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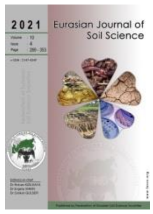

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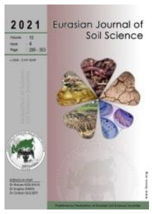

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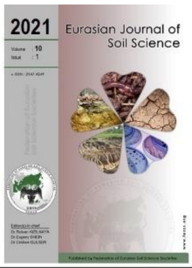

Reduced plant uptake of PAHs from soil amended with sunflower husk biochar

Svetlana Sushkova [a,*], Tatiana Minkina [a], Tamara Dudnikova [a], Andrey Barbashev [a], Yuryi Popov [a], Vishnu Rajput [a], Tatiana Bauer [a], Olga Nazarenko [b], Rıdvan Kızılkaya [c,d]

[a] Southern Federal University, Academy of Biology and Biotechnology D.I. Ivanovsky, Rostov-on-Don, Russia
[b] Federal State Budgetary Institution State Center of the Agrochemical Service Rostov, Rostov-on-Don, Russia
[c] Ondokuz Mayıs University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Samsun, Turkey
[d] Agrobigen Research & Development Trade Ltd.Co, Samsun Technopark, Samsun, Turkey

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Author(s)

S. Sushkova *
T. Minkina
T. Dudnikova
A. Barbashev
Y. Popov
V. Rajput
T. Bauer
O. Nazarenko
R. Kızılkaya

* Corresponding author

Abstract

Biochar effect on the polycyclic aromatic hydrocarbons (PAHs) uptake by spring barley (*Hordeum Sativum*) was studied in model experiment conditions with Haplic Chernozem spiked by the high doses of benzo[a]pyrene (BaP) (400, 800 and 1200 μg kg^{-1}), as the main marker of PAHs contamination. The relevance of the study is due to the BaP stability in natural environments and its carcinogenicity in relation to all living organisms. The express method of subcritical water extraction was used for BaP extraction from samples. The soil contamination by BaP contributed to the PAHs accumulation in soil and plants uptake from the polluted soil. It was found the 1% biochar application dose in the variant with 400 μg kg^{-1} contamination decreased the alone BaP and total PAHs content in soil and spring barley up to 50% compared to the contaminated variant. In soil contaminated with 800 μg kg^{-1} the 5% of biochar application led to the BaP content decreasing in the soil up to 56% and in the plants to 40-60%. Application of 5% biochar in the soil polluted with 1200 μg kg^{-1} led to the BaP and total PAHs content decreasing in soil up to 47% and 30%, respectively, plants the BaP content decreased up to 37-48%. Biochar 5% amendment effectiveness has been shown on the plants grown on the highly toxic variant contaminated with 1200 μg kg^{-1} BaP. The earing phase was inhibited in the spring barley plants growth at the most contaminated soil of the model experiment, whereas biochar application into the soil promoted the successful formation of the corn. The used biochar showed a high sorption capacity and its effectiveness under the soil remediation contaminated with BaP.

Keywords: Carbon sorbent, vegetation experiment, remediation of soil, plant uptake, PAHs.

Introduction

Benzo[a]pyrene (BaP) is one of the most dangerous organic contaminants in the environment and the most toxic, carcinogenic, and mutagenic representatives of the polycyclic aromatic hydrocarbons (PAHs), first-class dangerous compound (Tobiszewski and Namieśnik, 2012; IARC, 2020; Abdel-Shafy and Mansour, 2016). Its background content in Haplic Chernozems varies at the level of 15–25 μg kg^{-1}, which is due to the increased content of highly condensed organic matter in the Chernozems soil and its soil microflora specific composition (Sushkova et al., 2017). Absorbing on the soil surface as a pollutant the BaP is involved in the

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processes of distribution in the soil-plant system (Tsibart and Gennadiev, 2013). Plants are an integral component of terrestrial ecosystems and are exposed to the entire group of pollutants, and almost 45% of all PAHs released into the environment uptake in plants (Kang et al., 2010). The complex of soil - plants interaction is an important object of environmental pollution control by BaP. Since soils and plants function in close relationship with each other, a whole system arises that connects the processes of accumulation and transformation of BaP in soils and plants (Li et al., 2015).

Currently, there are no full-fledged investigations of the mechanisms of the BaP uptake by plants, especially, connected with reducing BaP bioavailability to plants. BaP can sorb on the root cell walls, for example, which is reported by Pretorius (2018). BaP sorption processes proceed according to the lipophilicity of the compound molecule (Kang et al., 2010). It is noted that BaP diffusion into the plant root is very slow (Li et al., 2015). It has been shown that the BaP content in cereals could exceed 1 μg kg^{-1} (Li et al, 2016; Liu et al, 2017; Tian et al., 2018). This process can relate to the level of soil contamination by the toxicant (Ni at al., 2017; Chen et al., 2019). Also, it was established that the plant species have a different tolerance to BaP contamination. For example, cereal plants are capable to accumulate BaP up to 30-40% of their total content in the soil (Roszko et al., 2020). Thus, the assessment of soil toxicity to different species of plants is complicated by the pollutant's uptake selectivity (Ni et al., 2017). The same level of BaP contamination led to the different degree of soil toxicity for each agricultural crop (Iljina et al, 2020). Thus, the costs of reclamation of such soils can be unjustifiably overestimated or, conversely, underestimated, which increases the relevance of research in this direction (Kuppusamy et al., 2017; Li et al., 2020).

There are several sorbents that can be used for soil remediation under polyaromatic hydrocarbons contamination (Carvalho et al., 2015). This soil remediation technology is not only low-cost, easy to use, but highly effective both in terms of selective removal of pollutants and in terms of restoring soil quality (Lima, 2018). Carbon sorbents, such as biochar, are the most often used for soil remediation (Vardhan et al., 2019; Kołtowski et al., 2017). The global production of biochar alone reaches hundreds of thousand tons per year (Eeshwarasinghe et al., 2019). Biochar is characterized by a large specific pore surface area, and high stability in soil (Qin et al., 2013; Huggins et al., 2016; Liu et al., 2017; Yakovleva et al., 2017).

The purpose of this research was to establish the reduced spring barley (*Hordeum Sativum*) plant uptake of PAHs from soil amended with biochar in the artificially contaminated Haplic Chernozem. The mechanisms of BaP uptake will be determined for spring barley as the most common agricultural plant in the Chernozem distribution area. Thus, this model experiment will provide unique information about the consequences of technogenic contamination in the Chernozem zone of the South of Russia that will help to correctly choose the ways of remediation and cultivating the agricultural crops in this region.

Material and Methods

Model experiment design

A model laboratory experiment was laid to study the biochar effect on the PAHs uptake by spring barley (*Hordeum Sativum*). The soil (0-20 cm) of the specially protected natural area "Persianovskyi Preserve Steppe" was used sampled from the territory located at the Rostov Region, southern Russia (Figure 1), which is represented by Haplic Chernozem (Table 1). Haplic Chernozem soils occupy a significant area in the large agro-industrial regions of southern Russia, such as the Rostov Region and Krasnodar Krai territory.

Table 1. Physical and chemical properties of Haplic Chernozem (0-20 cm)

Silt particles (<0.02 mm), %	Clay particles (<0.002 mm), %	$C_{org.}$, %	pH	$CaCO_3$, %	Ca^{2+} +Mg^{2+}, mmol(+)/100g	CEC, mmol(+)/100 g
48.1±1.4*	28.6±1.2	3.7±0.3	7.3±0.1	0.3±0.1	35.0±3.0	37.1±2.9

± the standard deviation (SD)

The selected soil was cleaned of plant residues and other inclusions, grounded in a porcelain mortar, and passed through a sieve with a hole diameter of 1 mm. Air-dry soil 4 kg was placed in the vegetative vessels, and an aqueous solution of BaP in acetonitrile (400, 800 and 1200 μg kg^{-1}) was added to the soil surface. The choice of doses of pollutants was determined by the existing levels of soil pollution in the Rostov Region (Sushkova et al., 2020). After the application of pollutants, the soil was incubated for 1 week, and then biochar was added in doses from 1% and 5% of the total soil volume.

The moisture content was maintained at 60% of the total field moisture capacity during the entire incubation period in the soil of the phytotoxicity model laboratory experiment (GOST RISO 22030-2009).

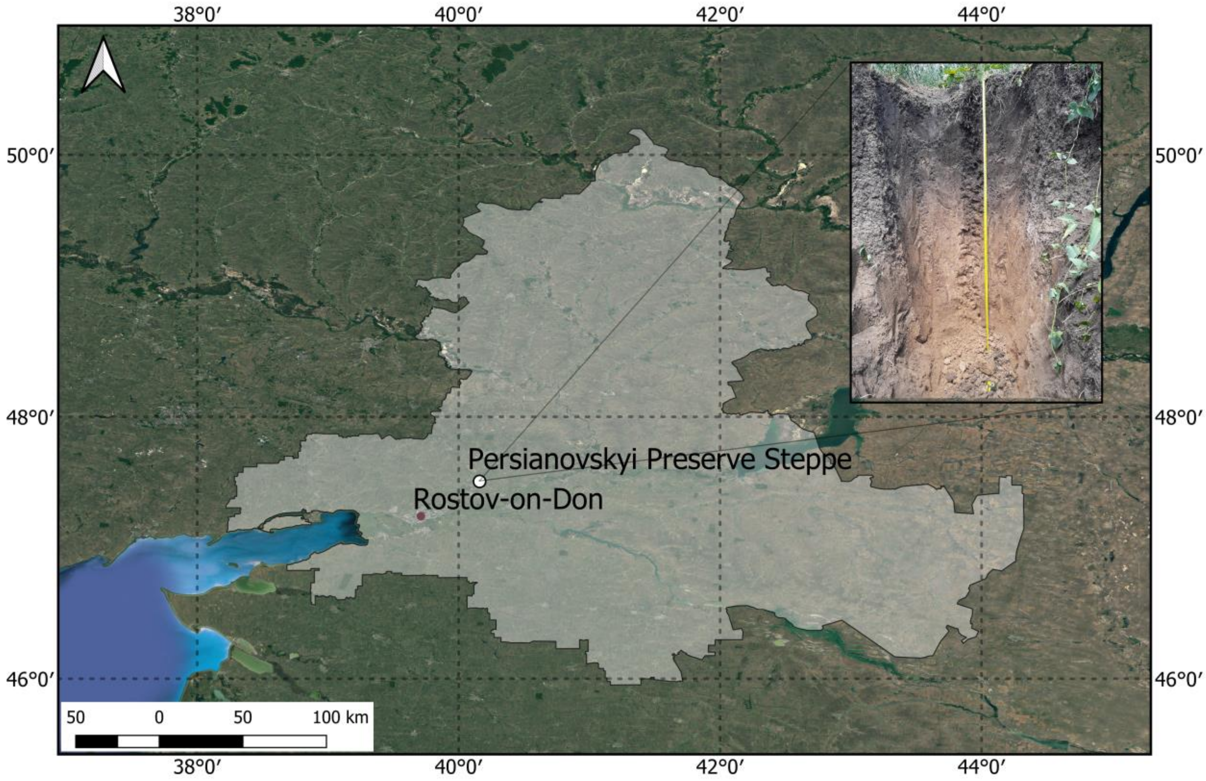

Figure 1. Soil sampling map.

After one month of incubating the soil with sorbents, the seeds of spring barley of the two-row variety Ratnik (*Hordeum Sativum*) of the Poaceae Family, which is one of the main grain crops grown in the Rostov region, were sown in the amount of 20 seeds per cup. Plants were sampled 3 months after sowing, and the PAHs content was measured in the soil, roots, stem, and corn of spring barley. The experiment was settled in 3 replications.

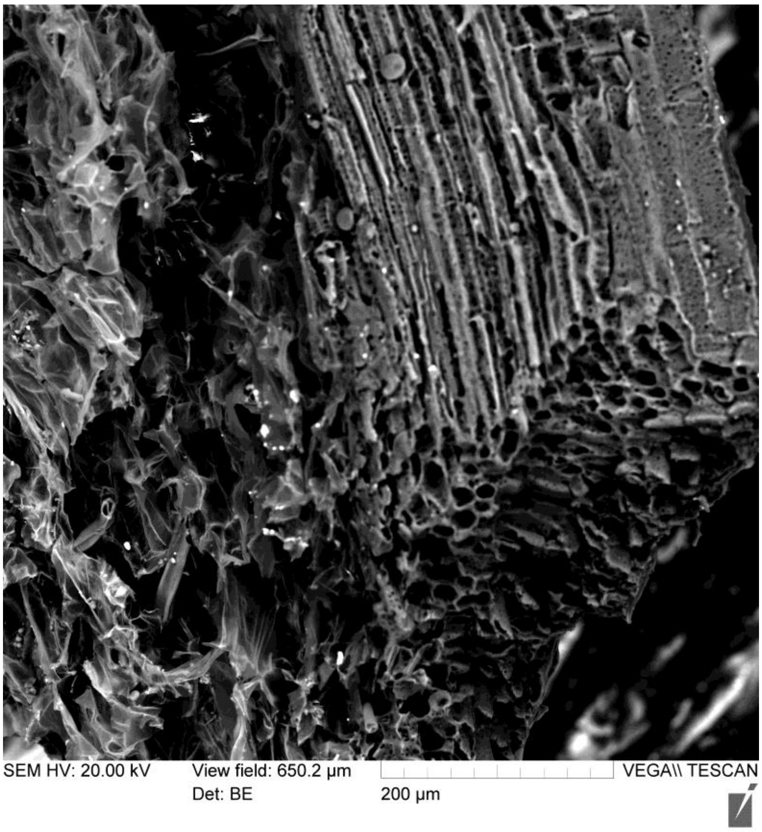

Figure 2. SEM image of the biochar from the regional sunflower husk.

Biochar made from sunflower husks at a pyrolysis temperature of 500°C was used for the model experiment. Scanning electron microscopy image of the biochar surface shows the various pores structure perspective for the PAHs adsorption from the soil (Figure 2).

Extraction of BaP from soil and plant samples

BaP was extracted from soils and plants using the method of subcritical water extraction (Sushkova et al., 2016). A 1 g weighed portion of soil/plants (roots, stem, corn) was placed in an extraction cartridge, and 8 ml of bidistilled water was added after it was tightly screwed on both sides with bolts. A pressure gauge with a built-in emergency pressure relief valve was connected to the cartridge so that the pressure inside the cartridge was equal 55-60 atm. The cartridge was installed in a thermostat and heated to 250°C for 30 min. After cooling the system, the cartridge was unscrewed, the contents were filtered 3 times through a paper filter with a blue ribbon into a glass conical flask to a clear solution, each time rinsing the filter with 2 ml of distilled water. From the resulting aqueous extract, BaP was re-extracted three times with n-hexane (analytical grade). For that purpose, the 5 ml of hexane was poured into the flask, closed with a glass stopper, and shaken on a shaker for 15 minutes. Separation of layers was carried out on a 50 ml separating funnel sequentially in three stages with the next portion of hexane. The combined hexane extract was passed through a funnel with calcined anhydrous sodium sulfate, after which the extract was evaporated in a pear-shaped flask on a rotary evaporator at a water bath temperature of 40°C to a dry residue. The resulting residue was dissolved in 1 ml of acetonitrile with stirring for 30 minutes, and the concentration of BaP in the extract was determined by HPLC. The completeness of BaP extraction was determined by the addition method, for which a soil sample of 1 g was placed in a flask for a rotary evaporator and a certain amount of a standard BaP solution in acetonitrile was added based on the creation of a BaP concentration in the sample of 2, 4, 6, 8, 16, or 32 μg kg^{-1}. After evaporation of the solvent for 30 min under room conditions, the analyte was kept at 7°C for 24 hours, and then the samples were analyzed by a high-performance liquid chromatography (HPLC) according to the certified procedures (MUK 4.1.1274-03. 4.1; ISO 13877-2005) using the system with fluorometric detection 1260 Infinity Agilent (USA).

The PAHs content was determined by the standardized external method (Procedure of measurements ..., 2008). The following equation was used to express the PAH's content:

$$C_s = k\, S_i \times C_{st} \times V / (S_{st} \times m), \quad (1)$$

where C_s and C_{st} are the PAH concentrations determined in the soil sample and the standard PAH solution, respectively (μg kg^{-1}). S_{st} and S_i correspond to areas under the peak of PAH standard solution, and studied sample, respectively. V, k, and m are assigned to the acetonitrile volume used for extraction (mL), the recovery factor for PAH from the sample and the mass sample (g), respectively. The efficiency of target PAHs extraction from soils was calculated using a spike matrix.

Samples were analyzed for 16 PAHs (naphthalene; chrysene; phenanthrene; benzo[k]fluoranthene; anthracene; a cenaphthylene; pyrene; fluoranthene; biphenyl; benzo[a]anthracene; benzo[b]fluoranthene; acenaphthene; benzo[a]pyrene; dibenzo[a,h]anthracene; fluorine; benzo[g,h,i]perylene) with an Agilent 1260 (Germany) Infinity high-performance liquid chromatography (HPLC) equipped with a fluorescence detector following the ISO 13859:2014 requirements. The HPLC system was coupled to reversed-phase column Hypersil BDS C18 (150 × 4.6 mm, 5 μm) with a mixture of acetonitrile and ultrapure water as the mobile phase. Compounds were identified according to the retention time recorded by the corresponding analytical standard samples. HPLC grade acetonitrile (99.9%, analytical grade), anhydrous Na_2SO_4, n-hexane (99%, analytical grade), ethanol (96%, analytical grade), potassium hydrate (98%, analytical grade), and NaOH (97%, analytical grade), were used in the analysis. All research results were performed in 3-fold analytical replication.

Statistical analysis of the results

SigmaPlot 12.5 and Statistica 10 were used for processing data. The reliability of differences between the experimental variants was assessed using the Student's t-test, with a fixed p-level <0.05. The relationships between the variables were estimated using linear regression with a fixed p-level <0.001.

Results and Discussion

BaP content in the soil of the model experiment

After 4 months of model experiment contamination, it has been established that contamination leads to BaP and priority PAHs accumulation in the soil of the model experiment (Figure 3). The content of BaP in Persianovskyi Preserve Steppe as control soil amounted to 17.6±0.7 μg kg^{-1} that is lower MPC (GN 2.1.7.2041-06. 2.1.7, 2006), total PAHs content was at the level 230.5±11.1 μg kg^{-1} that corresponds to our previous research data (Sushkova et al., 2017; Sushkova et al., 2020). BaP content in soil contaminated with doses of 400 μg kg^{-1}, 800 μg kg^{-1} and 1200 μg kg^{-1} promoted an increase of the BaP content in the soil to

355.6±13.3 μg kg^{-1}, 620.1±32.2 μg kg^{-1} and 870.1±44.3 μg kg^{-1}, respectively, as well, it was established a total PAHs content increasing in the BaP contaminated soil up to 660.9±31.7 μg kg^{-1}, 1154.5±58.3 μg kg^{-1} and 1350.7±68.9 μg kg^{-1}, respectively.

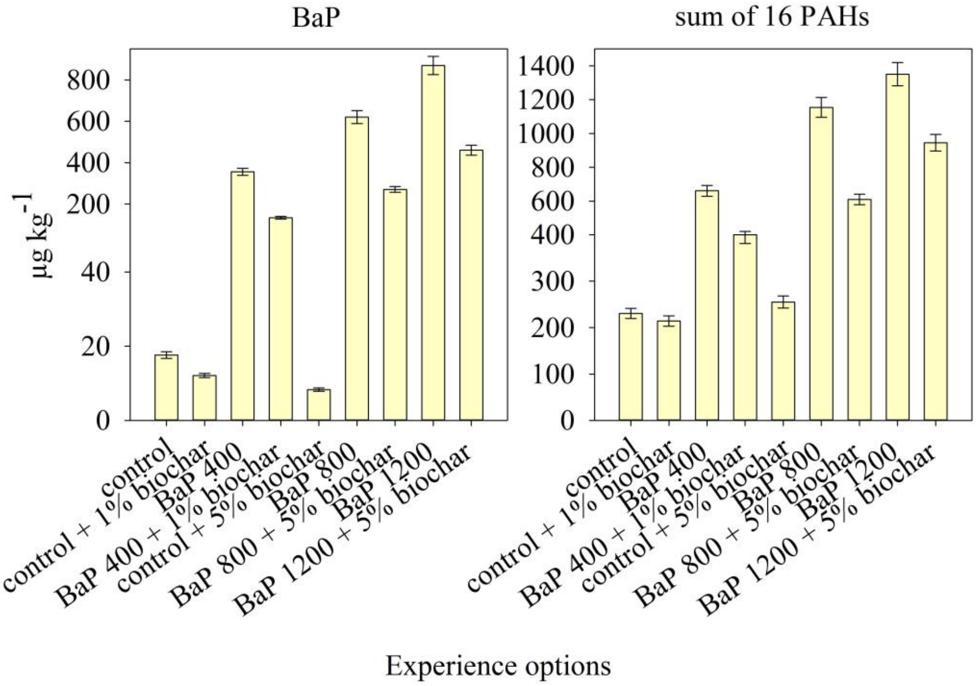

Figure 3. Benzo[a]pyrene and sum of total PAHs content in the soil of model vegetation experiment

The biochar application doses were divided to the pollution level and chose as 1% of the mass volume for variants with uncontaminated control soil and soil contaminated by 400 μg kg^{-1} BaP. The close relations were found by Vasilyeva et al. (2020). The biochar 5% was applied in the soil contaminated with 800 μg kg^{-1} BaP and 1200 μg kg^{-1} BaP. Biochar 1% amendment to soil decreased BaP content in control soil to 31%, and 5% biochar application decreased BaP content to 53%, compared to control, whereas the total PAHs content did not show any representative changes in its content under the biochar application. As well as 5% biochar application led to 10% increasing of the total PAHs content in the control soil which is most probably caused by pyrogenic PAHs presence in the biochar structure forming during the pyrolysis process (Hale et al., 2011).

The application of biochar at a dose of 1% into the soil contaminated with 400 μg kg^{-1} BaP was accompanied by a decrease in the BaP content up to 62.5% and in the total PAHs content up to 39%. In the variant contaminated with 800 μg kg^{-1} BaP the application of 5% of biochar led to the BaP content decreasing up to 56% from its initial content in the soil, and total PAHs content decreased to 47%. Application of 5% biochar in the soil contaminated by 1200 μg kg^{-1} BaP effect on the BaP and total PAHs content decreasing to 47% and 30%, respectively. The data received associated with the study reported by Bonaglia et al. (2020), shows a possibility of polyaromatic hydrocarbons microbial degradation under the biochar application in the petroleum-contaminated soil. Chen and Chen (2009) estimated the perspectives of biochar using for some PAHs sorption that approves the biochar efficiency using for the PAHs contaminated soil remediation.

BaP content in the plants of the model experiment

The BaP and total PAHs content were determined for all parts of the spring barley plants, such as roots, stem, and corn. Since BaP has a toxic effect, barley plants have not reached the heading stage in the variant contaminated with 1200 μg kg^{-1}. Soil contamination by BaP showed the spring barley uptake as BaP, as total PAHs from the soil.

BaP accumulation in roots of control plants was 0.9±0.1 μg kg^{-1}, total PAHs content was at the level 105.4±4.3 μg kg^{-1}; for stem the BaP content was 0.4±0.03 μg kg^{-1}, total PAHs content was 40.9±2.5 μg kg^{-1}; for corn BaP content estimated at the level 0.1±0.02 μg kg^{-1}, total PAHs content was 15.5±1.6 μg kg^{-1} (Figure 4). Contamination with 400 μg kg^{-1} BaP showed an increased BaP and total PAHs content in the spring barley roots 115.2±6.0 μg kg^{-1}, total PAHs content was at the level 291.6±14.0 μg kg^{-1}; in stem BaP content increased up to 36.0±1.7μg kg^{-1}, total PAHs content was at the level 106.3±5.0 μg kg^{-1}; in corn BaP content was 12.6±0.6 μg kg^{-1}, total PAHs content estimated as 54.3±2.6 μg kg^{-1}. The data received corresponding to the data Manzetti (2013).

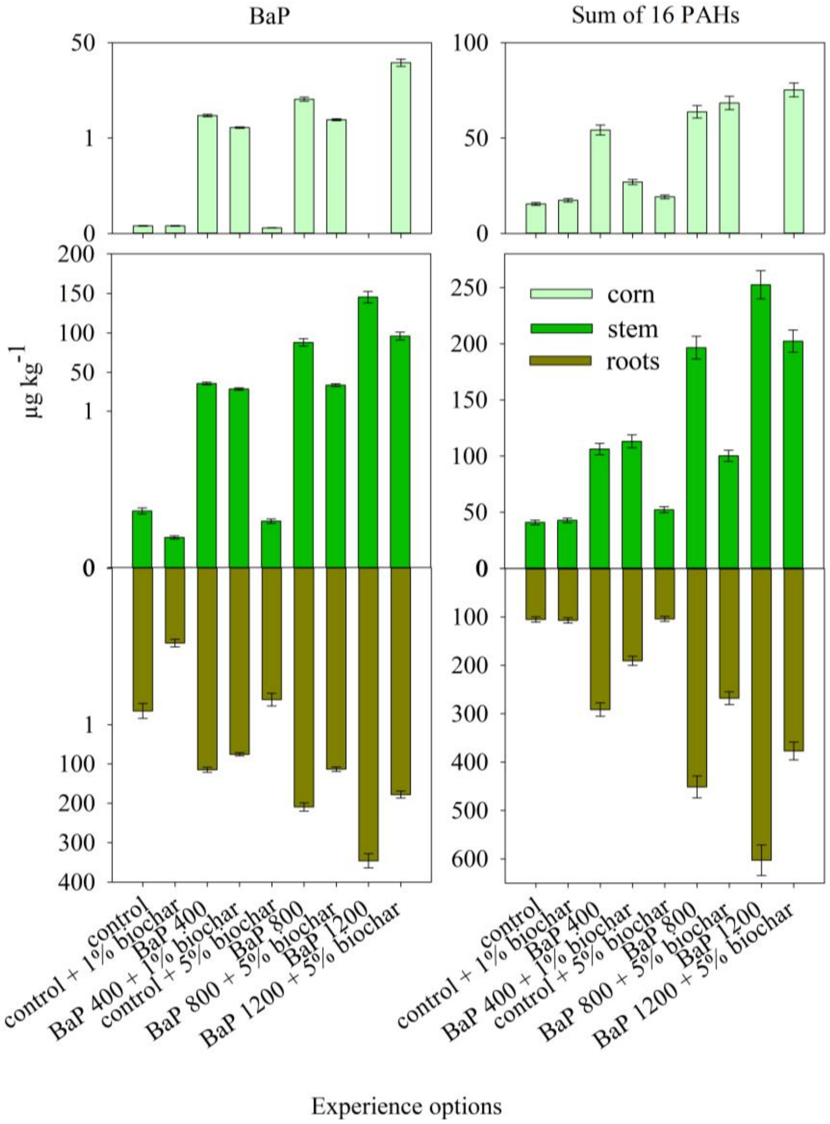

Figure 4. Benzo[a]pyrene and sum of total PAHs content in the roots, stem and corn of spring barley plants of model vegetation experiment.

Biochar 1% application in the control uncontaminated soil led to the BaP decreasing in the spring barley roots and stem up to 50% compared to control, in the corn there was no difference. Applying the 5% of biochar did not affect the BaP and total PAHs content in plants.

It was found the 1% biochar application in the variant contaminated with 400 μg kg^{-1} BaP affected the toxicant uptake by spring barley plant. BaP content in roots, as well as total PAHs content decreased up to 34% compared to contaminated variant, in stem there was found 19% decreasing of BaP content and in corn the content of BaP and PAHs decreased to 50%, that shows a high effectiveness of biochar application in the above-mentioned dose (Figure 5).

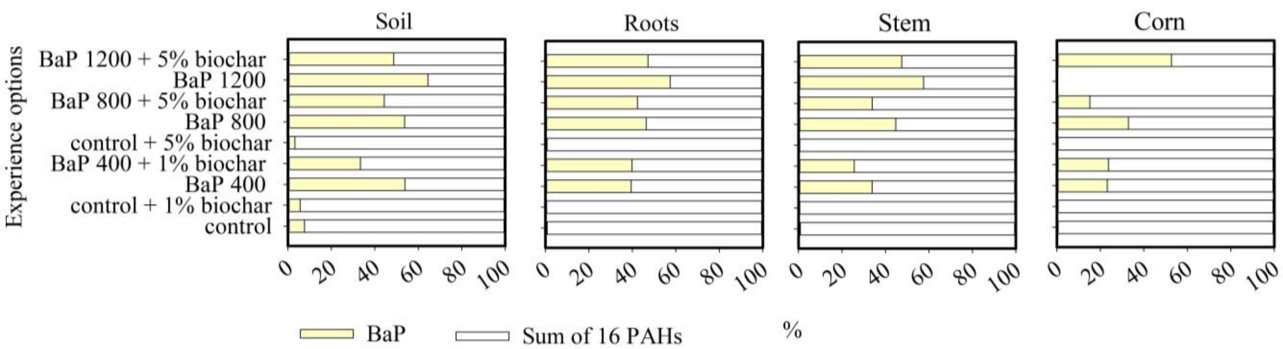

Figure 5. Benzo[a]pyrene to total PAHs content % relation in the different parts of spring barley plants and contaminated soil.

Soil contamination with 800 μg kg^{-1} BaP led to an intensive BaP and total PAHs uptake by the spring barley roots 209.4±10.5 μg kg^{-1}, total PAHs content was at the level 451.4±22.6 μg kg^{-1}; in stem BaP content increased up to 88.0±4.6 μg kg^{-1}, total PAHs content was at the level 196.5±10.2 μg kg^{-1}; in corn BaP content was 21.0±1.1 μg kg^{-1}, total PAHs content estimated as 63.7±3.3 μg kg^{-1}.

In the variant contaminated with 800 μg kg^{-1} BaP the application of 5% of biochar led to the BaP and total PAHs content decreasing in roots up to 40-46% from the plants of contaminated variant. In stem there was established BaP and total PAHs content decreasing up to 49-61%, and in corn only BaP content decreased up to 49%.

BaP application in the soil in amount of 1200 μg kg^{-1} BaP showed the extremely high level of toxicant uptake by plants. The content of BaP and total PAHs in roots was 346.0±18.2 μg kg^{-1}, and 602.9±31.7 μg kg^{-1}, respectively. For stem this level were found at the level 145.3±7.1 μg kg^{-1}, and 252.5±12.5 μg kg^{-1}, respectively. Corn is this variant didn't form that is connected with a high BaP toxicity for this contamination level.

Biochar amendment 5% showed a high effectiveness, because on the variant 1200 μg kg^{-1} BaP + biochar 5% the corn successfully formed. BaP and total PAHs content decreased in roots up to 37-48% from the plants of contaminated variant. In stem there was established BaP and total PAHs content decreasing up to 19-33%.

The differences in the BaP uptake by spring barley plants can be described by their relations roots/soil, stem/roots and stem/corn (Iljina et al., 2020). In all contaminated variants there was found a gradual decreasing of the bioaccumulation coefficients after biochar application that shows an improvement of plant barrier mechanisms to BaP and total PAHs content during the remediation process using 1% and 5% of biochar under the different contamination level (Figure 6).

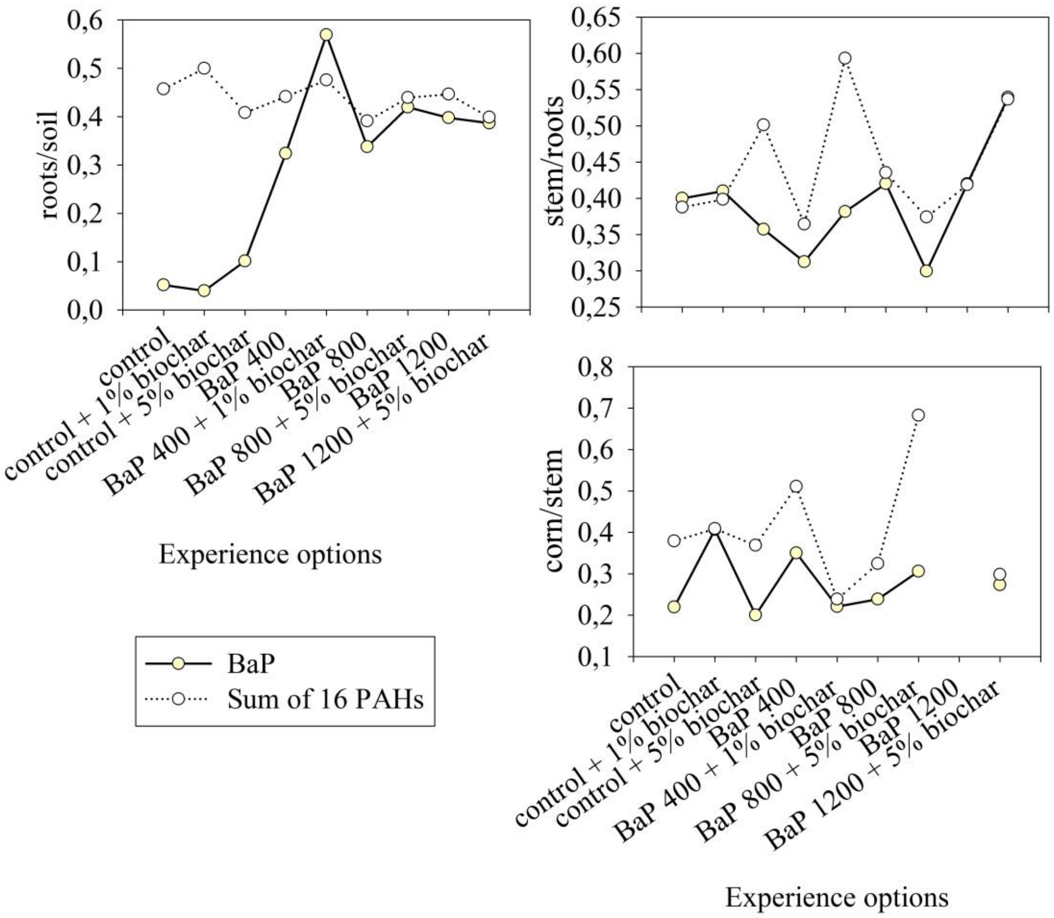

Figure 6. Bioaccumulation coefficients of benzo[a]pyrene and sum of total PAHs for soil and different parts of spring barley.

Conclusion

Thus, it was found that the soil contamination with a concentration of 400, 800 and 1200 μg kg^{-1} contributed to the BaP and total PAHs accumulation in soil and all plants. All contaminated doses promoted the inhibition in the growth characteristics of plants. The effect intensified with an increase in the pollutant concentration. The highest degree inhibition of the spring barley has been observed at the variant with BaP application 1200 μg kg^{-1}.

