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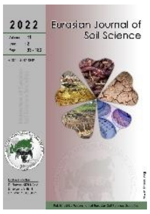

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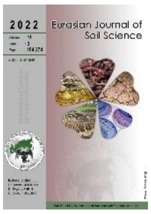

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CONTENTS

Eurasian Journal of Soil Science 2022, 11(3), 184 - 197

Eurasian Journal of Soil Science

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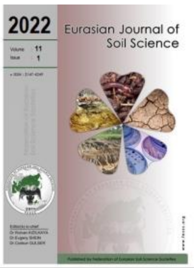

Biogeoaccumulation of zinc in hybrid rice (*Oryza sativa* L.) in an Inceptisol amended with soil zinc application and its bioavailability to human being

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Abstract

Soil Zn amended is an efficient agronomical Zn biofortification approach in rice. However, it is still need to know if higher rate of Zn over recommended dose can influence other essential nutrient uptake, high accumulation of Zn in soils and health risk for human consumption. This study was conducted by taking ten treatments (T_1: control, T_2: RDF, T_3: RDF + 1.25 mg kg^{-1}, T_4: RDF + 2.5 mg kg^{-1}, T_5: RDF + 3.75 mg kg^{-1}, T_6: RDF + 5 mg kg^{-1}, T_7: RDF + 6.25 mg kg^{-1}, T_8: RDF + 7.5 mg kg^{-1}, T_9: RDF + 8.75 mg kg^{-1}, T_{10}: RDF + 10 mg kg^{-1}) on hybrid rice in Zn (1.20 mg kg^{-1}) enriched soil. The findings have shown that 6.25 mg kg^{-1} Zn application significantly increased crop growth and grain concentrations of N, K, Zn, Cu and Fe by 71.4, 125, 78.9, 28.5 and 2.4%, respectively. Nutrient harvest index was significantly affected by ranged between 29.1–36.4%. Application of Zn at 6.25 mg kg^{-1} (T_7) recorded the highest Zn concentration in grain (28.2 mg kg^{-1}) and bioavailability of the fortified Zn (2.05 mg Zn day^{-1}). The lowest phytatic acid concentration in grain was recorded in T_8 (RDF + Zn at 7.5 mg kg^{-1}) and after that a significant increase was observed. Transfer coefficient was inversely behaving with Zn application and ranged between 6.03–18.0 grain. The average daily intake of Zn was ranged between 0.075–0.118 mg^{-1} kg^{-1} day. Across different treatments the Zn build-up factor, geo-accumulation index and soil enrichment factor was ranged between 0.98–4.90, -0.61–1.70 and 0.24–1.82, respectively in post-harvest soil. In conclusion, agronomic biofortification of Zn through soil applications at 6.25 mg Zn kg^{-1} was a sustainable way to improving growth and grain Zn, N, K, Cu and Fe uptake of hybrid rice to meet human recruitment.

Keywords: Nutrient harvest index, rice, zinc, zinc balance sheet, Zn build-up factor.

Introduction

The most frequent nutritional problems, particularly in developing nations, are zinc (Zn), vitamin A, and iron (Fe) deficiencies (Welch and Graham, 2005). It was estimated that 17.3% of people of worldwide, and 500,000 children die due to nutritional deficiency of Zn (Wessells and Brown, 2012). Mostly pregnant

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woman and children are affected by Zn deficiency (Jatav et al., 2019). In human, Zn plays vital role in many important catalytic, structural, and regulatory functions like enzymes catalytic activity that are involved in DNA replication, cell division, energy metabolism, growth, structure stability of protein, regulation of antioxidants, leptin and insulin signaling. Moreover, Zn deficiency in human causes malfunctioning of the immunity system, male hypogonadism, appetite loss, skin lesions, poor wound healing, diarrhea, delayed sexual maturity, slower growth rate (Prasad, 2013). In plants, Zn plays a key role in the proper functioning of different biochemical pathways by activating a broad range of enzymes and proteins. These are primarily concerned with carbohydrate, auxin and amino acid metabolism, reactive oxygen species (ROS) detoxification, pollen formation, the integrity of cellular membranes, and disease resistance (Hafeez et al., 2013). However, in a deficient Zn situation, crop yields may be decreased by 20% without any noticeable symptoms (Cakmak, 2000; Broadley et al., 2007).

Zinc bioavailability in soil affects plant uptake of Zn and also human and animal nutrition of Zn. The deficiency of Zn affects almost one third of the global population (Hotz and Brown, 2004) and is the fifth leading cause of human mortality in developing countries (Cakmak and Kutman, 2018). In early life stages, Zn deficiency may influence embryogenesis, hypogonadism, increased vulnerability to serious diseases, and decreased mental development. There is no Zn reservoir in the human body, therefore, the bioavailability of Zn through food or supplements must be regularly provided in order to avoid its deficiency. Nutrient fortification is a preferred way of addressing the problem of the undernourished rural population. However, majority of rural populations are unable to obtain a variety of diets, supplements and commercially fortified foods. Bio-fortification is a method of enhancing the bio-availability of vital elements in edible crop portions through agronomic action or genetic selection (White and Broadly, 2011).

Wheat and rice are major staple food crops of India which constitute about 60–70% of daily calorie uptake. The rice grain is very low in Zn and contains anti-nutrition compounds like phytates which reduced bioavaility of Zn (Kumar et al., 2017). Cereals normally comprise low Zn *i.e.,* 15–30 mg Zn kg^{-1} compared to a sufficient 40–60 mg Zn kg^{-1} concentration for better nutrition but, preferred over others being staple food of a large population. Zinc bio-fortification of rice could save lives in most populous countries like India and China between 1.6–2.3 million DALYs (disability-adjusted life year) and 0.4–1.5 million DALYs (De Steur et al., 2012) per year, respectively. Genetic bio-fortification of food crops faces several challenges and takes many years to get beneficial effects. Agronomic bio-fortification is, therefore, a feasible approach for developing nations, based on micronutrient-dense cultivar exploitation (Sharma et al., 2017). Zinc should be applied as seed coating, soil application or, foliar spray at higher quantity for fortification of crop with Zn and for better translocation of Zn to grain from soil (Singh and Prasad, 2014). Das et al. (2018) noted that Zn application will not only correct crop Zn deficiency and enhance crop yield and productivity, but also help to increase Zn concentration and reduce anti-nutrient (phytate) concentration in grain.

Rice is the second largest staple food in the world and cultivated in more than 100 countries. India is the world's largest producer and fourth largest exporter of rice (FAO, 2017). Generally, after polishing white rice contain low quantities of Zn (11–16 µg g^{-1}), thus consumption of rice fails to satisfy the estimated average requirement (EAR) of Zn in human being (Mayer et al., 2011). It was reported that increase of 12 µg Zn g^{-1} of milled rice could provide at least 25% of the Zn EAR for pre-school children (Alloway, 2009). Zn nutritional allowance for babies is 3–5 mg day^{-1}, while for kids 1–10 years of age it is 10 mg day-1, for males 12 mg day^{-1} and for lactating females 16–19 mg day^{-1} (Alloway, 2009). It is well documented that agronomic bio-fortification of cereals is one of feasible way to eradicate this Zn malnutrition problem (Cakmak 2008; Cakmak and Kutman, 2018). Zn fertilizer application in soil increases the Zn build-up in soil and food grain but causes other nutrient imbalances due to antagonistic interaction among the micronutrient cations (Jiao et al. 2012).

Excessive Zn buildup in soil promotes crop uptake, resulting in an unexpectedly high Zn concentration in grain (Noulas et al., 2018; Wongsasuluk et al., 2018). In contrast to higher Zn concentration in soil can affect crop absorption of other micronutrients. (Kolašinac et al., 2018). Our hypothesis is that different levels of Zn fertilizer application, especially at higher rate than soil test value in hybrid rice would increase the uptake of Zn by plant, and thus, represents a better Zn nutritional security. The precise objectives of this study were: (i) to evaluate the different levels of Zn soil application on uptake of major and micronutrient in rice, (ii) to assess the Zn balance sheet, Zn accumulation indices in soil-plant system and average daily intake of Zn through consumption of rice grain that was came from different level of Zn applications. As a result, this study will lead to a better understanding of Zn bio-fortification in paddy in relation to various zinc-soil-plant indices.

Material and Methods

Study area

A pot experiment was conducted during the *kharif* (July – November) seasons of 2018–19 at Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India (25^{0}26’N, 82^{0}99’ E, 80.7 m above sea level). The climate is semi-arid to sub-humid, hot summers and simply pleasant cold winters. The mean annual rainfall is 1100 mm. The meteorological observation recorded during rice growth period 2018–19 (*kharif* season) was presented in Figure 1. The experimental soil was sandy clay loam in texture with 58.3% sand, 25.7% silt and 16.0% clay. The soil had pH 7.71, EC 0.13 dS m^{-1}, soil organic carbon (SOC) 8.01 g kg^{-1} and DTPA-extractable Zn 1.20 mg kg^{-1} (Table 1).

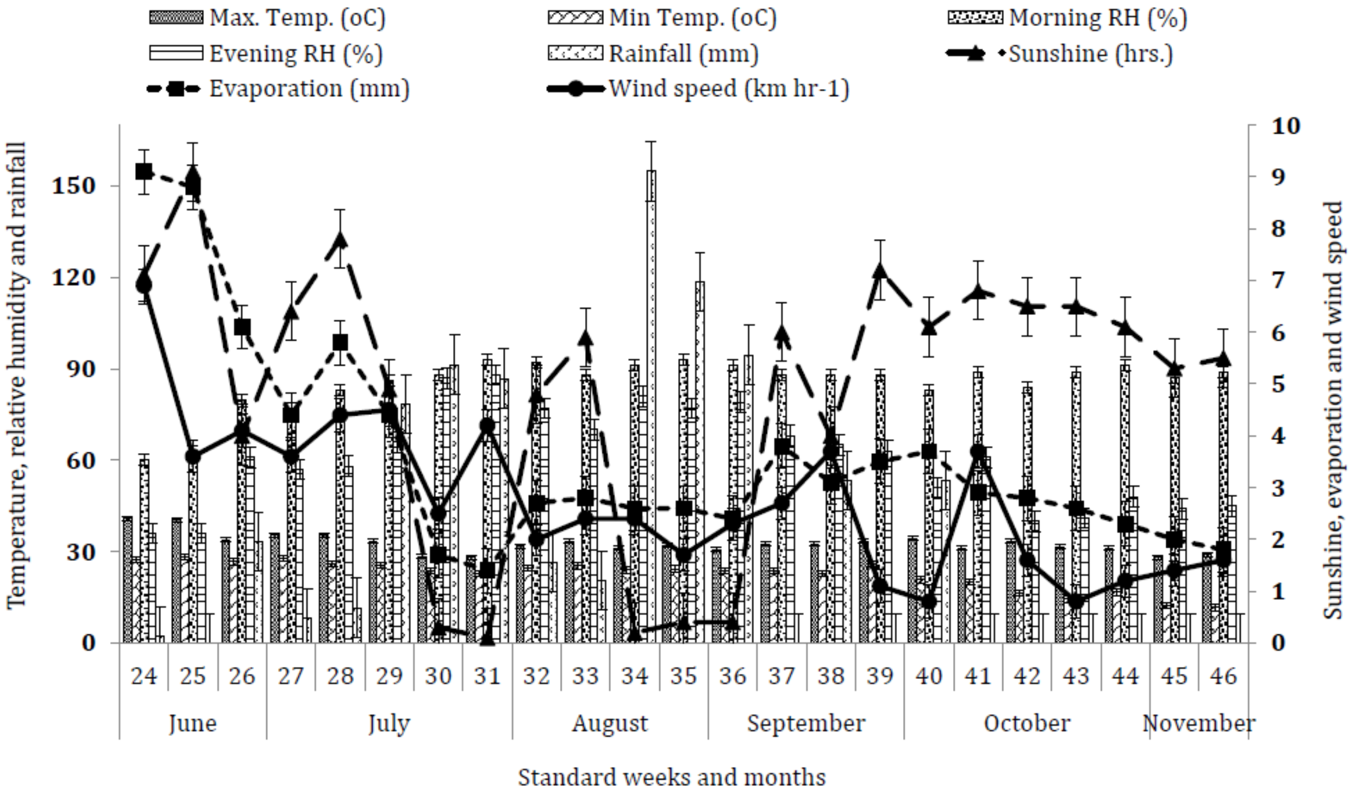

Figure 1. Meteorological data during rice growing period (June to November, 2018)
Error bars identifies standard error of mean of different treatments

Experimental setup

The completely randomized experimental design (CRD) employed with ten treatments *i.e.,* T_1: control (no fertilizer), T_2: recommended dose of fertilizer (RDF), T_3: RDF + Zn @ 1.25 mg kg^{-1}, T_4: RDF + Zn @ 2.5 mg kg^{-1}, T_5: RDF + Zn @ 3.75 mg kg^{-1}, T_6: RDF + Zn @ 5 mg kg^{-1}, T_7: RDF + Zn @ 6.25 mg kg^{-1}, T_8: RDF + Zn @ 7.5 mg kg^{-1}, T_9: RDF + Zn @ 8.75 mg kg^{-1}, T_{10}: RDF + Zn @ 10 mg kg^{-1} with three replications and Arize® H 6444 (hybrid rice) was taken as test crop. Standard practices were followed for the cultivation of rice and it was harvested in the second half of October in both study years. State (Utter Pradesh) recommended dose of fertilizer (N, P and K) for hybrid rice was 150, 60 and 60 kg ha^{-1}, respectively. Urea, di-ammonium phosphate and muriate of potash was the source of RDF and 50% N and 100% of P and K was applied in solution form before transplanting of rice and remaining 50% of N was applied in two equal splits at 30 and 60 days after transplanting. Zinc fertilization was done as soil application through zinc sulphate ($ZnSO_4.7H_2O$) in solution form.

Table 1. Initial properties of experimental soil

Parameters	Values
pH (1:2.5; soil:water)	7.71
EC (dS m^{-1})	0.13
Organic carbon (g kg^{-1})	8.01
N (mg kg^{-1})	138
P (mg kg^{-1})	11.0
K (mg kg^{-1})	89.5
S (mg kg^{-1})	14.6
Zn (mg kg^{-1})	1.20
Fe (mg kg^{-1})	45.2
Mn (mg kg^{-1})	13.2
Cu (mg kg^{-1})	5.20
B (mg kg^{-1})	0.29
Sand (%)	58.3
Silt (%)	25.7
Clay (%)	16.0
Texture	Sandy clay loam

Plant and soil analysis

Different growth attributing characteristics like plant height, number of tillers, greenness index (SPAD value) were recorded at 30, 60 and 90 days after transplanting. The yield attributing characters were measured at maturity *viz.,* average number of panicles per pot, number of grains per panicle and panicle length. Rice was harvested at 120 days after transplanting. Then plant samples were washed in 0.2% liquid detergent solution followed by 0.1 N hydrochloric acid (HCl) solution and de-ionized water. The plant samples were kept in hot air oven at 70 °C till the constant weight was gain. The grain yield, straw yield and 1000 grain weight per pot was recorded. Initial and post-harvest soil samples were analyzed for mechanical analysis of soil by using international pipet method (Piper, 1966), pH and electrical conductivity (EC) by (Sparks, 1996), organic carbon by method of Walkley and Black (1934), DTPA-extractable Zn, Cu, Mn and Fe (Lindsay and Norwell, 1978) by AAS (Agilent FS 240, 2019). The plant samples (grain and straw) were wet digested in a di-acid mixture and analyzed for P, K, Fe, Cu, Mn and Zn using AAS (Agilent FS 240, 2019) by Tandon (2001).

Nutrient uptake

$$\text{Nutrient uptake (mg pot}^{-1}\text{)} = \frac{N_C \times Y}{1000}$$

Where, Nc is nutrient content (mg kg^{-1}) and Y is biomass (g pot^{-1}).

Nutrient harvest index

Uptake of a particular nutrient is the product of the grain yield and concentration of respective nutrient in grain. Total nutrient uptake is the sum of nutrient uptake of grain and straw.

Nutrient harvest index (NHI) of different nutrients was calculated using Das et al. (2010) equation:

$$\text{NHI (\%)} = \frac{\text{Uptake of particular nutrient by grain kg/ha}}{\text{Total uptake of that nutrient in biomass kg/ha}}$$

The transfer coefficient (TC), the enrichment factor (EF), and the geo-accumulation index (Igeo) were calculated in order to assess the degree of Zn enrichment in soil and the plant's ability to accumulate Zn from soils and translocate it from roots to grain.

Estimation of phytate concentration and bioavailability of Zn

The technique outlined by Dai et al. (2007) was used to determined phytic acid from the rice sample. Miller et al. (2007) provided a trivariate model of Zn absorption for quantitative assessment of Zn bioavailability.

$$\text{TAZ} = 0.5 \times \left\{ \left(A_{MAX} + \text{TDZ} + \left(1 + \frac{\text{TDP}}{K_P}\right) \right) - \sqrt{\left(\text{AMAX} + \text{TDZ} + \left(1 + \frac{\text{TDP}}{K_P}\right) \right)^2 - (4A_{MAX} \times \text{TDZ})} \right\}$$

Where, Maximum absorption (A_{MAX}) = 0.091, Equilibrium dissociation constant of Zn–receptor binding reaction (K_R) = 0.680 and Equilibrium dissociation constant of Zn–phytate binding reaction (K_P) =0.033, respectively. These values were calculated based on Zn homeostasis in human intestine. The total daily-absorbed Zn (TAZ) (mg Zn day^{-1}) is a function of total daily dietary phytate (TDP) (mmol phytate day^{-1}) and total daily dietary Zn (TDZ) (mmol Zn day^{-1}). Here, TAZ value was calculated based on an adult human's average daily intake of 300 g of rice grain.

Transfer coefficient (TC)

The transfer coefficient (TC) of Zn is the ratio of the Zn concentration in the plant with respect to Zn concentration in soil (mg kg^{-1}) (Adamczyk-Szabela et al., 2017). It explains the ability of the plant to accumulate Zn with respect to its soil concentration.

The plant enrichment factor (PEF)

Plant enrichment factor (PEF) is used to evaluate the levels of Zn concentration and accumulation in plants growing on Zn treated soil to plants growing on control soil (Kisku et al., 2000). It's the ratio of Zn concentration in Zn-treated soil/plant to Zn concentration in control condition. PEF values larger than 1 indicate increased Zn availability and distribution in Zn treated soil is in higher compared to their reference values (control).

Zinc balance sheet

Balance sheet of Zn in soil was determined by using the formula (Bera and Ghosh, 2013).

$$B_{Zn} = Y_{Zn} - (X-A) - Zn$$

Where B = Balance sheet of nutrient, Y_{Zn} = Zn Uptake by crop, X= Zn concentration in Initial the soil, A = Zn concentration in post-harvest soil, Zn = Zn added through fertilizer.

Average daily intake

The average daily intake (ADI) of Zn was calculated using standard formula (Khan et al., 2008).

$$\mathrm{ADI} = \frac{C_{Zn} \times \mathrm{Dfood\ intake}}{\mathrm{average\ BW}}$$

where C_{Zn} is the Zn concentration in polish white rice, Dfood intake is the average daily rice intake (0.300 kg person^{-1} day^{-1}) and BWaverage weight represent body weight of the individuals (70 kg for adults) (Khan et al., 2010; GFBIC, 2014; Doabi et al., 2018).

Zinc build up factor

Zinc build up factor is the ratio obtained by dividing the concentration of Zn in soil by their background values (control):

$\mathrm{BF} = CS/C\mathrm{RefS}$

where CS is Zn concentrations in Zn treated soil and CRefS is background Zn concentration in control soil (Shaheen et al., 2017).

The index of geo-accumulation (Igeo)

The index of geo-accumulation (Igeo) which also assesses the Zn potential in soil is calculated as

$$\mathrm{Igeo} = \log2(C_{Zn}/\ 1.5\mathrm{Bn})$$

where C_{Zn} is Zn concentration in the Zn fertilizer treated soil and Bn is the geochemical background concentration of the Zn (Antoniadis et al., 2017).

Soil Enrichment factor

$$SEF = (Zn\mathrm{S}/\ \mathrm{FeS})\ /\ (Zn\mathrm{RefS}\ /\mathrm{FeRefS})$$

Where *Zn*S is the Zn concertation in Zn treated soil, whereas FeS is the Fe concentration on their respective treatment. Similarly, ZnrefS and FeRefS is the Zn and Fe concentration in control pot.

The level of enrichment of metal is then classified as EF < 2 (marked as “deficient enrichment”), EF=2–5 (“moderate enrichment”), EF=5–20 (“significant enrichment”), EF=20–40 (“very high enrichment”), and EF > 40 (“extremely high enrichment”) (Liu et al., 2005).

Statistical analysis

Using SPSS version 16.0 software, the data were statistically analyzed using one-way analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was used to see if the difference between the treatments was significant at $p \leq 0.05$.

Results and Discussion

Uptake of macro- and micro-nutrients

Based on the result of different level of Zn fertilization had a significant effect on uptake of macronutrient (Table 2). The uptake of N, P and K by hybrid rice grain ranged from 0.18–0.60, 0.04–0.11 and 0.04–0.10 g pot^{-1}, respectively. While macronutrient (N, P and K) uptake in straw varied from 0.11 to 0.28, 0.06–0.09 and 0.37 to 0.99 g pot^{-1} respectively. Significantly higher total nitrogen (N) uptake was recorded in 6.25 mg Zn kg^{-1} (T_7) (67.9 % increase over RDF). Similarly, higher total K uptake was recorded at 6.25 mg Zn kg^{-1} (T_7) (61.2 % over RDF), While higher total P uptake by hybrid rice was recorded at 2.5 mg Zn kg^{-1} T_4 (5% over RDF). All Zn treatments, 6.25 mg Zn kg^{-1} (T_7) were recorded most efficient in total macronutrient uptake by hybrid rice. The Zn uptake by grain, straw and total uptake varied between 0.34–1.02, 0.83–2.05 and 1.18–3.06 mg pot^{-1}, respectively (Table 3). The treatment received T_5, T_9, and T_{10} were statistically at per with each other. The maximum total Zn uptake was recorded in T_7, which 58.5% higher over RDF.

Table 2. Effect of zinc application on macronutrients uptake (g pot^{-1}) in various plant parts of rice (Different letters for each parameter show significant difference at $p \leq 0.05$ by Duncan's Multiple Range Test)

Treatment	N (g pot^{-1})		P (g pot^{-1})		K (g pot^{-1})	
	Grain	Straw	Grain	Straw	Grain	Straw
T_1 (Control)	0.18^{g}	0.11^{g}	0.04^{f}	0.06^{c}	0.04^{f}	0.37^{h}
T_2 (RDF*)	0.35^{ef}	0.18^{f}	0.09^{bc}	0.10^{a}	0.06^{e}	0.55^{f}
T_3 (RDF + $Zn_{1.25}$)	0.38^{de}	0.20^{e}	0.10^{ab}	0.09^{a}	0.07^{de}	0.58^{ef}
T_4 (RDF + $Zn_{2.5}$)	0.44^{c}	0.22^{cd}	0.11^{a}	0.09^{a}	0.08^{c}	0.61^{de}
T_5 (RDF + $Zn_{3.75}$)	0.48^{b}	0.26^{c}	0.10^{ab}	0.09^{a}	0.08^{b}	0.70^{bc}
T_6 (RDF + $Zn_{5.0}$)	0.55^{b}	0.26^{b}	0.09^{bc}	0.08^{b}	0.10^{a}	0.71^{b}
T_7 (RDF + $Zn_{6.25}$)	0.60^{a}	0.28^{a}	0.09^{bc}	0.08^{b}	0.09^{a}	0.91^{a}
T_8 (RDF + $Zn_{7.5}$)	0.46^{cd}	0.22^{cd}	0.07^{e}	0.07^{bc}	0.07^{d}	0.64^{cd}
T_9 (RDF + $Zn_{8.75}$)	0.44^{c}	0.17^{f}	0.08^{d}	0.06^{c}	0.07^{d}	0.47^{g}
T_{10} (RDF + $Zn_{10.0}$)	0.41^{c}	0.17^{f}	0.09^{bc}	0.08^{b}	0.07^{d}	0.47^{g}
SEm ±	0.01	0.01	0.003	0.003	0.002	0.02
CD ($p \leq 0.05$)	0.03	0.02	0.01	0.01	0.005	0.06

*Recommended dose of fertilizer

While total uptake of Cu, Fe, Mn and B varied between 0.18–0.44, 6.49–12.8, 4.92–10.1 and 0.65–1.27 mg pot^{-1} (Table 3). Significantly higher grain and straw uptake of Cu, Fe, Mn and B was recorded in T_6 (21 mg pot^{-1}) and T_4 (2.67 mg pot^{-1}), T_4 (1.95 mg pot^{-1}) and T_3 (11.0 mg pot^{-1}), T_3 (0.76 mg pot^{-1}) and T_5 (9.46 2.67 mg pot^{-1}), T_5 (2.67 mg pot^{-1}) and T_4 (2.67 mg pot^{-1}). Above 6.25 mg kg^{-1} of Zn soil application, total uptake of Cu, Fe and B was significantly decreased over RDF. Among all treatment T_4 was recorded most efficient in micronutrient (except Zn) uptake by hybrid rice. Nutrient uptake being functions of dry matter production and partly due to increase in nutrient concentration. The highest total uptake of nutrients (N, K, Zn, Fe and Mn) was recorded at 6.25 mg kg^{-1} and the lowest uptake at control.

Table 3. Effect of zinc application on micronutrients uptake (g pot^{-1}) in various plant parts of rice (Different letters for each parameter show significant difference at $p \leq 0.05$ by Duncan's Multiple Range Test)

Treatment	Zn (mg pot^{-1})		Cu (mg pot^{-1})		Fe (mg pot^{-1})		Mn (mg pot^{-1})		B (mg pot^{-1})	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
T_1 (Control)	0.34^{h}	0.83^{f}	0.09^{c}	0.09^{e}	0.72^{f}	5.77^{d}	0.28^{e}	4.64^{e}	0.29^{c}	0.36^{c}
T_2 (RDF*)	0.57^{g}	1.38^{de}	0.14^{c}	0.18^{dc}	1.64^{c}	10.4^{ab}	0.56^{c}	8.27^{bc}	0.50^{ab}	0.55^{ab}
T_3 (RDF + $Zn_{1.25}$)	0.63^{fg}	1.52^{cd}	0.18^{a}	0.26^{a}	1.79^{abc}	11.0^{a}	0.73^{a}	8.92^{ab}	0.50^{ab}	0.64^{a}
T_4 (RDF + $Zn_{2.5}$)	0.72^{de}	1.65^{c}	0.20^{a}	0.24^{ab}	1.97^{a}	10.3^{ab}	0.67^{ab}	8.87^{ab}	0.53^{a}	0.73^{a}
T_5 (RDF + $Zn_{3.75}$)	0.79^{cd}	1.87^{b}	0.20^{a}	0.22^{b}	1.84^{ab}	10.6^{a}	0.63^{bc}	9.46^{a}	0.57^{a}	0.59^{ab}
T_6 (RDF + $Zn_{5.0}$)	0.92^{b}	1.87^{b}	0.21^{a}	0.21^{bc}	1.68^{bc}	10.2^{ab}	0.59^{bc}	7.98^{bc}	0.53^{a}	0.67^{a}
T_7 (RDF + $Zn_{6.25}$)	1.02^{a}	2.05^{a}	0.18^{a}	0.19^{c}	1.63^{c}	10.9^{a}	0.64^{bc}	9.38^{a}	0.53^{a}	0.54^{ab}
T_8 (RDF + $Zn_{7.5}$)	0.84^{c}	1.64^{c}	0.15^{b}	0.16^{d}	1.40^{d}	9.61^{b}	0.48^{cd}	7.52^{c}	0.41^{b}	0.48^{bc}
T_9 (RDF + $Zn_{8.75}$)	0.74^{de}	1.29^{e}	0.15^{b}	0.11^{e}	1.22^{de}	7.67^{c}	0.47^{cd}	5.53^{cd}	0.42^{b}	0.39^{bc}
T_{10} (RDF + $Zn_{10.0}$)	0.67^{ef}	1.25^{e}	0.15^{b}	0.12^{e}	1.16^{e}	7.34^{c}	0.44^{d}	5.56^{cd}	0.43^{b}	0.41^{bc}
SEm ±	0.02	0.05	0.01	0.01	0.06	0.31	0.03	0.29	0.02	0.02
CD ($p \leq 0.05$)	0.07	0.15	0.02	0.02	0.17	0.92	0.08	0.87	0.05	0.06

*Recommended dose of fertilizer

Results might be because of increased nutrient concentrations in rice grain and straw, as well as increased yield of rice grain and straw. It has been noted that optimal Zn levels can improve rice nutrients uptake; our results agreed with previous research (Li et al., 2007; Dash et al., 2010; Rutkowska et al., 2014; Xue et al., 2014). The synergistic impact of Zn and N is mostly related to increased Zn availability in soil because N main responsible for soil acidity (Pooniya et al., 2018). Zinc fertilizer also had a significant impact on P absorption in rice grain and straw. This could be related to an increase in N levels in plants caused by $ZnSO_4.7H_2O$, as well as higher Basmati rice yields, which resulted in enhanced total P uptake. These results are in conformity with the findings of Pooniya and Shivay, (2013); Shivay et al. (2015). It might be due to antagonistic effect of P with Zn. Gohil et al. (2017) reported that in case of P uptake, lower rate Zn application recorded significantly more uptake of P. However, their application at higher levels significantly reduced the P uptake by both grain and straw portion of rice. Gohil et al. (2017) reported that Mn and Cu uptake was significantly affected by Zn fertilization.

Nutrient harvest index

Nutrient harvest index was calculated by taking nutrient uptake of particular nutrient. Table 4 revealed that different level of Zn soil application had a significant effect on nutrient harvest index of different nutrient. Results indicated that among different treatments; application of RDF + Zn 8.75 mg kg^{-1} in to soil has higher macro and micronutrient harvest index compared to other treatments. Among macronutrient (N, P and K), NHI value of N (72.5%) is greater than P (55.1%) followed by K (12. 4%). Where as in micronutrients, NHI value of Cu is higher than B followed by Zn, Fe and Mn. The values of nutrients nitrogen, phosphorus, potassium, iron, zinc, copper, manganese, and boron (N, P, K, Fe, Zn, Cu, Mn, and B) harvest index were greater under 7.5 mg kg^{-1} Zn application treatments (Table 4). The higher nutrient harvest index with respect to control could be attributable to the fact that under sustainable nutrient supply conditions, the plant seeks to take more from the soil and converts the most towards seeds in order to complete the life-cycle. Dass et al. (2010) reported the nutrient harvest index of N, P and K in rice. Kumar et al. (2015) reported the Nutrient harvest index concept in okra.

Table 4. Effect of zinc application on nutrient harvest index (%) of rice (Different letters for each parameter show significant difference at $p \leq 0.05$ by Duncan's Multiple Range Test)

Treatment	N (%)	P (%)	K (%)	Zn (%)	Cu (%)	Fe (%)	Mn (%)	B (%)
T$_1$ (Control)	61.8^{d}	37.7^{c}	8.52^{f}	29.5^{d}	51.0^{bc}	11.1^{c}	5.73^{d}	44.4^{cde}
T$_2$ (RDF*)	65.8^{c}	49.9^{bc}	10.4^{cde}	29.2^{d}	43.2^{de}	13.6^{b}	6.40^{b}	47.7^{abcd}
T$_3$ (RDF + Zn$_{1.25}$)	65.8^{c}	52.0^{ab}	10.6^{cd}	29.1^{d}	41.2^{e}	13.9^{b}	7.60^{ab}	44.0^{de}
T$_4$ (RDF + Zn$_{2.5}$)	65.8^{c}	54.7^{b}	11.3^{abc}	30.5^{cd}	46.4^{cde}	15.9^{a}	7.07^{abc}	42.1^{e}
T$_5$ (RDF + Zn$_{3.75}$)	64.9^{c}	51.0^{abc}	10.8^{bcd}	29.8^{d}	47.4^{cde}	14.8^{ab}	6.22^{cd}	49.1^{abc}
T$_6$ (RDF + Zn$_{5.0}$)	68.1^{bc}	52.1^{ab}	11.9^{ab}	33.0^{bc}	50.1^{bc}	14.2^{ab}	6.84^{abcd}	44.1^{de}
T$_7$ (RDF + Zn$_{6.25}$)	68.1^{bc}	52.4^{ab}	9.41^{ef}	33.2^{bc}	48.6^{cd}	12.9^{bc}	6.37^{bcd}	49.6^{ab}
T$_8$ (RDF + Zn$_{7.5}$)	67.8^{bc}	46.9^{b}	9.81^{de}	33.8^{ab}	47.8^{cd}	12.7^{bc}	6.01^{cd}	46.0^{bcde}
T$_9$ (RDF + Zn$_{8.75}$)	72.5^{a}	55.1^{a}	12.4^{a}	36.4^{a}	58.1^{a}	13.8^{b}	7.98a	51.6^{a}
T$_{10}$ (RDF + Zn$_{10.0}$)	70.8^{ab}	52.3^{ab}	12.1^{a}	35.1^{ab}	55.2^{ab}	13.7^{b}	7.29^{abc}	50.9^{a}
SEm ±	0.99	1.33	0.37	0.90	5.76	0.64	0.39	4.30
CD ($p \leq 0.05$)	2.92	3.91	1.08	2.66	1.95	1.88	1.16	1.46

*Recommended dose of fertilizer

Zn balance sheet

Zinc balance sheet were calculated and presented in the Table 5. In all the treatments, except for control and RDF, Zn balances were negative. The results of our study showed that The Apparent Zn balance was highly negative at higher doses of Zn festination. The higher negative value of nutrient balance was recorded at T$_{10}$ and least value was at control. Additional application of Zn influences the soil properties in various ways. So, Zn exceeds the amount of higher negative balance.

The higher negative Zn balance recorded under the treatment where higher amount of Zn fertilization applied. It is one of the important micronutrients available to plants through diffusion and mass flow in the soil environment. The availability of Zn, as well as its uptake and utilization by rice, are thus intimately tied to productivity, but are influenced by a variety of abiotic and biotic factors in the soil-plant system, such as cultivar, nutrient input, soil and climatic condition. This might be due to the fact that higher amount of Zn application helps in buildup Zn concentration in soil and plant. Similar type of result was recorded by Bera and Ghosh (2013).

Table 5. Effect of zinc application on zinc balance sheet in rice (Different letters for each parameter show significant difference at $p \leq 0.05$ by Duncan's Multiple Range Test)

Treatment	Zn input (mg pot^{-1}) [A]			Zn output (mg pot^{-1}) [B]			Apparent Zn balance (mg pot^{-1}) [A - B]
	Initial soil Zn [a]	Soil Zn applied [b]	Total Zn in soil [a + b]	Total Zn uptake [c]	Soil Zn after harvest [d]	Total Zn output [c + d]	
T$_1$ (Control)	12.0	0	12.0	1.18^{f}	11.7^{f}	12.9^{f}	0.94^{a}
T$_2$ (RDF*)	12.0	0	12.0	1.95^{e}	10.7^{f}	12.7^{f}	0.67^{a}
T$_3$ (RDF + Zn$_{1.25}$)	12.0	11.1	23.1	2.15^{d}	15.8^{ef}	18.0^{ef}	-5.10^{b}
T$_4$ (RDF + Zn$_{2.5}$)	12.0	22.3	34.3	2.37^{c}	19.6^{def}	21.9^{de}	-12.3^{c}
T$_5$ (RDF + Zn$_{3.75}$)	12.0	33.4	45.4	2.67^{b}	24.3^{cde}	27.0^{d}	-18.4^{d}
T$_6$ (RDF + Zn$_{5.0}$)	12.0	44.6	56.6	2.79^{b}	26.4^{cd}	29.2^{cd}	-27.4^{e}
T$_7$ (RDF + Zn$_{6.25}$)	12.0	55.8	67.8	3.06^{a}	32.9^{c}	36.0^{c}	-31.8^{fg}
T$_8$ (RDF + Zn$_{7.5}$)	12.0	66.6	78.6	2.48^{c}	46.9^{b}	49.4^{b}	-29.2^{ef}
T$_9$ (RDF + Zn$_{8.75}$)	12.0	78.1	90.1	2.03^{de}	53.1^{ab}	55.2^{ab}	-34.9^{g}
T$_{10}$ (RDF + Zn$_{10.0}$)	12.0	89.3	101.3	1.92^{e}	58.7^{a}	60.6^{a}	-40.7^{h}
SEm ±	-	-	-	0.06	2.82	2.81	1.09
CD ($p \leq 0.05$)	-	-	-	0.19	8.34	8.30	3.24

*Recommended dose of fertilizer

Phytate concentration and Zn bioavailability

The maximum reduction in phytate concentration was recorded in the treatment receiving soil application of 7.5 mg kg^{-1} (T$_8$) which is 27% lower than RDF (T$_2$) (Table 6) and a significant increase was noticed at higher dosed of Zn application (T$_9$ and T$_{10}$). It may be due to the dilution effect caused with increase in yield by application of Zn fertilizers. However, at higher levels of Zn application (T$_9$ and T$_{10}$), the phytate concentration increased significantly in grain, this might be due to the reduction in grain yield of rice. The range of phytate: zinc molar ratio was ranges from 20–41.2 (Table 6). The lowest phytate: zinc molar ratio was noticed in soil when 6.25 mg Zn kg^{-1} was applied in soil (T$_7$). The phytate: zinc molar is indicative of higher Zn availability with corresponding decrease in phytate: Zn molar ratio. The decrease in phytate: the zinc molar ratio was not only dependent on the decrease in the concentration of phytic acid (PA), but also due to the greater concentration of Zn in rice grains. Various levels of Zn implementation significantly impacted total absorbed zinc (TAZ) in rice grain (Table 6). Its concentration increased progressively with application of Zn in soil. The maximum TAZ was observed in treatment receiving 6.25 mg Zn kg^{-1} (T$_7$) *i.e.,* 2.05 mg Zn day^{-1} and the minimum was in RDF (T$_2$). The TAZ significantly increased with increasing doses of Zn application up to 7.5 mg Zn kg^{-1} and then significantly reduced.

Table 6. Effect of zinc application on phytate, Zn, phytate: Zn molar ratios and total absorbed zinc (TAZ) in rice grain (Different letters for each parameter show significant difference at $p \leq 0.05$ by Duncan's Multiple Range Test)

Treatments	Phytate (mg kg^{-1})	Zn (mg kg^{-1})	Phytate: Zn molar ratios	TAZ (mg Zn day^{-1})
T$_1$ (Control)	7260^{d}	17.5^{f}	41.2^{f}	1.27^{e}
T$_2$ (RDF*)	6177^{b}	19.5^{ef}	31.4^{e}	1.49^{d}
T$_3$ (RDF + Zn$_{1.25}$)	5930^{ab}	21.3^{de}	27.6^{cd}	1.62^{bc}
T$_4$ (RDF + Zn$_{2.5}$)	5922^{ab}	21.9^{d}	26.8^{cd}	1.66^{bc}
T$_5$ (RDF + Zn$_{3.75}$)	5769^{a}	23.5^{cd}	24.3^{bc}	1.77^{b}
T$_6$ (RDF + Zn$_{5.0}$)	5706^{a}	26.0^{ab}	21.7^{ab}	1.93^{a}
T$_7$ (RDF + Zn$_{6.25}$)	5671^{a}	28.2^{a}	20.0^{a}	2.05^{a}
T$_8$ (RDF + Zn$_{17.5}$	5649^{a}	27.6^{a}	20.3^{a}	2.02^{a}
T$_9$ (RDF + Zn$_{8.75}$)	6788^{c}	25.2^{bc}	26.8^{cd}	1.75^{bc}
T$_{10}$ (RDF + Zn$_{10.0}$)	6829^{c}	23.2^{cd}	29.2^{de}	1.64^{c}
SEm ±	112	0.77	1.06	0.05
CD ($p \leq 0.05$)	332	2.26	3.12	0.14

*Recommended dose of fertilizer

According to Wei et al. (2012), Zn treatment increases Zn concentration and decreases phytic acid, resulting in higher bioavailable Zn accumulation in rice grains. Wang et al. (2021) also revealed that phytate content in rice grain decreases as the dose of the application increases. Yaseen and Hussain (2021) and Akram et al. (2020) suggested that applying Zn fertiliser to the soil might significantly decrease the phytate: zinc molar ratio. Previous research Karmakar et al. (2020) has discovered a negative correlation between bioavailable Zn and phytic acid in grain. Yatou et al. (2018) observed that there had been no direct relationship between phytic acid content and Zn content. Various studies revealed close relationships between Zn and other essential nutrient in rice grain (Zhang et al., 2018). The fraction of soluble Zn released from the rice grain after digestion is referred to as bioavailability of Zn. Bioavailability of Zn has been found to be influenced by Zn solubility, digestion phase, and dietary components (Zhang et al., 2020). Wei et al. (2012) also observed that Zn bio-fortification enhanced the quantity of soluble Zn in rice grain, so the total Zn concentration is the most important factor in determining Zn bioavailability. The phytate to Zn molar ratio seems to be a good indicator of Zn bioavailability. The World Health Organization estimates that, When the molar ratio of phytic acid to zinc is more than 15, less than 15% of Zn from food is absorbed (Tsakirpaloglou et al., 2019). This indicated that increasing Zn content while lowering phytic acid concentration in rice grain can improve Zn bioavailability.

