Czech University of Life Sciences Prague

Faculty of Agrobiology, Food and Natural Resources

Department of Agro-Environmental Chemistry and Plant Nutrition

Uptake of nutrients by wheat and maize from soil treated with ash from biomass incineration

Diploma Thesis

Author: BSc. Niguss Solomon Hailegnaw

Supervisor: prof. Ing. Pavel Tlustoš, CSc.

Declaration

I declare that this diploma thesis work on Uptake of nutrients by wheat and maize from soil treated with ash from biomass incineration is my own work and all the sources I cited in it are listed in References.

Prague, 8th April 2015 ……..

Niguss Solomon

Acknowledgement

I would like to express my deep gratitude to prof. Ing. Pavel Tlustoš, CSc. and Ing. Filip Mercl my research supervisors, for their patient supervision, enthusiastic encouragement and useful reviews of this research work.

I would also like to extend my thanks to laboratory technicians of Agro-environmental Chemistry and Plant Nutrition Department for their appreciated support while my laboratory work and also many thanks go to Ing. Ivana Perná, Ph.D. for providing XRF analysis of investigated ashes. It is also my pleasure to thanks my parents, sweetheart (Fevan Shewangizaw) and friends who were with me while undertaking this study.

Above of all, I would like to thank almighty God, who gave me strength, patience and ability to carry out this thesis work.

Uptake of nutrients by wheat and maize from soil treated with ash from biomass incineration

Summary

It is evident that biomass ash has the capacity to fertilize agricultural soil which can be deficient in available nutrients. Biomass ash contains nutrients which are removed by plant during their growth. But availability of nutrients from biomass ash is low as compared to chemical fertilizer. Due to this reason, there is an effort to find mechanism of increasing nutrient availability from biomass ashes. In this study efficiency of two inoculant products of plant growth promoting rhizobacteria (PGPR) in solubilizing or increasing availability of nutrients from biomass ash in soil-plant system were tested. The study was conducted on loamy cambisol collected from arable land of Humpolec, Czech Republic with two types of ash which are straw and wood origin and two commercial PGPR. Wheat (*Triticum aestivum* L., variety Aranka) and maize (*Zea mays* L. variety Colisée) were grown in pots filled with a mixture of soil and ash. Soil solution was collected every two weeks (6 times) to analyze soil available nutrients and low molecular weight organic acids. Plant biomass was harvested separately and content of macro-nutrients was determined. The results revealed increment of total biomass by 12.7%, 13.5% and grain yield by 11.9%, 14.1% of wheat after application of straw and wood ash respectively. In addition straw ash increased leaves dry weight of wheat by 21.9%. In maize straw ash increased biomass yield by 10.6%. Amount of available P, K, Ca and Mg in straw ash treated soil solution was twice higher than wood ash. Soil solution of both ashes was dominated by lactate and followed by acetate, formate and oxalate. From this it can be suggested that the release of nutrients especially Ca and Mg from SA was strongly influenced by oxalate which need further study. When we see tested PGPRs they were not effective in causing any positive effect on biomass yield or availability of nutrient in soil solution. Finally from our results we concluded that biomass ash can be used to fertilize nutrient deficient agricultural land but biomass ashes of different origin are strongly differing in their fertilizing capacity and also individual crops differ in their use efficiency of available nutrients from biomass ash.

Keywords: Wheat, maize, ash, plant-grow-promoting rhizobacteria, root exudates.

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1. Introduction

Economic development of modern society depends on field crops for human consumption, feed of animals and as an industrial raw materials for the production of clothing, energy, plastic and other products. Among these wheat, rice and maize are world dominant food crops and their global production is much higher than production of other field crops (Chandrasekaran et al., 2010). Soil fertility plays the major role in crop production. To achieve high crop productivity supplementing adequate amount of nutrients for the crop is essential and this can be done by adding chemical fertilizer or by recycling of plant nutrients (Fageria et al., 2011).

Use of an ash from biomass incineration as the fertilizer for crop production is one way of recycling plant nutrients. Nowadays, there is high amount of ash production from biomass due to high demand of alternative and renewable energy sources. Most of the mineral nutrients, which are removed during growth of plant, will remain in the ash after combustion of biomass. These elements mainly include P, K, Ca, Mg, Cu and Zn (Sander and Andren, 1997). It is important to use biomass ash as a fertilizer because it contains these essential nutrients and this recycling of nutrients from biomass ash can save nutrient resources by reducing the use of commercial fertilizer and also have advantage from point of proper disposal of this enlarged amount of ash production. Low availability of nutrients from application of biomass ash is generally accepted. So, there is an effort to find a way how to increase solubility of nutrients from biomass ash. One way may be an application of plant-grow-promoting rhizobacteria.

2. Scientific hypothesis and objectives

2.1. Objective

The aim of this study is to evaluate the abilities of wheat and maize plants to take up macronutrients from two different biomass ashes. The effect of plant-grow-promoting rhizobacteria on the yield of plant biomass and their effect on the production of root exudates will be investigated.

2.2. Hypothesis

Release of nutrients from ash in soil environment and their uptake by plant species is affected by the ash origin as well as plant species. Both release and uptake of nutrients can be enhanced by inoculation of plant-grow-promoting rhizobacteria.