It was studied the optimal doses of biochar application for the soil remediation under the high and ultrahigh levels of BaP contamination for decreasing of the plants uptake. The dose of sorbent application was 1% for soil contamination with BaP in concentration 400 μg kg^{-1}. The BaP level decreased in soil and plants up to 50% compared to the contaminated variant. In soil contaminated with 800 μg kg^{-1} the dose 5% of biochar contributed to the BaP content decreasing in the soil and plants up to 60%. Application of 5% biochar in the soil contaminated by 1200 μg kg^{-1} effected on the PAHs content decreasing up to 47% that shows a high sorption capacity of biochar and its effectiveness under the soil remediation process.

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Effects of deforestation on soil properties and organic carbon stock of a hillslope position land in Black Sea Region of Turkey

Coşkun Gülser [a,*], İmanverdi Ekberli [a], Füsun Gülser [b],

[a] Ondokuz Mayıs University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Samsun, Turkey
[b] Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Van, Turkey

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Author(s)

C. Gülser *
İ. Ekberli
F. Gülser

* Corresponding author

Abstract

The effects of deforestation on soil properties and soil organic C (SOC) stock in adjacent pasture and forest areas located on the same hillslope position were investigated. The soil properties measured in the forest site had higher coefficient of variation compared with pasture site. Deforestation made almost 50 years ago significantly increased bulk density (Db), soil pH, exch. Mg and significantly decreased total porosity (F), gravimetric moisture content (W), EC, exch. K, soil organic matter (OM), SOC stock in 0-15 cm soil depth in pasture site. Mean Db increased fom 1.24 g/cm^3 in forest to 1.42 g/cm^3 in pasture while mean values of OM and SOC stock depleted from 4.41% and 30.70 ton/ha in forest to 1.89% and 15.55 ton/ha in pasture, respectively. The reduction ratios in some soil properties by changing land use type from forest to pasture were determined in the following order; OM (57.14%) > exch. K (55.88%) > SOC (49.35%) > EC (45.65%) > W (28.96%) > exch. Na (18.75%) > F (11.32%) > exch. Ca (4.07%). The values of OM and SOC stock had significant positive relations with F, W, EC, exch. K, and significant negative relations with Db, pH, exch. Mg. After deforestation, abandoning cultivated land to pasture had negative impact on the soil properties and depleted SOC stock in 0-15 cm soil layer.

Keywords: Land use, pasture, deforestation, soil organic C, soil properties.

Introduction

Clearing forests for agricultural use is a historical cause of deforestation in many areas of the world (Gorte and Sheikh, 2010). Deforestation has negative impacts on global climate change, loss in biodiversity and environmental sustainability. In the most study, it was reported that after deforestation and subsequent land use including pasture deteriorate soil physical, chemical and biological properties (Lu et al., 2002; Sahani and Behera, 2001; Rasiah et al., 2004; Zhang et al., 2004).

Many soil quality parameters interact each other related with land use, land characteristics and management practices (Karaca and Gülser, 2015; Karaca et al., 2018; Doğan and Gülser, 2019; Doğan and Gülser, 2020; Karaca et al., 2021). Gülser et al. (2020) found that under a hot and dry climatic condition, reduced tillage system decreased bulk density and increased total porosity with conserving soil OM due to preventing from rapid mineralization. The land use changes, including conversion of forestland into cropland, pasture land, and settlement areas generally cause soil degradation, soil organic carbon (SOC) and nutrient losses (Wang et al. 2001; Dinesh et al., 2003). The loss of soil OC due to deforestation has negative effects on soil quality parameters by influencing soil physical, chemical and biological, properties (Sun et al., 2015; Qi et al., 2018). Vera et al. (2007) studied the effect of deforestation and replacement by pasture in the rainforests of the Venezuelan Andes on soil properties. They reported that there were major differences in the microstructure

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and porosity, as well as in the characteristics of the fine fraction and organic constituents, together with other soil properties in the natural or altered forest landscape. Lemenih et al. (2005) reported that soil OC and total N contents in the surface soil layer under cultivation in 53 years decreased significantly compared with the natural forest. Koutika et al. (1997) studied on organic matter dynamics and aggregation in soils under rain forest and pastures of increasing age for 7, 12 and 17 years in the eastern Amazon Basin. They found that the effects of deforestation and pasture establishment on both clay and carbon contents were not significant, whereas significant differences were recorded for aggregate fabric porosity, water retention and reduction in total porosity with increasing pasture age. Organic material addition to soil increases basal soil respiration (BSR), aggregate stability and soil OC content which is significantly related with EC, total porosity, and bulk density (Gülser and Candemir, 2015; Gülser et al., 2017).

Many researchers indicated that the most important effect on soil organic carbon (SOC) stock rate is the change of land use type (Guo and Gifford, 2002; Liu et al., 2002; Don et al., 2007; Assefa et al., 2017). It has been reported in many studies that the conversion of natural vegetation to cropland often causes to a reduction of SOC stocks (Hajabbasi et al. 1997; Liu et al., 2002; Assefa et al., 2017). Del Galdo et al. (2003) determined that SOC content in the top 10 cm layer of cropland soils was 48% lower than that of permanent grassland soils.

Saikh et al. (1998) reported that deforestation in the tropic areas has a serious problem in soil because of the reduction in cation exchange capacity (CEC) and the consequent losses of nutrients from the soils. They found that grasslands and cultivated lands have statistically similar CEC but levels are higher in deciduous forest soils and the highest in evergreen forest soils in where only the soil organic C can significantly increase the CEC. Hajabbasi et al. (1997) found that deforestation and subsequently tillage practices resulted in 20% increase in bulk density, 50% decrease in organic matter and total nitrogen, between 10 and 15% decrease in soluble ions when compared the undisturbed forest soil.

Clearance of forests for agricultural purpose and grazing is widespread, especially in highlands of Turkey (Celik, 2005). There are several cultivation areas abandoned to pasture lands after deforestation in Black Sea Region of Turkey. Therefore, the objective of this study was to compare the long term effects of deforestation on soil properties and SOC stock in adjacent pasture and forest areas located on the same hillslope position in Samsun-Turkey.

Material and Methods

Two different lands were selected including a natural oak forest (*Quercus brontii*) (41^0 25,800' N, $36^0$10,425' E) and a pasture ($41^0$21,534' N, $36^0$10,449' E) land on 25% hillslope position about 320 m altitude with an average annual temperature of 14,6^{0}C and 720 mm of precipitation in Atakum, Samsun-Turkey. The soil in the study area is formed on basalt parent material. Forest clearance had been made more than 50 years ago by the local farmers to gain a field for cultivation. The field was abandoned as a pasture field later.

Disturbed and undisturbed six surface soil samples (0-15 cm) for each land use type were taken from the adjacent pasture and forest lands located on the same hillslope position. The soil depth is about less than 20 cm in both land uses. Bulk density (Db) values were determined on undisturbed soil core samples, and total porosity values were calculated using the following equation; F = [1 – (Db/2.65)] (Demiralay, 1993). After air drying the samples, soil particle size distribution was determined according to Bouyoucos hydrometer method (Demiralay, 1993), soil reaction (pH) and electrical conductivity ($EC_{25°C}$) values in 1:1 soil:water suspension, soil organic matter (OM) by 'Walkley-Black' method and exchangeable cations (Ca, Mg, K, Na) by ammonia acetate extraction method (Kacar, 1994). Soil organic C (SOC) stock in both land use was estimated for a volume of soil (1500 m^3/ha) in 15 cm soil depth using the equation given below,

$$\text{SOC (ton/ha)} = \text{volume of soil } (m^3/\text{ha}) \times \text{Db } (\text{ton}/m^3) \times (\text{OM } (\%)/172.4)$$

Descriptive statistical analyses including mean, standard deviation, coefficient of variation (CV), skewness and kurtosis, and one way analysis of variance (ANOVA) were done using SPSS statistical programme.

Results and Discussion

The soil textural class in both land use was clay loam according to the particle size distribution of soils in forest (28.12% clay, 26.97% silt, 44.90% sand) and pasture (35.03% clay, 22.37% silt, 42.59% sand) lands. Descriptive statistical results of the soil physical and chemical properties of both land uses are given in Table 1. Gravimetric water content and SOC stock values showed the highest standard deviation in forest and pasture land, respectively. In both land use types, coefficient of variation (CV) values, except exch. K and Na in forest, were lower than 35%. Although it shows the homogeneity of samples and the accuracy of experiment (Ogunkunle and Eghaghara, 2007), the CV values of the properties determined in forest land always higher than that in pasture land (Figure 1). It indicates that according to the measured soil properties, the soil samples from forest site had less homogeneity or higher variability than that from pasture site. While the soil pH had the lowest CV, exchangeable K content generally had the highest CV among the soil properties.

Table 1. Descriptive statistics of some soil properties in forest and pasture sites.

	Land use	Minimum	Maximum	Mean	Std. Dev.	CV, %	Skewness	Kurtosis
Db, g/cm³	Forest	1,04	1,44	1,24	0,18	14,17	0,13	-2,26
	Pasture	1,37	1,53	1,42	0,06	4,21	1,91	3,86
F	Forest	0,46	0,61	0,53	0,07	12,53	-0,04	-2,29
	Pasture	0,42	0,48	0,47	0,02	5,04	-1,88	3,59
W, %	Forest	22,00	36,65	27,14	5,85	21,54	0,84	-0,31
	Pasture	16,45	20,83	19,28	1,53	7,93	-1,55	2,79
pH (1:1)	Forest	6,44	6,75	6,56	0,10	1,57	1,30	2,97
	Pasture	6,77	7,02	6,88	0,10	1,46	0,25	-1,25
EC, dS/m	Forest	0,32	0,63	0,46	0,14	30,82	0,50	-2,22
	Pasture	0,23	0,28	0,25	0,02	7,38	1,24	1,17
OM, %	Forest	3,20	5,86	4,41	1,21	27,42	0,11	-2,92
	Pasture	1,59	2,19	1,89	0,23	12,04	0,31	-1,14
SOC, ton/ha	Forest	25,28	39,38	30,70	5,16	16,81	0,93	0,52
	Pasture	12,76	19,41	15,55	2,44	15,70	0,76	-0,32
Ca, cmol/kg	Forest	20,91	27,24	22,38	2,41	10,79	2,30	5,42
	Pasture	18,95	24,39	21,47	2,02	9,39	0,27	-1,09
Mg, cmol/kg	Forest	8,65	11,53	9,80	1,20	12,27	0,60	-1,51
	Pasture	10,48	13,76	12,10	1,51	12,48	0,11	-2,80
K, cmol/kg	Forest	0,21	1,03	0,68	0,29	43,38	-0,64	-0,04
	Pasture	0,21	0,49	0,30	0,10	32,50	1,82	3,87
Na, cmol/kg	Forest	0,16	0,57	0,32	0,14	44,19	1,28	2,47
	Pasture	0,21	0,34	0,26	0,06	21,76	0,75	-1,65

CV: Coefficient of variation, Db: Bulk density, F: Total porosity, W: Gravimetric water content, OM: Organic matter, SOC: Soil organic carbon stock in 15 cm soil depth.

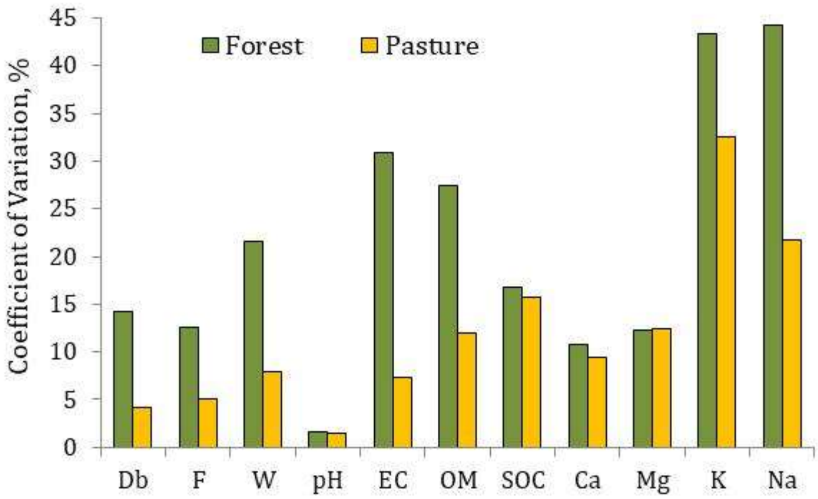

Figure 1. Comparison of the coefficient of variation values of soil properties in forest and pasture sites.

The variance analyses results for the long term effect of deforestation on soil properties and SOC stock in adjacent pasture and forest areas located on same hillslope land position are given in Table 2. Except, exch. Ca and Na content, the soil properties measured in forest site were significantly different from that in pasture site. While W, pH, EC, OM and OC stock measured in forest and pasture sites were significantly different each other at 1% level, Db, F, exch. Mg and K contents were significantly different each other at 5% level.

Table 2. The F-test values of one way variance analyses results for soil properties in different land use type.

Db	F	W	pH	EC	OM	SOC	Ca	Mg	K	Na
5.45*	5.58*	10.14**	29.10**	13.06**	25.19**	42.26**	0.50	8.50*	8.77*	0.85

**significant at 1% level, *significant at 5% level, Db: Bulk density, F: Total porosity, W: Gravimetric water content, OM: Organic matter, SOC: Soil organic carbon stock in 15 cm soil depth.

Effect of land use change on some soil physical properties

The percent changes in mean values of soil properties due to changing the land use from forest to pasture are given in Figure 2. Deforestation caused significant increases in Db, pH and exch. Mg values while it significantly decreased the other soil properties in pasture soil. The mean Db value significantly increased from 1.24 g/cm^3 in forest site to 1.42 g/cm^3 in pasture site with a 14.52% increment while the mean F value significantly decreased from 0.53 in forest site to 0.47 in pasture site with an 11.32% reduction. Chen et al. (2009) reported that the increase in macro pores significantly lowers bulk density, and the mean macro pores is the highest in the forest soils and lowest in the cultivated and grassland soils. Hajabbasi et al. (1997) determined that the surface soil (0-30 cm) of forest and deforested sites had the lowest (1.13 g/cm^3) and the highest (1.28 g/cm^3) bulk density, respectively. Similarly, many researchers reported that bulk density in the forest at surface soil layer was lower than that in cultivated plots and the pasture (Rasiah et al., 2004; Puget and Lal, 2005; Assefa et al., 2017). The mean W content (27.14%) in pasture site had a 28.96% reduction compared with forest site (19.28%). Chen et al. (2009) reported that when the forest was cut to become an agricultural field or bare soils, soil moisture content would be reduced by 32.1%. Magliano et al. (2017) found that at similar bulk density values after changing land use, pastures had a 20% reduction in volumetric water content at field capacity (16.3%) compared with woodlands (19.7%).

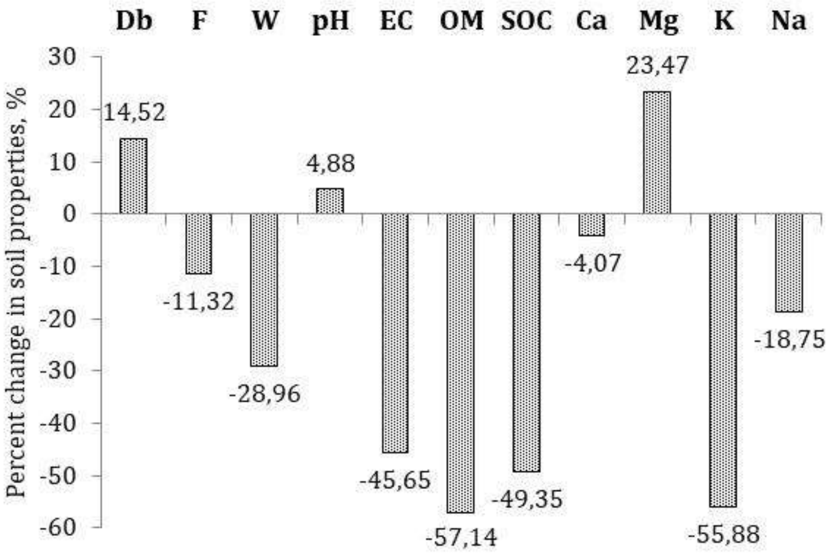

Figure 2. The percent changes in mean values of soil properties after deforestation.

Effect of land use change on some soil chemical properties and SOC stock

The mean soil pH value significantly increased from 6.56 in forest site to 6.88 in pasture site with a 4.88% increment while the mean EC value significantly decreased from 0.46 dS/m in forest site to 0.25 dS/m in pasture site with a 45.65% reduction. Liu et al. (2002) found that deforestation caused an increase in soil pH from 5.67 in forest to 6.27 in cultivated site while soil nutrients decreased in cultivated site of humid mountainous areas.

Soil OM content significantly decreased from 4.41% in forest to 1.89% in pasture site. After deforestation and abandoning cultivated land to pasture, soil OM showed the highest percentage reduction among the soil properties with 57.14%. Similarly, SOC stock in 15 cm surface soil depth significantly decreased from 30.70 ton/ha to 15.55 ton/ha with 49.35% depletion after conversion of forest to pasture site. Assefa et al. (2017) found that 60% of the total SOC in forest was determined in the upper 10 cm soil depth, and total SOC varied between land use systems with ranging from 3.1 kg C/m^2 in croplands to 23.9 kg C/m^2 in natural forest. Khormali et al. (2009) reported that the average OC stock (184.8 ton/ha) of the 0–60 cm depth of the forest was almost 3 times higher than that (58.8 ton/ha) of the deforestation site. Soil tillage affects the soil OM contents associated with aggregates and SOC sequestration in no till is greater than conventional tillage due to slower turnover of macro aggregates in no till (Six et al., 1999). Organic matter in cultivated soils has less physical protection than that in the uncultivated soils due to removal of biomass during land clearing and also reduced tillage system conserves soil OM with preventing from rapid mineralization (Barber, 1995; Gülser et al., 2020). Bruce et al. (1999) reported that OC losses in former grassland and forest soils by conventional tillage practices ranged from 20 to 50% of the initial content of cultivation zone within the first 40–50 years.

Exch. Mg content only significantly increased from forest (9.80 cmol/kg) to pasture (12.10 cmol/kg) site with a rate of 23.47%, while exchangeable Ca, K and Na decreased from forest (22.38, 0.68 and 0.32 cmol/kg) to pasture (21.47, 0.30 and 0.26 cmol/kg) site with the rates of 4.07%, 55.88% and 18.75%, respectively. Sharma et al. (2009) determined that Mg content was greatest (4.71 cmol/kg) in agroforestry system, whereas the least (2.46 cmol/kg) amount was recorded in arable land. Hajabbasi et al. (1997) similarly found that Mg content of the surface soil for the deforested site was significantly higher than the forest and cultivated forest sites. They also indicated that Ca contents of the all three land use sites were significantly similar, but the average K content of the forest site was significantly higher than the other sites. In this study, exch. K content showed the highest decrease among the other cations. Khormali et al. (2009) reported that available K content had significant decrease in the deforestation site (54.6 mg K/kg) compared to that of forest site (64.2 mg K/kg). Sharma et al. (2009) also found that mean exchangeable K was greatest (0.23 cmol/kg) in agroforestry system and least (0.15 cmol/kg) in arable land.

Correlations among the soil properties

The relationships among the soil properties are given in Table 3. The soil OM content gave higher correlations with the all soil properties compared with the SOC stock in surface soil (0-15 cm). Soil OM content had significant positive correlations with SOC, F, W, EC, K, and significant negative correlations with Db, pH, Mg. Increasing soil OM content in the forest site decreased soil pH, bulk density, and increased EC, exch. K, total porosity, soil moisture content compared with that in the pasture site. The similar relationships among the soil properties had been found in many researches (Gülser, 2006; Candemir and Gülser, 2010; Demir and Gülser, 2015; Karaca et al., 2018; Demir and Gülser, 2021).

Table 3. The correlation matrix among the soil properties.

	F	W	pH	EC	OM	SOC	Ca	Mg	K	Na
Db	-0.999**	-0.941**	0.701*	-0.930**	-0.836**	-0.683*	-0.478	0.647*	-0.694*	0.251
F		0.946**	-0.709**	0.936**	0.836**	0.683*	0.493	-0.640*	0.700*	-0.236
W			-0.761**	0.965**	0.888**	0.775**	0.363	-0.670*	0.759**	-0.183
pH				-0.798**	-0.842**	-0.838**	-0.445	0.608*	-0.659*	-0.025
EC					0.920**	0.819**	0.466	-0.720**	0.852**	-0.102
OM						0.969**	0.247	-0.834**	0.886**	-0.064
SOC							0.135	-0.824**	0.858**	0.061
Ca								0.132	0.296	0.217
Mg									-0.714**	0.244
K										0.061

** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level

Conclusion

The soil properties measured in the forest site showed the higher CV values than that in pasture site. Changing the land use type from forest to pasture after deforestation on the same hillslope position significantly increased Db, soil pH, exch. Mg and significantly lowered F, W, EC, exch. K, OM, SOC stock in 0-15 cm soil depth in pasture site. The highest decrease was obtained in soil OM content with 57.14%. Mean SOC stock in pasture site also decreased 49.35% after deforestation. The soil OM and SOC stock values similarly gave significant positive relations with F, W, EC, exch. K, and significant negative relations with Db, pH, exch. Mg content. Changing land use type or deforestation had negative impact on the soil properties and depleted SOC stock in 0-15 cm soil layer of pasture soil, and deteriorated most soil physical and chemical properties. This study shows that soils under forest vegetation should not be cleared or cultivated for agricultural production and should be protected for sustainable soil management especially on deep slope positions.

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Responses of potato (*Solanum tuberosum* L.) varieties to NPK fertilization on tuber yield in the Southeast of Kazakhstan

Yerbol Alimkhanov [a,*], Rakhimzhan Yeleshev [a], Bibigul Yertayeva [b], Akbope Aitbayeva [b]

[a] Kazakh National Agrarian Research University, Almaty, Kazakhstan
[b] Kazakh Research Institute of Fruit and Vegetable Growing, Kainar, Kazakhstan

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Author(s)

Y. Alimkhanov *
R. Yeleshev
B. Yertayeva
A. Aitbayeva

* Corresponding author

Abstract

In this study, field experiment was conducted to assess the effect of different doses of NPK on tuber yield on the potato (*Solanum tuberosum* L.) varieties such as Inovator, Gala, Aladin and Tyanshansky in foothill zone of the southeast of Kazakhstan. Five different combinations of NPK with control were studied. Results revealed that the fertilizer application increased the total tuber yield, the longest plant, number of main stem, number of leaves plant and average tuber number on the potato significantly over control. The highest tuber yield was obtained when 25% more than the recommended fertilizer dose with manure was applied at all potato varieties. Aladin potato variety significantly yielded higher than the other varieties tested at the same time in this experiment.

Keywords: Potato, fertilization, manure, tuber yield, potato varieties.

Introduction

Potatoes are an important crop in the Central Asian Republics (Loebenstein and Manadilova, 2003). They are the second most important crop in Kazakhstan, after wheat, grown on about 205.000 ha. In Kazakhstan, the largest potato grower in the Central Asian Republics, yields average 19,5 tons ha-1 which is very low in comparison to other potato producing countries like 50.4 tha-1 in New Zealand, 49.7 tha-1 in USA and 33.5 tha-1 in the Turkey in 2018. (Potatopro, 2020). Potatoes in Kazakhstan are planted in April-May, depending on the region and weather and harvested in September- October.

Fertility is one of the controllable major factors that affect the yield and quality of potatoes (Dubetz and Bole, 1975). Supply of nutrients plays an important role in growth and yield. Nitrogen is an essential constituent of protein and chlorophyll, where phosphorus fertilization contributes to early crop development and tuberization and enhances tuber maturation, whereas potassium influences both yield and tuber quality and also enhances plant resistance to withstand stress against drought and frost (Nizamuddin et al., 2003). In order to obtain good yield, modern varieties of different crops require relatively high quantity of fertilizer compared to the traditional cultivars (McArthur and McCord, 2017). However, the economic condition of Kazakhstan farmers often does not support them to use required quantity of fertilizers due to its high cost. On the other hand, the organic matter content of most of the soils of Kazakhstan is very low (<2.5%) as compared to desired (3% and above) levels (Takata et al., 2007; Causarano et al., 2011). Therefore, it becomes an immense need to formulate an optimum fertilizer recommendation that would produce satisfactory yields and would maintain soil health to ensure sustainable crop production. One of the alternatives to economize the use of chemical fertilizer is to incorporate farmyard manure in combination with chemical NPK fertilizers (Black and White, 1973; Zhang, 2009; Li et al., 2017). Bandyopadhyay et al.

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(2010) showed that mixed use of farmyard manure (cow dung) and chemical fertilizer improved soil organic carbon content compared to the use of chemical fertilizer (NPK) alone.

Different potato varieties (improved, imported or local) characterized by diverse forms, plant height and skin colour are found in Kazakhstan. In recent years, many foreign varieties of potatoes have been imported to Kazakhstan. However, these varieties are not yet fully adapted to the conditions of Kazakhstan. Therefore, the issues of adaptability of foreign highly productive potato varieties to the conditions of the irrigated zone of Kazakhstan are relevant and are of scientific and practical interest. Foreign potato varieties are highly intensive, i.e. the formation of high yields requires the use of very high rates of mineral fertilizers and the repeated use of pesticides against pests, diseases and weeds. In this aspect, it is important to study the responsiveness of different potato varieties to fertilization and determine their most optimal rates. The objective of this study was to determine the influence of applying different levels of NPK fertilizer on tuber yield on the potato (*Solanum tuberosum* L.) varieties in foothill zone of the southeast of Kazakhstan.

Material and Methods

Description of the Study Sites

The experiment was conducted at the Regional Branch "Kainar" of the LLP "Kazakh Research Institute of Fruit and Vegetable Growing" (merger of two institutions (fruit growing and vegetable growing)), foothill zone of the southeast of Kazakhstan (43°09'32.8"N 76°26'57.3"E) during the growing season 2019 with a view to finding out the suitable variety as well as determining the optimum dose of fertilizer of potato. The locations of the evaluations were characterized by the continental climate (large daily and annual fluctuations in air temperature, characterized by cold winters and long hot summers), the air temperature reaches minimum values in January (-32,-35^{0}C), and maximum values in July (37-43^{0}C). The warm period lasts 240-275 days, the frost-free period is 140-170 days and an annual amount of precipitation is 250 – 600 mm.

The soil belongs to the general soil type of dark chestnut. The land was medium high with loamy. Before conducting the experiment, the soil sample was analyzed from Kazakh National Agrarian Research University. The soil was characteristically slightly alkaline (pH 7.3-7.4), soil organic matter 2.9-3.0% (moderate), total N 0.18-0.20% (high), available P_2O_5 35-40 mg kg-1 (moderate), available K_2O 360-390 mg kg^{-1} (low), cation exchange capacity 20-21 me 100g^{-1} soil, bulk density 1.1-1.2 gr cm^3, field capacity 26.6%.

Treatments and Experimental Design

The experiment was performed using a completely randomized block design with four replications. The experimental unit was 50 m^2 (6 m x 8.3m). The sources of fertilizers used were ammonium nitrate 34.5% N, double superphosphate 46%P_2O_5 and potassium sulphate 56% K_2O. The experimental field was prepared in accordance with a standard practice used by RB Kainar of LLP Kazakh Research Institute of Fruit and Vegetable Growing. The land was disk ploughed, harrowed, and leveled with a tractor. Then ridging was done by hand. Fertilizer was applied using grain drill. Other agronomic practices and data collection were conducted based on the recommendations of Kazakh Research Institute of Fruit and Vegetable Growing. Four potato varieties (Inovator (Netherlands), Gala (Germany), Aladin (Netherlands) and Tyanshansky (Kazakhstan)) were combined with four fertilizer treatments. The trial was implemented on May (17th) and harvested on September (27th) 2019. Trial was well protected against insects and weeds during the season.

Table 1. Treatment description and nutrient rates used in the field experiment

Treatments	Nutrient Rate (kg ha^{-1})			Manure (t/ha)
	N	P	K	
T1 = Absolute control	0	0	0	0
T2 = Recommended fertilizer dose (for 30 t ha^{-1} yield)	150	90	120	0
T3 = 25% more than the recommended fertilizer dose	190	110	150	0
T4 = 50% more than the recommended fertilizer dose	225	135	180	0
T5= 25% more than the recommended fertilizer dose with manure (40 t ha^{-1})	190	110	150	40

Variables evaluated

Total tuber yield (t ha^{-1}): Total tuber yield was calculated as the sum of the weights of marketable and unmarketable tubers from the net plot area and transformed to ton per hectare.

Number of leaves per plant: The number of leaves per plant were determined by counting the number from ten plants (hills) in each plot before the start of tuber formation and averaged.

Number of main stem/hill: Data on Number of main stem/hill was recorded as the average stem number counted from ten hills per plot at 50% flowering. Only stems that had directly grown from the mother tuber and acted as an independent plant above the soil were considered as main stems. Stems branching from other stems above the soil were not considered as main stems.

Average tuber number/hill: Average tuber number/hill was recorded at harvest as the actual number of tubers collected from 20 middle row plants in each plot and calculated as an average tuber number.

Statistical analysis was performed using analysis of variance and LSD test using the SPSS package program.

Results and Discussion

The total tuber yields of potato are presented in Table 2 for the performance of potato varieties. In foothill zone of the southeast of Kazakhstan, significant differences between the four varieties tested were detected in 2019. Aladin potato variety significantly yielded higher than the other three varieties tested at the same time in experimental site. The tuber yield and means of the growth traits of potato varieties differed significantly due to NPK fertilization (Table 2). At all potato varieties, Application of NPK fertilization significantly ($P< 0.05$) influenced the total tuber yield (TTY), the longest plant (PLH), Number of main stem (NMS), Number of leaves plant (NLP) and Average tuber number (ATN) compared to untreated (control) plants. Similar results were obtained by Gunarto et al. (1985), Yousaf et al. (1999), Magnusson (2002) and Li et al. (2019) on several vegetable crops. Numerous studies have reported that inorganic NPK fertilizer increased growth in some species by enhancing nitrogen, phosphorus and potassium uptake (Shehu, 2014; Gülser et al., 2019). The yield of potatoes is a function of the number of tubers produced and the average weight per tuber. Besides yield, tuber size distribution and specific gravity are also important to the producer and the processor. N promotes cell elongation and vegetative growth whereas P is involved in root cell division and thus root growth. Adhikari and Rana (2017) reported that K was also important in cell division. Kołodziejczyk (2014) showed that N fertilizer increased the number of tubers set and allowed them to develop rapidly. Rosen and Bierman (2008) and Daoui et al (2014) reported a similar response from P. Nizamuddin et al. (2003) and Manolov et al. (2006) reported that K fertilizer increased the number of tubers as well as tuber size.

Table 2. Tuber yield and yield contributing characters of potato (*Solanum tuberosum* L.) varieties

Treatments	Traits				
	TTY	PLH	NMS	NLP	ATN
Variety Tyanshansky					
T1	22,3a	54a	4,8a	97a	9,5a
T2	26,0b	67b	5,3b	116b	11,6b
T3	31,1c	70c	5,5b	123c	12,9c
T4	35,2d	75d	5,5b	128d	14,0d
T5	36,4d	78e	6,0c	134e	14,7e
Mean	30,2	68,8	5,4	119,6	12,5
Variety Aladin					
T1	24,5a	60a	5,4 a	105a	12,4a
T2	28,4b	72b	5,7b	120b	15,7b
T3	33,5c	77c	6,0c	131c	16,5b
T4	37,9d	80d	6,2d	136d	17,9c
T5	40,8e	84e	6,3d	145e	18,4c
Mean	33,0	74,6	5,9	127,4	16,2
Variety Gala					
T1	21,4a	48a	4,5a	94a	9,2a
T2	25,7b	56b	4,7b	109b	10,8b
T3	30,5c	61c	5,0c	122c	13,1c
T4	34,4d	66d	5,3d	130d	13,6c
T5	35,3d	70e	5,5d	132d	14,2d
Mean	29,5	60,2	5,0	117,4	12,2
Variety Inovator					
T1	23,2a	57a	5,0a	102a	11,0a
T2	27,2b	68b	5,4b	114b	13,5b
T3	31,8c	72c	5,6b	128c	16,4c
T4	35,6d	79d	6,0c	139d	17,1d
T5	38,4e	82e	6,1c	143e	17,5d
Mean	31,2	71,6	5,6	125,2	15,1

TTY: Total tuber yield (t ha^{-1}), PLH: Plant height, cm; NMS: Number of main stem; NLP: Number of leaves per plant, ATN: Average tuber number

TTY, PLH, NMS, NPL and ATN were recorded from 25% more than the recommended fertilizer dose with manure (T5) based on average yield goal and it was statistically similar to all potato (*Solanum tuberosum* L.) varieties (Table 2). A similar study by Black and White (1973) showed that barnyard manure (9.7 t ha^{-1}) combined with chemical fertilizer resulted in high increase in total soil N. Moreover, it is believed that potato is more stable under combined organic and inorganic fertilization treatment compared with inorganic fertilization alone. The application of manure and chemical fertilizers in this experiment promoted yield response compared to mineral fertilized. Furthermore, the crop yield is also promoted with this fertilization treatment. This finding is in agreement with that one of Zhang (2009). That a combination of chemical fertilization and organic nutrient source gave maize higher yield than when applied separately. A similar study by Bandyopadhyay et al. (2010) found that manure application with chemical fertilizer causes higher yield in soybean, available nutrients and soil organic matter compared with those found under mineral fertilizer treatment.

Conclusion

It can be concluded that integrated use of organic manure and recommended dose of NPK fertilizers resulted in significant improvement in tuber yields of potato and quality despite being an active practice in nutrient management. Organic manure used with NPK fertilizers can maintain tuber yields of potato and meet the nutrient requirements to grow. Using organic manure from castoff and applying it in an intensive cropping system can be considered as an essential measure to decrease the potential risk of water pollution caused by castoff. In sustainable agriculture, the integrated use of organic manure and NPK fertilizers is necessary to create a healthy soil environment in the long run. This method produces a significant yield of crops compared to the application of organic manure or NPK fertilizers alone. Hence, combining the use of organic manure with NPK fertilizers is the right approach to sustainable agriculture.