Zinc transfer coefficient and plant enrichment factor

The translocation coefficient of Zn in grain and straw were significantly different among the treatment (Figure 2). The maximum TC for grain and straw was reported at T$_3$, which was 2.27 and 15.2**%** higher over RDF. With rising the level of Zn application, translocation coefficient in grain and straw were significantly reduced. At higher level of Zn application (10.0 mg kg^{-1}), the TC value for grain and straw was 65.7 and 75.6% decreased over RDF. The TC value of Zn for grain was less than straw observed among all the treatment. It was also observed TC value of grain was less than straw. In this case, values higher than 1 indicate that the plant effectively translocate metals from root to the aboveground plant part. The plant enrichment factor of Zn for grain and straw was significantly increased with increased in application rate (Figure 2). It was varied between 0.85-2.50 for grain and for straw was 0.86-3.86. Among all the treatment, the maximum Zn plant enrichment factor was observed at T10 (3.84 for straw and 2.50 in grain)**.** The transfer factor (TC) was indicated the amount of metal accumulated from for soil-to-edible parts of rice. Initially by raising the rate of Zn up to 2.5 mg kg^{-1} after that TC value decreased (Figure 2). This might be due to higher rate of Zn applications get adsorbed in to soil. The plant uptake significant amount of Zn if the TC is greater than 1, TC equal to 1 indicated that the plant uptake optimum amount of Zn and TC less than 1 indicates that the Zn uptake is insignificant level (Olowoyo et al., 2010). It was recorded the plant enrichment factor in straw was higher than grain. Vymazal and Brezinova (2015) reported that Zn Sequestration increased from top to bottom in plant. Plants precultured without a Zn supply had much lower 65Zn translocation than those precultured with an adequate Zn supply, according to a radio-labeled Zn (65Zn) experiment (Gupta et al., 2016; Erenoglu et al., 2011), indicating that plant Zn fertilizer has an impact on Zn translocation to grain. But rate of translocation is higher in control then Zn treated plant.

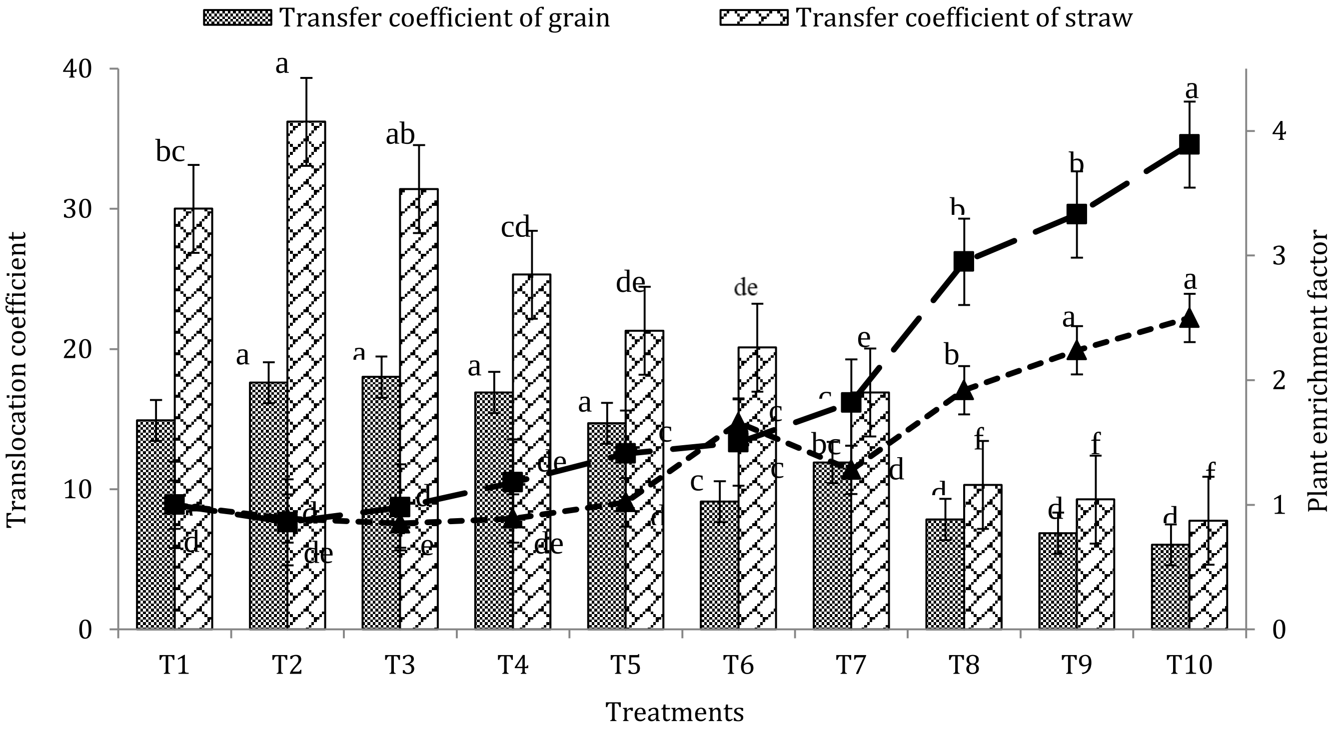

Figure 2. Effect of zinc application on Zn transfer coefficient from soil to grain and straw and plant enrichment factor of Zn in grain and straw of rice (Different letters for each parameter show significant difference at p ≤ 0.05 by Duncan's Multiple Range Test)

Error bars identifies standard error of mean of different treatments

Soil Zn indices

Zn build up index actually indicate the quantity of metal build up in soil matrix. in the present study the buildup factor was found to be increased with increased in Zn application rate. The Zn build up factor varied in between 0.98 – 4.90 among various treatments. Igeo is the quantitative measure used for the assessment of contamination level of Zn through addition of Zn fertilizer. (Figure 3) indicate the Igeo level of all the treatment from control to T_3 fell under the category of unpolluted Class-I for Zn (Igeo < 0), from T_4 –T_6 fell under the class-II (0< Igeo < 1). While remaining treatment fell under moderate pollution categories. Figure 3 illustrated the EF value for Zn in post-harvest Zn treated soil. Across the treatments EF value for Zn was ranged between 0.24-1.82. Zinc was applied to ameliorating the Zn deficiency, biofortification of Zn and higher production. The I-geo values of Zn in paddy soil around were ranged between -0.19 – 1.70 (Class 1 and 2, Antoniadis et al., 2017). At 8.75 and 10 mg kg^{-1} Zn application, I-geo values were around 2, indicating moderate Zn contamination. Lei et al. (2015) also reported that Zn had the highest concentration in paddy soils and its I-geo values ranged between moderates to sever contamination. Soil enrichment factor is helpful in understanding the influence of extreme Zn fertilization. According to the Chen et al. (2017), EF < 1 indicates no enrichment, EF< 3 is minor enrichment. Among these treatments, from control to T7, the EF value fall below <1, while from T_8 to T_9 the EF value was < 3. From this observation it was shown that higher level of Zn treated soil is little toxic to both plant and human. Among all the treatment it came under deficient enrichment. It indicated less hazardous to human being.

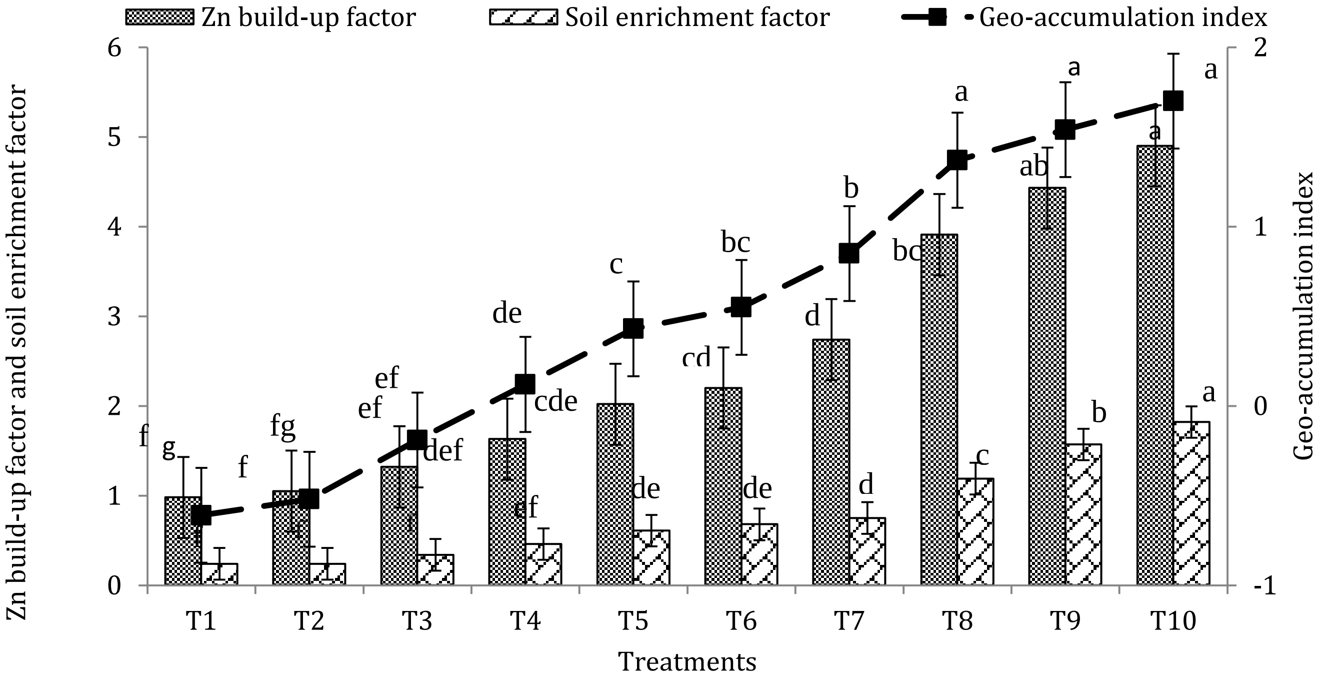

Figure 3. Effect of zinc application on Zn build-up factor, geo accumulation index and soil enrichment factor of zinc in post-harvest soil (Different letters for each parameter show significant difference at p ≤ 0.05 by Duncan's Multiple Range Test) Error bars identifies standard error of mean of different treatments

Dietary intake of Zn

Assuming that an adult man consumes 300 g of cooked rice in their daily diet, the amount of bioavailable Zn (ADI) in cooked rice prepared from Zn treated treatment varied from 0.071 to 0.121 mg kg^{-1} (Figure 4). Among the different treatment, the maximum average daily intake of Zn was recorded at T_7. In T_7, the ADI value of Zn fortified grain was 44% over RDF treated grain. Average daily Zn intake by adult human by consuming Zn fortified grain was presented in the Figure 4. The maximum levels Zn in foods is ≤50.0 mg kg^{-1} dry weight (Ministry of Health, 1991). Daily intakes of Zn in this study through rice were less than the recommended values of Zn for adult, which is 11 mg (Institute of Medicine, Food and Nutrition Board, 2001). It might be due to dietary zinc bioavailability was affected by the phytate content in the grain. Zinc is primary concentrated in the aleurone and embryonic parts of cereals and very small quantity in endosperm. Zinc content in the endosperm round 10 mg Zn kg^{-1}, whereas in embryo and aleurone layer have more than 100 mg Zn kg^{-1} (Cakmak and Kutman, 2018).

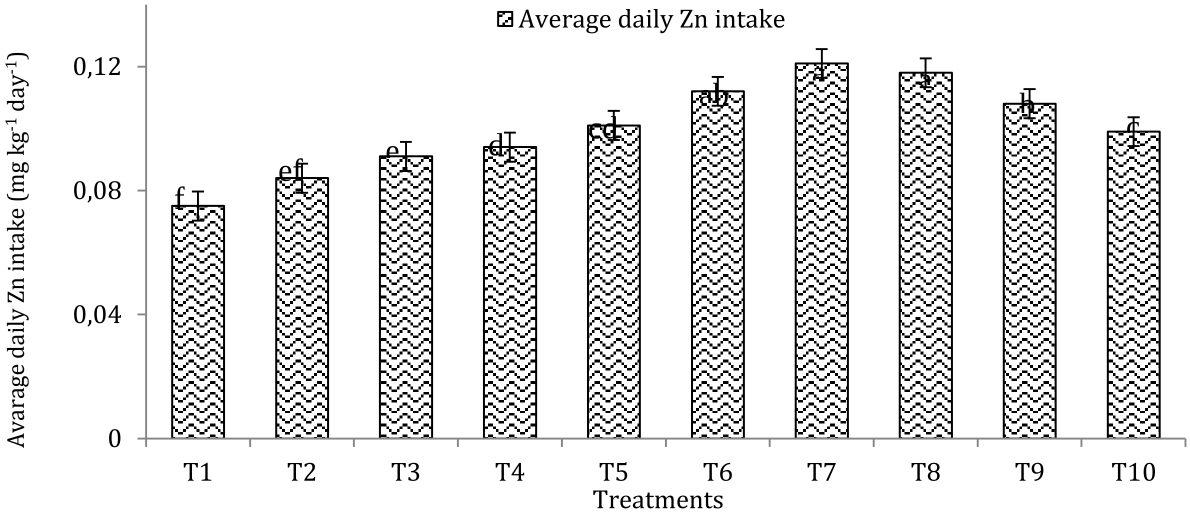

Figure 4. Effect of zinc application on average daily Zn intake by adult human by consuming Zn fortified grain (Different letters for each parameter show significant difference at $p \leq 0.05$ by Duncan's Multiple Range Test)
Error bars identifies standard error of mean of different treatments

Correlations

The correlation between among different zinc indices in soil, plant, and human presented in Table 7 at 0.95 and 0.01 confidence levels ($p < 0.05$ and $p < 0.01$) using ANOVA are presented in Table 7. The results show that Pearson correlation with 2-tailed test showed that, at 0.05 level ($p < 0.05$) positive significant relationships existed between average daily intake – grain Zn content (r = 1.00) and 0.01 level ($p < 0.01$) positive significant relationships existed between post-harvest soil Zn content – plant enrichment factor in grain and straw (r = 0.946, 0.989), Zn build-up factor – plant enrichment factor in grain and straw (r = 0.946, 0.989), Geo-accumulation index-grain Zn content (r = 0.756), Geo-accumulation index – plant enrichment factor in grain and straw (r = 0.898, 0.937) and soil enrichment factor – plant enrichment factor in grain and straw (r = 0.951, 0.989). This was suggesting that higher the Zn indices value in soil higher amount of Zn sequestered in plant. At 0.01 level ($p < 0.01$) there is also negative significant correlation between Zn soil indices – transfer coefficient. The negative correlation might be attributed to the higher Zn content in postharvest soil signify less translocation. At level 0.01 level ($p < 0.01$) there is also positive significant correlation between Average daily intake-soil Zn indices (r= 0.551, 0.551, 0.686 and 0.487), Average daily intake-Zn uptake in grain and straw (r = 0.920, 0.681) and Average daily intake was negatively correlated with Transfer coefficient (r= -0.554, -0661, $p < 0.01$).

Table 7. Pearson's correlation coefficients (r) among different zinc indices in soil, plant and human

Parameters	Zn uptake		Phytate content	Total absorbed zinc	Transfer coefficient		Enrichment factor		Average daily intake
	Grain	Straw			Grain	Straw	Grain	Straw	
Soil Zn after harvest	0.37*	0.003	0.14	0.48*	-0.88**	-0.93**	0.95**	0.99**	0.55**
Zn build-up factor	0.37*	0.003	0.14	0.48*	-0.88**	-0.93**	0.95**	0.99**	0.55**
Geo-accumulation index	0.54**	0.190	-0.04	0.63**	-0.89**	-0.97**	0.90**	0.94**	0.69**
Soil enrichment factor	0.31	-0.08	0.24	0.38*	-0.86**	-0.90**	0.95**	0.99**	0.49**
Apparent Zn balance	-0.67**	-0.33	-0.33	-0.70**	0.83**	0.91**	-0.82**	-0.83**	-0.77**
Average daily intake	0.92**	0.68**	0.68**	0.48*	-0.55**	-0.66**	0.45*	0.46*	1

*significance at $p < 0.05$ level and **significance at $p < 0.01$ level

Conclusion

Application of Zn enriched soil on macro- and micro-nutrient uptake in cereal grain, risk assessment of human health and further on the average daily intake. Zn fertilizer application at optimum quantity can achieve the Zn biofortification aim for hybrid rice grain while producing no hazardous impact to soil health. At 6.25 mg kg^{-1} Zn application was a sustainable approach for growth, nutrient uptake, Zn bioavailability and average daily intake of Zn at low economic lost without causing hazardous impact on soil and plant. The current study's findings will assist farmers in optimizing Zn fertilizer management in crop production in the future.

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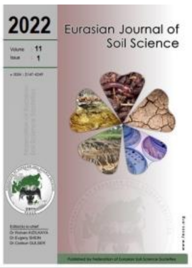

Effect of *Bacillus megaterium* var. *phosphaticum* applied together with rock phosphate on wheat yield and some soil properties in a calcareous soil

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Abstract

This study aims to determine the effect of *Bacillus megaterium var. phosphaticum* applied together with rock phosphate on the yield of wheat grown in calcareous soil, some biological properties of soils and phosphorus fractions in the soil under greenhouse conditions. Considering the P fixation capacity of the soil used in the experiments and the amount of P present in the soil, the trial subjects were created based on randomized block designs with 3 replications, depending on whether 0, 25, 50, 75 and 100% of the P required to be given to the wheat plant was met from rock phosphate and whether it was bacterial or not, and finally wheat was grown. In the harvested plants, grain and stem weights were determined, grain and stem P contents were analysed and the amounts removed with grain and stem were calculated. Dehydrogenase (DHA) and phosphatase (PA) enzyme activities were performed in the soil samples taken after harvest. Soluble and loosely bound-P, Calcium-bound-P (Ca-P), Reductant soluble-P (RS-P) fractions and Olsen-P were determined in soil samples taken before planting and after harvest. The percent reduction in the fractions was calculated by using the pre-sowing and post-harvest values of these samples. According to the results, *Bacillus megaterium* DSM 3228 strain inoculated with rock phosphate increased grain and stem yield, grain and stem P content, and P amount removed by grain and stem of wheat. These parameters were found to be higher at high doses of P applied as rock phosphate. Inoculation increased the DHA and PA values of the soils. A decrease in P fraction forms with low solubility was determined by inoculation, some of this phosphorus was removed by plants and some of it was retained in the soil in different forms.

Keywords: *Bacillus mageterium* var. *phosphaticum*, enzyme activity, inoculation, inorganic P fraction, wheat.

Introduction

Phosphorus (light-bearing) is the second major macronutrient essential for plant growth and development and plays a role in basic biological functions such as cell division, synthesis of nucleic acids, photosynthesis and respiration, as well as energy transfer, fat, sugar and starch formation. Naturally, the amount of phosphorus in various soils generally varies between 0.01-0.15%, but not all of this is in a form suitable for plant use. Phosphorus requirement in vegetative organs for normal plant development varies between 0.3-0.5% in dry matter content, and in the case that the phosphorus content of plants is usually 0.1% or less, the plant suffers from phosphorus deficiency.

All over the world, phosphorus chemical fertilizers are used in traditional agriculture to eliminate phosphorus deficiency and to obtain the highest yield in the product. Due to the difference in the amount,

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type and application times of the applied fertilizers and the lack of knowledge of the practitioners in this field, the health of living things and the environment can be adversely affected by phosphorus fertilizer applications. Phosphates were put in the same class as nitrate as an important pollutant, particularly in the last quarter of the 20th century. Regardless of its source, most of the dissolved phosphates are retained in the soil, and the part that can reach the sea due to the reasons such as erosion is deposited and imprisoned for millions of years (Correll, 1998; Daniel et al., 1998). In addition, the use of phosphorus fertilizers causes some heavy metals such as Cd, Cr, Pb and Ni in the structure of fertilizers to permeate into the soil and plant structure, and may have negative effects on soil and environmental health (Huang and Jin, 2008; Atafar, 2010). Due to the high costs arising from raw materials and intermediate inputs in fertilizer production in our country in recent years, fertilizer production has decreased and its import has increased. Further research is needed to ensure less use of these fertilizers and increase their effectiveness in order to reduce the import of raw materials, intermediate inputs and fertilizers in fertilizer production and to minimize the negative effects of chemical fertilizers on the environmental health.

Due to the negative effects and costs of chemical fertilizers on living things and the environment, the need for the use of more natural resources such as rock phosphate, which can be an alternative to these fertilizers, has arisen. However, the limited solubility of rock phosphate and the low rate of release limit the use of this material in agriculture. There are various factors affecting the utilization of raw phosphates by cultivated plants. In particular, soil reaction is the most important soil feature in the solubility of rock phosphate (Kanabo and Gilkes, 1987; Bolan and Hedley, 1990). Studies have shown that there has been an increase in crop yield with the application of raw rock phosphate in acid-reaction soils (Chien and Menon, 1995). On the other hand, it was determined that the raw rock phosphate application on calcareous alkaline reaction soils did not have a significant effect on the phosphorus nutrition of the plant (Çağatay et al., 1973).

It is known that microorganisms dissolve insoluble phosphate by producing organic acids (malic acid, acetic acid, indoleacetic acid) and by chelating oxoacids from sugar (Dawwam et al., 2013; Khan et al., 2016; Behera et al., 2017; Pande et al., 2017) and many microorganisms with the ability to dissolve phosphorus have been identified by various researchers (Chunga et al., 2005; Fernández et al., 2007; Iyer et al. 2017). It has been demonstrated by studies that inoculation of seeds or soil with phosphate-solving bacteria (PSB) increases the solubility of fixed soil phosphorus and phosphates applied as fertilizers, resulting in higher crop yields (Batool and Iqbal, 2019). In addition, it has been pointed out that the use of rock phosphate as phosphorus fertilizer and its solubility with microorganisms can be an alternative to costly chemical fertilizers (Kaur and Reddy, 2015).

The current study aims to determine the effect of *Bacillus mageterium var. phosphaticum* applied together with rock phosphate on the yield of wheat grown in calcareous soil, some biological properties of soil and phosphorus fractions in the soil under greenhouse conditions.

Material and Methods

Material

In this study, bacteria that dissolve phosphorus as material, rock phosphate as phosphorus source and wheat as plant were used. The microorganism *Bacillus megaterium var. phosphaticum* is isolate of DSM 3228, obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b 38124 Braunschweig, Germany). Rock phosphate was obtained from domestic sources in Turkey and rock phosphates from Mardin Mazı Mountain were used for this purpose. Altındane variety of wheat (Triticum aestium) was used as the test plant in the experiments. A soil with high lime content and low phosphorus content was used in the experiments.

Soil used in the trials was determined as silty loam (Sand: 38.28%; Clay: 11.5%; Silt: 50.21%) and bulk density was 1.25 gr.cm^{-3}, field capacity was 32.21% and wilting point was 16.21%. In addition, its pH (1:1, Soil : Water suspension) was 7.80; Electrical conductivity was (1:1, Soil : Water suspension) 0.502 dSm^{-1}; Lime content was determined as 42.6%, organic Matter was 0.90% and available P content was 3.04 mg kg^{-1}. The pH value of the rock phosphate used as a phosphorus source was 8.22, the total phosphorus content was 27%, the water-soluble phosphorus was 0.08%, and the water + citrate-soluble phosphorus values were 0.081%.

The trial subjects were created based on randomized block designs with 3 replications, depending on whether 0, 25, 50, 75 and 100% of the P required to be given to the wheat plant was met from rock phosphate and whether it was bacterial or not considering the P fixation capacity of the soil used in the experiments and the amount of P present in the soil.

For the application of bacteria in the experiment, lyophilized cultures of the mentioned bacteria were activated under completely aseptic conditions, and liquid culture of bacteria was formed in Nutrient agar (peptone 5 g, meat extract 3 g, 10 mg $MnSO_4.H_2O$ L^{-1}, pH=7). The greenhouse experiment was established and carried out in the light, temperature etc controlled research greenhouse in the Black Sea Agricultural Research Institute (at 25^0C, 350 ppm CO_2, 8 hours dark and 16 hours light conditions). In the greenhouse experiment, different doses of rock phosphate placed in pots of 5 kg soil (<4 mm) over the dry weight and bacterial applications were applied to each pot separately according to the trial subjects. Bacteria inoculation level was applied to the soil at 5 ml (10^8 CFU. mL^{-1}) per seed. Wheat seeds were sown by hand, 15 in each pot. After the first emergence of the seeds from the soil, thinning was made to have 10 plants in each pot. Nitrogen and potassium required for plants without any phosphorus fertilization during the experiment were added in liquid form according to the results of soil analysis. The moisture content of the soil was kept at the field capacity level; for this purpose, the water lost from the soil by various ways was completed with pure water by taking the weights every day.

Analysis Methods

Grain and stem weights were determined by harvesting the plants that reached grain maturity, P contents of grain and stem were analysed, and the amounts removed with grain and stem were calculated. Dehydrogenase and phosphatase enzyme activities were performed in the soil samples taken after the harvest. Soluble and loosely bound-P, Calcium bound P (Ca-P), Reductant soluble-P(RS-P) fractions and Olsen-P were determined in the soil samples taken before planting and after harvest (Table 1). The % reduction in fractions was calculated with the following formula using the pre-planting and post-harvest values of these samples:

$$\Delta Pi = (C_{preplanting} - C_{postharvest}) \times 100 / C_{preplanting}$$

Table 1. Analyses applied to soil samples taken at the end of the trials

Plant Analyses	Method
Grain weight	Gravimetrically (Jones, 2001)
Stem weight	Gravimetrically (Jones, 2001)
Grain and Stem P Amount	The total P amount in dry-burned soil samples was determined by the vanadomolybdophosphoric yellow photometric method (Jones, 2001)
Soil Biological Analyses	
Dehydrogenase activity	Spectrophotometric determination of the colour formed as a result of the conversion of TTC entering the cell into TPF with the effect of the dehydrogenase enzyme in the cell (Pepper et al., 1995)
Phosphatase enzyme activity	Decomposition of disodium *p*-nitrophenylphosphate with the effect of phosphatase enzymes and spectrophotometric determination of the released *p*-nitrophenol (Tabatabai and Bremner,1969)
Inorganic Phosphorus fractions	
Soluble and loosely bound P	Extraction with 1M NH_4Cl solution (Self-Davis et al., 2009)
Calcium-bound P (Ca-P)	Extraction with 0.25 M H_2SO_4 solution (Self-Davis et al., 2009)
Reductant soluble P(RS-P)	Extraction Na-dithionite- Na-citrate solution (Self-Davis et al., 2009)
Olsen P	0.5 N $NaHCO_3$ extraction (Olsen et.al., 1954)

Inorganic phosphorus fractions were done according to the method proposed by Kuo (1996). The procedure used for calcareous soils has been described in detail by Self-Davis et al. (2009). In the first step, soluble and weak Al and Fe bound phosphates are separated or labile phosphates (NaOH/NaCl) are extracted. In the second step, it is extracted in soluble reductant (occluded phosphorus bound to Fe and Al oxides and pedogenic Ca-phosphates with reduced availability)/(Na citrate-bicarbonate-dithionite-extracted-CDB). In the third step, the fraction of phosphates bound to calcium of primary minerals -apatite group, hardly soluble phosphates (HCl extracted) are extracted.

Evaluation of Obtained Results and Statistical Analysis

The trials were analysed in the ANOVA statistical program according to the split plot trial design in the randomized plot experimental design, and the differences were classified according to Duncan and LDS (0,05).

Results and Discussion

Effect of treatments on grain and stem yield and removed P amount

Table 2 shows the changes in grain yield, P content and P amount removed with grain of wheat by *Bacillus megaterium* DSM 3228 strain inoculated with rock phosphate. Both the application of rock phosphate and inoculation affected the grain yield of the wheat statistically. The highest yield was obtained from the application of 75% of the P required to be given to the wheat in the subjects with and without inoculation. Microorganism inoculation was the subject that increased the grain yield more than the subject without inoculation. Rock phosphate application affected the grain P content of wheat statistically and the highest grain P value was obtained from 100% application of P required to be given to the wheat plant. However the inoculation application, did not cause a statistical difference in the grain P values of the plant. Both the rock phosphate application and inoculation caused a statistical difference in the P content removed by the plant grain. The highest P value removed with the grain was obtained from the 75% dose application of the P required to be given to the wheat. The P value removed by grain was found to be higher in the inoculated subjects than in the non-inoculated subjects.

Table 2. Grain yield, grain P content and removed P amount by grain of wheat

	Grain yield (gr pot $^{-1}$)			Grain P (%)			P removed by grain (mg pot $^{-1}$)		
	-DSM 3228	+DSM 3228	Mean	-DSM 3228	+DSM 3228	Mean	-DSM 3228	+DSM 3228	Mean
0	3.088	4.050	3.733 B	0.129	0.129	0.129 C	3.971	5.212	4.592 C
25	3.087	4.683	3.887 B	0.193	0.175	0.185 B	5.967	8.193	7.080 B
50	3.487	4.360	3.923 B	0.195	0.195	0.197 AB	6.801	8.482	7.642 B
75	4.313	5.230	4.771 A	0.189	0.202	0.195 B	8.163	10.51	9.337 A
100	3.330	4.151	3.740 B	0.206	0.209	0.208 A	6.851	8.690	7.770 B
Mean	3.461 B	4.495 A		0.182	0182		6.351 B	8.218 A	
		LSD$_{0.05}$			LSD$_{0.05}$			LSD$_{0.05}$	
Inoculation (I)		0.284*						0.650*	
P doses (P)		0.378*			0.0126*			0.879*	
I*P									

Changes caused by *Bacillus megaterium* DSM 3228 strain inoculated with rock phosphate in stem yield, stem P content and the amount of P removed by stem of wheat are given in Table 3. Both rock phosphate application and inoculation affected the stem yield of wheat statistically. The highest yield was obtained from the application of 100% of the P required to be given to the wheat in the subjects with and without inoculation. Microorganism inoculation was the subject that increased the stem yield more than the non-inoculated subject. Rock phosphate application did not affect the stem P content of wheat statistically, whereas inoculation resulted in a statistically significant difference. Inoculation was the application that increased the stem P content of the plant more. Both the rock phosphate application and inoculation caused a statistical difference in the P content removed by the plant stem. The highest P value removed by the stem was obtained from the 100% application of the P required to be given to the wheat. The P value removed by the stem was found to be higher in the inoculated subjects than in the non-inoculated subjects. Many studies have shown that the application of phosphorus-solving microorganisms alone or in combination with any source of phosphorus provides increases in the development, growth parameters and P removal of various plants (Mamta et al., 2010; Singh and Reddy, 2011; Gupta et al., 2012; Hussain et al., 2019).

Table 3. Stem yield, Stem P content and removed P amount by stem of wheat

	Stem Yield, gr pot^{-1}			Stem P, %			P removed by stem, mg pot^{-1}		
	-DSM 3228	+DSM 3228	Mean	-DSM 3228	+DSM 3228	Mean	-DSM 3228	+DSM 3228	Mean
0	2.843	3.658	3.250 C	0.022	0.036	0.0267	0.633	1.300	0.967 B
25	3.127	3.975	3.550 BC	0.025	0.026	0.0267	0.796	1.018	0.908 B
50	2.920	4.044	3.483 BC	0.025	0.025	0.0267	0.733	1.004	0.870 B
75	3.680	4.138	3.908 B	0.023	0.027	0.0250	0.835	1.137	0.987 B
100	4.293	4.526	4.410 A	0.026	0.029	0.0283	1.103	1.307	1.207 A
Mean	3.373 B	4.068 A		0.024 B	0.028 A		0.820 B	1.153 A	
		LSD$_{0.05}$			LSD$_{0.05}$			LSD$_{0.05}$	
Inoculation (I)		0.379*			0.0033*			0.121*	
P doses(P)		0.463*						0.156*	
I*P									

Table 4 shows the change caused by the *Bacillus megaterium* DSM 3228 strain inoculated with rock phosphate in the DHA and PA of the soil samples taken after harvest. Inoculation affected DHA statistically, and rock phosphate doses were found to be statistically ineffective. The subjects with inoculation were the application that increased the DHA activity of the soils more than the subjects that were not inoculated. Both the application of rock phosphate and inoculation affected the PA of the soils taken after the harvest of the wheat statistically. Phosphatase activity was found to be higher in inoculated subjects compared to non-inoculated subjects. The highest phosphatase activity was obtained from 75% application of P required to be given to the wheat plant. This was followed by 25% and 50% applications, and the lowest PA value was obtained from the subject in which no fertilizer was applied.

Dehydrogenase activity is not independent of the host microbial cell; therefore it is considered a good indicator of total microbial activity (Masciandaro et al., 2000; Kızılkaya, 2008). Phosphatase activity is an extracellular enzyme, that is, it is synthesized by microorganisms and accumulates in the soil, where it is synthesized. This enzyme is effective in organic and inorganic phosphorus availability in the soil and is widely used in the evaluation of the biological activity of soils (Amador et al., 1997; Kızılkaya and Hepşen, 2004). It has been demonstrated by previous studies that the inoculation of microorganisms into the soil or seed that promote plant growth, alone or together with any P source, causes changes in the biological properties of the soil (Kızılkaya and Bayraklı, 2005; Singh and Reddy, 2011; Kaur and Reddy, 2014; Chaya and Bijoy, 2015).

Table 4. Dehydrogenase activity (DHA) and Phosphatase activity (PA) activity of soil samples taken after harvest

	Dehydrogenase activity (DHA) μg TPF g^{-1} soil 24h 25^{0}C			Phosphatase activity (PA) μg p-nitrophenol g^{-1} soil		
	-DSM 3228	+DSM 3228	Mean	-DSM 3228	+DSM 3228	Mean
0	2.551	2.952	2.751	111.9	120.4	116.1 C
25	2.750	3.499	3.123	131.8	123.2	127.5 AB
50	2.702	2.864	2.783	123.5	130.9	127.2 AB
75	3.204	2.990	3.095	124.9	144.6	134.7 A
100	2.321	2.757	2.541	112.9	138.6	125.7 B
Mean	2.706	3.012		121.0	131.5	
	LSD$_{0.05}$			LSD$_{0.05}$		
Inoculation (I)		0.268*			7.509*	
Phosphor doses(P)					8.204*	
I*P						

In this study, P fractions the sequential analysis of inorganic phosphorus proposed by Kuo (1996) were modified for application to calcareous soils (Self-Davis et al., 2009). In this approach, fractionation procedures are based on the different solubility of various inorganic P forms in various extracts. With NaOH/NaCl extraction, some of both Al-P and Fe-P as well as the soluble loosely bound P were extracted. Reductant soluble P retained in the matrices of aggregates/minerals was extracted by CDB (Na citrate-bicarbonate-dithionite) extraction, and finally, poorly soluble phosphates and especially the Ca-P fraction were extracted using HCl (Milić, 2019).

P added to the soils as rock phosphate increased the inorganic phosphorus fractions of the soils compared to the control (Table 5). Studies have shown that phosphorus added to soils in different forms increases the amount of phosphorus in the soils (Wang et al., 2010; Audette et al., 2016; Mahmoud et al., 2018). The highest phosphorus amounts were determined as the Ca-P fraction both in the pre-planting and post-harvest periods. The order of the P fractions in the soil were as Ca-P > reductant-P > Soluble and loosely bound-P. In the studies, it has stated that the dominant form in calcareous soils is Ca-P (Solis and Torrent, 1989; Shen et al., 2004; Kızılkaya et al., 2007). P fractions in the pre-planting soil samples were higher than the amount of P fractions in the post-harvest soil samples (except reductant-P) in the inoculated and non-inoculated treatments. The P values in the post-harvest soil samples may have been lower due to some chemical reactions and/or plant uptake in the soil. The relationships between plant P uptake and inorganic P forms in calcareous soils are not clearly defined. Plant P uptake is most closely associated with Ca-P (Kamprath and Watson, 1980), resin extractable P (Yang et al., 1990), and citrate-bicarbonate P (RS-P) (Solis and Torrent, 1989). Samadi (2006) reported significant positive relationships between Ca_2-P, Al-P, Fe-P and Ca10-P fractions and plant P uptake. Compared with the pre-planting P fractions, the Ca-P fraction had lower values in the inoculated subjects than being in the non-inoculated. When the reduction rates in the fraction are examined, it is seen that the percentage of decrease in the inoculated subjects is higher. On the other hand, there was an increase in reductant-P values when compared to the pre-planting P fractions, and this increase was higher in the inoculated subjects. The ability of plants to utilize acid-soluble P (Ca-P) fractions has been attributed to acidification of the rhizosphere (Grinsted et al., 1982; Ahmad et al., 2018). Phosphorus-solving microorganisms can secrete various acids and provide the dissolution of Ca-P by lowering the pH of the soil and rhizosphere region. *Bacillus mageterium var. phosphaticum* is a very important phosphorus dissolving bacterium, especially capable of dissolving calcium phosphate. It is known that *Bacillus* strains produce lactic, isovaleric, isobutyric and acetic acid mixtures and these play an important role in phosphorus solubility. Other organic acids as glycolic, oxalic, maleic, and succinic acids have also been identified as phosphate solvents and are produced by bacteria that dissolve phosphorus (Banik and Dey 1982, Illmer and Schinner, 1992). Bacteria secrete these organic acids and solubilize insoluble inorganic phosphate in the form of tricalcium, dicalcium, rock phosphate and hydroxy apatite (Goldstein 1986, 1995; Güneş et al., 2013). According to Oberson et al. (2001), microorganisms constitute highly important dynamic reserves of potentially useful nutrients for plants. Microorganisms play an important role in the conversion of organic phosphorus in the soil by secreting phosphatase enzyme and they also allow moderately unstable forms from phosphate compounds to pass into solution. Gong et.al (2014) reported that *Penicillium oxalicum* I1 (PI1) isolate, a fungus with high phosphorus dissolving ability, converted a wide variety of insoluble phosphates such as $Ca_8H_2(PO_4)_6.5H_2O$, $AlPO_4$, $FePO_4$ and $Ca_{10}(PO_4)_6(OH)_2$ in soil into soluble $CaHPO_4$ and this isolate prevented the conversion of $CaHPO_4$ into insoluble form.