3. Literature Review

3.1. Plant nutrients

Plants are composed of essential nutrients in which their amount differ from plant to plant depending on hybrid of variety, stage of growth, interaction among elements and that of soil factor (soil moisture, movement of ion, temperature, soil pH, tillage, and compaction) and specific elemental essentiality (Benton, 2012). These nutrients include Carbon (C), Hydrogen (H), Oxygen (O), Nitrogen (N), Phosphorous (P), Sulphur (S), Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe), Manganese (Mn), Copper (Cu), Molybdenum (Mo), Boron (B), Zink (Zn), Chlorine (Cl) and Nickel (Ni) (Osman, 2013). Based on the level of their importance to plant nutrition they are referred to as either a macronutrient or a micronutrient. Macronutrients are nutrients which found and required in relatively higher amounts as compared to micronutrients and they include C, H, O, N, P, S, K, Ca and Mg from which C, H and O constitute 90–95% of the dry matter weight of plant. And that of micronutrients includes Fe, Mn, Cu, Zn, Mo, B, Cl and Ni. Sometimes content of macronutrients in plant tissue can be a thousand times greater than the content of micronutrients (Kumar and Sharma, 2013). Generally these nutrients are presented in different amounts in plants for example that of macronutrients account 1000 mg/kg of dry matter or more and that of micronutrients accounts 100 mg/kg of dry matter base or less (Fageria, 2011).

Each of these essential nutrients performs different biochemical and biophysical function within the plant. For example the main function of mineral nutrients such as nitrogen, sulfur and phosphorous, which serve as constituents of protein and nucleic acid, are quite evident and readily described. Other nutrients, such as magnesium and micronutrients (except chlorine), may function as constituents of organic structures, predominantly of enzyme molecules, where they are either directly or indirectly involved in the catalytic function of the enzymes. Potassium and chlorine function involve mainly in osmoregulation (e.g., in vacuoles), the maintenance of electrochemical equilibrium in cell and their concentrations, and the regulation of enzyme activities. Naturally because of their low concentrations, micronutrients do not play a direct role in either osmoregulation or the maintenance of the electrochemical equilibrium (Marschner, 1995). In this review, macronutrients especially N, P, K, Ca and Mg will be further discussed.

3.1.1. Nitrogen (N)

Amount of nitrogen in soils ranges from 0.02 to 0.2% N by weight. And it is mainly occur in soil in organic form from which only 2% is available for plant. The most important inorganic form of N which plants absorb directly from soil solution is nitrate ion NO_3^- and the ammonium ion NH_4^+ (Benton, 2003). Soil pH, soil moisture, soil temperature, C/N ratio of the organic matter and soil texture will affect mineralization of organic nitrogen (Loll, 1983). In most well drained and moist agricultural soils having pH 6.0 or higher, nitrification will occur rapidly. But in that of acidic soil nitrification is inhibited by acidity and NH_4^+ will become dominantly absorbed by plant. Nitrification mainly occur under temperature ranging from 5 to 40°C and water content ranging from 50%-67% of the water holding capacity (Foth and Ellis, 1988). Application of N fertilizer will also greatly affect pH in the rhizosphere because nitrogen is the nutrient required in large quantities. When ammonium is the major nitrogen source more protons must be extruded which will reduce rhizosphere pH. Similarly when nitrate is the main nitrogen source, the rhizosphere pH tends to increase slightly. An additional cause of the decline in rhizosphere pH when ammonium is the source of N is that for each N that is incorporated into amino acids, one H^+ is produced. Production of H^+ is greater with ammonia because it is exclusively assimilated by roots but that of nitrate is assimilated partly in the roots and partly in the leaves (Lambert et. al, 1998).

In plants nitrogen is the most abundant mineral nutrient which ranges between 1.0 and 6.0% of dry weight of many crops. High N levels, however, can cause growth stimulations, that may produce deficiencies of other elements (if not supplied additionally) due to dilution effects (Benton, 2003). It is a part of the chlorophyll (the green pigment in leaves) and it is an essential constituent of all proteins, which is responsible for the dark green color of stem and leaves (vegetative growth), for vigorous growth, branching, leaf production, size enlargement, and yield formation (Chandrasekaran et al., 2010).

3.1.2. Phosphorus (P)

Content of phosphorus in soils ranges from 0.01-0.05 % by dry weight and it occurs in both, mineral and organic forms. Organic P is tied into the structure of the compounds and it is

unavailable to plants until the organic material decomposes. P is mainly taken up by plants in the form of dihydrogen phosphate ($H_2PO_4^-$) and sometimes plants may take up hydrogen phosphate (HPO_4^{2-}) at high pH value because of its dominance in solution having pH value above 7.2. PO_4^{3-} ion and H_3PO_4 molecules are not accessible for plants uptake because of their occurrence at very high and very low pH respectively (Troeh and Thompson, 2005). Most favourable pH for P availability is from nearly neutral to slightly acidic. Under mild alkaline condition, soluble phosphorous reverts to hydroxyl apatite $Ca_5(PO_4)_3OH$, then P deficiency will occur due to low solubility of calcium phosphate at pH value near to 8. P has positive interaction with N, K, and Mg (Fageria, 2009*).* For example if we see Ca, an increase in Ca content in soil solution increases P uptake, possibly because Ca stimulates the transport of P at the mitochondrial membranes. Concentration of P in mature leaves ranges from 2000 to 5000 mg/kg by dry weight. P content in actively growing plant parts is higher due to intense anabolism which requires multiple energy transfer reactions involving ATP (Benton, 2003). And also it is more concentrated in the reproductive parts because seeds must contain enough amount of phosphorous to satisfy P requirements until roots are formed. Most of time, seed crops contain the largest percentage of P as compared to forage crops. P concentration in many plants ranges from 800 to 1600 mg/kg in straw and 2700 to 7000 mg/kg in grain. For example amount of phosphorus is 3800 and 800 mg/kg in grain and straw of wheat respectively and that of maize contain 2700 mg/kg and 1000 mg/kg in grain and stover respectively (Smith et al., 1998).

