some yield components and crude protein concentration in the dry steppe zone of Eastern Kazakhstan. At harvesting, hay production (fresh and dry weight), seeding rate (SER), shrub diameter (SHD), height of generative shoots (HGS), length of root leaves (LRL), weight per bush (WEB), percentage of leaves and vegetative shoots (LVS) and crude protein concentration of crested wheatgrass (*Agropyron cristatum*) were determined. NPK fertilizer treatments increased hay production, SER, SHD, HGS, LRL, WEB, LVS and crude protein concentration. The results showed that crested wheatgrass at the $N_{80}P_{50}K_{40}$ treatments achieved a higher hay production and some yield components of crested wheatgrass (*Agropyron cristatum*) in the dry steppe zone of Eastern Kazakhstan than other NPK treatment and control.

Keywords: Crested wheatgrass, fertilization, hay production, crude protein concentration.

Introduction

Agriculture plays an essential role in Kazakhstan's economic, social and environmental development. Once considered the breadbasket of the Soviet Union, Kazakhstan still suffers from the effects of agricultural and environmental mismanagement during the Soviet era. Over a third of Kazakhstanis' livelihoods depend directly or indirectly on the country's extensive rangelands for food, fodder, fuel and medicinal plants. Widespread stockbreeding reflects the country's nomadic tradition, with around 75 percent of all agricultural land used for grazing. While sheep breeding dominates the sector, cattle, pig, horse and camel rearing are important sources of food and income (Privacyshield, 2022). In Kazakhstan livestock has always been a major focus in the agricultural sector and forage production is very important because the forage is the basic source of energy for the growth and maintenance of livestock and increase their products (Tazhibaeva et al., 2014). This attitude is clearly reflected on poor output and performance of animals resulting from poor quality of forages and the problems of over and under grazing. Overgrazing of natural pasture and improper conservation measures lead to reduction of rangelands. Most of the animals in Kazakhstan are greatly dependent on the natural vegetation as their major source of feed for maintenance and production. The possible solution to support the natural pastures is to establish and develop the pastures and encourage the utilization of agricultural by-products and residues that are produced in huge amounts for animals feeding in the Kazakhstan. The most important forage crop under dry steppe zone of Eastern Kazakhstan is crested

doi : https://doi.org/10.18393/ejss.1162364
: http://ejss.fesss.org/10.18393/ejss.1162364
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

wheatgrass and it is commonly used to natural pastures (Cheng and Nakamura, 2007). Crested wheatgrass is a long-life, perennial and cool season with extensive root systems forage plant. It is one of the most hardy and drought-tolerant plants among the grasses (Asay and Jensen, 1996). It grows itself in rangelands in dry steppe zone of Eastern Kazakhstan. It grows early in the spring and becomes ready for grazing and the animals eat it willingly. That's the reason why it is in short supply in our rangelands despite the fact that its homeland is Eastern Kazakhstan in Eurasia.

Forage production can be improved through fertilizer application, management of grazing, and control of weeds (McCarthy et al., 2016). The effects of fertilizers on forage production are examined through plot experiments, which attempt to simulate the real production systems (Delevatti et al., 2019). Most studies on grazing strategies aim to improve forage production and animal performance and are based on pasture height. And also, the nutrient contents of the forage have an important role in animal feeding. The factors influencing the nutritive value of forage are many, and the degree to which they are interrelated may vary considerably from one area to another. These factors may include, alone or in combination, plant type, climate, season, weather, soil type and fertility, soil moisture, leaf to stem ratio, and physiological and morphological characteristics, and may change depending on whether the plants are annuals perennials, grasses or legumes (Türk et al., 2009).

The importance of cultivated forage crops in dry steppe zone of Eastern Kazakhstan continues to increase. Each year more stockmen are turning to, or extending, established cultivated forage crops for their winter feed requirements. Unfortunately, many of the hay fields in dry steppe zone are composed of grasses only, notwithstanding recommendations to seed grass-legume mixtures. Grass alone becomes sod-bound quickly and resultant hay yields are low. This is attributable to an insufficient supply of available nutrient contents in an area where the climate is usually the main uncontrollable limiting factor. Thus, it seems important to learn as much as possible about the factors which can be controlled. Although fertilizer trials on cultivated grasses for hay in dry climates have been, and are being conducted, few results have been published. The aim of this research was to determine the effects of NPK fertilization on hay production and some yield components such as seeding rate, shrub diameter, height of generative shoots, length of root leaves, weight per bush and percentage of leaves and vegetative shoots of wheatgrass (*Agropyron cristatum*) plants in the dry steppe zone of Eastern Kazakhstan.

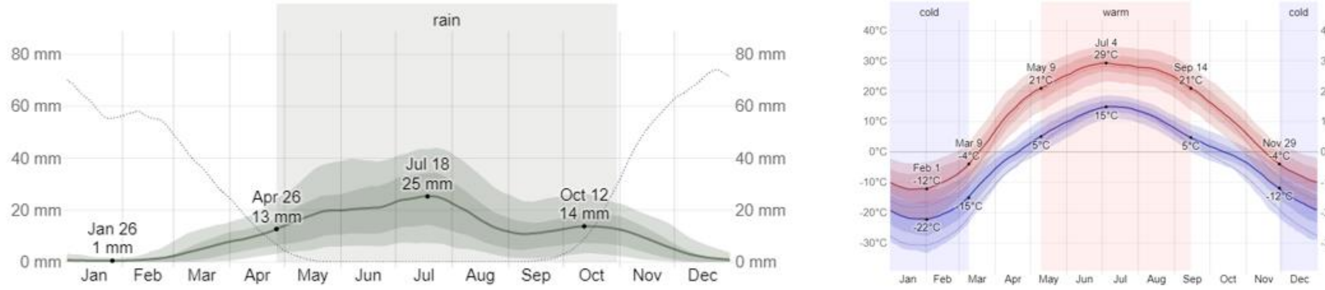
Material and Methods

Study Area

Kazakhstan is the world's ninth largest country with an area of 2.72 million km^2. It is mainly characterized by arid and semiarid conditions (Eisfelder et al., 2012). The main crop-producing areas of Kazakhstan are concentrated in the steppes in the northern parts of the country. This broad agroecological area consists of forest-steppe, steppe, dry steppe, semi-desert and desert. It is highly vulnerable to droughts (Broka et al., 2016). The steppe zone occupies 1.10 million km^2 or about 28% of Kazakhstan and is subdivided into 3 sub-zones: the moderately dry and warm zone of feather grass and various other grasses, the moderately dry and warm zone of tipchakovo and feather grass, and the dry, moderately hot wood and feather grass zone. The steppe zone has been transformed mostly by human activities. Large-scale ploughing of the land in the period of virgin land cultivation (1954-1960) led to the destruction of most of the main types of steppes. More than 38 million ha of land have been ploughed in the steppe zones. These include about 90% of the rich feather steppes and various grass valley steppes, 50-60% of the dry steppes in the plains, 30% of the low-hill steppes and 10-15 % of the small hill steppes. The remaining steppe lands in these sub-zones (stony, complex steppes on saline soils) have been significantly affected by overgrazing. The feather grass steppe has been invaded by typchakovyi (*Festuca valesiaca),* avstryisko feather grass (*Artemisia austriaca),* weeds and various grass communities (UN, 2000). East Kazakhstan region is located in the eastern part of Kazakhstan, on the border with Russia and China (Figure 1). The field experiment was located on peasant farm "Lana" of Beskaragai district, Earst Kazakhstan.

Figure 1. Study area

Experimental site has a dry steppe zone, with the mean annual temperature of 4°C and long-term average precipitation is 133.1 mm (Figure 2). The warm season lasts for 4.1 months, from May 9 to September 14, with an average daily high temperature above 21°C. The hottest month of the year in research area is July, with an average high of 29°C and low of 15°C. The cold season lasts for 3.4 months, from November 29 to March 9, with an average daily high temperature below -4°C. The coldest month of the year in research area is January, with an average low of -21°C and high of -12°C. A wet day is one with at least 1.00 millimeters of liquid or liquid-equivalent precipitation. The chance of wet days in research area varies throughout the year. The wetter season lasts 6.4 months, from May 6 to November 18, with a greater than 13% chance of a given day being a wet day. The month with the most wet days in research area is July, with an average of 5.7 days with at least 1.00 millimeters of precipitation. The drier season lasts 5.6 months, from November 18 to May 6. The month with the fewest wet days in research area is February, with an average of 1.9 days with at least 1.00 millimeters of precipitation. Among wet days, we distinguish between those that experience rain alone, snow alone, or a mixture of the two. Based on this categorization, the most common form of precipitation in research area changes throughout the year. Rain alone is the most common for 8.3 months, from March 13 to November 22. The month with the most days of rain alone in research area is July, with an average of 5.7 days. Snow alone is the most common for 3.6 months, from November 22 to March 13. The month with the most days of snow alone in research area is December, with an average of 2.2 days.



(A) (B)

Figure 2. Monthly average temperature (A) and distribution of precipitation (B) of the experimental area

Experimental Design

The experiments were carried out in 2018, 2019 and 2020 at the peasant farm "Lana" of Beskaragai district, East Kazakhstan. The experimental design was a randomized complete block with three treatments (Control-$N_0P_0K_0$, $N_{60}P_{40}K_{30}$, and $N_{80}P_{50}K_{40}$). These treatments were independent to each other. Plot size was 100 ha. The treatments were applied to the same plot areas for three successive years (2018, 2019, and 2020). For the fertilized treatment, fertilizers were applied with granule fertilizer spreader machine to the experimental plots at the end of May in the early growing period every year. The sources of fertilizers used were urea 46% N, double superphosphate 47% P_2O_5 and potassium chloride 60% K_2O. Soil samples were taken one times a year in spring (May) season between 2018 and 2020. After the soil samples were air dried and passed through a sieve with 2 mm size opening, some soil characteristics were determined as follows; total organic matter contents, soil reaction (pH), mineral-N, available phosphorus and available potassium as described by GOST 26213-2021, GOST 26423-85, GOST R 53219-2008 and GOST 26205-91.

Plant measurements and sampling

Measurements of three randomly selected quadrats (0.5 m × 0.5 m) were averaged in each plot with three replicates. Quadrats were established in the middle of each plot at least 10 m from the edge. All crested wheatgrass (*Agropyron cristatum*) plants were recorded, measured and clipped. Sampling and measurements were carried out following a consistent manner one times on 24 August. Plant height was measured on 10 plant individuals per species within each quadrat. After the harvest, the plants were trod and evaluated individually to determine the Forage fresh weights (FFW), Forage dry weights (FDW), Seeding rate (SER), Shrub diameter (SHD), Length of root leaves (LRL), Weight per bush (WEB), Percentage of leaves and vegetative shoots (LVS). After being harvested, the chickpeas was dried and milled and the crude protein content concentrations were determined.