Table 5. P fractions of soils taken before planting and after harvest, % decrease in Olsen-P values

		Post-harvest		Reduction in Fraction (%)	
	Pre-planting	+DSM 3228	- DSM 3228	+DSM 3228	- DSM 3228
		Soluble and loosely bound P (mg kg^{-1})			
0	2.595	2.539	2.031	2	22
25	2.595	2.539	2.116	2	18
50	2.883	1.354	1.947	53	32
75	3.325	1.524	2.285	54	31
100	3.748	1.778	2.539	53	32
		Ca-P (mg kg^{-1})			
0	186	156	159	16	15
25	193	170	171	12	11
50	199	180	185	10	7
75	225	196	200	13	11
100	235	210	215	11	9
		Reductant-P (mg kg^{-1})			
0	9.625	10.70	9.513	-11	1
25	12.70	13.38	11.59	-5	9
50	6.930	9.513	7.135	-37	-3
75	8.855	8.918	10.11	-1	-14
100	8.085	17.540	16.35	-117	-102
		Olsen-P (mg kg^{-1})			
0	3.104	2.324	2.462	25	21
25	3.235	2.324	2.321	28	28
50	3.133	2.380	2.480	24	21
75	4.040	3.201	3.447	21	15
100	3.635	2.954	3.242	19	11

Conclusion

In this study, *Bacillus megaterium* DSM 3228 strain inoculated with rock phosphate increased grain and stem yield, grain and stem P content, and P amount removed by grain and stem of wheat. These parameters were found to be higher at higher doses of P applied as rock phosphate. Inoculation increased the DHA and PA values of the soils. A decrease was determined in P fraction forms with low solubility by inoculation, some of this phosphorus was removed by plants and some remained in the soil as other forms. It is considered that inoculated strain and some acids secreted by the plant roots are effective in this conversion. In calcareous soils, the solubility of more natural resources such as rock phosphate can be achieved by applying the *Bacillus megaterium* DSM 3228 strain. Conducting these trials with different phosphorus-solving bacteria and for many years will provide us more information about the behaviour of P fractions in the soil.

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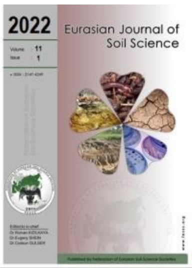

Impact of NPK fertilization on hazelnut yield and soil chemical-microbiological properties of Hazelnut Orchards in Western Georgia

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Abstract

In this study, the effects of ground and foliar applications of the NPK fertilizers in hazelnut orchards on the soil chemical and microbiological properties and hazelnut yield were investigated. The fertilization practicesses from ground were done two times using NPK (20:10:10 +trace elements) on March and May while the fertilization practices from leaf were done three times using NK (15:12 +trace elements) on May, June and July at six different hazelnut orchards located on Samegrelo, Guria and Adjara regions in Western Georgia in 2018. The alkaline characterized fertilizer applications from soil generally increased soil reaction (pH), nutrient contents and EC values in different magnitude depends on the soil characteristics of locations. The lowest soil pH (4,40) and EC (0,107 dS m^{-1}) values showed the highest increment (10,7% and 77,6%, respectively) over the control. The basal soil respiration and C_{mic} values of all hazelnut orchards were generally increased by the NPK ferilization. Increasing soil pH and EC by the fertilization also increased CA and DHA activity. The mean values of percent increase in yield and yield parameters by the NPK fertilization were obtained as 8,3% in yield, 13,3% in shelled nut weight, 10,0% in kernel weight and 5,1% in percent kernel efficiency. The hazelnut yield value had significant positive correlation with soil pH (0,669*), EC (0,652*) and C_{mic} (0,620*) values. The foliar fertilization and improving the soil properties of hazelnut orchards by the application of alkaline characteristic NPK fertilizer from soil increased hazelnut yield and yield parameters compare with the farmer applications or control treatments. The increments in soil microbiological properties and nutrients are considered as a desirable result in terms of sustainable soil management and plant nutrition for hazelnut orchards.

Keywords: Hazelnut, soil, fertilization, microbiological properties

Introduction

Hazelnut is one of the most widely cultivated hard-shelled fruit in the world. The main top of the hazelnut producer countries are Turkey, Italy, Spain, USA, Georgia, Azerbaijan, China, Iran, Chile, Australia and France. The world hazelnut production has come close to one million tons in recent years. Hazelnut cultivation in Georgia is carried approximately 26 thousand hectares. Hazelnut plantations occupied 8-10 times more territory than in Soviet times and it has a high economic value for Georgia which exportes hazelnuts to the EU countries in large quantities. The hazelnut production is mainly taking place in Western Georgia, including Samegrelo, Guria, Adjara, and Imereti regions (Mirotadze, 2004). Agricultural productivity, which is influenced by many factors such as climate, irrigation, fertilization, cultural practices and soil quality, is

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ensured by the optimum formation of multifaceted conditions. Especially in hazelnut cultivation, where cultural practices are utilized at a minimum, the importance of agricultural practices increases still more. Moreover, in recent years, hazelnut came to bear great importance as raw material for the food industry besides its quality as an agricultural product. Increasing the efficiency and quality values of hazelnut, which is an important export product of Georgia, is also vital for meeting the economic needs of the regional producer (Mirotadze, 2004; Chanishvili, 2019).

In hazelnut farming, producer characteristics, agricultural production practices, productivity parameters need to be established together. Fertilization activities carried out in hazelnut fields change all soil properties and will contribute to improvement of yield. Olsen et al. (2000) studied the effect of isotopically labeled nitrogen on the uptake, storage, and remobilization of N in hazelnut trees for both ground and foliar N applications at various timings. They reported that hazelnut trees rely heavily on stored N reserves to fuel leaf and nut production and new growth in early spring, and also ground application of N in June was less efficient than March applications. Bignami et al. (2004) reported that in the adult hazelnut orchard, nuts represented 46% of the biomass and the highest N content in the leaves is in spring, it reduces during the growing season and N accumulation in shoots startes only after harvest. Snare (2008) indicated that hazelnuts benefit from a balanced nutritional program such as annual applications of a complete NPK fertilizer and N, K, B are the elements most commonly found deficient in hazelnuts. Tous et al. (2004) reported that in a mature hazelnut orchard in north-east Spain, the greater amount of N (100 kg N/ha) resulted in significantly lower production and kernel yield from hazelnut trees, whereas a B foliar and Fe chelate treatment increased production. Applying 50 kg N/ha when the leaf N level was about 2.4% of the dry mass in July and two B foliar sprays with soil Fe chelates in spring was the optimum fertilization obtaining higher production and better nut quality. Nicolosi et al. (2009) studied the effect of foliar fertilization on hazelnuts growing with applying a 8.5% organic nitrogen fertilizer and an NPK fertilizer (20:20:20) plus chelated micro-elements (3%) at three different times during the vegetation. Treatments had significantly higher kernel weight and nut size as compared to the control plants. Wei and Zhai (2010) found that the NPK contents in the hazelnut fruit continued to increase from kernel growth stage till nut maturity stage, in which the P content in the hazelnut fruit decreased dramatically due to the great consumption in the kernel development and less absorption. They reported that NPK in fruits had a significant or very significant positive correlation in the development course of hazelnut fruit and there was a dynamic equilibrium of coordination among the three elements.

Many studies were conducted in order to sustain hazelnut farming and increase productivity. Works aimed at improving the efficiency of soils often take into account the physical and chemical properties of the soils, but biological properties are as important and often ignored. Soil fertility is not only dependent on the physical conditions of the soil and the level of nutrients, but also closely related to the density of biological phenomena. The biological characteristics of soils are an important indicator of soil fertility, and also soil health and quality. Ding et al. (2016) reported that soil microbial community size was enhanced by the application of inorganic fertilizer and manure. They found that aoil microbial diversity was decreased by inorganic fertilizer and increased by the incorporation of inorganic fertilizer with manure. Basal soil respiration represents a fundamental component of the soil carbon cycle (Raich and Schlesinger, 1992) and is an indicator of soil carbon storage, soil biological activity and overall soil quality (Ewel et al., 1987). In general, soil respiration depends on the respiration of plant roots and soil microorganisms. Environmental factors such as soil temperature and soil humidity are known to have a significant effect on the seasonal dynamics of soil respiration (Lloyd and Taylor, 1994). Microbial biomass C plays a crucial role; as it is a much easier and faster determining method than microscopes or other coating methods in determining the mass of bacterial and fungal populations; and in terms of its effect on the disintegration of organic matter (Powlson and Brookes, 1987) and, in relation to it, the continuity of the nutrient cycle (Jenkinson and Parry, 1989).

The objective of this study was to investigate the changes in hazelnut yield, soil chemical and microbiological properties as a result of the ground and foliar applications of the NPK fertilizers in hazelnut orchards located on different regions in Western Georgia.

Material and Methods

Study sites

This study was carried out at the hazelnut orchards located on Samegrelo, Guria and Adjara regions in Western Georgia with different topographical positions in 2018 (Figure 1). The hazelnut orchards were selected for consistency in soil texture (clay to sandy clay loam) and slope (0–3%). The six different hazelnut orchards were on acidic soils and not irrigated. All sites have a history of chemical fertilizer use (e.g., diammonium phosphate, triple super phosphate and calcium ammonium nitrate) and pesticide applications for 10–15 years. During the field experiment, monthly precipitation and average temperature for the experimental locations are given in Figure 2. After transporting the soil samples to the laboratory, they were prepared for analysis by drying under shade, removing crop roots and stones by hand, smashing and sieving from a sieve having 2mm size opening. The soil physico-chemical properties were determined based on standard methods as follows: particle size distribution by the hydrometer method (Bouyoucos, 1962), $CaCO_3$ content by the volumetric method (Martin and Reeve, 1955), pH in 1:1 (w/v) in soil: water suspension by pH - meter (Rowell, 1996), electrical conductivity (EC) in the same soil suspension by EC - meter (Rowell, 1996). Whole soil samples were sieved through a 150 µm mesh to determine the total organic matter (SOM) by the wet oxidation method (Walkley-Black) with $K_2Cr_2O_7$ (Rowell, 1996).

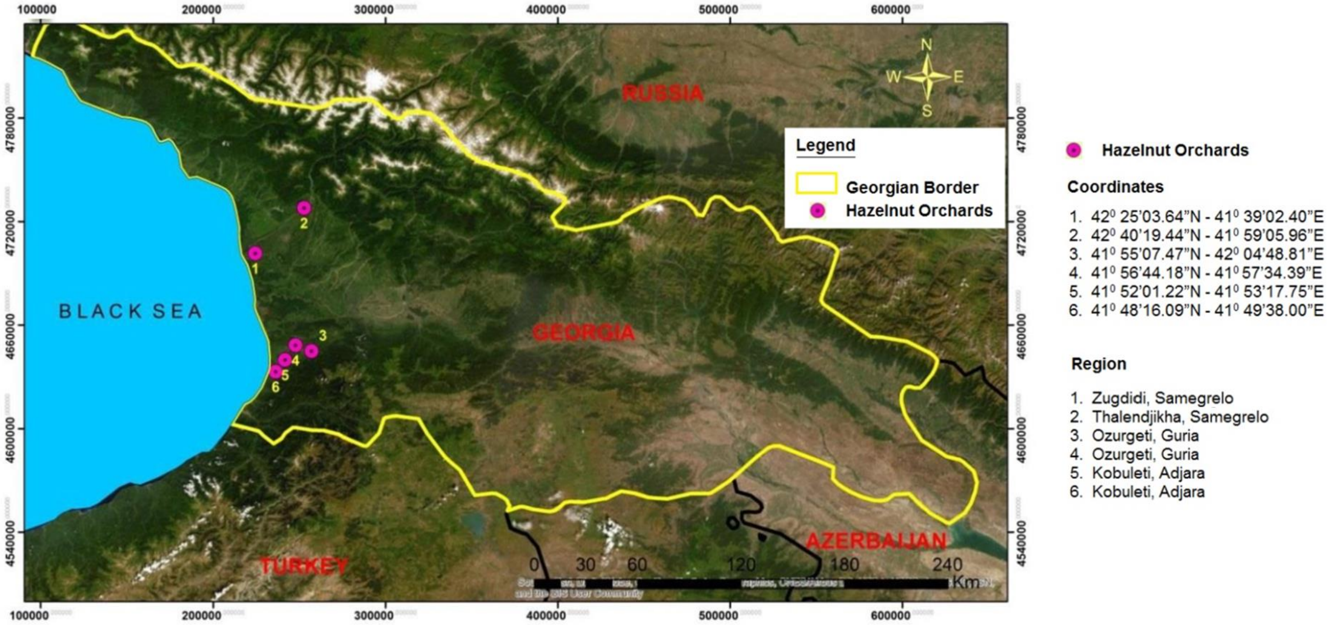


Figure 1. Locations of the hazelnut orchards

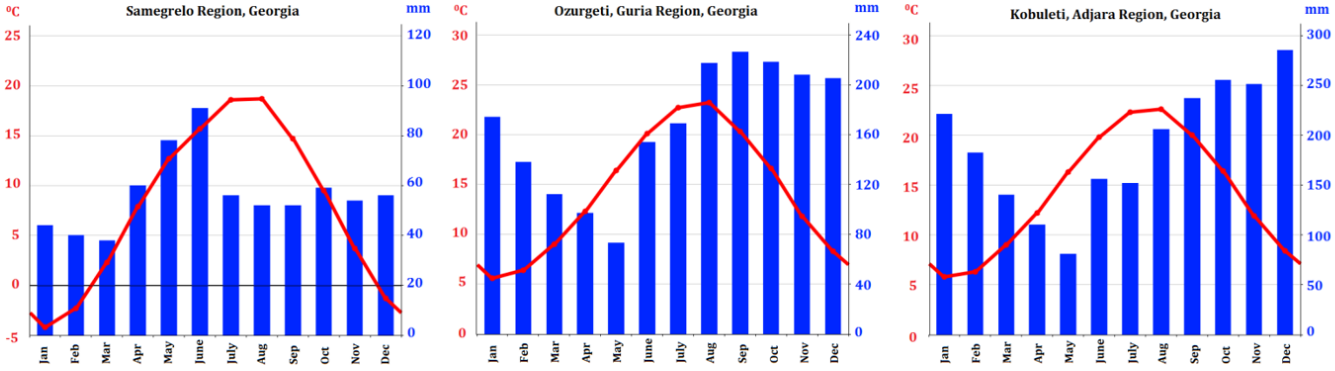


Figure 2. Montly average values of precipitation and temperature in the three locations of Western Georgia.

Fertilizers used in this study

The fertilizers used in this study were produced spesifically for hazelnut plant. The specific properties of the fertilizers are given below;

The fertilizer produced for soil application contains 20% N, 10% P_2O_5 and 10% K_2O (+trace elements): i) pH is over 7.50, and helps to neutralize soil acidity if regularly used, and helps to reduce or remove using "Agricultural Lime" in acidic soils, ii) It is a slow release fertilizer, melts easily and difficult to leach from soil profile, iii) It can provide essential nutrients for the hazelnut plant for a vegetation period if application is made in correct dose and timing.

The fertilizer produced for foliar application contains 15% N, and 12% K_2O (+trace elements): i) The pH level in the application dose are adjusted between 5.0-5.5 at that pH the leaf absorption is the highest, ii) The foliar fertilizer can be used with other physiological acid reaction pesticides such as hazelnut worm, which greatly harms the hazelnut plant, iii) The fertilizer is solid and completely melts in water solution, does not leave any residue/remnants in the fertilizer/disinfection engine, iv) Microelement contents within the fertilizer are chelated with EDTA to uptake esily by the plant and to get a greater effect.

Experimental design

Both fertilizations from soil and leaf were used in this experiment. The details of soil and foliar fertilization are given below.

Fertilization from ground: NPK (20:10:10 + trace elements) fertilizer was applied to band in two different period as follow; the first application was made at the end of February- start of March as 1000 g/tree (includes 12 or 15 hazelnut plants in each tree) (250 kg/ha for row planting) and the second application was made at the end of May and start of June; 750 g/tree (200 kg/ha for row planting).

Fertilization from leaf: The foliar fertilization was applied three times in the following periods: 500 g/100 L within April, 750 g/100 L at the end of May and 750 g/100 L at the end of June - start of July.

The experiments were conducted in six different hazelnut orchards located in Samegrelo, Guria and Adjara Regions of Georgia. In each hazelnut orchard, 80 hazelnut trees (about 0,2 ha) were fertilized from soil and leaf in the recommended doses and periods given above. The yield and yield parameters (shelled nut weight, kernel weight and percent kernel efficiency) of hazelnut fertilized both from soil and leaf were determined and compared with the trees under the routine fertilization by the farmers used as a control treatments during the harvest. Percent kernel efficiency was estimated with dividing the kernel weight by the shelled nut weight. At the end of the harvest, soil samples were taken from the hazelnut orchards in each location. The following analyses were made in the soil samples.

Soil nutrient contents and some chemical properties

To analyze of some nutrient contents and chemical properties of soil samples, crop residues, root fragments and stones larger than 2 mm had been removed from air-dried soil samples and stored at room temperature after passing from 2 mm sieve. Soil nutrient contents were determined by the following methods: total N content was determined by digestion and subsequent measurement by the Kjeldahl method (Bremner, 1965), available P content was determined by 0.03 M NH_4F (Bray and Kurtz, 1945), exchangeable Ca, Mg, Na, K contents were determined by 1N NH_4OAc extraction (Rowell, 1996), pH was detrmined in 1:1 (w/v) in soil:water suspension by pH - meter (Rowell, 1996), electrical conductivity (EC) was determined in the same soil suspension by EC - meter (Rowell, 1996).

Soil microbiological characteristics

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

Microbial biomass carbon: Microbial biomass carbon (Cmic) was determined by the substrate-induced respiration method by Anderson and Domsch (1978). A moist soil sample equivalent to 100 g oven-dry soil was amended with a powder mixture containing 400 mg glucose. The CO_2 production rate was measured hourly using the method described by Anderson (1982). The pattern of respiratory response was recorded for 4 h. Microbial biomass carbon (Cmic) was calculated from the maximum initial respiratory response in terms of mg C g^{-1} soil as 40.04 mg CO_2 g^{-1} +3,75. Three replicates of each soil sample were tested. Data was expressed as mg CO_2-C 100 g^{-1} dry soil 1 h^{-1}.

Dehydrogenase activity: Dehydrogenase activity (DHA) was determined according to Pepper et al. (1995). Six grams of soil, 30 mg glucose, 1 ml of 3% 2,3,5-triphenyltetrazoliumchlorid (TTC) solution and 2.5 ml pure water were added. The samples were incubated for 24 h at 37°C. The formation of 1, 3, 5 triphenylformazan (TPF) was determined spectrophotometrically at 485 nm, and the results were expressed as µg TPF g^{-1} dry sample.

Catalase activity: Catalase activity (CA) was measured by the Beck method (Beck, 1971). Ten ml of phosphate buffer (pH, 7) and 5 ml of a 3% H_2O_2 substrate solution were added to 5 g of soil. The volume (ml) of O_2 released within 3 minutes at 200°C was determined. Three replicates of each sample were tested, and controls were tested in the same way, but with the addition of 2 ml of 6.5% (w/v) NaN_3. Results were expressed as ml O_2 g^{-1} dry soil.

The relationships between hazelnut yield parameters and the other factors were determined using the statistical software package SPSS. The relationships between soil microbiological and chemical properties were also estimated mainly by using principal component analysis.

Results and Discussion

Some soil properties of the orchards

The soil properties of hazelnut orchards are given in Table 1. Although the soil properties in the orchards show great differences in texture and organic matter content, all soils have acidic reactions (<6.5), nonsaline (<0.980 dS m^{-1}), and low in lime content ($CaCO_3$ < 5%).

Table 1. Some soil properties of the hazelnut orchards

Location Numbers of hazelnut orchards	Particle size distribution				$CaCO_3$, %	pH (1:1)	EC, dS m^{-1}	SOM, %
	Clay, %	Silt, %	Sand, %	Class				
1L: Zugdidi, Samegrelo	18,80	31,96	49,24	L	3,62	5,51	0,847	0,97
2L: Thalendjikha, Samegrelo	43,15	37,70	19,15	C	3,22	4,54	0,618	1,58
3L: Ozurgeti, Guria	19,65	21,20	59,15	SL	3,42	6,01	0,445	2,77
4L: Ozurgeti, Guria	38,79	29,29	31,92	CL	3,30	4,40	0,107	3,54
5L: Kobuleti, Adjara	39,66	23,51	36,83	CL	2,84	4,78	0,349	1,79
6L: Kobuleti, Adjara	22,99	20,27	56,74	SCL	2,37	4,54	0,532	5,67

Effect of both soil and foliar fertilization on nutrient contents and some chemical soil soil properties of the hazelnut orchards comparing with the farmer applications as a control are given in Table 2. The fertilizers applied from soil generally increased soil nutrient contents and EC values. Also, the soil fertilizer having a physiological alkaline reaction increased soil pH. The 4th location having the lowest soil pH (4,40) and EC (0,107 dS m^{-1}) values showed the highest increment (10,7% and 77,6%, respectively) over the control by the fertilization.The 3rd location having a coarse soil textuture class (SL) had the highets increment in av. P (192,8%) and exch. K (54,8%) contents over the control by the fertilization. The highest increase in total N (26,1%) over the control was observed in the 2nd location including clay texture and low soil OM content. The increments in soil nutrients compared with control applications are considered as a desirable result in terms of sustainable soil management and plant nutrition for hazelnut orchards. Adeniyan et al. (2011) determined that the application of NPK (15:15:15) fertilizer enhanced availability of soil nutrients and cation exchange capacity considerably in acid soils and increased dry matter of maize. Wang et al. (2015) reported that soil nutrient contents with application of chemical fertilizers were generally significantly higher than those of the control without fertilization.

Table 2. The effect of fertilization on nutrient content and chemical properties of hazelnut orchard soils

Location No of the orchards		pH (1:1)	EC, dS m^{-1}	Total N, %	Av. P, mg kg^{-1}	Exchangeable cations, cmol kg^{-1}		
						Ca	Mg	K
1L	F	5,57	0,110	0,23	3,72	5,45	1,10	0,35
	C	5,51	0,085	0,23	4,24	5,51	0,84	0,29
2L	F	4,78	0,087	0,29	10,01	4,34	0,87	4,24
	C	4,54	0,062	0,23	7,26	4,54	0,61	3,72
3L	F	6,12	0,484	0,47	10,13	5,16	4,49	0,48
	C	6,01	0,445	0,41	3,46	6,01	1,18	0,31
4L	F	4,87	0,190	0,45	26,65	4,30	3,90	3,46
	C	4,40	0,107	0,54	11,58	4,40	1,07	2,75
5L	F	5,01	0,046	0,38	4,58	4,46	4,84	4,58
	C	4,78	0,035	0,32	1,94	4,78	0,84	3,50
6L	F	4,80	0,067	0,70	3,50	4,80	0,67	2,65
	C	4,54	0,053	0,73	2,75	4,54	0,53	1,94

F: Both soil and foliar fertilization; C: Farmer application as a control

Some microbiological soil properties of the orchards

The changes in microbiological properties of hazelnut orchard soils by the fertilization are given in Table 3. Soil microbiological characteristics of all hazelnut orchards were increased by the NPK ferilization. The highest value for BSR (0,424 µg CO_2-C g^{-1}) and C_{mic} (79,093 mg CO_2-C 100 g^{-1}) were obtained in the 6th location having the highest soil OM content (5,67%). However, the highest percent increment for BSR (142,2%) and C_{mic} (138,3%) over the control by the fertilization was determined in the 1st location having the lowest soil OM content (0,97%). It indicates that NPK fertilization was more effective in increasing BSR and C_{mic} at lower soil OM level. It is known that the soil properties such as texture, organic matter content, root density and microbial biomass affect the size of soil respiration (Haynes and Gower, 1995; Kelting et al, 1998; Raich and Tufekcioglu, 2000). Iovieno et al. (2009) reported that soil respiration and enzyme activities increased in compost-treated soils due to consequence of both microbial growth and stimulation of microbial activity by enhanced resource availability, as well as of changes in microbial community composition. Microbial biomass C enables the determination of the functional diversity of soil communities (Lupwayi et al., 1998, Altieri, 1999); and the causal effects on plant production and long-term soil management status. Zhang et al. (2017) found that application of inorganic fertilizer increased soil C_{mic} concentration.

The highest value for CA (220,742 ml O_2 g^{-1}) and DHA (2,704 µg TPF g^{-1}) were detrmined in the 4th location having the highest percent increase in soil pH (10,7%) and EC (77,6%) values over the control by the NPK fertilization. Increasing soil pH and EC by the fertilization increased CA and DHA activity in 4th location. Samuel et al. (2019) determined that catalase and dehydrogenase enzymatic activities were generally higher in NPK fertilized plots than in the unfertilized plot. Dehydrogenase activity reflects the total oxidative activity range of the soil's microflora and can ultimately be a good indicator of microbiological activity in the soil (Skujins, 1973). Dehydrogenase activities are not associated with microbial population and degradation of hydrocarbons, but are widely used to reflect microbial oxidative activity of soils. The enzyme catalase (H_2O_2:H_2O_2-oxidoreductase, EC 1.11.1.6.) is an enzyme that catalyzes the reaction of hydrogen peroxide (H_2O_2) to water and molecular oxygen. The enzyme catalase is closely related to the presence of the aerobic microbial population in the soil and the efficiency of the soil, and the determination of the enzyme catalase enzyme is used as an indicator in the assessment of the aerobic microorganism population available in the soil (Garcia and Hernandez, 1997). Hydrogen peroxide (H_2O_2) occurs in the respiratory processes of living organisms and at the end of various biochemical processes in which organic matter is oxidized (Weetall et al., 1965; Trevors, 1984).

Table 3. The effect of fertilization on biological properties of hazelnut orchard soils

Location No of the orchards		BSR, µg CO_2-C g^{-1}	C_{mic}, mg CO_2-C 100 g^{-1}	CA, ml O_2 g^{-1}	DHA, µg TPF g^{-1}
1L	F	0,281	74,85	117,22	0,573
	C	0,116	31,41	78,91	0,308
2L	F	0,216	60,93	105,62	1,780
	C	0,207	32,63	57,15	0,197
3L	F	0,197	69,91	96,90	0,363
	C	0,186	68,87	82,73	0,276
4L	F	0,419	72,32	220,74	2,704
	C	0,229	48,93	110,79	0,576
5L	F	0,191	42,90	92,19	1,119
	C	0,190	41,80	44,56	1,066
6L	F	0,424	79,09	164,47	1,503
	C	0,303	50,78	120,63	0,883

F: Both soil and foliar fertilization; C: Farmer application as a control

The effect of fertilization on hazelnut yield and yield parameters

The results of the fertilization effects on yield and yield parameters of hazelnut obtained form the six different hazelnut orchards are given in Table 4. The yield and yield parameters in all hazelnut orchards increased by the fertilization from soil and leaf together compared with the control treatments (Figure 3). The mean values of pecent increase in yield and yield parameters over the control by the fertilization were obtained as 8,3% in yield, 13,3% in shelled nut weight, 10,0% in kernel weight and 5,1% in percent kernel efficiency. Ellena et al. (2012) studied on the application of different rates of NPK foliar nutrients on fruit yields and quality characteristic and found that there were significant differences between the treatments in yield, nut weighs, and kernel weighs compared with the untreated control.

The hazelnut yield values varied beteen 2,70 kg/tree in control application of 2nd location and 4,20 kg/tree in the fertilizer application of 3rd location. The highest percent increase (12,9%) in yield by the fertilization was determined in the 1st location. The shelled nut weight values varied beteen 2,41 g in control application of 5th location and 3,23 g in the fertilizer application of 3rd location. The highest percent increase in av. P (192,8%) and exch. K (54,8%) contents was also determined in the soil of 3rd location by the fertilization over the control. Increses in av. P and exch. K content in soil by the fertilization improved yield and shelled nut weight of hazelnut. The kernel weight values varied beteen 1,16 g in control application of 3rd location and 1,47 g in the fertilizer application of 3rd and 4th locations. Chen et al. (2014) studied the effects of foliar

N, P, and K applications on yield and fruit quality of hazelnut and reported that only a foliar N application at shoot growing stage significantly increased yield and single grain weight.

The highest percent increase (26,7%) in kernel weight by the fertilization was also determined in the 3rd location. The percent kernel efficiency values varied beteen 42,00% in control application of 2nd location and 53,80% in the fertilizer application of 5th location. The highest percent increase in shelled nut weight (22,8%) and in percent kernel efficiency (8,3%) by the fertilization was determined in the 6th location which had the highest total N and soil OM contents. In many researches on hazelnut, it has been reported that nut weight varied due to their genetic constitution of cultivar, crop load, cultural practices and regions (Bostan and Islam, 1999; Aziz et al.i 2007; Silva et al., 2007). Milošević and Milošević (2017) determined size and features of twelve different hazelnut varieties and reported that nut weight varied between 1,32 g and 4,00 g, kernel weight between 0,53 g and 1,76 g, and percent kernel between 36,47% and 49,59%.

Table 4. The effects of fertilization on yield and yield parameters of hazelnut.

Location No of the orchards		Yield (kg/tree)	Weight of shelled hazelnut, g	Kernel weight of hazelnut, g	Percent kernel efficiency, %
1L	F	3,50	3,20	1,40	45,00
	C	3,10	2,90	1,37	43,50
2L	F	3,00	2,80	1,20	43,00
	C	2,70	2,43	1,20	42,00
3L	F	4,20	3,23	1,47	45,40
	C	4,00	2,64	1,16	43,70
4L	F	3,20	3,03	1,47	49,20
	C	3,00	3,00	1,43	47,00
5L	F	3,50	2,60	1,40	53,80
	C	3,40	2,41	1,21	50,00
6L	F	3,90	3,18	1,41	48,10
	C	3,50	2,59	1,25	44,40
Mean	F	3,55	3,01	1,39	47,42
	C	3,28	2,66	1,27	45,10

F: Both soil and foliar fertilization; C: Farmer application as a control

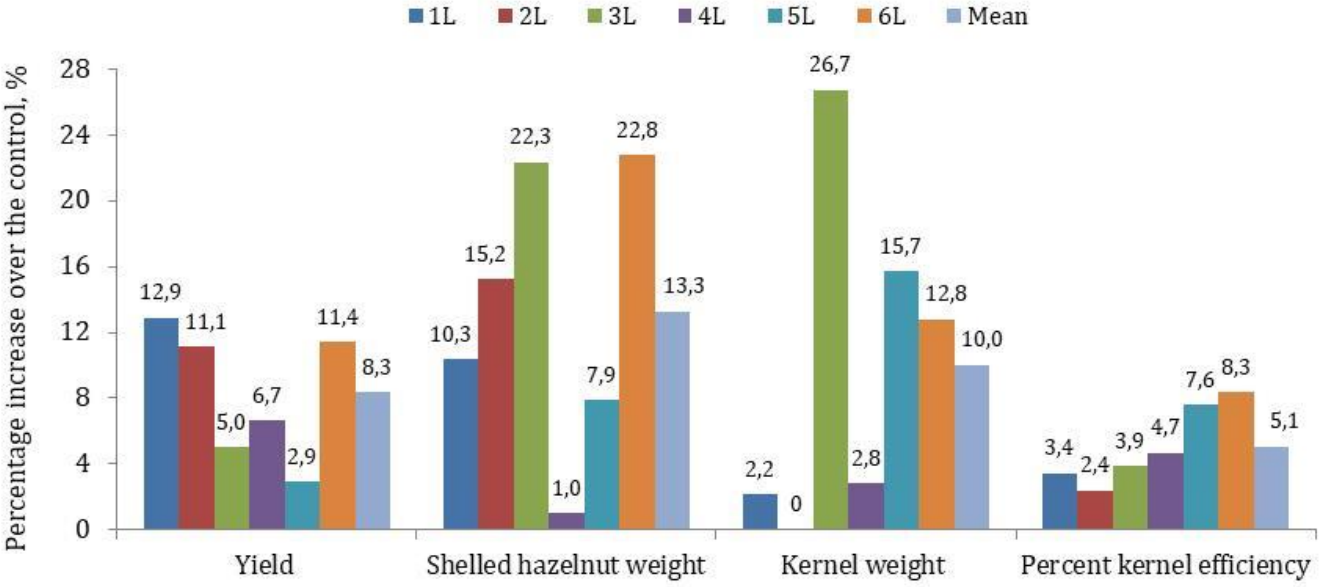


Figure 3. Effect of both ground and foliar fertilization on yield and some yield parameters compare with the farmer application as a control. (Locations; 1L: Zugdidi-Samegrelo, 2L: Thalendjikha-Samegrelo, 3L: Ozurgeti- Guria, 4L: Ozurgeti-Guria, 5L: Kobuleti-Adjara, 6L: Kobuleti-Adjara)

Relationships among the yield parameters and soil properties

A correlation matrix among the soil properties and the yield parameters is given in Table 5. The yield value had significant positive correlation with soil pH (0,669*), EC (0,652*) and Cmic (0,620*) values. It indicates that increasing of soil reaction, nutrient contents and microbial activity of hazelnut orchards due to the application of alkaline characteristic NPK fertilizer increased hazelnut yield values. Exch. Mg content had significant positive correlations with kernel weight (0,586*) and percent kernel efficiency (0,581*).

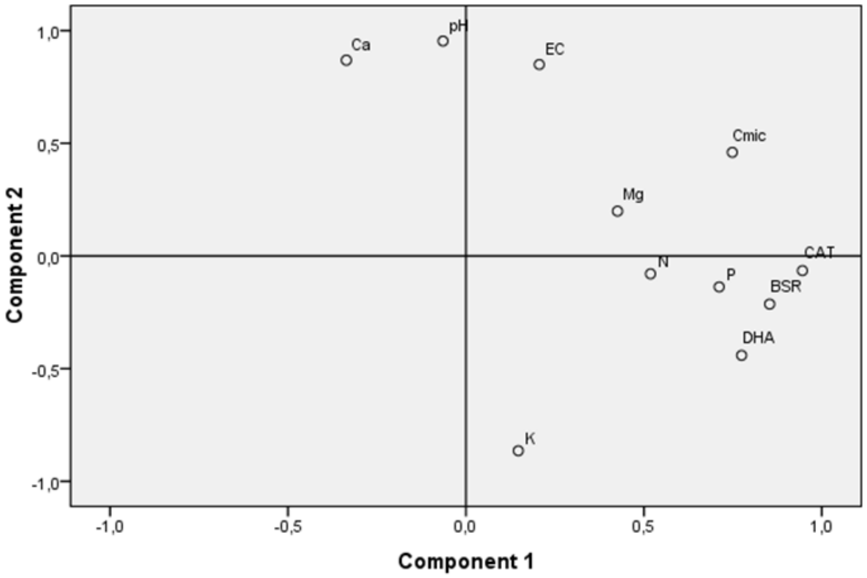
Increasing soil microbiological properties generally increased hazelnut yield parameters. Bodaghabadi et al. (2019) found that there was a relatively strong correspondence between yield and soil properties in pistachio orchards, clay, EC, K and B were negatively related, but sand and $CaCO_3$ significantly positive correlated with yield. Kainer et al. (2007) reported that Brazilian nut productivity had a positive relationship with cation exchange capacity levels and a weakly negative correlation with extractable P levels.

In PC1, CAT is the greatest contributor to the PC as given by the factor loading (Figure 4). A further 6 variables had highly weighted factor loadings, namely BSR, DHA, Cmic, P, N and Mg. Soil pH was the best representative of PC2 having the highest factor loading. While the the total N content had a significant positive correlation with BSR (0,591*), the available P content showed significant positive correlations with CAT (0,680*) and DHA (0,638*) (Table 5). Soil pH was one the most important soil properties which was influenced by the application of alkaline characterixezed NPK fertilizer from acidic soil (Figure 4). Soil EC and Ca had also highly weighted factor loadings positively in PC2. Soil microbial properties improved due to NPK fertilization generally had significant positive correlations eachother (Table 5). Singh et al. (2018) reported that there were significant improvements in microbial counts, microbial biomass carbon, soil respiration, soil enzymes and soil organic carbon with fertilization in lentil growth.

Table 5. Relationships among the soil properties and hazelnut yield parameters.

	Nut	Kernel	Keff.	pH	EC	N	P	K	Ca	Mg	BSR	Cmic	CAT	DHA
Yield	0,362	0,206	0,197	0,669*	0,652*	0,435	-0,260	-0,505	0,521	0,335	0,146	0,620*	0,113	-0,118
Nut		0,781**	-0,019	0,363	0,323	0,211	0,333	-0,440	0,166	0,240	0,416	0,685*	0,594*	0,161
Kernel			0,438	0,137	0,100	0,236	0,451	-0,139	-0,139	0,586*	0,368	0,328	0,579*	0,234
Keff.				-0,184	-0,228	0,215	0,125	0,467	-0,356	0,581*	0,241	0,040	0,235	0,445
pH					0,792**	-0,263	-0,140	-0,755**	0,835**	0,337	-0,332	0,362	-0,142	-0,379
EC						0,058	0,193	-0,571	0,556	0,397	-0,139	0,476	0,030	-0,243
N							0,029	0,009	-0,255	0,013	0,591*	0,370	0,487	0,231
P								0,266	-0,449	0,468	0,432	0,271	0,680*	0,638*
K									-0,860**	0,141	0,195	-0,274	0,082	0,564
Ca										-0,160	-0,368	0,193	-0,309	-0,589*
Mg											0,041	0,188	0,280	0,272
BSR												0,653*	0,873**	0,685*
Cmic													0,654*	0,388
CAT														0,749**

*significant at 0,05 level, **significant at 0,01 level.



	Component	
	1	2
pH	-0,064	0,954
EC	0,206	0,850
N	0,519	-0,079
P	0,712	-0,137
Ca	-0,336	0,869
Mg	0,426	0,199
K	0,147	-0,864
BSR	0,853	-0,214
Cmic	0,749	0,460
CAT	0,946	-0,065
DHA	0,775	-0,441

Figure 4. Rotated component matrix from the soil properties obtained from the hazelnut orchards.

Conclusion

At the end of the experiments conducted in six different hazelnut orchards in Georgia, the fertilizer applications improved soil chemical and biological properties and increased hazelnut yield and yield parameters. The fertilizer having alkaline reaction applied from soil increased soil pH, nutrient contents and EC values in all hazelnut orchards. Basal soil respiration, Cmic, DHA and CAT enzyme activities of soils in the hazelnut orchards were also increased by the soil application of NPK fertilizer. Total hazelnut yieled, shelled nut and kernel yields and percentage of kernel efficiency generally increased over the control in all orchards by the soil and foliar application of NPK fertilizers. It can be concluded that foliar fertilization and increasing of soil reaction, nutrient contents and microbial activity of hazelnut orchards due to the application of alkaline characteristic NPK fertilizer from soil increased hazelnut yield and yield parameters compare with the farmer applications or control treatments.

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Eurasian Journal of Soil Science 2022, 11(3), 216 - 224

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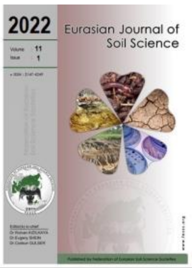

Relative potential of *Rhizobium* sp for improving the rice-wheat crop in the semi-arid regions

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Abstract

Soil Microbiologists have been concentrating on manipulation of rhizosphere microbes in cereals, but many researchers have reported that rhizobia can act as plant growth promoting rhizobacteria (PGPR). *Rhizobium* species impacted the crop ontogeny by root / endophytic colonization, producing phytohormones, efficient nutrient use and nutrient solubilization / mineralization. Field studies were performed at Soil Bacteriology Section and Soil Chemistry Section, Faisalabad to assess the comparative potential of *Rhizobium* species for promoting the growth, yield of wheat and rice. Auxin biosynthesis potential of isolates of *Rhizobium* species (mung (*Vigna radiata*), berseem (*Trifolium alexandrinum*), chickpea (*Cicer arietinum*), lentil (*Lens culinaris*) and peanut (*Arachis hypogaea*)) was determined and isolates of each species having higher values were used for field experiments. Assay for root / shoot elongation, root colonization in plates were carried out under controlled conditions. The rhizosphere soil of wheat and rice were assayed for the Indole Acedic Acid (IAA) content 15 and 30 days after germination / transplanting, respectively. Results revealed that significant increase was observed in the yield parameters of wheat and rice. Highest wheat grains were produced i.e., 4917 kg ha^{-1} with *Rhizobium sp of mungbean* (Mb$_3$) followed by 4823 with *Rhizobium sp of berseem* (Br$_3$) than control i.e., 4500 kg ha^{-1}. Similarly, the maximum paddy yield i.e., 4667 kg ha^{-1} with *Rhizobium sp of mungbean* (Mb$_3$) followed by 4625 *Rhizobium sp of berseem* (Br$_3$) inoculation was obtained as compared to control i.e., 4208 kg ha^{-1}. Other physical parameters of wheat and rice also showed positive response to inoculation and have elevated levels of IAA in the rhizosphere of inoculated treatments. Results clearly demonstrated that *Rhizobium* species increased the yield of rice and wheat.