3.1.3. Potassium (K)

Potassium in soils is presented in four different forms firstly as structural component of primary and secondary minerals; secondly as fixed K in the lattices of clay minerals; thirdly as adsorbed and exchangeable ions at the surfaces of soil colloids; and fourthly as solutes of soil solutions (Benton, 2003). Total content of K in most soils is in between 2500 mg/kg and 5000 mg/kg. However, only 0.1 to 2% of total soil K is readily available to plants. Hence potassium ions move from one category to another when removal or addition of K^+ disturbs the equilibrium within soil K^+ pool (Fageria, 2003). The relative presence of K, Ca, and Mg will influence concentration of each individual cation within the plant. There is a strong mutual antagonism between K and Ca and also Mg and NH_4^+ tends to depress K by plants. Nitrate content, soil

moisture and temperatures have effect on movement of K from the soil to the roots. K is the most abundant cation in plant tissues. It ranges from 15,000 to 40,000 mg/kg on a dry weight basis in recently and fully developed leaves of plant and as high as 60,000 to 80,000 mg/kg in the stem tissues of some vegetable crops (Benton, 2003). Potassium is readily taken up by plant roots from the soil solution in the form of the K^+ ion. K is the most important ion with respect to its physiological and biological function. Potassium will increase leaf area and leaf chlorophyll content, and delay leaf senescence then this will contributes to greater canopy photosynthesis and crop growth. K also controls water loss from plants, plays a crucial role in translocation of photosynthesis and also promotes mobilization of stored material and control activation of various enzymes (Kumar and Sharma, 2013).

3.1.4. Calcium (Ca)

Most soils of humid temperate region contains about 10,000-20,000 mg/kg of calcium (Troeh and Thompson, 2005). Generally calcium content in higher plants ranges from 5,000 to 30,000 mg/kg in dry weight base. This high uptake is mainly due to its high availability in soil solution not due to its efficiency of uptake method as it is only absorbed by young root tips. Ca is taken up by plant in the form of Ca^{2+} ions from the soil solution. Its main sources are Ca-carbonate and gypsum in some soils. Uptake of calcium by plant can be depressed by presence of ions like H^+, K^+, Na^+, Mg^{2+}, Al^{3+} and also its significantly affected by soil moisture level and reduced plant evapotranspiration (Benton, 2012). Calcium plays a very significant role in cell elongation and cell division. It is also important in maintaining cell wall integrity, activation of enzyme, osmoregulation, maintenance of cation–anion balance in cells and bio membrane maintenance (Kumar and Sharma, 2013).

3.1.5. Magnesium (Mg)

Magnesium concentration in soils vary widely due to variation in soil weathering and parent materials, Mengel et al. (2001) indicated Mg^{2+} content of most soils as 5,000 mg/kg for clay soils and 500 mg/kg for sandy soil. Based on Benton (2003) the normal concentration of Mg in plants ranges from 1500 to 4000 mg/kg. Mg is presented in soil in three different fractions; non

exchangeable, exchangeable and water soluble. From this non exchangeable form of Mg is the largest fraction and it can be dissolved at high level of H^+ concentration (pH lower than 3) if it is present in the form of carbonate. The exchangeable Mg^{2+}, which has the highest importance in the supply of plants accounts 5% of the total Mg (Feigenbaum et al., 1981). Plants will take up Mg from soil solution in the form of Mg^{2+}. Mg is highly mobile in soil, and this high mobility of Mg in soil result in high concentration of Mg in the soil solution which contributes for the mass flow to plant Mg nutrition and exposed Mg for leaching in considerable amounts. Uptake of Mg^{2+} is highly affected by K^+, NH_4^+, Ca^{2+}, and Na (Benton, 2003). Basically increasing concentration of Ca to a certain level will increase uptake rate of Mg. This is due to the reason that slight increase in concentration Ca in the nutrient solution will rapidly restores the membrane functionality, so that leakage will be reduced and the uptake of other cations enhanced. But further increasing of Ca concentrations in the nutrient solution will turns the positive synergistic effect of the nutrients into an antagonistic cation competition for uptake (Gransee and Fuhrs, 2013). Concentration of Mg may be also higher in plant which is supplied by low level of K^+ nutrition (Grimme et al., 1974). Mg is a constituent of chlorophyll and acts as the coordinating metal ion in the chlorophyll molecule. Thus it is involved in CO_2 assimilation and protein synthesis. Mg also regulates cellular pH and cation–anion balance and it activates several enzymes (Kumar and Sharma, 2013).

3.2. Composition and properties of wheat and maize

3.2.1. Wheat

Wheat is world's most widely cultivated food crop which can be grown in wide range of environment including the cold tract of the far north by tolerating severe cold and snow. Wheat can be cultivated from sea level to as high as 3300 m. The optimum temperature range for ideal germination of wheat seed is 20–25°C, though the seed can germinate in the temperature range of 3.5–35°C. It can be grown in regions where rainfall varies from 250–1500 mm/year. The wheat plants require medium (50–60%) humidity for their growth. But at time of maturity, crop requires less humidity and warm season. At the time of maturity, the plants require 14–15°C. It is grown in a variety of soils. Well drained loam and clay loams are good for wheat. However, good crop of wheat is raised in sandy loams and black soils also. Soils should be neutral in pH reaction.

Heavy soils with good drainage are also suitable for wheat cultivation under dry condition (Chandrasekaran et al., 2010). In most wheat-growing areas, farmers grow the crop on the same land every year. This produces soil fertility problems and increase water and wind erosion. Wheat is harvested when its moisture level is not more than 14 % (Benton, 2003).

3.2.1.1. Nutrient composition

Composition of individual nutrient is different between parts of plant for example grain contain high amount of N and P as compared to other parts of plant and that of K, Ca and Mg are higher in the top part (Fageria et al., 2011). The average wheat content of N, P and K as Limin et al. (2013) determined were 21200 mg/kg, 5600 mg/kg and 4300 mg/kg (oven-dry weight) respectively in grain and were 5700 mg/kg, 1400 mg/kg, and 15500 mg/kg respectively in straw. About 74% of N and 78% of P in above-ground plant are presented in the grain, and 79% of K in the straw. Therefore, grain was the primary pool for N and P, and straw for K. And Smith et al., (1998) determined content P in wheat grain and straw as 0.38% and 0.08% respectively.