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Physiological behavior of olive (*Olea europaea* L.) varieties under different foliage nutrition and irrigation regimes in the hyper-arid zone

Sherif El-Gioushy [a], Ibrahim Dobiea [a,b], Botir Khaitov [c,*], Aziz Karimov [c], Reda Zewail [d]

[a] Department of Horticulture, Faculty of Agriculture, Benha University, Egypt
[b] Department of Plant Production, Desert Research Center, Egypt
[c] International Center for Biosaline Agriculture (ICBA), Regional office for Central Asia and the South Caucasus, Tashkent, Uzbekistan
[d] Department of Botany, Faculty of Agriculture, Benha University, Egypt

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Author(s)

S. El-Gioushy
I. Dobiea
B. Khaitov *
A. Karimov
R. Zewail

* Corresponding author

Abstract

Olive (*Olea europaea*) is an emblematic tree in the Mediterranean regions that grows an integral part of the area. The Mediterranean vegetation often undergoes challenging periods of severe drought stress, which causes significant impairment to olive trees' growth and production performance. The practical study was designed to follow up the effect of three irrigation regimes (50%, 75% and 100% ETc) in combination with four doses (0, 2, 4 and 6 g/L) of Lithovit ($CaCO_3$+$MgCO_3$, micronized calcium carbonate) on growth performance, yield, and fruit quality of two olive (Picual and Manzanello) varieties during 2017 and 2018 growing seasons. Regardless Lithovit doses, irrigation regime 100% of ETc exhibited the highest values of leaf water content, leaf relative water content, total chlorophyll and N, P, K, Ca and Mg concentrations of both olive varieties compared to the other watering regimes. Interestingly, proline content in the fruit was enhanced with increasing water deficit (50% of ETc) and Lithovit dose (6 g/L). However, the highest yield and fruit oil content were obtained by the combination of 75% ETc irrigation level and Lithovit treatment at a rate of 4 g/L in both olive varieties. This study contributes to developing olive production technologies, thereby ensuring sustainable olive culture farming with high-quality yield in hyper-arid zones.

Keywords: Olive, Lithovit, drought, irrigation requirements, chemical aspects, water relations, fruit quality, fruit yield.

Introduction

Olive trees were widely grown throughout the Mediterranean basin for around 5000 years. Olives can thrive and produce in arid, semi-arid and new reclaimed areas, as it can resist abiotic stresses such as drought, fluctuation in temperature, salinity, drought (Xiloyannis et al., 1999; Shaheen et al., 2011).

Egypt is a one of the largest olive producer in the world, whereas, in the 2018-2019 growing season the country produced around 450,000 tons of olives, of which approximately 100,000 tons were exported (FAO, 2020). There is a prediction that increasing olive production in Egyptian desert lands will bring much needed added value to the sector through upgrading olive cultivation technology.

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However, drought stress is one of the main challenges that constrain growing olives in Egypt's newly reclaimed areas. Deficit irrigation can seriously impair the growth and production of olives. Although olive trees control water loss by transpiration effectively and can withstand intense internal water deficit, however, its growth and fruit quality gradually reduce with increasing water stress (Lavee et al., 1990). Previous studies also indicated that water stress has an irreconcilable role in olive growth and productivity, seriously deteriorating vegetative and generative performance (Xiloyannis et al., 1999; Tangu, 2014). On the contrary, sufficient water supply certainly increases vegetative growth, yield, and olives' fruit quality (Asik et al., 2014).

According to the climate change forecast scenarios of the Intergovernmental Panel on Climate Changes (IPCC), air temperature will rise and precipitation trends will alter, contributing to enhanced evaporative indicators and diminishing supply of irrigation. In addition, night-time temperature will rise to a larger degree than day-time temperature, whereas, the intensity of drought and heat waves are expected to increase (Stocker et al., 2019). These environmental factors have adverse pleiotropic effects on olive growth and production. Specifically, the water shortage has negative impacts on water connections, nutrient absorption, carbon assimilation, canopy dimension, oxidative pathways, phenology and reproduction processes of olive trees (Bacelar et al., 2006; Brito et al., 2019).

New agro-technologies help plants to alleviate abiotic stresses, which usually cause a big challenge for olive production. As a new material – Lithovit, a new technological fine powder created by tribiodynamic activation and micronization, was found to alleviate a negative impact of drought stress. In addition, some nutrients also contain in this product, including trace elements that influence physiological processes, growth, vitality and quality of the fruit crop as well as increase the resistance to abiotic stresses (Hamoda et al., 2016). Micronized calcium carbonate (Lithovit) fertilizer is a natural stone that was converted to a fine powder in special mills. This material as a foliar application leads to decompose its particles and release among other substances, especially calcium oxide (CaO) and carbon dioxide (CO_2) at high concentration in the intercellular compartment inside the leaves as well as on leaves surface which penetrates directly through the stomata (Kumar et al., 2013). The process of elevating CO_2 in intercellular compartment and leave surface leads to close stomata and reduce photosynthesis efficiently due to diffused carbon dioxide inside the leaves, so plant decreases transpiration rate and reduces water requirement due to high drought tolerance (Bunce, 2003; Ainsworth and Rogers, 2007; Carmen et al., 2014). Recent studies showed the metabolic changes and efficiency of Lithovit treatment on the productivity of various crops, including soybean (Abd El-Nabi and Eid, 2018), wheat (Morsy et al., 2018), onion (Abdelghafar et al., 2016), potato (Farouk, 2015) and many other crops.

To date, only a few studies were dedicated to the combined effect of irrigation regime and Lithovit treatment to olive performance under the arid environment. Thus, this study was initiated to follow up the effect of spraying Lithovit ($CaCO_3$ & $MgCO_3$) on water relations, chemical compositions and yield quality of Picual and Manzanello varieties of olive under severe water stress conditions of the desert area.

Material and Methods

Experiment area and design

This study was carried out at Wadi El-Natron of a private orchard at Wadi El-Natron, El Behera governorate, Egypt (30° 31' 05" N and 30° 07' 34" E). Surface soil samples were taken and air dried for carrying out a chemical analysis which presented in the Tables 1. The experimental field soil is considered sandy soil, consisting coarse sand 15.4%, medium sand 38.1%, fine sand 32.2%, very fine sand 6.5% and silt+clay 0.36%. The experiment was started in December in both two successive growing seasons (2017 and 2018). Two popular olive varieties, Picual and Manzanello, with an age of 11 years old were studied which were grown by vegetative multiplication. The stand density was 333 tree per hectare. Seventy-two bearing trees were selected and divided into 12 different treatments. Each treatment was divided into three replicates and two trees for each of them. These selected trees were treated with three irrigation levels (50, 75 and 100% of ETc) and four concentrations of micronized calcium carbonate (Lithovit) (0, 2, 4 and 6 g/L) which were sprayed as the foliar application in the first week of February, May and August.

Table 1. Soil chemical analysis

Soil layer	Ca^{2+}, mg/kg	Mg^{2+}, mg/kg	Na^{+}, mg/kg	K^{+}, mg/kg	CO_3^{2-}, mg/kg	HCO_3^{-}, mg/kg	Cl^{-}, mg/kg	SO_4^{2-}, mg/kg	pH	EC, ds/m	TDS, mg/L
0-30 cm	25.988	2.518	5.218	0.383	Nil	0.42	30.389	4.828	7.6	3.34	2334.3
30-50 cm	25.850	2.603	5.152	0.382	Nil	0.35	31.423	3.482	7.48	3.22	2243.1

Irrigation water was pumped out of a well and kept for a two hours before using for watering through a drip irrigation system. The well-water exhibited the following chemical indicators: EC 1617 µS/cm, TDS 641.5 mg/l, pH7.7, cations: Ca^{2+} 1.638, Mg^{2+} 1.467, Na^{+} 8.696, K^{+} 0.077 mg/kg, anions: CO_3^{2-} 0.799, HCO_3^{-} 2.599, SO_4^{2-} 0.262, Cl^{-} 7.668 mg/kg.

Climatic Data

El Behera governorate, Egypt is situated in an arid zone where the long-term annual average rainfall does not exceed 7 mm and a growing season average rainfall is around 4-9.5 mm (Table 2). Despite some rainfall (2-4.3 mm) at the beginning of spring, no rain is expected in the summer period. Whereas crop evapotranspiration (ETc) reached the maximum level in June with values of 5.967 and 4.42 mm/day, respectively of the 2017-2018 experiment years (Table 3). In this hyper-arid condition, crop growth is only possible with applying an appropriate irrigation regime (Table 4).

Table 2. Weather data on air temperature, rainfall and relative humidity of the study area, El Behera governorate, Egypt

	Month of the year											
Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Air temperature (⁰C)												
2017	15.1	17.5	22.0	29.1	34.5	38.7	39.8	39.8	36,6	31.3	23.3	16.9
2018	15.2	17.3	21.6	29.7	34.0	38.0	39.2	39.3	36.4	30.9	22.2	15.9
LTA	15.2	17.4	21.8	29.4	34.3	38.4	39.5	39.6	36.5	31.1	22.8	16.4
Rainfall (mm)												
2017	1.0	1.0	1.0	1.0	0	0	0	0	0	0	0	0
2018	1.5	1.5	2.0	2.3	0	0	0	0	0	0	0	2
LTA	1.3	1.3	1.5	1.7	0	0	0	0	0	0	0	1
Relative humidity (%)												
2017	52.0	44.0	39.0	31.0	29.0	31.0	36.0	38.0	43.0	46.0	51.0	55.0
2018	54.0	46.0	41.0	33.0	28.0	32.0	37.0	37.0	44.0	45.0	52.0	54.0
LTA	53.0	45.0	40.0	32.0	28.5	31.5	36.5	37.5	43.5	45.5	51.5	54.5
Evapotranspiration mm/day												
2017	1.43	1.95	3.37	4.69	5.43	5.96	5.60	5.23	4.55	5.60	2.48	1.95
2018	1.43	1.94	3.37	4.17	4.42	4.42	3.60	3.36	2.92	3.34	2.30	1.81
LTA	1.43	1.94	3.37	4.17	4.42	4.42	3.60	3.36	2.92	3.34	2.30	1.81
Crop Coefficient (KC)												
2017	0.50	0.50	0.65	0.68	0.68	0.68	0.70	0.70	0.70	0.70	0.70	0.70
2018	0.50	0.50	0.65	0.60	0.55	0.50	0.45	0.45	0.45	0.65	0.65	0.65
LTA	0.50	0.50	0,50	0.64	0.61	0.59	0.57	0.57	0.57	0.67	0.67	0.67

LTA: Long-term average
Source: Meteorological Station of Damanhur, Bahira Governorate, Egypt.

Table 3. Kc and ETc. in El-Behara, by using climwatt and cropwatt programs.

	First season		Second season	
	Kc	ETc mm/day	Kc	ETc mm/day
January	0.50	1.43000	0.50	1.43000
February	0.50	1.94500	0.50	1.94500
March	0.65	3.37350	0.65	3.37350
April	0.68	4.69125	0.60	4.17000
May	0.68	5.43375	0.55	4.42750
June	0.68	5.96700	0.50	4.42000
July	0.70	5.60700	0.45	3.60450
August	0.70	5.23600	0.45	3.36600
September	0.70	4.55000	0.45	2.92500
October	0.70	3.60500	0.65	3.34750
November	0.70	2.48500	0.65	2.30750
December	0.70	1.95300	0.65	1.81350

Table 4. Irrigation water levels, dates and intervals.

Months	First season						Second season					
	Irrigation requirement L/ day			Irrigation duration	Irrigation interval days	Number of irrigation time/month	Irrigation requirement L/ day			Irrigation duration	Irrigation interval days	Number of irrigation time/month
	100%	75%	50%	hours/ day			100%	75%	50%	hours/ day		
January	23.32	17.49	11.66	0.25	9	3	23.3	17.5	11.7	0.25	9	3
February	31.72	23.79	15.86	0.34	6	5	31.7	23.8	15.9	0.34	6	5
March	55.02	41.27	27.51	0.60	4	7	55.0	41.3	27.5	0.60	4	7
April	76.51	57.38	38.26	0.83	3	10	68.0	51.0	34.0	0.74	3	10
May	88.61	66.46	44.31	0.96	2	15	72.2	54.2	36.1	0.78	3	10
June	97.31	72.98	48.66	1.06	2	15	72.1	54.1	36.0	0.78	3	10
July	91.44	68.58	45.72	0.99	2	15	58.8	44.1	29.4	0.64	3	10
August	85.39	64.04	42.70	0.93	2	15	54.9	41.2	27.4	0.60	4	7
September	74.20	55.65	37.10	0.81	3	10	47.7	35.8	23.9	0.52	4	7
October	58.79	44.09	29.40	0.64	3	10	54.6	40.9	27.3	0.59	4	7
November	40.53	30.40	20.27	0.44	5	6	37.6	28.2	18.8	0.41	5	6
December	31.85	23.89	15.93	0.35	6	5	29.6	22.2	14.8	0.32	7	4

Meteorological data was determined by using climwatt and cropwatt programs to calculate reference evapotranspiration.

ETc calculated as follow:

$$ETc = ETo \times Kc$$

ETc : crop evapotranspiration
ETo : reference crop evapotranspiration
Kc : crop coefficient

Lithovit composition

Natural CO_2 as a Nano-foliar fertilizer in the form of Lithovit (a Nano $CaCO_3$) is a new top quality nanotechnological nanopowder created by tribodynamic activation and micronization. The main constituents of Lithovit® were illustrated as follow: Calcium carbonate 79.19, Sulphate 0.33, Nitrogen 0.06, Iron 1.31, Phosphate 0.01, Zinc 0.005, Potassium oxide 0.21, Manganese 0.014, Magnesium carbonate 4.62, Copper 0.002, Selinium dioxide 11.41, Clay 0.79. Lithovit 2, 4, and 6 g/L concentrations were prepared by mixing with sterile water two hours before the treatment. 10 L of suspension with appropriate concentration were sprayed per olive tree.

Determinations of water relation

Leaf water content (L.W.C.)

The leaves were excised before dawn and fresh weight of the leaf samples was determined. Then, the samples transferred to the oven at 72-75^{0}C., until constant weight. The samples were weighed again after the drying process. This procedure was repeated after 10, 15 and 20 days of the last spray in each experiment in both season and the average data were presented.

Leaf water content was calculated according to the following equation:

$$LWC = \frac{Fw - dw}{Fw} \times 100$$

Fw = fresh weight of leaves

Dw = dry weight of leaves

Leaf relative water content (L R.W.C)

10 leave samples were prepared to determine fresh weight immediately after removal from the trees. These samples were placed into a closed container filled with distilled water and kept until they reached constant weight (about 48 hours after) in a shade place. The leaves were surface dried with a blotting paper and weighted to determine their turgid weight. The dry weight of the discs was determined after 24 hours; this procedure was repeated after 10, 13 and 20 days of the last spray in each experimental season and the average data were presented.

Leaf water relative content was calculated according to the following equation:

$$L.W.R.C = \frac{Fw - Dw}{Tw - Dw} \times 100$$

Tw = Turgid weight

Chemical analysis

The leaf proline content was estimated according to the method described by Bates et al. (1973). Total carbohydrate was estimated according to the method described by Smith et al. (1964).

The percentage of moisture and oil content of fruits

The fresh fruit samples were dried in an oven at 60°C until constant weight then moisture percentage was calculated. Oil content percentage was estimated from the fresh fruit samples by extracting the oil using hexan at 60-80°C boiling points by Soxhlet fat extraction apparatus as described in the A.O.A.C. (1990).

Total chlorophyll contents (SPAD)

Total chlorophyll contents on the 5th or the 6th leaves of ten shoots per tree were determined at the end of November in the experimental field by using Minolta chlorophyll meter "SPAD 502" (Wood et al., 1993).

Leaf mineral content

Samples of 30 leaves for each replication were collected from the first full mature leaves (5th – 7th leaves from shoot tips) in mid of October. The leaves were washed with distilled water then dried using an electric oven at 60-70°C until constant weight, then were ground in a stainless-steel mill. Wet digestion was done by using concentrated sulphoric acid and hydrogen peroxide according to Parkinson and Allen (1975). Total nitrogen content was determined by modified the Micro-kjeldahl method as described by Plummer (1971). Phosphorus percentage was determined by the method of Truog and Meyer (1929).

The percentages of potassium, calcium and magnesium were measured by using Atomic Absorption spectrophotometer (Model 3300, MS-DOS, detection limit is 3 s, µg/L, PerkinElmer Inc., USA) according to the method described by Chapman and Pratt (1961).

Fruit weight and yield

Fruit weight was measured by using a balance for 20 fruits per tree. Fruit yield was recorded as kg/tree by using a digital balance.

Statistical analysis

The results in each parameter were exposed to proper statistical analysis of variance for a split plot design with two factors by using statistics computer program (Anonymous, 2008) with three replicates each of them includes average of two trees values. The irrigation regimes were considered as the main plot and the Lithovit treatments as sub plot. Duncan's multiple range tests was used for comparison between means. Different alphabet letters in the column significantly differed at (0.05) level of significance (Duncan, 1955). The same trees were used throughout both experimental seasons.

Results

Water relations in leaves and fruits

Averaged across irrigation treatments, the highest ETc irrigation regime (100%) exhibited the greatest values of leaf water content, relative water content, total chlorophyll and fruit moisture in both Manzanello and Picual olive varieties (Tables 5 and 6). As the subplot effect, the Lithovit treatments significantly enhanced the above-mentioned vegetative parameters of both olive varieties at all ETc irrigation levels, indicating positive effects of the foliage nutrition.

The above-mentioned physiological parameters declined gradually with decreasing ETc irrigation levels and Lithovit application rates. The main plot (ETc irrigation regime) effect was significant for all these parameters, while the subplot (Lithovit treatments) effect values did not reach to significant level at the closed doses.

The highest leaf water content was recorded at an irrigation level of 100% ETc was interacted with 6 g/L Lithovit treatment with values 55.68 and 57.13% in Manzanello and 55.20 and 58.96% in Picual, respectively in the first and second seasons. Conversely, the lowest leaf water content was found at an irrigation level of 50% ETc was used without Lithovit treatment, reducing the values by 54.2 and 41.4% in Manzanello and by 65.5 and 73.6% in Picual, respectively in the first and second seasons. These results were not significantly differed from the values attained at an irrigation regime of 50% ETc when was interacted with 2 g/L Lithovit treatment in both olive varieties, confirming slight changes among the closed Lithovit rates. Whereas, there were the substantial difference in the determined value of both olive varieties when was compared between irrigation levels of 50%, 75% and 100% ETc, proving water deficit is the decisive factor for olive growth performance under the arid zone.

Table 5. Effect of ETc and Lithovit applications on water relations of Manzanello.

Treatments		Leaf water content		Relative water content		Total chlorophyll		Fruit moisture (%)	
ETc	Lith	2017	2018	2017	2018	2017	2018	2017	2018
					Manzanello				
	0 g/L	51.77b	52.16b	57.51b	74.56b	70.33bc	78.83c	51.24bc	53.43a
100%	2 g/L	53.52ab	54.61ac	59.01ab	77.43ab	73.70b	79.27c	52.47ab	54.19a
	4 g/L	54.82ab	56.30ab	60.04ab	79.27ab	74.80ab	80.67b	52.06a	53.37a
	6 g/L	55.68a	57.13a	61.90a	80.61a	75.23a	82.17a	52.86a	54.90a
	0 g/L	46.30c	46.48c	52.90d	57.70c	68.57cd	78.57c	48.90cd	50.17bc
75%	2 g/L	46.83c	46.91c	53.63cd	62.47bc	70.17c	78.60c	50.23c	50.29bc
	4 g/L	46.91c	48.69bc	54.07c	64.14bc	71.30bc	78.60c	51.40bc	51.53bc
	6 g/L	47.04c	49.57bc	54.23c	65.53bc	73.53b	79.03c	51.82ab	51.14bc
	0 g/L	36.11d	40.39e	43.51f	43.14e	67.47d	70.27f	46.24e	48.95c
50%	2 g/L	37.69d	42.13de	47.45ef	46.17f	68.50cd	73.27e	47.24de	48.72c
	4 g/L	38.46d	43.62de	49.32e	51.30d	69.17cd	74.10de	49.15cd	49.93bc
	6 g/L	38.70d	44.32d	49.04e	52.27d-f	71.87c	74.90d	49.67cd	50.23bc

Means followed by the same letters (S) in each column are not significantly different at 5% level.

Table 6. Effect of ETc and Lithovit applications on water relations of Picual.

Treatments		Leaf water content		Relative water content		Total chlorophyll		Fruit moisture (%)	
ETc	Lith	2017	2018	2017	2018	2017	2018	2017	2018
					Picual				
100%	0 g/L	51.49bc	54.29bc	62.21c	70.99b	68.27cd	70.80c	59.05ab	60.14b
	2 g/L	53.01b	55.62b	64.08b	72.13ab	69.63c	71.93c	59.40ab	61.21ab
	4 g/L	54.07ab	57.29ab	65.41ab	72.69ab	72.40ab	74.00b	60.11ab	61.34ab
	6 g/L	55.20a	58.96a	66.46a	74.72a	74.47a	76.93a	61.12a	61.82a
75%	0 g/L	46.54 e	48.70d	50.44e	57.54d	67.53d	68.53d	56.27bc	56.95c
	2 g/L	47.14d	48.81d	52.23d	59.16cd	68.43cd	71.33c	52.76e	58.74bc
	4 g/L	48.95cd	49.70d	52.82d	59.73cd	70.30ab	73.93b	57.26bc	58.92bc
	6 g/L	49.38c	50.52c	53.57d	62.17c	72.80ab	75.80ab	58.35b	59.81bc
50%	0 g/L	33.35f	33.96f	39.42g	47.41fg	65.73g	64.37f	52.51e	54.92d
	2 g/L	34.62f	36.40ef	41.05e	49.02f	66.07de	66.10e	53.46de	55.72cd
	4 g/L	34.61f	36.71ef	42.39fe	51.38ef	67.83cd	67.33d	55.17d	58.13bc
	6 g/L	35.18f	38.29e	43.69f	53.61e	69.17cd	70.77c	57.12bc	57.62bc

Means followed by the same letters (S) in each column are not significantly different at 5% level.

A similar trend was observed in leaf relative water content irrespective of different treatments in both seasons. The interaction between 100% ETc irrigation regime of and 6 g/L Lithovit treatment resulted in the highest leaf relative water content with values of 61.90% and 80.61% in Manzanello and 66.46% and 74.72% in Picual, respectively in the first and second seasons.

Reasonably significance was observed on fruit moisture content that Lithovit at a rate of 6 g/L and 100% ETc irrigation had the maximum fruit moisture content in Manzanello with values of 52.86 and 54.90% and in Picual with values of 61.12% and 61.82%, respectively in the first and second seasons.

Total chlorophyll values were also more influenced by the irrigation regime than the Lithovit treatments. The highest values were recorded in the well-watered treatment (100% ETc) coming together with 6 g/L Lithovit application. Whereas the lowest readings were noted under the severely water-stressed (50% ETc) treatment combined without (0 g/L) Lithovit spray in both olive varieties. The differences between the highest and lowest values were 11.5% and 16.9% in Manzanello and 13.3% and 19.5% in Picual as compared to the control, respectively in the first and second seasons. Although the impact of these treatments showed a similar tendency, a significant difference was revealed in chlorophyll content values between these two olive varieties in majority same treatments, showing variety-specific reactions.

Chemical composition of olive leaves

Lithovit significantly enhanced olive leaf N, P, K, Ca and Mg concentrations, however, the highest readings were detected at 100% ETc irrigation level in both olive varieties (Tables 7 and 8). The leaf nutrients contents increased with increasing irrigation level. The Lithovit effect was not significant at the closed doses in most cases. Averaged across the main plot values, the leaf chemical content was significantly increased at the irrigation regime of 100% ETc as compared to 50% ETc: N 50.4% and 87.1%, P 52.3% and 116.7%, K 99.3% and 56.3%, Ca 23.9% and 28.3%, Mg 104.5% and 75.0% in Manzanello, respectively in the two seasons. Likewise, similar increments under the same treatment were revealed in Picual, N 72.4% and 50.9%, P 27.3% and 77.8%, K 57.5% and 52.8%, Ca 27.3% and 49.2%, Mg 75.0% and 81.8%, regardless of the Lithovit treatments.

Table 7. Effect of ETc and Lithovit applications on chemical content of Manzanello.

Treatments		N, %		P, %		K, %		Ca, %		Mg, %	
ETc	Lith	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
						Manzanello					
100%	**0 g/L**	1.45c	1.69c	0.18b	0.31b	1.19b	1.47bc	1.87b	1.93c	0.19d	0.20d
	2 g/L	1.47bc	1.72b	0.19a	0.33a	1.54a	1.88a	1.90c	1.97bc	0.21c	0.21cd
	4 g/L	1.48b	1.74b	0.16cd	0.29c	1.45a	1.82a	1.92ab	2.01a	0.24b	0.23b
	6 g/L	1.51a	1.81a	0.14ef	0.24d	1.24b	1.38c	1.93a	2.02a	0.26a	0.27a
75%	**0 g/L**	1.28f	1.42f	0.15de	0.21f	0.87d	1.05de	1.69f	1.70f	0.15f	0.16g
	2 g/L	1.32e	1.44ef	0.16c	0.23e	1.25b	1.71ab	1.72e	1.73ef	0.17e	0.18f
	4 g/L	1.35d	1.45e	0.15cd	0.19g	1.02c	1.30cd	1.75d	1.75e	0.19d	0.19e
	6 g/L	1.35d	1.51d	0.13f	0.17h	0.64 e	0.70f	1.76d	1.81d	0.21c	0.22 c
50%	**0 g/L**	0.95i	0.12i	0.11g	0.14i	0.76d	0.88ef	1.51i	1.51h	0.09i	0.11i
	2 g/L	0.98h	1.17h	0.13f	0.16h	0.60ef	1.48bc	1.53hi	1.51h	0.11h	0.13h
	4 g/L	0.99h	1.19h	0.11g	0.14u	0.85d	1.23cd	1.55gh	1.57g	0.12g	0.14h
	6 g/L	1.01g	1.24g	0.09f	0.10j	0.51f	0.60f	1.56g	1.59g	0.12g	0.14h

Means followed by the same letters (S) in each column are not significantly different at 5% level.

Table 8. Effect of ETc and Lithovit applications on chemical content of Picual olives

Treatments		N, %		P, %		K, %		Ca, %		Mg, %	
ETc	Lith	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
						Picual					
100%	**0 g/L**	1.40c	1.71c	0.18b	0.31b	1.47c	1.74b	1.87c	1.92c	0.24c	0.25d
	2 g/L	1.65b	1.74b	0.19a	0.33a	1.79a	2.00a	1.90bc	1.97c	0.26b	0.27bc
	4 g/L	1.68a	1.76b	0.16cd	0.29c	1.62b	1.81ab	1.92b	2.11b	0.26b	0.28b
	6 g/L	1.69a	1.80a	0.14ef	0.24d	1.41 c	1.57bd	1.96a	2.32a	0.28a	0.30a
75%	**0 g/L**	1.27g	1.42f	0.15de	0.21f	1.13d	1.38d	1.69f	1.70de	0.18gh	0.21g
	2 g/L	1.33f	1.47e	0.16c	0.23e	1.21d	1.17e	1.72e	1.73d	0.19ef	0.22f
	4 g/L	1.37e	1.48e	0.15cd	0.19g	1.13d	1.44bd	1.75d	1.75d	0.20e	0.24e
	6 g/L	1.40d	1.52d	0.13f	0.17h	0.86 f	1.07fg	1.77d	1.78d	0.22d	0.26cd
50%	**0 g/L**	0.95j	1.15j	0.11g	0.14i	0.89f	0.99fg	1.50i	1.51ef	0.14j	0.14j
	2 g/L	0.97i	1.18i	0.13f	0.16h	1.00e	1.18dg	1.53h	1.54f	0.15i	0.16i
	4 g/L	0.99i	1.21h	0.11g	0.14u	0.93ef	1.03fg	1.55gh	1.57f	0.17h	0.18h
	6 g/L	1.01h	1.23g	0.09f	0.10j	0.76g	0.91g	1.58g	1.60f	0.18fg	0.18h

Means followed by the same letters (S) in each column are not significantly different at 5% level.

However, the Lithovit treatment at various doses promoted irrigation regimes' effect on leaf chemical content with more obvious indicators than without Lithovit treatment.

The highest N content was detected under the combined application of 100% ETc with 6 g/L Lithovit treatment with values 1.51 and 1.81% in Manzanello and 1.69 and 1.80% in Picual, respectively the first and second seasons. The leaf N contents of these two olive varieties at this combined treatment were significantly higher by 49.5% and 46.0% in Manzanello, by 66.3% and 43.1% in Picual, respectively in the two seasons than those at irrigation level of 50% ETc was used without the Lithovit treatment.

Similarly, leaf Ca and Mg contents were higher under combined application of 100% ETc with 6 g/L Lithovit treatment with values of Ca 1.93% and 2.02%, Mg 0.26 and 0.27% in Manzanello, Ca 1.96 and 2.32, Mg 0.28 and 0.30 in Picual, respectively in the first and second seasons.

The highest P and K contents were found at the irrigation level of 100% ETc was coming together with 2 g/L Lithovit treatment, exhibiting P 0.19% and 0.33%, K 1.54% and 1.88% in Manzanello, P 0.19% and 0.33%, K 1.79% and 2.0% in Picual, respectively in the two seasons.

Whereas, the lowest N, P, K, Ca and Mg concentrations in olive leaf were detected following the application of 50% ETc coupled with 0 g/L Lithovit treatment.

Regardless of Lithovit addition, the plant nutrients (N, P, K, Ca and Mg) contents were higher under 100% ETc irrigation level which was significantly higher than those of other irrigation regimes, confirming the effectiveness of the sufficient irrigation for olive nutritional balance.

Fruit chemical content and yield

The increase of fruit quality and yield was substantially influenced by the interaction of irrigation level and Lithovit application doses in both olive varieties (Tables 9 and 10).

Table 9. Effect of ETc and Lithovit applications on fruit quality and yield of Manzanello.

Treatments		Fruit oil content (%)		Total carbohydrates (%)		Proline content (ppm)		Fruit weight (g)		Fruit yield (kg/ tree)	
ETc	Lith	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
		Manzanello									
100%	0 g/L	38.14f	39.12ef	3.17f	11.29de	14.60g	8.43h	5.04c	5.07f	54.75f	27.83e
	2 g/L	38.47ef	40.23bc	5.03c	13.98ab	14.80g	10.70g	5.62b	5.87d	61.67d	33.67d
	4 g/L	39.02ef	39.65cd	4.05de	12.91bc	16.30f	13.57f	5.71b	6.86b	76.33c	37.83c
	6 g/L	38.01f	38.17f	3.73e	12.55cd	17.37f	15.15e	6.90a	7.93a	56.17e	33.67d
75%	0 g/L	42.15ab	42.76ab	6.90b	12.79bc	19.90e	14.61ef	4.35d	4.57g	62.83d	33.87d
	2 g/L	42.45ab	42.91ab	8.42a	15.23a	21.10de	19.20d	4.87c	4.97fg	75.17ab	41.60b
	4 g/L	43.37a	44.17a	7.31b	14.83ab	22.40d	20.32d	5.72b	5.77de	75.33a	45.43a
	6 g/L	42.78ab	42.65ab	6.77b	13.19bc	25.67c	21.90c	6.02b	6.40c	66.83b	38.33c
50%	0 g/L	39.53d	40.12c	2.41g	8.33g	27.07bc	22.70bc	2.38f	4.00h	19.67i	14.77f
	2 g/L	40.50bc	41.69bc	4.66cd	11.29de	28.17b	23.50b	3.71e	4.17h	25.83g	17.60f
	4 g/L	41.20b	40.78bc	3.50ef	10.03ef	30.63ab	25.37ab	4.07de	4.47g	25.67g	17.10f
	6 g/L	40.01c	39.20d	3.71ef	9.17fg	32.10a	26.23a	4.37d	5.27e	24.17h	15.27f

Means followed by the same letters (S) in each column are not significantly different at 5% level.

Table 10. Effect of ETc and Lithovit applications on fruit quality and yield of Picual.

Treatments		Fruit oil content (%)		Total carbohydrates (%)		Proline content (ppm)		Fruit weight (g)		Fruit yield (kg/ tree)	
ETc	Lith	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
		Picual									
100%	0 g/L	41.13bc	39.21f	3.77d	14.82de	12.92i	10.27h	5.03f	5.47g	38.83h	26.93f
	2 g/L	42.14ab	40.21ef	4.70bc	19.18b	14.70h	13.51g	5.83c	5.47d	48.33f	33.83c
	4 g/L	41.78b	40.07ef	3.78de	16.90cd	18.49g	14.86fg	6.54b	6.90b	52.17e	31.27d
	6 g/L	40.84d	40.76de	3.71d	15.16de	19.76fg	17.30de	7.84a	8.13a	45.67g	29.33e
75%	0 g/L	42.24ab	43.08b	3.74d	15.54ce	18.81g	10.27h	5.25e	5.67h	54.50d	31.10de
	2 g/L	43.39a	44.78a	5.45a	23.29a	21.40ef	15.68ef	5.51d	5.80g	62.67b	38.10b
	4 g/L	42.98ab	42.84bc	5.34ab	16.58cd	22.51e	17.84d	6.03c	6.53g	65.17a	40.60a
	6 g/L	41.83b	42.83bc	4.64bc	16.18cd	24.73d	19.73 c	6.62b	6.67f	58.17c	34.77c
50%	0 g/L	41.13cd	42.09b-d	4.28c	9.69g	26.22c	20.54bc	3.67i	4.77ef	18.00k	11.10i
	2 g/L	42.14ab	42.77bc	4.49bc	14.31e	26.97bc	21.08bc	4.18h	5.33e	22.33i	17.20g
	4 g/L	41.78b	42.03b-d	3.46de	12.14f	28.62ab	21.62ab	4.63g	5.37cd	20.67j	16.27g
	6 g/L	40.84d	41.22c-e	3.15e	11.22fg	29.92a	22.43 a	5.05f	5.63c	19.83j	14.17h

Means followed by the same letters (S) in each column are not significantly different at 5% level.

There was no significant difference in fruit oil content in the closed doses of Lithovit treatment as a sub-plot effect, however, the main plot effect was permanently significant at all irrigation levels.

The highest oil content was detected at 75% of ETc irrigation level was interacted with 4 g/L Lithovit treatment with values of 43.37 and 44.17% in Manzanello, whereas, this parameter in Picual was obtained at

75% of ETc irrigation level combined with 2 g/L Lithovit with values of 43.39 and 44.78%, respectively in the first and second seasons.

Total carbohydrates percentage was significantly varied regarding the interaction between irrigation levels and spraying Lithovit. The highest readings of total carbohydrates percentage were noted under the combined treatment of 75% of ETc irrigation and 2 g/L Lithovit spray with values of 8.42 and 15.23% in Manzanello as well as 5.45 and 23.29% in Picual, respectively in the first and second seasons.

Proline content substantially increased with decreasing irrigation level, however, an opposite increasing trend was observed regarding Lithovit doses in both olive varieties, as well as, Lithovit application doses were significant in most cases. The highest proline content was obtained at 50% of ETc irrigation level combined with 6 g/L Lithovit application in Manzanello with values of 32.10 and 26.23 ppm and in Picual with values of 29.92 and 22.43 ppm, respectively in the first and second seasons. Whereas, the lowest proline content in both olive varieties was revealed at 100% of ETc irrigation level when interacted with 0 g/L Lithovit application, decreasing the above-mentioned parameter by 119.9% and 211.2% in Manzarello and 131.6% and 118.4% in Picual, respectively in the first and second seasons.