Keywords: *Rhizobium* species, IAA equivalents, PGPR, Interaction, wheat, rice.

Introduction

Biofertilizer or microbial inoculants are the substance that either solid carrier based or liquid based contains beneficial microbes that have participated in different functions for promoting plant growth. Biofertilizer are environment friendly, inexpensive (cost effective) and reasonable source that have potential role in the plant growth promotion. The microbial inoculants perform different functions for plant growth viz. by improving

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soil conditions, solubilizing/mineralizing/mobilizing nutrients, restricting pathogens, supplementing/ compensating mineral fertilizers, enhancing nutrient use efficiency, protecting plant micro-environment i.e., rhizosphere from pollutants, abolishing harmful substances, effective in arid, semi-arid or adverse soil conditions and ultimately the higher yield of plants and healthy returns for farmers. Application of mineral fertilizers results in readymade supply of nutrients to plants and on the other hand significant portion of added fertilizer is fixed or lost (Sessitsch et al., 2002; Ibiene et al., 2012; Vargas et al., 2017). The efficient microbes are responsible to restore the soil fertility status by biological means and thus act as restoring agents of soil fertility. The continuous or prolonged and excessive usage of chemical or mineral fertilizers deteriorates/degrades soil, water and air (Vejan et al., 2016; Vargas et al., 2017). Further, injudicious use of mineral fertilizers results in environmental pollution (Lin et al., 2019). Biofertilizer are usually carrier-based formulations of beneficial microbes. The carriers of biofertilizer are carbon source i.e., compost, muds, manures, lignite, leaf molds, peat and water etc. Biofertilizer improve soil fertility, enhance soil water holding capacity, and act as agents of climate change (Vejan et al., 2016; Rachel et al., 2018).

Soil microbes can mobilize soil nutrients, transform nutrients into available forms; store water-soluble nutrients in available forms, tender the plants balanced nutrients, maintain soil aeration required for the roots; improve nutrient uptake/efficiency, provide nutrients during the growing season and especially during growth critical periods of plants (Reddy, 2014; Rachel et al., 2018). Soil microbes arbitrate in various beneficial soil processes like nutrient fixation/mobilization, biodegrade agro-chemicals, improve soil conditions, and suppress soil pathogens (Lupwayi et al., 2011; Przygocka-Cyna and Grzebisz, 2018). Due to competition of crop growers to produce more and more yields to get maximum return enhances the usage of mineral fertilizers and results in ill effect on soil and produce have turn their attention to use biofertilizer in integration with mineral fertilizers (Savci, 2012; Rachel et al., 2018).

Injudicious use of mineral fertilizers to feed the rising population also poses severe threat to environment. Under the prevailing circumstances, the eco-friendly approach to use microbial inoculants is valid option and inspiring the end users (Hardoim et al., 2008; Noreen et al., 2012). The various means i.e., fixing nutrients (nitrogen), solubilizing / mobilizing nutrients, releasing plant hormones, vitamins and antibiotics, inducing stress resistance etc. opted by PGPR for plant growth promotion (Sinha et al., 2014; Bhat et al., 2019). The beneficial rhizosphere microbes are involved in transforming nutrients and supplementing the mineral fertilizers and results in better crop yields (Singh et al., 2011; Vargas et al., 2017). The fixation / losses of nutrients (~60-90%) and about 10-40% is available for plants and microbial inoculants have prime significance in this regard and enhance crop growth/yield and healthy environment (Adesemoye and Kloepper, 2009; Lin et al., 2019).

The best explored PGPR belong to genus i.e., *Rhizobium*. The valuable effect of *Rhizobium* species is recognized splendidly in legumes and non-legumes. The *Rhizobium* sp. due to its root colonizing capability could be used efficiently as potential PGPR (Gouda et al., 2017; Vargas et al., 2017). Species of *Rhizobium* responsible for symbiosis with legumes also reported as asymbiotic means that these species can behave as PGPR in non-legumes (Dobbelaere et al., 2003; Hussain et al., 2009, 2016, 2018). The rhizosphere microbes produce metabolites i.e., primary and secondary for stress relief is well demonstrated and documented (Verbon and Liberman, 2016; Rachel et al., 2018). The exploration of *Rhizobium* species as potential PGPR in non-legumes such as cereals is also established and provides principal rank in agricultural system and can become tool for food security in sustained way (Sessitsch et al., 2002; Gouda et al., 2017; Vargas et al., 2017).

Rhizobium species improve the crop yields by improving root system architecture, increasing lateral roots by producing growth hormones, repressing plant pathogens by releasing antibiotics/siderophores, solubilizing /mobilizing inorganic/organic fixed nutrients, diminishing adverse effects of biotic stresses by producing ACC deaminase, volatile organic compounds and lytic enzymes, and role in systemic resistance (Hardoim et al., 2008; Mehboob et al., 2008; Vargas et al., 2017). Plant nutritionists are in search of such roles by the most promising microbes of agriculture system i.e., *Rhizobium*. Role of *Rhizobium* species in bio-control has also been described (Pacheco-Villalobos et al., 2016; Ullah et al., 2017a; Vargas et al., 2017). *Rhizobium* species can modulate the endogenous plant hormones. It has also been demonstrated that *Rhizobium* sp. influence the various plants processes (Zahir et al., 2010a; Qureshi et al., 2013; Datta and Chakrabartty, 2014; Lin et al., 2019). The use of *Rhizobium* sp. in improving crop growth has been documented by numerous researchers and obtained marvelous results (Hussain et al., 2009; 2016, 2018, 2019; Zahir et al., 2010a,b; Ullah et al., 2017 a,b; Vragas et al., 2017). Studies were aimed to evaluate the relative potential of *Rhizobium* species for the growth and yield promotion of wheat and rice.

Material and Methods

Isolation and Screening of Isolates

Isolations were carried out from the nodules of particular legumes viz. (mungbean, berseem, chickpea, lentil and peanut) on YEM (yeast extract mannitol) (Vincent, 1970). The YEM medium was autoclaved at 15-20 psi pressure and 121^{o}C temperature for 20-30 minutes. The autoclaved YEM medium was plated aseptically in laminar air flow cabinets and exposed to UV light for 30 minutes. The nodules were detached from the roots of legume plants, washed with tap water and then 4-5 washings with autoclaved distilled water (Russell et al., 1982). The nodule sap was collected by puncturing the nodules with sterilized forceps, streaked on YEM and incubated at 28±2^{o}C for 48-72 hours. The bacterial colonies were purified on fresh plates for few times. After repeated purification, the purified growth was preserved at 5±1^{o}C in eppendorf tubes for further studies. The isolates of each specie have been checked for plant infectivity test to assess that isolate was *Rhizobium* sp of that specific legume. The isolates showed promising results have been screened for different biochemical characteristics.

Determination of IAA Equivalents

The IAA content/equivalents with and without L-tryptophan (L-TRP) were analyzed for three isolates of each *Rhizobium* specie. The test tubes containing sterilized general-purpose medium (GPM) inoculated with each bacterial isolate and incubated at 28±2°C for one week. The incubated medium was centrifuged @10000 rpm for 15-20 minutes and filtered. The IAA equivalents were determined using Salkowski's reagent (Sarwar et al., 1992). Then performed the biochemical testing viz. Congo red, organic acid production (BTB test), urease for each isolate. Isolates showed the high IAA content/equivalents (Mb$_3$, Br$_3$, Cp$_3$, Lt$_3$ and Pt$_3$) with and without L-TRP were chosen for field studies (Table 1).

Table 1. Different stats/traits of rhizobium species under study.

Rhizobium species	Isolates	L-TRP* [-] (µg mL^{-1})	L-TRP [+] (µg mL^{-1})	Congo red test	BTB* test	Urease test	Root colonization in Rice X 10^4
Mung bean (MB)	Mb$_1$	2.91	3.52				
	Mb$_2$	2.28	3.90				
	Mb$_3$	3.25	4.36	+ve	+ve	+ve	38
Berseem (BR)	Br$_1$	2.09	2.83				
	Br$_2$	2.16	2.64				
	Br$_3$	3.21	3.93	+ve	+ve	+ve	32
Chickpea (CP)	Cp$_1$	2.50	3.55				
	Cp$_2$	2.75	4.30				
	Cp$_3$	3.34	4.19	+ve	+ve	+ve	33
Lentil (LT)	Lt$_1$	2.53	3.24				
	Lt$_2$	2.91	3.96				
	Lt$_3$	3.28	4.23	+ve	+ve	+ve	29
Peanut (PN)	Pt$_1$	1.97	3.09				
	Pt$_2$	2.25	3.00				
	Pt$_3$	2.63	4.07	-ve	+ve	-ve	22

L-TRP* [-]: without L-tryptophan; L-TRP [+]: with L-tryptophan;

Note: Different isolates have been assessed for auxin biosynthesis with and without L-TRP. The biochemical tests have also been carried out and isolate having higher values of IAA equivalents have been mentioned here. The root colonization assay of rice roots of above isolates has been given in the table.

Plate Experiment

The different isolates of *Rhizobium* species were assayed for root colonization of rice in a plate experiment under controlled conditions. The seeds of rice were treated with different tested *Rhizobium* species as seed coating. The rice seeds were moistened with sterilized distilled water at regular intervals. After one week of germination of rice, the seedlings were placed in sterilized distilled water. The serial dilutions of each isolate (treatment) were prepared and inoculated the already prepared plates of yeast extract mannitol agar medium (YEM) and after 48 hours of incubation at 28±2 ^{o}C. The bacterial count was carried on colony counter. The root/shoot elongation assay of wheat under axenic conditions for the selected bacterial isolates (Mb$_3$, Br$_3$, Cp$_3$, Lt$_3$ and Pt$_3$) was performed. Recorded the root/shoot length/mass after one week of germination. Then analyzed the IAA content/equivalents from root/shoot part after incubating for a week. The root/shoot (1.0 g each) was sterilized separately and placed in the sterilized GPM containing tubes, squashed, agitated repeatedly and incubated for a week. The supernatant was collected after centrifugation of medium at 1000 rpm and utilized for determination of IAA equivalents (Sarwar et al., 1992).

Inoculum Preparation

The yeast extract mannitol (YEM) broth without agar was prepared and autoclaved 15-20 psi pressure and 121°C temperature for 30 minutes. Inoculation of sterilized medium was carried out by specific isolates (Mb_3, Br_3, Cp_3, Lt_3 and Pt_3) separately and incubated at 28±2 for 3 days.

Field Studies

Field studies on wheat and rice were conducted at Soil Bacteriology and Soil Chemistry research area Ayub Agri. Research Institute, Faisalabad with pH 7.88-7.90, EC 1.52-2.0 dS m^{-1}, N 0.032-0.035% and available P 7.14-7.40 mg $kg^{-1}$and organic matter 0.70%, respectively. Plate experiment for wheat and rice for root-shoot elongation and root colonization for bacterium was carried out, respectively. Uniform fertilizer dose i.e., 120-100-60 kg NPK ha^{-1} was applied to wheat while 110-66-62 kg NPK ha^{-1} to rice was applied. Isolates of Rhizobia (mung, berseem, chickpea, lentil and peanut) viz. (Mb_3, Br_3, Cp_3, Lt_3 and Pt_3) were applied as seed coating for 30 minutes to wheat while seedlings of rice were dipped in the suspension of inoculum while control was placed for comparison. There were six treatments in which one control and five inoculation levels with five isolates of *Rhizobium* sp (Mb_3, Br_3, Cp_3, Lt_3 and Pt_3) with three repeats laid out in randomized complete block design (RCBD). Then determined the IAA equivalents from the rhizosphere soil of wheat and rice after 15 and 30 days of germination/transplanting. Wheat and rice were harvested at maturity and recorded yield parameters. Dried the grain and straw samples in oven at 70 °C for 60 minutes and analyzed for N and P. The analyses of soil samples for available P and N at harvest were performed (Bremner and Mulvaney, 1982; Olsen and Sommers, 1982).

Statistical analysis

The statistical analyses were performed using analysis of variance (ANOVA) following Randomized Complete Block Design (RCBD). The statistical analysis was performed using Statistix v8.1 software. The significance was assessed by Least Significance Difference (LSD) test for comparing means at probability level $p \leq 0.05$ (Steel et al., 1997).

Results

Results presented in tables and graphs revealed that *Rhizobium* species enhanced the yield attributes of both crops i.e., wheat and rice. Isolates of *Rhizobium* sp exerted more affirmative effect than the control of both crops. Isolates of *Rhizobium* species either tested in lab or field environment quite amazingly. Results presented in Table 1 clearly demonstrated that three isolates of each host species produced IAA equivalents with and without L-TRP and each isolate showed that the effect was more pronounced with L-TRP (Table 1). The biochemical testing of these isolates was carried out (Table 1). The isolates having higher IAA equivalents with and without L-TRP were observed with Mb_3, Br_3, Cp_3, Lt_3, and Pt_3 as shown in the Table 1. The values of IAA equivalents were observed by Mb_3, Br_3, Cp_3, Lt_3, and Pt_3 i.e., 3.25, 3.21, 3.34, 3.28 and 2.63 µg mL^{-1} and value was enhanced to 4.36, 3.93, 4.19, 4.23 and 4.07 µg mL^{-1} with application of L-TRP, respectively. The root colonization was performed in plate experiment and bacterial count was carried out by standard dilution plate technique. Results presented in Table 1 revealed that different isolates showed variable response for bacterial root colonization. The highest count was observed with Mb_3 i.e., 38×10^4 while lowest was observed with Pt_3 i.e., 22×10^4.

Data regarding root/shoot parameters of wheat and IAA equivalents in root/shoot (Table 2) demonstrated that isolates improved the root/shoot considerably. The root/shoot length / mass significantly higher than control with bacterial isolates. The maximum root/shoot length / mass was observed with isolate (Mb_3) i.e., 14.0, 13.5 cm and 1.26, 2.65 g as compared to control i.e., 10.50, 11.25 cm and 1.07, 1.87 g, respectively. The isolate Mb_3 showed maximum IAA equivalents in root/shoot i.e., 1.81, 2.09 µg g^{-1}, respectively compared to control.

Results relating to biomass/grain or paddy yield of wheat and rice (Figure 1 and 2) explicitly exhibited that inoculation of *Rhizobium* species increased the wheat and rice yield significantly. Each isolate of *Rhizobium* species increased the biomass/paddy of rice and biomass/grain yield of wheat and maximum biomass/paddy and biomass/grains was produced with Br_3 and Mb_3 i.e., 24.20/4.63 and 13.80/4.92 t ha^{-1} in comparison to control i.e., 19.60/4.21 and 12.13/4.50 t ha^{-1}, respectively. The biomass/paddy yield of rice with Mb_3, Br_3 and Cp_3 showed statistically at par with slight variations. The lowest biomass/paddy and biomass/grains yield with *Rhizobium* sp (Pt_3) but it was also higher than control. Percent increase in biomass and paddy yield with different *Rhizobium* species (Mb_3, Br_3, Cp_3, Lt_3, and Pt_3) was observed (21.58, 23.47, 16.99, 14.95, and 8.67%) and (10.93, 9.98, 7.83, 4.99 and 2.85%), respectively. Percent increase in biomass and grain yield of wheat with different *Rhizobium* species (Mb_3, Br_3, Cp_3, Lt_3, and Pt_3) was observed (13.77, 11.13, 9.40, 7.58 and 6.51%) and (9.33, 7.11, 4.89, 1.78, 1.56%), respectively.

Table 2. Root-shoot elongation assay of wheat as affected by different treatments.

Treatments	Shoot length (cm)	Root length (cm)	Shoot Mass (g)	Root mass (g)	IAA in shoot (µg g^{-1})	IAA in root (µg g^{-1})
Control	11.25 d	10.50 d	1.87 d	1.07 c	1.17 d	0.86 d
Rhizobium sp (MB) (Mb_3)	13.50 a	14.00 a	2.65 a	1.26 a	2.09 a	1.81 a
Rhizobium sp (BR) (Br_3)	12.75 b	13.25 b	2.39 ab	1.17 b	1.95 ab	1.64 ab
Rhizobium sp (CP) (Cp_3)	12.55 b	13.00 b	2.32 bc	1.15 b	1.64 bc	1.53 b
Rhizobium sp (LT) (Lt_3)	12.25 bc	11.75 c	2.18 bc	1.12 bc	1.61 bc	1.25 c
Rhizobium sp (PN) (Pt_3)	11.75 cd	10.75 d	2.07 cd	1.10 bc	1.53 cd	1.17 c
LSD	0.7063	0.6841	0.2843	0.0674	0.3601	0.1757

* Mean values with different letter(s) show significant difference ($P \leq 0.05$) as Least Significance Difference Test

Note: The bioassay or root/shoot elongation assay of wheat was carried out and IAA equivalents in root/shoot has been determined and mentioned.

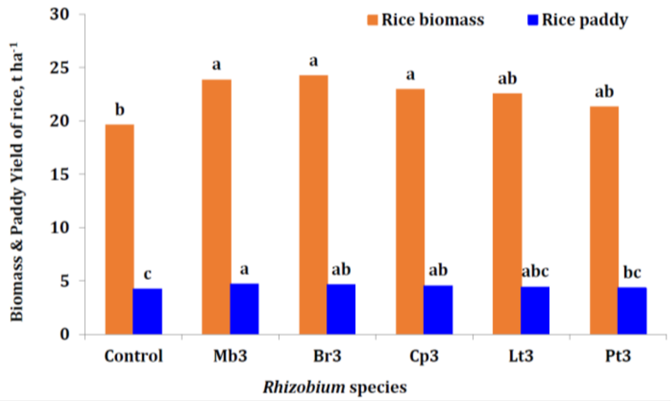

Figure 1. The biomass/paddy yield of rice

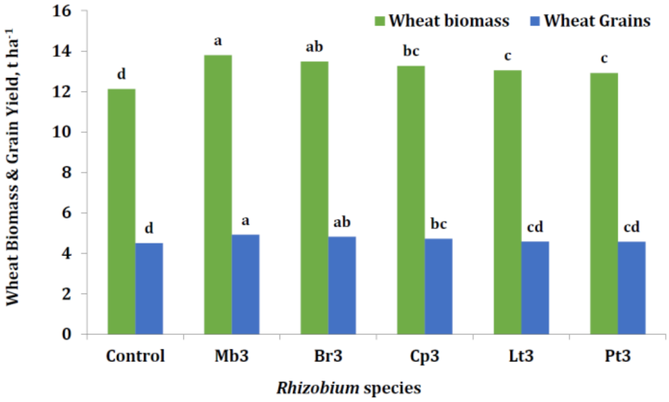

Figure 2. The biomass/grain yield of wheat

Results regarding IAA contents in the rhizosphere soil of rice and wheat after 15 and 30 days transplanting of rice/germination of wheat (Figure 3 and 4) clearly showed that bacterial inoculation of *Rhizobium* species increased the IAA content significantly. Isolates of *Rhizobium* species increased the IAA equivalents in rice and highest IAA was observed with Cp_3 after 15 days of transplanting i.e., 2.50 and 1.62 with Mb_3 after 30 days of transplanting in rice as compared to control i.e., 1.88 and 1.21 µg g^{-1}, respectively. Whereas in wheat, *Rhizobium* species enhanced the IAA content and maximum value was obtained (3.14 µg g^{-1}) after 15 days of germination while after 30 days of germination maximum value was obtained with Cp_3 i.e., 1.83 µg g^{-1} as compared to control (1.78 and 1.36 µg g^{-1}), respectively. The minimum value of IAA content after 15-days and 30-days transplanting/germination was obtained with Pt_3.

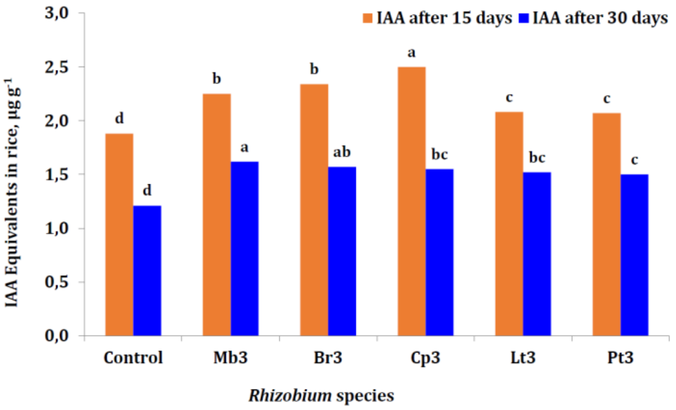

Figure 3. IAA Equivalents in rhizosphere soil of rice after 15 and 30 days of transplanting

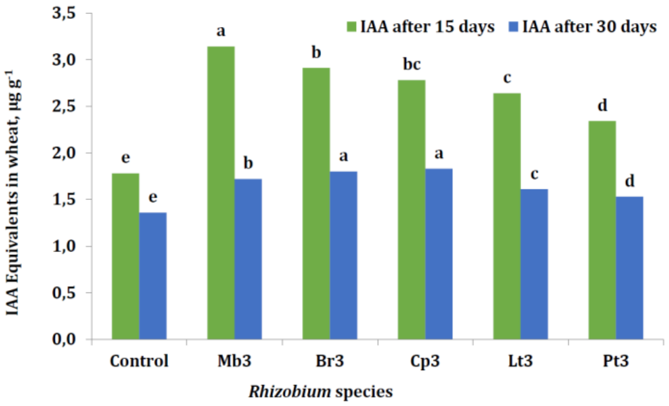

Figure 4. IAA Equivalents in rhizosphere soil of wheat after 15 and 30 days of germination

Results regarding physical attributes i.e., plant height, tillers m^{-2} and 1000 paddy/grain weight in rice and wheat (Table 3). Results clearly demonstrated that *Rhizobium* species enhanced the yield attributes significantly. The maximum plant height, no. of tiller m^{-2} and 1000 paddy weight of rice was obtained with Mb_3 i.e., 118.5, 293, 27.07 while maximum parameters in wheat i.e., 121.1, 466 and 48.70 was obtained and that was significantly higher than control.

Table 3. Rice and wheat yield parameters as affected by different treatments.

Treatments	Rice			Wheat		
	Plant height (cm)	Tillers m^{-2}	1000-Paddy weight (g)	Plant height (cm)	Tillers m^{-2}	1000-Grain weight (g)
Control	111.8 cd	277 c	25.10 c	109.2 c	387 f	42.58 e
Rhizobium sp (MB) (Mb_3)	118.5 a	293 a	27.07 a	121.1 a	466 b	48.70 a
Rhizobium sp (BR) (Br_3)	115.1 abc	289 ab	26.60 a	119.4 a	487 a	47.31 b
Rhizobium sp (CP) (Cp_3)	116.9 ab	286 abc	25.73 b	115.1 b	434 c	45.10 c
Rhizobium sp (LT) (Lt_3)	113.5 bcd	281 bc	25.60 bc	113.5 b	415 d	44.54 cd
Rhizobium sp (PN) (Pt_3)	110.1 d	280 bc	25.50 bc	111.8 bc	402 e	43.62 de
LSD	4.689	10.279	0.5048	3.9238	13.224	1.1317

* Mean values with different letter(s) show significant difference ($P \leq 0.05$) as Least Significance Difference Test
Note: The rice and wheat growth/yield parameters recorded at harvesting of both crops was mentioned in this table.

Results regarding plant and grain N, P content of wheat and soil parameters (Table 4) clearly demonstrated the bacterial isolates improved the grain and straw N, P content significantly. *Rhizobium* species isolates (Mb_3) produced the maximum wheat grain N, P content i.e., 1.648, 0.417% and wheat straw N, P content i.e., 0.337, 0.148% that is significantly higher than control (1.596, 0.321 and 0.282, 0.110%), respectively. The soil parameters tested i.e., Available P and soil N showed that soil N had significant higher values in inoculated treatments than control while statistically non-significant higher values was also observed in soil available P. The highest soil N available P was observed with Mb_3 i.e., 0.034% and 10.78 ppm than control i.e., 0.030% and 6.86 ppm, respectively.

Table 4. Influence of treatments on N, P content of wheat and soil analysis at harvest.

Treatments	Grain N (%)	Grain P (%)	Straw N (%)	Straw P (%)	Avail. P (ppm)	Soil N (%)
Control	1.596 d	0.321 e	0.282 c	0.110 d	6.86	0.030 b
Rhizobium sp (MB) (Mb_3)	1.648 a	0.417 a	0.337 a	0.148 a	10.78	0.034 a
Rhizobium sp (BR) (Br_3)	1.639 ab	0.386 b	0.327 a	0.140 ab	9.8	0.032 ab
Rhizobium sp (CP) (Cp_3)	1.629 b	0.369 bc	0.311 b	0.133 abc	8.82	0.032 ab
Rhizobium sp (LT) (Lt_3)	1.615 c	0.359 cd	0.307 b	0.128 bc	8.82	0.031 ab
Rhizobium sp (PN) (Pt_3)	1.609 c	0.335 de	0.301 b	0.122 cd	7.84	0.030 b
LSD	0.013	0.0237	0.0133	0.0159	5.2587	0.003

* Mean values with different letter(s) show significant difference ($P \leq 0.05$) as Least Significance Difference Test
Note: The table 4 contains wheat grain N and P content and soil N and available P at harvest.

Results regarding plant and grain N, P content of rice and soil parameters (Table 5) clearly showed that isolates of *Rhizobium* species improved the grain and straw N, P content significantly. *Rhizobium* species isolates (Mb_3) produced the maximum grain N, P content i.e., 1.40, 0.345% and straw N, P content i.e., 0.51, 0.138% that is significantly higher than control (1.33, 0.309 and 0.46, 0.107%), respectively. The tested soil parameters i.e., Available P and soil N showed that soil N was significantly higher in inoculated treatments than control. The highest soil N and available P was observed with Mb_3 i.e., 0.041% and 9.303 ppm than control i.e., 0.036% and 7.367 ppm, respectively.

Table 5. Influence of treatments on N, P content of rice and soil analysis at harvest.

Treatments	Grain N (%)	Grain P (%)	Straw N (%)	Straw P (%)	Avail. P (ppm)	Soil N (%)
Control	1.33 d	0.309 c	0.46 c	0.107 d	7.367 c	0.036 d
Rhizobium sp (MB) (Mb_3)	1.40 a	0.345 a	0.51 a	0.138 ab	9.303 ab	0.041 a
Rhizobium sp (BR) (Br_3)	1.38 b	0.337 a	0.50 a	0.145 a	9.787 a	0.040 ab
Rhizobium sp (CP) (Cp_3)	1.37 b	0.325 b	0.50 ab	0.132 bc	9.303 ab	0.039 bc
Rhizobium sp (LT) (Lt_3)	1.36 c	0.321 bc	0.49 ab	0.129 bc	9.300 ab	0.038 bcd
Rhizobium sp (PN) (Pt_3)	1.35 c	0.313 c	0.48 b	0.122 c	7.85 bc	0.037 cd
LSD	0.014	0.0119	0.0176	0.0102	1.674	0.002

* Mean values with different letter(s) show significant difference ($P \leq 0.05$) as Least Significance Difference Test
Note: The table 5 contains rice grain N and P content and soil N and available P at harvest.

Discussion

Isolates of *Rhizobium* species influenced the wheat and rice growth parameters positively while variable response of isolates was observed. The biosynthesis of auxins was observed with and without L-TRP in different isolates of *Rhizobium* species. Five isolates of each *Rhizobium* sp were characterized. Application of L-TRP has more assenting effect on IAA content than without L-TRP. Isolates (Mb_3, Br_3, Cp_3, Lt_3 and Pt_3) were selected on the basis of IAA equivalents. Isolates showed considerable increase in IAA equivalents when L-TRP was applied exogenously. The bacterial isolate Mb_3 produced elevated values of IAA in the presence/absence of L-TRP and biosynthesis of auxin with and without precursor as observed by several researchers (Zahir et al., 2010a, b; Hussain et al., 2013; Qureshi et al., 2013; Verbon and Liberman, 2016).

The bioassay (plate experiment) of bacterial isolates was carried out to assess the growth promoting traits of wheat. The root-shoot elongation assays (bioassay) clearly showed that bacterial isolates boosted the root/shoot length/mass and IAA content in root/shoot. The bacterial isolates exhibited more IAA content in root/shoot (Zahir et al., 2004). The lab bioassay of rice for root colonizing capability of rice showed that bacterial colonized the rice root and showed variable response (Naher et al., 2009; Ullah et al., 2017a, b; Rachel et al., 2018).

Field studies on rice and wheat illustrated higher growth and yield attributes, IAA in the rhizosphere soil, N, P content in grain or straw and soil parameters after harvest might be attributed to the biosynthesis of hormones changing the root architecture, more roots/shoot length / mass results in better crop growth and ultimately yield of crops (Dazzo and Yanni, 2006; Mehboob et al., 2011; Hussain et al., 2014 a,b). The bacterial inoculation due to its root colonizing capability boosted growth and yield of cereals might be ascribed as the hormone/siderophore production, improvement in nutrient uptake and inducing systemic resistance (Akhtar et al., 2013; Vargas et al., 2017; Rachel et al., 2018). The bacterial inoculation increased the plant ontogeny owing to release of auxins and amended the endogenous hormone status and ethylene level repression (Ullah et al., 2017 a,b). The improvement of wheat and rice yield and their yield components by *Rhizobium* inoculation might be accredited to several mechanisms viz. production of biologically active substances, siderophores and organic acids, suppression of plant pathogens and higher nutrient uptake (Mehboob et al., 2011; Parthiban et al., 2016; Vargas et al., 2017).

The bacterial inoculation improved the yield parameters by producing primary and secondary volatiles and enhanced auxin biosynthesis in the rhizosphere (Hossain and Martensson, 2008; Hussain et al., 2014 a,b; Ullah et al., 2017b). The microbial activities in the rhizosphere soil were boosted and impacted the crop growth positively and produced economical yields (Zahir et al., 2010; Gopalakrishnan et al., 2015). Microbial inoculation of cereals improved the yields and bettered nutrient content in plant parts owing to the expanded root system, more lateral roots, better nutrient acquisition to sustain the crop yields (Mehboob et al., 2011; Parthiban et al., 2016).

Inoculation expanded the root surface area by producing the lateral roots for better nutrient acquisition (Naher et al., 2009). The increase in grain and straw N and P might be due to better nutrient mobilization by bacterial inoculation (Ullah et al., 2017b). The rhizosphere microbes having the potential of solubilizing insoluble nutrients improved the nutrient content in plant parts and enhanced the quality of grains and straw (Hemissi et al., 2011; Parthiban et al., 2016; Mukhongo et al., 2017). Isolates released the organic acids that solubilized the phosphates, lowered the rhizosphere soil pH and results in better uptake of nutrients by plants reported by numerous researchers (Berger et al., 2013; Nagy and Pinte, 2015; Parthiban et al., 2016; Mukhongo et al., 2017). The increased levels of N and P content in grain and straw of wheat and rice due to bacteria inoculation owing to enhance root surface area and growth due the presence of plant hormones in the rhizosphere produced by microbes (Hussain et al., 2009; Rachel et al., 2018). The increased levels of soil nitrogen and available P might be ascribed by the solubilization of phosphates, production of organic acids, more root exudations due to microbial activities or microbe meditated processes (Ullah et al., 2017a,b; Vargas et al., 2017; Przygocka-Cyna and Grzebisz, 2018). The interaction of bacterial isolates and roots might enhance the root mass and respiration results in more water and nutrient uptake in shoots and grains. Microbial inoculation improved the crop growth and yield owing to the more nutrient intake by plants, more photosynthetic activity by plants and release of hormones (Parthiban et al., 2016; Przygocka-Cyna and Grzebisz, 2018).

Studies deduced that *Rhizobium* sp can be considered as effective PGPR in non-legumes after thorough screening. *Rhizobium* species responded positively in wheat and rice and may be used as bacterial inoculants for the field crops.

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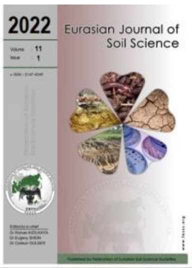

Contrasting rice management systems – Site-specific effects on soil parameters

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Abstract

Conventional rice production systems (CRPS) with continuous flooding demand much water. While population growth increases the demand for rice and, consequently, water consumption, agricultural production needs to reduce its water demand. The System of Rice Intensification (SRI) is promoted as an alternative cropland management strategy to sustainably maintain rice yields while optimizing water use. Here, we aimed at investigating whether different management translates into differences in soil parameters. To this end, the two contrasting rice production systems were compared on the same soil types, at four different study sites of D.I. Yogyakarta Province, Indonesia. Crop yields were estimated, and soils were analysed for soil total soil organic carbon (TOC), total nitrogen (TN), dissolved organic carbon (DOC), macro-aggregate stability, and a fungal biomarker (ergosterol) indicative of oxidative soil conditions. Rice yields in the study area were between 6.7 and 9 t ha^{-1}. For TOC, the combined effect of management and site was significant; in particular, in Kulonprogo and Bantul, SRI significantly exceeded CRPS' TOC values. However, a significant management effect was observed for ergosterol and DOC concentrations. Significantly higher ergosterol concentrations in SRI vs CRPS were found in Sleman and Bantul. DOC was significantly higher under SRI compared to CRPS only in Sleman. DOC and ergosterol were most responsive to management and were improved in SRI systems. The observed site-specific effects suggest the importance to consider the prevailing site conditions for adapting management strategies.

Keywords: System of rice intensification (SRI), water use efficiency, soil parameters, on-site farm studies, Indonesia.

Introduction

Worldwide 50-60% of the population uses rice as their primary staple crop (FAO, 2013), in Indonesia even 90% (Kurniadiningsih and Legowo, 2012). Increased demand for rice contributes to increasing worldwide water demand. Competition among alternative uses for surface and groundwater is starting to affect the agricultural sector. As the largest agricultural consumer of irrigation water worldwide with 24-30 % of the total freshwater withdrawals (Gathorne-Hardy et al., 2016), the rice sector was under increasing pressure to economize water use (Uphoff, 2011). As an anthropogenic source of methane (CH_4) emissions, rice paddies also contribute to global warming (Suryavanshi et al., 2013). Hence, the system of rice intensification (SRI)

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has being promoted as an alternative resource management strategy in wide areas of eastern Asia, and particularly in Indonesia (Dobermann, 2004). SRI focuses on water and nutrient use efficiency to increase rice yields while reducing external inputs (Stoop et al., 2002; Uphoff, 2003; Krupnik et al., 2012). Additionally, efficient irrigation water management such as intermittent drainage can reduce CH_4 emissions from paddy soil (Suryavanshi et al., 2013). SRI was reported to have a positive impact on grain yield (Maftukhah et al., 2015), directly benefitting both, subsistence and semi-commercial farming households. Conventional flooded rice production and SRI have several distinct features: SRI favours younger seedlings, uses wider spacing of plants, emphasizes compost or other organic fertilizers, and improves water use efficiency as compared to conventional methods (Uphoff, 1999; Uphoff et al., 2011; Gathorne-Hardy et al., 2016). Reducing demand for water and seeds, as well as, greenhouse gas emissions make SRI a promising alternative production system that is affordable to poor and marginalized communities and farmers facing water scarcity.

A more controversial claim is that the productivity of the system increases through positive synergetic interactions among the SRI practices (Stoop et al., 2002; Uphoff, 2011). Application of organic fertilizers could maintain rice yields under SRI (Dawe et al., 2003; Tsujimoto et al., 2009); intermittent drainage and intensive irrigation during SRI cultivation lead to mineralization of the accumulated soil organic matter, providing more nutrients for rice. This practice also improved the development of the root system and enhanced plant growth (Tsujimoto et al., 2009). The management of the rice fields could also affect soil properties. Tang et al. (2011) reported that adding organic fertilizer in rice fields can promote macro-aggregate formation and improve soil stability. Intact soil aggregates improve physical and chemical processes in soil. These processes are influenced by different factors including environmental conditions, soil management, agronomic practices, and soil properties (Zhang et al., 2016). Understanding how management practices in rice farming influence soil properties is important for sustainable production.

This study aimed at investigating soil parameters as potential indicators for changes in management systems. We hypothesized that different management systems (CRPS vs. SRI) are reflected in changes in soil parameters. Four test sites in D.I. Yogyakarta, where SRI and CRPS are practiced in proximity by smallholder farmers, were established as demonstration farms to educate SRI practices to farmers under a program of the Irrigation section, Indonesian Ministry of Public Work. These test sites were selected to study the influence of different management practices on comparable fields on the same soil types.

Material and Methods

Experimental site

The four study sites are located in the districts of Gunungkidul, Kulonprogo, Bantul, and Sleman in D.I. Yogyakarta, Java Island, Indonesia (Figure 1). Most of the farmers in these areas are smallholders owning 0.5–1 ha of agricultural land. The sites differ in soil types with Leptosol in Gunungkidul and Kulonprogo, Ferralsol in Bantul, and Vertisol in Sleman (Table 1, FAO, 2015). The soil physical characteristics of each site and production system are described in Table 2. These sites were selected because SRI and CRPS are being used next to each other on the same soil types. Crop yields were estimated by the farmers for each field (Table 3).

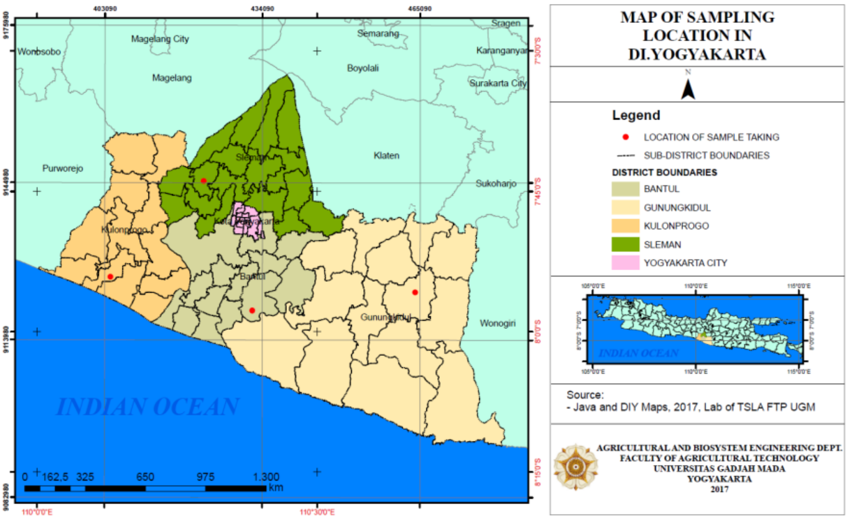

Figure 1. Location of study sites in D.I. Yogyakarta, Java Island, Indonesia. The four sites Gunungkidul, Kulonprogo, Bantul, Sleman are indicated by red points.

Table 1. Description of study sites summarizing locations, GPS data, soil type, and available irrigation schemes.

Site	Gunungkidul (G)	Kulonprogo (K)	Bantul (B)	Sleman (S)
GPS coordinates	7°55’49.42” S 110°40’30.56” E	7°54’8.93” S 110°07’53.01” E	7°43’55.01” S 110°17’51.28” E	7°57’45.82” S 110°23’04.30” E
Soil type	Leptosol	Leptosol	Ferralsol	Vertisol
Water availability	all year	No irrigation from June - Oct	Less water from Jun - Oct	all year

Table 2. Particle density (ρ_s), bulk density (ρ_b), and texture for soils under System of Rice Intensification (SRI) and Conventional Rice Production Systems (CRPS) at the study sites.

Site		Physical soil characteristics					Texture
		ρ_s	ρ_b	Particle size distribution			
		[g cm^{-3}]	[g cm^{-3}]	% Sand	% Clay	% Silt	
Gunungkidul	SRI	2.31	1.63	23	25	51	Silt Loam
	CRPS	2.03	1.59	23	26	52	Silt Loam
Kulonprogo	SRI	1.99	1.62	36	40	21	Clay
	CRPS	2.01	1.52	31	53	16	Clay
Bantul	SRI	2.17	1.36	14	45	41	Silty clay
	CRPS	2.35	1.57	17	41	43	Silty clay loam
Sleman	SRI	1.90	1.50	26	39	35	Clay loam
	CRPS	2.29	1.72	18	45	37	Clay

Table 3. Overview of soil management practices at the study sites. The cultivation type is given: System of Rice Intensification (SRI), or Conventional Rice Production Systems (CRPS). Fertilizer application rates for all schemes are given for compost, urea, and NPK (nitrogen, phosphate, and potassium fertilizer). ZA is ammonium sulphate (nitrogen 21 w/w%, sulphur 24 w/w%). ΣC is the sum of (organic) carbon from the fertilizers added (in t ha^{-1}) and ΣN sum of nitrogen from the fertilizers added (in t ha^{-1}). Yields (in t ha^{-1}) refer to the cropping season.