3.2.1.2. Nutrient requirement

Crops require nutrients in different amount depending on soil, climate, yield potential of the cultivar, cropping system and stage of growth (Fageria et al., 2011). For example wheat requires nitrogen mainly at the grain filling phase and that of potassium is mainly taken up in the vegetative phase (Mengel et. al., 2001). In the period of 60-100 days after planting of wheat, the plant required as much as 3.90; 0.69; 7.47; 0.60; 0.35 and 0.32 kg $ha^{-1}day^{-1}$ of N, P, K, Ca, Mg and S, respectively (Preez and Bennie, 1991). Based on Roy et al. (2006) a crop of wheat producing 6.7 tonnes grain/ha absorbed an average of 200 kg N, 24.2 kg P and 209 kg K/ha. Under subtropical Indian conditions, a crop producing 4.6 tonnes grains and 6.9 tonnes straw absorbed 128 kg N, 20 kg P, 182 kg K, 27 kg Ca, 19 kg Mg, 22 kg S, 1.8 kg Fe, 0.5 kg Zn, 0.5 kg Mn and 0.15 kg Cu. It is highest during the maximum vegetative growth in spring. More than 80 % of the nutrients are taken up by ear emergence (Fageria et al., 2011).

3.2.1.3. Nutrient deficiency

Deficiencies of essential nutrients have more or less similar deficiency symptoms. Nitrogen deficiency leads to stunted plants with thin, spindly stems and short, erect leaves. If deficiency is severe tiller then grain production will reduce highly. Deficiency of P is more noticeable in young plants. Mild deficiencies cause stunted growth, more severe deficiencies produce small, light green plants that have short, erect leaves, stout stems, and often develop orange, red, or purple areas. Then tiller production and grain yield will reduce even by mild deficiencies. K Deficient plants are stunted and have thin, spindly stems and pale green, yellow-tipped foliage. The lower leaves may wilt and lie on the surface of the soil or may die and turn brown if the deficiency is severe. If deficiency persists or becomes severe, many young tillers die before producing heads, while mature tillers produce small heads that set few grains (Benton, 2003).

3.2.1.4. Exudation of low-molecular-weight organic acids

In study which was conducted by Kravchenko et al. (2014) the major organic acids identified in wheat root exudates were malic (55.3%), succinic acids (27.2%), citric acid (11.6%) and oxalic acids 2.4%. Organic root exudates solubilize unavailable soil P, Ca, Fe and Al phosphates (Dakora and Phillips, 2002). It was also confirmed by work of Khademi et al. (2010) in which organic acid increased P mobilization. Also Hens (2003) reported the increment in solubility of both organic and inorganic P by exudation of oxalate. Based on findings of Dotaniya et al. (2013) it was only oxalic acid which is exuded by wheat roots and its concentration was increased as level of phosphorus decreased. This is one of the mechanisms in adapting P deficiency. Neumann (1999) reported the increased exudation of carboxylates and citric acid in response to P deficiency. Deficiency of Zn induced exudation of organic acids by the roots of wheat plant, mainly malic and citric acids which were efficient in the release Zn from Fe-Mn oxides (Maqsood et al., 2001).

Organic acid exudation is also mechanism of avoiding Cu and Al toxicity. Severe Cu - Al stress on wheat seedling induced exudation of organic acid mainly malate and citrate (Niana et al., 2002).

3.2.2. Maize

Maize is one of the most important cereal crops in world agricultural economy both as food for human and feed for animals. Because of its higher yield potential compared to other cereals, it is called as "Queen of Cereals". It is originally a tropical crop which is a C_4 short day plant (Chandrasekaran et al., 2010). Maize can grow in most mild and tropical regions (30 to 55° latitude) mainly in latitudes below 47° and have with best yield in air temperatures range from 21 to 27°C and the optimum mean air temperature is below 19°C. Evapotranspiration ranges from 0.20 to 0.25 cm per day for young plants and up to 0.48 cm per day during the reproduction stage. It will produce best when rotated with other crops like legumes which can add N to the soil for the next corn crop and reduce the potential for pests and diseases. The best plant growth occurs on soils with pH levels from 6.0 to 7.0 and moderate to high fertility. Maize is best adapted to well-drained sandy loam to silt loam soil. Water stagnation is extremely harmful to the crop, therefore proper drainage is must. Maize cannot thrive on heavy soils especially on low lands with suitable pH of 5.5 up to 7.5 (Chandrasekaran et al., 2010).

3.2.2.1. Nutrient composition

Setiyono et al. (2010) observed variation in nutrient concentrations both grain and straw due to the wide range of environmental and management conditions. In grain the lowest and the highest content of N, P and K was 4,900–19,600 mg/kg, 600-5,200 mg/kg and 1,000-9,700 mg/kg respectively and in stover it was 2,200-19,600 mg/kg, 100-4200 mg/kg and 1,500-41,700 mg/kg respectively. When we see their average concentration in grain it were 13,300 mg N kg^{-1}, 3,600 mg P kg^{-1}, 4,080 mg K kg^{-1}, and in straw were 7,700 mg N kg^{-1}, 1,800 mg P kg^{-1}, 1,300 mg K kg^{-1}.

3.2.2.2. Nutrient requirement

Based on finding of Xu et al. (2013) the minimum and maximum internal nutrient efficiencies (IE, kg grain per kg nutrient in the above-ground plant dry matter) were 36 and 89 kg grain per kg N, 135 and 558 kg grain per kg P, 30 and 132 kg grain per kg K for spring maize, 31 and 70 kg grain per kg N, 108 and 435 kg grain per kg P, 32 and 110 kg grain per kg K for summer maize. To produce 1000 kg of maize grain yield, 16.9 kg N, 3.5 kg P and 15.3 kg K were

required by above-ground dry matter of maize. And for summer maize, 20.3 kg N, 4.4 kg P, 15.9 kg K were needed to produce 1000 kg maize grain in the linear part. Over half of the N and P and 80% of the K for best growth are required before the reproductive stage. Requirement of N varied from 45 to 100 kg ha^{-1} for corn which is planted after a legume crop and 145 to 170 kg ha^{-1} for corn which is planted after non leguminous crop (Benton, 2003).