A significant difference was observed in olive varieties' fruit weight value regarding the irrigation levels and Lithovit treatments. The highest fruit weight parameter was recorded at 100% of ETc irrigation when interacted with 6 g/L Lithovit in Manzanello (6.90 and 7.93 g) and Picual (7.84 and 8.13 g) in both seasons, respectively. Whereas, the lowest fruit yield was detected at 50% of ETc irrigation combined with 0 g/L Lithovit treatment in Manzanello (2.38 and 4.00 g) and Picual (3.67 and 4.77 g), showing a 2-fold decrease due to the water deficit and Lithovit absence.

Regarding olive fruit yield as a main component of the study, significant interactions were observed between irrigation levels and Lithovit doses. The highest fruit yield of Manzanello (75.33 and 45.43 kg/tree) and Picual (65.17 and 36.14 kg/tree) was achieved at 75% of ETc irrigation combined with 4 g/L Lithovit compared to other treatments, respectively in the two seasons. Whereas, the lowest yield was recorded at 50% of ETc irrigation level was integrated with 0 g/L Lithovit application in Manzanello (19.67 and 14.77 kg/tree) and Picual (18.00 and 11.10 kg/tree), decreasing by 137.6% and 163.2% in Manzanello and 167.8% and 150.8% in Picual compared to the highest values, respectively in the two seasons.

Discussion

Results show substantial differences in the vegetative and generative parameters of the two olive varieties due to the alternative bearing during the two growing seasons. The values of these measured parameters in the first season were higher than those in the second season. A Lithovit treatment at a rate of 6 g/L recorded the highest values of leaf water content and leaf relative water content in Picual and Manzanello, which is highly likely due to the enhanced assimilation and dissimilation process CO_2 in the intercellular compartment and on the leaf surface. Subsequently, it led to close stomata according to Kumar et al. (2013) and decreased the transpiration rate and enhanced photosynthesis. With regard to the irrigation levels, the fruit moisture content of Picual and Manzanello significantly increased with an increasing water supply and 100% of Etc irrigation regime surpassed the other levels in both seasons. Same results were obtained by Ben Rouina et al. (2007) and Boughalleb and Hajlaoui (2011). Thus, these might be the reason for improving fruits' physical characteristics.

An application of Lithovit at a 6 g/L led to maximising the value of total chlorophyll in the first and second seasons. These results in agreement with those obtained by Sabina (2013); Shallan et al. (2016); Hamoda et al. (2016); Abd El-Nabi et al. (2017); Abd El-Nabi and Eid (2018); Ghatas and Mohamed (2018); Fathelrahman et al. (2020). Irrigation level at 100% of ETc stimulated the fruit moisture and total chlorophyll of both varieties, while irrigation at 50% of ETc recorded an opposite trend. Several researchers reported that spraying dolomite nano-particles might play an influential role in enhancing chlorophyll content because of its components (Abd El-Nabi et al., 2017; Ghatas and Mohamed, 2018). Furthermore, the reduction of these parameters was related to the degree of water content, as these parameters decreased gradually with increasing water stress. The negative effect of prolonged water stress reduces the plant cell's water content and relative water content, which affects the rate of cell expansion and ultimate cell size. Drought affects not only physiological processes but also deteriorates biochemical processes. Thus, drought stress caused a reduction in vegetative growth parameters and it depends on the severity of the drought. The reduction of chlorophyll pigment due to water stress might be reflected on degradation and reduction of olives growth and production. These results are in harmony with Sharma (2006); Arzani et al. (2009);

Guerfel et al. (2009); Boughalleb and Hajlaoui (2011); Shaheen et al. (2011); Tangu (2014) and Rosecrance et al. (2015).

An application of Lithovit at a rate of 6 g/L recorded the highest values of leaves N, Ca and Mg contents of both varieties compared to other treatments, while spraying at 2 g/L increased the values of P and K of both varieties in the two seasons. These results were in harmony with Maswada and Abd El-Rahman (2014); Gatas and Mohamed (2018). Water stress at 50% of ETc led to a decrease in the values of leaf mineral contents such as N, P, K, Ca and Mg of both varieties in both seasons. These elements increased gradually with increasing irrigation levels up to 100% of ETc. These results agree with those reported by Benlloch-González et al. (2008) and Shaheen et al. (2011).

Exposure water deficit led to a decrease in total carbohydrates of both olive varieties. These results may be explained by the reason for the degradation and reduction of olives metabolic processes. Also, water deficit in plants is associated with deteriorating physiological, biological, assimilation, and dissimilation processes under water stress conditions. Similar results were proved by Tombesi et al. (1986); Arji and Arzani (2008); Lelago and Tadele (2019).

Regarding the fruit oil content of both varieties, 75% of ETc recorded the highest values of this parameter. These results were in line with previous reports of Berenguer et al. (2004). Results proved that the drought level had a significant effect on the proline content of both varieties. Exposure olives to drought conditions increased proline content. Accumulation of organic compounds like amino acids in cells cytoplasm plays a major role in osmotic adjustment in plants. It is not the only important role of proline accumulation but also proline stores carbon and nitrogen without damaging. Proline is accumulated to higher levels than other amino acids in many plants under drought conditions. This might conclude that olive performance was excellent and the proline accumulation saved olives alive under prolonged drought and environmental stress conditions. It does not mean that olives did not get affected under water stress conditions, but olives adapt excellence under water stress. These findings are in partial agreement with Ennajeh et al. (2006); Arji and Arzani (2008); Bacelar et al. (2009) and Shaheen et al. (2011).

Lithovit treatment at a rate of 4 g/L led to increasing fruit oil and fruit yield of both varieties under 75% of ETc irrigation regime, while 6 g/L dose recorded the highest values of fruit proline content under 50% of ETc irrigation regime. These results are very close with the facts declared by Wang et al. (2013); Carmen et al. (2014); Maswada and Abd El-Rahman (2014); Abo-Sedera et al. (2016); Shallan et al. (2016); Ghatas and Mohamed (2018) and Abdel Nabi and Eid (2018).

While the water deficit increased the amount of proline but decreased the levels of carbohydrates, fruit oil content, fruit weight and fruit yield. These results indicated that water is a key factor for olive survival and productivity in the arid zone. In general, the highest yield was obtained at an irrigation level of 75% ETc which were significantly higher than those of 50% and 100% ETc irrigation regimes, confirming the effectiveness of the sufficient water supply for olive culture in the hyper-arid condition. Furthermore, the additional Lithovit treatments significantly contributed to improving the physiological and morphological attributes of both olive varieties.

Conclusion

A Lithovit treatment as foliage nutrition at a 6 g/L rate combined with an irrigation level of 100% ETc recorded the highest values of leaf water content, leaf relative water content, total chlorophyll and N, P, K, Ca and Mg contents of the two studied olive varieties compared to other treatment combinations.

However, the highest olive yield was obtained at 75% of ETc irrigation regime together with Lithovit treatment at a 4 g/L rate on the Picual and Manzanello varieties. This is the best treatment to balance between vegetative growth and fruiting. Moreover, this positive variance among the irrigation levels and Lithovit concentrations may reduce the hardness of alternative bearing.

Finally, this research indicates that water stress is a vital factor limiting olive production in the Mediterranean basin; sufficient water supply enhances plant growth, while foliage nutrition reinforces the physiological performance of olive plants grown in the hyper-arid zone.

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Comparison of different types of fertilizers on growth, yield and quality properties of watermelon (*Citrullus lanatus*) in the Southeast of Kazakhstan

Akbope Aitbayeva [a,*], Berik Zorzhanov [a,b], Zharas Mamyrbekov [a,b], Damira Absatarova [a], Birzhan Rakhymzhanov [b], Meruert Koshmagambetova [a]

[a] Kazakh Research Institute of Fruit and Vegetable Growing, Kainar, Kazakhstan
[b] Kazakh National Agrarian Research University, Almaty, Kazakhstan

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Author(s)

A. Aitbayeva *
B. Zorzhanov
Z. Mamyrbekov
D. Absatarova
B. Rakhymzhanov
M. Koshmagambetova

* Corresponding author

Abstract

Over the years, the use of organic materials in farming has reduced due to the increase in the use of chemical fertilizers which are rich in readily available plant nutrients. Intensive use of chemical fertilizers may have depressing effect on yield of watermelon. The field experiment were conducted at the Experimental Clinic of the Laboratory "Selection of vegetable and melon crops" and in the laboratory "Biosafety and Biocontrol of vegetable and melon crops" of Regional Branch "Kainar" of the LLP "Kazakh Research Institute of Fruit and Vegetable Growing" which is located in the foothill zone of the southeast of Kazakhstan, to study the effects of different types of organic fertilizers (cow manure, poultry manure and biohumus) and recommended chemical fertilizer (NPK) on the characteristics of watermelon (growth parameters, yield and quality) of southeast of Kazakhstan. There were significant differences among the treatments in relation to fruit yield of watermelon, growth paremeters and quality properties (dry matter, total sugar, Vitamin C and NO_3-N). Recommended fertilizer dose ($N_{90}P_{60}K_{60}$) had the highest fruit yield of watermelon and growth paremeters followed by manure applied at 40 t ha^{-1}. All the fertilizer treatments had higher yield of watermelon than control. From this study, the use of manure as an organic fertilizer in the cultivation of watermelon could be used as alternative to chemical fertilizer. It is recommended that manure at 40 t ha^{-1} be adopted for watermelon cultivation in in the Southeast of Kazakhstan.

Keywords: Watermelon, fertilizer, fertilization, manure, yield.

Introduction

Organic farming is an eco-friendly means of production based on sustainable productivity that doesn't use artificial components such as chemical fertilizers or pesticides. Organic food is food produced using methods that do not include modern artificial additives such as pesticides and chemical fertilizers, does not contain genetically modified organisms and is not treated with radiation, industrial solvents or chemical food additives. Unfortunately, in the World, currently, there is only about 1% of organic farming (Duram, 2005; Hansen, 2010). In Asia, the top three organic farming nations by land area are China, India, and Kazakhstan. Kazakhstan has about 291,203 ha of land used for organic farming. Organic farming in Kazakhstan is becoming increasingly popular due to the growing demand for organic products, availability of large tracts of agricultural land, and comparative advantage such as low labor costs. Organic farmers engage in both horticulture and cattle farming some of which are exported. Watermelon cultivation is one of the main agricultural sectors in Kazakhstan that is largely come into play to organic farming.

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Watermelon (Citrullus lanatus) is a member of the cucurbit family (Cucurbitaceae), which also includes cantaloupes, cucumbers, pumpkins, squash, zucchini and butternuts. It is one of the most widely cultivated crops in the world. Its global consumption is greater than that of any other cucurbit (Schaffer and Paris, 2016). Watermelons have the greatest world production of any cucurbit, exceeding 63 million tonnes according to the FAO. Not surprisingly, China is by far the largest producer of watermelons, with over 38 million tonnes. The principal watermelon producing countries in Asia are China, Turkey, Iran and Kazakhstan. China produces over 50% of the world supply. Kazakhstan produced 1.2 million tons of watermelon (12th largest producer in the world) in 2018. Watermelon is a good cash crop in Kazakhstan with very good market opportunities, particularly in urban areas (FAO, 2020).

In organic farming, the soil becomes rich in nutrients; therefore, crops grow healthy and can be resistant to pests and diseases, making the quality of the products more nutritious, tastier and contain substances that are good for health. In order to improve the yield of watermelon, the nutrient contents should be increased to increase the soil fertility. One of the ways of improving soil fertility is by maintaining its organic matter. The organic matter content in soil can be increased by the addition of organic wastes (Gülser and Candemir, 2015) such as municipal solid waste, food waste, biowaste, manure, sewage sludge, etc. The quality of soil and improvement of soil health can be restored by incorporation of recycling organic wastes in the soil (Kızılkaya, 2005, 2008). Debiase et al. (2016) reported that urban areas produce a huge amount of degradable organic wastes such as Municipal solid waste and sewage sludge. These wastes contain several macro- and micro-nutrients that can be used as potential organic fertilizers for crop production. Goss et al. (2013) are of the same opinion. Several studies have been done related to organic amendments on improving soil physical, chemical, and biological properties, providing essential plant nutrients to stimulate plant growth and yield (Kızılkaya and Hepşen, 2004, 2007; Candemir and Gülser, 2010; Gülser et al., 2017; Kızılkaya et al., 2021). Rodriguez-Vila et al. (2016) found that organic amendments sustain soil properties by increasing organic matter, nutrient content, microbial activity and thus increase crop growth and yield. Organic wastes not only influence soil properties, but also play a great role in the growth and development of plants and thus improve agricultural productivity. Studies by Papafilippaki et al. (2015) and Mbarki et al. (2008) recorded positive responses of spiny chicory and Ryegrass to organic waste application. Debiase et al. (2016) reported that incorporated organic wastes gave 32% higher yields than an unfertilized field. Moreover, the authors con-firmed that SS application ensured 12% higher yield compared to municipal solid waste addition of a wheat field. These amendments not only increase crop yields, but also minimize the risk of nitrogen leaching from the soil. There is paucity of information on the use of organic wastes for the production of watermelon in southeast of Kazakhstan.

This study was carried out to evaluate the effects of different types of organic fertilizers (cow manure, poultry manure and biohumus) and recommended chemical fertilizer (NPK) on the characteristics of watermelon (growth parameters, yield and quality) of southeast of Kazakhstan.

Material and Methods

Description of the Study Sites

The experiments were conducted at the Experimental Clinic of the Laboratory "Selection of vegetable and melon crops" and in the laboratory "Biosafety and Biocontrol of vegetable and melon crops" of Regional Branch "Kainar" of the LLP "Kazakh Research Institute of Fruit and Vegetable Growing" which is located in the foothill zone of the southeast of Kazakhstan (43°09'32.8"N 76°26'57.3"E) North Slope of Zailiyskiy Alatau Mountains (Altitude : 1000-1050 m) during the growing season 2019-2020 with a view to finding out the Watermelon expo as well as determining the different organic fertilizers such as biohumus, manure, poultry manure, grain straw, biohumus and recommended chemical NPK fertilizers. The locations of the evaluations were characterized by the continental climate (large daily and annual fluctuations in air temperature, characterized by cold winters and long hot summers), the air temperature reaches minimum values in January (-32,-35^{0}C), and maximum values in July (37-43^{0}C). The warm period lasts 240-275 days, the frost-free period is 140-170 days and an annual amount of precipitation is 350-600 mm.

The soil belongs to the general soil type of dark chestnut. The land was medium high with loamy. Before conducting the experiment, the soil sample was analyzed from Kazakh Research Institute of Soil Science and Agricultural Chemistry named after U. Uspanov. The soil was characteristically slightly alkaline (pH 7.3-7.4), soil organic matter 2.9-3.0% (moderate), total N 0.18-0.20% (high), available P_2O_5 35-40 mg kg^{-1} (moderate), available K_2O 360-390 mg kg^{-1} (low), cation exchange capacity 20-21 me 100g^{-1} soil, bulk density 1.1-1.2 gr cm^{3}, field capacity 26.6%.

The sources of chemical fertilizers utilized were: ammonium nitrate 34.5% N, double superphosphate 46%P_2O_5 and potassium chloride 60% K_2O. The source of the organic fertilizer utilized was biohumus, manure, poultry manure, grain straw, biohumus, whose contents of organic matter, nitrogen, phosphorus and potassium are presented in Table 1.

Table 1. Composition of organic wastes, measured variables

Organic materials	Organic matter, %	C:N	N, %	P_2O_5, %	K_2O, %
Manure	21	24,4	0,50	0,25	0,60
Poultry manure	40	14,5	1,60	1,50	0,80
Grain Straw	35	40,6	0,50	0,25	0,80
Biohumus	40	10,5	2,20	1,80	1,60

Treatments and Experimental Design

The experiment was performed using a completely randomized block design with four replications. The soil was ploughed, harrowed, and flat seedbeds measuring 10 m x 3,5 m (35 m^2) were made. Each plot was separated from the other by a one-metre alley. Fertilizer was applied using grain drill. The design of the experiment was a randomized complete block replicated thrice. Treatments comprised control, biohumus, manure, poultry manure, grain straw, biohumus and recommended chemical NPK fertilizers. The experimental field was prepared in accordance with a standard practice used by RB Kainar of LLP Kazakh Research Institute of Fruit and Vegetable Growing. Other agronomic practices and data collection were conducted based on the recommendations ($N_{90}P_{60}K_{60}$) of Kazakh Research Institute of Fruit and Vegetable Growing. The experiment was performed with the following 8 treatments.

- T1: Control (non-fertilization)
- T2: Recommended fertilizer dose ($N_{90}P_{60}K_{60}$)
- T3: Biohumus (10 t ha^{-1})
- T4: Biohumus (15 t ha^{-1})
- T5: Manure (40 t ha^{-1})
- T6: Poultry manure (5 t ha^{-1})
- T7: Poultry manure (10 t ha^{-1})
- T8: Grain Straw (3 t ha^{-1}) + Recommended fertilizer dose ($N_{90}P_{60}K_{60}$)

The trial was implemented on May and the harvest began in the first decade of August and Trial was well protected against insects and weeds during the season. Watermelon expo was planted on 26 May 2019 and 22 May 2020. The size of each elemental plot was 35 m^2 and included seven planting rows 1.5 m apart with eight plants per row, with a distance of 1.5 m between plants. The irrigation system, which was similar to that used by farmers in the area, consisted of one drip line per crop row and emitters of 2 L/h, 0.5 m apart.

Watermelons were harvested by hand when the fruit matured. The watermelons were picked by experienced persons and in general fruit were considered mature when the tendril nearest to fruit start to dry, and color of fruit on the bottom side changed from creamy white to yellowish. Fruits were measured and weighed during harvest and total yield and phenological observations were determined. In addition, the total sugar content was determined using the Bertrand method, Vitamin C (Ascorbic acid) content was determined titrimetric method using 2,6-Dichlorophenol indophenol (DCPIP), nitrate content was determined potentiometrically with ion-selective electrodes (AOAC, 2005).

Results and Discussion

Yield and growth parameters

The effects of the organic and chemical fertilizer on the fruit yield are shown in Table 1. The fruit yield of watermelon ranged from 16.84 t ha^{-1} to 25.64 t ha^{-1}, with an average of 23.13 t ha^{-1} (Table 1). The average fruit yield in this study area was higher than the 41.7 t ha^{-1} in the whole China, and much higher than the world yield of 32.1 t ha^{-1} in 2018. The reason for these significant differences was the diversity of watermelon varieties, climate, and field management (Gusmini and Wehner, 2005). There were no significant differences among treatments for yield of watermelon between 2019 and 2020. Recommended fertilizer dose ($N_{90}P_{60}K_{60}$) with chemical fertilizers and organic wastes used at various rates increased the yield of watermelon. However, recommended fertilizer dose (T2) has shown a great influence on the yield of watermelon followed by Manure (T5). The lowest nutrients recorded by the control experiment might be the reason why the yield parameters were very low compared with other treatments. Biohumus (T3 and T4) and poultry manure (T6 and T7) were similar in their effect on yield of watermelon and the difference was significant ($P < 0.05$) as shown in Table 1. The results are in total agreement with those obtained by Massri and Labban (2014) who found that chemical fertilizers ($N_{20}P_{40}K_{25}$) had positive impact on watermelon productivity. In their study, chemical fertilizer gave around 11 t ha^{-1} which are very close to our result. Abul-Soud et al. (2010) also observed an increase in average fruit weight when levels of livestock manure and liquid pig manure were applied to the soil. However, melon plants growing in soils containing different sources and levels of organic matter including manure.

Table 1. Effect of different types of fertilizers on fruit yield of watermelon (Citrullus lanatus)

Treatments	Fruit Yield, t/ha			Increase in fruit yield	
	2019	2020	Average	t/ha	%
T1	17.00a	16.68a	16.84	-	-
T2	26.19d	25.08c	25.64	8.80	52.23
T3	23.51b	22.40b	22.96	6.12	36.31
T4	24.94c	23.83bc	24.39	7.55	44.80
T5	25.96cd	24.95c	25.46	8.62	51.16
T6	23.84bc	22.73bc	23.29	6.45	38.27
T7	24.44bc	23.33bc	23.89	7.05	41.83
T8	23.14b	22.03b	22.59	5.75	34.12
LSD $_{\alpha=0.05}$	1.18	1.71			

T1: Control (non-fertilization); -T2: Recommended fertilizer dose (N90P60K60); T3: Biohumus (10 t ha^{-1}); T4: Biohumus (15 t ha^{-1}); T5: Manure (40 t ha^{-1}); T6: Poultry manure (5 t ha^{-1}); T7: Poultry manure (10 t ha^{-1}); T8: Grain Straw (3 t ha^{-1}) + Recommended fertilizer dose ($N_{90}P_{60}K_{60}$)

In Table 2, different types of fertilizers did significantly influence growth parameters of watermelon (Citrullus lanatus) during the fruit formation in both years. Different types of fertilizers influenced higher value in 2020 than in 2019. The control (T1) consistently produced the smallest growth parameters of watermelon such as lenght of the main shoot, quantity of stems, base thickness, intermode lenght, leaf width, leaf lenght, quantity of inflorescences, quantity of fruits, fruit diameter and average weight of the 1st fruit in each year. Recommended fertilizer dose (T2) has shown a great influence on growth parameters of watermelon followed by Manure (T5).

Table 2. Effect of different types of fertilizers on growth parameters of watermelon (Citrullus lanatus) during the fruit formatiion

Treatments	Years	Length of the main shoot, cm	Quantity of stems, pcs.	Base thickness cm	Internode length, cm	Leafstick length, cm	Leaf width, cm	Leaf length, cm	Quantity of inflorescences, pcs.	Quantity of fruits, pcs.	Fruit diameter, cm	Average weight of the 1st fruit, g
T1	2019	2.07	4.75	1.39	5.55	5.55	5.55	5.55	19.35	1.89	13.89	598.95
	2020	2.19	4.85	1.44	5.97	6.16	7.57	9.69	19.45	1.95	14.86	599.35
	Average	2.13	4.80	1.42	5.76	5.86	6.60	7.62	19.40	1.92	14.38	599.15
T2	2019	2.05	5.25	1.60	7.35	7.35	7.35	7.35	24.95	2.25	17.90	1146.9
	2020	2.72	5.30	1.79	7.49	7.82	9.22	12.17	25.05	2.35	18.70	1147.1
	Average	2.39	5.28	1.70	7.42	7.59	8.30	9.76	25.00	2.30	18.30	1147.0
T3	2019	2.45	5.05	1.45	6.55	6.55	6.55	6.55	20.50	2.05	16.90	865.90
	2020	2.55	5.00	1.53	6.64	6.38	8.02	10.66	21.55	2.10	17.06	866.20
	Average	2.50	5.03	1.49	6.60	6.47	7.30	8.61	21.03	2.08	16.98	866.05
T4	2019	2.55	5.05	1.45	6.70	6.70	6.70	6.70	24.08	2.15	18.75	1117.70
	2020	2.64	5.15	1.62	6.81	7.16	8.40	11.1	24.20	2.35	18.90	1118.10
	Average	2.60	5.10	1.54	6.76	6.93	7.60	8.91	24.14	2.25	18.83	1117.90
T5	2019	2.70	5.05	1.55	7.00	7.00	7.00	7.00	24.75	2.25	18.18	1046.65
	2020	2.80	5.20	1.67	7.22	7.28	8.68	11.2	24.85	2.35	18.28	1047.70
	Average	2.75	5.13	1.61	7.11	7.14	7.80	9.10	24.80	2.30	18.23	1047.18
T6	2019	2.39	4.75	1.39	5.85	5.85	5.85	5.85	21.35	1.90	17.00	833.70
	2020	2.49	4.90	1.49	6.56	6.29	7.90	10.46	21.45	2.00	17.10	834.10
	Average	2.44	4.83	1.44	6.21	6.07	6.90	8.16	21.40	1.95	17.05	833.90
T7	2019	2.49	5.00	1.45	6.05	6.05	6.05	6.05	21.65	2.05	18.10	918.80
	2020	2.59	5.15	1.55	6.73	6.84	7.99	10.84	21.75	2.25	18.24	919.70
	Average	2.54	5.08	1.50	6.39	6.45	7.00	8.45	21.70	2.15	18.17	919.25
T8	2019	2.47	4.95	1.40	6.85	6.85	6.85	6.85	20.75	2.08	18.05	919.85
	2020	2.57	5.10	1.54	6.91	6.95	7.99	10.46	20.85	2.15	18.20	920.95
	Average	2.52	5.03	1.47	6.88	6.90	7.40	8.66	20.80	2.12	18.13	920.40

T1: Control (non-fertilization); -T2: Recommended fertilizer dose (N90P60K60); T3: Biohumus (10 t ha^{-1}); T4: Biohumus (15 t ha^{-1}); T5: Manure (40 t ha^{-1}); T6: Poultry manure (5 t ha^{-1}); T7: Poultry manure (10 t ha^{-1}); T8: Grain Straw (3 t ha^{-1}) + Recommended fertilizer dose ($N_{90}P_{60}K_{60}$)

Quality properties

The quality indicators of watermelon fruits such as dry matter, total sugar, Vitamin C and NO_3-N as affected by treatments are shown in Table 3. Vitamin C is an important water-soluble vitamin that had being implicated in many life processes apart from its antioxidant property (Chambial et al., 2010). Recommended fertilizer dose (T2) had generally highest dry matter, total sugar, Vitamin C and NO_3-N compared with the other treatments. Poultry manure (T7) and Manure (T5) came second and it was significantly better than the other treatments. The highest NO_3-N obtained from recommended fertilizer dose (T2) treatment may probably due to faster release of nutrient contents of NPK than those of other treatment. Similar reports have been made on faster nutrient release from inorganic fertilizers compared to organic nutrients sources when used for the production of vegetables, cereal and tree crops (Adeoye et al., 2008; Ainika et al., 2012). The lowest nutrients recorded by the control experiment might be the reason why the yield parameters were very low compared with other treatments. In this research results showed that the content of NO_3-N in watermelon products was much lower than the maximum permissible concentration (60 mg kg^{-1} for watermelon) on variants where organic fertilizers were applied to the watermelon 39-62 mg kg^{-1}. In experiments where Recommended NPK fertilizers were used, the excess of NO_3-N is more than 3 times the maximum permissible concentration. Thus, these fruits could be dangerous for consumption. Tamme et al. (2006) recommended that the average nitrate content in watermelon was 95 mg kg^{-1}. The results of our investigations showed that (Table 3) the content of nitrates in other treatments (organic fertilizers) was less than the maximum level for nitrates as recommended by the Tamme et al (2006), 95 mg kg^{-1}. This means that the watermelons are safe for consumption.

Table 3. Effect of different types of fertilizers on quality properties of watermelon (Citrullus lanatus)

Treatments	Dry matter, %			Total sugar, %			Vitamin C, mg in 100g			NO_3-N, mg / kg		
	2019	2020	Average	2019	2020	Average	2019	2020	Average	2019	2020	Average
T1	8.3	9.2	8.8	12.82	13.62	13.22	5.48	6.48	5.98	15	10	12.5
T2	12.6	13.1	12.9	16.98	17.98	17.48	8.54	8.64	8.59	180	196	188.0
T3	10.5	11.9	11.2	15.88	16.98	16.43	7.10	7.20	7.15	40	38	39.0
T4	11.4	12.4	11.9	17.29	18.20	17.75	7.84	7.95	7.90	62	55	58.5
T5	12.4	13.3	12.9	16.82	17.52	17.17	8.53	8.64	8.59	65	59	62.0
T6	11.0	12.0	11.5	15.70	16.60	16.15	7.85	7.92	7.89	56	51	53.5
T7	12.9	13.1	13.0	16.62	17.52	17.07	8.54	8.64	8.59	63	57	60.0
T8	12.0	13.0	12.5	16.30	17.10	16.70	7.88	7.92	7.90	63	57	60.0

T1: Control (non-fertilization); -T2: Recommended fertilizer dose (N90P60K60); T3: Biohumus (10 t ha^{-1}); T4: Biohumus (15 t ha^{-1}); T5: Manure (40 t ha^{-1}); T6: Poultry manure (5 t ha^{-1}); T7: Poultry manure (10 t ha^{-1}); T8: Grain Straw (3 t ha^{-1}) + Recommended fertilizer dose ($N_{90}P_{60}K_{60}$)

Conclusion

This study showed that manure at 40 t ha^{-1} gave the best performance among all other organic fertilizers used due to its impact on the growth and yield of watermelon and its quality properties. Given its superior responses, manure applied at a rate of 40 t ha^{-1} could serve as alternative to recommended chemical fertilizer. It is therefore reasonable to recommend the use of organic fertilizer 40 t ha^{-1} cultivation of watermelon in the Southeast of Kazakhstan.

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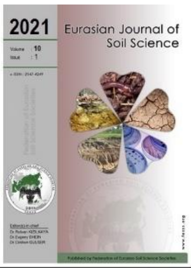

The role of the ornithogenic factor in soil formation on the Antarctic oasis territory Bunger Hills (East Antarctica)

Timur Nizamutdinov [a,*], Mikhail Andreev [b], Evgeny Abakumov [a]

[a] Saint-Petersburg State University, Faculty of Biology, Department of Applied Ecology, Saint-Petersburg, Russia
[b] Komarov Botanical Institute of the Russian Academy of Sciences, Saint-Petersburg, Russia

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Author(s)

T. Nizamutdinov *
M. Andreev
E. Abakumov

* Corresponding author

Abstract

The study focuses on the ornithogenic factor of soil formation in Antarctic conditions. Since the traditional soil formation processes in Antarctic conditions are very limited, the relevance of studying the role of the ornithogenic factor is increasing. This article provides a comparative study of nutrient content and values of some physico-chemical parameters between ornithogenic and non-ornithogenic soils sampled at terrestrial ecosystems of the Antarctic oasis Bunger Hills (Knox Coast, Wilkes Land). The levels of key biogenic elements content have been estimated with special reference to ornitogenic factor of soil formation. A high content of available forms of phosphorus and potassium in ornithogenic and non-ornithogenic soils was found. According to the results of statistical analysis, we can see that the content of nutritional elements has a close significant correlation relationship ($p<0.05$). The analysis of variance showed that the content of available phosphorus and potassium varies weakly between soils of ornithogenic and non-ornithogenic genesis. The greatest variability depending on soil-forming processes is noted for basal respiration, pH and available forms of nitrogen.

Keywords: Antarctic soils, ornitogenic soils, nutrients.

Introduction

The investigation of the soil cover of the Antarctic continent and subantarctic islands is basically aimed to understanding and estimating the dynamics of anthropogenic impact and the current condition of soils in the vicinity of the Antarctic Research Stations. There are numerous works devoted to the assessment of chemical pollution of soils and soil-like bodies in the territory of Bellingshausen (Abakumov et al., 2014), Scott Base (Saul et al., 2005), McMurdo (Campbell et al., 1998), Russkaya, Leningradskaya, Akademik Fedorov (Abakumov et al., 2014) stations and their vicinities. The soils of King George Island (Vlasov et al., 2005), Livingston Island (Kostova et al., 2015), the Schirmacher Oasis (Lepane et al., 2018), the South Shetland Islands , the Larsemann Hills (Mergelov, 2014), and many other areas free of cover glaciation, which are subjected to anthropogenic influence in scientific research of various kinds, have been extensively studied.

The soils of the continental ice-free part of Antarctica, where there are no permanent research stations, are much less studied due to the inaccessibility of these territories and the extreme climate conditions (Beyer et al., 2000; Hopkins et al., 2006; Zazovskaya et al., 2015). Although it is possible that it is in isolated areas that take place unique and unexplored to this day processes of soil formation. The surface area of ice free zones,

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or as they are often called - Antarctic Oasis's, is variates from 1 to 5% of the whole territory of the Sixth Continent (Dolgikh et al., 2015).

Soil zonal distribution in Antarctica is traceable only in sub-Antarctic (including island) and coastal territories; in the continental part, the soil cover is represented by intrazonal soil formations of different genesis (Campbell and Claridge, 1987; Bockheim and Hall, 2002). One of the essential factors for the existence of soil zonality in Antarctica is the degree of moisture supply; the moistest places inhabited by various mosses and lichens are the areas of soil formation in its classical sense (Ugolini and Bockheim, 2008).

Goryachin (2012) notes that Antarctica as a whole has no complete soil cover, and identifies so-called "pedospheric islands" that are not influenced by Latitudinal Zonation and their development depends more on "pedospheric islands" size and the influence of glaciers on them (Goryachkin, 2019). Some researchers have an opinion that the ahumic – according to Tedrow (1966) soils of Antarctica are not "true" soils, in the classical sense laid down by Dokuchaev and his predecessors (Tedrow and Ugolini, 1966).

The main argument is that these soils are weakly developed in terms of solum differentiation and have no real genetic horizons of organic matter accumulation, which is characteristic of "true soils" and are mechanically mixed substrates of mineral sediments and organic matter of zoogenic genesis. However, even if one agrees with the argument, these formations can be classified as soil-like bodies (Goryachkin, 2019) (typical for formations of aeolian origin on glaciers - cryoconites), based on the works of Sokolov, Goryachin, Targulyan (Targulyan, 1971; Sokolov, 1993; Goryachkin, 2019; Goryachkin et al., 2019).

Broadly speaking, the soil cover of the Antarctic continent is characterized by a hyperskeletic texture with a predominance of coarse grained fractions and a high sand percentage in the fine earth (Bockheim, 2014).

Although erosion and aeolian process are the main soil forming process in Antarctica (Abakumov, 2011), there are less pronounced processes, particularly the processes of organic matter accumulation and alteration of mineral components (Antarctic tundra), also the processes of salinization and cryoturbation (Antarctic deserts) (Glazovskaya, 1958, Bockheim, 2015), as well as the processes of endolithic soil formation (Mergelov et al., 2012).

Coastal areas are characterized by processes of formation of subaquatic soils, caused by periodic or permanent underflooding of local erosion bases, as a result of thawing of the glacial cover (Nikitin and Semenov, 2020). The existence of large colonies of various bird species (penguins, skuas, petrels) is the cause of zoogenic or ornithogenic soil formation, which is observed not only in coastal areas, but also in continental ecosystems isolated by glaciers (Heine and Speir, 1989; Emslie et al., 2014; Abakumov et al., 2016; Abakumov et al., 2019).

The ornithogenic factor of soil formation in Antarctic conditions is, in our opinion, one of the most interesting. Ornithogenic soils are the result of specific processes of zoogenic soil formation, which is characteristic mainly for the ecosystems of the southern hemisphere (Syroechkovsky, 2019). Climatic conditions are limiting the processes of humification in the classical sense, since the vegetation cover of the continent is represented by spots of distribution of lower plant-forms in the most humid areas (Abakumov, 2019).