Site	Cultivation	SRI Start	Fertilizer application rates [t ha^{-1}]				ΣC [t ha^{-1}]	ΣN [t ha^{-1}]	Rice yield [t ha^{-1}]
			Compost	Urea	NPK	remarks			
Gunungkidul	SRI	2000	15	0.10	0.15		3.42	0.32	7.50
	CRPS	---		0.20	0.20	0.10 ZA	0.04	0.14	6.10
Kulonprogo	SRI	2003	10	0.15	0.10		2.30	0.25	9.00
	CRPS	---		0.30	0.20		0.06	0.17	8.60
Bantul	SRI	2006	20	0.15	0.05		4.56	0.41	8.00
	CRPS	---		0.20	0.10	0.10 ZA	0.04	0.13	6.80
Sleman	SRI	2007	10		0.10		2.27	0.18	8.00
	CRPS	---		0.20	0.20		0.04	0.12	6.70

Management systems

Some farmers in this area have been using SRI since 2000, while others are still producing rice in a conventional way. Land management is fully comparable among the sites, the cropping patterns were the same (rice-rice-secondary crop (*palawija*)). For both systems (SRI and CRPS), land was prepared under wet condition using a hand tractor. Plowing and harrowing were carried out during land preparation to attain puddled soils as described by De Datta (1983). In this study, a local rice variety (*Oryza sativa* var. Javanica (Rojolele)) was cultivated by the farmers for its high economic value. SRI uses young seedlings (7–10 days) and single seedlings with wider spacing (30 x 30 cm), while CRPS uses older seedlings (20–25 days) and three to four seedlings with smaller spacing (25 x 25 cm).

While SRI systems are irrigated intermittently, CRPS fields are continuously flooded. In both systems, the fields are flooded to a water depth of 3-5 cm for the first ten days after transplanting to optimize seedling development and control weeds. In CRPS, fields remained flooded with water 5-10 cm deep during the entire period of rice growth. Contrarily, in SRI fields, water levels were reduced to 1 cm above soil surface ten days after transplanting and kept at this level for sufficient oxygen supply until the generative stage. At the generative stage, water was increased to 5 cm to support grain formation, then continued with 1 cm after grains had appeared. For nutrient management, CRPS farmers followed the recommendations of the Indonesian government for synthetic fertilizers (Kementerian Pertanian, 2007). SRI farmers applied both compost and a reduced amount of synthetic fertilizer compared to CRPS farmers (Table 3).

Soil sampling and analyses

Soil samples were collected in 2016 during the vegetative phase at a depth of 0-15 cm (topsoil) in three replicates per field. Field sizes were approximately 50 m^2. Total soil organic carbon (TOC) and total nitrogen (TN) were analysed by dry combustion elementary analysis (the soils did not contain carbonates) according to ISO 10694 (2009), using a Carlo Erba CNA 1500 chromatographic separation system equipped with a thermo-conductivity detector.

The particle size distribution in mineral soil material was determined by sieving and sedimentation following ISO 11277 (2009). Particle density (ρ_s) was determined using a pycnometer. The bulk density (ρ_b) was determined using the wax method which is used in Indonesia (Agus et al., 2006) based on Russell and Balcerek (1944). Dissolved organic carbon (DOC) was determined in a 1:10 w/v extract of soil in water and analysed using a UV-VIS spectrophotometer (Agilent 8453 Diode Array UV-VIS), at a wavelength of 254 nm (Brandstetter et al., 1996).

Ergosterol, a biomarker for fungal biomass, was extracted according to (Gong et al., 2001), with minor modifications as described recently in (Ferretti et al., 2018).

Ultrasonic aggregate stability (USAS) of macro-aggregates (2000 – 250 µm) was determined at 17.4 J mL^{-1} ultrasound energy applied with an ultrasonic device (Bandelin Sonopuls HD 2200). Sonification was performed with 10 g soil in 200 ml water (Mentler and Mayer, 2004; Schomakers et al., 2011). The suspension was subsequently sieved through 250 µm mesh for further calculation of macro-aggregate stability. The sand fraction was collected after sonification with 630 J mL^{-1}.

Data evaluation and statistical analyses

Data were analysed using SigmaPlot 11.0 and summarized as means ± standard deviation (SD), except for TOC (median). The statistical significance of differences in soil parameters among different management systems and sites was analysed by ANOVA, using the Tukey post hoc test ($p < 0.05$). Differences between SRI and CRPS in the same village were tested using the t-test ($p < 0.05$). In the case of TOC levels, statistical significance was determined using the Mann-Whitney-U-test. In addition, all CRPS sites were compared to all SRI sites using t-test ($p < 0.05$), except for USAS, where only Gunungkidul and Kulonprogo were compared according to different management systems.

Results and Discussion

Our results show that, some of the investigated soil parameters can be used to distinguish between the two production systems; however, for others, site-specific effects seem to dominate. For example, TOC showed site-specific management effects, and was significantly higher under SRI than under CRPS in Kulonprogo and Bantul ($p < 0.05$, Figure 2a), while in Sleman and Gunungkidul there was no significant difference between the production systems. The higher amounts of TOC under SRI in Kulonprogo and Bantul are likely due to the application of compost, the duration since when SRI management was implemented and the respective application rate of compost (Table 3). Yang et al (2005) previously reported that TOC was significantly higher when a combination of organic and inorganic fertilizers was applied in rice fields under intermittent irrigation. Even though more aerobic conditions under SRI favor C losses from the soil via mineralization due to the higher redox potential (Dobermann, 2004), the regular compost amendment that is part of the SRI management resulted in higher TOC concentrations.

Since crop yields in both systems were comparable, we can conclude that SRI can be implemented in the studied sites. The higher N inputs (Table 3) for SRI may compensate for lower plant availability (Uphoff, 2003). In our study, this has resulted in significantly elevated soil TN concentrations for Bantul and Sleman (Figure 2c, d). The turnover (mineralization) and potential losses of N were probably exceptionally high in the SRI of Gunungkidul and Kulonprogo. Hence, our results demonstrate that SRI-managed fields show many benefits over CRPS, with major drawbacks in fertilizer requirements, as also shown in another study by Thakur et al. (2022). Divergent findings in other studies might be due to differences in compared soil types rather than reflecting management differences. Bertora et al. (2018) reported that the influence of management in rice cultivation on C and N content could be strongly dependent on soil properties (soil mineralogy, soil organic carbon content) and pore-water sampling depth. The mean C/N ratios for SRI and CRPS-soils were comparable (16.9 and 16.7) and showed no statistically significant differences between the production systems (Figure 2e).

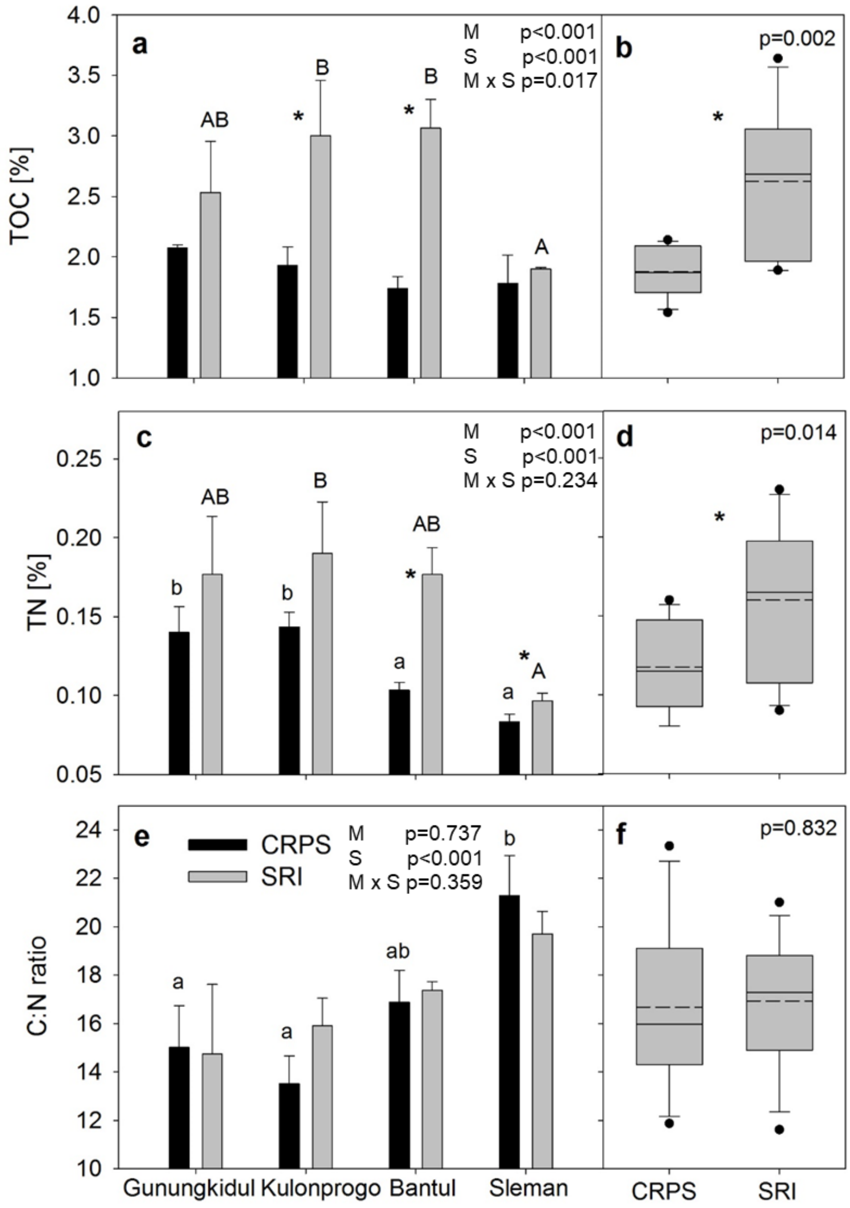

Figure 2 (a, b). Total soil organic carbon (TOC), (c, d) Total soil nitrogen (TN), (e, f) soil carbon to nitrogen (C/N) ratio in different crop management systems. Black bars indicate conventional management (CRPS), and grey bars depict system of rice intensification (SRI) at the four different locations in D.I. Yogyakarta, Java Island, Indonesia. In (a), (c) and (e), mean values of the different locations are shown, ANOVA analysis using the Tukey post-hoc test was performed for all sites. If a power of 0.8 could be reached, statistical significance ($p < 0.05$) is indicated using uppercase letters (SRI sites) or lowercase letters (CRPS sites); p-values are given in the upper right corner, for management (M), site (S), and their combined effects (MxS). Asterisks (*) indicate statistically significant differences between individual sites of different management in the same village as tested using the Mann-Whitney-U-test (a), or the t-test (c, e). In (b, d, f), box-plots of all sites of the same management system are compared (b: TOC, d: TN, f: C:N ratio). The dashed and solid lines indicate mean and median, while dot depict outliers. Statistical significance of differences was tested using the t-test; p-values are given in the upper right corner.

DOC is a bulk parameter that quantifies C solubility in water and indicates an easily available C fraction. In soils used for rice cultivation, this fraction has been suggested to result from rhizodeposition (Shen et al., 2015). Jones et al. (2014) described that the quantity of DOC depends on vegetation and soil type. We found a significant management effect on DOC concentration, which was generally higher in SRI, however, only significantly different in Sleman ($p < 0.05$, Figure 3a,b). In the other sites, the differences were not significant most likely due to weathering and soil formation (Lilienfein et al., 2004) and also high data variability. A higher DOC concentration may reflect increased rice growth and indicate more root deposits and plant residues under SRI compared to CRPS. Bertora et al. (2018) demonstrated that root exudates, plant residues, and soil organic carbon (SOC) are important sources of DOC in soil. Several studies have shown that the degradation of crop residues significantly contributes to DOC (Said-Pullicino et al., 2016), and is considered as a soil quality indicator (Wang et al., 2015).

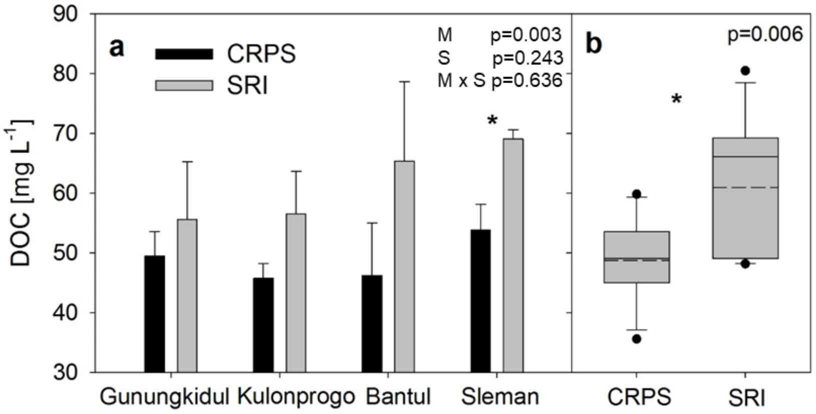

Figure 3. Dissolved organic carbon (DOC) in soils under different crop management systems. Black bars indicate conventional management (CRPS), and grey bars depict system of rice intensification (SRI) at the four different locations in D.I. Yogyakarta, Java Island, Indonesia. In (a), mean values of the different locations are shown, ANOVA-analysis was performed for all sites; p-values are given in the upper right corner, for management (M), site (S), and their combined effects (MxS). Asterisks (*) indicate statistically significant differences between individual sites of different management in the same village as tested using the t-test. In (b), box-plots of all sites of the same management system are compared. The dashed and solid lines indicate mean and median, while dot depict outliers. Statistical significance of differences was tested using the t-test; p-value is given in the upper right corner.

Ergosterol, the predominant sterol found in most fungi, is considered an indicator of fungal biomass (Srzednicki et al., 2004). The presence of fungi, in turn, signals aerobic conditions, and the fungi contribute to soil aggregation and stabilization (Ritz and Young, 2004). Again, management effects on ergosterol concentrations were detected, however, significantly higher concentrations in SRI only for Bantul and Sleman (1.81±0.28 and 1.83±0.05 mg kg^{-1}, respectively) (Figure 4a, b). The higher ergosterol was likely due to the addition of organic fertilizer and more oxidative conditions under SRI management. Interestingly, DOC and ergosterol are the only two parameters where the factorial ANOVA resulted in significant management effects, supporting higher DOC and ergosterol with SRI management. In addition, there is a significant positive correlation (r=0.532, p = 0.0074), between DOC and ergosterol. The latter has also been reported for a soil tillage experiment, where a direct positive effect of DOC for viable fungi was found (Sae-Tun et al. in revision). The authors further note a direct positive effect of viable fungi on soil aggregate stability.

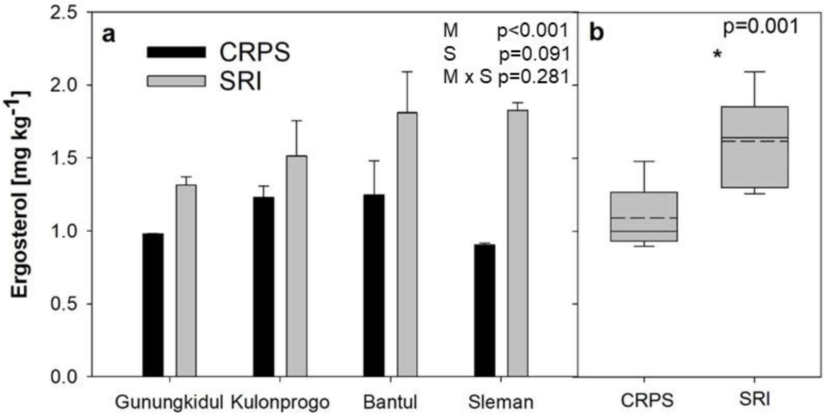

Figure 4 (a, b). Soil ergosterol concentration in different crop management systems, black bars indicate conventional management (CRPS), and grey bars depict system of rice intensification (SRI) at the four different locations in D.I. Yogyakarta, Java Island, Indonesia. In (a), mean values of the different locations are shown, ANOVA-analysis was performed for all sites; p-values are given in the upper right corner, for management (M), site (S), and their combined effects (MxS). In (b), box-plots of all sites of the same management system are compared. The dashed and solid lines indicate mean and median. Statistical significance of differences was tested using the t-test; p-values are given in the upper right corner.

Soil macro-aggregate stability is an integrative indicator of soil management changes (Mustafa et al., 2020). A higher USAS indicates better conditions for root system development, and fungal development, especially important for crop rotations in rice production systems (Menete et al., 2008). Hyphae build an extensive network extending beyond the rhizosphere and penetrate soil pores more intensively than fine roots or root hairs because of their smaller hyphal diameter (Bodner et al., 2021).

The Leptosols in Kulonprogo and Gunungkidul exhibited significantly more stable macro-aggregates under SRI conditions compared to CRPS ($p < 0.05$, Figure 5). The USAS results of Bantul and Sleman are not shown. The Vertisol (Sleman) in paddy culture is fully dispersed during the vegetation period (when the sampling was conducted), hence, no significant macro-aggregate fraction can be determined. The Ferralsol (Bantul) is dominated by micro-aggregates due to cementation by iron oxides, and ultrasound energy is too weak to disperse this cementation (also in the macro-aggregate fraction). Similarly, Yang et al (2005) found aggregates to be more water-stable under intermittent irrigation than under conditions of waterlogging (like in CRPS). This may protect organic carbon by a physicochemical mechanism in paddy soil, and protection might be further enhanced by the application of organic fertilizers. Intermittent irrigation is believed to improve oxygen supply to rice roots, decreasing aerenchym development and causing a stronger, healthier root system with potential advantages for nutrient uptake (Stoop et al., 2002). Under CRPS schemes, crop rotation is challenging and requires assiduous tillage; in contrast, soils under SRI management lend themselves much more to crop rotation because of their improved physical soil structure (macro-aggregate stability), as found for the two Leptosols in our study (Figure 5). Further, the application of organic manure and crop straw improves macro-aggregation and significantly promotes soil fertility (Bandyopadhyay et al., 2010; Huang et al., 2010).

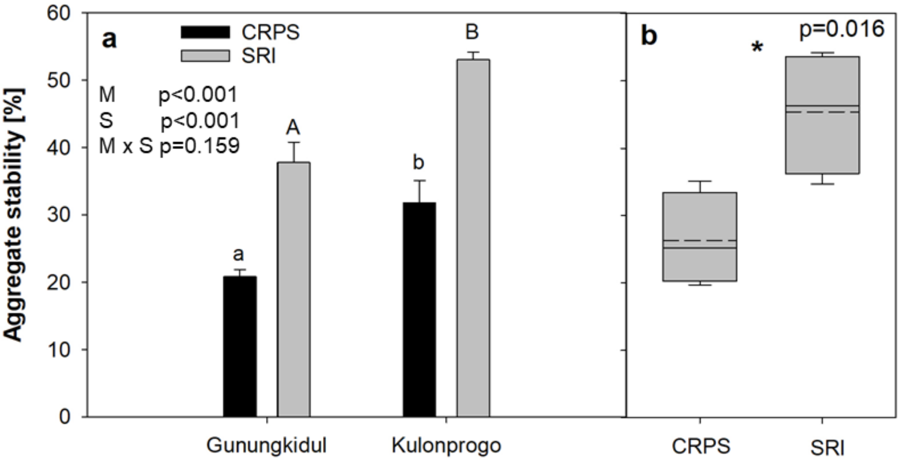

Figure 5 (a, b). Macro-aggregate stability in different crop management systems, black bars indicate conventional management (CRPS), and grey bars depict system of rice intensification (SRI) at Gunungkidul and Kulonprogo (D.I. Yogyakarta), Java Island, Indonesia. In (a), mean values of different locations are shown, ANOVA analysis using the Tukey post-hoc test was performed for all sites. If a power of 0.8 could be reached, statistical significance ($p < 0.05$) is indicated using uppercase letters (SRI sites) or lowercase letters (CRPS sites); p-values are given in the upper left corner, management (M), site (S), and their combined effects (MxS). In (b), box-plots of all sites of the same management system are compared. The dashed and solid lines indicate mean and median. Statistical significance of differences was tested using the t-test; p-values are given in the upper right corner.

The International Rice Research Institute (IRRI) showed that continuous rice cropping systems, like CRPS, lead to a significant reduction in productivity, due to a loss of organic carbon and microbial biomass (Reichardt et al., 1997). Considering that Indonesia is expected to be highly vulnerable to climate change, the present study shows that SRI could be a viable alternative rice production system to address this issue. Rendering soil conditions more aerobic could improve soil aggregation and stabilization, and thus facilitate C sequestration and contribute to climate change mitigation. Previous studies on SRI reported a deeper rooting system, which further supports SRI as more resilient than conventional systems (Uphoff and Thakur, 2019; Thakur et al., 2022).

Conclusion

As water becomes increasingly scarce, the necessity for less water-intensive production systems like SRI grows. This system is a viable option to ensure sustainable rice production under a changing climate and increased pressure to produce for a growing population, as in the present study SRI could increase DOC and ergosterol concentrations as well as sustain rice yield. The site-specific effects observed in our study suggest the importance to consider the prevailing site conditions for adapting management strategies.

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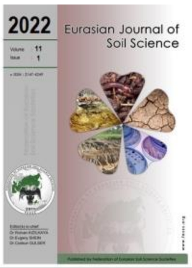

Effects of long-term tea (*Camellia sinensis*) cultivation on the earthworm populations in northern Iran

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Abstract

The earthworms' abundance is usually one of the main indicators of healthy and productive soils. However, agricultural management practices affect the earthworm population and activities. Although there is a lot of information that shows the relation between land use/land cover change and earthworms activity, very little is known about these effects under tea cultivation. Thus the current study was done to determine the effects of long-term tea cultivation on the earthworm's population and abundance in the tea plantations of Iran to distinguish effects of these practices on soil properties concerning earthworms. Hence, 58 locations of tea cultivations were randomly selected in Guilan and Mazandaran province. Earthworm were sampled by manually excavating and sorting four 30×30 cm pits by 30 cm deep in each location. Earthworms were enumerated in the field and taken to the lab for identification. Once identified, the earthworms will dry in the oven at 60°C for 48h and the dry weight registers. Some physicochemical properties of the mineral soils were determined in the laboratory. The finding indicated that the earthworms were only observed in the two from 58 locations: Bazkiagorab (Lahijan) and Shekarposhteh (Tonekabon). Three species as *Perelia kaznakovi*, *Aporrectodea trapezoides*, and *Dendrobaena veneta* were recorded from Bazkiagorab but only *P. kaznakovi* was identified in Shekarposhteh. The total population of all identified earthworms was 22 and 3 m^{-2} in the Bazkiagorab and Shekarposhteh, respectively. Results of the physicochemical analysis showed that 35% and 51% of the soils had a pH less than4.5 and organic carbon less than2%, respectively. Available phosphorus and potassium in 80% and 65% of the soils were less than 25 and 150 mg/kg, respectively. It can be concluded that monoculture and long-term tea cultivations had a negative effect on the earthworm population, in addition, it has strongly acidified the soil. It is recommended that native nitrogen-fixing trees mixed planted with tea, and more attention should be paid to nutrient Best Management Practices in tea plantations.

Keywords: Iran, acid soil, *Perelia kaznakovi*, *Aporrectodea trapezoides*, *Dendrobaena veneta*.

Introduction

Soil earthworms are one of the most important soil macrofaunae that lead to the transformation of materials into another physical stage. Soil earthworms, by eating up the particles of plant and soil residues, are a part of food chain affect important soil quality indicators such as the structure and dynamics of organic matter (Pulleman, 2002). They contribute to decaying organic matter and to incorporate them into soil. Although their drilling activities improve soil structure and increase soil aeration (Edwards and Lofty, 1972).

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The abundance of earthworms is related to the characteristics of soil and climate. Earthworms are usually absent at very acidic pH (less than 3.5) and they rarely found at pH less than 4.5 (Curry, 1998). The low number of earthworms in acidic soils has been linked to a lack of Ca^{2+} ions in acidic soils, trees and shrubs produce unpalatable and low quality foods and a C/N ratio>60, which is undesirable for earthworms. The quality of plant residual has a greater impact on the population of earthworms than the abundance and stock of soil organic matter (Boström and Lofs-Holmin, 1986). In addition to the availability of nutrients, the quality of leaf litters is affected by the content of carbohydrates, the concentration of phenolic compounds (especially tannins), etc. (Graff and Satchell, 1967).

Camellia sinensis (L.) Kuntze has been imported to Iran for more than 100 years. Nowadays, the area of plantation may reach to over 25000 ha (ITO, 2018). In recent years, excessive use of nitrogenous fertilizers, leaching of nutrients from the soil, and failure to use soil modifiers such as lime and dolomite have reduced the pH of soils in tea gardens (Shirinfekr, 2018). Jamatia and Chaudhary (2017a,b) reported that out of 17 species of earthworms observed, 13 and 15 species of earthworms were recorded in managed tea Cultivations and destroyed tea cultivations, respectively. Density (56 m^{-1}) and biomass (27 g m^{-1}) of non-native species were significantly higher in the managed tea gardens than the destroyed ones (23 m^{-1}, 17 g m^{-1}). Luthfiyah (2014) reported that the diversity of earthworms in the three tea gardens of research stations is low where *Pontoscolex* sp. had the highest density. Correlation between the density of earthworms and physical and chemical properties was observed in tea fields of PTPN XII region. Soil moisture and pH have a positive effect on the population and density of earthworms. Jamatia and Chaudhuri (2017a,b) identified 15 species of earthworms from four families by study on the structure of earthworm community in the tea Cultivations of four Tripura regions of India. Overall, the average density and biomass of earthworms, regardless of their age group, was 212 m^{-1} and 51.7 g m^{-1}, respectively.

Due to the sustainable agriculture approach and the use of biological materials in tea plantations, the population and abundance of earthworms in these climatic conditions should be investigated to determine their potential for improving the physicochemical and biological properties of the soil. This aim might be achievable by rearing and releasing of the most suitable species in the tea plantations of north Iran as there is no similar report. So far in Iran, the effect of tea cultivation on earthworm abundance as one of the most important indicators of soil quality has not been reported. This study was therefore aimed at determining the population of earthworms., identify the species resistant to acidic soil, and their relationship with soil characteristics under long term tea plantation in north of Iran.

Material and Methods

Study Sites

This study was conducted in Iranian tea cultivations in the north of Iran. This area is located in Guilan and Mazandaran provinces, between the latitudes 37°12’39” to 37°50’15” N and longitudes 49°55’57” to 50°45’31” E. The minimum and maximum altitudes of the study area are -6 m and 485 m above sea level respectively. Mean annual precipitation and temperature are 1186 mm and 17.5°C, respectively.

More than 100-y ago, these areas were covered by Hyrcanian forests trees. Later on, that tea plants (*Camellia sinensis*) imported from India and introduced in two provinces, these forests were clearly cut and changed to the tea plantations. Seven study locations in different conditions were selected as Table 1. Fiftyeight sites (Table 1) were randomly selected and their coordinate were recorded using GPS (Figure 1).

Table 1. Distribution the number of samples in different locations

Region	Visited site	Sampled sites
Astaneh Ashrafieh	11	2
Lahijan	34	7
Langrood	15	6
Roodsar	53	28
Amlash	35	10
Tonekabon	7	2
Fooman	6	3
Total	161	58

Methods

At each study site the earthworm density, earthworm biomass, earthworm species, earthworm ecological group and soil parameters were measured. On each site 1 kg of soil samples for chemical analysis were collected from four points placed at the apices of a 10-m side square. Soil samples were air-dried, ground through a 2 mm sieve and analyzed for soil pH (1:1 w/v) in a soil: water suspension, organic carbon using Walkley-Black method, available P content by Bray-1 method, and available potassium by 1N NH_4OAc method (Motsara and Roy, 2008).

Figure 1. Locations of soils sampled

Earthworms were collected in spring of three years ago using four random samples (30cm×30cm×30cm) with 15 m distance between the pits. By removing the litter layer, the soil was gradually dug to a depth of 0-30 cm using a shovel. The soil from each plot was spread on polyethylene sheets and hand-sorted (Thielemann, 1986). The earthworms were enumerated, collected and were brought into the glass jars with sufficient amount of moist soil to the laboratory.

In the laboratory, the collected earthworms were washed, weighed, and sexual maturity and body color were recorded. The earthworms were fixed with 70% alcohol and morphologically identified according to the available keys (Edwards and Lofty 1972; Latif et al., 2009; Reynolds and Mısırlıoğlu, 2018) using a stereomicroscope.

Pearson's correlation analysis was performed between various physico-chemical parameters of soil and earthworm density. All the analyses are done with the help of SPSS 18 software program (SPSS, 2018).

Results

Earthworm density, biomass and ecological group

The earthworms were observed only in the two locations of 58 location at Lahijan and Tonekabon counties (Table 2). These specimens were identified as *Perelia kaznakovi, Aporrectodea trapezoides*, and *Dendrobaena veneta* based on key morphological characteristics such as body length and diameter, length, and position of Clitellum (Table 3). Three species were collected in Bazkiagorab (Lahijan) while only one species observed in Shekarposhteh (Tonekabon). Mean biomass of the earthworms varied from 0.65-1.38 g m^{-1} and 0-1.5 g m^{-1} in Bazkiagorab and Shekarposhteh, respectively. The population of earthworms in the Tonekabon region was 3 m^{-1} while it was determined 22 m^{-1} in Lahijan region (Table 3).

Table 2. Abundance and biomass of collected earthworm

Species	Lahijan		Tonekabon	
	Density	Biomass	Density	Biomass
	ind.m^{-2}	gm^{-2}	ind.m^{-2}	gm^{-2}
Perelia kaznakovi	9	1.38	0	0
Aporrectodea trapezoides	5	1.26	3	1.5
Dendrobaena veneta	8	0.65	0	0
UTM(X,Y)	4120199	406786	4074973	477472

Table 3. Morphological characteristics of collected earthworm

Species	Ecological group	Body Length(mm)	Diameter(mm)	Clitellum position
Perelia kaznakovi	Endogeic	55-68	5-6	27-35
Aporrectodea trapezoides	Endogeic	80-140	3-6	26-35
Dendrobaena veneta	Epigeic	50-155	4-8	27-33

Perelia kaznakovi

Aporrectodea trapezoides

Dendrobaena veneta

Soil Physico-chemical properties

Descriptive statistics of the physical and chemical properties measured in soil samples are presented in Table 4. The results indicated that 34% of the soils have the pH less than 4.5 and the pH of 12% of the sampled soils was more than 6.0, which is not suitable for optimal tea growth which needs to be reclaimed (Figure 2). In the soils sampled from Tonekabon and Fooman, the pH is more than 5, and in other soils of the dominant pH is less than 5.0.

Table 4. Descriptive statistics of measured soil parameters

Characteristics	pH	OC %	P, mgkg^{-1}	K, mgkg^{-1}	Sand, %	Clay, %
mean	4.78	%	23.70	147	23	51
median	4.75	1.95	5.4	122	23	49
Mode	4.19	2.20	62	67	23	47
Min.	3.40	0.78	0	14	6	22
Max.	6.10	3.67	268	692	50	81
Sd	0.61	0.66	49.57	112	9.35	12.98
variance	0.37	0.43	2414	12628	87	168
Range	2.70	2.89	268	678	44	59
No.	58	58	58	58	58	58

Also, 52% of the studied soils have less than 2% organic carbon, which indicates the depletion of soil carbon, the non-consumption of organic fertilizers, and no return of plant residues to the soil. Organic carbon was appropriate just in 9% of soils (Figure 2). It was determined that 69% of soils have severe deficiencies due to the amount of available phosphorus so it may be inferred a high response of the tea plants to phosphate fertilization. On average, phosphorus deficiency is observed in 10% of soils although almost 17% of the sampled soils required a little phosphate fertilizer application (Figure 3).

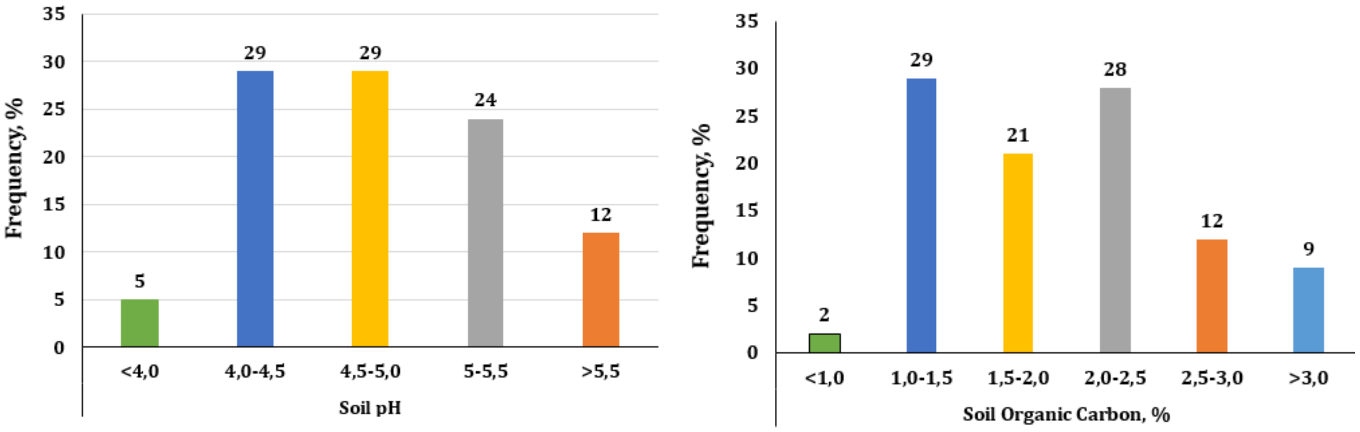

Figure 2. Distribution of soil pH and SOC in the studied soil

Almost, 65% of the studied soils had less than 150 mg K kg^{-1}, indicating a severe potassium deficiency and depletion. In 19% of soils the response of tea plants may be positive to potassium fertilizers, and in 16% of the soils, there is no need to use potassium fertilizers (Figure 3).

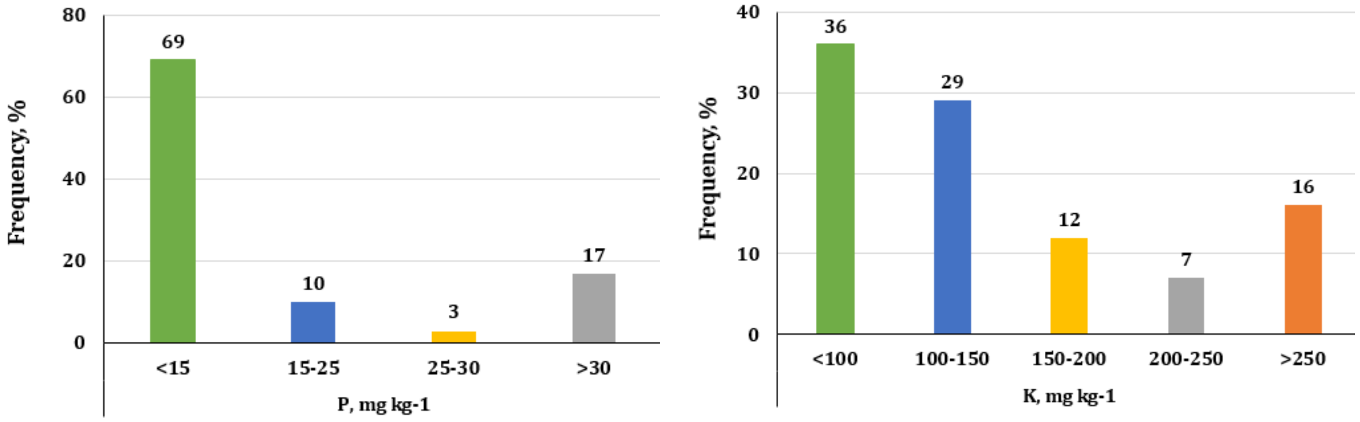

Figure 3. Distribution of available P and K in the studied soil

The texture of more than 50% of the studied soils is suitable for tea cultivation. The amount of clay in 22% of soils was more than 33%, which can reduce the growth of tea by creating unfavorable conditions, and the need for corrective operations such as embedding drainage system and lightening soil texture was necessary (Figure 4).

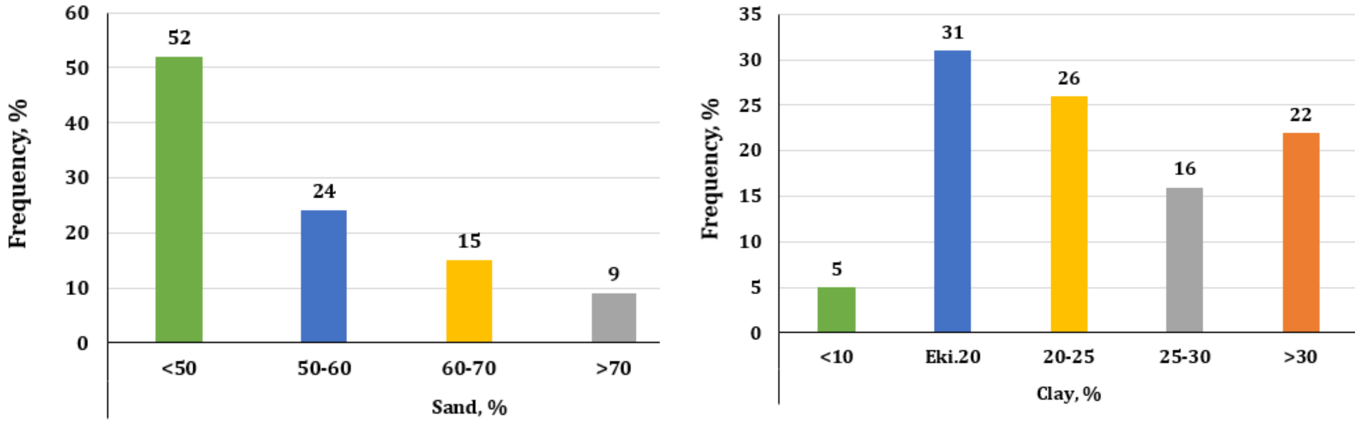

Figure 4. Distribution of soil sand content in the studied soil

Discussion

Earthworm's abundance

Earthworms was absent in majority of the present studied soils. A maximum of three species was observed in an area, indicating severe ecosystem degradation and adverse conditions for growth and survival of the earthworms in Iranian tea plantations. Earthworms are one of the biological indicators to evaluate soil quality and unbalanced physical and chemical properties of soils, it is necessary to improve soil conditions. The number of earthworm's species in each soil is the simplest way to measure species diversity, which usually varies between 4-14 species (Edwards and Bohlen, 1996). Latif et al. (2009) reported *D. veneta* and *P. kaznakovi* species from the central Alborz Mountains in Haraz and the Chalous River (Karaj to Chalous).

According to Radaei and Izadi (2016), the maximum and minimum population of earthworm at the forest of northern Iran, found in alder and spruce stands. Sinha et al. (2010), reported two ecological groups of earthworms in the soils of tea ecosystems. A single ecological system with the same ecological status in the tea garden has fewer ecological earthworms than the forest ecosystem with different ecological niches.

Jamatia and Chaudhuri (2017) reported that endogenic species were generally predominant in the tea ecosystems. In their study, 12 out of 15 observed species were endogenous, similar to our study. According to Harbowy et al. (2017), the absence of epigeic species and a small number of endogenic species (3 out of 15 species) in the tea soil may be attributed to their low plant diversity and high polyphenolic residues.

Tea leaf residues are not popular with earthworms due to their high polyphenol content (Harbowy et al., 2017). According to Edward and Bohlen (1996), the amount of leaf polyphenols had a negative relationship with leaf utilization by earthworm. Jamatia and Chaudhari (2017a,b) reported a positive and significant correlation between earthworm density with pH and organic carbon of soil.

Chaudhuri and Nath (2011) found a correlation between density and biomass of earthworm with soluble sugars, polyphenols, flavonoids and lignin in the leaf residues of rubber cultivation.