For the crude protein content, the methodology proposed by Kjeldahl was used, finding the value of the total nitrogen (N) of the sample and later, converting to crude protein by the factor 6.25 - using three samples to cultivate in each of the tests, and each sample weighing 0.5 grams of dry and ground material (GOST 10846-91).

Results and Discussion

The soil pH was 7.77 (alkaline reaction), soil organic matter content was 2.59% (moderate), Easily hydrolysable nitrogen (NO_3-N+NH_4-N) was 39,2 mg kg^{-1}, available phosphorus was 26 mg kg^{-1} and available potassium was 740 mg kg^{-1} in experimental site.

Hay production (fresh and dry weight) crested wheatgrass (*Agropyron cristatum*) according to NPK fertilizer treatments are shown in Figure 3. The results showed that the effects NPK fertilization rates were significant in 2018, 2019 and 2020 years (Figure 3). Years were shown separately, because differences of years were significant all parameters of crested wheatgrass. The 4-year average yield increased with increasing levels of NPK, irrespective of the source of NPK. The highest yields were obtained in 2020 when the precipitation, particularly during the month of May and June, was high. The increases attributable to NPK treatments were good for crested wheatgrass. Generally, the highest hay production obtained by the N_{80} P_{50} K_{40} treatment in all years. The lowest yield was observed in the controls. Over the mean of the three years, fresh matter yield ranged from 1.46 to 1.57 ton ha^{-1} in the plots treated with NPK. Dry matter production in the control plot was 0.52 ton ha^{-1}, and NPK fertilizer treatments increased the dry matter production of the plots by about 28.9% (in $N_{60}P_{40}K_{30}$) and 40.4% (in $N_{80}P_{50}K_{40}$) in the present study. In various studies (Baenziger and Knowles, 1969; Lawrence and Knipfel, 1981; Jefferson and Cutforth, 2005), hay production of crested wheatgrass ranged from 1.28-10.19 ton ha^{-1} depending on growth environment, N fertilizer application, and cultivar. Walton (1983) described that fertilizers are normally used to increase forage yield and quality, but since plant tissue reflects the mineral constituents of the soil in which the plants are grown, quality is also greatly influenced. The herbage is especially responsive to the Ca, P, K, S, and N content of the soil.

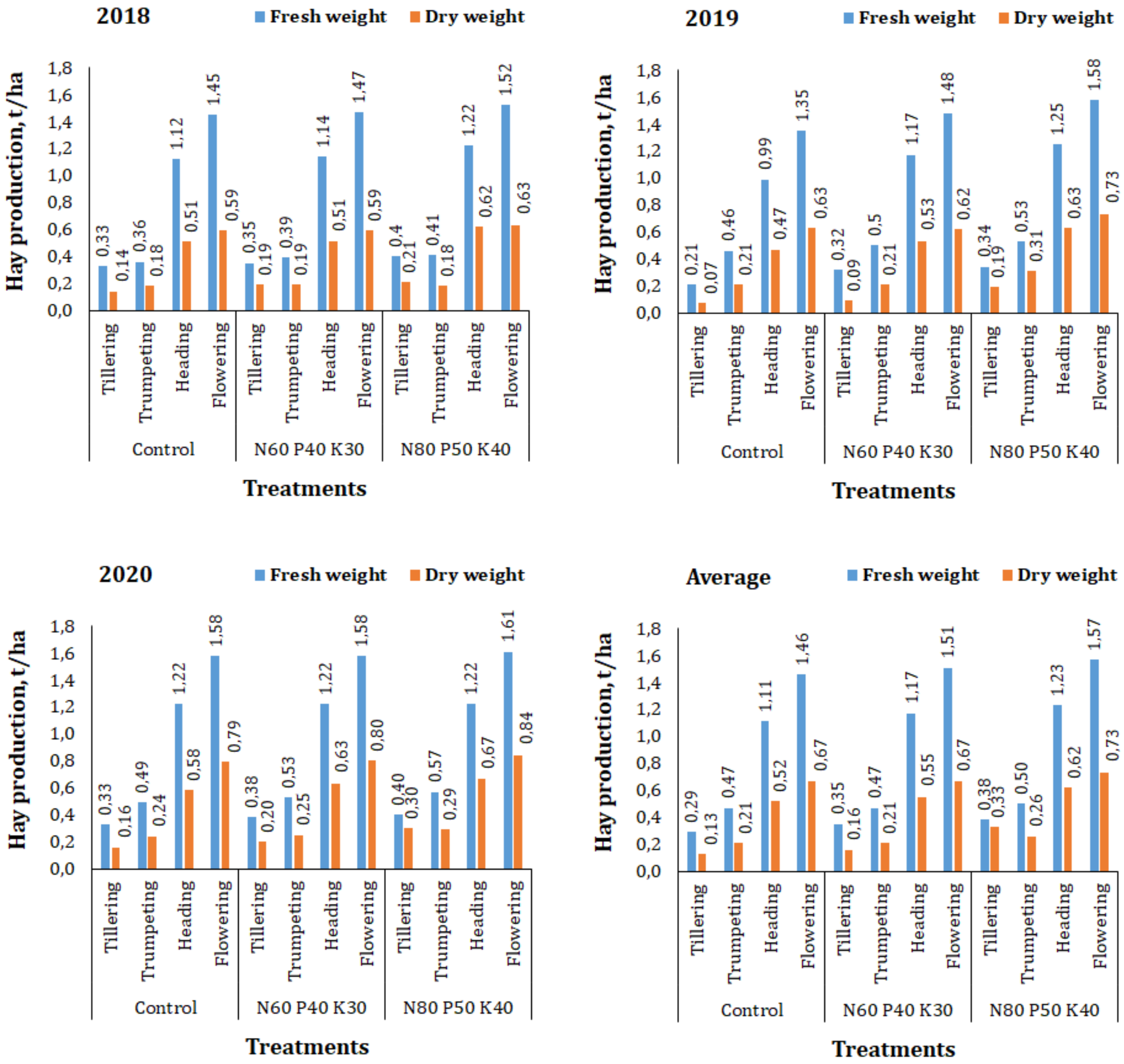


Figure 3. The effect of NPK fertilization rates on hay production (frest and dry weight) of crested wheatgrass (*Agropyron cristatum*) at different phenological stages during 2018, 2019 and 2020.

When species are grown in a pure stand, the effect of these minerals on the plant is direct. The uptake of nutrient was also affected by soil properties such as salinity and soil texture and forms of fertilizer treated (Irshad et al., 2002). Lauriault et al. (2002) reported that N is the most important fertilizer nutrient required for growing grasses. Increase in hay production due to N application is well documented by many authors (Power, 1986; Hall et al., 2003; Scarbrough et al., 2004). It was also found that the increase in NPK fertilizer level resulted in high hay production (fresh and dry forage yield) compared to the control. This is attributed to the fact that nitrogen increases the photosynthetic capacity of growing plants, which enhances growth to produce adequate dry matter. It is observed from the results of growth attributes, the fertilizer increased number of leaves per plant, plant fresh and dry weight, and leaf area index. Consequently higher yield could be expected at higher NPK fertilization level. This finding is in agreement with the finding of several research workers about the effect of nitrogen and phosphorus on yield of different forage grasses (Cowan et al., 1995; Buerkert et al., 2001).

The effect of NPK fertilization rates on Seeding rate (SER), Shrub diameter (SHD), Height of generative shoots (HGS), Length of root leaves (LRL), Weight per bush (WEB), Percentage of leaves and vegetative shoots (LVS) of crested wheatgrass (*Agropyron cristatum*) in 2018, 2019 and 2020 years is presented in Table 1. During all years there was significant difference in SER, SHD, HGS, LRL, WEB and LVS of crested wheatgrass between the different levels of NPK fertilization rate. The highest SHD, HGS, LRL, WEB and LVS were obtained at the higher NPK fertilization level ($N_{80}P_{50}K_{40}$) with highest width. On the contrary, the highest SER was obtained at the higher NPK fertilization level with the lowest width. The results revealed that the effect of NPK fertilization rates on SER, SHD, HGS, LRL, WEB and LVS were significant in all counting occasions during the experimental period. The increase in fertilizer level led to increase the SER, SHD, HGS, LRL, WEB and LVS. These results are in agreement with those reported by Kilcher (1958), Wilkinson and Langdale (1974), McCaughey and Simons (1996) and Türk et al. (2009) who stated that fertilization rates influence the growth attributes of grass.

For all three years, there was significant effect of NPK fertilizer treatments on crude protein concentration (Figure 4). In present study, increasing NPK treatments resulted in an increase in crude protein concentration. Generally, the highest crude protein concentration obtained by the $N_{80}P_{50}K_{40}$ treatment (9.45% in 2018, 10.04% in 2019 and 9.54% in 2020). The lowest crude protein concentration was observed in the controls. Crude protein is the amount of nitrogen from both protein and non-protein sources in the forage and is predictive of available protein. Crude protein concentration (%) of crested wheatgrass ranged from 14.4–22.8 at vegetative stage, 12.2-19.3 at boot stage, 11.9-12.4 at heading stage, 10.3-11.4 at anthesis , 6.5-10.2 5 at seed development stage, and 3.9-9.0 at seed maturity stage (Whitman et al., 1951; Glover et al., 2004; Biligetu et al., 2014).

In this research, increasing NPK fertilization led to slight increase in crude protein percentage. This result emphasized the fact that nitrogen plays a great role in synthesis of protein. Also phosphorus plays an important role in photosynthesis processes to produce protein and remobilization of sugar to starch. Similar results regarding the increased crude protein due to fertilizer application were obtained by several researchers. Brima and Abusuwar (2020) reported that nitrogen fertilization increased the crude protein of Rhodes grass.