3.2.2.3. Nutrient deficiency

Maize is very sensitive to N supply and even mild deficiencies severely reduce growth. Nitrogen deficient young plants are stunted and have thin, spindly stems and pale green to yellow, short, erect leaves. If the deficiency persists then it will have small ears and the depression of kernel size severely reduce grain yields. Phosphorous deficiencies cause reduced growth but few clearly recognizable leaf symptoms. When the deficiency is severe purple or purple- red colour will develop starting from the margin of older leaves. A deficient plant may produce only one small ear containing fewer, smaller kernels than usual. Grain yield is often severely reduced. That of K deficient plant will show stunted growth; plants have short, thin stems and pale green foliage. In severe deficiencies, plants become very stunted with short, spindly stems, pale green young leaves, and dead old leaves that hang down around the lower stems. Potassium deficiency severely reduces grain yield. Calcium deficiency produces very stunted plants with very short stems and stout; the foliage is green, often distorted, and appears torn and ragged. If the deficiency persists, young leaves have difficulty emerging fully and unrolling, and shoots may die before reaching maturity (Benton, 2003).

3.2.2.4. Exudation of low-molecular-weight organic acids

In three different variety of maize, exposure to aluminium stimulated exudation of oxalic acid (Kidd et al., 2001). It is also supported by work of Chaffai and Marzouk (2009) where exudation of citrate and in general organic acids was related to the degree of Al stress. Maize plants which were planted under P-deficient soil condition exuded high amount of acid phosphatase as well (Gaume et al, 2001). Exudation of organic acids increased solubility of soil nutrients e.g. oxalate increased P uptake and resulted in two fold accumulation of P in maize shoot as compared to the control (Strom et al., 2002).

3.3. Ash from biomass incineration

World biomass production is estimated to be 146 billion tonnes per year (Demirbas, 2001) and from this if only 7 billion tonnes burned then total production of ash per year is estimated around 476 million tonnes with 6,8 % mean ash yield (Vassilev et al., 2000), biomass may differ in their ash production, for example bark/wood chips fuels contain 2.2-2.5% ash and the straw fuel 4.2% (Etiegni and Campbell, 1991). Due to high production of ash worldwide and its valuable nutrient content, it is important to utilize biomass ash to create effective nutrient cycles in agriculture by which we can save nutrient resources. Biomass ash contains carbonates, sulphates, silicates, and phosphates (Vassilev et al., 2013). So ash can be effective source of nutrients for plant for example application of ash on loamy sandy soil increased crop P uptake as well as the readily plant available P pools in soil in comparison to the control (Schiemenz and Löbermann, 2010). Application of biomass ash to the soil exhibited remarkable effect on plant content of P, Ca, K and Mg (Demeyer et al., 2001; Park et al., 2005). Application of wood ash also facilitated Scots pines growth and also increased formation of stem biomass (Mandre et al., 2006). It has also increased yield of barley by 50% (Patterson et al., 2004).

Physical and chemical properties of ash are different from ash to ash depending on temperature, type of combusted material like whether it is wood or straw, part of plant combusted (wood, bark or leaf), type of soil and climate, condition of combustion, condition of collection and storage (Demeyer et al., 2001). The main properties of ash from biomass incineration specifically wood and straw ash are described below.

3.3.1. Straw ash

Straw fuel burning will produce 4.2% ash, in which most of the inorganic elements taken up during the growth of cereals remained. These residual elements include plant nutrients such as P, K, Ca and Mg and also Cu and Zn (Tan, 1994). Based on finding of Olanders and Steenar (1995) the burning temperature was 500°C in which ash dominated by calcium, silicon and potassium. Potassium was in the form of crystalline compounds with low melting points. Piekarczyk et al. (2011*)* reported the elemental composition of straw ash as 155.7 g kg^{-1} of K, 124.0 g kg^{-1} of Ca, 15.1 g kg^{-1} of P and 7.3 g kg^{-1} of Mg. And also he concluded that percentage

of available P and K for plant in straw ash is comparable with that of fertilizers. For example amount of P was 1.3% in wheat straw ash, 1.7 % in barely straw ash, 1.6 % in rye straw ash and 2.1% in rape straw ash (Sander and Andren, 1997). As it is reported by Schiemenz and Löbermann (2010) solubility of P from straw ash in water was low but about 80% of P was soluble in citric acid. Even if P solubility in water is low, its fertilizing effect was comparable to that of highly soluble P fertilizers such as triple superphosphate and supply of straw ash resulted in an increased uptake of P by cultivated crops and in an increase soil P pools (total P, water-soluble P, double-lactate-soluble P, and oxalate-soluble P).

3.3.2. Wood ash

Wood ash is produced as a waste product during wood combustion at power plant and paper industry. It is less expensive than conventional fertilization based on liming and mineral fertilization. Use of this waste product as a fertilizer is also good management of waste ash in addition to its use as source of nutrient for plant (Ferreiro et al., 2011). Content of wood ash mainly depends on species of plant used, origin of ash whether it is from bark or wood and combustion process (Zimmermann and Frey, 2002). Based on report of Etitgni and Campbell (1991), the yield of ash decreased approximately by 45% when the combustion temperature increased from 538 to 1093°C. In addition to this amount of nutrient like potassium, sodium, zinc and carbonate content decreased with temperature. When we look variation between sources of ash, Sano et al. (2013) proved this variation in content of nutrient between bark and wood ash, where concentration of Na, Al and Si was higher in bark ashes while that of K became higher in wood ashes. From all nutrient elements, K showed high water solubility and its plant-availability Ulery et al. (1993) but in the bark ash K content was low and that of Ca and Mg had intermediate solubility while P was less soluble in both water and acetic acid. Generally speaking wood ash contains K, Ca and also has significant amount of P and Mg (Park et al., 2004). As Park et al. (2005) find out total content of N, P, K, Ca, Mg, and Na in wood ash was 0.03–0.09%, 0.5–0.6%, 1.6–3.6%, 7.1–15.5%, 0.6–1.3%, 0.27–0.30% respectively. Wood ash application showed increment of extractable P, K, Ca, and Mg concentration in soil. After ash is applied availability of K will increase immediately with no long term effect. But that of P will not result in an immediate availability due to its low solubility and it will become available over long period of