Under conditions of deficit of organic matter of plant formation, accumulation of organic matter in the soil profile is possible during zoogenic-ornitogenic soil formation processes (Heine and Speir, 1989; Simas et al. 2007). Especially relevant are these processes in isolated areas of continental Antarctica or on the islands of the Subantarctic. The origin of ornithogenic soils is caused by the transformation of bird food into organic matter, followed by the transfer of this substance to nesting sites and accumulation in the soil cover in the form of guano (Ugolini, 1972; Abakumov, 2014b).

Ornithogenic soils are intrazonal and their formation strongly depends on the population of birds and the specifics of their migratory activity (Parnikoza et al., 2015). Birds, by transferring the genetic material of small invertebrates and plants, provide an opportunity for the development of mosses, lichens and algae to grow in isolated Antarctic oasis (Parnikoza et al., 2012; Abakumov et al., 2020b).

The most important of the features of ornithogenic soil formation depend on the character of the terrain on which guano accumulates. The process of migration of organic matter of ornithogenic origin on loose bedrock allows absorption of the lowest soil horizons and active mineralization across the full soil profile. On higher-density crystalline rocks, the processes of vertical migration are limited, resulting in the formation of a large ornitokoprogenic horizon weakly influenced by mineralization. The intensity of mineralization is the higher the more the content of fine-grained material in the underlying rocks (Abakumov, 2019; Lupachev and Abakumov, 2013; Alekseev and Abakumov, 2020).

According to some researches devoted to ornithogenic soil formation in Antarctic conditions, it is noted that biogenic processes in ornithogenic soils contribute to the accumulation of Phosphorus Forms in the soil profile (Simas et al., 2007).

The processes of ornithogenic soil formation cardinally alter the morphological structure of the soil profile. It is important to note that soils formed under the influence of the ornithogenic factor cannot be classified as "soil-like bodies", since mineralization of organic matter and formation of organogenic horizons in the soil profile take place (Abakumov, 2014a).

This article is aimed to identify the chemical parameters of ornitogenic factor of soil formation on the territory of the Bunger Hills oasis (Antarctica, Wilkes Land, Knox Coast). To identify the significance of ornithogenic soil formation, we conducted a set of laboratory analyses of soil samples. And using statistical methods, we tried to identify differences between ornithogenic and non-ornithogenic intrazonal soils.

Material and Methods

Regional setting

The Bunger Hills are an ice-free region of low hills and deep glacial lakes covering 400 km^2 along the coast of Wilkes Land, East Antarctica (located at approximately 100°45’ E, 66°17’ S), and separated from the Southern Ocean by the Shackleton Ice Shelf. Its total area including marine basin and islands is about 950 km^2 (length – ca. 50 km, width – ca. 20 km) (Tucker et al., 2017).

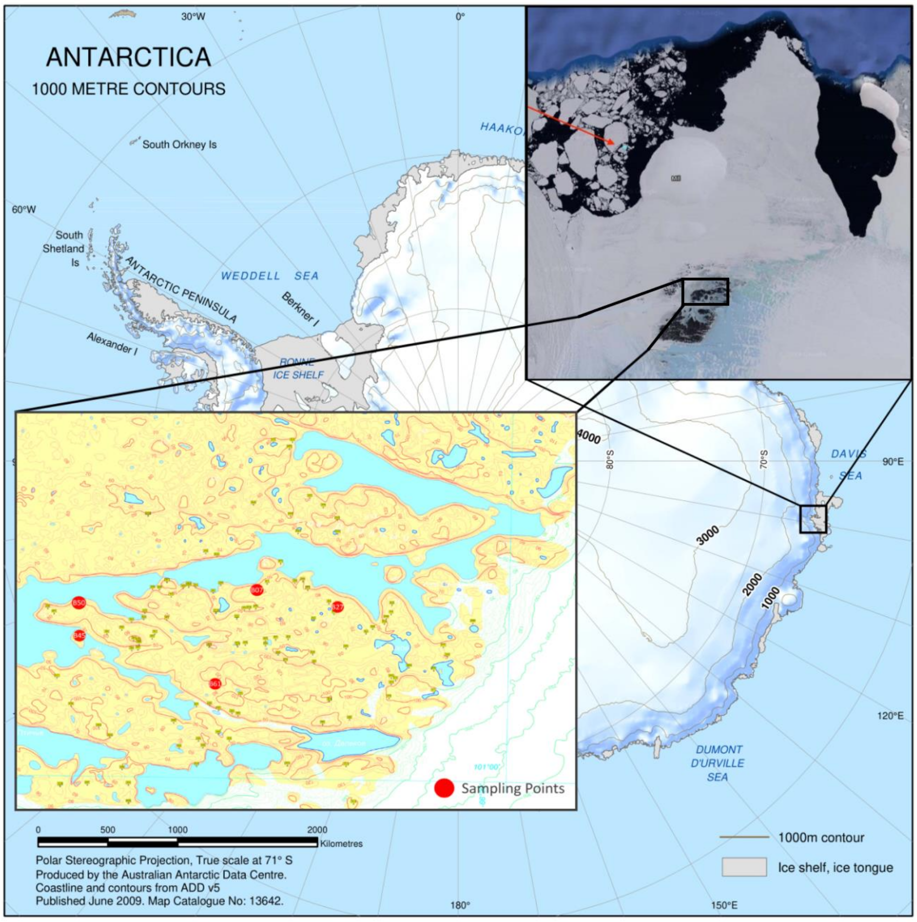


Figure 1. Sampling site map

The field work was conducted at the south-eastern side of the mainland’s part of the Bunger Hills oasis between big and long lake Figurnoe and the ice cap (Figure 2). The work of the field party was organized by the Polar Marine Geosurvey Expedition" (PMGE) during 65 Russian Antarctic Expedition (30.12.2019-14.03.2020). Soil samples collecting was made contemporary with the investigation of the lichen flora and vegetation of the oasis as an extension of previously made study (Andreev, 1990 and in prep.).

Rocks on the cost of subglacial lake

Hilly landscape near Tikhoe Lake

Depression with small lake in lake's valley

Field camp near small lake

Figure 2. Bunger Hills. (Photos: M. Andreev).

40 soil samples (21 ornithogenic and 19 non-ornithogenic) were collected in 5 sampling sites. Detailed map of points and sampling sites characteristics can be seen in Figure 1 and Table 1.

Table 1. Sampling site characteristics

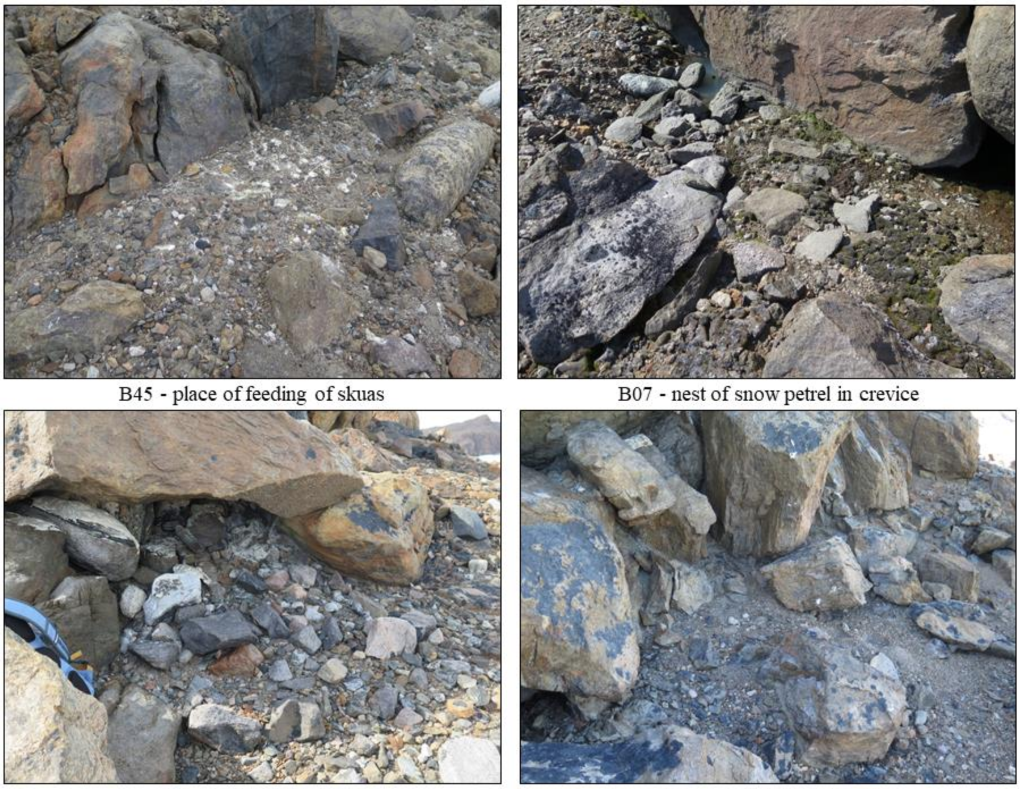
Sample code	Plot name	Sampling site description	Meters above sea level	S	E
B07	Antarctica, Wilkes Land, Knox Coast, Bunger Hills	Land to the South of the Lake Figurnoe, a rock ca. 6 m high, with a nest of snow petrel in crevice.	52 m	66 18.171	100 54.280
B27	Antarctica, Wilkes Land, Knox Coast, Bunger Hills	Land to the South of the Lake Figurnoe,, "Lakes Valley", small depression between rocks, flat bottom, a nest of snow petrels in rocks.	71 m	66 18.315	100 56.088
B45	Antarctica, Wilkes Land, Knox Coast, Bunger Hills	Land to the South of the Lake Figurnoe, gentle slope of the hill on an isthmus to the "Black Peninsula", moraine, feeding place of skuas.	19 m	66 18.614	100 50.211
B50	Antarctica, Wilkes Land, Knox Coast, Bunger Hills	Land to the South of the Lake Figurnoe, hill's top on long peninsula, black rocks, nest of snow petrels.	61 m	66 18.293	100 50.206
B61	Antarctica, Wilkes Land, Knox Coast, Bunger Hills	Land to the South of the Lake Figurnoe, at the river between lakes Dalekoe and Figurnoe, the rock on hill's top, southern slope, nest of snow petrel.	125 m	66 19.064	100 53.335

All samples during the field seasone were saved in plastic bags in box on fresh air at external temperature (first – from 0 to +3-5°C, later – below zero), and transported to Saint-Petersburg university laboratories within scientific vessel "Akademik Fedorov" at the temperature below zero. After air drying all the samples were grounded and passed through a 2 mm mesh screen.

Oceanites oceanicus (Wilson's storm petrel), *Pagodroma nivea* (Snow petrel) *Stercorarius maccormicki* (South polar skua) are the following bird species that inhabit the Bunger oasis (Leishman et al., 2020).

Pagodroma nivea nests found anywhere in southern Bunger Hills where crevices are present in cliffs (Gibson, 2000). As Bulavintsev (1993) notes, the population of *Pagodroma nivea* in the banger oasis numbers more than 1,000 individuals (Bulavintsev et al., 1993), and their nests are characterized by the characteristic accumulation and solidification of petrel stomach oil for this species (Hiller et al., 1995).

Stercorarius maccormicki are highly visible, as they are relatively large, congregate in small groups and are noisy. Gibson (2000) estimated that there were about 50 individuals in the banger oasis (Gibson, 2000). Most often they can be spotted in feeding areas where many feathers and remnants of their prey (petrel shells) are scattered. Some most typical of the identified bird habitats (at the sampling sites) are presented in the Figure 3.



B45 - place of feeding of skuas B07 - nest of snow petrel in crevice

B50 - nest of snow petrels B61 - nest of snow petrels

Figure 3. Birds habitat places in Bunger Hills

Oceanites oceanicus nests are mainly located at a height and are hidden in crevices and folds of the terrain. Therefore, sampling of ornithogenic soils was carried out directly near the nesting sites from a depth of 7 - 10 cm, and background (non-ornithogenic) soils were sampled down the slope at a distance of 15 - 20 meters from the nesting sites. A large amount of organic material was found at the *Stercorarius maccormicki* feeding sites, so background samples of non-ornitogenic soils were sampled at a distance of 50 - 100 meters.

Laboratory analysis

All laboratory activities on chemical and physical analysis of soil were conducted at 22.5°C in soil fine earth. Basal respiration of soil was estimated by measuring CO_2 in Sodium Hydroxide. Incubation of CO_2 was conducted for 10 days in plastic sealed containers (Jenkinson and Powlson, 1976). The pH of soil was determined by potentiometric method using pH-meter (pH-meter - millivolt meter pH-150MA, Belarus). Soil solution was prepared in the ratio of 1:2,5 with water or 1M $CaCl_2$ (for mineral soils the optimal soil weight for solution preparation is 8 g) (Black, 1965). The particle size distribution of the soil was determined by the sedimentation method (Jackson and Saeger Jr, 1935; Kachinskiy, 1958). The texture class of soil was determined on the base of particle size disctribution analyzes.

Key parameters of soil nutrient state have been determined by the standard procedures recorded in GOST 54650–2011 (for evaluation of available phosphorus and potassium contents) and GOST 26489–85 (for evaluation of ammonium nitrogen content). The procedure for measuring ammonium nitrate requires its extraction from the soil with potassium chloride solution. Quantitative determination of ammonium is carried out using photometry of colored solutions (EPA, 1993). The determination of the available forms of phosphorus and potassium is based on the extraction of the compounds described above with hydrochloric acid (acid concentration 0.2 mol/L). After extraction, the quantitative determination of mobile phosphorus and potassium compounds is carried out by photometry methods (Sparks et al., 2020).

Total organic carbon and nitrogen were determined with a C-H-N analyzer (Euro EA3028-HT, Italy) of Research Park at St. Petersburg University.

Results and Discussion

Key chemical and physical properties of soil samples

The results of the laboratory analysis of soil samples are shown in Table 2. Based on the data obtained on basal respiration of soils, acidity, and nutrient content, it is not possible to conclude findings about cardinal differences between ornithogenic and non-ornithogenic soils. The terrain in the areas adjacent to the glacier is shallow, deeply dissected, in the southern and western parts is more smooth. The highest altitude is 168 m above sea level. Prevailing heights 50-80 m above sea level. The ridges and slopes of hills are covered with loose eluvial-deluvial and moraine deposits (Simonov, 1971).

Table 2. Chemical and physical properties of soil samples

Sampling code	Sampling point	Basal respiration, CO_2/100g soils per day	pH (H_2O)	pH ($CaCl_2$)	Available P	Available K	NH_4-N	NO_3-N	Particle-size distribution
					mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	
Non-ornitogenic soils									
B27	NR2/10	69.14	8	nd	801	203	6.46	1.05	Loamy Sand
	NR2/11	69.14	7.96	nd	1083	263	12.3	1.98	
	NR2/7	88.00	8.85	nd	726	217	3.26	3.03	
	NR2/8	56.57	8.46	nd	863	341	12.8	7.64	
	NR2/9	37.71	8.55	nd	946	230	4.59	0.79	
B45	NR3/10	69.14	9.71	nd	797	304	4.8	1.62	Sandy Loam
	NR3/11	69.14	7.89	nd	759	737	5.39	7.16	
	NR3/7	81.71	8.6	nd	635	350	7.95	1.84	
	NR3/8	56.57	8.26	nd	693	415	10	19.4	
	NR3/9	37.71	8.72	nd	755	433	15.3	5.93	
B50	NR4/10	31.43	7.8	nd	851	309	16.5	2.55	Loamy Sand
	NR4/11	94.29	8.57	nd	913	359	7.63	4.65	
	NR4/7	69.14	8.25	nd	747	290	4.48	1.45	
	NR4/8	56.57	7.59	nd	875	244	12.6	2.2	
	NR4/9	37.71	8.44	nd	780	392	11.8	5.84	
B61	NR5/10	62.86	7.47	nd	1390	341	12.1	8.96	Loamy Sand
	NR5/11	75.43	6.55	4.28	929	382	5.76	2.55	
	NR5/7	62.86	6.74	5.81	913	194	6.4	0.97	
	NR5/9	56.57	7.31	nd	15870	852	754.1	18.9	
Ornitogenic soils									
B27	OR2/1	94.29	8.25	nd	763	253	10.8	3.42	Loamy Sand
	OR2/2	62.86	8.05	nd	1407	134	34.4	10.1	
	OR2/4	75.43	7.55	nd	938	369	10.6	6.24	
	OR2/3	37.71	8.25	nd	730	143	8.7	1.89	
	OR2/5	119.43	6.24	5.8	1232	235	25.8	22.2	
B45	OR3/1	56.57	7.86	nd	1075	401	19.2	15.9	Sandy Loam
	OR3/2	100.57	7.22	nd	813	276	19.3	12	
	OR3/3	56.57	8.33	nd	1083	212	22.9	17.6	
	OR3/4	62.86	7.56	nd	639	143	3.36	7.11	
	OR3/5	69.14	7.91	nd	1212	359	26	37.3	
B50	OR4/1	69.14	8.15	nd	1091	322	6.62	11.2	Sandy Loam
	OR4/3	75.43	7.75	nd	751	226	14.8	5.31	
	OR4/4	169.71	8.32	nd	1469	433	7.85	17.4	
	OR4/5	106.86	7.58	nd	950	253	9.18	1.93	
	OR4/2	50.29	8.13	nd	1311	364	12.5	15.9	
B61	OR5/1	75.43	4.4	3.5	1274	157	56.6	13.4	Sand
	OR5/2	106.86	5.26	4.61	3303	392	321.3	26.1	
	OR5/3	69.14	5.74	4.59	2377	382	283.9	19.8	
	OR5/4	69.14	5.76	5.61	8525	1290	714.1	60.9	
	OR5/5	88.00	5.52	3.98	1909	115	36.3	7.33	
	OR5/8	37.71	6.63	5.22	838	138	28.1	4.83	

The level of soil basal respiration variates sharply in all the obtained data, no clear differences in the level of CO_2 emission depending on soil type are observed. Several local minimums and maximums for soil types are identified. For non-ornitogenic soils, the maximum basal respiration value is 94.29 mg CO_2/100g soil per day (sample NR 4/11, area B50), and the minimum is 31 mg CO_2/100g soil per day (sample NR 4/10, area B50). For soils of ornithogenic genesis, the highest soil CO_2 emission level was 169.71 mg CO_2/100g soil per day (sample OR 4/4, zone B50) and the minimum was 37.71 mg CO_2/100g soil per day (sample OR 2/3, area B50). It is important to note that the minimums and maximums of soil respiration levels occur in the same sampling area (B50).

Non-ornitogenic soils are characterized by predominantly alkaline and weakly alkaline reaction (the average value of pH H_2O 8.09 for all nonornitogenic soil samples). The dynamics of acidity reaction for soils of ornithogenic genesis is rather different. From strongly acidic and weakly acidic (pH H_2O 4.4 – 6.63) B61 area to weakly acidic and weakly alkaline (pH 5.8 – 7.62) in all other samples. pH in salt suspension were lower (by 1 on average) for all soil samples, which may indicate a reserve of acidity in soil colloids, associated with the processes of destruction of organic matter (Thomas, 1996).

Among the major nutrients, the most notable is the content of available phosphorus in all soil samples. The concentration of phosphorus varies greatly (635 – 15870 mg×kg^{-1}), interestingly, the highest concentration was recorded in soil of non-ornitogenic genesis (15870 mg×kg^{-1}, sample NR 5/9 area B61). We can also note a high content of available phosphorus in ornithogenic soils of predominantly sandy particle size distribution (area B61). Some researchers have already noted an increased content of available phosphorus in ornithogenic soils and noted the formation of phosphorus - containing minerals in them (Tatur and Barczuk, 1985; Tatur and Keck, 1990; Simas et al. 2007; Abakumov, 2018). In works devoted to the influence of birds on soil formation noted that the high content of available forms of phosphorus and potassium is one of the main signs of the processes of ornithogenic soil formation (Simas et al. 2007; Simas et al. 2008; Abakumov et al., 2021).

The content of available potassium is also highly variation (115 – 1290 mg kg^{-1}). The maximum values were recorded in the ornitogenic soil of sandy particle size distribution (1290 mg kg^{-1} sample OR5/4, area B61).

It should be noted 2 soil samples with extremely high content of nutrients, these are samples NR5/9 and OR5/4 in area B61 of non-ornithogenic and ornithogenic genesis, respectively. In these soils the content of available forms of phosphorus, potassium and nitrogen is the highest, in comparison with all other soil samples studied, at that they have different character of soil formation, presumably.

We have calculated C/N ratios for estimation of levels of soil organic matter enrichment by nitrogen. This value reflects the influence of many factors on the soil, especially climate and soil formation features (Miller et al., 2004; Yamashita et al., 2006; Lou et al., 2012). Thus, the lower the ratio, the higher the enrichment of the soil with nitrogen. Since for this territory the nitrogen inflow is mainly due to the influence of ornithogenic factor, the C/N ratio for this territory can be used as an indicator of the degree of activity of ornithogenic soil formation.

The C/N value allows one to understand what processes are currently occurring in the soil, mobilization or mineralization. It is known that at the $C/N < 20$ the processes of nitrogen mineralization prevail, and at the $C/N > 30$ there is immobilization of nitrogen in the soil profile (Janssen, 1996; Semenov, 2020). However, this classification is more applicable to soils in their classical sense, in primitive ahumic soils the C/N ratio can be used to estimate the degree of nitrogen deficiency. As can be seen from Table 3, for most of the investigated soil samples the C/N ratio is essentially lower than 20, except for samples OR4/2 and OR5/4 for which the values are 22.2 and 28.78, respectively. The low values of the C/N ratio are explained by the inflow of nitrogen into the soil due to the influence of the ornithogenic factor, as shown in Table 2, soils are quite rich in nitrogen compounds.

Based on the results, we can conclude that there is no nitrogen deficiency in almost all of the studied soils in the study area. Low ratio values indicate significant nitrogen input to the soil.

Statistical analysis

Based on the obtained data set on the content of soil nutrients, basal respiration and soil pH, Spearman rank correlation coefficient matrix was calculated (Table 4).

As can be seen from the table above, a significant correlation is observed between the various nutritional elements. The strongest correlation was found between available phosphorus and ammonium nitrogen content, as well as between anmonium and nitrate nitrogen content.

Table 3. C and N content of soils and C/N ratio values.

Sampling area	Soil samples	C, %	N, %	C/N*
		Non-ornitogenic soils		
B45	NR3/10	0.42	0.14	2.97
	NR3/11	1.21	0.91	1.32
	NR3/9	0.22	0.04	5.03
B50	NR4/10	1.47	0.43	3.38
	NR4/7	1.42	1.14	1.25
	NR4/8	1.18	0.91	1.29
B61	NR5/7	1.44	1.12	1.28
	NR5/9	2.80	1.49	1.88
				Ornitogenic soils
B27	OR2/2	0.31	0.08	3.59
	OR2/5	3.00	1.16	2.59
B45	OR3/1	0.26	0.11	2.25
	OR3/2	1.60	1.04	1.54
	OR3/3	0.11	0.05	1.96
	OR3/4	0.98	0.86	1.14
B50	OR4/5	1.23	0.81	1.52
	OR4/2	0.22	0.01	22.20
B61	OR5/1	2.36	0.44	5.39
	OR5/2	3.33	0.51	6.50
	OR5/3	1.37	0.41	3.41
	OR5/4	3.19	0.75	4.12
	OR5/5	0.57	0.02	28.78
	OR5/8	0.44	0.14	2.99

* - mass ratio

Table 4. Spearman Rank Order Correlations Marked correlations are significant at $p < 0.05$

	Basal respiration	Available Phosphorus	Available Potassium	Ammonium Nitrogen	Nitrate Nitrogen
Basal respiration	1.00				
Available Phosphorus	0.17	1.00			
Available Potassium	0.00	0.18	1.00		
Ammonium Nitrogen	-0.05	0.62	0.10	1.00	
Nitrate Nitrogen	0.13	0.58	0.42	0.67	1.00

Analysis of the mean values by ANOVA (Table 5 and Figure 5) revealed significant differences for all the obtained soil parameters depending on the genesis of soil formation. Significance was confirmed by Fisher's test with a P value of 0.0007.

Table 5. ANOVA Multivariate Tests of Significance. Sigma-restricted parameterization. Effective hypothesis decomposition

	F	p
Ornitogenic vs non-ornitogenic	4.9592	0.0007

More detailed differences between the average values of all studied soil parameters are presented in Figure 4. The variability of the mean values clearly shows that the main contribution to the overall variability is made by the parameters of basal respiration, pH (H_2O) and the content of ammonium and nitrate forms of nitrogen. The average content of available phosphorus and potassium practically does not change depending on the genesis type of soil formation.

Conclusion

A Lithovit treatment as foliage nutrition at a 6 g/L rate combined with an irrigation level of 100% ETc Data obtained, showed that the processes of ornithogenic soil formation on the territory of the Bunger oasis have a fingerprint in both types of location: directly and indirectly faced to geochemical effect of birds. All the soils surveyed are characterized by high content of available forms of nutrients, which is typical for ornithogenic soils. The high content of available phosphorus, whose high concentrations in ornithogenic soils were also recorded by other researchers, is particularly remarkable. Non-ornitogenic soils are characterized by predominantly alkaline and weakly alkaline reaction of the soil solution The soil solution reaction of ornithogenic soils is more variable, ranging from strongly acidic to weakly alkaline. Soils of sandy texture type are characterized by an acidic reaction of the soil solution. Based on the C/N values, we can conclude about no nitrogen deficiency in the studied soils.

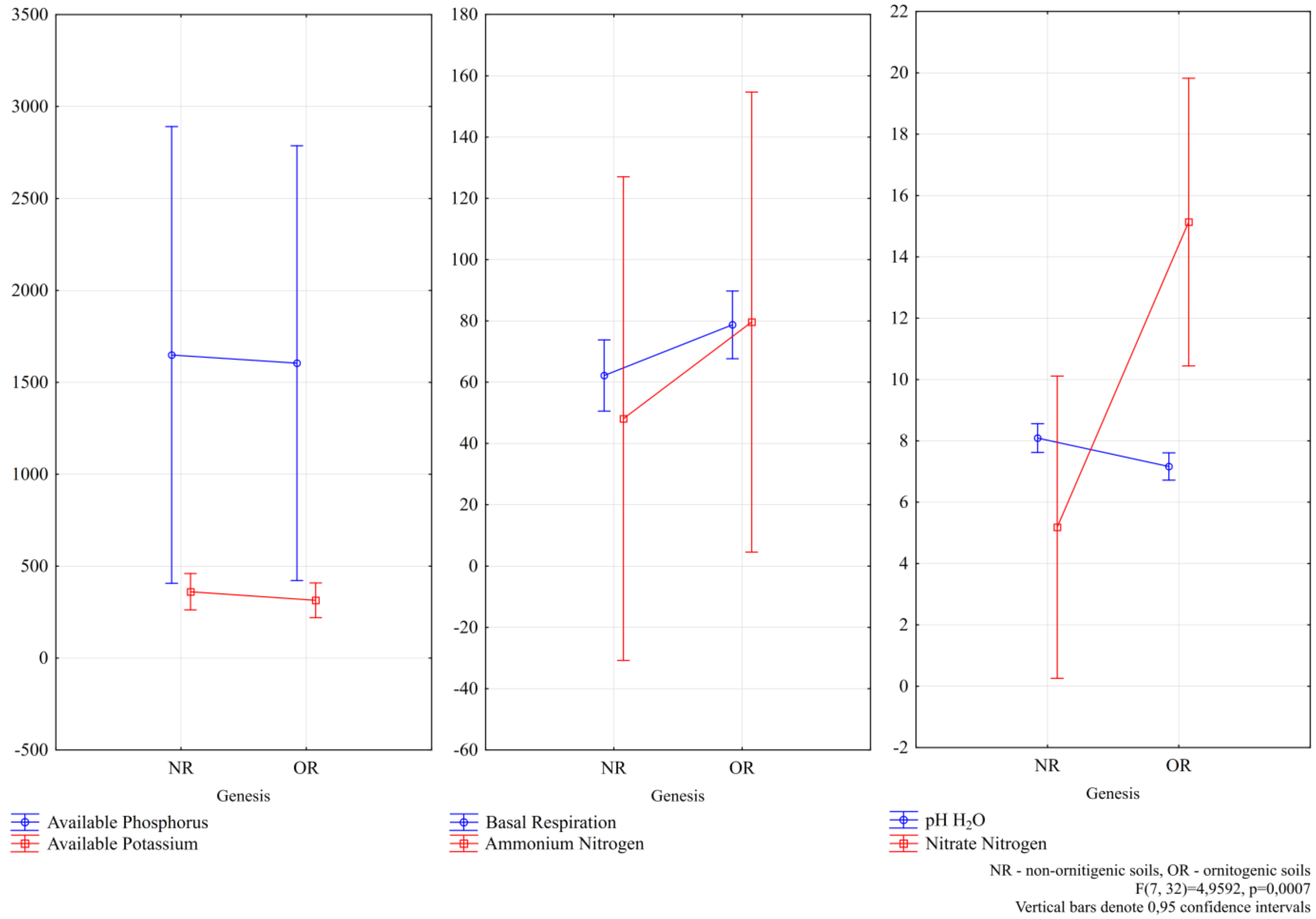


Figure 4. Visualization of variance analysis (ANOVA) of soil properties.

Although to the significant differences between ornithogenic and non-ornithogenic soils obtained by analysis of variance (ANOVA), we cannot clearly distinguish the studied soils by the type of soil forming factor. Even though the samples under study differ in the content of some parameters. The content of available phosphorus and potassium, according to the results of analysis of variance, practically does not change in soils of ornithogenic and non-ornithogenic genesis. As was said above, ornithogenic soil formation contributes to the accumulation of this element in the soil profile. Therefore, we can conclude that the influence of the ornithogenic factor applies equally to all the soils we studied.

The nesting places of Oceanites oceanicus (Wilson's storm petrel) and Pagodroma nivea (Snow petrel) are located mainly on highlands, and due to the settled type of nesting of these species, the organic material of their guano accumulates in the soil profile and then laterally migrates along the slope. The migration of organic matter is possible due to the appearance of some liquid precipitation in the area and the predominantly sandy particle size distribution of soils. Permafrost limits the migration of organic matter into the deeper horizons, which results in the horizontal transfer of organic matter over a wide area. So, the fingerprint of bird activity is evident for all the soils of the studied area, including current ornitogenic soils and geochemically subordinated soils of adjacent areas.

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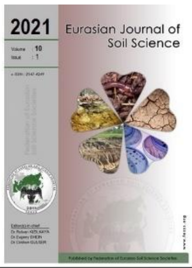

Soil organic matter composition of forest Rendzinas in West Bulgaria

Biser Hristov [a,b,*], Ivaylo Kirilov [b], Pavel Pavlov [a]

[a] University of Forestry, 10 Kl. Ohridski Blvd., Sofia 1797, Bulgaria
[b] N. Poushkarov Institute of Soil Science, Agrotechnologies and Plant Protection, Sofia, Bulgaria

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Author(s)

B. Hristov *
I. Kirilov
P. Pavlov

* Corresponding author

Abstract

The paper deals with of the relations between the forest ecosystem, calcareous rocks and content and composition of soil organic matter in the Golo Bardo area in West Bulgaria. In that area Rendzinas are used mainly for forestry, viticulture and pastures. A specific study based on the accumulation of soil organic matter according to the data on the composition of the soil organic acids. The research on the soil organic matter composition provided information on the course of the humification processes in studied soils. Soil organic matter accumulation in Rendzinas depends on different factors, such as land use and climatic conditions. Soil has rapid transformations of organic residues and strongly decomposed plant tissue predominates. There are often signs of active work of the soil mesofauna. Studied soils have good reserves of organic carbon high degree of humification. The organic carbon content varies in a wide range from 0.8 to 7.2 %. The humic acids prevail over fulvic acids, and the degree of humification is higher in deeper horizons. According to the C/N ratio the humus type is Mull in almost all horizons. Studied soils are biologically active that have favorable impact over the soil structure and vegetation. Rendzinas have high potential of organic carbon sequestration.

Keywords: Fulvic acids, humic acids, Rendzina, soil organic matter.

Introduction

Golo Bardo is located in Western Bulgaria, between the Pernik and the Radomir valleys. It is a small undivided, oblong mountain with steep slopes and a broad flat ridge with afforested areas. Accoding to Belivanova (1997) the mountain is built up by Triassic sediments, which usually are limestones and dolomites. Its highest peak is Vetrushka (1158 m a.s.l.). On the slopes of Ostritsa Peak there is a reserve of the same name, one of the oldest, designed to protect rare species of grass and shrubs.

The soil cover of in the area were studied for the first time by Koynov (1943), he mentioned about red-brown humus-carbonate soils over limestone at the foot of Golo Bardo mountain. Such soils are spread in foothills and mountain areas in the same region. The main soil type of the region is Rendzina that could be classified as Rendzic Leptosol or Rendzic Phaeozem according to IUSS Working Group (2015) soil classification system, but also in that area could be found Leptosols, shallow Phaeozems and Cambisols. These soils are mainly under forest. In many places deep soil could turn into shallow Rendzinas deeper up to 20–30 cm due to soil erosion. These soils are typical for the region of Pernik district and near the town of Radomir in Western Bulgaria. Calcareous soils are spread over 12 % of soil resources in the world (FAO, 1996).

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Rendzians formation and distribution is mainly related to the mineral composition and chemical of the calcareous rock, as well as to its physical, chemical and mineral properties (Donov, 1993; Artinova 2014; Bogdanov et al.; 2015, Filcheva and Malinova 2015; Filcheva and Sarafov 2015; Ilinkin et al., 2017).

The average content of soil organic matter (SOM) in the surface horizon of uncultivated soils in lowland and mountainous areas is from 5 to 7 % up to 13.5 % at above 800 m a.s.l. Differences in organic matter content are determined mainly by the altitude and vegatatopn cover (Bachvarova et al., 2005). Schreier and Lavkulich (1985) mentioned that these soils are with polygenetic origin. They have developed under conditions where humic acid accumulation is greater than decomposition as a result of the limestone-rich environment, and where a portion of the organic component might be derived from residues of bedrock weathering.

Carbonate content varies over a wide range (10–50 %). To be classified as Rendzinas soil forming materials should contain more than 40 % carbonates. Rendzinas are soils with high sorption capacity and the soil reaction is neutral and slightly alkaline from 7 to 8.4 pH in water. The high calcium content could have negative affect over nutrient assimilation. It is necessary to improve the nutrient content in the soils to meet the requirements for vegetation (Ilinkin et al., 2017). The use of humus-carbonate soils for cultivation of agricultural crops leads to changes only in humus content and humus reserves, especially in the orchard (Filcheva, 2007).