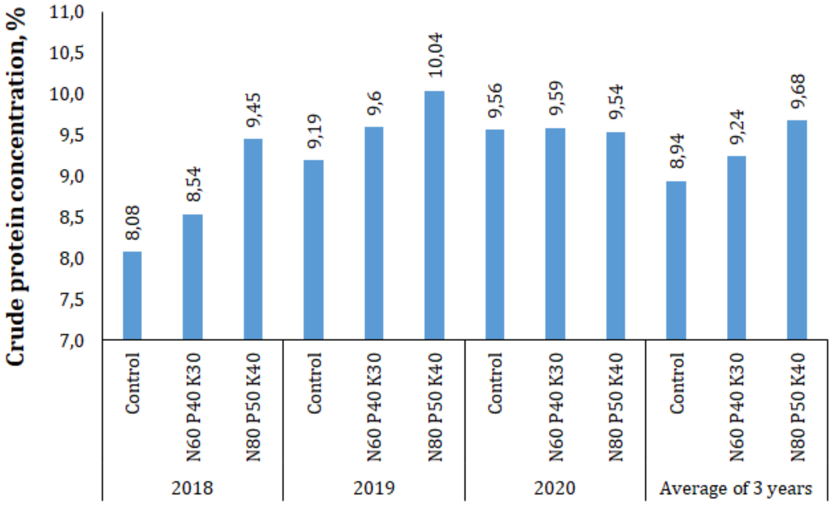


Figure 4. The effect of NPK fertilization rates on crude protein concentration of crested wheatgrass (*Agropyron cristatum*) during 2018, 2019 and 2020.

Table 1. The effect of NPK fertilization rates on Seeding rate (SHD), Shrub diameter (SHD), Height of generative shoots (HGS), Length of root leaves (LRL), Weight per bush (WEB), Percentage of leaves and vegetative shoots (LVS) of crested wheatgrass (*Agropyron cristatum*) during 2018, 2019 and 2020.

Year	Treatments	Width, cm	SER,mln/ha	SHD, cm	HGS, cm	LRL,cm	WEB, gr	LVS
2018	Control- $N_0P_0K_0$	10	3,0	2,5	45,5	13,0	3,8	89,3
		22	1,8	3,2	48,3	14,9	4,3	89,3
		38	0,8	3,5	48,0	14,9	4,5	88,3
		48	0,8	3,3	49,1	13,0	5,8	89,0
	$N_{60}P_{40}K_{30}$	13	3,5	3,5	50,0	14,8	4,4	99,2
		28	2,0	4,0	49,0	15,8	5,0	98,0
		42	1,2	4,2	51,3	14,8	5,5	98,3
		54	1,0	4,0	52,0	14,0	6,0	98,4
	$N_{80}P_{50}K_{40}$	15	4,0	3,9	50,4	15,1	4,4	99,8
		30	2,0	4,2	49,5	16,0	5,6	98,4
		45	1,3	4,4	51,6	15,5	5,8	98,8
		60	1,0	4,6	52,3	14,9	6,3	98,8
2019	Control- $N_0P_0K_0$	10	3,0	3,0	45,2	15,0	3,0	97,0
		20	1,0	3,2	43,0	15,0	5,8	98,2
		30	0,8	4,5	40,0	15,0	5,9	95,3
		40	0,5	4,3	40,3	16,3	6,0	93,5
	$N_{60}P_{40}K_{30}$	13	3,8	4,0	49,0	15,8	4,9	97,3
		27	1,9	4,1	52,3	15,8	5,8	98,0
		41	1,2	4,9	40,3	15,9	5,9	96,0
		54	0,7	4,8	50,0	16,7	6,9	94,8
	$N_{80}P_{50}K_{40}$	15	4,0	4,4	52,7	16,6	5,4	98,6
		30	2,0	4,7	56,7	17,3	6,1	98,1
		45	1,3	5,1	49,4	16,3	6,4	96,4
		60	1,0	4,8	52,3	17,1	7,1	95,7
2020	Control- $N_0P_0K_0$	11	3,5	11,0	54,8	20,1	19,3	90,1
		23	1,7	12,3	48,0	19,3	20,3	78,9
		43	1,2	11,7	49,3	17,3	25,7	79,3
		58	0,7	12,7	49,1	18,0	23,5	78,3
	$N_{60}P_{40}K_{30}$	15	4,0	12,0	55,1	22,0	24,1	90,1
		27	1,9	13,0	57,0	20,3	26,3	85,9
		43	1,2	12,1	55,0	23,0	28,3	85,3
		57	1,0	13,4	57,3	22,5	29,4	80,0
	$N_{80}P_{50}K_{40}$	15	4,0	12,1	55,4	22,2	24,5	90,6
		30	2,0	13,4	57,0	20,5	26,2	86,4
		45	1,3	12,6	55,4	23,2	28,5	85,4
		60	1,0	13,7	57,4	22,7	29,5	80,0
Average of 3 years	Control- $N_0P_0K_0$	10	3,2	5,5	48,5	16,0	8,7	92,1
		22	1,5	6,2	46,4	16,4	10,1	88,8
		37	0,9	6,6	45,8	15,7	12,0	87,6
		49	0,7	6,8	46,2	15,8	11,8	86,9
	$N_{60}P_{40}K_{30}$	14	3,8	6,5	51,4	17,5	11,1	95,5
		27	1,9	7,0	52,8	17,3	12,4	94,0
		42	1,2	7,1	48,9	17,9	13,2	93,2
		55	0,9	7,4	53,1	17,7	14,1	91,1
	$N_{80}P_{50}K_{40}$	15	4,0	6,8	52,8	18,0	11,4	96,3
		30	2,0	7,4	54,4	17,9	12,6	94,3
		45	1,3	7,4	52,1	18,3	13,6	93,5
		60	1,0	7,7	54,0	18,2	14,3	91,5

SER: Seeding rate, SHD: Shrub diameter, HGS: Height of generative shoots, LRL: Length of root leaves, WEB: Weight per bush, LVS: Percentage of leaves and vegetative shoots

Conclusion

Summarizing the results about the influence of NPK fertilizers on hay production and some yield components of crested wheatgrass (*Agropyron cristatum*) in the dry steppe zone of Eastern Kazakhstan, it is concluded that grass quality indices were mostly influenced by the rate of NPK fertilizer, by what the crude protein concentration in grass dry matter and its total yield per hectare increased considerably. The results of this study clearly indicate hay production, yield components and crude protein content was higher in $N_{80}P_{50}K_{40}$ treatment compared with $N_{60}P_{40}K_{30}$ treatment and control plots. At the end of the 3-year research conducted in dry steppe zone conditions of Eastern Kazakhstan, $N_{80}P_{50}K_{40}$ treatment is recommended for high hay production, yield components and crude protein concentration in crested wheatgrass.

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Eurasian Journal of Soil Science 2022, 11(4), 345 - 352

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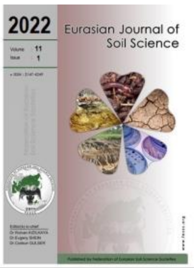

The effect of bio-humus on Cardinal grape yield (*Vitis vinifera* L.) and nutrient contents of dark brown soil using drip irrigation systems under the open field conditions

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Article Info

Received : 12.03.2022
Accepted : 02.09.2022
Available online : 07.09.2022

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Abstract

The aim of this study was to investigate impact of bio-humus on Cardinal grape yield (*Vitis vinifera* L.) and nutrient contents of dark brown soil using drip irrigation systems under the open field conditions in the vineyard region of Azerbaijan. A field experiment was conducted in a Cardinal Vineyard farm located in Shamakhi district, Azerbaijan between May and October 2021. An experiment with one dose of bio-humus treatment (5 t.ha^{-1}) and three replications, with a plot size of 1 ha treatment was used. There were performed drip irrigation, starting from May 15 up to September 15, every 15 days. The soil sampling and measurements carried out after harvest the application of bio-humus in soil and the soil samples were collected from depth of 20 cm. The results showed that addition of bio-humus increased fresh berryweight yield, contents of organic matter, total N, available P, and available micronurtient (Fe, Cu, Zn and Mn) in soil compared with control plots. The soils treated with bio-humus had significantly more EC in comparison to unamended plots. The addition of bio-humus in soil resulted in increase of soil pH.

Keywords: Bio-humus, grape yield, soil nutrients, vermicompost.

Introduction

The continuous decomposition of organic matter in cultivated soils of arid and semiarid regions may lead to soil degradation with a consequence of inability to ensure a sustainable production (Aggelides and Londra, 2000). The application of organic materials could be a way of solving two problems, the materials disposal and the correction of the low organic matter content of many agricultural soils. While chemical fertilizers are important input to enhance crop productivity, over reliance on chemical fertilizers is associated with decline in some soil properties and crop yields over time (Hepperly et al., 2009). Hence, integrated use of chemical fertilizers with organic materials is a sustainable approach for efficient nutrient usage which enhances efficiency of the chemical fertilizers while reducing nutrient losses (Candemir and Gülser, 2010; Schoebitz and Vidal, 2016). Organic material treatments could contribute significantly to the improvement of the soil organic matter content in the long term (Barral et al., 2009) and hence to the physicochemical and biological quality of the soil (Ozores-Hampton et al., 2011; Gülser et al., 2015, 2017; Cercioğlu, 2019; Yakupoğlu et al., 2021). Conventional organic materials such as farmyard manure and compost are widely used for these purposes (Ferreras et al., 2006; Herencia et al., 2007). However, in recent years, vermicompost, in many countries of the former Soviet Union such as Azerbaijan, Kazakhstan and Russian Federation, vermicompost is called bio-humus, has been emerged as an alternative to conventional organic fertilizers due to its additional benefits.

Bio-humus has been the subject of several studies related to its utilization in agriculture. The main objectives in majority of these studies have been its effect on plant growth and yield (Arancon et al., 2003; Kalantari et al., 2011; Kızılkaya et al., 2012). There are also some studies focusing on the relationship between bio-humus

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: https://doi.org/10.18393/ejss.1172178
: http://ejss.fesss.org/10.18393/ejss.1172178
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

and some soil properties under various soil conditions (Arancon et al., 2006; Gopinath et al., 2011; Doan et al., 2013; Lazcano et al., 2013). However, most of these studies were under controlled conditions in greenhouse or incubator. Therefore, in terms of soil parameters, how Bio-humus performs in field conditions is open to speculation due to lack of information. This is especially important for vineyard region in Azerbaijan whose soils are typically dark brown soil. Even though there are some studies investigating potential of bio-humus for plant growth and yield in the region (Umetov and Xudaybergenov, 2017), to our knowledge, there is no detailed study focusing on the effect of bio-humus on grape yield and nutrient contents of soil in this region.

Therefore, the aim of this study was to investigate effect of bio-humus on grape yield and nutrient contents of dark brown soil using drip irrigation systems under the open field conditions in the vineyard region of Azerbaijan.