Soil moisture and temperature in tea Cultivations have a significance positive and negative correlations with earthworm density and biomass, respectively. Because the body of earthworms is soft, they need moist conditions to survival, maintenance of hydrostatic pressure and prevention from drying out (Najar and Khan 2014). Because, soil compaction was evident and tillage operations were not carried out for a long time, the slow rate of water infiltration, irregular distribution of rainfall and increasing hot days of the year (Kahneh et al. 2019) create unfavorable conditions led to decrease the population of earthworms in Iranian tea gardens.

Since calcium is essential for the growth and the reproduction of earthworms. Deficiency and leaching of this element in the tea soil and excessive consumption of nitrogen fertilizers are among the factors affecting elimination of earthworms.

Therefore, it seems that tea cultivation has reduced the number of earthworms that feed on carcasses and reduced the variety of worms in tea cultivations. However, the presence of some native species in the soil of tea cultivations indicates presence the resistant native species that can be active with foreign species.

Change in Soil Properties

In the current study, 34% of sampled soils had acidic to very acidic pH. also Chien et al. (2019) reported that out of 12 tea gardens tested in Vietnam, four soils have pH less than 3.5 and six have pH = 3.5 (H_2O). Karak et al. (2015) observed that the pH of surface soil in the study areas of Indian tea gardens varied from 3.61-6.81. According to Zhu et al. (2013), the secretion of organic acids from the roots of the tea plant can acidify the soil pH gradually decreased with increasing age of tea plants. Therefore, one of the most important factors in reducing pH of the studied soils can be: i. Excessive consumption of nitrogen fertilizers (urea) in tea gardens. İi. The age of tea plants in most gardens and iii. leaching of nutrients, especially calcium and magnesium from the soil.

The organic carbon of the studied soils showed the ranges between 0.78% and 3.67%. Karak et al. (2017), also reported low levels of organic soil carbon in the tea Cultivations of India and Rwanda. Karak et al. (2015), concluded that low levels of organic carbon in tea soil can be due to low plant residues and high yields of young shoots.

In our study, almost 69% of the sampled soils contained very little or high amount of phosphorus. Chien et al. (2019), reported that the available soil phosphorus-Brya2 in some Vietnamese tea was 54-1107 mg kg^{-1} with an average of 535 mg kg^{-1}. Isobe et al. (2017), reported that the available phosphorus- Brya2 varied from 0 to 260 mg per 100 g in the tea cultivation in highlands in Japan, but most soils have less than 25 mg per 100 g of soil available phosphorus.

The amount of available potassium was less than 150 $mgkg^{-1}$ in 65% of the sampled soils. Ruan et al. (1999, 2013) declared the availability of potassium less than 100 mg kg^{-1} in the soils of Chinese tea plantations. Accordingly, out of 3396 samples of soil tested, 2513 samples had less than 100 mg kg^{-1} of potassium and the average potassium in these soils was 80 mg kg^{-1}.

Moreover, 45% of the soils had 10-20 clay and only 3% of the soils had more than 30% clay. Chien et al. (2019), also reported a soil clay content of 10-32% in Vietnamese tea. They considered the amount of clay to be 18% for the grouping of tea soils and concluded that if leaf quality depends on the growth, yield and yield components of the tea plant, soil texture and related characteristics could be one of the important factors. Therefore, it can be concluded that tea cultivation has reduced the number and abundance of earthworms in Iranian tea Cultivations. However, the presence of some native species in the tea Cultivations indicates that there is a resistant native species in these soils that can be used with foreign species for further research. Also mixed plantation of N_2-fixing tree with tea and nutrient Best Management Practices is recommended in tea plantations

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Eurasian Journal of Soil Science 2022, 11(3), 241- 247

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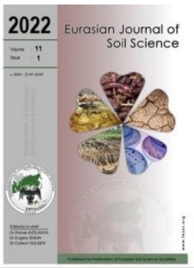

Effects of different polymer hydrogels on moisture capacity of sandy soil

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Abstract

In arid and semi-arid regions, efficient utilization of available water necessitates the adaptation of appropriate water management practices. One such approach is through soil conditioners like polymer hydrogels. The application of polymer hydrogels aids efficient management of water in agricultural production by increasing water holding capacity and improving water conservation of sandy soils. This has led to practical applications of these materials particularly in arid regions and countries, where water is the limiting factor for plant production. Therefore, the ultimate objective of this study was to address the impacts of different polymer hydrogels such as potassium polyacrylate (PH1), starch-acrylonitrile (PH2), starch-acrylic-acid (PH3) and polyacrylic acid (PH4) on the moisture capacity of sandy soils from sand dune. The sandy soils contained >95% sand. Maximum rate of water absorption of polymers (PH1, PH2, PH3 and PH4) were 174, 38.75, 21.7 and 201.1 times their weight respectively. Four polymer hydrogels with three treatments (0.25:0.75, 0.5:0.5 and 0.75:0.25; v/v) were used in the experiment with four replication. With respect to the untreated soil, addition of polymer hydrogels increased significantly full moisture capacity (FMC) and smallest moisture capacity (SMC) for for all polymer: sand mixtures. PH1 recorded highest FMC and SMC than all four polymers. The results suggest that addition of a potassium polyacrylate to sandy soils is more effective polymer hydrogel at increasing moisture capacity in sandy soils.

Keywords: Polymer hydrogel, sandy soil, desert, moisture capacity.

Introduction

Desertification due to wind erosion (deflation process) has touched the semi-desert and desert landscapes of Central Asia and Kazakhstan. The problem of desertification in Central Asia is more serious; 75% of the territory in Kazakhstan, 60% of the territory in Uzbekistan, and 66% of the territory in Turkmenistan are prone to antropogenic desertification (Gulnara et al., 2014). Among the states of Central Asia, Kazakhstan possesses the largest area 'captured' by desertification processes - 179.9 million ha. In fact, about 66% of the country's territory is subject to degradation. The total area of Kazakhstan's non-irrigated arable land is 24 million hectares, of which desert lands make up 10.4 million ha. More than 17 million ha have been withdrawn from the category of arable lands due to loss of humus, salinization, low productivity of land, chemical pollution, and erosion (UNDP, 2004). Degradation is especially pronounced in arid pastures with sandy or sandy loam soils, the area of which in the Kazakhstan is more than 25 million ha, which includes one of the largest sand massifs of the Southern Balkhash region (7.3 million ha). Unsuitable land practices and irrigation use of natural resources and environmental pollution lead to land degradation and desertification in Southern Balkhash region, Kazakhstan. The problem of soil degradation and desertification is the most problem of land agriculture development in this region, Kazakhstan (Almaganbetov and Grigoruk, 2008). Soil

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moisture is an important parameter of the hydrological cycle of terrestrial ecosystems (Bindlish et al., 2003; Song et al., 2007; Schneider et al., 2008), and also is one of the most important ecological factors in sandy ecosystems, where its shows significant variation (Chen et al., 2011; Wagner et al., 2003). Thus, decreasing soil water content is a main driving force in the development of desertification (Berndtsson and Chen, 1994; Yao et al., 2013).

Sandy soils are characterized by low water holding capacity and excessive drainage of rain and irrigation water below the root zone, leading to low water, poor water and fertilizer use efficiency by crops. Therefore, development of non-traditional new technologies to conserve water is becoming important for attaining a sustainable economic growth, especially in agriculture producing countries (Nada and Blumenstein, 2015). Hydrogel is one of the most popular materials, in addition to increasing water holding capacity for agricultural applications having also been used to reduce water runoff and increase infiltration rates in field. The efficiency of the technology is highly suited for farmers growing crops under rained and limited water availability areas (Kumar et al., 2020). Cross-linked Polymer hydrogels with hydrophilic properties have attracted much interest in this area as they can increase water holding capacity of sandy textured soils (Li et al., 2014) and thus increase plant growth (Nada and Blumenstein, 2015). Polymer hydrogels with high molecular weight and high negative charge that absorb a significant amount of water, up to 2000 g water g^{-1}, therefore are considered very suitable for applications in agriculture (Zhang et al., 2006; Yang et al., 2014; Guilherme et al., 2015).

The performance of polymer hydrogels depends on the soil and crop types. The addition of polymer in saline soil had positive effects on plant growth, yield, and available moisture content in corn (Dorraji et al., 2010). Likewise, better performance in sandy loam soils over the clay and clay loam soils has been reported (Narjary et al., 2012).

Most of the research carried out so far has focused on results obtained following the application of hydrogels to sandy soils or soils with a lighter mechanical composition. The effect of hydrogels on sandy soils can be expected to be particularly obvious due to their low productive humidity capacity. The objective of this paper was to determine using a pot trial the influence of different polymer hydrogels such as starch-acrylonitrile, starch-acrylic-acid, polyacrylic acid and potassium polyacrylate on the moisture capacity of sandy soils from sand dune.

Material and Methods

Soils in deserts are very thin; humus content in them is meager. The mean soil types in the Southern Balkhash region, Kazakhstan are sandy desert, gray-brown, takyr-like, and solonchaks. The sandy desert soils dominate in the Southern Pre Balkhash deserts. The research site was conducted in the Southern Pre-Balkhash deserts (Gulnura et al., 2014). Study site is located from a fine-grained dune located 5 km from the village of Bakbakty on the right side of the road towards the district center of Bakanas (N44°33"54', E076°41"12'), Southern Balkhash region. The height of the dune from the base is about 5 m. It was formed on the territory of the parking lot of the peasant farm "Tarshelov", engaged in animal husbandry (Figure 1). The surface of the sand dune is broken and exposed due to the daily cattle run of 135 cattle. The grazed area of the farm is 300 ha.

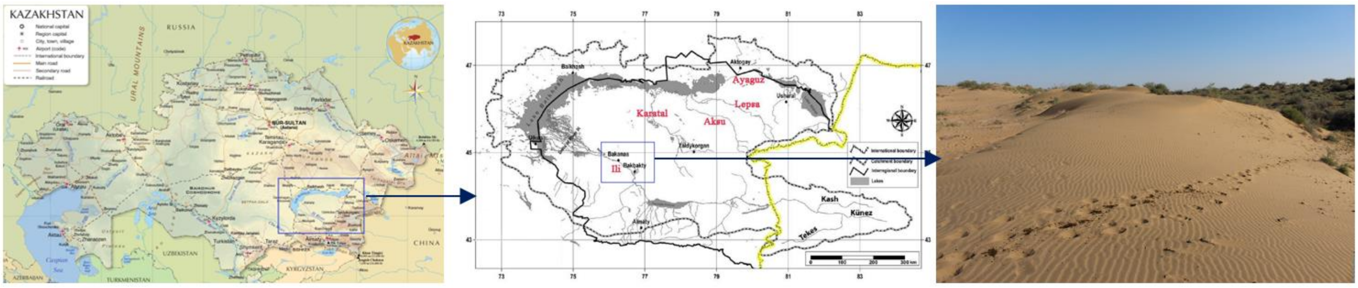


Figure 1. Study area

Climate

The climate of deserts in Kazakhstan, Central Asia, and elsewhere in the world are characterized by high air temperatures and a long dry period during the summer. Climate of the southern Pre-Balkash is continental, arid, characterized by daily and annual variations of air temperature, and high levels of solar radiation. The average mean temperature is about 33°C highs in July and the average mean temperature is −8°C in January. Average precipitation is 175 mm per year (Figure 2).

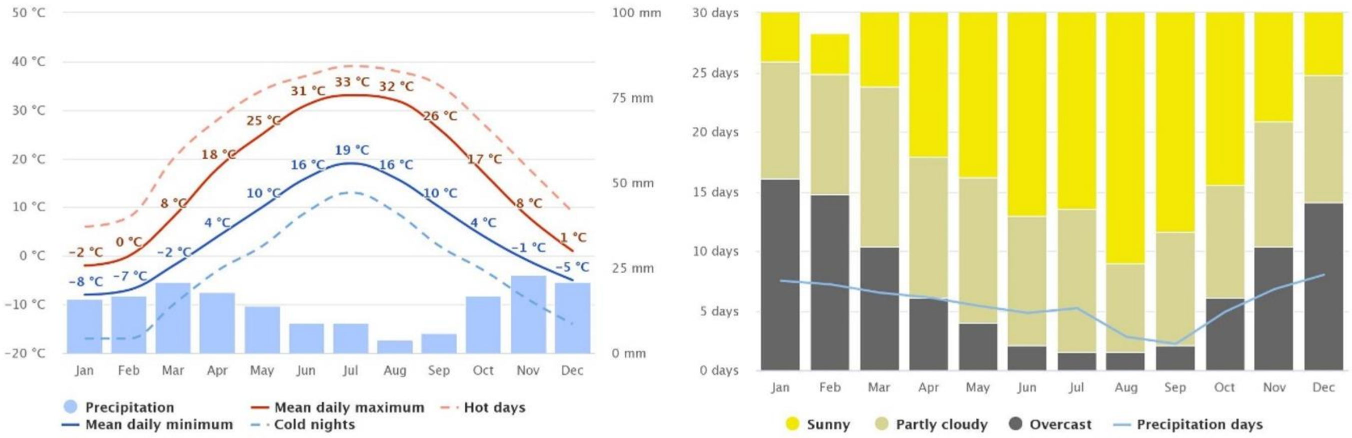


Figure 2. Climate conditions of the study area, Southern Balkhash region, Kazakhstan

Sandy Soil Sampling

Sandy soil samples were collected from 0-20, 20-40, 40-60, 60-80 and 80-100 cm of sand dune. Hygroscopic moisture content of samples determined according to Prakash et al. (2016) and The granulometric composition of samples was determined by the pipette method (Day, 1965). To Seasonal moisture regime of a sand dune, samples were repeatedly taken from the soil depth of 0-20, 20-40 and 40-60 cm to cover the whole spring and summer season of research site (May, June, July, August and September). Two different locations (erosive zone by wind erosion and deposition zone) were selected for sampling. Soil samples were transported to the laboratory and moisture contents of sand samples determined according to Gardner (1965).

Polymer hydrogels

The soil amendments used were four hydrogels. These were "starch-acrylonitrile", "starch-acrylic-acid" and "polyacrylic acid" synthesized in Kazakh National Agrarian Research University by chemists group. "potassium polyacrylate" is supplied ready to use. Polymer hydrogels degree of swelling and moisture capacity is determined by the difference in weight and volume between dry hydrogel samples and water-soaked samples. To establish the maximum swelling of hydrogels, samples of air-dry polymer hydrogel with a known total weight were taken according to a measurement of mass or a change in mass provides quantitative information about Gravimetric methods after 120 min. (Bassett et al., 1981).

Laboratory experiment

Polymer hydrogels have an amazing ability to absorb and retain exceptionally large quantities of water. When it comes in contact with water it swells to form a gel. Sandy soil samples (0-20, 20-40, 40-60, 60-80 and 80-100 cm) of sand dune was used in the experiment. The sandy soils contained >95% sand. Four polymer hydrogels (starch-acrylonitrile, starch-acrylic-acid, and polyacrylic acid and potassium polyacrylate) with three treatments (0.25:0.75, 0.5:0.5 and 0.75:0.25; v/v) were used in the experiment with four replication. The volumetric weight of the mixture of hydrogel and sand was calculated for a volume of 175 cm^3 dishes, taking into account the degree of swelling of hydrogels in free water space and the volumetric weight of each component of the mixture (sand and hydrogel) was 1.51 and 0.40 g/cm^3, respectively. Then evenly mixed calculated mixtures of hydrogel and sand filled the vessels according to their volume ratio (0.25:0.75, 0.5:0.5 and 0.75:0.25). After that, the mixtures were compacted by gently tapping the bottom of the vessels on the table. Then plastic dishes with a mixture of polymer hydrogel and sand were immersed in a special bath with water so that the water was at the level of the mixture in the vessels. After that, the vessel covers were closed to prevent physical evaporation from the surface of the mixture and left in this form until the next day. Water was transferred through the pores of the paper in the mixture, and its complete saturation took place. Thus, these cylinders were moistened to such a state until all the pores were filled with water. After reaching the specified state, the vessels, after wiping water from their walls, were weighed on electronic scales until its mass was constant.

To determine the SMC (smallest moisture capacity), i.e. the volume of water remaining after the gravity water drained, the vessels with the mixture soaked to PV were pulled out of the bath and left for some time until the water stopped dripping from the bottom of the vessels. Upon reaching the specified state, the cylinders were weighed and the weight of moisture of the corresponding SMC was determined by the difference in mass between the air-dry and the current one.

The value of FMC (full moisture capacity) and SMC separately of sand and a mixture consisting of polymer hydrogels of various water characteristics and a sandy substrate was determined as a percentage of absolutely dry sand by the formula (Romashchenko et al., 2019):

$$W_{\text{FMC and LMC}} = ((d\text{-}c+a)/(c\text{-}a\text{-}b)) \text{ x } 100$$

d : the weight of the cup with a mixture of hydrogel and sand, saturated to FMC or LMC, g
c : the weight of the cup with a mixture of hydrogel and sand before moistening, g
a : the amount of water in the sand sample before saturation;
b : the mass of an empty cylindrical plastic vessel and filter paper installed on its bottom, g

Results and Discussion

Partical size distribution of Sand Dune

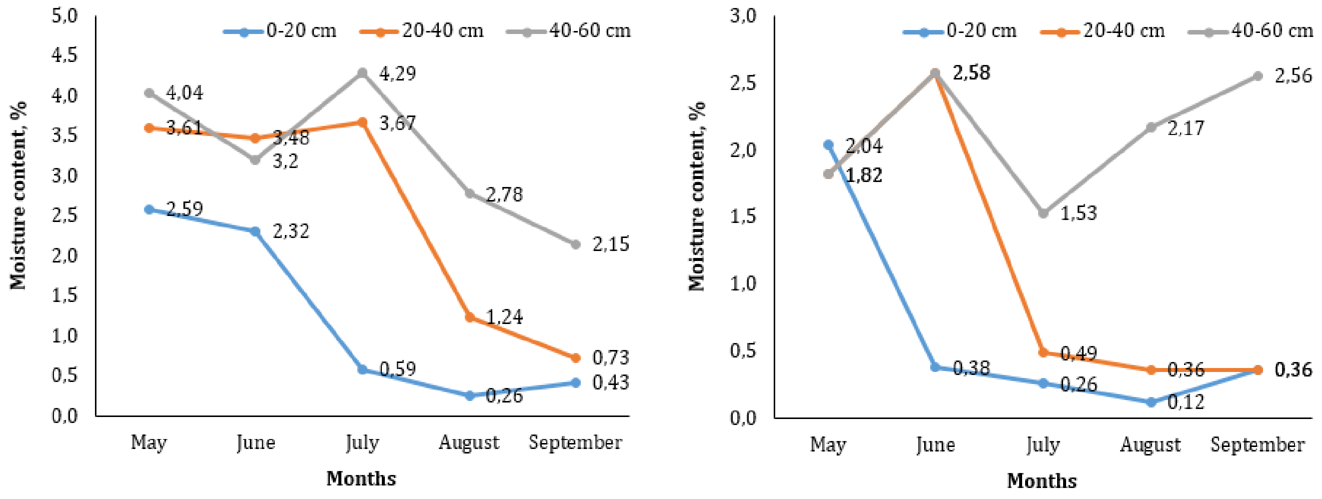
Partical size distribution and hygroscopic moisture for all the different depth from sand dune are given Table 1. The soil was characterized by low organic matter and low water holding capacity. Percentages by weight of medium sand (1,00-0,25mm), fine sand (0,25-0,05mm), coarse silt (0,05-0,01 mm), medium silt (0,01-0,005 mm), fine silt (0,005-0,001 mm) and clay (<0,001 mm) were calculated by weight in accordance to Russian soil partical size classification system. As shown in Table 2, 85,6–89,7% of the sand samples were mainly distributed within the range of 0.25–0.05 mm (fine sand), indicating that only 10.1-14.4% had sizes greater than 0.25 mm and less than 0.05 mm. It can be seen that the trend of particle size distribution and hygroscopic moisture is similar in all depths. This agrees with the findings of Yuan et al. (2008) and You et al. (2022) suggests the predominance of fine sand in the particle composition of the Aeolian sand deposits found in Yulin Area, China.

Table 1. Particle Size distribution and hygroscopic moisture contents of samples

Sampling depth, cm	Hygroscopic moisture, %	Particle Size distribution, mm					
		Sand		Silt			Clay
		1,00-0,25 mm	0,25-0,05 mm	0,05-0,01 mm	0,01-0,005 mm	0,005-0,001 mm	<0,001 mm
0-20	0,32	8,287	85,694	3,612	0,401	0,803	1,204
20-40	0,38	9,155	85,625	2,409	1,205	1,205	0,402
40-60	0,32	8,086	86,697	2,006	1,605	0,803	0,803
60-80	0,28	7,260	87,124	2,407	0,802	1,604	0,802
80-100	0,30	4,514	89,870	2,407	2,006	0,802	0,401

Changes of the moisture contents of the sand dunes

Since the moisture status of the profiles in erosive zone and deposition zone is influenced by the rainfall received during the rainy season, soil moisture contents during the rainless period (August) are particular interest. Sand dune in erosive zone, reasonably high soil moisture encountered within 20-40 and 40-60 cm depth, while sand dune in deposition zone, moisture content below the permanent wilting point may be found (Figure 3).



Erosive zone **Deposition zone**

Figure 3. Changes of the moisture contents of the sand dunes in erosive and deposition zone

Thus sand dune in erosive zone offer better soil moisture condition for plant establishment and growth and also support the usefulness of deep planting in shifting sand dune in research area. Deep planting also provides favorable temperature for root growth during the summer at the early stages of plantation. In this study, moisture regime of the sand dunes showed that in the month of May in its windward part, the moisture content of the sand was relatively in optimal content for normal growth and development of *Psammophytes*. Psammophyte a plant that thrives in shifting sands, primarily in deserts. *Psammophytes* are marked by a number of adaptations that enable them to exist on wind-blown sands. In such an environment, the plants are often covered with sand, or their root system is exposed. *Psammophytes* are encountered not only in deserts but also along seas and large lakes and in sands along rivers (Elymus giganteus, sand fescue, sharp-leaved willow). *Psammophytes* are often used to stabilize sandy soils (For The Great Soviet Encyclopedia, 2021).

Polymer hydrogels

Polymer hydrogels showed rapid initial hydration followed by a progressive decrease in the rate of absorption towards the point of equilibrium. All polymers had a similar pattern in the rate of water absorption. The results of the swelling properties of the polymer hydrogels used in this study are given in Figure 4. The Figure 4 shows that the average absorption of water by potassium polyacrylate (PH1), starch-acrylonitrile (PH2), starch-acrylic-acid (PH3) and polyacrylic acid (PH4) was found to be 174, 38.75, 21.7 and 201.1 times of its weight respectively. The equilibrium swelling tests in distilled water showed that PH4 displayed higher swelling ratios when compared with PH2, PH3 and PH1 (Figure 4). This may be due to its high molecular weight materials that can absorb as much as several hundred times its weight. Chaudhry et al. (1994) revealed that average water absorption by polymer (Aquasorb) after 120 min was 130 times its weight. Hydrogels may absorb water from 10-20% up to thousands of times their dry weight. The character of the water in a hydrogel can determine the overall permeation of nutrients into and cellular products out of the gel. When a dry hydrogel starts absorbing water, the first water molecules entering the matrix will hydrate the polar, hydrophilic groups, leading to primary bound water. As the polar groups are hydrated, the network swells, and hydrophobic groups are exposed, which further interact with water molecules, leading to hydrophobically bound water, or secondary bound water. Primary and secondary bound water are often combined and simply called the total bound water. After the polar and hydrophobic sites have interacted with and bound water molecules, the network will imbibe additional water, due to the osmotic driving force of the network chains towards infinite dilution. This additional swelling is opposed by the covalent or physical crosslink, leading to an elastic network retraction force. Thus, the hydrogel will reach an equilibrium swelling level (Kumar et al., 2020).

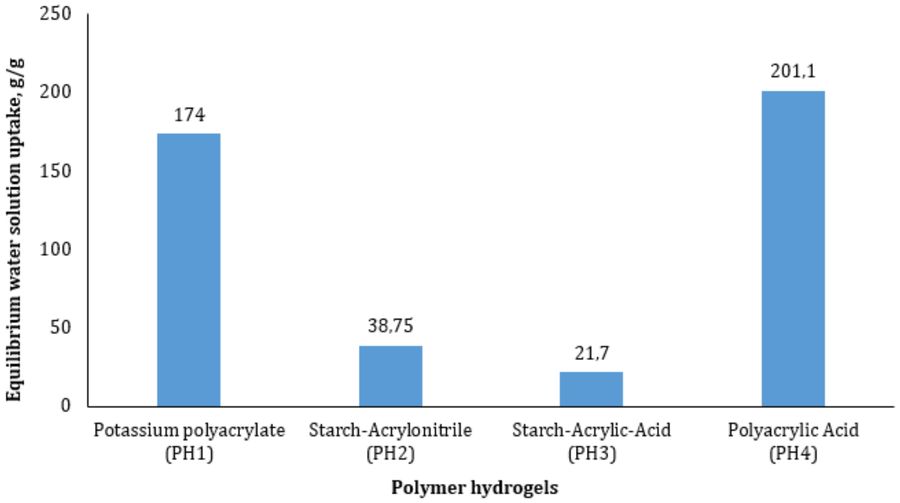


Figure 4. Polymer hydrogels equilibrium swelling properties in distilled water.

Moisture Capacity

The moisture content of treated sand samples was studied. Generally, the full and smallest moisture capacity of the sand samples increased with increasing amounts of polymer hydrogels in the samples. However, the water holding capacity of the samples did not increase linearly with increasing amounts of polymers in the samples. This increase in water retention can reduce the amount of water otherwise lost by deep percolation. FMC is the maximum possible amount of gravitational water that can be contained by the soil while filling all the voids. SMC is the amount of capillary-hanged water retained by the soil after the expiration of excess liquid water (Romashchenko et al., 2019).

Mixing the sandy soil with the polymer hydrogels showed high pronounced effect on its values of FMC, SMC and FMC-SMC (Figure 5). The values of these parameters in the polymer hydrogels amended soils relative to the non-amended control were shown in Figure 5. With respect to the untreated soil, addition of polymer hydrogels increased significantly FMC, SMC and FMC-SMC for all polymer: sand mixtures. PH1 recorded highest FMC and SMC than all four polymers. FMC was 38.54, 59.10 and 119.12 respectively and the SMC was 35.20, 54.03 and 105.24 at 0.25:0.75, 0.5:0.5 and 0.75:0.25 (v/v) polymer: sand mixtures. While, untreated soil observed with the lowest values of FMC and SMC. These results are in agreement with those obtained by Akhter et al. (2004), Dorraji et al. (2010) and Nada and Blumenstein (2015).

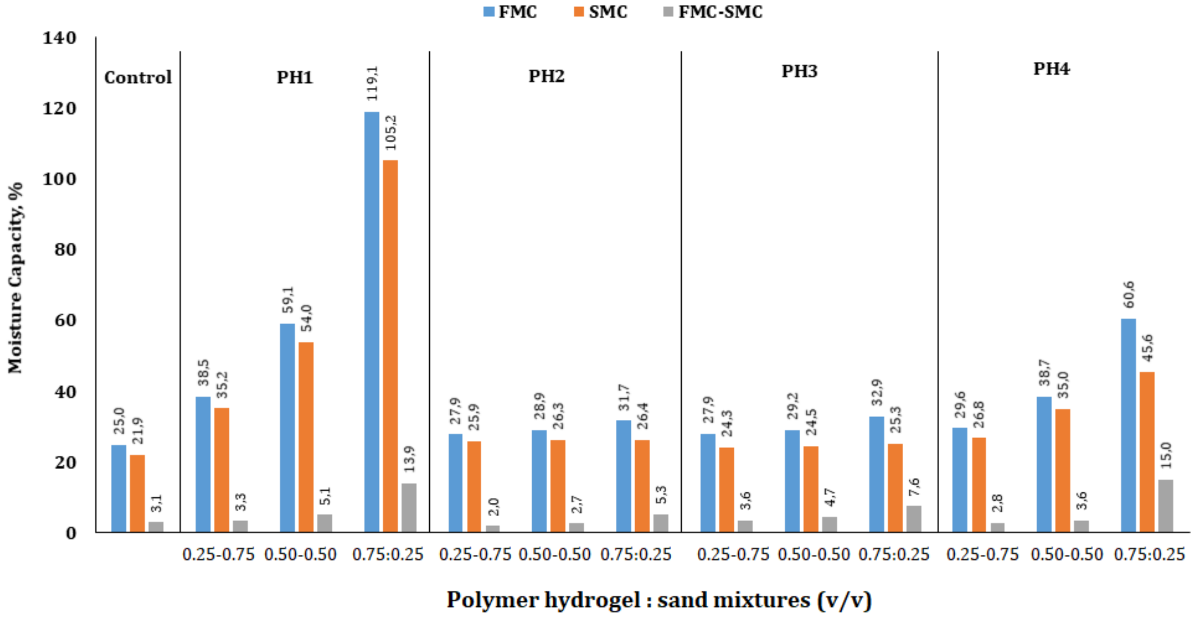


Figure 5. Different polymer hydrogel:sand mixtures (v/v) on moisture capacity

Conclusion

Desert sandy soils in Kazakhstan are widespread in the sandy pastures of the Southern Balkhash region, which, due to anthropogenic degradation, turned into mobile dunes in a short time, which began to bring inconvenience to the living population in places of their active manifestation. The study of sandy deserts in Southern Balkhash region allows qualitative assessment of the modern deflation processes intensity in this area. The sands of our study region are most affected by the deflation process. They consist mainly of fine sand (0.25-0.05 mm), the proportion of which ranges from 87.6 to 94.6% in the meter thickness. They have an eternal shortage of moisture, but despite this they are the soils of valuable pastures of the region's distilling livestock. The survival rate of seedlings of sand-strengthening forest shrubs due to the hot climate, high seasonal mobility of the relief of mobile sands and their low humidity is very low, and the creation of optimal soil moisture by irrigation, due to the low moisture capacity of sand, water scarcity and the difficulty of delivering it to the planting sites of seedlings in the summer months is almost impossible. Therefore, for the restoration of the soil and vegetation cover of sand dunes, the autonomous improvement of their water regime under the existing norms of atmospheric precipitation is relevant. Therefore, polymer hydrogels, which significantly increase the moisture capacity of not only sandy soils, but also sands, were promising in this regard. Our results suggest that mixing Polymer hydrogels with sandy soils could improve their moisture capacities. In addition, it was determined that Potassium polyacrylate recorded highest full and smallest moisture capacity than the starch-acrylonitrile, starch-acrylic-acid and polyacrylic acid on sand from sand dune compared to the untreated control treatment. However, further comparisons between the polymer hydrogels and soil physico-chemical properties and their influence on the polymers and the plant production are necessary on field conditions in this area.

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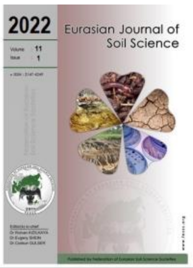

Response of selected physical properties of Fluvisols to tillage implements and frequencies at Haramaya, Eastern Ethiopia

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Abstract

The study was conducted to evaluate the effects of tillage implements and frequencies on selected physical properties of Fluvisols at Haramaya University, Eastern Ethiopia, during the 2013 cropping season. Soil bulk and particle density, total porosity, texture, and soil water retention were analyzed immediately (within 72 hours) and one month after tillage for samples collected from 0-20 and 20-40 cm depths. The experiment was laid out in a split-plot design with treatment combinations consisting of three levels of tillage frequencies (0, 2 and 4) and two tillage implements, oxen-drawn traditional *Maresha* and disc plows, with three replications. Results indicated that the mean bulk density values were significantly different ($P \leq 0.05$) at plow layers (0-20 cm). It ranged from 1.68 g cm^{-3} for disc plows at two passes to 1.72 g cm^{-3} for zero tillage and disc plows at four passes one month after tillage at a depth of 21-40 cm. Tillage with a disc plow at increased frequencies decreased total porosity, while oxen-drawn *Maresha* increased total porosity. Insignificant differences ($P \leq 0.05$) in mean values of particle size distribution were observed except for percent clay content immediately after tillage with disc plows at two passes, which showed significant highest mean value (26.30%). Tillage by traditional *Maresha* resulted in more water holding capacity at increased tillage frequencies. Tillage practice using disc plows at two passes significantly affected the bulk density, total porosity, and soil water retention characteristics. In conclusion, tillage implements and frequencies have shown a negative effect on the physical properties of Fluvisols by disrupting the structure of the soil at surface and subsurface depths, resulting in varying levels of impact on soil bulk density, total porosity, and soil water retention characteristics. Therefore, it is recommended to use the tillage implements at reduced frequencies for less disruption of soil properties while performing soil tilth for agricultural production.

Keywords: Tillage, *Maresha,* Disc plow, Implements, Haramaya.

Introduction

Tillage is the physically manipulating and managing soil for the purposes of managing previous crop leftover, preparing a seedbed for planting, controlling competing plants, and incorporating fertilizers and other crop production inputs. It entails of breaking the earth's compact surface to alter the soil's state to a particular depth and loosen the soil mass to allow crop roots to enter and spread into the soil (Wolkowski, 1996; Sahay, 2008). Tillage implements type and frequency alter the physical properties of soil, causing changes in biological activity, which in turn affects the soil's chemical properties and nutrients availability (Sundermeier et al., 2011; Iqbal et al., 2013). As a result, effective tillage is employed to create essential soil physical conditions for producing reasonably yields and maintaining acceptable soil quality (Kishor et al., 2013).

Tillage plays an important part in high and lucrative yields in modern agricultural production, where extensive mechanization has a beneficial financial impact, because it gives the soil a good growth environment for crops.

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However, it was discovered that it was negatively influencing soil conditions around the world by destroying soil structure, losing nutrients, and polluting the environment (Montgomery, 2007; Tayari and Jamshidi, 2011; Boloor et al., 2013). Soil compaction can be induced by a variety of tillage implements and the amount of passes used in ploughing, especially in intense tillage and heavy gear (Seladji et al., 2010). Approximately 80% of Africa's farmed land is prepared by hand using human power, 16% with animal draught power, and 4% with equipment power. Because Ethiopia is an agrarian country, tillage is a common concern (Chanyalew et al., 2010). According to Temesgen et al., (2009), smallholder farmers in Ethiopia use an ard plow called *Maresha* to implement traditional tillage practices. Land degradation (development of a plow pan) and inadequate rainwater utilization have resulted in reduced agricultural productivity due to traditional tillage practices that require repeated cultivations with the *Maresha* plow.

The *Maresha* (a primitive animal-drawn plow made of wood and steel) only penetrates the soil to a shallow depth (less than 20 cm). Tillage with tractor passes also causes undesirable changes in soil properties, which are strongly linked to changes in physical qualities of soil like porosity and bulk density, which further raises mechanical impedance, limiting oxygen, water, and nutrient availability (Lipiec and Steniewski, 1995; Coelho et al., 2000). Multiple passes during conventional tillage can result in up to 70% of the field being trafficked, which has implications for soil compaction (Raper, 2005). Increased soil tillage frequency creates excessively localized pulverization, which causes structural damage (Souchere et al., 1998).

Because the usage of various tillage implements is increasing over time, it is vital to research the impacts of tillage implements and frequencies on selected soil physical attributes under Ethiopian conditions. Therefore, at Haramaya University in Eastern Ethiopia, a study was conducted to evaluate the impact of tillage implement and frequency of ploughing on selected physical attributes of Fluvisols.

Material and Methods

A description of the study area

The research was conducted during the 2013 cropping season at Haramaya University research field which is located in eastern Ethiopia at Haramaya, which is 508 km east of the capital city, Addis Ababa. The experimental site is geographically located between latitudes of 9° 24' 54.45''N and 9° 24' 55.83''N, and between longitudes of 42° 01'56.49''E and 42° 02' 02.23''E. The average altitude is about 2021 meters above sea level, with an annual rainfall of 909.1 mm from January to August of 2013. The mean annual (2004-2013) rainfall of the area is about 810.7 mm with the short rainy season, locally called *Bega* (March to April), and the long rainy season, also called *Kiremt* (June to October) (Simane et al., 1998). The annual mean minimum and maximum temperatures are 11.4 and 24.7 °C, respectively, with monthly values ranging between 4.6 and 14.4, and 23.1 and 25.7 °C (Figure 1). The soil type of the study site is characterized as a Fluvisols (Tamire, 1973) that is sandy clay loam in texture. Due to continuous nutrient depletion resulting from intense soil erosion, cereal mono-cropping and complete removal of crop residues, the soil becomes infertile. It contains low organic matter content (1.5-2.0 %) (Zewdie, 1994).

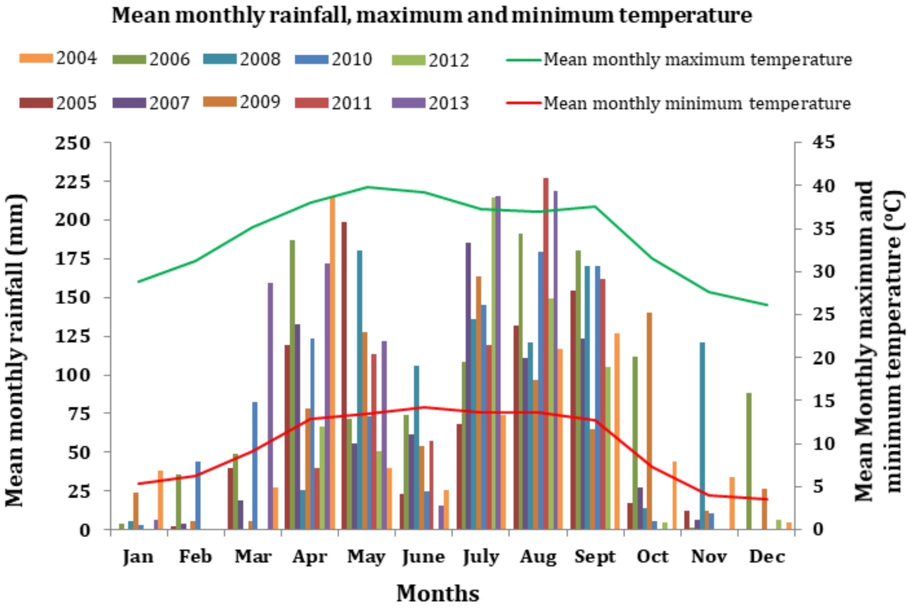

Figure 1. Mean (2004-2013) annual rainfall, maximum and minimum temperatures of Haramaya as recorded at the Haramaya Meteorological Station.

Experimental treatments and design

Two types of tillage implements, including traditional *Maresha* and the disc plow, served as main plot factors, with three tillage frequencies (zero, two, and four) serving as subplot factors and the treatments combinations were used as experimental treatments (Table 1). A split-plot design with three replications was used to lay out the treatment combinations. The disc plow was pulled by Landini 8860 Model tractor with a 62 horse power (hp), while the local *Maresha* was drawn by pair of oxen, for total of five treatment combinations. Without tillage, the zero tillage (ZT) was utilized as a control without tillage practice. Each plot area in a block was 15 m x 6 m in size. Treatments with multiple tillage frequencies were operated every 14 days. The soil samples were collected the plow layer (0-20 cm) and the subsurface layer (21-40 cm) for certain parameters. Tillage depth was achieved with the disc plow implement using a depth control wheel and with the *Maresha* implement by adjusting the *Maresha's* tying unit and controlling the plow during the plow.

Table 1. Treatments

Treatments code	Description
ZT	Zero tillage, no till
ODM2	Oxen drawn *Maresha* at two times plough
ODM4	Oxen drawn *Maresha* at four times plough
DP2	Disc plow at two passes
DP4	Disc plow at four passes

Soil sampling

To assess the status of selected soil properties, samples were collected randomly using a zigzag pattern from the entire experimental field. Accordingly, six disturbed sub-samples were used to make one composite sample. Similarly, three core samples each from the surface (0-20 cm) and subsurface (21-40 cm) layers were collected. The disturbed samples were collected using an auger, while the undisturbed samples were collected using core samplers. The samples were collected immediately (within 72 hours after plowing) and one month after the plowing operations (set up specifically to evaluate the effects of ploughing on soil physical parameters over time due to the modifications caused by climatic and environmental factors, especially the natural succession of wetting and drying cycles of the soil with subsequent progressive compaction) of the respective treatments. There is evidence that there are significant interactions between tillage and time for all properties, indicating that the tillage effect changes with time (Hu et al., 2018). Three randomly selected subsamples from tilled surface were collected from the entire plot. The air-dried soil samples were then ground and allowed to pass through a 2 mm size and analyzed at the Haramaya University soil physics and chemistry laboratories.