time after effect of weathering and organic acids (Sano et al., 2013). In study of evaluating the effect of wood ash on the biomass production and nutrient status of young silver birch (*Betula pendula,* Roth); silver birch on wood ash treated soil showed an increase in concentration of P, K and Ca in leaves and also obtained higher biomass yield with better annual height increment as compared to control without wood ash addition. There was no great difference between the effects of the ash doses. However, usually the higher dose resulted in higher P, K and Ca concentrations in leaves (Kikamagi et al., 2013). Wood ash treatment on pasture land also increased pasture yield by 100% and that of feed value by 60% by increasing proportions of white clover and ryegrass (Ferreiro et al., 2011). From application of wood ash plants can benefit (increased biomass production and favoured plant growth) from Ca and K supplementation, from change in soil chemistry and reduced Al and Mn toxicity (Nkana et al., 1998). Reduction of potentially toxic element (Cd by 60%, Zn by 50%, and Pb by 45%) and increment in concentration of major nutrient in wheat which were planted in contaminated soil also reported by Ochecova et al. (2014). But Erich (1991) confirmed in his results the week solubility of P from wood ash and the large portion of the dissolved P is probably immobilized in the soil. This conclusion was also supported by the finding of Erich and Ohno (1992).

Application of wood ash (8 t ha^{-1}) on an acid forest soil showed an increase in soil pH and quantities of exchangeable cations (Ca, Mg, and K), application of wood ash also showed a significant increase in microbial activity due to increased growth rate of microorganisms in the soil environment by addition of nutrients and increase of soil pH (Zimmermann and Frey, 2002). After soil treatments with wood ash there was an increase in the pH of soil and soil water of all treatments while pH and concentrations of extractable nutrients of the untreated soils of control sample plots showed no change (Mandre et al., 2006).

3.3.3. Availability of nutrient from biomass ash

It is well documented that biomass ash have significant amount of essential plant nutrients but their uptake by plant is very low due to their low solubility. For example as it described by Naylor and Schmidt (1986) from wood stove ash only 51% K was available for plants. P availability in wood ash is also 28 to 70% lower than that of commercial fertilizer (Erich and Ohno, 1992). This low solubility of nutrients from biomass ash can be improved or increased by

different methods like carbonation of biomass ash using CO_2 before application of ash to the soil which will reduce high alkalinity of ash (Dong et al., 2014), re-burning of ash; there was an increase in the amount of Ca, K and P from 12-18%, 6.5-8.9 and 0.6-0.95 respectively at the re-burning temperature of 550°C, increasing dose of ash to be applied to achieve desired concentration of nutrient required by the plant (Tan and Lagerkvist, 2011), colonization of ash by ectomycorrhizal fungi which mobilized nutrients in ash under field condition (Mahmood et al., 2001) and inoculation with plant growth promoting rhizobacteria also increased availability of soil nutrients (Vessey et al., 2003; Nadeem et al., 2014; Lugtenberg et al., 2002; Cakmakci et al., 2006).

3.4. Plant Growth Promoting Rhizobacteria

Vessey et al. (2003) defined Plant growth promoting bacteria (PGPR) as wide diversity of bacteria which grow in, or around plant roots and when they grow in association with host plant, this results in stimulation of plant growth through different mechanisms. They facilitate plant growth by regulating nutritional and hormonal balance, producing plant growth regulators, solubilizing nutrients and also by inducing resistance against plant pathogens (Nadeem et al., 2014). PGPR increase plant growth, speed up seed germination, improve seedling emergence, responses to external stress factors, protect plants from disease and root growth pattern (Lugtenberg et al., 2002). Inoculation of wheat seed by *Pseudomonas aeruginosa* improved the uptake of P and N with an increase in leaf chlorophyll, total soluble protein and plant biomass production. Also this analysis showed that a disclose Zn concentration in root and shoot of wheat that was inoculated with *P. aeruginosa* as compared to wheat which is grown under Zn stress without inoculation (Faisa et al., 2014). These growth promoting bacteria also showed significant increase in growth of sugar beet, sugar yield and weight of leaf and root (Cakmakci et al., 2006).

Seed inoculation of fingermillet (*Elosine coracana*), maize (*Zea mays*), amaranth (*Amaranthus hypochondriacus*), buckwheat (*Fagopyrum esculentum*), frenchbean (*Phaseolus vulgaris*) with P-solubilizing *Bacillus* species indicated increase in grain and vegetative yield of these common crops (Sudhansu, 1998). Plant growth promoting bacteria have an ability to change nutritionally important elements from unavailable to available form (Vessey, 2003). For example Rendig and Taylor (1989) reported the solubility of calcite to soluble supplies of Ca^{2+}

and Dakora and Phillips (2002) release P from organic compound. On a study which is conducted by Verma et al. (2010) on chickpea a positive influence of plant growth promoting rhizobacteria and *Rhizobium* sp. BHURC01 has shown on nodulation, plant biomass, nitrogen and phosphorus in nodule, grain, straw and yield related parameter were recorded in two year of field experiments. Ahemad and Kibret (2014) who studied mechanisms and applications of plant growth promoting rhizobacteria also reported that these bacteria have importance in facilitating plant growth directly or indirectly by assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents. Gunes et al. (2014) concluded that these plant growth promoting bacteria have the ability to decrease the global dependence on agricultural chemicals by promoting plant growth through increasing macro- and micro-nutrient availability with exudates being more beneficial under environmental or stress condition.