Material and Methods

Site Description

The field experiment was located in one of the agricultural districts, Shamakhi, Azerbaijan (Figure 1). The study area is vineyard region in Azerbaijan. This region is located between 40°37′49″N and 48°38′29″E. Total areas of individual wine growing districts are much smaller than the areas of the subregions such as Avakhil and Pirgulu. Conversely, areas under vineyards are far smaller than the distribution of each wine growing district, as well as areas that have the potential for wine growing production. These areas have been increasing in recent years. The climate in study site is clasified as classified as continental climate, with a daily average air temperature of 17 °C and an annual precipitation of 600 mm (Figure 2). The eye altitude of the study site is 8.96 km.

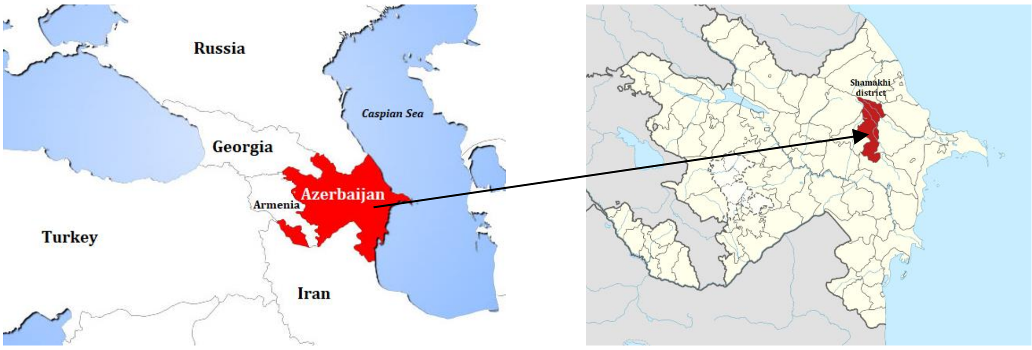

Figure 1. Study area

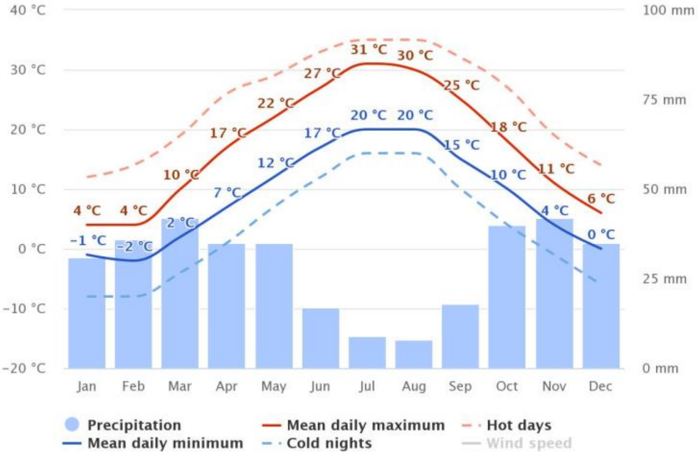

Figure 2. Monthly average temperature (^{0}C) and distribution of precipitation (mm) of the experimental area.

Bio-humus (Cattle manure vermicompost)

Bio humus was obtained from cattle manure by worm composting in a container way using earthworms 'Eisenia Fetida'. Vermicomposting duration – 2,5 months. Aqueous extract of bio humus was prepared in 6 m^3 tanks, mixing it with water at a rate of 6:10 and using a compressor for drip irrigation. The filtered aqueous extract was a dark brown odorless liquid with neutral to slightly alkaline medium reaction. The properties of the bio-humus were expressed on a dry weight basis and were analyzed by standard procedures as given in Ryan et al. (2001) and Jones et al. (1991).

Vineyard

Although it has a wide range of grape varieties in Shamakhi district, Azerbaijan, it is mostly well-known for its table grape varieties used in production of high quality. The most widespread table vineyard varieties are "Prima", "Cardinal", "Alfonz Lavelle" and "Danuta". In this study we selected "Cardinal" grape variety (*Vitis vinifera* L.) . Cardinal is certainly one of the most popular vineyard varieties of table grapes and one of the best and most proven red table grapes early due to its appearance and flavour, as well as its hardiness in withstanding handling and shipping. This grape used as a typical table grape for eating and making raisins. Grains of grapes Cardinal are large, oval-shaped and slightly raised longitudinal ribs. The skin is dark violet, medium thick, fragile. The flesh is crunchy and palatable with a mild muscat flavor (with good ripening). The grains are uneven in size. With low value variations and viral diseased vines often uneven coloring of the skin of the nipple, a tendency to strongly bunch stem necrosis and millerandage.

Irrigation

Many irrigation systems such as flood, sprinkler and drip are suitable for vineyard use. In study site in Shamakhi district, climate is continental climate and water management is a key issue for agriculture. So, in present study we selected drip irrigation system. The big reason why drip irrigation is so common among these vineyards is due to that fact that drip irrigation has a high irrigation efficiency. Drip irrigation puts the water directly where the irrigator wants it, and does not lose much water to evaporation. Drip systems are typically the most efficient and save a lot of water that other systems might lose to inefficacies. In this research, subsurface drip irrigation with two drip line positioned equidistantly between adjacent vine rows and at 35 cm deep. The drip line was mechanically installed before vineyard establishment, deep enough to prevent damage by tillage, but sufficiently shallow to supply water to the root zone without wetting the soil surface.

Experimental Procedure

A field experiment was conducted in a Cardinal Vineyard farm located in Shamakhi district, Azerbaijan between May and September 2021. The soil at the site is classified as dark brown soil. Basic soil physico-chemical properties were determined according to the Rowell (1996). The experimental plot was situated in a uniform flat land with 3-4° sloping gradient, 636 m elevation, and a planting density of 1000 vine bushes ha^{-1}. Vines were trained according to Tent system with 2 m height. Drip irrigation system was constructed since 2021. An experiment with one dose of bio-humus treatment (5 $t.ha^{-1}$) and three replications, with a plot size of 1 ha treatment was used. There were performed drip irrigation, starting from May 15 up to September 15, every 15 days. The other cultural practices were the same as for the other part of the vineyard.

Soil sampling and analyses

After harvest in September 15, the soil samples collected from depth of 20 cm were naturally air-dried, milled and passed through 2.0 mm sieve. Soil pH and EC (Electrical Conductivity) value was determined by the potentiometric method in a 1:1 suspension of soil in dH_2O using a pH meter and EC meter, respectively according to Jackson (1973) and Rhoades (1996).Soil organic matter was determined by modified Walkley-Black method (Walkley and Black, 1934). Total Nitrogen (N) by the modified Kjeldahl method (Bremner, 1965), available Phosphorus was determined by the 0.5M NaHCO3 extraction method (Olsen and Sommers, 1982), available micronutrients (Fe, Cu, Zn and Mn) content were determined by the DTPA+TEA+$CaCl_2$ extraction method (Lindsay and Norvell, 1978).

Grape yield

Data are reported from the 2021 vegetative season during harvest, the productive grape yield as a fresh berryweight and green bush density were gravimetrically determined.

Results and Discussion

Soil Properties

The experimental site is characterized by a dark brown soil with an slightly acidic soil reaction. These soils have equal amounts of silt, sand and clay particles giving them a loamy texture. As there is space between the soil particles for air and water to pass through it, this means that dark brown soils are well drained making them very fertile and ideal for agricultural practices. The soils of study are has 29.22% sand, 34.44% silt and 36.34%% clay fraction and soil textural class was named as loamy. Also, soil properties in experimental field are given in Table 1. The soil were medium in organic matter content (1.71-3%), low in electrical conductivity (<0.98 dS m−1), medium in total N (0.15-0.25%), high in available P (>18 $mg.kg^{-1}$). The soil were medium in available Fe (2.5–4.5 $mg.kg^{-1}$), high in available Cu (>0.2 $mg.kg^{-1}$), medium in available Zn (0.7–2.4 $mg.kg^{-1}$) and medium in available Mn (14–50 $mg.kg^{-1}$).

Table 1. Soil properties in experimental field

Properties		Value
Texture	Sand, %	29.22
	Silt, %	34.44
	Clay, %	36.34
	Texture class	Loamy
pH (1:1)		6.70
EC (1:1), dSm^{-1}		0.86
Organic matter, %		2,01
Total N, %		0.23
Available P, mg.kg^{-1}		21.56
Available micronutrients	Fe, mg.kg^{-1}	3.99
	Cu, mg.kg^{-1}	4.02
	Zn, mg.kg^{-1}	1.04
	Mn, mg.kg^{-1}	16.09

Bio-humus properties

The bio-humus contained high levels of macro- (N, P, K, Ca and Mg) and micronutrients (Fe, Cu, Zn and Mn), and organic matter (Table 3). However, it also had high levels of Na (1045 mg kg^{-1}) with slightly high EC value (1675 µmhos.cm^{-1}) and pH values of 7.10 (Table 2), indicating that is a non-saline organic product of neutral reaction. These values are in the expected range in stabilized and mature compost. It is important to state that the chemical properties of bio-humus are variable depending on the type and state of decomposition and storage time of the subproducts used for its elaboration.

The organic matter had values of 48.1%, which was in the range considered as adequate in this type of compost. The C/N ratio was 5.1, indicating that is a bio-humus (vermicompost) stable and mature, since according to some authors (Ndegwa and Thompson, 2000; Majlessi et al., 2012) it meets the established value for this characteristic (C/N = 15 or less). This is a direct estimation of the biological degradable fractions of C and N in the organic substrates (Jiménez and García, 1992), plus it is an index for celerity of substrate decomposition and later mineralization of their components. Similar to previous studies (Padmavathiamma et al., 2008; Fornes et al., 2012), the vermicompost or bio-humus has many favorable properties including high content of organic matter, and macro- and micronutrients, in spite of the high EC value due to its high contents of Na. This suggests that a high application rate as soil amendment is not recommended because of the salinity stress it might cause. Even after harvesting, soil bio-humus amendments had high contents of nutrient and organic matter. The results indicate that bio-humus could be used to improve soil fertility as a soil amendment.