Methods of soil analysis

Particle size distribution was determined using the Bouyoucos hydrometer method (Bouyoucos, 1936). The textural class was determined using textural triangular procedures as laid out in the USDA system (Shirazi and Boersma, 1984; Gerakis and Baer, 1999). Bulk density was determined using the core method (Blake and Hartge, 1986a) and particle density was measured by the Pycnometer method (Blake and Hartge, 1986b), Total porosity (TP) was calculated from soil bulk (ρb) and particle (ρp) densities using equation (1) (Hall et al., 1977).

$$TP = (1 - \frac{\rho b}{\rho p}) * 100 \qquad \text{Equation (1)}$$

The water contents were tested at seven matric potentials, namely 0, -3, -5, -7, -10, -33.3, and -1500 KPa, to establish a typical water retention curve. A sand suction table was used to evaluate the water content at matric potentials of (0, -3, -5, -7, and -10 KPa) (Stakman et al., 1969). Gravimetrically, the water contents corresponding to these matric potentials were determined. The bulk density was used to transform the gravimetric water content determined at each matric potential into volumetric water content as shown in equation (2).

$$\theta = w \times \frac{\rho b}{\rho_w} \qquad \text{Equation (2)}$$

Where θ is the volumetric water content (v/v); *w* is the gravimetric water content (w/w); ρb is bulk density (g cm^{-3}), and ρw is the density of water (≅ 1 g cm^{-3}).
Available water capacity (*AWC*) (mm m^{-1}) was computed using equation (3) (Barthakur and Baruah, 1998):

$$AWC = 1000\,(\theta_{FC} - \theta_{PWP}) \qquad \text{Equation (3)}$$

Statistical data analysis

The selected soil physical properties measured were subjected to analysis of variance appropriate to the experimental design (Gomez and Gomez, 1984) using statistical analysis system institute package (SAS, 2008). Multiple comparisons of treatment mean values were made by the least significant difference (LSD) test method. In all analyses, a probability of error of P $\leq$ 0.05) was considered significant.

Results and Discussion

Soil analysis

Soil analysis result before the experiment (Table 2) indicated that soil has higher proportions of sand (57 %) with textural class of sandy clay loam. The soil pH was 7.62 which is rated as moderately alkaline (Mamo, 1991) with a soil organic carbon content of 0.78% which is rated as low (Debele, 1980).

Table 2. Selected physicochemical characteristics of the experimental soil before the experiment

Parameters	Soil depth	Values
Textural class	0-20 cm	Sandy clay loam
Sand (%)	0-20 cm	57.00
Silt (%)	0-20 cm	21.00
Clay (%)	0-20 cm	22.00
pH (H_2O)	0-20 cm	7.62
Organic carbon (%)	0-20 cm	0.78
Particle density (g cm^{-3})	0-20 cm	2.49
Bulk density (g cm^{-3})	0-20 cm	1.56
	21-40 cm	1.64
Total porosity (%)	0-20 cm	37.00
	21-40 cm	34.00
Water content(% w/w)	0-20 cm	5.12
	21-40 cm	4.66

Particle size distribution

Tillage implements and frequencies significantly affected percent clay content ($P \leq 0.05$) immediately after tillage, but not one month after tillage (Table 3). The highest and the lowest mean percent clay contents were 26.3% and 17.67% for disc plow at two passes and oxen-drawn *Maresha* plow immediately after twice tillage, respectively. In the same way, the mean values of 24.33 % and 20.00 % disc plow at two passes (DP2) and oxen-drawn *Maresha* plow twice (ODM2), respectively were recorded one month after tillage. The results obtained were in line with the finding of Canarache (1991) who reported that tillage could increase the proportion of clay content in soil. This could be due to tillage equipment moving finer soil particles around within the soil profile (Hajabbasi, 2005).

Tillage implements and frequencies did not bring about significant variations ($P \leq 0.05$) in silt and sand content immediately after and one month after tillage operations. But, the sand content showed a slight reduction in mean values for the oxen-drawn *Maresha* plough with increased tillage frequencies. These slight differences in the mean values were probably due to changes in mean percent clay (Table 3).

The differences in soil textural classes after the tillage experiment were not apparently seen as compared to soil test results before the experiment. The result was in line with the work of Sauwa et al., (2013), indicating that tillage treatments had no significant effect on the particle size distribution of the soil at the plow layer. The textural classes of the plow layers of all plots were sandy clay loam one month after tillage. However, immediately after tillage, soils under zero tillage and ODM2 were sandy loam in texture. Therefore, the textural classes of the soil were not changed under this experiment except for the slight increments in percent clay content. These results were in line with the work of Hajabbasi (2005) and USAID (2008), indicating that the differences might be due to the mixing of soil layers by repeated soil turning and sorting as a result of tillage implements and frequencies. It also suggests that the soil tillage by DP2 showed a relatively higher proportion of clay (Table 3).

Table 3. Mean particle size distribution variations immediately and one month after tillage

Treatment	Particle size distribution (%) immediately after tillage			STC	Particle size distribution (%) one month after tillage			STC
	Sand	Silt	Clay		Sand	Silt	Clay	
ZT	62.67	18.00	19.33^{bc}	SL	62.00	14.67	23.33	SCL
ODM2	66.00	16.33	17.67^{c}	SL	64.67	15.33	20.00	SCL
ODM4	59.67	15.33	25.00^{ab}	SCL	63.33	16.00	20.67	SCL
DP2	61.67	12.00	26.30^{a}	SCL	58.33	17.33	24.33	SCL
DP4	63.30	15.33	20.33^{abc}	SCL	61.67	15.33	23.00	SCL
SE (±)	1.31	1.07	1.13		0.92	0.45	0.68	
LSD (0.05)	NS	NS	6.02		NS	NS	NS	
CV (%)	5.66	19.34	14.71		5.07	10.18	10.89	

Note: Means for specific soil parameters followed by the same letter (s) within a column are not significantly different at P 0.05; SE = standard error, LSD = least significant difference, STC = Soil textural class, SL = Sandy loam, SCL = sandy clay loam, NS = Non significantly different, CV = Coefficient of variation, % = Percentage

Bulk density

The tillage implements and frequencies resulted in significant differences ($P \leq 0.05$) in bulk density values at the plow layer (0-20 cm) both immediately and one month after tillage (Table 4), but the differences were not significant for the depth below the plow layer (21-40 cm). According to Ji et al. (2013), shallow tillage (up to 20 cm depth) is referred to as the plow layer, whereas tillage beyond this depth is considered deep tillage. Thus, the impact of tillage implements and frequencies on soil bulk density values was found to be significant at those specified depths of tillage. The mean bulk density values for the traditional *Maresha* implement and that of zero tillage were relatively higher than those for the tractor pulled implements. These differences might be due to the shallow tillage capacity of the oxen-drawn *Maresha* plow that probably resulted in less soil disturbance. The highest bulk density observed in the control treatment is probably due to the undisturbed soil, which is in line with the work of Monneveux et al. (2006), which indicated that zero tillage is associated with increased soil bulk density.

The results also showed that the tractor powered disc plow at two passes decreased the mean bulk density values, unlike the other tillage treatments, and this reduction might be due to heavy soil pulverization with the lesser frequency of tillage. Oxen-drawn *Maresha* at increased frequencies of tillage slightly decreased the mean bulk density of the soil. The increase in mean bulk density values under the disc plow at four passes of tillage might be attributed to the compaction caused by the effects of axle load and tire traffic, leading to shear and vertical soil stresses, as has been evidenced by the research reports of Abu-Hamdeh (2004) and Sarker et al. (2011).

The lowest mean bulk density value of 1.59 g cm-3 was reported for disc plow at two passes one month after tillage, whereas the tilled layer had the greatest mean value of 1.72 g cm-3 for ZT and DP4. The sand content of the soil was not significantly influenced by tillage equipment and frequencies immediately after and one month after tillage (Tables 3 and 4). The irregular decline in mean sand content values for *Maresha* plow tools immediately and one month after plowing was also recorded, indicating that there were generally proportional increments and decrements in sand and bulk density.

However, with increased frequency tillage under disc plow implement, a steady drop in mean sand and bulk density values were found both immediately and one month following tillage. The findings also revealed that sand content had a direct effect on soil bulk density under various tillage implements and frequencies, which is consistent with the findings of Aşkın and Özdemir (2003) and Tanveera et al. (2016), who found that sand content was the most effective soil fraction in influencing soil bulk density.

At the depth of 21-40 cm, the highest mean value of 1.72 g cm^{-3} was observed for ZT and DP4, which is against the finding of Qin et al. (2006) and Sornpoon and Jayasuriya (2013), who reported that bulk density was significantly higher in the zero tillage than in the tilled conditions. However, disc plow at two passes resulted in the lowest mean values of 1.68 g cm^{-3} among the other treatments. Even though the mean values of bulk density were significantly different, all mean values showed that the soils had a bulk density greater than 1.60 g cm^{-3}. It might be related to prone to compaction (Greenland and Lal, 1979; USDA, 2008; Mada et al., 2013). The mean bulk density values for the tilled layers of all the tillage implements and frequencies were lower for plots immediately after tillage as compared to one month after tillage. The mean bulk density values at the depth of 21-40 cm for most treatments showed an increment in mean values one month after tillage, which is in line with the finding of Nath (2014). The probable reason for this might be the soil condition created by tillage implements and frequencies where recently tilled soils tend to have lower bulk density compared to conditions before tillage. In line with this finding, different researchers (Nuhu and Tashiwa, 2006; Agbede et al., 2009; Ramzan et al., 2012; Ramzan et al., 2014), revealed that the number of passes of a tractor and traditional plow during tillage creates hardpan by compacting and pressing the agricultural land, which leads to the increased bulk density that finally brings decreased total porosity. Furthermore, Ahmad et al. (2009) reported that plowing with the same implement at the same depth for a long period affected soil physical properties such as bulk density and total porosity.

Table 4. Mean bulk and particle densities, and total porosities immediately and one month after tillage practice

	Immediately after tillage					One month after tillage			
	BD (g cm^{-3})		PD (g cm^{-3})	TP (%)		BD (g cm^{-3})		TP (%)	
Treatment	0-20cm	21-40 cm	0-20 cm	0-20 cm	21-40 cm	0-20 cm	21-40 cm	0-20 cm	21-40 cm
ZT	1.67^{a}	1.70	2.58	37.11^{b}	35.85	1.68^{a}	1.72	36.60^{b}	35.09
ODM2	1.65^{a}	1.69	2.51	37.86^{b}	36.23	1.64^{a}	1.70	37.99^{b}	35.72
ODM4	1.64^{a}	1.67	2.55	38.24^{b}	37.11	1.68^{a}	1.71	36.60^{b}	35.34
DP2	1.55^{b}	1.67	2.62	41.51^{a}	37.11	1.59^{b}	1.68	40.00^{a}	36.60
DP4	1.63^{a}	1.70	2.58	38.37^{b}	35.72	1.67^{a}	1.72	36.98^{b}	35.22
SE (±)	0.11	0.11	0.03	0.44	0.42	0.01	0.01	0.28	0.21
LSD(0.05)	0.043	NS	NS	1.62	NS	0.039	NS	1.48	NS
CV (%)	1.40	1.90	4.78	2.24	3.33	1.26	0.96	2.09	1.74

Note: Means within a column for specific soil parameters followed by the same letter(s) are not significantly different from each other at $P \leq 0.05$; SE= Standard Error, LSD = Least Significant Difference, BD= Bulk Density, PD = Particle Density, TP = Total Porosity, NS = Non Significantly Different, CV = Coefficient of Variation, % = Percentage

Particle density

The tillage implements and frequencies did not bring about significant differences in mean particle density. The lowest and the highest mean values of 2.51 g cm^{-3} and 2.62 g cm^{-3} were recorded for ODM2 and DP2, respectively (Table 4). The mean values were below the average particle density values for mineral soils (2.67 g cm^{-3}). The results showed that the effects of tillage implements and frequencies were non-significant on soil particle density and this might be explained by the fact that particle density is the same as the specific gravity of the soil's mineral materials, excluding the pore spaces. This result is in agreement with those reported by Brady and Weil (2008) that revealed the particle densities of most mineral soils vary between the narrow limits of 2.0–2.75g cm^{-3}. Several studies have shown that particle density of soils do not vary unless there are highly significant differences in soil organic carbon contents (Rühlmann et al., 2006; Martínez et al., 2008).

Total porosity

Following the pattern of bulk and particle densities, the total porosity significantly varied among the treatments for plow layers both immediately and one month after tillage (Table 4). Immediately after tillage, disc plow at two passes and zero tillage resulted in the highest and lowest mean values of total porosity of 41.51 % and 37.10 %, respectively, for the plow layer. At a depth of 21-40 cm, all treatments showed non-significant differences in mean total porosity both immediately after and one month after tillage. The total porosity under tillage with traditional oxen-drawn *Maresha* implements increased with increased frequencies of tillage. Unlikely, the tractor powered tillage implements decreased the total porosity with an increased number of passes through the plow layer. This decrease in mean values of total porosity under motorized implements could be due to the general increase in mean values of bulk density as a result of the repeated heavy wheel traffic causing compaction on the soil which was in line with the findings of Jamshidi et al. (2013) and Miriti et al. (2013).

The lowest mean total porosity value for zero tillage at the plow layer could be attributed to minimal or no soil disturbance, allowing the present soil bulk density to be maintained. According to Foth (1990), the disc plow's highest mean total porosity value at two passes could be attributable to the lowest mean bulk density of the soil during disc plow tillage at two passes, which is likely produced by the disc plow's mechanical disturbance. In addition, Anken et al. (2004) found that disc plow tillage resulted in higher overall porosity than zero tillage.

The mean total porosity values for all treatments revealed a slight decrease for all treatments one month following tillage. At the plow layer, the disc plow with two passes resulted in the highest mean total porosity value of 40.00 percent. At a depth of 21–40 cm, the mean total porosity values were lower than in the plow layers. The drop in mean total porosity values at a depth of 21–40 cm could be attributable to compaction caused by the weight of underlying soil layers and severe machinery loads during multiple passes, which likely raised the mean bulk density of the soil. The results showed that tillage implements and frequencies had a substantial impact on total porosity mean values. The highest mean values of total porosity were found at the plow layer as well as at a depth of 21–40 cm when tillage was done with a disc plow in two passes. This could be because disc plow plowing at two passes creates more porous soil. The soils were more porous just after tillage than they were a month later. The lowest mean total porosity values in zero-tilled soil could be owing to higher soil compaction caused by hard pan, which could lower the number of pores in the soil. This was in contrast to the findings of Tangyuan et al. (2009), who found that zero-tillage soil had higher overall porosity than tilled soil.

Soil water retention characteristics

Soil water content at field capacity (FC) and permanent wilting point (PWP), as well as available water content (AWC), were significantly affected by tillage implements and frequencies immediately after and one month after tillage (Table 5). Tillage by traditional oxen-drawn *Maresha* resulted in more water holding capacity at increased tillage frequencies, while tillage with tractor powered implements at an increased number of passes decreased the water content held by the soil (Figures 1 and 2). The soil water retention characteristic curve also showed that tillage implements and frequencies significantly affected the soil water retention characteristics at each suction. Four frequencies of oxen-drawn *Maresha* tillage resulted in finer soil particles, which increased soil pore space and water retention.

Table 5. Mean variations in soil water contents at field capacity, permanent wilting points and available water

	Immediately after tillage				One month after tillage			
	FC (v/v)	PWP (v/v)	AWC (mm m^{-1})	IR (cm hr^{-1})	FC (v/v)	PWP (v/v)	AWC (mm m^{-1})	IR (cm hr^{-1})
ZT	0.174^{a}	0.073^{c}	101.920^{a}	7.470^{d}	0.076^{b}	0.049^{bc}	26.915^{b}	20.570^{a}
ODM2	0.183^{a}	0.151^{a}	32.550^{b}	15.130^{bc}	0.062^{b}	0.044^{c}	18.197^{b}	7.700^{d}
ODM4	0.208^{a}	0.114^{b}	94.070^{a}	19.530^{ab}	0.123^{a}	0.070^{a}	52.847^{a}	17.200^{b}
DP2	0.178^{a}	0.104^{b}	74.660^{a}	22.700^{a}	0.137^{a}	0.064^{ab}	72.787^{a}	12.500^{c}
DP4	0.126^{b}	0.107^{b}	18.470^{b}	10.270^{cd}	0.065^{b}	0.042^{c}	22.485^{b}	2.930^{e}
SE (±)	0.008	0.007	0.009	1.699	0.009	0.004	0.006	1.713
LSD(0.05)	0.037	0.031	30.006	5.93	0.021	0.018	20.161	3.458
CV (%)	11.35	15.01	24.77	20.98	12.08	17.69	27.71	15.25

Note: Means within a column for specific soil parameters followed by the same letter(s) are not significantly different from each other at $P \leq 0.05$; SE= Standard Error, LSD = Least Significant Difference, CV = Coefficient of Variation, FC = Field capacity, PWP = Permanent Wilting Point, AWC = Available Water Content, IR = Basic Infiltration Rate

The decrease in available water content of the soil tilled by disc plow at four passes, on the other hand, could be owing to an increase in mean bulk density as a result of increased compaction. Furthermore, repeated disc runs on a soil can cause a decrease in mean total porosity values, which can squeeze bigger pores into smaller ones. This result matched the findings of Abu-Hamdeh (2004), who found that the kind of tillage implements and frequency had an effect on the soil water retention characteristics curve and water holding capacity of the soil.

Immediately after tillage, the highest and lowest mean values of water content at field capacity for ODM4 and DP4 were 0.208 cm^3 and 0.126 cm^3, respectively. Tillage with a disc plow in four passes lowered the water content at field capacity substantially. For DP2 and ODM2, the highest and lowest mean water content at field capacity one month after tillage were 0.137 cm^3 and 0.062 cm^3, respectively. This could be attributed to the disc plow's soil loosening impact, which reduced soil bulk density, resulting in larger pores and the ability to hold more water at lower frequencies. Gbadamosi (2013) found that two passes of conventional tillage with a disc plow increased soil water content and lowered soil bulk density. Moreover, Reynolds et al. (2009) revealed that at the plow layer, the water content at a field capacity of greater than 0.14 cm^3 was required in sandy clay loam soils to meet the plant water requirements. However, the more traditional threshold level of soil water content greater than 0.10 cm^3 was typically recommended for agricultural soils in order to reduce the incidence of yield-reducing aeration deficits in the root zone. Accordingly, the results of all treatments immediately after tillage showed good water content for crop growth. But, one month after tillage, only the disc plow at two passes and the oxen-drawn Maresha at four frequencies brought significantly different water content at field capacity, which was ideal for crop growth.

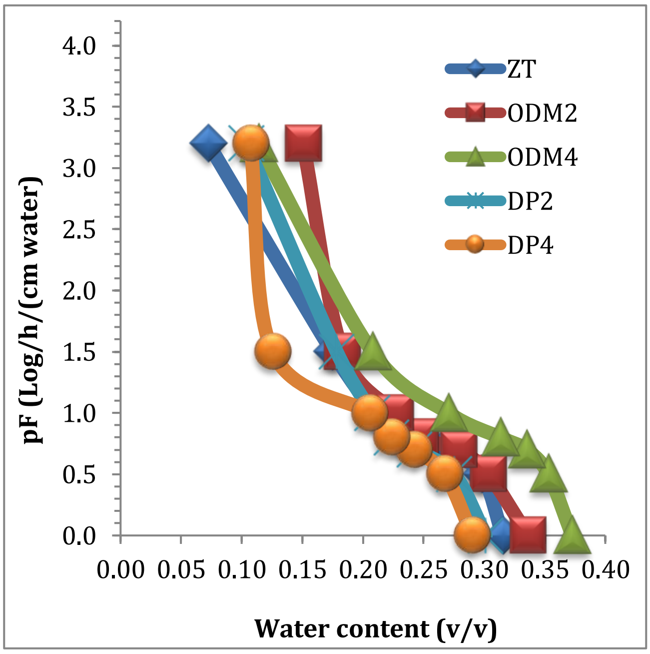

Figure 2. Effects of tillage implements and frequencies on soil water retention characteristics curve immediately after tillage

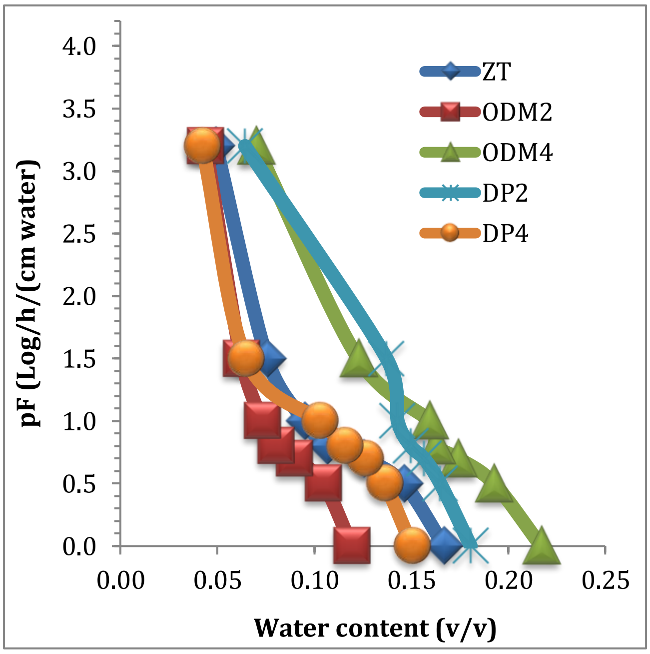

Figure 3. Effects of tillage implements and frequencies on soil water retention characteristics curve one month after tillage

At the permanent wilting point, there was a considerable change in mean water content values. The water content at the permanent wilting point was substantially higher in oxen-drawn *Maresha* at frequency of four, which could be linked to the relatively higher clay content (Table 3) one month after tillage. Because the water content at permanent wilting points pertains to the soil's ability to retain and provide water to plant roots, treatments with higher water content at permanent wilting points are likely to provide and replenish more plant-available water to plant roots.

Available water content (AWC) was significantly different immediately after and one month after tillage. Tillage by ODM4 resulted in the highest available water contents of 94.07 mm m^{-1} at field capacity (Figures 1 and 2). One month after tillage, the highest available water content was 72.80 mm m^{-1} for DP2. The treatment of ODM2 revealed the lowest mean values of 18.197 mm m^{-1} which could be associated with more compaction due to the relatively highest bulk density and low total porosity (Table 4), which resulted in variations of water content at field capacity and permanent wilting points.

In general, the variations in AWC among the treatments were due to the variations in water contents at field capacity and permanent wilting capacity which was in line with the findings of Hodnett and Tomasella (2002). These could, in turn, might be due to differences in bulk densities, sand and clay contents resulting in differences in water held by the soil (Table 3, 4, and 5). The effect of the tillage implements and frequencies on soil water characteristics curve also indicated that tillage under frequent plough by oxen-drawn *Maresha* hold more water at field capacity immediately after tillage, but disc plow at two passes hold more water at field capacity one month after tillage (Figure 2 and 3).

Despite the fact that the mean values of available water contents were significantly different, all treatments had available water contents lower than the average value for ideal crop growth in sandy clay loam soil, as indicated by Saxton and Rawls (2006). According to Reynolds et al. (2009), available water content of ≥ 0.20 cm^3 cm^{-3} was regarded optimum for root growth and functioning, while $0.15 \leq AWC < 0.20$ cm^3 cm^{-3} was good, $0.10 \leq AWC < 0.15$ cm^3 cm^{-3} was limited, and $AWC < 0.10$ cm^3 cm^{-3} was poor. The results showed that all treatments had inadequate available water contents, with the exception of zero tillage, which had restricted available water contents immediately after tillage. However, one month after tillage, all treatments had mean water content values that could be categorized as limited.

Conclusion

The results of the study indicated that disc plow tillage at two passes affected most of the examined soil physical parameters considerably ($P \leq 0.05$) immediately and one month after tillage. All of the treatments' bulk density values for the plow layer were lower immediately after tillage, and the mean values below the tilled layer for most treatments exhibited an increase one month after tillage. Due to substantial soil pulverization at lower frequencies, disc plows with a frequency of two dramatically reduced bulk density and total porosity.

The variation in particle density was non-significant. With the exception of percent clay contents (highest mean value of 26.30%) immediately after plowing by disc plows at two passes, insignificant changes in mean values of particle size distribution were observed. Apart from the minor increases in percent clay content one month following tillage, the soil's textural class remained sandy clay loam. However, soils under zero tillage and oxen drawn *Maresha* at two passes were sandy loam in texture immediately after cultivation. Water contents at field capacity and permanent wilting point was significantly different due to the variations in bulk density and percent clay content, resulting in considerable variances in available water content. At increasing tillage frequency, traditional *Maresha* tillage resulted in more water retention capacity. Among the treatments, soil under zero tillage followed by four oxen-drawn *Maresha's* revealed greater available water content immediately after tillage, but one month later, a two-pass disc plow shown much higher available water content, which could be attributed to the soil's increased clay content, which may hold more water. Furthermore, plowing with a disc plow in two passes had a significant impact on bulk density, total porosity, and soil water retention characteristics.

In conclusion, tillage instruments and frequencies have a negative impact on the physical properties of Fluvisols by altering the soil structure at surface and subsurface depths, resulting in various degrees of impact on soil bulk density, total porosity, and water retention capacity. Therefore, for less disruption of soil properties while performing soil tilth for agricultural production, it is recommended to use tillage implements at lower frequency.

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Eurasian Journal of Soil Science 2022, 11(3), 259 - 265

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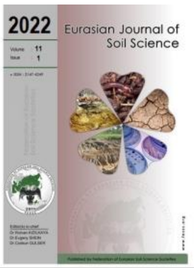

The effects of two Fe-EDDHA chelated fertilizers on dry matter production and Fe uptake of tomato seedlings and Fe forms of a calcareous soil

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Abstract

The present study was carried out to investigate the effects of two different ratios of Fe-EDDHA chelated fertilizers, (F1:4.8% and F2:6%) having the same amount of 6% soluble Fe content, on dry matter production and Fe uptake of tomato seedlings at different growth periods and Fe forms of a calcareous soil. The experiment was conducted in a factorial experimental design using Fe-EDDHA chelated fertilizers and the plant growth periods (10, 20, 30 and 40 days after seedling) with three replicates under the greenhouse conditions. The results indicated that the dry matter content, Fe uptake, chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid contents in plants generally increased over the control with increasing the growth periods. The plant dry matter contents were higher in F1 than F2 fertilization. The plant Fe uptakes in F1 treatment during the growth periods were also higher than that in F2 treatment. The carotenoid content and the chlorophyll formations in terms of both chlorophyll-a, chlorophyll-b were higher in F2 fertilization at the 20th day and higher in F1 fertilization at the 40th day. The DTPA-Fe and exchangeable-Fe contents in soil samples generally decreased while the organically bounded-Fe content in soil samples increased with increasing growth periods. It can be suggested that 4,8% of Fe-EDDHA fertilizer is more effective on Fe uptake when compared with 6% of Fe-EDDHA chelated Fe fertilizer. Therefore, F1 fertilizer can be used when chlorosis is seen on plants in calcareous soils. On the other hand, F2 fertilizer can be used if long-term Fe fertilization is desired. The differences in effectiveness between Fe-EDDHA chelated fertilizers having the same amount of water-soluble Fe content may be occurred due to differences in their chelating formulas.

Keywords: Tomato, Fe-EDDHA, Fe forms, seedlings.

Introduction

Iron is one of the basic nutritional elements for plant growth and development and affects both quality and yield parameters in plant production. Iron has a role of catalyst in chlorophyll formation. Therefore, plants cannot synthesize chlorophyll in iron deficient conditions, and yellowing (chlorosis) is observed in the veins between the leaves. When the plant is exposed to iron deficiency during the growing period, even if a short time, plant grows slowly, yield decreases, and the plant becomes more sensitive to stress conditions (Sainju et al, 2002; Fernández and Ebert, 2005). In recent years, several studies have shown that iron deficiency can cause yield losses in various plants (Takahashi, 2001; Jin et al., 2009; Ravet et al., 2009, 2012).

Iron deficiency in plants can be caused by the lack of iron in the growing media and the iron in the environment in a form that the plant cannot benefit from. Iron deficiency is seen in soils with high pH and lime content, especially in arid and semi-arid climates. The bicarbonate ion in calcareous soils prevents the movement of the accumulated iron from the roots to the leaves which directly affects its availability by

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buffering the soil pH (Elkins and Fichtner, 2012). The crucial factors decreases the effectiveness of iron such as; high pH, oxidation-reduction reactions, the richness of phosphate and carbonate in the environment, oxygen deficiency in the root area, antagonistic relationship due to high concentrations of manganese, zinc, or copper (Fernández and Ebert, 2005; Chohura et al., 2007).

To eliminate iron deficiency in plants, the most useful iron source is iron-chelate complexes. Chelated fertilizers are well soluble in water, have low dissociation constant, and show a stable structure (Wreesmann, 1996). These ferrous fertilizers are gradually transferred to the soil solution, or they can be kept in the form of organo-mineral complexes. Besides the effectiveness of the fertilizers used causally related to the chelating agent, the other most common chelating agents include EDTA, DTPA, and EDDHA (Lucena, 2003). Especially when soil pH level is higher than 7.2, many chelated iron fertilizers become ineffective. However, EDDHA chelated iron fertilizers are not affected by this situation due to the stable structure of this chelate. It prevents iron from precipitating even when soil pH rises above 9 (Fageria et al., 1990; Forner-Gina and Ancillo, 2011). Gülser et al. (2019) reported that different iron sources and application doses significantly affected plant growth criteria in soybean seedlings. They found that the highest shoot development was determined in 15 ppm nano-Fe application compared with the $FeSO_4.7H_2O$ and Fe-EDDHA treatments, and nano-Fe applications were more effective on seedling growth.

Among many vegetables, tomato is considered as one of the most available source of carotenoids. Carotenoids are pigments found in plants that give the colors between light yellow and red. It contains antioxidant properties, which are effective in preventing or delaying cancer (Stahl and Sies, 2005; Krumbein et al., 2012). Wala et al. (2022) reported that supraoptimal Fe-HBED supplementation significantly increased in xanthophylls and β-carotene contents in tomato plant which is very desirable for food quality.

Many researchers indicated that the relative efficiency of the different Fe chelates to remediate the Fe deficiency in plants has not been explored in depth, mainly due to difficulties in evaluation originating in the lack of purity of commercial products used in the studies (Lucena 2003; Álvarez-Fernández et al., 2005). Schenkeveld et al. (2010) reported that understanding the behavior of the FeEDDHA components in the soil-plant system as a function of time and dosage is important to relate this behavior to Fe uptake by plants. The aim of this study was to investigate the effects of two different ratios of EDDHA Fe chelated fertilizers (4.8% Fe-EDDHA and 6.0% Fe-EDDHA) on DTPA extractable, exchangeable and organically bounded-Fe contents in a calcareous soil, and also dry matter production, Fe uptake, chlorophyll and carotenoid contents of tomato plant seedlings at different growth periods.

Material and Methods

The experiment was carried out fertilizing tomato sedlings with two different Fe-EDDHA chealted sources in the greenhouse of Soil Science and Plant Nutrition Department in Agricultural Faculty of Ondokuz Mayıs University, Samsun-Turkey. After the soil sample was sieved through a 4 mm sieve, 4.5 kg of soil was weighed into plastic pots without drainage holes, and a tomato seedling was planted in each pot on March 2019. The experiment was conducted with fertilization treatments (C: control without fertilization and two different ratios of EDDHA chelated Fe fertilizers (F1: 4.8% Fe-EDDHA (Sidero) and F2: 6.0% Fe-EDDHA (Fe-Sequestrene), containing the same amount of water-soluble Fe content (6%)) and four different plant growth periods (10, 20, 30 and 40 days) in a factorial experimental design with three replicates. The Fe fertilization was applied as 13.6 mg Fe/kg soil for each pot. During the study, the pots were weighed daily, and soil moisture level was kept at field capacity. The tomato plants in pots were harvested after the 10, 20, 30 and 40 days after seedling. Soil particle size distribution was determined according to Bouyoucos hydrometer method (Demiralay, 1993), soil reaction (pH) and electrical conductivity (EC) values in 1:1 soil:water suspension, lime content by Scheibler calcimeter method, organic matter (OM) content by 'Walkley-Black' method and exchangeable cations (Ca, Mg, K, Na) by 1 N ammonia acetate extraction method, total N by Kjeldahl method, available phosphorus by Olsen Method (Kacar, 1994), and DTPA extractable Fe, Cu, Mn and Zn contents using atomic absorption spectrophotometer (Lindsay and Norwell, 1978). The basic properties of the soil used in this experiment are given in Table 1.

Table 1. Some chemical and physical properties of the soil.

Texture	OM	$CaCO_3$	pH	EC	Total N	P	Ca	Mg	Na	K	Fe	Cu	Mn	Zn
	%		(1:1)	µS/cm	%	ppm	me/100g				ppm			
Sandy loam	0,9	14,5	7,8	212,7	0,063	12,2	20,3	5,0	0,28	0,25	8,0	0,26	0,5	0,27

The Fe fractions in soil samples were determined as follows;

i) DTPA exchangeable Fe 1:2 (w/v) soil:solution mixture of DTPA (Lindsay and Norvell, 1978),

ii) Exchangeable-Fe 1:4 (w/v) soil:solution mixture of 1M Mg $(NO_3)_2$ and

iii) Organically bounded-Fe 1:2 (w/v) soil:solution mixture was extracted with 0.7M NaOCl (Shuman, 1985) and iron contents were determined by atomic absorption spectrophotometer.

In plant analyses, 0.2 g fresh leaf sample was taken after harvesting and the chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoid contents were determined according to Witham et al. (1971). After drying the plant samples at 65°C with aeration until reaching a constant weight, 0.5g dried plant sample was weighed, and dry ashing was carried out in a furnace at 550°C for 4-8 hours. The ash was dissolved in hydrochloric acid (HCl) and the iron content was determined using atomic absorption spectrophotometer (Jones et al., 1991).

The variance analysis of the data was determined according to Yurtseven (1984) in a factorial experimental design, and LSD test was used to compare the mean values of the results.

Results and Discussion

The Fe fertilization had significant effects on some plant properties and Fe fractions in soil at different growth periods (Table 2). The dry matter amounts of tomato seedlings increased by the Fe fertilization (Figure 1A). Both Fe fertilizer applications increased the dry matter ammounts over the control. While the dry matter amount was the lowest in the F1 application (0.79 g) at the 10th day, the highest dry matter amount (5.97 g) was also obtained in the F1 application at the 40th day. Chohura (2007) examined the effect of different chelated irons on the yield and quality of tomato plants and reported that there was a difference between iron chelates and increased the tomato yield. Similarly, many researchers indicated that Fe fertilization increased the tomato growth and dry matter production (Karaman et al., 2012; El-Desouky et al., 2021).

Table 2. The effects of Fe-EDDHA fertilizers (F), harvest periods and their interactions on some plant and soil properties

LSD	Dry matter	Chlorophyll A	Chlorophyll B	Total Chlorophyll	Carotenoid	Fe uptake	DTPA-Fe	Exc.-Fe	Org.Fe
	g/plant	mg/g fresh matter				mg/g plant	mg/kg soil		
F	0,410*	0,137**	0,068**	0,855**	0,029**	0,127**	1,098**	0,592**	0,690**
HP	0,473**	0,158**	0,079**	0,987**	0,033**	0,147*	1,268**	0,684**	0,797**
F*HP	0,820*	0,275**	0,136**	1,710**	0,058**	0,255**	2,197**	1,185**	ns

**;significant at 0.01 level, *;significant at 0.05 level, ns; non-significant, F; Fertilizers, HP: Harvest period.
Exc.Fe : Exchangeable-Fe ; Org.Fe : Organically bounded-Fe

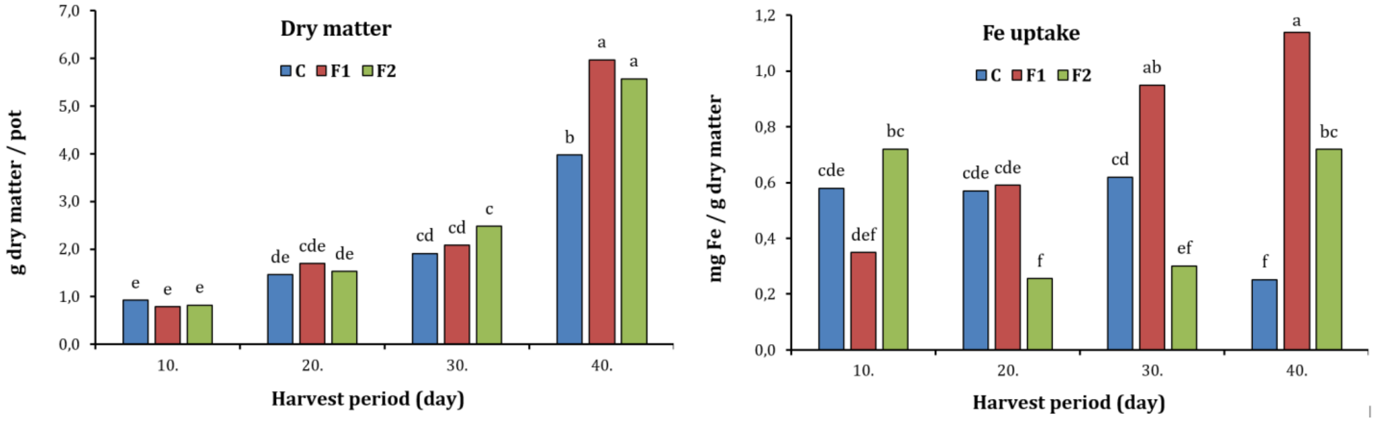

Figure 1. The effects of Fe fertilizations on the amount of dry matter (A) and the iron uptake (B) of the tomato plant at different growth periods. C: Control, F1: 4.8% Fe-EDDHA, F2: 6.0% Fe-EDDHA

Fe uptake values by the plants significantly influenced by the fertilization, period and interaction between fertilization and harvest period, statistically (Table 2). At the end of the 10th day, the highest iron intake occurred in the F2 application containing 6% chelated iron. While there was no statistical difference between the C and F1 treatment, the lowest iron uptake was in the F2 treatment at the end of the 20th day. The highest iron uptake was observed in the F1 application on the 30th and 40th days. In general, iron uptake of tomato plants increased with chelated iron fertilization applied to the soil compared to the control. This can be explained by the fact that EDDHA chelate is effective in most soils, as stated in previous studies (Sekhon, 2003; Gil-Ortiz and Bautista-Carrascosa, 2004).