3.4.1. Factors affecting activities of plant growth promoting bacteria

According to Latour et al. (1996) performance of plant growth promoting rhizobacteria severely influenced by environmental factors such soil types. Soil type has a marked influence on the rhizosphere microflora of maize, whereas cultivar type does not have role. Some studies indicate that the soil types are the dominating factor responsible for the diversity of the bacterial populations associated with plant roots. Egamberdiyeva (2007) in his study proved their effectiveness on nutrient deficient soil as compared to nutrient rich soil. The bacterial inoculation showed much better stimulatory effect on plant growth and nitrogen (N), phosphorus (P) and potassium (K) uptake of maize in nutrient deficient Calcisol and their stimulatory efficiency reduced in relatively rich loamy sand soil. Root exudation of organic acids, carbohydrates, and amino acids and the sloughing of polysaccharides increase substantially when phosphate have low availability (Lambert et al., 1998). Vessey (2003) on his study reported that degree of relationship between the PGPR and the host plant have effect on enhancement of the nutrient status of their host plant growth, which implies that they needs to be in an intimate relationship with their host plant even if this degree of intimacy between the PGPR and the host plant depend on where and how the PGPR colonizes the host plant. Zabihi et al. (2011) also described

difference of effectiveness between different species of bacteria. In their study *Pseudomonas* strains differ in their ability to enhance plant growth and yield, and the strains with the higher PGPR activities (for example *P. putida* 108) are more effective on the growth and yield of wheat, however, the amount of crop production is optimum when such bioinoculants are used in combination with P fertilization. Number of this growth promoting bacteria will be decreased by application of nitrogen fertilizer for example there was reduction in population of *B. polymyxa* when N fertilization was applied, this is due to competitive suppression of diazotrophs by non-fixing bacteria probably induced by high amount of N (Gouzou et al., 1995).

3.4.2. *Bacillus amyloliquefaciens* FZB42 (Rhizovital)

Based on Idriss et al. (2002) *Bacillus amyloliquefaciens* can stimulate plant growth and also has the ability to produce secondary metabolites that suppress soil-borne plant pathogens. An auxin which is produced by *B. amyloliquefaciens* FZB42 is a major component of its interaction with the root system of crop like *Triticum aestivum*. This mechanism stimulates a greater rate of early growth of both seminal and lateral roots in *Triticum aestivum*. So this large root system will increase uptake of water and nutrients. For example it is beneficial along conventional phosphate fertilisation (Talboys, 2014). However, based on this study root capacity to take up phosphate (Pi) from soils with a low Pi concentration was reduced due to *Bacillus amyloliquefacizens* FZB42 auxin production.

3.4.3. Bacto_PROF (*Bacillus licheniformis, B. megaterium, B.polymyxa, B. pumilis, B. subtilis, Trichoderma harzianum*)

Inoculation of *Pinus pinea* by *Bacillus licheniformis* and *B. pumilus* promoted growth of *Pinus pinea* seedlings but these studies also demonstrated that a combination of *Bacillus licheniformis* and *B. pumilus* strains does not necessarily produced an additive effect (Probanza, 2002). Based on finding of Zaidi (2006), treatment of *Brassica juncea* by *Bacillus subtilis* strain *SJ 101* exhibited solubilization of inorganic phosphate. Tomato which is treated with *Bacillus subtilis BEB-lSbs* (BS13) have got higher fruit weight, yield per plant, length of fruit and also texture of red fruits is improved as compared to tomato plant which is not treated (Mena, 2007).

Grain yield of sorghum which is pelleted with *Bacillus subtilis*, with phosphorous fertilization was 2291 kg/ha and that of sorghum grain which is only fertilized with phosphorous was 1987 kg/ha which is 304 kg/ha greater than that of untreated one (Broadbent, 1977). Seed inoculation of *Arabidopsis thaliana* and *Phaseolus vulgaris* by *Bacillus megaterium* strain promoted growth and development of seedlings (Ortíz-Castro, 2008).

4. Material and Methods

4.1. Soil and biomass ash collection

Loam (Cambisol) was brought from arable agricultural land of Humpolec having the following characteristics: 30% (w/w) sand, 48% (w/w) silt, 22% (w/w) clay, pH ($CaCl_2$) 4.8 (Ochecova et al., 2014). Contents of plant-available nutrients, extractable by Mehlich 3 solution (Mehlich, 1984) were: Ca 1543 mg/kg, K 112 mg/kg, Mg 116 mg/kg, P 100 mg/kg. Soil was collected from upper horizon (Ap), then air dried and sieved thru 1 cm stainless sieve. Two types of biomass ash, cereal straw (SA) and wood ash (WA) were collected from two industrial combustion plants in Czech Republic and analyzed by the same methods as soil samples. Total element contents were determined by X-ray fluorescence (XRF) analysis, which was previously described by Szakova et al. (2013). Specific characteristics of ashes used are described in the following Table 1.

Character	Straw Ash	Wood Ash
pH ($CaCl_2$)	10.2 ± 0.01	11.2 ± 0.01
Total Ca (XRF)	56,460 ± 160	117,789 ± 200
Total K (XRF)	159,900 ± 200	58,938 ± 170
Total Mg (XRF)	9,030 ± 160	17,478 ± 280
Total P (XRF)	13,610 ± 20	10,195 ± 50
Available Ca (Mehlich 3)	5,931 ± 438	23,360 ± 1071
Available K (Mehlich 3)	37,464 ± 2,889	5,096 ± 224
Available Mg (Mehlich 3)	838 ± 75	2,607 ± 65
Available P (Mehlich 3)	1,977 ± 45	249 ± 10

Table 1 - Characteristics of straw and wood ash (mg/kg). All values represent means ± standard deviation (n=2).