Table 2. Chemical characteristics of Bio-humus (cattle manure vermicompost)

Properties	Value
pH (1:10)	7.1
EC (1:10), µmhos·cm^{-1}	1675.0
Organic matter, %	48.1
C/N	5.1
Total N, %	5.6
Total P, mg.kg^{-1}	675.0
Total Fe, mg.kg^{-1}	4543.0
Total Cu, mg.kg^{-1}	4.7
Total Zn, mg.kg^{-1}	156.0
Total Mn, mg.kg^{-1}	180.0

Changes in pH, EC and organic matter in soil

Bio-humus treatments increased pH values of soil. The pH of soil was 6.70 in control treatment and the value increased to 7.30 (Figure 3a). This trend may be the result of the high base content in the compost and its large capacity to absorb free protons (H^{+}) in the soils, this result is similar to that reported by Cox et al. (2001). Romaniuk et al. (2011) reported that in low pH soils, 20 Mg ha^{-1} vermicompost treatment increased soil pH from 6,06 to 6,45. Such an increase was attributed to greater pH of vermicompost than the soil. The study conducted by Mabuhay et al (2006) agreed with these results; they found that soil pH increased when organic and chemical fertilizers were applied to agricultural lands. Nastri et al (2009) observed very slight soil pH response to addition of either organic or inorganic fertilizers. Giannakis et al (2014) reported that compost application increased soil pH from 7.80 to 8.10 and 8.20 in the 50 and100 t ha^{-1} application rates, respectively, at the 0-15 cm soil layer.

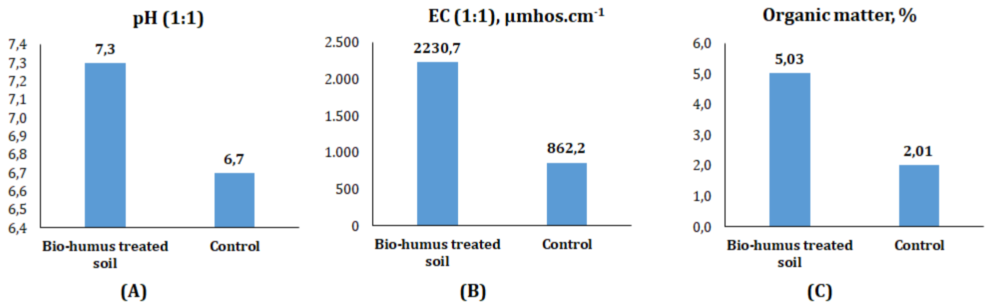

Figure 3. Changes in pH (A), EC (B) and organic matter content (C) in soil

The electrical conductivity (EC) in bio-humus was 1.6 dSm^{-1}. The soils amended with bio-humus had significantly higher EC than the untreated control soils (Figure 3b); this trend may be due to the high amount of nutrients in the applied compost. The soil EC increased with vermicompost in soil as reported by Atiyeh et al. (2001) with pig manure vermicompost substituted into Metro-Mix 360. The EC of vermicompost depends on the raw materials used for vermicomposting and their ion concentration (Atiyeh et al., 2002). Several researchers reported that addition of organic manure and compost to the soils significantly increased electrical conductivity (Candemir and Gulser, 2010; Morugán-Coronado et al., 2011; Cercioglu et al 2012; Mahmoud and Ibrahim, 2012).

Experimental bio-humus had an organic matter content of about 48%. The soils amended with bio-humus had significantly higher organic matter in soil than the untreated control soils (Figure 3c). The soil organic matter content was increased because of high organic matter content of bio-humus.. The increase in the levels of soil organic matter was expected, since, organic sources have the ability of increasing soil organic matter content. Many researchers reported that addition of organic matters on soil also increased organic matter content in soil (Cercioglu et al., 2012; Kızılkaya et al., 2012, 2020).

Changes in Nitrogen and Phosphorus content in soil

The results showed that the total N and available P concentration in soil was significantly affected by bio-humus treatments (Figure 4a). These effects may have been caused by the high content of these elements in the bio-humus (Table 2). The soils treated with bio-humus at the rate of 5 t ha^{-1} had more total N compared to soils without bio-humus treatment. Bio-humus might have produced more residual N in soil than those in control plots.

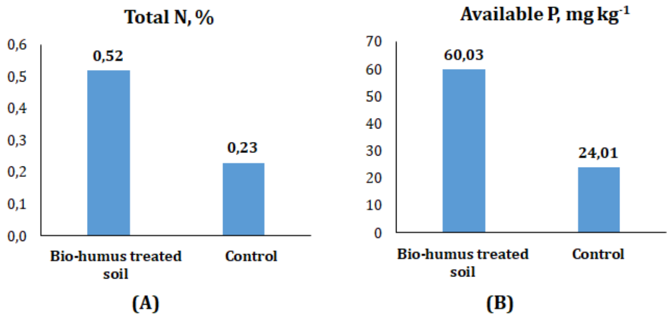

Figure 4. Changes in Total N (A) and available P (B) content in soil

Soils treated with bio-humus had significantly more available P as compared to control plots (Figure 4b). This implied that the continuous inputs of P to the soil were probably from slow release from bio-humus and release of P was due largely to the activity of soil microorganisms (Arancon et al., 2006). Marinari et al. (2000) showed similar increases in soil P after application of organic amendments such as vermicompost and/ or bio-humus. The enhancement of phosphatase activity and physical breakdown of material resulted in greater mineralization (Sharpley and Syres, 1977). In this experiment the more available P probably could have contributed to decrease of soil pH caused from application of bio-humus.

Changes in available micronutrients in soil

The data on effect of bio-humus treatment on available micronutrients (Fe, Cu, Zn and Mn) in soil are presented in Figure 5a,b,c,d. The results showed that the available micronutrients concentration in soil was significantly affected by bio-humus treatments. The total Zn content, pH and organic matter addition on soil affect the Zn availability (Alloway, 1993; Karaca et al., 2010). The application of bio-humus increased the available micronutrient concentration, almost 2.5 times as compared with the control. Bio-humus contain micronutrients (Table 2) due to the supplements in animal feeds, which could increase soil Fe, Cu, Zn and Mn contents. When organic materials are applied to soils to improve organic matter status, they also supply soils with nutrients including macro- and micronutrient concentrations in soil (Tiwari et al., 2004; Kızılkaya, 2004; Zakir et al., 2012). Our data are in agreement with this fact. On the other hand, these findings contradict some previous reports suggesting that especially nutrients in vermicompost is released more gradually in available form in soil (Nethra et al., 1999; Lazcano et al., 2008).

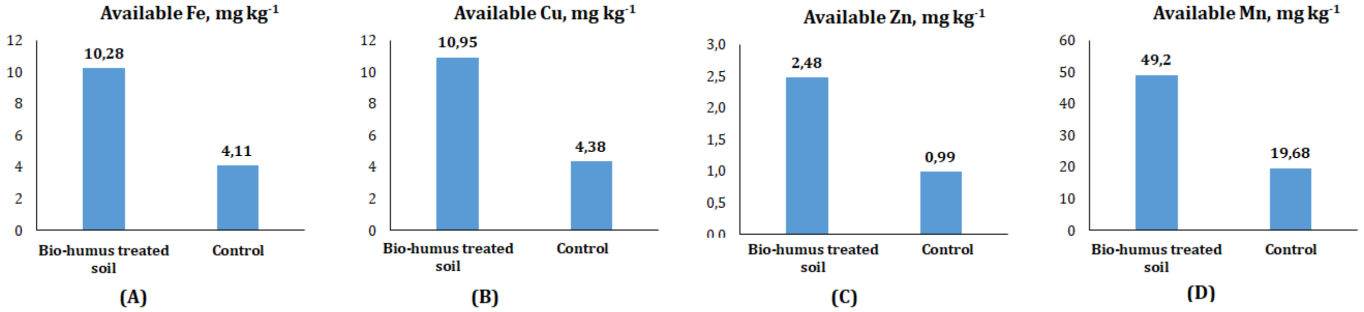

Figure 5. Changes in available Fe (A), available Cu (B), available Zn (C) and available Mn (D) content in soil

Changes in Grape yield

Results indicated that application of bio-humus showed a significant effect on the fresh berryweight yield, green bush density and tield duration (Figure 6a,b,c). All changing in soil properties show itself in grape yield, too. Fresh berryweight yield values significantly varied between 11.86 and 26.25 t ha^{-1} with an increase of 221% over the control. Green bush density became 41% more than before being fertilized and yield duration lasts 6 month that is twice more than the initial duration. Before fertilizing productivity increased in early autumn. Higher Fresh berryweight and green bush density were found after the treatments bio-humus, which is consistent with previous research showing that crop plants had increased height after vermicompost was applied (Atik and Yılmaz, 2014). This result could be due to the higher macro- and micronutrient content in soil caused by applying bio-humus in this experiment. And also, different types of phytohormones have been found in vermicomposts (bio-humus) (Zhang et al., 2014; Scaglia et al., 2016), and these phytohormones can significantly improve fruit quality. The use of organic fertilizer was shown to increase soil organic carbon and soil fertility, consequently resulting in a larger yield trend compared to a balanced chemical fertilizer (Gong et al., 2011).

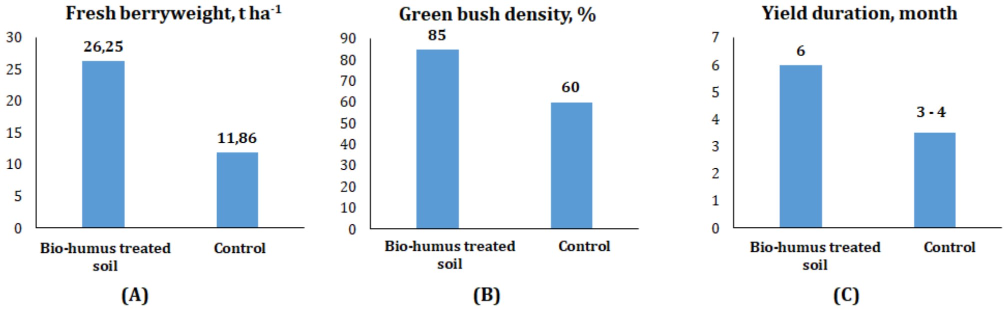

Figure 6. Changes in fresh berryweight yield (A), green bush density (B) and tield duration (C)

Conclusion

In conclusion, our results suggest that bio-humus improved organic matter and available nutrient contents of soil, thereby increasing fresh berry weight yield, and yield quality compared with control. Moreover, considering the higher electrical conductivity (EC) and pH achieved by applying bio-humus could be a better recommendation for dark brown soil using drip irrigation systems. However, more field studies are still needed to confirm our results. These studies should be designed to elucidate the long-term effects of bio-humus on nutrient contents and cycling on different soil types, to increase grape yields under sustainable production systems. The final goal is to optimize its application rates in the field to maximize yields and quality.