According Shishkov and Kolev (2014) the organic matter content and composition of Rednzinas has an advanced stage of humification. The SOM content is between 4 to 10 % in uncultivated land and it is less in arable land. Humus is entirely composed of humic acids bounded with calcium. The C/N ratio is about 8–12 and indicates humus enrichment with nitrogen. Rendzina soil characteristics (total N content and humus fractional composition) are very important because they are closely related to soil-forming factors and processes (Radmanovic et al., 2020).

Figure 1. Typical soil profile of Rendzina, under Black pine forest.

The aim of this study is to analyze soil organic matter content and composition, as well as the factors of humus formation in the Golo Bardo Mountain, in order to facilitate the planning and implementation of activities related to preserve soil organic carbon stocks.

Material and Methods

Description of the study area

The study area is Golo Bardo Mountain, which is located at 25 km south west direction from the city of Sofia, the capital of Bulgaria (Figure 2). The mountain covers from northwest to southeast area about 25 km long and its width is 6-7 km. Its slopes gradually sink into the Pernik and Radomir valleys. On the west side Struma River separates it from the Cherna Gora Mountain. The upper side reaches the Struma River again from side of Vitosha Mountain.

The Golo Bardo Mountain has average altitude about 1000 - 1100 m, and its slopes are slightly sloping, deforested and furrowed by ravines. The mountains rocks are usually Triassic limestone, dolomite and marl. The surface and underground karst has a massive development, and many karst springs could be found. The climate is temperate continental. Almost the entire mountain is drained by small and short tributaries of the Struma River.

Figure 2. West Bulgaria and Golo Bardo Mountain, study area

Soil analysis

Eight soil profiles were morphologically described and 26 soil samples were analysed. Bulgarian soil classification defines them as Typical and Skeletic Rendzians (Teoharov, 2019). According IUSS Working Group (2015) classification studied soils are Rendzic Leptosols, Calcaric Leptosol, Rendzic Phaeozems and Endocalcic Phaeozems. Soil organic matter composition was determined by the method of Kononova-Belchikova (Kononova, 1966; Filcheva and Tsadilas, 2002; Hristova et al., 2016; Filcheva et al., 2018). For total nitrogen is used Kjeldahl method (Arinushkina, 1961). Microsoft Excel 2017 is used for graphics and statistics.

Results and Discussion

Studied Rendzinas are usually shallow soils with A-AC-CR profile, well-formed humus layer with good fine granular structure to crumb structure with different content of rock fragments in the profile. Soil texture of Rendzinas are mainly sandy-clay, sandy-loam, silty loam with different contents of the biger particals of limestone. Soils are with loose structure, warm and well-aerated soils. A considerable part of them have an unfavorable water regime due to their shallow profile. In many places soil cover is cut by rocks.

Under the natural vegetation, predominantly forest and pastures, in which the studied soils are formed accumulate significant quantities of humus with favorable composition and properties (Table 1). In the complex processes of transformation of plant residues into soil organic matter, it may turn out that in addition to the possibility of differences in the properties of the litter of one species, some fractions of debris undergo certain changes in some circumstances and different changes (Handley, 1954).

Table 1. Content and composition of soil organic matter

Soil profile	Soil horizon depth, cm	Total C, %	Organic carbon, % Extracted with 0.1M $Na_4P_2O_7$+0.1M NaOH			Ch/Cf	Organic carbon, % Fractions of humic acids, %		Degree of humification	Unextr. organic carbon, %	Extracted organic carbon with 0.1N H_2SO_4, %	Optic characteristics (E_4/E_6)		Extracted organic carbon with NaOH, %	Total N, %	C/N
			Total C, %	Humic acids, %,	Fulvic acids, %		Free or bound with R_2O_3, %	Bound with Ca^{2+}, %	$\frac{Ch*100}{C\ total}$			Total humic acids	Free humic acids			
1	Akh 0–5	7.14	2.52	1.37	1.15	1.19	62.04	39.96	19.19	4.62	0.09	5.86	6.73	1.63	0.407	17.54
1	Ak 5–20	2.53	0.78	0.41	0.37	1.11	0.00	100.00	16.21	1.75	0.06	4.47	-	0.28	0.255	9.92
1	ACk 20–38	1.26	1.18	0.50	0.68	0.73	66.00	34.00	39.68	0.08	0.14	4.78	5.04	0.66	0.153	8.24
1	Ck 38–70	0.50	0.14	0.10	0.04	2.50	0.00	100.00	20.00	0.36	0.02	6.00	-	0.08	0.06	8.33
2	ACk 0–19	5.35	1.39	0.52	0.87	0.60	78.85	21.15	9.72	3.96	0.15	4.86	2.68	0.65	0.465	11.51
3	Ah 0–10	6.00	1.43	0.63	0.80	0.89	52.38	47.62	10.50	4.57	0.12	4.57	4.60	0.70	0.598	10.03
3	A1 10–22	3.40	1.06	0.52	0.54	0.96	36.54	63.46	15.29	2.34	0.00	4.33	4.07	0.40	0.407	8.35
3	ACk 22–43	2.89	0.57	0.45	0.12	3.75	0.00	100.00	15.57	2.32	0.05	4.04	-	0.27	0.354	8.16
3	Ck1 43–60	1.97	0.57	0.26	0.31	0.84	0.00	100.00	13.20	1.40	0.05	2.78	-	0.19	0.235	8.38
3	Ck2 60–100	1.14	0.32	0.19	0.13	1.46	0.00	100.00	16.67	0.82	0.03	4.03	-	0.11	0.119	9.58
4	Ah 0–10	7.80	2.27	1.27	1.00	1.27	68.50	31.50	16.28	5.53	0.08	4.78	7.02	1.54	0.487	10.02
4	ACk 10–28	4.08	1.09	0.58	0.51	1.14	32.76	67.24	14.22	2.99	0.07	3.96	7.15	0.39	0.385	10.6
4	CRk 28–70	2.86	0.84	0.52	0.32	1.63	30.77	69.23	18.18	2.02	0.06	4.11	2.96	0.26	0.349	8.19
5	AChk 0–14	3.47	1.00	0.58	0.42	1.38	0.00	100.00	16.71	2.47	0.05	4.02	-	0.30	0.338	10.27
6	A_h 0–5	2.68	0.73	0.46	0.27	1.70	39.13	60.87	17.16	1.95	0.09	4.34	3.43	0.24	0.265	10.11
6	A_k 15–30	1.85	0.47	0.30	0.17	1.76	0.00	100.00	16.22	1.38	0.08	4.14	-	0.22	0.196	9.44
6	AC_k2 30–50	2.09	0.52	0.33	0.19	1.74	0.00	100.00	15.79	1.57	0.09	4.32	-	0.23	0.211	9.90
6	AC_k 50–80	1.13	0.30	0.18	0.12	1.50	0.00	100.00	15.93	0.83	0.06	3.63	-	0.16	0.137	8.25
7	Akh 0–9	6.63	1.82	1.02	0.80	1.28	34.41	65.69	15.38	4.81	0.21	4.31	4.46	0.73	0.522	12.70
7	Ak 9–32	4.20	1.15	0.84	0.31	2.71	0.00	100.00	20.00	3.05	0.16	3.95	-	0.30	0.394	10.66
7	ACk1 32–45	3.29	1.04	0.71	0.33	2.15	0.00	100.00	21.58	2.25	0.11	3.93	-	0.20	0.336	9.79
7	ACk2 45–61	2.86	0.92	0.63	0.29	2.17	0.00	100.00	22.03	1.94	0.09	3.87	-	0.17	0.292	9.79
7	Ck1 61–90	2.36	0.70	0.50	0.20	2.50	0.00	100.00	21.19	1.66	0.09	3.83	-	0.12	0.236	10.00
7	Ck2 90–120	2.09	0.74	0.47	0.27	1.74	0.00	100.00	22.49	1.35	0.08	3.94	-	0.16	0.214	9.77
8	Ak 0–11	5.33	1.20	0.80	0.40	2.00	33.75	66.25	15.01	4.13	0.17	4.63	3.84	0.45	0.511	10.43
8	ACk 11–30	3.52	0.73	0.45	0.28	1.61	0.00	100.00	12.78	2.79	0.09	4.32	-	0.23	0.374	9.41

The soil-forming effect of the place where the organic residues decompose and the particle size composition of the soils control the rate of decomposition and humification of the organic residues.

These processes of plant residues decaying and mineralization of the constituent elements are an important issue for the carbon cycle. Dead plants and leaves are the primary items to degrade the processes in the surface horizon. In the forests above the limestone, the litter is subsequently incorporated into the deep soil by earthworms (Scheu and Wolters, 1991).

Visual observation shows that in these soils rapid transformations of organic residues and strongly decomposed plant tissue predominate. There are often signs of active work of the soil mesofauna. Humus is dark-coloured, according to Munsell Color (1994) is ususally with values – 10YR 3/3; 10YR 3/4 which is drak olive brown and olive brown. The surface horizon contains carbonates, its reaction is neutral or slightly alkaline. Surface soil horizons are naturally active soils that contain high amount of stable forms of organic compounds which favorably affects the soil structure and plant vegetation.

Rendzinas have well-formed humus horizon and humus accumulation is up to a depth of 50 cm. The distribution of organic matter usually is decreasing in depth. The organic carbon content is the highest in the surface of the humus-accumulative horizon. Depending on the conditions of humification and the participation of vegetation, organic carbon content varies in quite wide range and reaches 5–7.2 % (Table 1 and Figure 3) In rich carbonate soil-forming materials it is less than 0.5 % (Ck horizon). The average content of organic carbon is 3.4 % in whole profile (Table 2). Organic content classification of Gurov and Artinova (2004) shows that studied Rendzinas have very high amount of humus content. Similarly, as in the case of other soils, the organic carbon stock as well as accumulation of organic matter in different forms in Rendzinas depend on many factors, such as land use, vegetation and climatic conditions.

An important factor forming the sequestration of carbon in calcareous soils is the type of parent rock, which determines the content of calcium and magnesium and the amount and type of clay minerals. All the processes described above lead to the creation of specific morphological properties of Rendzinas with a deep black surface horizon, as well as certain conditions - and specific feature of organic compounds (Wasak et al., 2018).

The type of humus is fulvic-humic to humic, the ratio Ch/Cf is from 0.6 to 3.75 (Figure 4). The degree of humification is high in the almost all soil horizons (10–22 %). Humic acids in deepest part of soil profile are bound to calcium and the degree of condensation of the aromatic nuclei is high (E2:E6 3–6), the involvement of the aggressive fraction of fulvic-acids is insignificant – 5–7 %.

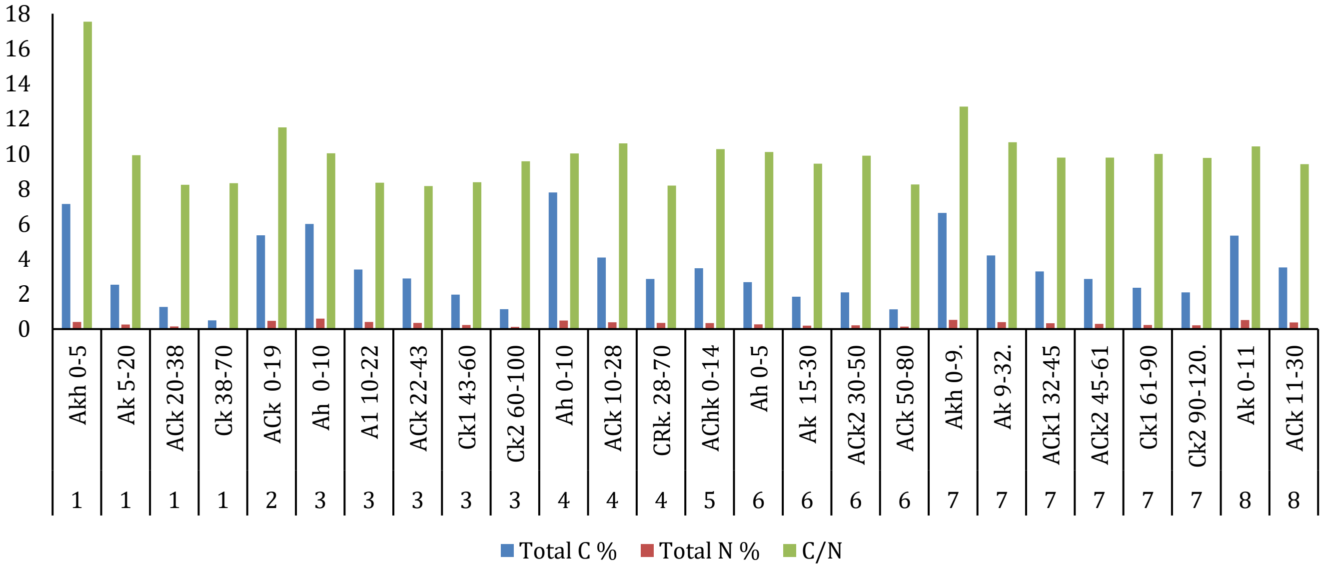

Figure 3. Total organic carbon (C, %), total nitrogen (N, %) and ratio C/N

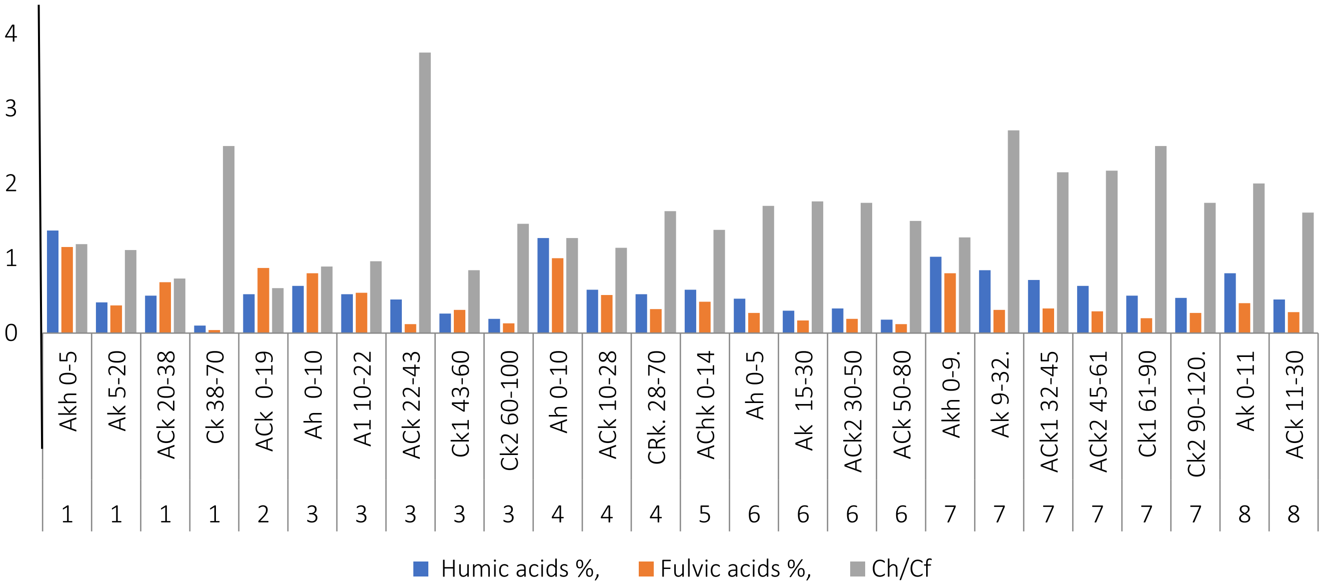

Figure 4. Extracted humic and fulvic acids (%), and ratio humic acid/fulvic acids (Ch/Cf)

According to Kononova (1966), the humus is 'Second type' and it is typical for Phaeozems, Rendzinas, Chernozems and Kastanozems. humic/fulvic (Ch/Cf) acid ratio is upper than 1. Extent of aromatic nuclei condensation is high in humic acids, which cause their hydrophobic properties and inability to creation of chelates. Unusually favoralble humic acids are strongly bounded with mineral components of soil in this type of humus.

In most cases the soil horizons humic acids are 100 % bounded with calcium. In some surface horizons, humic acids are free or bound with sesquioxide R_2O_3. The reason for that is surface litter of plant residues in which calcium is insufficient and fulvic acids are much more.

In all soil horizons (except in profile 1) the type of humus according C:N ratio is Mull (<14). This type of ratio is specific characteristic for forests, or grasslands with temperate climate. The porous, crumbly humus rapidly decomposes and becomes well mixed into the mineral soil, so that distinct layers are not apparent. Where free $CaCO_3$ occurred in the Ah-horizon, a mull forest horizon had developed even on coarse sand and the associated herb flora differed only slightly from that occurring on very acid mull.

According to Delecour (1980), the mull type of humus generally form under trees with more nutrient-rich litter, where gut-passage through soil animals followed by predominantly bacterial decomposition creates granular structures

Table 2. Descriptive statistics of soil organic matter content.

	Total C, %	Total N, %	C/N	Extract Humic acids, %	Extract Fulvic acids, %	Ch/Cf
Mean	3.40	0.32	9.98	0.56	0.42	1.63
Standard Error	0.38	0.03	0.37	0.06	0.06	0.14
Median	2.88	0.34	9.85	0.51	0.32	1.56
Mode	2.86	0.41	9.79	0.52	0.80	2.50
Standard Deviation	1.93	0.14	1.89	0.30	0.29	0.71
Sample Variance	3.74	0.02	3.58	0.09	0.09	0.50
Range	7.30	0.54	9.38	1.27	1.11	3.15
Minimum	0.50	0.06	8.16	0.10	0.04	0.60
Maximum	7.80	0.60	17.54	1.37	1.15	3.75

The correlation matrix in table 3 shows very good relation between main soil organic matter values. There are very high positive correlation (0.90), between total organic carbon and total nitrogen content, that's explain the mull type of humus, the high enrichment of soil organic matter with nitrogen. Extracted humic and fulvic acids also have good correlation with organic carbon and nitrogen.

Table 3. Correlation between main soil organic matter values.

	Total C	Ex. Humic acids, ,	Ex. Fulvic acids	Humification	Total N
Total C,	*				
Ex. Humic acids,	**0.89**	*			
Ex. Fulvic acids,	**0.84**	**0.79**	*		
Humification	-0.35	0.03	-0.04	*	
Total N	**0.90**	**0.71**	**0.67**	-0.45	*

*In bold: positive correlation between values

Conclusion

Studied soils, mainly Rendzinas, have shallow profile over weathered calcareous rock with high number of skeletal fragments, and well-formed dark colored surface rich of humus horizon. The amount of soil organic carbon in the surface horizon is high, up to 7.8 %. The average content of organic carbon is 3.4 % in whole profile. The type of humus is humic to humic-fulvic-, and the ratio Ch/Cf is from 0.6 to 3.75. The degree of humification is high in the almost all horizons. Humic acids are predominantly bounded with calcium and optical properties are with high density. In some surface horizons, humic acids are free or bound with sesquioxide, because of organic litter of plant residues in the surface. According C:N ratio the type of humus is mull (under 15), in all soil horizons, except one. Enrichment of soil organic matter with nitrogen is high, that is proved also with high correlation between total organic carbon and total nitrogen content. .

Studied soils are biological active with high amount of stable forms of humus and that have favorable impact over the soil structure and vegetation. Rendzinas have high potential of organic carbon sequestration.

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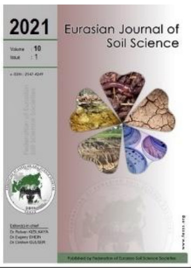

Yield and yield components of five tomato varieties (*Solanum lycopersicum*) as influenced by chemical NPK fertilizer applications under chestnut soil conditions

Aliya Maxotova [a,*], Elmira Nurbayeva [b], Temirzhan Aitbayev [a], Balseker Nurgaliyeva [c]

[a] Kazakh National Agrarian Research University, Almaty, Kazakhstan
[b] Kazakh Research Institute of Fruit and Vegetable Growing, Kainar, Kazakhstan
[c] Kazakhstan University of Innovation and Telekomunication Systems, Uralsk, Kazakhstan

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Author(s)

A. Maxotova *
E. Nurbayeva
T. Aitbayev
B. Nurgaliyeva

* Corresponding author

Abstract

The tomato is an important fruit, both fresh and processed, for human nutrition worldwide, and plays a significant role in agriculture. Especially in the intensive agricultural system where chemical fertilizers are used, little is known the impact of chemical NPK fertilizer applications on the yield of tomato under chestnut soil conditions. The objective of this study was to investigate the effects of four types of NPK fertilizer applications ($N_{120}P_{90}K_{60}$, $N_{150}P_{120}K_{90}$, $N_{180}P_{150}K_{120}$, $N_{210}P_{180}K_{150}$) on the yield and yield parameters of 5 different tomato varieties (Ogonyok 777, Barin, Hybrid Shuruk, Hybrid SC-2121 and Hybrid Falcon) under chestnut soil conditions in in the Southeast of Kazakhstan. According to field experiment results, there were significant differences among the treatments in relation to yield and yield parameters (plant height, number of stems, number of leaves, and number of fetus after planting) of tomato varieties. In foothill zone of the southeast of Kazakhstan, Hybrid Shuruk and Hybrid SC-2121 tomato varieties significantly yielded higher than the other three varieties tested at the same time under chestnut soil conditions. And also, it was determined that the best outputs tended to be obtained with $N_{210}P_{180}K_{150}$ fertilizer dose.

Keywords: Tomato, tomato varieties, NPK fertilizer, chestnut soil, fertilization.

Introduction

Fertilizers, which are indispensable and the most important material input in modern agricultural production (Chen et al., 2018; Li et al., 2019a), have played a vital role in improving the yield and quality of crops (Mahajan and Singh, 2006; Rajput and Patel, 2006; Hebbar et al., 2014). To date, numerous studies have explored the effects of fertilizer application rate on crop growth, yield, and quality (Mahajan and Singh, 2006; Castellanos et al., 2012; Bilalis et al., 2018). For example, Du et al. (2019) determined that the yield of tomato increased with a rising fertilizer application rate up to a point.

Tomato is one of the most important vegetables worldwide. As it is a relatively short duration crop and gives a high yield, it is economically attractive and the area under cultivation is increasing daily (Kalbani et al., 2016). Yields of field tomatoes usually range between 40 and 100 t ha^{-1} depending on the location, growing season, the cultivar used and crop management practices (Heuvelink and Dorais, 2005). The main reasons for differences in yield in fields tomato crops are well known. Ecological conditions such as soil types, cultivated tomatoes varieties, climate conditions, and fertilizer and pesticide applications are important factors in determining tomato yield and quality (Huat et al., 2013; Bilalis et al., 2018; Ouansafi et al., 2019; Litskas et al., 2019). However, fewer studies have focused on field-grown tomato crops for fresh

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consumption and those that did were mostly conducted in temperate regions in developed countries (Clark et al., 1999; Scholberg et al., 2000). Few studies have dealt with the factors that affect the production of field-grown tomatoes in chestnut soils. And also, little is known about the impact of chemical NPK fertilizer applications on yield of different tomato varieties (*Solanum lycopersicum*) under chestnut soil conditions. Chestnut soils a soil type occurring in arid steppes. The soils cover large areas of Turkey, Mongolia, northern China, the United States, and Kazakhstan (Saparov, 2014; Yertayeva et al., 2018; 2019). The climate in the chestnut soil zone is continental and arid. The genetic and zonal properties of chestnut soils include deficient drainage, a shortage of productive moisture, alkalinity, and soil heterogeneity. The parent material consists chiefly of calcareous deposits with a predominance of loess like loams, calcareous sandy loams, loesses, calcareous sands, sandy loams, and alluvium. Chestnut soils contain carbonates and, in most cases, gypsum in the lower part of the profile. The presence of readily soluble salts causes the alkalinity of chestnut soils.

The aim of our experiment was to investigate chemical NPK fertilizer applications for five tomato varieties (*Solanum lycopersicum*) on chestnut soil conditions in the Southeast of Kazakhstan.

Material and Methods

Description of the Study Sites

The experiment was conducted at the Regional Branch "Kainar" of the LLP "Kazakh Research Institute of Fruit and Vegetable Growing" which is located in the foothill zone of the southeast of Kazakhstan (43°09'32.8"N 76°26'57.3"E) North Slope of Zailiyskiy Alatau Mountains (Altitude: 1000-1050 m) during the growing season 2019-2020 with a view to finding out the Watermelon expo as well as determining the optimum dose of fertilizer of tomato. The locations of the evaluations were characterized by the continental climate (large daily and annual fluctuations in air temperature, characterized by cold winters and long hot summers), the air temperature reaches minimum values in January (-32,-35^{0}C), and maximum values in July (37-43^{0}C). The warm period lasts 240-275 days, the frost-free period is 140-170 days and an annual amount of precipitation is 350-600 mm.

The soil belongs to the general soil type of dark chestnut. The land was medium high with loamy. Before conducting the experiment, the soil sample was analyzed from Kazakh National Agrarian Research University. The soil was characteristically slightly alkaline (pH 7.3-7.4), soil organic matter 2.9-3.0% (moderate), total N 0.18-0.20% (high), available P_2O_5 35-40 mg kg^{-1} (moderate), available K_2O 360-390 mg kg^{-1} (low), cation exchange capacity 20-21 me 100g^{-1} soil, bulk density 1.1-1.2 gr cm^{3}, field capacity 26.6%.

Treatments and Experimental Design

The experiment was performed using a completely randomized block design with four replications. The experimental unit was 35 m^2 (3,5 m x 10 m). The sources of fertilizers used were urea 46% N, triple superphosphate 44% P_2O_5 and potassium sulphate 50% K2O. The experimental field was prepared in accordance with a standard practice used by RB Kainar of LLP Kazakh Research Institute of Fruit and Vegetable Growing. The land was disk ploughed, harrowed, and leveled with a tractor. Then ridging was done by hand. Fertilizer was applied using grain drill. Other agronomic practices and data collection were conducted based on the recommendations of Kazakh Research Institute of Fruit and Vegetable Growing. Five tomato varieties (Ogonyok 777 (Kazakhstan), Barin (Russia), Hybrid Shuruk (Netherlands), Hybrid SC-2121 (Turkey) and Hybrid Falcon (Turkey)) were combined with four fertilizer treatments. The transplanting was made on 25 May 2019 for field experiment. The tomato field was irrigated at the interval of five to six days depending on the prevailing weather conditions throughout the crop cycle, and harvesting was carried out from the third decade of August to the second decade of September. Trial was well protected against insects and weeds during the season. Full dose of given phosphorus and potassium fertilizer treatment was added at the time of transplanting and Urea was applied in three equal splits, 1/3 at transplanting and 1/3 at 20 days after transplanting, and the remaining 1/3rd was applied 40 days after transplanting. Both urea and phosphate fertilizers were placed alongside the ridge in the plating rows about 5 cm away from the transplanted to ensure that there would be no direct contact with the soil particles below the plant and to reduce fixation and N leaching.

Table 1. Treatment description and nutrient rates used in the field experiment

Treatments	Nutrient Rate (kg ha^{-1})		
	N	P	K
T1 = Absolute control	0	0	0
T2 = $N_{120}P_{90}K_{60}$	120	90	60
T3 = $N_{150}P_{120}K_{90}$ (recommended fertilizer dose)	150	120	90
T4 = $N_{180}P_{150}K_{120}$	180	150	120
T5= $N_{210}P_{180}K_{150}$	210	180	150

Data on growth and canopy characteristics such as plant height, number of stem, number of leaves, and number of fetus were measured from 10 randomly selected plants per plot. Yield of tomato at harvest were measured from sample fruits using digital balance and total yield (tons/ha) were assessed.

Results and Discussion

Effect of different levels of NPK fertilizers on yield parameters and yield of five tomato varieties was evaluated (Table 2). According to the Table 2, there was a significant difference between controls of each variety on yield of tomato, plant height, number of stems, number of leaves, and number of fetus after planting. In foothill zone of the southeast of Kazakhstan, Hybrid Shuruk (Netherlands) and Hybrid SC-2121 (Turkey) tomato varieties significantly yielded higher than the other three varieties tested at the same time in experimental site. The yield of tomato and yield parameters of tomato varieties differed significantly due to NPK fertilization (Table 2). At all potato varieties, Application of NPK fertilization significantly influenced the tomato yield, plant height, number of stems, number of leaves, number of fetus compared to untreated (control) plants. The best outputs tended to be obtained with $N_{210}P_{180}K_{150}$ (T5). Similar results were obtained by Gunarto et al. (1985), Yousaf et al. (1999), Magnusson (2002) and Li et al. (2019b) on several vegetable crops. Numerous studies have reported that inorganic NPK fertilizer increased growth in some species by enhancing nitrogen, phosphorus and potassium uptake (Shehu, 2014; Gülser et al., 2019).

Table 2. Effect of different NPK fertilizer doses on different vegetative parameters and yield of five tomato varieties

Treatments	Tomato plant height, depth,cm	Number of stems plant^{-1}	Number of leaves plant^{-1}	Number of fetus plant^{-1}	Total tomato yield, t ha^{-1}
Variety Ogonyok 777 (Kazakhstan).					
T1 = Control	40.7	3.5	17.6	10.2	27.1
T2 = $N_{120}P_{90}K_{60}$	45.2	3.8	20.3	11.4	30.1
T3 = $N_{150}P_{120}K_{90}$	48.3	4.0	21.7	12.5	32.2
T4 = $N_{180}P_{150}K_{120}$	56.0	4.0	23.5	12.8	36.4
T5 = $N_{210}P_{180}K_{150}$	61.4	4.1	24.2	13.6	40.0
Variety Barin (Russia)					
T1 = Control	39.5	3.4	18.0	8.5	26.7
T2 = $N_{120}P_{90}K_{60}$	42.5	3.5	20.1	9.0	29.4
T3 = $N_{150}P_{120}K_{90}$	44.7	3.8	20.2	9.1	34.2
T4 = $N_{180}P_{150}K_{120}$	50.2	3.9	21.4	11.4	37.3
T5 = $N_{210}P_{180}K_{150}$	53.8	3.9	22.6	12.7	40.5
Hybrid Shuruk (Netherlands)					
T1 = Control	45.3	3.0	16.8	9.0	28.5
T2 = $N_{120}P_{90}K_{60}$	50.5	3.1	18.4	9.5	32.6
T3 = $N_{150}P_{120}K_{90}$	53.6	3.0	19.3	9.6	37.7
T4 = $N_{180}P_{150}K_{120}$	58.4	3.2	22.5	10.7	41.2
T5 = $N_{210}P_{180}K_{150}$	62.7	3.4	25.0	11.8	45.5
Hybrid SC-2121 (Turkey)					
T1 = Control	38.6	3.2	19.2	7.8	30.2
T2 = $N_{120}P_{90}K_{60}$	42.1	3.7	23.0	8.6	33.4
T3 = $N_{150}P_{120}K_{90}$	43.6	3.9	24.1	8.9	36.5
T4 = $N_{180}P_{150}K_{120}$	47.8	3.8	25.6	9.4	40.6
T5 = $N_{210}P_{180}K_{150}$	50.2	4.0	26.7	10.3	44.6
Hybrid Falcon (Turkey)					
T1 = Control	41.0	3.0	17.4	8.1	27.0
T2 = $N_{120}P_{90}K_{60}$	44.3	3.2	18.4	8.7	30.3
T3 = $N_{150}P_{120}K_{90}$	45.7	3.3	19.0	9.0	32.5
T4 = $N_{180}P_{150}K_{120}$	50.4	3.5	21.7	9.8	38.0
T5 = $N_{210}P_{180}K_{150}$	53.5	3.7	23.8	10.5	42.7

Plant nutrition is one of the most important factors that increase plant production. Nitrogen (N) is the most recognized in plant for its presence in the structure of the protein molecule. Accordingly, N plays an important role in synthesis of the plant constituents through the action of different enzymes. Phosphorus (P) is required in large quantities in young cells, such as shoots and root tips, where metabolism is high and cell division is rapid. P aids in root development, flower initiation, seed and fruit development. P_2O_5 has been shown to reduce disease incidence in some plants and has been found to improve the quality of certain crops. Potassium (K) is an important macronutrient and the most abundant cation in higher plants. K has been the target of some researchers mainly because it is essential for enzyme activation (Wiedenhoeft, 2006;

Maynard and Hochmuth, 2007; Barker and Pilbeam, 2007). As per the previous results vegetative characteristics of all five varieties were increased with increase in NPK levels.

Results indicated that integrated supply of plant nutrients through chemical NPK fertilizer, played a significant role in sustaining soil fertility and crop productivity in terms of vegetative and reproductive growth. Several researchers have demonstrated the beneficial effect of combined use of chemical fertilizers to mitigate the deficiency of many secondary and micronutrients in fields that continuously received only N, P and K fertilizers for a few years, without any micronutrient. Also it is evident that the excessive use of synthetic agrochemicals in crop production and in soil fertility management causes detrimental effect on plant growth, make residue toxicity and environmental pollution. Yields of field tomatoes usually range between 40 and 100 t ha^{-1} (Heuvelink and Dorais, 2005). In this study, the fruit yield of tomato ranged from 27 t ha^{-1} to 45.5 t ha^{-1}, with an average of 35 t ha^{-1} (Table 2). The average fruit yield in this study area was higher than the 69 t ha^{-1} in the whole Turkey, 59 t ha^{-1} in China, and 61 t ha^{-1} in Japan. The reason for these significant differences was the climate, field management and soil types.

Conclusion

Five tomato varieties (Ogonyok 777 (Kazakhstan), Barin (Russia), Hybrid Shuruk (Netherlands), Hybrid SC-2121 (Turkey) and Hybrid Falcon (Turkey)) and five treatments (Control, $N_{120}P_{90}K_{60}$, $N_{150}P_{120}K_{90}$, $N_{180}P_{150}K_{120}$ and $N_{210}P_{180}K_{150}$) were used to investigate effects on yield and yield parameters of tomato under chestnut soil conditions in the Southeast of Kazakhstan. It was evident that increased levels of NPK levels resulted higher growth performance in all five tomato varieties than control. The T5 treatment ($N_{210}P_{180}K_{150}$) had a greater tomato yield than the other treatments [control without any nutrient supply (T1), and recommended fertilizer dose' treatment (T3, $N_{150}P_{120}K_{90}$]. This means that the typical NPK fertilization in this area (chestnut soil conditions in the Southeast of Kazakhstan) is not adequate. In general, Hybrid Shuruk (Netherlands) and Hybrid SC-2121 (Turkey) tomato varieties significantly yielded higher than the other three varieties tested at the same time in chestnut soil condition in Southeast of Kazakhstan.