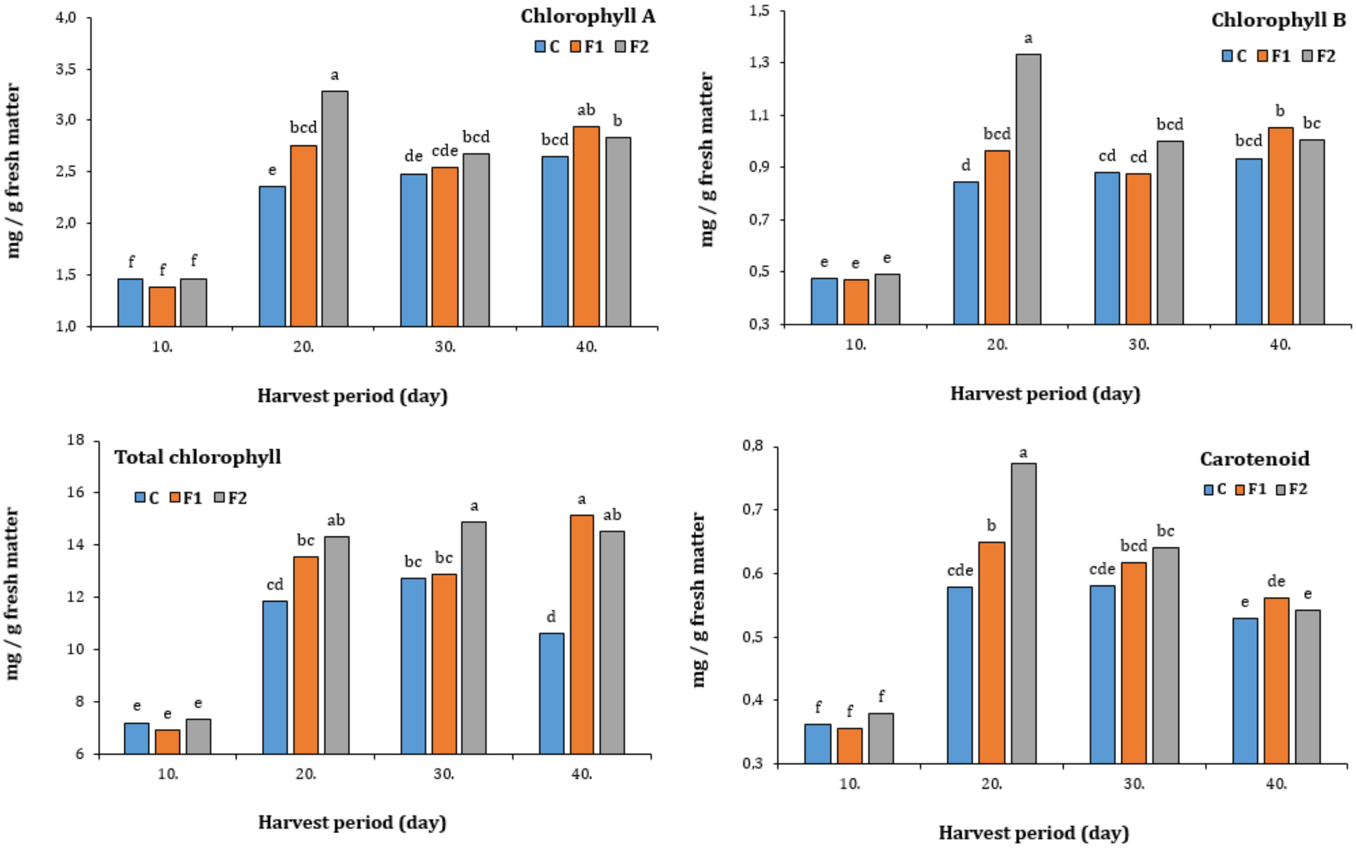

Figure 2. The effects of Fe fertilizations on the amount of chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids of the tomato plant at different growth periods. C: Control, F1: 4.8% Fe-EDDHA, F2: 6.0% Fe-EDDHA

The effects of different Fe fertilizations on chlorophyll-a and chlorophyll-b contents related with growing periods were statistically significant (Figure 2 A and B). The lowest amount of chlorophyll-a was determined as [1.37 mg (g fresh plant)$^{-1}$] in F1 application from the plants harvested on the 10th day, while the highest chlorophyll-a amount was determined as [3.29 mg (g fresh plant)$^{-1}$] in F2 application from the plants harvested on the 20th day. The lowest chlorophyll-b amount was determined as [0.47 mg (g fresh plant)$^{-1}$] in control and F1 applications in the plants harvested on the 10th day. In comparison, when the plants were harvested on 20th day, the highest chlorophyll-b content was recorded as [1.33 mg (g fresh plant)$^{-1}$] in F2 applications.

The total chlorophyll content of tomato plants were also significantly influenced by the Fe fertilizations and growing period (Figure 2 C). While the lowest total chlorophyll amount was determined as [6.95 mg (g fresh plant)$^{-1}$] in the plant harvested on the 10th day of the F1 application, the highest one was determined as [15.15 mg (g fresh plant)$^{-1}$] on the 40th day of the F1 application. In both Fe fertilization treatments, chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoid amounts were increased compared with the control treatment. The reason behind F2 application increased more than F1 application could be explained by the fact that the amount of chelated iron content of F2 fertilizer (6%) was higher than F1 fertilizer (4.8%). Erdal et al., 2013 found that although there was increase in the amount of chlorophyll-a in the plant, it was statistically insignificant as they applied different doses of ferrous fertilizers to the bean plant. However, they recorded statistically significant increases in chlorophyll-b and total chlorophyll values. Leaf chlorophyll content is the method best suited to assess plant Fe status (Abadía et al., 2004). Terry and Low (1982) reported that chlorophyll content is quantitatively related to the bound Fe content of the chloroplast lamellae, and Fe deficiency may reduce chlorophyll and lamellar Fe contents.

The effects of different Fe fertilizations on carotenoids contents related with growing periods were statistically significant (Figure 2 D). Carotenoids have important functional roles in plant physiology such as the protection of the photosynthetic systems against light energy excess through dissipating actions (Pogson and Rissler, 2000), energy transfer to chlorophyll related to the activity of the light-harvesting complex involving carotenoids (Ronen et al., 1999). The lowest amount of carotenoid [0.31 mg (g fresh plant)$^{-1}$] was obtained on the 10th day of harvesting from both control and F1 treatments. The highest carotenoid [0.72 mg (g fresh plant)$^{-1}$] was determined in plants harvested on the 20th day of F2 application. Borowski and Michalek (2011) stated that foliar application of iron salt increased carotenoid contents of French bean.

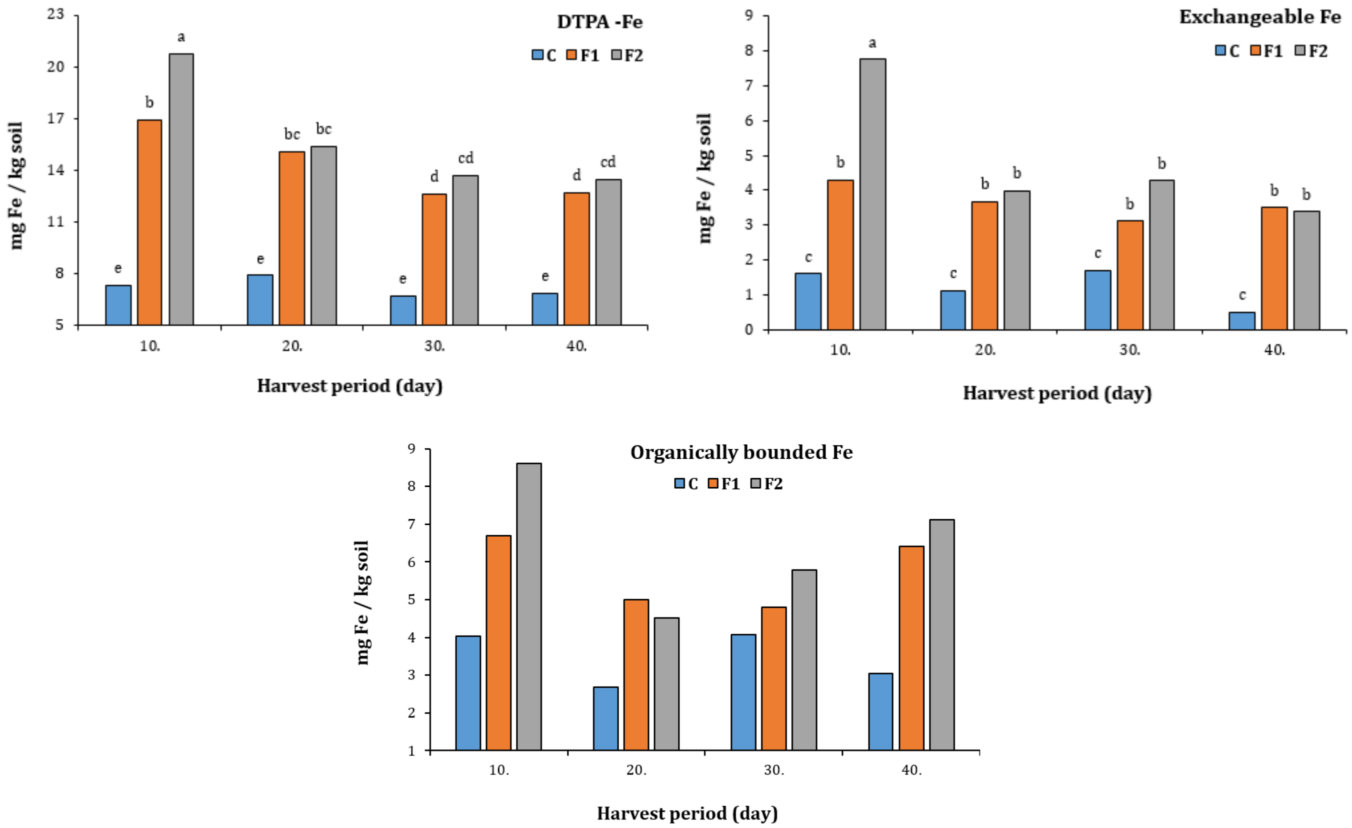

Figure 3. The effects of Fe fertilizations on DTPA extractable, exchangeable and organically bound forms of Fe contents in the soil sampled at different growth periods. C: Control, F1: 4.8% Fe-EDDHA, F2: 6.0% Fe-EDDHA.

In this study, the amount of DTPA extractable, exchangeable, and organically bounded-Fe amounts of soils were significantly influenced by the Fe fertilization treatments at the different growth periods (Figure 3). The Fe contents of soil samples in terms of all three forms (DTPA-Fe, exchangeable-Fe and organically bounded-Fe) were generally ordered as follow F2>F1>C for all growth periods. The lowest DTPA-Fe was found as [6.73 mg (kg soil)$^{-1}$] in the control soil sampled on the 10th day and the highest amount [20.77 mg (kg soil)$^{-1}$] was found in F2 application soil sampled on the 10th day. It had been seen that DTPA-Fe and exchangeable-Fe contents of soils decreased with increasing the growth periods.

The effects of Fe applications and sampling times on the exchangeable-Fe contents of soil samples were statistically significant. While the lowest exchangeable-Fe content [0.51 mg (kg soil)$^{-1}$)] was determined in the soil sampled on the 40th day of control application, the highest exchangeable-Fe content [7.76 mg (kg soil)$^{-1}$)] was determined in the soil sampled on the 10th day of the F2 application. Similar to DTPA-Fe contents, the F2 treatment increased the exchangeable-Fe contents in the soil samples greater than F1 treatment.

The organically bound Fe contents in all soil samples decreased after the 10th day, but these Fe contents in Fe fertilization treatments, especially in F2, increased from 20th to 40th day. While the lowest amount of organically bounded-Fe [2.68 mg (kg soil)$^{-1}$] was determined in the control soil at the 20th day, the highest amount was determined as [8.61 mg (kg soil)$^{-1}$] in the F2 application at the 10th day.

DTPA-Fe and exchangeable-Fe contents in soil samples were reduced after 10th day. This result can be explained by the use of DTPA- Fe and exchangeable-Fe in the soil by plants. Levesque and Mathur (1986) described that the most common plant available ferrous metal forms, as in other metals, are water-soluble and exchangeable-Fe forms. In this study DTPA-Fe and exchangeable-Fe contents in soil samples reduced with increasing plant Fe uptake and probably changing these forms to organically bounded-Fe forms during the growth periods. The organically bounded-Fe contents in soils reduced after the 10th days, but they gradually increased for F1 and F2 fertilizations until the 40th day. Schenkeveld et al. (2010) determined that FeEDDHA concentration in soil declined strongly within the first week with the Fe uptake by soybean plant and removal of FeEDDHA from the soil system displayed a similar trend with the Fe uptake by the plants in vegetative stages (3rd and 4th week) and the pods filling with seeds (6th week).

Conclusion

In this study, applications of two different Fe-EDDHA chelated fertilizers (F1:4.8% and F2:6%) having the 6% soluble Fe content were compared each other at different growth periods of tomato seedlings. The dry matter production of tomato seedlings increased with the F1 fertilization more than F2 fertilization, but this increase was not statistically significant. The plant Fe uptakes in F1 treatment during the growth periods, except 10th day, were generally higher than that in F1 treatment. The carotenoid content and the chlorophyll formations in terms of both chlorophyll-a, chlorophyll-b in the F2 fertilization were higher than the F1 fertilization and control treatment at the 20th day. However, these parameters were the highest in F1 fertilization at the 40th day.

While the DTPA-Fe and exchangeable-Fe contents in soil samples generally decreased, the organically bounded-Fe content in soil samples increased with increasing the growth periods. The all Fe forms determined in this study reduced after the 10th day, but organically bounded Fe content in soil samples gradually increased for F1 and F2 fertilizations during the following growth periods. Therefore, it can be concluded that when chlorosis is seen on plant leaves in calcareous soils, 4,8% of Fe-EDDHA chelated iron fertilizer (F1) can be used due to high Fe uptake by plant. If a long-term Fe fertilization is desired, 6% of Fe-EDDHA chelated iron fertilizer (F2) can be applied. The differences in soil and plant systems between F1 and F2 fertilizer applications may be occurred due to differences in their chelating formulas.

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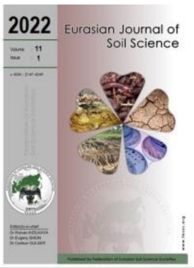

Study on the potential of silica-available based on types of soil on the productivity of paddy field in West Java Province, Indonesia

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Abstract

The Si-available (Si$_{ap}$) content in the soil of paddy fields is decreasing, so it will affect decreasing the productivity of paddy fields. Soil type maps can be used to estimate the potential Si$_{ap}$ content in paddy fields. The purpose of this study was to assess the productivity of the paddy field in West Java Province based on the Si$_{ap}$ potential in each region using maps of soil types and paddy productivity data. This research was conducted in West Java Province. The research was carried out from February 2021 to March 2021. The research method used was the descriptive research method. This research is secondary data analysis so that no field test is carried out. The validation of the data from the analysis was based on the literature from the previous researchers. The parameters measured in this study were: the distribution of paddy fields, the percentage of soil types in each paddy field, the average productivity of paddy field on each type of soil, the distribution of paddy productivity levels, the potential for Si$_{ap}$ to paddy productivity and map of the potential distribution of Si$_{ap}$ in West Java Province. Secondary data obtained were then analyzed using spatial analysis and descriptive analysis. The results of the spatial analysis show that 77% of paddy fields in West Java have medium Si$_{ap}$ potential, 17% low and 7% high. The results of the correlation analysis show that the productivity of paddy plants has a strong correlation ($r = 0.99$) to the Si$_{ap}$ of paddy soil. The soil maps can be used to estimate the potential of Si$_{ap}$ and the productivity of paddy plants. The Si application was recommended in paddy fields in the southern region of West Java Province.

Keywords: Land planning, paddy soil, productivity map, Si available.

Introduction

The National paddy filed productivity in 2019 based on data from the Central Bureau of Statistics (Badan Pusat Statistik, 2020) is 5.1 t/ha. The provinces of West Java, East Java and Central Java contributed to be the top three provinces with the highest paddy productivity in 2019, with average productivity of 5.8 t/ha. National paddy productivity and paddy productivity on the island of Java at the regional level of Southeast Asia, Indonesia, is still below the Vietnamese state's paddy crop's productivity, with the production of 5.88 t/ha (Foreign Agricultural Service, 2021). According to Adiningsih et al. (2000), the paddy fields productivity in Java is high, so increasing productivity is more challenging. The fertilization program recommended by the government still focuses on nitrogen (N), phosphorus (P) and potassium (K) fertilization (Husnain et al., 2020). Efforts to increase productivity on the island of Java, especially in West Java Province, need additional fertilization efforts and N, P and K. Silica (Si) is identified as a functional nutrient in paddy plants (Takahashi,

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1968). Some recent studies have strengthened the role of Si in paddy plants. Agostinho et al. (2017) and Pereira et al. (2004) stated that plants that were applied with Si fertilization had a high Si content in plant tissue and resulted in higher paddy productivity.

According to Wedepohl (1995), naturally, the Si content on the earth's surface is around 28% (Si-total), but the Si-available content is lower than the total Si content. According to Sumida (1992), Si available for paddy plants is a minimum of 300 mg SiO2/kg of soil. Paddy is a Si accumulator plant. Dobermann and Fairhurst (2000) stated that 480 kg of Si-available is removed from the soil of paddy field in a planting season. About 15% of Si-available paddy plants are removed out of the paddy fields. The process of transporting Si out of the fields in the form of husks and paddy straw. The Si content in paddy husks is about 10% of the biomass of the paddy plant. The management of paddy straw by burning can indirectly affect the available Si.

The burning of paddy straw in fields is a bad habit of most farmers in this country. The process of burning straw is considered more practical and makes it easier to cultivate the soil. The straw that is burned causes some nutrient loss of nutrients and they cannot be used by plants. The Si element in plant tissue will not be released into the air, but there is a change from Si-amorphous to Si-crystalline (Todkar et al., 2016). Several studies have shown that the use of paddy straw by fermentation as organic fertilizer can increase the paddy yields (Simarmata et al., 2016; Thammasom et al., 2016; Birnadi et al., 2019; Setiawati et al., 2020). The Si element available to paddy plants is in the amorphous form. Every time the harvest is continuously available, the Si reserves available in the long term will continue to decline. The continuously decreasing Si-available content causes the productivity of paddy plants to decrease. the effect of applying N, P and K fertilizers also decreases and the quality of the paddy produced decreases in protein and amino acid content (Liu et al., 2017; Nwajiaku et al., 2018).

The research conducted by Husnain et al. (2008) reported the Si availability of paddy soil varies in one area of the Citarum river basin. According to Dengiz (2020) soils in river basin areas show large variation even though over a short distance. The Si content is available in paddy soil, according to Liang et al. (2015), is influenced by basic material, soil type, land use, soil texture, soil pH, redox potential, organic matter content and environmental temperature. According to Savant et al. (1997), there is a relationship between soil types and available Si based on the USDA classification from the lowest available Si potential to the highest, namely Oxisols, Ultisols, Alfisols, Inceptisols, Vertisols and Mollisols. Identification of available Si potentials based on soil classification maps is an attempt to utilize soil type maps as a working map in land resource management planning (Grealish et al., 2015).

The scoring method or quantitative method is one of the methods in evaluating land resources. This method provides value to both qualitative data and quantitative data (Sitorus, 2010). The scoring is determined based on the results of the literature search. In the final assessment of this quantitative method, each parameter's score is added to obtain a total score. This total score is used to assess the potential status of Si in West Java Province. This study assessed the productivity of lowland paddy plants in each district/city based on the Si-available potential in each district/city. The research results are expected to provide recommendations for priority areas that require Si fertilization.

Material and Methods

Field description

The areas with the broadest paddy fields are Indramayu Regency, Karawang Regency and Subang Regency. The paddy field area in these three regions contributes 34% of the total paddy field area in West Java (Figure 1). Based on Indonesia's geomorphological map, the northern part of West Java is a depositional landform (alluvial plain). The central part is a volcanic landform and the southern part is a structural landform (Verstappen, 2014). The north coast region has rainfall less than 2000 mm per year based on morphoclimatic maps, including dry areas. However, the northern region of West Java Province is a lowland area through which rivers flow into the Java Sea. The availability of abundant water for irrigation water supports increased production throughout the year. The southern part of West Java and central West Java is included in the intermediate zone, where the average annual rainfall is above 2000 mm per year and below 3000 mm per year. A small proportion in the central region, especially in mountainous areas, has rainfall above 3000 mm per year (Perhumid).

Soil types in West Java paddy fields based on soil classification Dudal and Soepraptohardjo (1957) were dominated by Alluvial (35.28%) and Latosol (29.81%) types. The lowland paddy fields are dominated by Alluvial and Gleisol soils, while the paddy fields of latosol type are dry land that is tilled. The classification of soil types in paddy fields is determined by the classification of the soil from which it originates

(Hardjowigeno et al., 2004). Alluvial soil characteristics as young soil have varied textures, sticky consistency when wet and soil fertility is generally moderate to high. Moderate to high fertility variations are influenced by soil fertility on the upper slopes because alluvial soils are formed in river alluvial plains, coastal alluvial plains and basin areas (Sartohadi et al., 2014). The lower slope area is generally affected by soil deposition from the upper slope due to erosion. According to Hardjowigeno (2003), soil type Latosol has soil weathering characteristics, including advanced acid soil pH (4.5-5.5), low organic matter content and silica leaching.

This research was conducted from February 2021 till March 2021. The research location was in the administrative region of West Java Province at the geographic position (5˚50'- 7˚50' South Latitude and 104˚48'- 108˚ 48' East Longitude). The study area was illustrated in Figure 1. The materials and tools used in this research were:

Materials:

1. Data on paddy production and West Java Province's productivity in 2019 from the Central Bureau of Statistics (Badan Pusat Statistik, 2020).
2. Map of Indonesia's Earth (RBI) West Java Province from Geospatial Information Agency (2021).
3. Map of West Java Province soil types from the Regional Development Planning Agency of West Java Province.
4. Map of West Java province land cover from the Regional Development Planning Agency of West Java Province.

The equipment used is a laptop that contains the Arc Gis 10.1, SPSS 19, MS Office 2010 program.

Methods

This present study used the descriptive research method. The parameters observed in this study were: the distribution of paddy fields in West Java Province, the percentage of soil types in each paddy field, the average productivity of paddy plants on each type of soil, the distribution of paddy productivity levels, the potential for Si-available to paddy productivity and map of the potential distribution of Si-available in West Java Province. Secondary data obtained were then analyzed using spatial analysis, regression analysis and descriptive analysis presented in Table 4 and Figure 1 - 6. This research activiy is divided into several stages, namely secondary data collection, data input, data analysis and data presentation. The data collection process is carried out using digital exploration through the Central Statistics Agency, the Development Planning Agency, the Geospatial Information Agency through https://portal.ina-sdi.or.id/. The collected data is then processed using Arc GIS and MS Excel.

Data processing is in the following stages:

1. Overlay land use maps of paddy fields, administrative maps of West Java Province and maps of soil types. The overlay results map is then completed with paddy productivity data per district/city in the attribute table. The approach method used in filling paddy productivity data is the average productivity data from each district. The paddy field polygon data in the same administrative area have the same productivity value.
2. the next stage is the classification of paddy productivity levels in West Java into three classes: low, medium and high. The method of determining the interval for each class uses the following equation (Sudjana, 2005):
   - Interval = X max – X min
   - Number of classes = 3 (low, moderate and high)
   - Class length = $\frac{\text{Interval}}{\text{Number of classes}}$

Table 1. Paddy productivity category in West Java

Paddy Productivity (t/ha)	Class
$X \leq 5{,}4$	Low
$5{,}4 < X < 5{,}8$	Moderate
$X \geq 5{,}8$	High

3. Si's potential is determined by the scoring method, which is a method used to assign a value to each type of soil (Sitorus, 2010). The Si potential scoring refers to Liang et al. (2015) with modifications in scoring. The scoring is based on the USDA classification Histosol = 1, Oxisol = 2, Ultisol = 3, Alfisol = 4, Inceptisol = 5, Vertisol = 6 and Andisol = 7. Paddy productivity category in West Java.

Table 2. Si potential score based on soil type

Dudal and Soepraptohardjo (1957)	USDA*	Si-available Potential Score
Organosol	Histosols	1
Red Yellow Podsolik	Ultisols	3
Mediteran	Alfisols	4
Gleisol	Inceptisols	5
Litosol	Entisols	5
Alluvial	Entisols	5
Regosol	Entisols	5
Latosol	Inceptisols	5
Brown Podsolik	Inceptisols	5
Grumosol	Vertisols	6
Andosol	Andisols	7

Note: * The conversion of Dudal and Soepraptohardjo (1957) classification to USDA based on Subardja et al. (2014)

Data of the table for each soil type in the overlapping map are then scored according to soil types classification. The available Si potential was then classified into three categories, namely low, medium and high. Determination of the interval in each potential class Si - available using the equation from (Sudjana, 2005).

- Interval = X max – X min
- Number of classes = 3 (low, moderate and high)
- Class length $= \frac{\text{Interval}}{\text{Number of classes}}$

Table 3. Potency class Si-available

Si-available potential score	Class
$X \leq 4$	Low
$4 < X < 5$	Moderate
$X \geq 6$	High

4. Determination of paddy field area based on soil type and paddy field area based on available Si potential using spatial analysis. Spatial analysis is carried out by projecting maps from the World Geodetic System 1984 (WGS-84) coordinate system to The Universal Transverse Mercator (UTM) 48 S and 49 S. The UTM 48 S and 49 S projections are used because the administrative territory of West Java Province is located in two zones.

Results

The spatial analysis of paddy fields with the most significant area is concentrated in the Karawang, Subang and Indramayu regions. The deep green colour (Figure 1) in the northern part of West Java indicates vast paddy fields in that area. Meanwhile, in the southern part of West Java, there are fewer paddy fields.

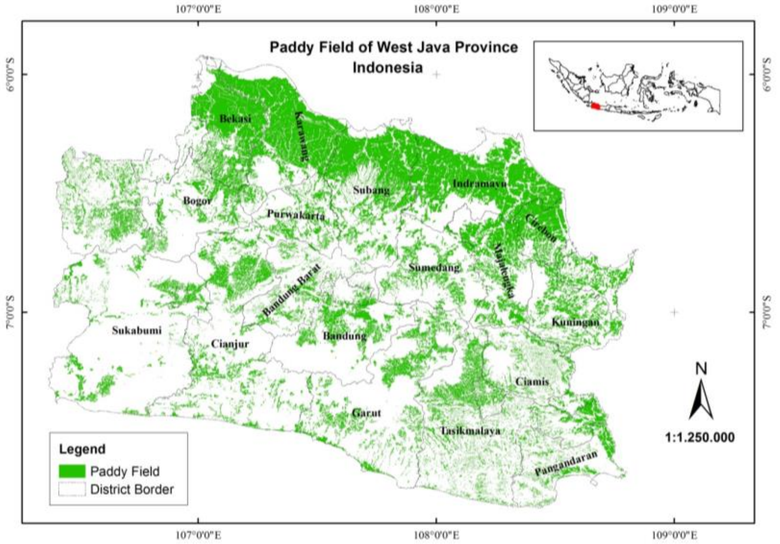

Figure 1. Output of spatial analysis paddy field West Java Province

Based on the soil type classification map Dudal and Soepraptohardjo (1957) from Regional Development Planning Agency (Bapeda) West Java Province shows that the paddy fields in West Java (Figure 2) with the most significant percentage are Alluvial soil types (35.28%).

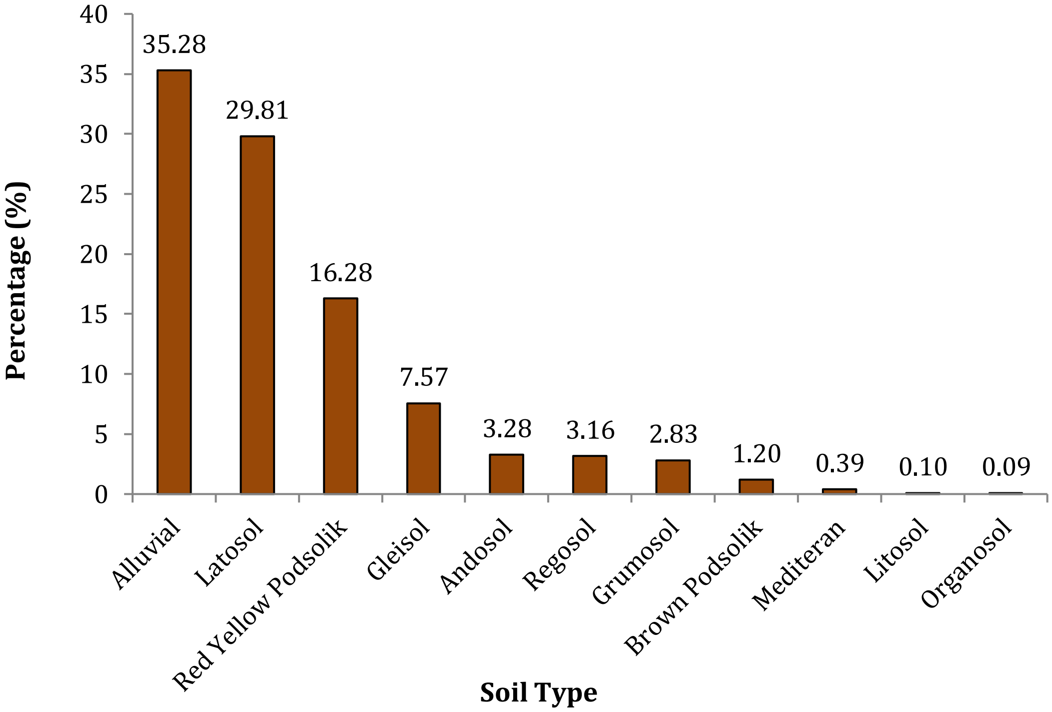

Figure 2. Percentage area of soil type West Java Province

The results of the analysis of paddy productivity data in 2019 from the Central Bureau of Statistics of West Java Province (Badan Pusat Statistik Provinsi Jawa Barat, 2020) and the soil type classification map based on Dudal and Soepraptohardjo (1957) from Bapeda West Java Province showed that Gleisol, Litosol andosol and Alluvial soil produced average paddy productivity of more than 5.8 t/ha (Figure 3).

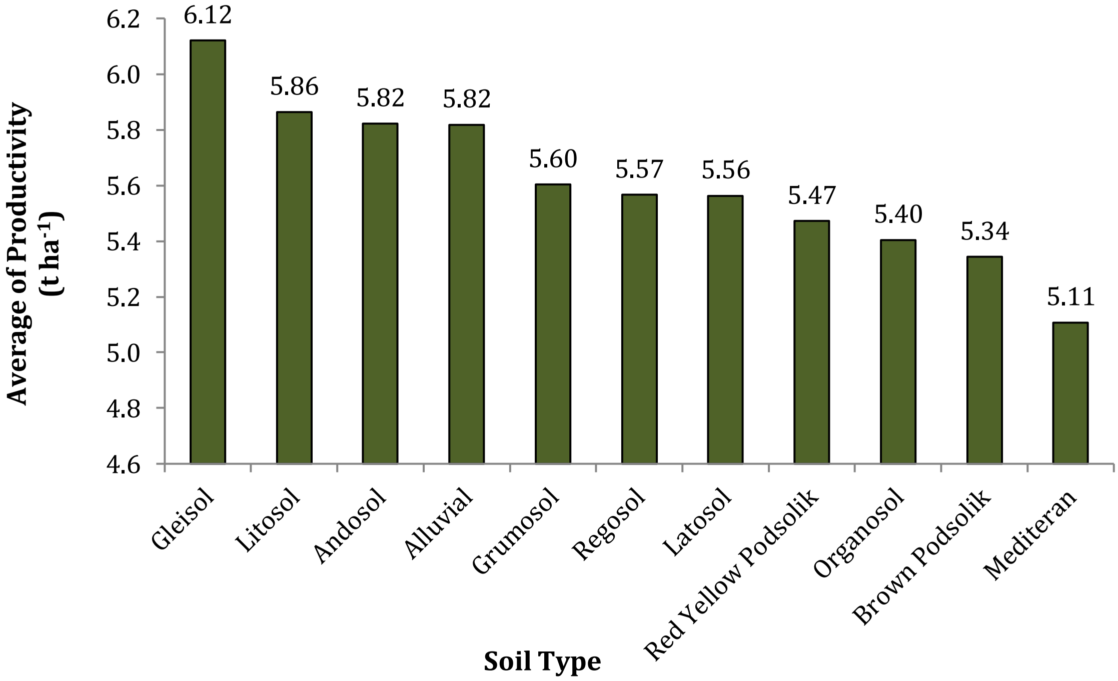

Figure 3. Graph of paddy productivity 2019 West Java Province based on soil type

The results of spatial analysis of paddy productivity levels in West Java Province (Figure 4 B) areas with a high average category of paddy productivity (> 5.9 t/ha) are primarily located in the northern part of West Java. The percentage of paddy fields in West Java with high productivity category is 44.78%, medium productivity is 35.65% and low productivity is 19.56%.

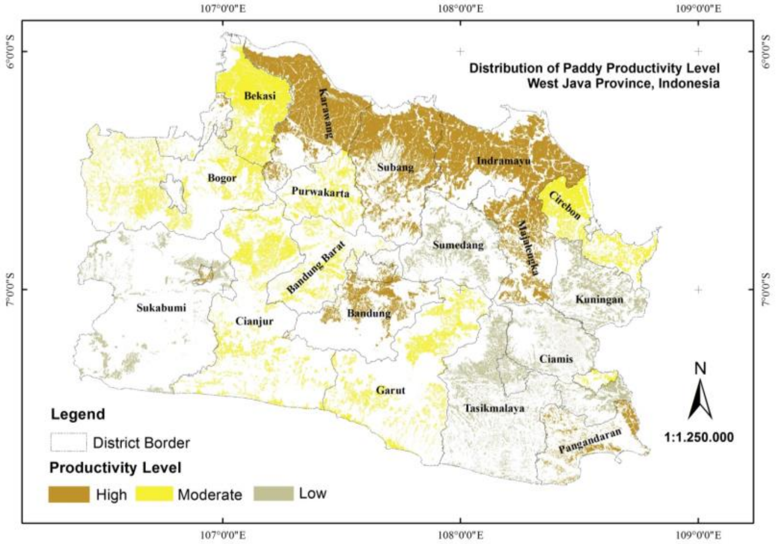

Figure 2. Output of spatial analysis (A) Soil type distribution of paddy field (B) Distribution of paddy productivity level

The results of the regression analysis of paddy productivity on the Si-available level (Figure 5) obtained a correlation coefficient of 0.99, which indicates a very high correlation (Sugiyono, 2012). The correlation coefficient value is positive, indicating that the increase in Si content will affect the increase in paddy productivity.

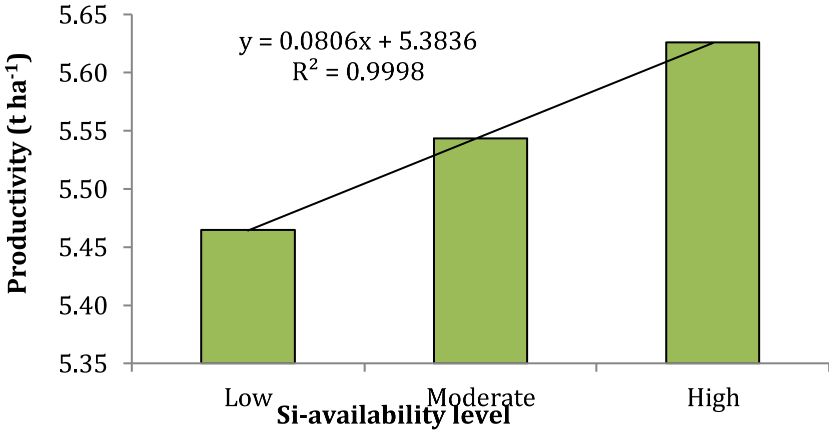

Figure 3. Graph of potential Si-availability level to paddy productivity

The spatial analysis results of the distribution of paddy productivity levels and Si-available levels (Figure 6) show that the productivity of medium and high paddy plants are in areas that have moderate and a high potential for Si-available. Meanwhile, areas that have low productivity are in areas that have low Si-available potential.

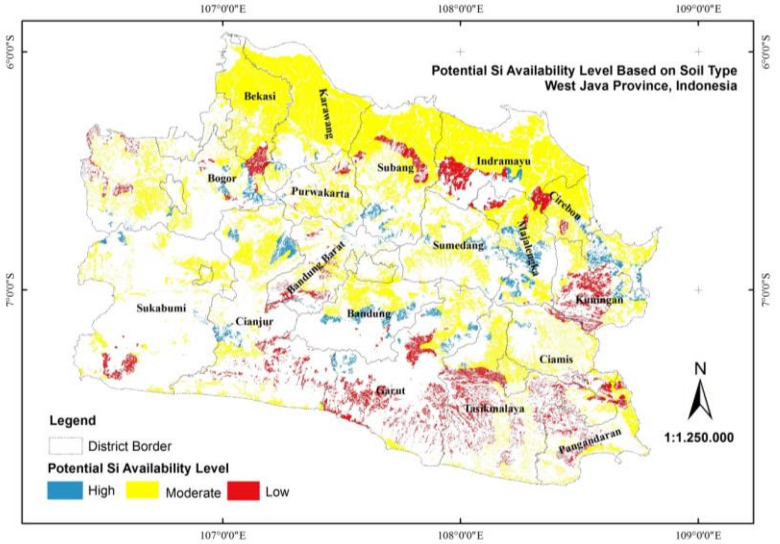

Figure 4. Distribution of Si-availability level based on soil type

The cross-tabulation of paddy field area based on productivity level and available Si level (Table 4) shows that low paddy productivity came from low to high Si-available paddy fields was 18.58%. The percentage of land with low productivity in paddy fields with high Si potential is 0.99%. Paddy productivity is being planted in paddy fields with a low to moderate Si-available potential of 32.62%. Medium paddy productivity in paddy fields with high Si-available potential is 3.03%. Paddy productivity is high in paddy fields with a low to medium Si-available potential of 42.04%. Paddy productivity is high in paddy fields with a Si-available potential of 2.75%.

Table 4. Cross-tabulation of the percentage of paddy field area based on the level of productivity and the level of Si-available

Paddy productivity level	Paddy field area (%)		
	Si-available potential level		
	Low	Moderate	High
Low	5.97	12.61	0.99
Moderate	6.83	25.79	3.03
High	3.82	38.22	2.75

Discussion

Forty-two percents of paddy fields in West Java Province are in the northern part, while the rest are scattered in the central and southern parts of West Java. The spatial analysis of soil types with productivity data (Figure 3 and 4) shows that the Gleisol soil type has the highest average productivity of 6.12 t/ha. The lowest is the Mediterranean type of 5.11 t/ha. Although Gleisol land has the highest productivity, it covers only 7.6% of the total paddy field area in West Java. The soil of Gleisols is constantly saturated with water and has a greyish colour. Soil types with the most significant percentage, namely Alluvial, Latosol and Red Yellow podsolic, have average paddy productivity of 5.82 t/ ha, 5.56 t/ha and 5.47 t/ha, respectively. The difference in productivity reduction from Alluvial soil type to Latosol soil type is 0.29 t/ha. The existence of paddy productivity differences in each type of soil provides information that paddy field management needs to adjust to the characteristics of the type of soil it originates. The Ministry has made the method of providing site-specific fertilizers of Agriculture of the Republic of Indonesia into a national program (Husnain et al., 2020). Soil type does not directly affect productivity, but different soil types have different characteristics, including soil fertility.

The regression analysis results (Figure 5) show a linear increase in paddy productivity with an increase in the potential of Si-available. According to Lavinsky et al. (2016) and Ning et al. (2016), fulfilling the Si needs of paddy plants in the reproductive phase will increase the number of seeds and weight of seeds. Meeting the need for available Si helps increase nutrient uptake of paddy plants N, P and K so that the efficiency of nutrient use and biomass formation also increases (Rao et al., 2019). The Si element in paddy plants and increasing nutrient uptake also helps plants minimize stress due to salinity, especially in paddy fields adjacent to coastal areas (Coskun et al., 2016).

The spatial analysis results (Figure 6) show that paddy fields with moderate and high paddy productivities are primarily located in northern West Java. Meanwhile, the southern part of West Java has a low level of productivity. The cross-tabulation of paddy field area based on the level of paddy productivity and the available Si potential level (Table 4) showed several anomalous conditions, including the percentage of land with high available Si potential, only 2.75%, which resulted in a high level of paddy productivity. Meanwhile, there is 3.82% of the land at the low Si-available potential, which produces high paddy productivity. The area of paddy fields with the potential level of Si-available in the medium category produces medium and high productivities levels of 64.01% of the total paddy field area in West Java. Alluvial and Latosol soil types with the most significant percentage affected the high land percentage with moderate Si-available potential.

Conclusion

The study shows that the productivity of paddy plants has a strong correlation (r=0.99) to the Si-available of the paddy soil. The soil map can be used to estimate the potential of Siap and the productivity of paddy plants. Further research is needed to determine the dose of Si fertilization based on the Si-available potential of paddy soil.

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