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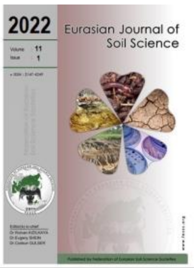

Assessing the effect of application of organic manures and grapevine pruned biomass on Thompson Seedless

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Article Info

Received : 02.06.2022
Accepted : 01.07.2022
Available online : 08.09.2022

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Abstract

Our soil continues to grapple with a number of familiar challenges like soil infertility, unfavourable soil conditions, and declining soil health as well as quality. These issues are caused by the ongoing crises of climate change, biodiversity loss, pollution, and excessive fertilizer usage alone in intensive cropping. Deterioration of soil health can be alleviated by application of organic fertilizers. With this background, the current experiment was conducted during 2013- 2016 to evaluate the effect of different organic sources *viz.* farm yard manure (FYM), green manure, press mud compost and grapevine pruning residue on Thompson Seedless and soil organic carbon content. Results indicated that maximum yield of 19.50 t/ha was obtained in T_3 (press mud @15ton/ha). The increase in yield was +10.36% and +4.62% over T_1 (only Fertigation schedule) and T_2 (FYM), respectively. Maximum petiole potassium concentration (1.63%) was recorded in T_3 at fruit bud differentiation stage. The soil organic carbon was highest in T_4 (FYM @7.5 ton/ha and Press mud @ 7.5 ton/ha) among all the treatments. The increase was +5.6%, +66.66% and +63.56% over T_1 in first, second and third year respectively. The gross returns (Rs. 319945/-), net profit (Rs. 121170/-) as well as cost benefit ratio (0.61) was maximum in case of press mud among all the organic sources. On the basis of obtained results, it can be concluded that use of press mud compost or press mud and FYM may be recommended as an organic fertilizer to improve yield and petiole nutrient content of Thompson Seedless as well as soil organic carbon content.

Keywords: Farm yard manure, press mud compost, green manure, petiole, grapes.

Introduction

The emblematic maternal relationship of human beings with fertile soil is intense, as 95 percent of global food production is supported by soil. The statistical figure anticipates a worldwide population of 9.6 billion people by the year 2050. Nourishing the burgeoning population with nutritious food will not only put an immense pressure on the present condition of soil but will also demand a healthy and fertile soil for healthy food (Euronews, 2022). Climate change, excessive usage of chemicals and fertilizers as well as desertification accentuates soil impoverishment, resulting into degraded soil health and declining soil quality (Pinamonti and Sicher, 2001; Belletti, 2002). Due to continuous soil degradation, in the present scenario, it is left with a very lesser amount of organic matter, which is almost eight times lesser than what is required for its health and proper functioning. According to FAO (2022) if the present situation persists, entire global population will be deprived of topsoil in the coming 60 years and according to the GBD (2017) *humans are not happy either.* At least, one in five early deaths occurs due to poor diet globally. The quality of food, water as well as air is very much affected by our soils. Intensive cultivation and unsustainable use of soil as well as water has led to

: https://doi.org/10.18393/ejss.1172771
: http://ejss.fesss.org/10.18393/ejss.1172771
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

productivity losses of $400 billion per year. As a result of this, we may expect an increase of 30% in food-price by 2035. Nowadays, about half of the populations rely on fertilizers for their alimentation. Farmers have been affected by the volatility of fertilizer purchase prices as well as high transportation costs in the last decades. It directly decreases profitability, strongly for productions where fertilizer account for a large part of production costs (Huang et al., 2009). Facing these challenges, other approaches for fertilization would benefit from being better known in order to feed a growing population.

The benefits of using farm yard manure (FYM) or press mud compost, green manuring, pruning residue in perennial crops such as grapevine has been demonstrated by several research reports (Garcia et al., 2018; Atwood and Wood, 2021). Viticulture is the study and practice of cultivating grapevines that produce grapes (Vitis vinifera *L.*). Variability in vineyards which is universal across the vine growing areas of the globe poses several challenges for grape growers (Delay et al., 2015). Therefore, it becomes essential for the growers to choose efficient practices aimed towards enhancing the yield of poor performing sections within vineyards, while maintaining soil health and economic sustainability. Balanced nutrition is one of the most important aspects for improving the vine productivity and nutrient content (Lester et al., 2007). Along with considering vine nutrient content, maintaining soil health as well as quality and increasing soil organic carbon content can considerably contribute to combat climate change. A good soil health is an essential pre-requisite to sustain plants, animals as well as human beings (Lehmann et al., 2020). In this context, combined application of organic manures *viz.* FYM, press mud compost, green manure which can improve soil physical, chemical as well as biological properties and grapevine pruning residue, which targets soil physical limitations, can be significant.

Organic fertilizers are plant and animal derived products that provide vital nutrients for the growth and development of crops. Organic manure plays an important role in soil through its active groups which have the ability to retain the inorganic elements in complex and chelated forms. The beneficial effects of organic fertilizers are involved in improving physical, chemical and biological characteristics of soil *viz.* increase in water holding capacity, improved soil structure, reduced bulk density, improved drainage, decrease in soil pH, increase in bacterial and fungal population as well as enzymatic activities (Mills and Fey, 2003). Organic matter on decomposition release organic acids that aid in dissolving essential nutrients and ensure their adequate supply to plants as well as improve the stability of soil aggregates (Cass and McGrath, 2004; Farooq et al., 2021). Therefore, soil organic matter is considered as the key to soil health and quality. There are a variety of organic fertilizers like FYM, dry leaf manure, press mud compost, green manure etc. (Joshi et al., 2010). Press mud, a soft, spongy, dark brown substance that helps preserve soil fertility and crop production, contains fibre, sucrose, coagulated colloids, and other biological substances. Use of green manures like sun hemp is a low cost effective technology which also conserves soil productivity (Korwa et al., 2006). The green manure crops provide a protective action against soil erosion and leaching. A large amount of grapevine pruning residue are generated by viticultural practices which is a serious concern regarding environmental as well as economic sustainability. (Liguori et al., 2013; Rondeau et al., 2013; Teixeira et al., 2014; Kammerer et al., 2014; Colantuono et al., 2017; Jesus et al., 2017). Several regulatory organisations have recently focused on the "waste" problem related to environmental sustainability (examples include European Commission Directives 1999/31/EC and 2008/98/EC).As a result, there is currently significant interest in using wine industry byproducts to satisfy the growing demand for environmentally friendly materials that can serve as vital sources of nutrients and bioactive chemicals for the food industries.

For various soil types and fruit crops, FYM has long been a crucial supply of organic matter in Indian agriculture. Grape vineyards were no exception. However, an attempt was made to partially or completely replace FYM with alternative cheaper sources of organic matter, such as press mud compost, green manure, and grapevine pruning residue in different treatment combinations, due to the higher cost and limited availability of FYM in the grape-growing regions. A better knowledge and understanding of whether or not, combined application of compost and grapevine pruning residue proves to have substantial benefits to the grape industry, would be of great significance to the farmers as well as researchers. If this practice proves to be economically sustainable, it would establish the ground work for application of organic manures into the vineyards. With this background, field experiments were carried out to see the effect of using various organic sources and pruned biomass on Thompson Seedless grapevine and soil health.

Material and Methods

Due to lesser availability of FYM in grape growing regions and its higher cost, an experiment was conducted for three successive years (2013-14 to 2015-16). The major objective was to replace FYM with other organic sources, such as press mud compost, green manure, and grapevine pruned biomass, either completely or partially. Recommended fertigation schedule was applied in all the treatments.

Vineyard site and plant material

The experiment was conducted in a vineyard situated in (40°58' N; 27°28' E; elevation 4 m a.s.l.) that was five years old. For carrying out the experiment, Thompson Seedless grafted on Dogridge rootstock was used. The rootstock was collected from nursery and the scion material was collected from vineyard blocks of ICAR-National Research Centre for Grapes, Pune.

Climate

The maximum as well as minimum temperature was 35.89 and 8.6°C during the experiment. Total rainfall was 512 mm and total pan evaporation was 1302 mm.

Soil

The soil of the experimental site was clayey (40% clay content). Recommended fertigation schedule *i.e.* 160 kg N, 50 kg P_2O_5 and 160 kg K_2O was followed in vineyard during the study period. Some of the important physico-chemical properties of initial experimental soil have been presented in Table 1.

Table 1. Some important physico-chemical properties of the initial experimental soils

Parameter	NRC for Grapes, Pune	Reference
Soil pH	8.13	Jackson (1967)
Soil EC (dS m^{-1})	0.65	Jackson (1967)
Soil texture	Clay loam	Bouyoucos (1962)
$CaCO_3$ (%)	2.74	Puri (1930)
Organic carbon (%)	1.11	Walkley and Black (1934)
Soil Available N (ppm)	179	Subbiah and Asija (1956)
Soil Available P (ppm)	32.83	Olsen (1954)
Soil Available K (ppm)	865.5	Hanway and Heidel (1952)
Soil Available Na (ppm)	1025	Hanway and Heidel (1952)
Soil Available Ca(ppm)	7884	Hanway and Heidel (1952)
Soil Available Mg (ppm)	2309	Hanway and Heidel (1952)

Treatments

The treatment details are as follows:

T_1: Control (no organic manure)

T_2: T_1+ Farm yard manure @ 15 ton/ha

T_3: T_1+ Press mud @ 15 ton/ha

T_4: T_1+ Farm yard manure @ 7.5 ton/ha+ Press mud @ 7.5 ton/ha

T_5: T_1+ Press mud @ 8.5 ton/ha + Pruned biomass @ 4 ton/ha + Green manure @ 2.5 ton/ha

The percent nutrient content in different sources of organic matter (Press mud, FYM, Green manure) has been presented in Table 2.

Table 2.Nutrient content in different sources of organic matter used

Sample	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)
Press mud	1.96	1.82	2.46	2.02	0.74	0.37	37.33	48.00	260.33	5846.00
FYM	1.50	0.57	0.92	1.27	0.71	0.22	33.67	85.33	271.00	11211.33
Green manure	40.15	0.47	3.35	1.89	1.88	0.32	17.25	74.10	7.25	178.95

Statistical analysis

Analysis of variance (ANOVA) was used for the statistical analysis. The statistical design used was randomized block design (Snedecor and Conchran, 1980). SPSS statistical software was used for the analysis (version 9.2; SAS Institute, Cary, NC). To distinguish between means from various treatments, the standard error of the mean was employed.