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Hygienization assessment during heap co-composting of Turkey manure and olive mill pomace

Rachid Aboutayeb [a,*], Soufiane El-mrini [b], Abdeljalil Zouhri [b], Omar Idrissi [a], Khalid Azim [c]

[a] National Institute of Agricultural Research (INRA), Regional Center, Settat, Morocco
[b] Hassan 1st University, College of Sciences and Techniques, Settat, Morocco
[c] National Institute of Agricultural Research (INRA), Regional Center of Agadir, Morocco

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Author(s)

R. Aboutayeb *
S. El-mrini
A. Zouhri
O. Idrissi
K. Azim

* Corresponding author

Abstract

This study aimed to investigate the co-composting time effectiveness as well as the effect of the initial Carbon/Nitrogen ratio (C/N)i variation on the hygienization of olive pomace and turkey manure. Six different heaps, at 3 levels of (C/N)i ratios: 20, 22 and 28, were installed and monitored during 6 months and assessed at three steps: At the beginning, the end of thermophilic-phase and the end of curing-phase. The microbial monitoring concerned 5 microbial pathogens contents, used as hygiene microbial indicators, namely: Sulphite-Reducing Anaerobes (SRA), Escherichia Coli (E. Coli), Total Aerobic Mesophilic Flora (TAMF), Staphylococci, and *Salmonella* spp. Initially, the mixtures showed high TAMF and *Staphylococci* loads. Meanwhile, SRA and E. coli populations were relatively low and *Salmonella* spp. was not detected. The microbial assessment showed a significant effect of composting time on the reduction of pathogens load, except for SRA where its population has increased significantly, while the $(C/N)_i$ had a non-significant effect on pathogen content of the end-product. The final values expressed as colony-forming unit per gram (CFU g^{-1}), were as follow: Sulfite-reducing Anaerobes (≤3.1 x 10^3 CFU g^{-1}), E. Coli germ used as an indicator of faecal contamination (<4 x 10^1 CFU g^{-1}), Total aerobic mesophilic flora (≤1.4 x 10^6 CFU g^{-1}), *Staphylococci* (<10 CFU g^{-1}) and non-detection of *Salmonella* spp. Finally, the seed germination tests were carried out on three different seeds: lentils (Lens culinaris), barley (Hordeum vulgare) and durum wheat (Triticum turgidum) showed that the use of the compost extract is favourable for seed germination with germination index (GI%) values exceeding 85%. These results confirm the non-phytotoxicity and maturity of the composts.

Keywords: Sanitation, pathogens, poultry manure, microbial hazard, germination index, olive by-products.

Introduction

Agro-food industries generate a considerable amount of organic wastes. They can be recycled as organic amendments after biological treatments (Tortosa et al., 2019). Nowadays, the rapid increase of biowaste production has become one of the most crucial issues in most countries around the world (Azim et al., 2017). Besides, intensive agricultural activities lead to soil fertility depletion, worsens soil erosion, and causes organic matter content reduction (Ramli et al., 2020). The olive crop is one of the main cultivation in Morocco and also in several Mediterranean countries and its socio-economic role is well known. Thus, olive production causes a serious environmental issue through the by-products generated from olive factories (Toledo et al., 2020). The three-phase system used for olive oil production generates huge amounts of by-

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products, namely olive mill wastewater (OMW) and olive mill pomace (OMP) within few months in a year according to Roig et al. (2006).

On the other hand, throughout the last decades, the poultry livestock has increased significantly in most countries, which has led to an increase in poultry manure production and has increased considerably the amount of all organic solid by-products and wastewaters (Assess et al., 2019; Toledo et al., 2020). According to Aboutayeb (2015), in the Chaouia-Ouardigha region in Morocco, quite 300,000 tons of turkey manure (TM) are produced yearly. The number of turkey livestock farms is up to 220 production units and the production capacity exceeds 5 million turkeys per breeding cycle (Aboutayeb, 2015). These intensive livestock systems cause ecological problems due to the production of huge amounts of manure, the spread of microbial pathogens and the release of putrid odours (Heinonen-Tanski et al., 2006).

While olive pomace is not usually associated with the risk of microbial pathogens, poultry manure could be a source of microbial hazard when it is spread as an organic amendment without former treatment. It contains several human pathogens species like *Escherichia Coli*, *Clostridium, Listeria and Salmonella* (Chen et al., 2014). Moreover, it provides favorable conditions for the proliferation of microbial pathogens, which worsens environmental pollution (Li et al., 2016).

In this regard, according to Bustamante et al. (2008), composting is defined as a biological process occurring in an aerobic environment leading to organic materials stabilisation and heat production. Composting has been presented as a promising technology and environmentally friendly technique to manage and recycle these biowastes, to obtain a quality compost used as an organic amendment soil fertility improvement (Azim et al., 2018; Assess et al., 2019). Among the various biological treatments, composting is perceived to be among the most promising practices due to its low cost and effectiveness to produce stable organic amendments according to de Mendonça Costa et al. (2016) and Soobhany et al. (2017). These end-products, called composts, could be used as organic amendments able to enhance soils physicochemical and biological properties (Chowdhury et al., 2013; Tortosa et al., 2019). Composting process, as biological treatment, could be used for biowastes processing to reduce its pathogenic potential (El Fels et al., 2014; Assess et al., 2019). Actually, it is a widely accepted technique for organic waste recycling into a stable organic material, with low pathogens loads and phytotoxicity. Compost is applied as an organic fertilizer and soil amendments to enhance soil fertility parameters (Huang et al., 2006; Toledo et al., 2020) and increase plant growth and yield production (Bustamante et al., 2008).

Hence, the biowaste reuse as compost on agricultural soils can play a vital role in increasing the sustainability of agricultural practices, especially in Mediterranean countries where soil organic matter (SOM) is generally low as mentioned by Assess et al (2019). Indeed, soils of semi-arid rainfed areas, such as those of the West-Asia and North-Africa regions have less than 1.5% SOM content (Azim et al., 2017; Aboutayeb et al., 2020).

Depending on the composting process monitoring and the raw material origin, composts may also contain pathogenic microorganisms (Bustamante et al, 2008). Instead, the raw manure or immature compost spreading can induce pathogenic microorganism dissemination according to Millner et al. (1994) and Beffa et al. (1996) such as *Salmonella* spp., *Listeria monocytogenes* and *E. coli*, (Zhao et al., 1995; Islam et al., 2005; Soobhany et al., 2017). Moreover, the composting process improperly monitored can be a vector of several pathogens species initially present in raw organic waste or those resulting from the risk of re-proliferation during composting (Chen et al., 2014). Hassen et al. (2001) and Bustamante et al. (2008) have registered the proliferation of Shigella, Enterobacter, Yersinia and Streptococci which could cause infections diseases for farmers and compost handlers. . Actually, growing demand for sanitized compost is observed which reflects the increased interest in the food safety and environmental issues (Pandey et al., 2016; Soobhany et al., 2017). In certain cases, the remaining pathogenic organisms in a compost pile has been attributed to 3 main factors: inequal heating temperatures among different parts of the compost heap: The surface, the middle and the bottom of the piles (Aboutayeb, 2015); Mixtures inadequately homogenized as mentioned by Elving et al. (2010), and cross-contamination due to infected working tools (Soobhany et al., 2017). Hygiene microbial indicators, such as faecal coliforms, *E. coli* and streptococci are generally monitored during the composting process to ensure compost quality production (Bustamante et al., 2008; Aboutayeb, 2015). The subsistence of pathogenic populations in compost piles remains less explored (Soobhany et al., 2017). Faecal coliforms are generally associated with the animal faeces such as poultry manure (Aboutayeb et al., 2013); this is why regulations have adopted fecal coliforms, especially in *E. coli*, as an indicator of potential fecal contamination to assess the hygienic quality of the final compost (Soobhany et al., 2017). Although the composting process is potentially effective to reduce pathogens loads and thus producing a sanitized composts (Soobhany et al., 2017), data on the relationship between pathogen reduction and composting duration remains unsettled and needs more attention. This study aims to assess the effect of the initial C/N ratio and Treatment-time of co-composting on the microbial characteristics of the final compost concerning different human pathogens and microbial groups used as hygienic microbial indicators.

Material and Methods

Raw materials

The turkey manure used to carry out this study was collected and transported to the composting experiment site from three Turkey farms located in the immediate vicinity of Settat province in North-West of Morocco. The composting site is located in the experimental station of Sidi Elaidi (altitude 230 m, 33.17° N, 7.40° W) belonging to the National Institute of Agricultural Research (INRA-Morocco). We have taken six composite samples for microbial analysis. The Olive mill pomace (OMP) was collected from a three-phase artisanal crushing unit (Maasras) in the Settat region. The durum wheat straw was used as a bulking agent for the composting process. Six heaps were prepared to obtain the initial C/N of 20, 22 and 28 in duplicate (2 heaps for each ratio) (Table 1). C/N was calculated using the formula OC(%)/TN(%) where OC(%) = OM(%)/1.73 (Table2).

Table 1. Composition (weight/weight) and (C/N)i of different mixtures

Heaps	Durum wheat straw	Turkey manure	Olive mill pomace	C/N	Height (m)	Width (m)	Length (m)
H1	10.0%	26.4%	63.6%	20	1.2	1.3	1.5
H2	10.0%	26.4%	63.6%	20	1.2	1.3	1.5
H3	60.0%	10.0%	30.0%	28	1.5	1.4	1.8
H4	60.0%	10.0%	30.0%	28	1.5	1.4	1.8
H5	20.0%	20.0%	60.0%	22	1.6	1.4	1.8
H6	20.0%	20.0%	60.0%	22	1.6	1.4	1.8

Composting process

Olive mill pomace and turkey manure were added to the durum wheat straw and the heaps were moistened if necessary and composted in aerobic conditions. The experiment of composting is carried out for six months. During the composting process, the heaps have been manually turned. Heap temperatures monitoring was carried out using a compost thermometer. A sample from each heap was collected in sterile plastic bags to serve for physicochemical and microbial analysis. The Organic carbon, organic matter and nitrogen contents properties of composts, at the initial and final time, are shown in Table 2.

Table 2. Physicochemical properties of composts at the initial and final time (TNK: total nitrogen, OM: organic matter, C/N: Carbon to nitrogen ratio).

	H1		H2		H3		H4		H5		H6	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
TNK (%)	1.82 ± 0.15	2.26 ± 0.03	1.84 ± 0.08	2.77 ± 0.06	1.36 ± 0.06	2.58 ± 0.03	1.21 ± 0.02	2.81 ± 0.01	1.65 ± 0.12	2.82 ± 0.05	1.63 ± 0.07	2.66 ± 0.00
OM (%)	61.72 ± 0.90	43.66 ± 0.98	58.96 ± 0.70	58.58 ± 1.31	66.37 ± 0.13	54.1 ± 1.31	58.96 ± 0.31	64.76 ± 1.75	62.65 ± 0.58	54.95 ± 0.11	59.04 ± 0.39	50.31 ± 2.30
C/N	19.73 ± 1.88	11.26 ± 0.4	18.65 ± 0.59	12.26 ± 0.54	28.34 ± 1.36	12.15 ± 0.43	28.17 ± 0.22	13.38 ± 0.31	22.10 ± 1.76	11.30 ± 0.22	21.02 ± 0.77	10.97 ± 0.50

Microbial Analysis

Microbial analyses were performed on the enumeration of 5 microorganisms considered as hygienic microbial indicators. The assessment of microorganism loads was expressed in colony-forming units per gram (CFU g^{-1}). The samples were analyzed in three stages: S1 (at the beginning of composting), S2 (after 3 and 6 weeks of composting for heaps 5,6 and heaps 1,2,3,4 respectively) and S3 (at the end of the composting experiment). The Total Aerobic Mesophilic Flora (TAMF) density was determined using Plate Count Agar (PCA) medium and incubation at 30° C for 72h (ISO 4833-1:2013). *E. coli* was assessed using the tryptone-bile-glucuronide medium, incubation at 44±1°C for 18 h to 24 h. (ISO 16649-2:2001). *Staphylococcus aureus* load was determined using rabbit plasma fibrinogen agar medium, then incubation at 37° C for 24h (ISO 6888-2:1999). Sulfite-reducing bacteria (characterized by typical black coloured colonies) were incubated under anaerobic conditions on agar plates using an iron-sulfite medium, then incubated at 37◦C ± 1°C for 24h to 48h (ISO 15213:2003). Finally, the determination of *Salmonella* presence was carried out, in 25g sample inoculated to buffered peptone water and incubated at 37 °C ± 1°C for 18 h ± 2 h, isolated then identified and confirmed following the protocol of ISO 6579-1:2017/AMD 1:2020.

Phytotoxicity test and germination index (GI)

The phytotoxicity test is based on the principle of the compost aqueous extract phytotoxicity towards the tested seeds. It involves placing the seeds of three different species (Durum wheat (*Triticum turgidum*), Barley (*Hordeum vulgare*) and Lentil (*Lens culinaris*), in a series of Petri dishes with filter paper impregnated with increasing doses of the compost extract: 25%, 50%, 75% and 100% (v/v) (three repetitions for each treatment). Another set of control (without compost aqueous extract) is prepared with distilled water and its germination index is considered as 100% for relative comparison with the treatments. The Petri dishes are placed in the germination chamber for 5 days at a temperature of 25°C and relative humidity of 85 to 90%. After 3 days the number of seeds germinated per petri dish was determined. The phytotoxicity levels of the compost extracts were determined according to a standard method (Zucconi et al., 1981). Index Germination was calculated using the following formula:

$$IG = \frac{(\text{Nmgg} * \text{Lr})}{\text{Nmggt} * \text{Lrt}}$$

Where
Nmgg : Number of germinated seeds;
Lr : Average length of the root;
Nmggt : Number of germinated seeds of the control;
Lrt : Average length of the witness root.

Statistical Analysis

The effect of the factors studied (C/N ratio and Time) on the evolution of microbial populations was evaluated by ANOVA 2 and Tukey's test. The results were carried out using SPSS software, Version 20.

Results and Discussion

Temperature monitoring

Temperature is one of the major parameters to assess the progress of the composting process as it indicates the rate of microbial activity (Manu et al., 2019). Many authors consider 45° C as the temperature limit between the mesophilic and thermophilic phase during composting (Albrecht, 2007; Pujol, 2012). A temperature above 45° C can hygienize the heaps by reducing pathogens loads (Aboutayeb et al., 2013). All the heaps have recorded (Figure 1) thermophilic phases with maximum temperatures of 62, 57.3, 60.1, 59.6, 56.6 and 61.3° C for heaps 1 to 6 respectively, indicating organic matter biodegradation. The short periods of the thermophilic phase characterized the heaps with a lower proportion of straw and low initial C/N (H1 and H2) who reached the thermophilic phase faster due to their high manure content, and therefore high initial microbial load. After thermophilic phase, the six heaps have entered the maturation phase where the temperature tends to the ambient one indicating the end of the composting process. There is a similarity between the temperature curves of the heaps with the same composition (H1, H3 and H5 with H2, H4 and H6 respectively).

Evolution of the microbial parameters

At the start, the initial mixtures showed a high load of *Staphylococci* and TAMF populations, due probably to non-compliance with good hygiene practices in the *Maasras* (Rouas et al., 2015) and given that TM is considered as natural host for many microbes (Bustamante et al., 2008). However, all the mixtures recorded no presence of *Salmonella* even if the manure is a natural host, which proves good hygiene control in the livestock house (Aboutayeb, 2015).

Sulphite reducers Anaerobes (SRA)

Several studies have focused on the pathogenic density decay as one of the most important factors through composting process according to Gale (2004) or after compost spreading on agricultural soils, for healthy risks assessment (Soobhany et al., 2017). The presence of Clostridium bacteria, a pathogenic SRA germ, can be used as a suitable indicator for other faecal pathogens (Bustamante et al., 2008). Except for H2, all the heaps recorded an increase at S2 of composting time (Table 3). Then, H1, H3, H4 and H6 recorded a reduction at the end of composting while H2 and H5 recorded a slight increase in SRA by the end of the process. The statistical test shows that only the composting time factor has a significant effect on the increase in SRA load but only between the S1 and S2 composting stages. The final reduction was not significant which is unusual for SRA considered as a strict anaerobic germ. It could be explained by the presence of certain SRA species that tolerate oxygen presence and able to generate ATP in an aerobic environment, or capable to reduce nitrates (Loubinoux, 2001). The final reduction of SRA, even if it is not significant, can be explained by the scarcity of labile organic matter during the curing phase, the aerobic conditions and the presence of nitrates (Aboutayeb, 2015).

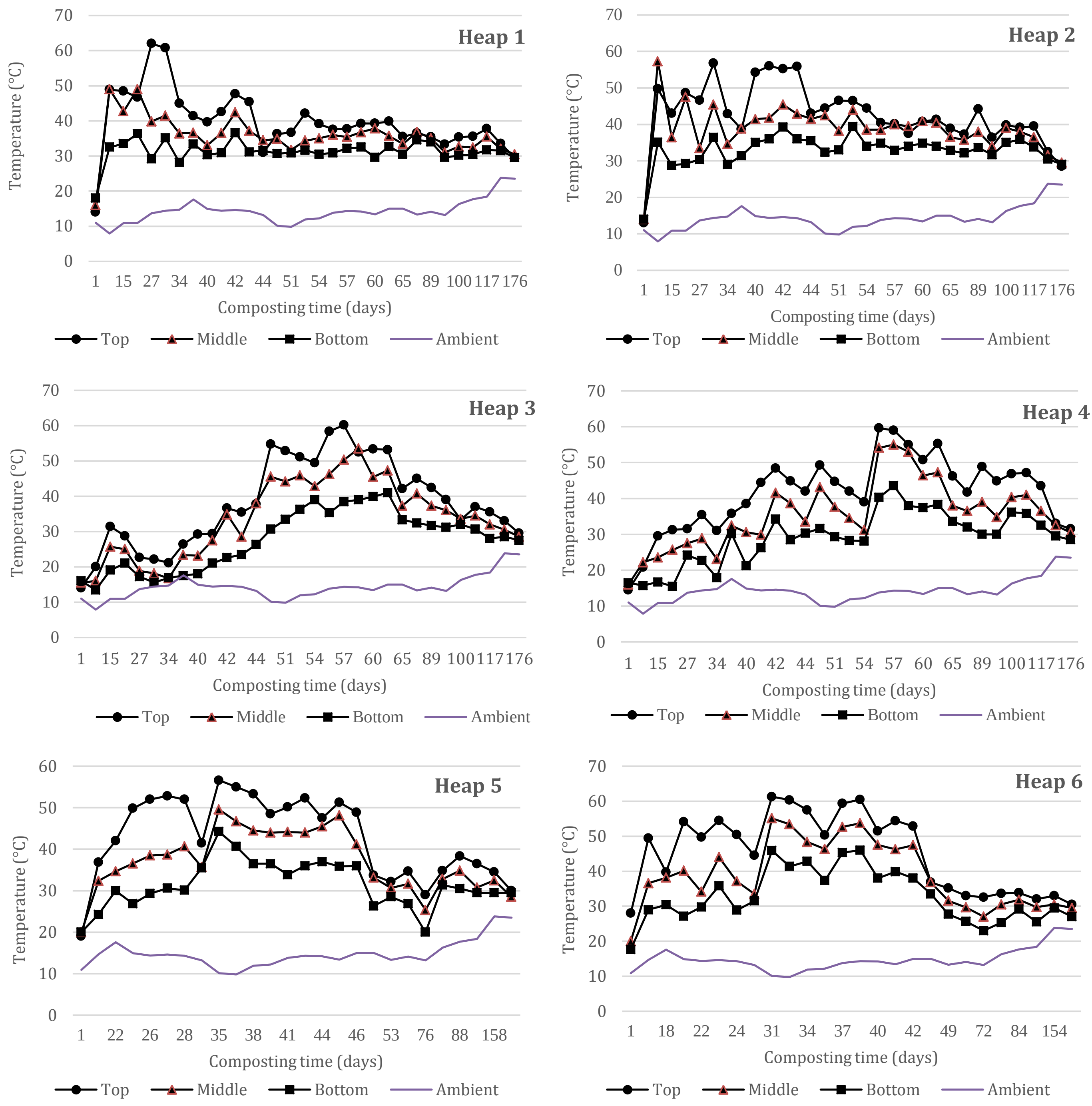

Figure 1. Temperature evolution during co-composting of olive pomace with turkey manure

The SRA loads in all heaps remain above 10^3 CFU g^{-1}. This result is consistent with those found by Bustamante et al. (2008) who mentioned that there is no enough hygienization in composting piles to ensure the total elimination of *Clostridium*. This result could be explained by the fact that *Clostridium* bacteria show resistance to several adverse conditions and are among the heat resistant bacteria as mentioned by Juneja and Marmer (1998) and Payment (1999). However, the distribution of SRA makes challenging the production of free-*Clostridium* composts even in aerobic conditions (Juneja et al., 2003; Bustamante et al., 2008). This resistance could be explained by spores ability to survive in harsh conditions and the remaining anaerobic spaces leading to SRA growth (Böhnel and Lube, 2000; Jones and Martin, 2003; Pourcher et al., 2005). These results are better than those found by Bustamante et al. (2008) who found higher levels of SRA in turned piles, in general exceeding 10^4 CFU g^{-1}. These findings may be due to higher temperature values, during the thermophilic phase, reached in turning heaps and thus a more effective elimination of SRA have been recorded.

Table 3. Monitoring of SRA and E.Coli content during the composting process. (S1 (at the beginning of composting), S2 (after 3 and 6 weeks of composting for heaps 5,6 and heaps 1,2,3,4 respectively) and S3 (at the end of the composting experiment)

Heaps	SRA			E.Coli		
	S1	S2	S3	S1	S2	S3
H1	2.4×10^2	2.10×10^4	2.00×10^3	2.0×10^2	1.90×10^4	<10
H2	2.4×10^2	1.0×10^2	2.40×10^3	2.0×10^2	3.30×10^5	<10
H3	9.0×10^2	4.80×10^4	1.70×10^3	1.0×10^2	1.30×10^4	<10
H4	9.0×10^2	1.60×10^4	2.90×10^3	1.0×10^2	7.8×10^2	<10
H5	2.0×10^2	1.60×10^3	2.40×10^3	1.3×10^2	1.10×10^4	<10
H6	2.0×10^2	1.10×10^5	3.10×10^3	1.3×10^2	5.70×10^5	<40

Escherichia coli

E. coli as an emerging pathogen is the main faecal coliform microorganism. It is usually considered as the most important pathogen to investigate in all processes that use or integrate faecal materials (Déportes et al., 1998; Hess et al., 2004; Bustamante et al., 2008). The presence of coliform bacteria reflects the hygienic quality level of soil and water in the environment. Its utilization as a hygienic indicator could bring several benefits due to the high detection frequency and easy revelation in faecal materials compared to other pathogens (Hassen et al., 2001).

Generally, the monitoring of the composting process showed the same evolution for both *E.coli* and fecal coliforms bacteria (Le Minor, 1984). The $(C/N)_i$ factor had a non-significant effect on *E.coli*; while the composting-time factor recorded a significant effect. *E.coli*, considered a thermotolerant germ, is not considered heat-resistant. Its growth limit temperature is 45.5°C (Vernozy-Rozand and Roze, 2003). At composting time S2 (Table 3), *E.coli* increased in all the heaps, given the favourable temperature because all the heaps were still in the mesophilic phase except H1 where the temperature exceeded 45° C but for just one day.

At the end of composting, *E.coli* was almost eliminated in all the heaps. *E.coli* was removed in H1 even though its temperature was < 36.7° C from S2 until composting end, which may be in agreement with the findings of Larney et al. (2003) who concluded that more than 99.9% of *E. coli* was removed, during the first 7 days of composting, at temperatures ranging from 33.5 to 41.5°C. The thermophilic temperature/time pairs of H1 to H6 were (43.5-45.8°C/4 days separately), (43.6-46.6°C/4 days), (43.7-47.1°C/14 days), (47-51.3°C/6 days), (42.7-50.1°C/11 days) and (44.7-54.1°C/14 days) respectively. These lethal time/ temperature pairs are in agreement with other studies findings: Total destruction of *E.coli* in manure at 45°C during 72 hours of composting (Lung et al., 2001), and 7-14 days at 67°C as stated by Johannessen et al. (2005). E. Coli regrowth was observed during the first weeks (between S1 to S2 time of composting) which could be usually attributed to recontamination phenomena or explained by the insufficiency of compost self-heating and composting time (Soobhany et al., 2017).

The reduction of *Escherichia coli* loads used as a faecal-contamination indicator was significant in all final heaps. The *E. Coli* population is compliant with the recommended limit (10^3 CFU g^{-1}) indicating the heap co-composting efficiency (Ros et al., 2006; Aboutayeb, 2015). This significant decrease could be explained by both a high temperature occurring in the heaps and aerobic conditions (Semenov et al., 2011).

This decay was likely the result of the high temperatures (thermophilic phase between 43.5°C and 54.1°C in all the heaps). Similar conclusions have been recorded by Hess et al (2004), who mentioned a decline, then an increase of *E.coli* population at 50°C. Other studies highlighted that even with temperatures reaching up to 66°C, the elimination of *E.coli* is not complete. According to De Bertoldi et al (1983) and confirmed by Assess et al. (2019), during the thermophilic stage, the recommended temperature is ranging between 40 and 65°C and temperatures above 55°C are required to eliminate coliforms considered as thermotolerant microorganisms. An important decrease in the *E.coli* population was recorded after the thermophilic phase in all the heaps. This finding complies with several previous works that have shown the ability of the composting process to eliminate E. coli (Mainoo et al., 2009; Aira et al., 2011).

TAMF

The effectiveness of the composting-time factor was high on the reduction of TAMF, especially between S2 and the end of composting (Table 4). Initially, all the heaps showed a high density of TAMF. At S2 time of composting, the density of TAMF increased for all heaps, which is consistent with the work of El Fels (2015) who reported that mesophilic bacteria growth continues even at 50°C. Then, TAMF was significantly reduced in the mature composts. This decrease was mainly due to the temperature increase during the thermophilic phase and the unfavourable conditions in the heap essentially due to the labile organic matter depletion" (Kalamdhad and Kazmi, 2009; Aboutayeb et al., 2013). The final density of TAMF which still quite important could be explained by its re-proliferation during the maturation phase characterized by the favorable temperature conditions for mesophilic microflora growth (El Fels et al., 2015).

Strict monitoring, especially of the temperature profile, should be performed to reduce contamination risks due to pathogens high loads essentially when raw animal material, considered as a natural host, was used. The outcomes of other studies were consistent with these results by the fact that mesophilic bacteria were predominant at the beginning of the co-composting process and their population remain around 10^5 and 10^6 CFU g^{-1} during the cooling phase (Hachicha et al., 2009; Assess et al., 2019).

Table 4. Monitoring of TAMF, *Staphylococci* and *Salmonella* content during the composting process (ND: not detected. D: detected)

Heaps	TAMF			*Staphylococci*			*Salmonella*		
	S1	S2	S3	S1	S2	S3	S1	S2	S3
H1	3.54×10^6	2.50×10^7	7.60×10^5	4.49×10^6	9.80×10^6	<10	ND	ND	ND
H2	3.54×10^6	2.00×10^7	2.70×10^4	4.49×10^6	<10	<10	ND	ND	ND
H3	1.43×10^6	1.50×10^7	1.40×10^6	1.70×10^6	<10	<10	ND	ND	ND
H4	1.43×10^6	1.60×10^7	2.10×10^5	1.70×10^6	2.10×10^6	<10	ND	ND	ND
H5	2.86×10^6	3.20×10^6	1.50×10^5	3.40×10^6	<10	<10	ND	ND	ND
H6	2.86×10^6	2.00×10^7	2.00×10^5	3.40×10^6	<10	<10	ND	D	ND

Staphylococci

Staphylococci is considered an optional aero-anaerobic microorganism widely known as one of the main causes of food biological hazards such as toxic infections (Vernozy-Rozand et al., 2004; Bustamante et al., 2008). *Staphylococci* growth occur under a large pH interval (4-9.3), requires temperature ranging between 7°C and 46°C and water activity (Aw) above 0.84 in aerobic conditions (Lamprell, 2003). Table 4 shows that in all heaps, the C/N factor has a no-significant effect on *Staphylococci* content while composting time has reduced significantly its load. It was almost eliminated by the end of the composting period (beneath 10 CFU g^{-1} for all the heaps).

The number of *Staphylococci* increased during the bio-oxidative phase in H1 and H4 then decreased at the maturation phase in all the heaps (beneath 10 CFU g^{-1}). These results could be explained by the temperature evolution (heating and cooling) in the bio-oxidative phase and scarcity of labile organic matter in addition to the competition with other microorganisms essentially fungi (molds and yeasts) present in the heaps. These results are in agreement with those found by Bustamante et al. (2008) where levels of *Staphylococci* detected were less than 3 CFU g^{-1} of compost. Some authors report that pathogens species could be inhibited by several compounds contained in the heap such as organic acids, ammonia and flavonoid compounds even though temperature remains the most effective factor (Ait Baddi et al., 2004; Hachicha et al., 2009).

These results are better than those found by Aboutayeb (2015) who concluded that the population of *Staphylococci* was significantly reduced through composting time; however, the remaining density (slightly higher than 10^4 CFU g^{-1}) could be due to the ubiquitous character of *Staphylococci*. Despite successful composting, the sanitary hazard remains probable due to a potential staphylococcus regrowth, especially in the peripheral parts (Albrecht, 2007; Sidhu et al., 2001; Aboutayeb et al., 2013).

Salmonella

Salmonella is a pathogenic bacteria of high concern in farming (Jamieson et al., 2002). It is related directly to the compost hygienic quality according to Brinton Jr and Droffner (1994) and Yanko et al. (1995). The risk related to this pathogen is increased because of its fast growing and ubiquitous presence (Hassen et al., 2001).

Several studies have concluded the possibility of the presence of *Salmonella* during biowaste composting even if the required time-temperature pairs were reached (Pourcher et al., 2005; Millner et al., 2014). As mentioned by Bustamante et al. (2008), *Salmonella* was detected in all animal biowaste which explains its presence in the composting pile containing poultry manure as a raw material. Even though *Salmonell*a is not among thermotolerant microorganisms, it could persist at a high temperature exceeding 50° C (Droffner et al., 1995).

The present work showed that *Salmonella* was completely absent in all the heaps by the end of the co-composting process (Table 4). This result is in agreement with those found by Soobhany et al. (2017) who concluded that *Salmonella spp.* decreased below the detection limit (Less than 1 most probable number per sample of 4 g). Similarly, it is consistent with the result of Aboutayeb (2015) who concluded the non-detection of *Salmonella* in all the heaps. In the sixth heap (H6), *Salmonella* was only detected at S2 of composting (Mesophilic phase). This finding is consistent with other studies which demonstrated that *Salmonella spp*. was able to regrow in compost, windrows as a post-contamination even if the composting process was well monitored (Erickson et al., 2010; Soobhany et al., 2017). The reason for the regrowth could be explained by the lack of homogeneous temperature profile in the heap or due to recontamination occurring through compost handling (PereiraNeto et al., 1986; Gerba et al., 1995). Other factors could also influence *Salmonella* growth particularly, nutrient availability and moisture content (Bustamante et al., 2008). As a consequence, the production of *Salmonella* free compost requires an effective and efficient composting process monitoring to avoid pathogens regrowth (Soobhany et al., 2017).

Germination Index (GI)

In order to assess the compost maturity, the phytotoxicity test is one of the most important criteria used for this purpose (Bargougui et al., 2019). It allows to state reliable conclusions about the potential importance of the end-product, compost, to be used as an alternative organic fertilizer on agricultural crops. The final composts were assessed to reveal their phytotoxic potential, through the germination indexes (GI%) determination. Seeds of 2 cereals and a legume were used, Durum wheat, Barley and Lentils respectively.

The compost extracts were prepared from the six mature compost samples obtained from the studied process once finished. The compost extracts recorded quite good seeds germination and the recorded results were as follow: 101,42% ± 20,32%, 112,22% ± 15,74% and 92,29% ± 18,43% for lentils, durum wheat and barley seeds respectively, thus indicating both the absence of any phytotoxic effect and so the maturity of the olive pomace and turkey manure final co-compost (Table 5).

Table 5. Germination index (GI) of lentils, durum wheat and barley at different doses of compost extract (v/v)

	Lentils	Wheat	Barley
GI 25%	99,70% ± 35,84%	124,37% ± 2,69%	95,90% ± 36,03%
GI 50%	116,94% ± 17,61%	109,11% ± 35,59%	102,60% ± 11,47%
GI 75%	84,27% ± 3,38%	103,09% ± 18,27%	93,43% ± 9,25%
GI 100%	104,76% ± 24,47%	112,31% ± 6,42%	77,25% ± 16 ,99%
GI average (%)	101,42% ± 20,32%	112,22% ± 15,74%	92,29% ± 18,43%

GI value exceeding 80% reflects the non-phytotoxicity effect of mature compost according to Hachicha et al. (2006) and Francou (2005). These high seeds germination indexes values could be due to the high quality of the obtained compost and seeds resistance to residual phytotoxic compounds indicating consistency with previous studies (Bargougui et al., 2019).

Conclusion

From a hygienic point of view, co-composting of TM and OMP could remarkably decrease, over 6 months follow-up, the pathogen loads in the end-product especially for TAMF, sulfite-reducing Anaerobes, *E. Coli*, and *Staphylococci*. Besides, the initial C/N ratio has a no-significant effect on microorganism populations. Consequently, co-composting treatment has been demonstrated to be an effective and sustainable process for biowaste valorization and organic material sanitization. This study revealed that the co-composting technique could contribute effectively to transform poultry manures and industrial olive-oil by-products into a valuable resource. Aerobic heap co-composting made it possible to produce a sanitized and non-phytotoxic end-product that could be used as a soil organic amendment. This bioprocess has prevented contamination and reduced the density of all studied pathogenic microorganisms and improved the GI of all studied seeds (wheat, barley and lentil) leading to control of both sanitary and phytotoxic hazards associated with compost production from contaminated organic wastes. Additionally, achieving high thermal values during the bio-oxidative phase was revealed insufficient; it is also crucial to monitor the whole co-composting process to ensure a safe final compost with low pathogen contents leading to minimize the microbial hazards. This fact leads to the reduction of environmental issues due to the mismanagement of different biowastes composting and therefore contributes to the sustainability of agricultural practices. These findings could be completed by further studies focusing on composting process sanitation and contamination risk assessment, in order to contribute to a sustainable development policy capable to recycle and reuse agro-food by products.