Results and Discussion

Effect of Organic sources on yield

Significantly highest yield of 19.50 t/ha was recorded in T_3 treatment (Press mud) over T_1 (only Fertigation schedule), however, it was on par with other treatments (Figure 1 and Table 3, 4 and 5). The increase was

+10.36% and +4.62% over T_1 (control) and T_2 (FYM) respectively. Similarly, an increase in +10.05 and +2.55 % was recorded in bunch weight in T_3 over T_1 and T_2 respectively. This may be ascribed to high sugar content and significant amount of organic carbon, macronutrients as well as micronutrients in press mud resulting into improved soil fertility and crop productivity (Liard et al., 2001; Abd El Hady et al., 2003; Banulekha, 2007; Joshi et al., 2010; Myburgh, 2013). An increase in yield was observed in first, second and third year as a result of compost application. It may be attributed to higher 100-berry weight and more bunch weight (Ahmed et al., 2000; Harhash and Abd EL-Nasser, 2000; Kassem and Marzouk, 2002). Various research reports revealed an increase in vine growth and yield per hectare due to addition of compost in vineyards (Rubio et al., 2013; Gaiotti et al., 2017; Ramos, 2017; Brunetto et al., 2018). Because compost has a narrow area of contact with the soil surface, it releases nitrogen slowly into the soil, allowing the grapevine to use more of the organic compost's nitrogen. (Korboulewsky et al., 2002; Morlat and Chassod, 2008; Bustamante et al., 2011). Since, nitrogen is considered essential for vegetative growth of plant, incorporation of compost results into higher yield of grapes. Addition of organic manures into the soil improves the microbiological activity in the root zone. It also aids to an increase in soil porosity, soil aggregates, water holding capacity, thereby contributing to vineyard health and productivity. Continuous and sustained supply of nutrients due to application of compost also contributes towards higher yield.

Among all the organic treatments, the maximum total soluble solids (TSS) was found in T_5 (FYM + Press mud + Pruned biomass + Green manuring) in pooled analysis which was 21.43°B (Figure 1). The increase was +1.18%, +3.38% and +1.99% over T_2, T_3 and T_4 respectively. High TSS in the present study was due to more release of nutrients which is involved in synthesis of carbohydrate and proteins as well as breakdown and transloaction of photosynthetic products (starch) from leaves to developing fruits and thereby increasing the total sugars. Also, press mud compost is rich in sugar resulting into more TSS. This may also be credited to increased population of microorganisms who might have contributed in the release of phytohormones *viz.* auxins, gibberellins and cytokinins due to their increased metabolic activity. The beneficial properties of grapevine leaves may be attributed to the phenolic compounds (phenolic acids, flavonols, mainly in the form of O-glycosides of quercetin and kaempferol and, to a lesser extent, by stilbenes (resveratrol), flavan-3-ols, and anthocyanins and secondary metabolites, correlated with antioxidant activity (Doshi et al., 2006; Monagas et al., 2006; Fernandes et al., 2013; Katalinic et al., 2013; Krol et al., 2014; Fontana et al., 2017; Barreales et al., 2019). Among all the organic treatments, the minimum acidity (6.56%) was recorded in T_3 (Press mud) followed by T_4 (FYM + press mud) in which it was 6.58%. The decrease was -2.6% and -2.4% over control (T_1) in which the acidity was 6.74% (Figure 1).

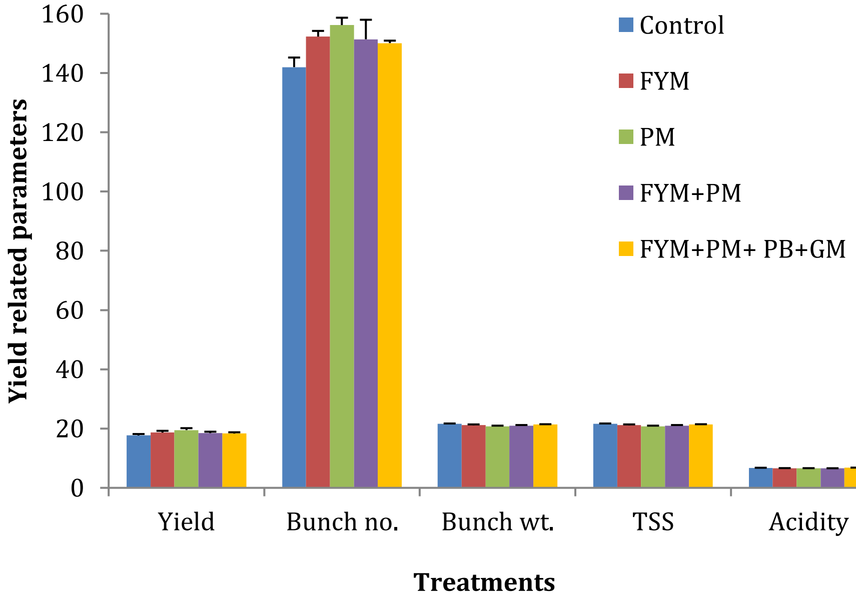

Figure 1. Effect of treatments on yield and yield related parameters (pooled data)

Table 3. Effect of treatments on yield and yield related parameters (First year)

Treatments	Yield (t/ha)	Bunch no.	Bunch wt.(g)	TSS (°B)	Acidity (%)
T1	17.65±0.41a	127.90±3.64a	20.43±0.41a	20.43±0.41a	6.73±0.05a
T2	18.80±0.68a	135.73±5.09a	20.48±0.12a	20.48±0.12a	6.63±0.05a
T3	19.05±0.59a	140.86±5.49a	20.30±0.34a	20.30±0.34a	6.65±0.12a
T4	18.97±0.75a	137.40±5.27a	20.95±0.32a	20.95±0.32a	6.60±0.04a
T5	17.90±0.38a	132.23±2.79a	20.55±0.23a	20.55±0.23a	6.88±0.13a

Table 4. Effect of treatments on yield and yield related parameters (Second year)

Treatments	Yield (t/ha)	Bunch no.	Bunch wt.(g)	TSS (°B)	Acidity (%)
T1	16.98±0.53a	13.02±6.56a	22.20±0.18b	22.20±0.19b	7.23±0.05a
T2	18.23±0.87a	137.52±8.95a	21.05±0.66ab	21.05±0.67ab	7.00±0.41a
T3	18.82±1.32a	140.35±9.15a	19.80±0.58a	19.80±0.58a	7.00±0.07a
T4	17.87±0.49a	131.23±12.48a	19.78±0.44a	19.78±0.44a	7.00±0.11a
T5	18.27±0.67a	137.19±2.47a	21.63±0.24b	21.63±0.24b	7.23±0.09a

Table 5. Effect of treatments on yield and yield related parameters (Third year)

Treatments	Yield(t/ha)	Bunch no.	Bunch wt.(g)	TSS (°B)	Acidity (%)
T1	18.38±1.24a	183.90±4.89a	22.15±0.19a	22.15±0.19a	6.28±0.11a
T2	18.88±1.24a	195.97±11.63a	22.00±14.72a	22.00±0.15a	6.23±0.10a
T3	20.63±1.62a	199.83±19.97a	22.08±0.08a	22.08±0.75a	6.03±0.08a
T4	18.58±0.89a	205.71±5.75a	22.30±0.07a	22.30±0.07a	6.13±0.05a
T5	18.79±1.31a	193.34±7.36a	22.13±0.15a	22.13±0.15a	6.25±0.06a

Effect of organic sources on petiole nutrient content

Maximum nitrogen (1.06%), phosphorus (0.46%), potassium (2.52%), and calcium (0.96%) concentration in petiole was obtained in T_5 at flowering stage whereas maximum potassium content (1.63%) at fruit bud differentiation was found in T_3 (Figure 2, 3 and Table 6, 7, 8, 9 and 10). The petiole potassium concentration was higher in those treatments where press mud compost was used either alone or with other organic sources. Exchangeable Ca^{2+} and Mg^{2+} also increased due to organic sources. These findings were in accordance with Ahmed et al. (2000); Morlat and Chassod (2008); Chan et al. (2010); Bustamante et al. (2011) and Rubio et al. (2013). This suggests that compost application can result in improved vine nutritional status. In vineyards, green manure can also be used as a nutrient source to restore nitrogen to grape (Cherr et al., 2006; Scneider and Huyghe, 2015; Garcia et al., 2018). Soil nitrogen absorption is greatly enhanced from blooming to veraison stages (Conradie, 1986). As grape nitrogen needs are the most important from bud burst to veraison and reach a peak at blooming, soil fertility here consists in a good availability of nutrients near blooming. Green manures including leguminous have already proved itself to provide nitrogen to grapes (Gontier, 2013). An increase in available phosphorus was observed due to organic manures incorporation. It may be attributed to competitive inhibition of phosphorus sorption due to the organic acids and anions released as a result of decomposition of organic matter (Korboulewsky et al., 2002; Calleja-Cervantes et al., 2015a, Wilson et al., 2016).