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Evaluation of municipal sewage sludge for Arbuscular mycorrhizal fungi inoculum production

Anju Tanwar [a,*], Ajay Singh [b], Ashok Aggarwal [c], Esha Jangra [c], Sergio T. Pichardo [d]

[a] Department of Botany, Government P.G. College, Ambala Cantt–133001, Haryana, India
[b] Department of Food Technology, Mata Gujri College, Fatehgarh Sahib, Punjab, India
[c] Department of Botany, Kurukshetra University, Kurukshetra–136119, Haryana, India
[d] Abraham Baldwin Agricultural College (ABAC), School of Agriculture and Natural Resources (SANR) Tifton, GA, USA

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Author(s)

A.Tanwar *
A.Singh
A.Aggarwal
E.Jangra
S.T.Pichardo

* Corresponding author

Abstract

This experiment was carried out to assess the effect of soil amendment with different concentrations of municipal sewage sludge (SS) as a substrate on inoculum production of two selected arbuscular mycorrhizal fungi (AMF) i.e., *Glomus mosseae* and *Acaulospora laevis*. The experiment was a 4 × 5 factorial design with four hosts including, maize (*Zea mays* L.), lemon grass (*Cymbopogon nardus* (L.) Rendle), palmarosa (*Cymbopogon martini* (Roxb.) Wats.) and *Sesbania* (*Sesbania aculeata* Poir.) and the following five SS concentrations 1) no substrate, 2) 25 g, 3) 50 g, 4) 75 g and 5) 200 g pot^{-1}) with five replications. After 90 days, the host roots and its rhizosphere soil were examined for fungal mycorrhization in terms of percent of root colonization and AMF spore quantification. Furthermore, we calculated the response of each host in terms of increase in plant height (cm), root length (cm), root, fresh shoots, and dry weight (g). Mycorrhization pattern showed moderate to abundant intraradical mycelium, extraradical mycelium, vesicles, and arbuscules in all the host plants. This pattern varied with a change in the input level of SS. The 75 g treatment obtained the maximum mycorrhization of both the AMF, while the highest input level was detrimental to AMF and host plants' survival. Among the tested hosts, lemon grass and maize had a tremendous increment in *G. mosseae* and *A. laevis* inoculum respectively. Consequently, 75 g SS with lemon grass is the most compatible host–substrate combination capable of maximum *G. mosseae* and *A. laevis* spore production and root colonization and so far, highlights the significance of an alternative, cost–effective and affordable carrier medium that can be adopted by farmers as sustainable cultural practices for on farm AMF inoculum production.

Keywords: *Acaulospora laevis*, Agricultural waste, *Glomus mosseae*, Sludge utilization strategy, Lemon grass.

Introduction

Among agricultural practices, plant microbe interactions in plant rhizosphere are the interesting environment friendly stimuli that contribute in the development of sustainable agriculture. (Mhlongo et al., 2018). Producing good quality food in a sustainable manner, while mitigating deleterious biotic as well as abiotic stress makes the use of Arbuscular mycorrhizal fungi (AMF) as preferred substitute and achievable production practice (Nzanza et al., 2011). Nevertheless AMF cannot complete their life cycle unless they receive fixed carbon (simple sugars) from their host plant and hence acts as an obligate symbionts (Smith and Read, 2008). AMF mediates trade of important ecological services with their host plants, in particular nutritional benefits together with increased mobilization of nutrients especially immobile P, strengthen water relations, increase plant tolerance against biotic and abiotic stress, soil structure and fertility (Whiteside et al., 2019). Prospects of utilizing native AMF strains in agriculture through diverse eco–friendly

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avenues such as manipulation of agro–practices, crop rotations and implementation of native AMF inoculum and their practical feasibility has been validated for adoption and recommendation as integrated crop production component (Maiti, 2011). Therefore, considerable effort has focused on finding ways to multiply these fungi for potential applications in agriculture.

Besides their obligate nature, large scale multiplication of AMF in conjunction with appropriate host is more often executed in substrate-based medium (small beds, pots, plots, large fields), substrate less medium (aeroponic) or *in vitro* conditions like transformed root culture (Kokkoris and Hart, 2019). The soil-based medium is the traditional, economical and highly preferred technique of AMF on large–scale (Kapoor et al., 2008; Selvakumar et al., 2018). Varieties of commercial inoculants are available in the market. However, due to expenses pertaining to maintenance, establishment and multiplication of pure cultures of AMF strains, the cost of carrier substrates, shipping and handling of inoculums has pushed the cost for a farmer even higher. Skill based training program on AMF isolation, establishment and multiplication can be provided by various government agencies to extension workers, agro-dealers and small-holder farmers. Consequently, farmers and nursery owners can conventionally multiply AMF in pots or small plots or nursery beds at their place by utilizing different hosts and substrates, whichever is available.

AMF inoculum encompasses spores, sporocarps, mycelium and colonized root segments (Gupta, 2017; Moreira et al., 2019). A nurse plant with high efficiency of mycorrhization and selection of suitable material for the multiplication and delivery of AMF spores and hyphae is essential. In general, large scale propagation of AMF, multiplication under pot conditions is regarded as the most appropriate system (Feldmann and Grotkass, 2002). Furthermore, this soil can be amended with various other substrate materials to speed up the propagation process (Douds and Schenck, 1990; Gómez–Bellot et al., 2020) like sugarcane bagasse (Tanwar et al., 2013), cattle manure, wood powder and wood (Rodrigues and Rodrigues, 2017), or poultry manure (Uko et al., 2020) to enhance plant growth. Henceforth, various other substrates could be tested for their use in AMF multiplication. The carrier substrate should be easily available one and skillfully selected to allow a stable environment to AMF. Besides it should not be deleterious for AMF, having extended inoculum shelf life, physically and chemically stable, easy to handle and composed of biodegradable and non polluting substances (Mukhongo et al., 2016). Among all, municipal sewage sludge (SS) is a residue from the biological wastewater treatment plants, where domestic waste predominates over industrial one and heavy metal level and pathogens are within acceptable range for agricultural use (Lobo and Filho, 2009).

Disposal of SS is a worldwide problem since it is produced in large volumes due to rapid urbanization. The contemporaneous methods for its disposal encompass dumping in sea, land filling, incineration and agricultural applications as soil fertilizer and soil improver (Pöykiö et al., 2019). Other management practices: include energy and heat recovery, biomass and nutrients (P) recovery and resource usage (Rosiek, 2020). Utilization of SS for agriculture perhaps offer an environmentally acceptable and agronomically favorable means of waste disposal since it is a good source of organic matter and plant nutrient that can have a beneficial effect on plant growth by conditioning soil via improving its biological, chemical and physical properties. Concomitantly, the main limitation of using this resource is its complex composition due to high moisture, ash, toxic heavy metals and organic contaminants which influences the safety of SS (Oladejo et al., 2019).

Soil amendment with SS can impart a sustainable secure practice for the safe disposal of this waste besides improving plant growth, while employing no environmental threats provided there is no heavy metal accumulation in the edible part or in the amended soils (Eid et al., 2020). Several experiments have been conducted on the utilization of SS for crop improvement and most of them have supported and emphasized its use in agriculture (Burducea et al., 2019; Gómez–Bellot et al., 2020). However, excessive application of SS is related to the toxicity of crops due to the aggregation of heavy metals and salt content in the soil (Bettiol and Ghini, 2011). Therefore, it should be used judiciously with an amount able to increase plant growth without showing any phytotoxic symptoms.

Likewise, the growth stage and physiology of the host plant plays an important role in AMF propagation thus, picking up a suitable host as a nurse plant for AMF production is needful (Simpson and Draft, 1990). Therefore, in the present investigation four host plants i.e., Maize (*Zea mays* L.), Lemon grass (*Cymbopogon nardus* (L.) Rendle), Palmarosa grass (*Cymbopogon martinii* (Roxb.) Wats.) and *Sesbania*/dhaincha (*Sesbania aculeata* Poir.) were tested for propagation of *Glomus mosseae* and *Acaulospora laevis* under different concentrations of SS with the following objectives: a) identify the role of the substrate in enhancing host root colonization and AMF sporulation, b) verify if the addition of AMF to the host plant positively affects its growth c) to identify the most compatible host–substrate combination capable of maximum AMF spore production and root colonization for farm recommendation.

Material and Methods

Experimental design

This experiment was conducted in a greenhouse at Kurukshetra University, Kurukshetra, India. The experiment was a 4×5 factorial design with two factors A and B in five replications, with 100 pots in total. Factor A was plant species with four plants type (a1 = maize, a2 = lemon grass, a3 = palmarosa grass and a4 = sesbania), while factor B was five SS concentrations (b1 = no substrate, b2 = 25 g, b3 = 50 g, b4 = 75 g and b5 = 200 g pot^{-1}).

Soil characteristics

The soil characteristics were: sand = 64.2%, silt = 21.81%, clay = 3.90%, pH = 6.8±0, EC = 0.25 dS·m^{-1}, organic carbon = 0.40%, total N = 0.042%, P = 0.000247 kg·m^{-2}, K = 0.217 kg·m^{-2} and S = 0.01480 mg·g^{-1}.

Preparation of initial AMF inoculum

The rhizosphere soil of vegetable crops were examined for the isolation of healthy AMF spores using the technique of wet sieving and decanting protocol (Gerdemann and Nicolson, 1963) and later the manual of Schenck and Perez (1990) was used for their identification. AMF strains (*Glomus mosseae* and *Acaulospora laevis*) were found to dominate in the samples and hence selected. Identified spores were initially cultivated for a period of 60 days in association with the Maize plant by using the funnel technique (Menge and Timmer, 1982) with sterilized soil: sand (3:1) mixture. Ahead, plant roots were analyzed for mycorrhizal colonization by the protocol of Phillips and Hayman (1970) and rhizosphere soil was analysed for the number of AM spores. This starter inoculum of respective AMF fungi was then employed for their mass multiplication with different hosts and SS concentrations under pot conditions.

Selection and preparation of substrate

SS cakes were obtained from Sewage Treatment Plant, Yamunanagar. It was grounded to fine powder and analyzed as listed in Table 1.

Table 1. Physical and chemical characteristics of sewage sludge

1.	pH	5.57	±	0.11
2.	EC (dS m^{-1})	5.87	±	0.04
3.	Chlorides (mg g^{-1})	1.33	±	0.15
4.	Hardness (mg g^{-1})	9.50	±	0.20
5.	Alkalinity (mg g^{-1})	27.00	±	0.50
6.	Ortho phosphate (mg g^{-1})	1.81	±	0
8.	Nitrate (mg g^{-1})	0.24	±	0

Selection of the host plant

Four different host plants (Maize, Lemon grass, Palmarosa grass and *Sesbania*) were tested for each AMF strain. All these plants are monocots and members of Poaceae (Maize, Lemon grass, Palmarosa grass) except for *Sesbania* that is a Leguminosae.

Experimental setup

The top layer of soil up to 3 cm was collected from a botanical garden. It was then dried under the sun, grinded to remove lumps of soil, strained through a sieve (2 mm). Soil was mixed with sand in the ratio of 3:1 (w/w). Prior to use, it was sterilized in an autoclave (121^{0}C, 30 min) for two consecutive days. Earthen pots (25.4 × 25 cm) were filled with four different concentrations of SS (no substrate, 25 g, 50 g, 75 g and, 200 g pot^{-1}) and sand: soil, to make a total volume of 2 kg. The control pots did not receive SS. It was followed by the addition of 200 g of respective AMF initial inoculum (*Glomus mosseae* and *Acaulospora laevis*) that consist of chopped colonized Maize root segments and its rhizosphere soil (350–420 spores 100 g^{-1}).

Healthy seeds of Maize and *Sesbania*, were disinfected (0.5 % sodium hypochlorite for 10 min), and then rinsed with sterilized deionized water. Five to ten seeds were sowed above the added inoculum. After 15 days, Maize and *Sesbania* plants were lessened to three plants per pot. Likewise, Lemon and Palmarosa grasses were acquired from Chaudhary Devi Lal Herbal Garden, Chuharpur, Yamunanagar (Haryana), India. The overhead foliage part of each grass was pruned to the height of 15 cm and, roots were disinfected with sodium hypochlorite (0.5 %). In each pot, one stiff upright tiller with rhizomatous rootstock was planted. Pots were watered daily along with the addition of Hoagland's solution @ 100 ml pot^{-1} (Hoagland and Arnon, 1950) (except KH_2PO_4) after every 15 days interval.

Plant harvest and its analysis

After 90 days of growth, vegetative growth response was determined. Increase in plant height (cm), root length (cm), roots, and shoots fresh weight (g) was determined first. Shoot and roots were dried with a hot air oven at 70 °C to get a constant dry weight of shoot and root (g). The percentage of AMF root colonization and AMF spores were quantified using the previously discussed methods.

Statistical analysis

The experimental data were analyzed with an analysis of variance, and means were separated with the least significant difference (LSD) test using the Statistical Package for Social Sciences (SPSS) (ver. 11.5, Chicago (IL), USA).

Results

As per the data presented in Tables 2 and 3 for analysis of variance, the various values for host types, sewage sludge concentration as well as their interaction on inoculum production of *G. mosseae* and *A. laevis* and plant growth were found significant (P<0.05).

Table 2. Analysis of variance for effect of host plant, substrate concentration and their interaction on inoculum production of *G. mosseae*.

Source	DF	AMF spore number	AMF root colonization (%)	Plant height (cm)	Above ground weight (g)		Root length (cm)	Root weight (g)	
					Fresh	Dry		Fresh	Dry
H	3	29.475**	65.765**	240.977**	534.782**	143.009**	31.879**	545.554**	185.725**
S	4	517.971**	554.520**	95.290**	188.405**	59.169**	268.770**	118.122**	59.817**
H × S	12	71.855**	90.023**	17.586**	31.973**	32.516**	24.127**	30.430**	28.654**

H: host type, S: substrate concentration, ** = significant at *P*<0.05 for various values, DF = degree of freedom

Table 3. Analysis of variance for effect of host plant, substrate concentration and their interaction on inoculum production of *A. laevis*.

Source	DF	AMF spore number	AMF root colonization (%)	Plant height (cm)	Above ground weight (g)		Root length (cm)	Root weight (g)	
					Fresh	Dry		Fresh	Dry
H	3	44.918**	61.009**	96.360**	347.735**	510.973**	856.422**	73.116**	25.283**
S	4	530.668**	338.639**	125.814**	132.929**	74.440**	191.917**	41.586**	42.257**
H × S	12	32.072**	33.835**	45.045**	59.323**	18.069**	42.235**	22.914**	35.992**

H: host type, S: substrate concentration, ** = significant at *P*<0.05 for various values, DF = degree of freedom

Mycorrhization pattern of *G. mosseae*

In all the selected host plants, mycorrhization in terms of percent mycorrhizal root colonization and number of AMF spores were recorded. Moderate to abundant intraradical, extraradical mycelium, vesicles and arbuscules were detected that vary with change in the input level of SS.

Great variation was noticed in *G. mosseae* spore number and root colonization level that ranges from 22.22 to 290.6 (AMF spore number) and 16.44 to 100 % (AMF colonization) with the lowest numerical values recorded in control plants without any substrate (Table 4). An increase in the input level of SS caused a steady increase in mycorrhization in all the plants up to 75 g addition level only and, any further increase, decreased the mycorrhization. Among all the hosts tested, the effect of SS (75 g) was significant and in the presence of Lemon grass had the greatest AMF spore number and colonized roots which also showed abundant vesicles. The second greatest increment in AMF spores (222.2±16.3) and level of colonization (95.32±4.48) was obtained in Maize with 75 g, followed by *Sesbania* with 50 g and Palmarosa grass with 75 g respectively, and sporulation and root colonization were correlated.

Plant growth characteristics with *G. mosseae*

A perusal of the data presented in Table 5 showed that an increase in the colonization level of plant roots increased plant height, shoot fresh and dry weight, root length, root fresh and, dry weight. It was noticed that an increase in the addition level of SS in the potting mixture showed positive results in terms of increasing mycorrhization and plant growth as well. However, a maximum addition level of 200 g was inhibitory to all the plants except for Lemon grass, where 200 g SS amended plants exhibited the highest plant growth unlike to the highest mycorrhization shown by 75 g supplemented plants. However, the values recorded with 200 g were higher as compared to unamended control.

Table 4. Efficacy of sewage sludge on mycorrhization pattern of *G. mosseae* with different hosts.

Host type	Substrate concentration (g pot^{-1})	AMF spore number	AMF root colonization (%)	Pattern of colonization		
				Mycelium	Vesicles	Arbuscules
Maize	0	35.65±3.76j	21.11±4.87f	+	-	-
	25	144.2±12.64g†	56.57±7.05c	+	+	-
	50	181.6±12.14f	68.50±5.48b	+	+	+
	75	222.2±16.30c	95.32±4.48a	++	+	++
	200	193.8±17.94d	44.50±5.70d	+	-	+
Lemon grass	0	36.22±1.87j	26.87±4.11f	+	+	-
	25	101.4±15.14g	55.71±8.21c	+	+	+
	50	126.6±19.30g	74.50±5.70d	+	++	+
	75	290.6±19.24a	100.0±0	+++	+	++
	200	181.2±16.41f	88.50±5.48b	+++	+++	+++
Palmarosa grass	0	22.22±7.43j	16.44±3.21f	+	-	-
	25	78.20±11.88i	28.20±3.82e	+	+	+
	50	124.4±24.15fg	54.32±8.76c	+	+	+
	75	160.2±18.61cd	86.16±5.64a	+++	+	++
	200	130.6±15.45fg	46.92±4.13d	+	-	+
Sesbania	0	50.56±6.23j	33.35±5.05f	+	-	-
	25	128.2±19.34fg	53.62±8.24c	+	+	+
	50	202.6±19.82b	94.82±5.23ab	+++	+	++
	75	197.2±14.41e	45.40±4.56d	+	-	-
	200	89.60±13.05h	27.26±4.25e	+	-	-

Each value is a mean of five replicates, ±: standard deviation, AMF: Arbuscular mycorrhizal Fungi, -: absent, +: scanty, ++: moderate, +++: abundant

†indicates the level of significance at *P*≤0.05 level. Means followed by same letter/s within a column are not significantly different over one another (Least significant difference test, *P*≤0.05).

Table 5: Efficacy of sewage sludge and *G. mosseae* on growth response of Maize, Lemon grass, Palmarosa grass and *Sesbania*.

Host type	Substrate concentration (g pot^{-1})	Plant height (cm)	Above ground weight (g)		Root length (cm)	Root weight (g)	
			Fresh	Dry		Fresh	Dry
Maize	0	21.28±2.29†	3.53±0.48	1.13±0.12	10.05±0.87	1.71±0.16	1.16±0.11
	25	30.48±2.77	4.17±0.19	1.43±0.20	18.16±1.57	2.23±0.17	1.27±0.13
	50	39.40±3.08	7.69±0.59	3.05±0.27	26.00±1.78	3.85±0.20	2.18±0.12
	75	41.36±2.14	10.4±0.85	3.64±0.52	31.66±2.46	4.99±0.24	2.87±0.21
	200	33.13±2.03	5.50±0.58	2.16±0.17	21.90±1.72	3.23±0.16	1.85±0.35
Lemon grass	0	50.48±4.24	10.2±0.79	3.56±0.19	10.22±1.63	6.27±0.86	2.21±0.30
	25	63.94±10.8	19.3±2.24	5.03±0.47	14.20±1.58	7.73±0.55	3.34±0.35
	50	76.60±8.11	25.9±1.71	5.19±0.34	22.20±2.18	9.17±0.76	3.91±0.17
	75	77.34±11.6	28.5±2.73	5.05±0.42	26.14±1.52	8.70±0.78	3.45±0.39
	200	88.70±7.10	32.7±2.72	6.97±0.26	30.22±1.55	12.3±1.58	4.79±0.42
Palmarosa grass	0	20.54±2.99	6.86±0.50	2.17±0.30	12.62±2.20	1.22±0.16	0.86±0.13
	25	38.42±2.47	10.3±1.16	4.24±0.49	17.82±2.10	2.85±0.35	1.14±0.10
	50	50.52±3.20	16.8±1.70	5.06±0.65	14.88±1.90	4.33±0.21	2.25±0.15
	75	56.48±2.86	23.1±2.22	6.92±0.55	25.22±3.23	6.69±0.56	3.04±0.33
	200	43.14±2.81	15.5±1.89	4.10±0.48	20.20±2.57	3.03±0.51	1.27±0.22
Sesbania	0	60.34±5.91	7.71±0.87	3.34±0.79	13.54±1.57	3.06±0.44	2.07±0.22
	25	80.40±5.10	12.2±1.25	5.57±0.83	25.98±2.43	3.58±0.20	2.32±0.31
	50	90.42±5.15	15.5±1.59	7.45±1.05	32.90±3.11	5.90±0.18	2.95±0.23
	75	68.56±3.70	11.5±1.58	5.60±0.71	25.55±3.67	3.96±0.47	2.50±0.63
	200	52.78±4.85	6.57±1.53	3.15±0.48	14.74±1.97	2.78±0.53	1.44±0.23

Each value is a mean of five replicates, ±: standard deviation

†indicates the level of significance at *P*≤0.05 level. Means followed by same letter/s within a column are not significantly different over one another (Least significant difference test, *P*≤0.05).

Mycorrhization pattern of *A. laevis*

While for the multiplication of *A. laevis*, a similar trend was depicted regarding the influence of substrate addition on mycorrhization (Table 6). According to the results in Table 6, the 75 g SS amendment obtained a significant AMF multiplication. A further inoculum addition had an inhibitory effect on the AMF. Meanwhile, 100 % colonized roots and maximum spores were obtained with 75 g SS on Maize, followed by Lemon grass, Sesbania, and Palmarosa. Results also revealed the presence of an extensive and abundant mycelium colonizing Maize, Lemon grass, and Sesbania roots, while abundant vesicles and arbuscules were formed in Maize and Sesbania roots only. Vesicle and arbuscule formation were not detected in some of the treatments having an excess of SS after 90 days of the experiment.

Table 6: Efficacy of sewage sludge on mycorrhization pattern of *A. laevis*.

Host type	Substrate concentration (g pot^{-1})	AMF spore number	AMF root colonization (%)	Pattern of colonization		
				Mycelium		
Maize	0	38.55±4.07k	25.87±3.02h	+	+	+
	25	173.6±14.11d†	44.50±5.70e	+	+	+
	50	164.0±37.14f	55.96±9.81d	+	++	+
	75	221.8±15.71a	100.0±0a	+++	+++	+++
	200	80.60±10.38i	32.86±4.86fg	+	+	-
Lemon grass	0	20.87±3.02k	16.40±2.77h	+	-	-
	25	130.0±15.93gh	54.35±4.32d	+	+	+
	50	179.0±19.49cd	78.82±7.96b	++	+	+
	75	208.6±15.61ab	84.00±8.94c	++	++	+
	200	65.00±10.91	36.26±7.36f	+	-	+
Palmarosa grass	0	23.33±2.66k	10.34±2.11h	+	-	-
	25	57.20±8.22j	26.16±5.44g	+	+	-
	50	112.8±15.05h	57.97±5.61d	+	+	+
	75	181.4±13.22c	71.00±4.18c	++	++	+
	200	169.0±10.58e	76.16±8.68bc	++	+	++
Sesbania	0	67.87±2.55k	33.67±3.45h	+	+	+
	25	150.4±11.97g	46.79±7.28e	+	+	+
	50	174.2±10.96d	86.45±8.19b	++	+	++
	75	204.2±16.16ab	87.32±3.67ab	++	+	++
	200	167.4±11.65ef	78.50±5.48c	++	+++	+++

Each value is a mean of five replicates, ±: standard deviation, AMF: Arbuscular mycorrhizal Fungi, -: absent, +: scanty, ++: moderate, +++: abundant

†indicates the level of significance at $P \leq 0.05$ level. Means followed by same letter/s within a column are not significantly different over one another (Least significant difference test, $P \leq 0.05$).

Plant growth characteristics with *A. laevis*

Likewise, with *A. laevis*, the results for plant growth characteristics were also in line with mycorrhization level (Table 7). Treatments showing highest mycorrhization also showed the highest plant growth parameters except for Palmarosa grass, in which instead of maximum mycorrhization shown by plants under 75 g SS addition, a maximum addition level of SS (200 g) showed the highest plant growth characteristics.

Overall, the results showed maximum benefits with 75 g of SS addition in Lemon grass for *G. mosseae* inoculum production while for *A. laevis*, Maize, and Lemon grass with 75 g SS promoted maximum increment in *A. laevis* propagules with the highest percent of colonization rate. Moreover, other hosts also exhibited a tremendous increase in inoculum intensity with different concentrations of substrate.

Discussion

Production of AMF inocula is as complex in development as tedious to propagate owing to the involvement of biotechnological expertise. In addition to it, consideration of related legal, ethical, educational and commercial requirements are also in line while working on it (Gianinnazi and Vosátka, 2004). In this investigation all the host plants were highly selective for the AMF multiplication. The tested host plants were either members of Poaceae or Leguminosae and formed effective AMF symbiosis which is ascribed to the high density of secondary roots supporting colonization of root and spore propagation as well (Bhowmik et al., 2015). Among all the tested host plants Lemon grass had the highest inoculum production level of *G. mosseae*. In comparison, Maize manifests maximum compatibility with *A. laevis* followed by Lemon grass. Vesicles and arbuscules were abundantly formed in all these plant roots showing the formation of efficient symbiosis between both the partners. Host mycorrhizal dependency is genetically fixed, and the level of mycorrhizal dependency can be expressed up to the extent of an individual, or as a gradient within an ecological niche and relevant environment context of the host (Feldmann et al., 2008). The suitability of some host plant species to stimulate AMF propagation could be due to the secretion of a wide variety of water-soluble and volatile organic compounds, endogenous hormone level characteristics helping as a stimulant, attractants, nutrient sources and even as genetic regulatory signals for AMF colonization (Koide and Schreiner, 1992). Research by Ryan and Graham (2002) and Liu and Wang (2003) support that a variation in root type, its anatomy and morphology, carbon biomass, and environmental interactions also influence AMF symbiosis.

Table 7: Efficacy of sewage sludge and *A. laevis* on growth response of Maize, Lemon grass, Palmarosa grass and *Sesbania*.

Host type	Substrate concentration (g pot^{-1})	Plant height (cm)	Above ground weight (g)		Root length (cm)	Root weight (g)	
			Fresh	Dry		Fresh	Dry
Maize	0	29.48±6.31†	2.54±0.51	0.95±0.14	12.7±1.89	2.15±0.23	0.85±0.21
	25	35.86±3.12	5.63±0.70	2.41±0.54	16.6±1.87	2.87±0.46	1.25±0.31
	50	40.78±3.81	8.34±0.77	3.49±0.41	21.0±2.54	4.69±0.91	1.92±0.24
	75	44.08±3.86	9.62±0.92	3.56±0.39	26.7±3.10	5.36±0.61	2.22±0.22
	200	30.40±3.67	2.93±0.56	1.22±0.22	18.4±1.60	3.04±0.91	1.14±0.15
Lemon grass	0	42.22±6.03	8.38±0.73	2.65±0.44	6.44±1.13	3.77±0.42	1.18±0.16
	25	54.72±5.08	23.6±3.94	3.59±0.46	12.7±1.51	5.28±0.45	2.44±0.46
	50	57.66±4.54	12.7±0.81	4.25±0.70	17.4±1.28	7.53±1.03	3.54±0.45
	75	65.98±4.24	23.0±2.17	5.28±0.64	10.8±1.00	5.54±0.60	2.64±0.48
	200	34.52±2.88	8.07±0.78	2.65±0.70	5.68±0.84	3.43±0.43	0.94±0.16
Palmarosa grass	0	17.48±1.63	7.44±0.34	2.56±0.49	36.8±2.87	2.98±0.22	0.87±0.17
	25	28.64±2.65	12.4±1.05	4.38±0.41	48.1±2.96	3.71±0.51	1.44±0.29
	50	36.32±2.50	11.4±2.00	4.86±0.36	45.7±2.96	4.35±0.52	1.37±0.43
	75	48.88±2.02	17.4±1.70	5.65±0.38	55.1±3.07	5.11±0.63	2.49±0.39
	200	56.26±2.42	22.8±1.02	5.99±0.28	62.9±3.19	5.59±0.58	2.89±0.39
Sesbania	0	22.40±2.83	2.75±0.33	0.97±0.22	4.68±0.89	2.30±0.42	0.99±0.23
	25	25.12±1.99	2.28±0.23	1.15±0.19	7.12±0.91	1.98±0.31	1.18±0.20
	50	21.86±2.70	3.89±0.22	1.42±0.34	8.06±1.09	2.54±0.36	1.03±0.21
	75	27.74±2.38	4.52±0.41	1.73±0.21	10.1±1.23	3.06±0.24	1.27±0.25
	200	36.40±2.40	4.99±0.40	2.20±0.43	13.4±1.58	4.00±0.41	1.63±0.25

Each value is a mean of five replicates, ±: standard deviation

†indicates the level of significance at $P \leq 0.05$ level. Means followed by same letter/s within a column are not significantly different over one another (Least significant difference test, $P \leq 0.05$).

Excessive *A. laevis* multiplication in association with Maize roots corroborate to the extensive and fast-growing root system of Maize, able to tolerate and adapt to the changing environmental conditions due to the addition of substrate (Mukerji et al., 2002). This result confirms the findings of Tahat et al. (2012), who also observed maximum spore number and colonized roots with Maize compared to Sorghum, Barley and Lentil. However, the results of this research are in contradiction with the findings of some other researchers including Al–Raddad (1995), Selvakumar et al. (2016), who reported the inability of Maize plant for AMF multiplication. Meanwhile, Lemon grass also showed tremendous increase in mycorrhizal inoculum after Maize which supports the results of Kaushish et al. (2011).

On comparing the inoculum production efficiency of all the hosts, Palmarosa showed a bit lesser tendency when utilized for the propagation of *A. laevis* which corroborates with our earlier results with sugarcane bagasse (Tanwar et al., 2013). This probably could be due to the short duration of the experiment which might be responsible for the low level of colonization observed in the Palmarosa plant. One more reason could be the poor production and limited secretion of photoassimilates to the AMF symbiont. Even though, efficient inoculum quantity was achieved by Palmarosa is sufficient to be used for multiplication purposes. *Sesbania* is a member of Leguminosae which are also believed to be a perfect host for AMF multiplication

(Gill and Singh, 2001). According to Kaushik et al. (2011), they also tried *Sesbania* for the multiplication of AMF and recorded maximum spore count with vermicompost substrate. In the present investigation, also *Sesbania* harbored excess spores efficiently colonizing its roots to be utilized for AM multiplication purposes.

The interaction between the host plant, AMF species, and type of growth substrate can be stimulatory or inhibitory to the AMF and host and amongst all, type and the concentration of substrate used is foremost for AMF multiplication. As per Moreira et al. (2019), regardless of the inoculum source, the percentage of viable spores exists greater at the layer of 0.00–0.05 m (76.32 %) compared to the layer of 0.05–0.10 m (72.05 %). Soil amendment up to this range of depth could be productive. This is more likely related to increased available nutrients, porosity and better water holding capacity of the added substrate. For AMF proliferation, a well-aerated substrate deficient in nutrient is considered to be good (Gaur and Adholeya, 2000). The addition of organic residues as a substrate along with soil is known to promote AMF sporulation hence leading to increased inoculum production (Rodrigues and Rodrigues, 2017). Likewise, the addition of SS helped to maintain appropriate soil moisture content, aeration, and space which in turn provide feasible environment for the germination and sporulation of AMF. A perusal of the data showed that the soil amendment with SS had a significant influence on root colonization by AMF, its sporulation and their combined effect on plant development. The addition of SS changes the soil chemical and physical properties which became more conducive for the sporulation of AMF. The soil pH at the time of this experiment was 6.8. Angle and Heckman (1986) stated that the addition of SS at a soil pH of 6.2 exhibited a higher degree of mycorrhizal colonization of soybean roots as compared to the addition of the same sludge at a lower pH 5.7. However, high input of SS proved detrimental for AMF survival which strongly supports the findings of del Val et al. (1999) that this concentration interferes with the growth and proliferation of mycelium and limits the formation of arbuscules.

It is envisaged from the results that the mycorrhizal spore number increased more for *G. mosseae* as compared to that of *A. laevis*. This corresponds to a change in the soil pH to slightly acidic by the added sludge (Ansari and Jaikishun, 2011), making conditions more conducive for *Glomus* sp. However, reduction of pH below 5.0 has been reported to be inhibitory to the *Glomus* survival (Carrenho et al., 2001). Apart from increasing inoculum production of AMF, the effect of the substrate on plant growth is another important issue. After the formation of perfect symbiosis through root colonization and the formation of arbuscules and vesicles, AMF started benefiting the host plant through increased nutrient supply, especially P, as observed in this study. It is believed that the addition of SS increased organic matter and nutrient fertility of the soil especially N, P, K, Ca, Mg, B, Mn, Cu, Mo and Zn, depending upon the specific nature of the sludge material (Dolgen et al., 2004; Haghighi, 2011).

In the present investigation, overall growth inhibition was observed at higher SS concentrations including plant height, plant dry matter except for Lemon grass and *Sesbania* with both the AMF. Excessive addition of SS is associated with root growth inhibition which is perhaps the most important indicator of SS toxicity (Oleszczuk et al., 2012). Micronutrients such as Zn, Cu and Mn can be toxic to AMF at higher concentrations. However, low concentration of these nutrients can increase the percentage of root colonization, extrametrical chlamydospore number, and the number of infective propagules (Sreenivasa and Bagyaraj, 1988). Besides this, increased plant growth on the addition of SS has also been documented by numerous researchers including Wei and Liu (2005), Aslantas et al. (2010). Moreover, Amir et al. (2019) documented seven times higher increased in the dry weight of *Metrosideros laurifolia* seedlings than the control plants while using AMF and SS together, which are characterized by improved mineral nutrition, a higher Ca/ Mg ratio, and reduced translocation of heavy metal as well and thereby improved ecological re-establishment of the studied area(ultramafic mine–degraded area). Consequently, soil supplementation with municipal SS can be employed as a source of fertilization for growing sweet basil, a high value crop, by directly affecting its plant growth and physiology (Burducea et al., 2019). Singh and Agrawal (2010) also recommended SS amendment in the soil at 6 kg m^{-2} and below for promoting mung bean yield and nutritional quality. Contrarily higher SS application leads to heavy metal accumulation of Cd, Pb and Ni in the seeds, which is a major cause of concern to human wellness. In this investigation also excessive addition of sludge showed an inhibitory effect and hence should be avoided. Our results are in accordance with those found by Sullivan et al. (2006), Tariq et al. (2012).

Conclusion

AMF inoculum production method employing trap host roots under pot conditions with carrier substrate is extensively used, since it is less artificial, highly economical and competent in producing highly–efficient inoculants of colonized plant roots on large (Schlemper and Stürmer, 2014). Excessive application of SS presents limitations in the AMF survival, sporulation, and reduced ability to colonize host roots and host growth as well. Nonetheless, SS should be viewed as a resource in lieu of a waste product considering its valuable properties and a broad spectrum of potential uses (Kicińska et al., 2019). All the studied plants behaved as highly mycorrhizal plants when propagated in association with appropriate doses of SS. One can utilize these plants for AMF (*G. mosseae* and *A. laevis*) multiplication. It is foremost to have an inoculum with the potential to colonize host roots, proliferate in the substrate and also supplement plant growth. Consequently, this inoculum also should be previously tested with host crops either in pots or under field conditions. Apart from this, one more uphill battle for mycorrhizologist is to grow awareness among consumers about the potential benefits of AMF technology for sustainable plant production.

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