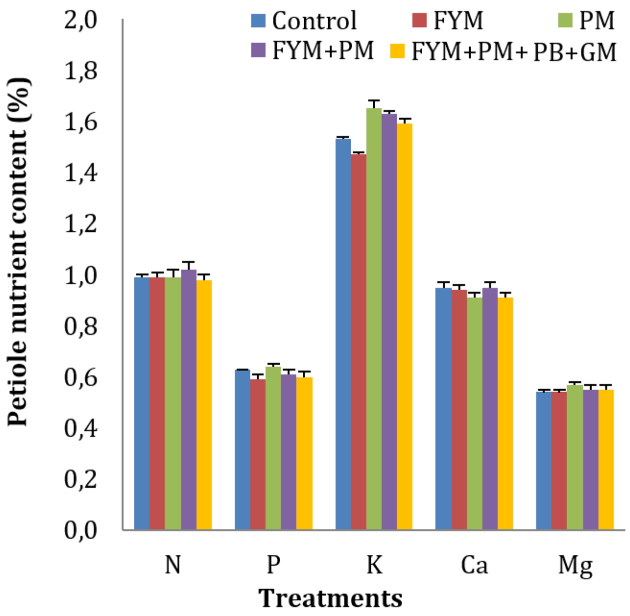

Figure 2. Effect of treatments on petiole nutrient content (%) at fruit bud differentiation stage (pooled data)

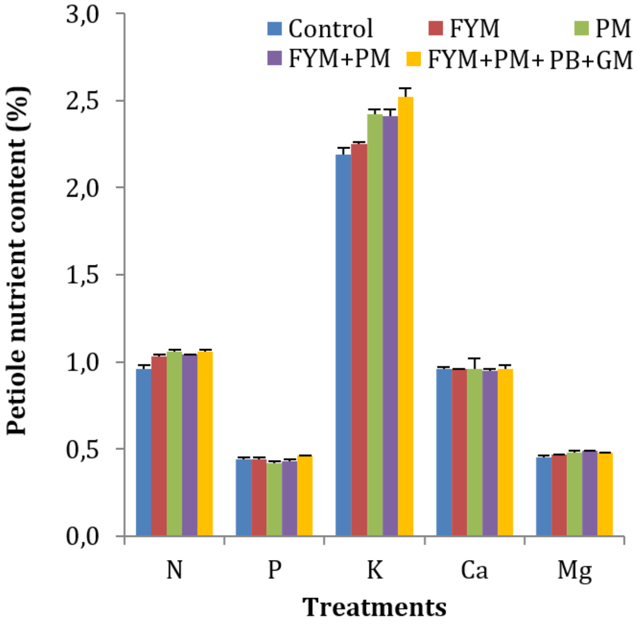

Figure 3. Effect of treatments on petiole nutrient content (%) at flowering stage (pooled data)

Table 6. Effect of treatments on petiole nutrient content (%) at fruit bud differentiation stage (First year)

Treatments	N	P	K	Ca	Mg
T1	0.87± 0.01a	0.69±0.02b	1.88±0.01a	0.78±0.00b	0.55±0.02c
T2	0.89± 0.03ab	0.63±0.02a	1.83±0.01a	0.75±0.00a	0.55±0.02b
T3	0.87± 0.02a	0.69±0.02b	2.05±0.07b	0.74±0.00a	0.62±0.02b
T4	0.95± 0.02b	0.64±0.02ab	2.07±0.01b	0.77±0.01b	0.60±0.05d
T5	0.88±0.02a	0.63±0.02ab	2.18±0.02c	0.75±0.01a	0.61±0.04a

Table 7. Effect of treatments on petiole nutrient content (%) at fruit bud differentiation stage (Second year)

Treatments	N	P	K	Ca	Mg
T1	1.09±0.04a	0.57±0.01a	1.19±0.02ab	1.13±0.04a	0.55±0.02a
T2	1.09±0.03a	0.57±0.02a	1.12±0.02a	1.12±0.04a	0.55±0.02a
T3	1.11±0.04a	0.59±0.03a	1.24±0.03b	1.08±0.03a	0.62±0.02a
T4	1.09±0.04a	0.59±0.24a	1.19±0.03ab	1.13±0.05a	0.60±0.46a
T5	1.08±0.02a	0.57±0.02a	1.18±0.04ab	1.07±0.04a	0.61±0.04a

Table 8. Effect of treatments on petiole nutrient content (%) at flowering stage (First year)

Treatments	N	P	K	Ca	Mg
T1	1.07±0.06a	0.45±0.01a	2.15±0.03a	1.38±0.03a	0.59±0.01a
T2	1.20±0.04b	0.44±0.01a	2.28±0.03b	1.39±0.01a	0.59±0.01a
T3	1.24±0.02b	0.44±0.01a	2.43±0.04b	1.40±0.01a	0.61±0.02ab
T4	1.24±0.01b	0.45±0.01a	2.37±0.08b	1.39±0.01a	0.63±0.01b
T5	1.26±0.01b	0.45±0.01a	2.32±0.05b	1.39±0.01a	0.63±0.01b

Table 9. Effect of treatments on petiole nutrient content (%) at flowering stage (Second year)

Treatments	N	P	K	Ca	Mg
T1	0.90±0.01a	0.32±0.01ab	2.52±0.08ab	0.76±0.01a	0.37±0.01a
T2	0.94±0.01ab	0.31±0.00a	2.50±0.01a	0.79±0.00b	0.39±0.01a
T3	0.97±0.01c	0.32±0.00b	2.67±0.03bc	0.76±0.01a	0.38±0.00a
T4	0.92±0.01ab	0.34±0.00c	2.73±0.02c	0.76±0.01a	0.39±0.01a
T5	0.94±0.02bc	0.35±0.01d	2.92±0.07d	0.76±0.01a	0.36±0.01a

Table 10. Effect of treatments on petiole nutrient content (%) at flowering stage (Third year)

Treatments	N	P	K	Ca	Mg
T1	0.91±0.01a	0.56±0.15ab	1.91± 0.03a	0.75±0.00a	0.38±0.08a
T2	0.95±0.01ab	0.57±0.03ab	1.96± 0.01ab	0.71±0.02a	0.43±0.01b
T3	0.98±0.02b	0.51±0.01a	2.16±0.06bc	0.72±0.01a	0.44±0.01b
T4	0.95±0.01ab	0.52±0.01ab	2.14± 0.08bc	0.71±0.01a	0.44±0.01b
T5	0.99±0.02b	0.57±0.01b	2.31± 0.12c	0.74±0.04a	0.43±0.01b

Effect of organic sources on soil organic carbon

The soil organic carbon was highest in T_4 (FYM @7.5 ton/ha plus Press mud @ 7.5 ton/ha) among all the treatments. The increase was +5.6%, +66.66% and +63.56% over T_1, +4.16%, +19.46% and +34.39% over T_2 (FYM) and +2.74%, +7.14%, +3.43% over T_3 (Press mud compost) in first, second and third year respectively (Figure 4).

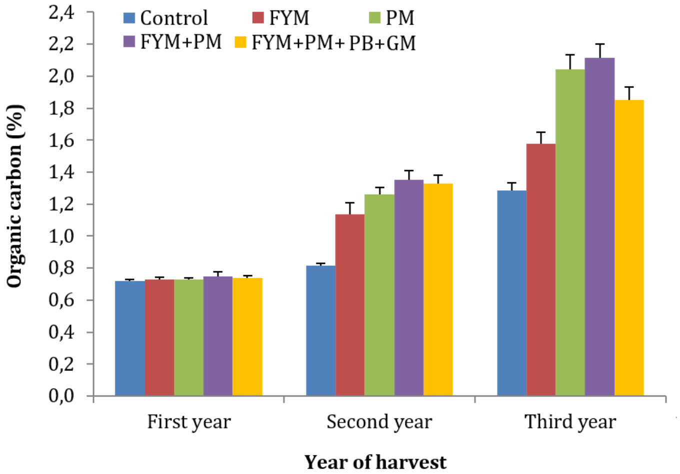

Figure 4. Effect of treatments on soil organic carbon (%)

In the treatments that supplied organic sources, the soil organic carbon accumulated significantly over T_1 (control). Similar results have been reported by Biala (2000); Pinamonti and Sicher (2001) and Martinez et al. (2018). Increased soil organic carbon content may result in improved soil aggregation, improved water holding capacity of soil as well as infilteration, reduced bulk density, improved porosity, increased microbial activity and potential soil carbon sequestration (Morlat and Chassod, 2008; Bustamante et al., 2011; Ramos, 2017, Martinez et al., 2018). More research is needed to have a better understanding about the effect of compost additions on soil carbon content in vineyards (Lazcano et al., 2020).

Effect of organic sources on cost benefit ratio

Form Table 11, it is clear that amongst all the organic sources, the gross returns (Rs.319945), net profit (Rs. 121170) and the cost-benefit ratio (0.611) was highest in T_3 (Press mud compost).These results obtained revealed that press mud compost or press mud compost plus other organic sources like FYM, green manure and pruned biomass can be used as one of the most economic sources of plant essential nutrients as well as soil organic carbon for sustainable grape production (Elsayed et al., 2008).

Table 11. Cost benefit ratio of the treatments

Treatments	Yield (t/acre)	Gross returns** (Rs)	Gross returns*** ($)	Recurring cost (Rs)	Recurring cost ($)	Net profit (Rs)	Net profit ($)	Cost benefit ratio (Net profit/ Recurring cost)
T1	7.16	286350.0	3722.55	164675	2140.78	121675.0	1581.78	0.739
T2	7.51	300488.2	3906.35	190775	2480.08	109713.2	1426.27	0.575
T3	7.99	319445.0	4152.79	198275	2577.58	121170.0	1575.21	0.611
T4	7.38	295141.4	3836.84	205775	2675.08	89366.4	1161.76	0.434
T5	7.50	300146.6	3901.91	205775	2675.08	94371.65	1226.83	0.459

** Sale price of produce @Rs 40 /kg

*** Sale price of produce @ $ 0.52/kg

Conclusion

From this study it is clear that adding press mud compost or press mud plus other organic manures in Thompson Seedless increased yield, bunch number, vine nutritional status and soil organic carbon content. The use of these organic sources is particularly complementary to the goals of organic viticulture. We need to implement good practices that are a combination of scientific and local knowledge for re-setting the balance and harmony of our soils. A balance should be maintained between organic matter accumulation and utilization to maintain soil fertility and to feed the global population. While compost application may demand immediate costs, the long-term financial benefits can be significant, as well as the benefits to the soil environment. Therefore, press mud compost alone or in combination with other organic amendments can be used as a cheaper source of organic fertilizer.

Future prospects: Despite having a lower concentration of nutrients, organic manures nevertheless contain many of the necessary elements for plant growth and release them over a longer time period. Therefore, they are advantageous over chemical fertilizers, which only provide plants with a limited number of nutrients for a short time. Additionally, the country's soil quality is declining as a result of the unbalanced use of fertilizers. These facts led the Ministry of Agriculture Development (MoAD) to introduce a number of organic intervention initiatives designed to improve soil health, reduce reliance on chemical fertilizers, and lower crop production costs. Vermi-composting, cattle shed development and the establishment of organic fertilizer plants, are a few of these that the nation has implemented in various Financial Years (FYs).These programmes are currently in the implementation phase. Although these initiatives have been found to be successful in educating farmers on the value of organic manure in improving soil health. The demonstration effect that these initiatives have had on the village level has motivated farmers who are not a part of the programmes to properly manage the organic manure produced at the home level. Some of the issues preventing the proper execution of programmes include harsh topography in hilly and mountainous areas, lack of availability in a timely manner, and installation of production plants far from the demand centres. Instead of constructing production facilities in dense metropolitan regions, it is preferable to encourage their establishment in and around demand centres, hilly areas, and mountainous locations.

Acknowledgment

VDR and TM would like to recognize the financial support of the Ministry of Education and Science of Russia (Agreement No. 075-15-2021-1363). Thank you so much for kindness and support

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