4.2. Experimental design

A pot experiment was undertaken in outdoor precipitation-controlled vegetation hall of the Department of Agro environmental Chemistry and Plant Nutrition. Plants of wheat (*Triticum*

aestivum L., variety Aranka) and maize (*Zea mays* L. variety Colisée) were grown in 6L plastic pots filled with mixture of soil and biomass ash. 5 kg (d.w.) of soil (sieved thru 1 cm stainless sieve) and 1% (50 g of biomass ash per pot) were mixed to the soil thoroughly and filled to the pots. Both ash treatments were moreover inoculated by PGPR (two types of commercially available bio inoculants of ABiTEP, Germany and TerraBioScience, Germany). Control treatments without ash addition were also established. The experiment was conducted in randomized block design in three replications. Detailed experimental design is shown in Table 2.

Cambisol								
Ash	**Cereal straw ash (SA)**			**Wood ash (WA)**			**Control**	**Control-PK**
PGPR	**BE3**	**NoBE 0**	**BE4**	**BE3**	**NoBE 0**	**BE4**	**0**	**0**
Fertilization	0.5g N	0.5g N	0.5g N	0.5g N	0.5g N	0.5g N	0.5g N	0.5g N+ 0.16 g P + 0.44 g K
Wheat pot No.	1,2,3	4,5,6	7,8,9	10,11,12	13,14,15	16,17,18	19,20,21	22,23,24
Maize pot No.	49,50,51	52,53,54	55,56,57	58,59,60	61,62,63	64,65,66	67,68,69	70,71,72

Table 2 - Experimental design.

4.3. Crop cultivation and fertilization

Thirty seeds of wheat on 10th April 2014 and 5 seed of maize on 23rd April 2014 were sown in the pot at depth of 2 and 5 centimeters respectively. All treatments were fertilized with 0.5 g N (NH_4NO_3) and also irrigated before sowing to promote proper germination of seeds. Irrigation was also applied after sowing on regular base at 60% of soil water holding capacity. Control PK treatments were established as a mineral fertilized treatment and were enriched by 0.16 g P and 0.44 g K (K_2HPO_4). Thinning of seedlings were applied 37 DAS (days after sowing) for wheat and 23 DAS for that of maize.

4.4. Inoculation

All plants were inoculated twice, initially at the time of sowing and then at the stage of third leaf development which was 29 DAS for wheat and 16 DAS for maize. BE3 treatments were inoculated using 200 ml of RhizoVital® 42 (ABiTEP, Germany) solution (1 ml of RhizoVital® per L). BE4 treatments were inoculated using 50 ml of Bacto_Prof (TerraBioScience, Germany) solution (2 g of Bacto_Prof per L).

4.5. Soil solution sampling

Samples of soil solution were collected every two weeks using Soil Moisture Samplers - Rhizons (Rhizosphere, Netherlands) which were installed in the pot while filling with soil. 20 ml syringes were used to create vacuum. 10 ml of soil solution were collected to measure nutrients in soil solution (plant available nutrients) and 0.5 ml for organic acid measurement. For each 0,5ml soil solution 10 µl of methanol were added to avoid microbial degradation of samples.

4.6. Crop harvest

Separated plant parts (leaves, stems and grain) of wheat and maize were harvested on 29th of July 2014 and 13th of August 2014 respectively and stored in separate bags. Roots of plants from each pot were washed thoroughly on wire mush by running demineralized water to remove soil particles then dried in open air. These all plant parts were oven dried at 60°C, dry weight was determined and were grinded with laboratory mill for further analysis.

4.7. Plant and soil solution analysis

From grinded plant parts 0.5 g were weighed then digested with 7 ml concentrated (65% v/v) HNO_3 (Analytika) and 2 ml (30% v/v) H_2O_2 (Analytika) in Ethos 1 microwave oven (Milestone). Then concentrations of nutrient were determined by optical emission spectrometer with inductively coupled plasma ICP-OES (Varian Vista Pro, Varian Australia) and that of higher potassium concentrations were determined using flame atomic absorption spectrometer F-AAS (Varian AA285S, Varian Australia).

Low molecular mass organic acid (especially lactic, acetic, formic and oxalic) were also determined by means of ion-exchange chromatography with suppressed conductivity. The ion chromatograph ICS 1600 (Dionex, USA) equipped with Ion Pac AS11-HC (Dionex, USA) guard and have analytical columns. Mass and speciation of available nutrients were determined by atomic spectrometry. And that of available nutrients in soil solution were determined by I CP-OES for Ca and Mg. But P and K were measured by ICP-MS and F-AAS respectively.

4.8. Statistical methods

Data were compared using one-way analysis of variance followed by *post-hoc* Fischer`s LSD test. All statistical analyses were done using the software STATISTICA 12. MS Excel 2010 was used for the calculation of means and standard deviations.

5. Results

5.1. Biomass yield

5.1.1. Wheat

Wheat								
Ash	**Straw ash (SA)**			**Wood ash (WA)**			**Control**	**Control-PK**
PGPR	**BE3**	**NoBe**	**BE4**	**BE3**	**NoBe**	**BE4**	**0**	**0**
Stems	$19,6^{d}$ (*2.4*)	$17,9^{ad}$ (*0.9*)	$17,0^{ab}$ (*1.4*)	$14,3^{c}$ (*1.0*)	$15,9^{abc}$ (*0.2*)	$16,6^{ab}$ (*0.8*)	$15,1^{bc}$ (*0.8*)	$17,7^{ad}$ (*0.3*)
Leaves	$7,5^{cd}$ (*0.8*)	$7,8^{d}$ (*0.3*)	$7,3^{bcd}$ (*0.3*)	$6,3^{a}$ (*0.3*)	$6,5^{ab}$ (*0.2*)	$6,8^{abc}$ (*0.4*)	$6,4^{a}$ (*0.3*)	$7,0^{abc}$ (*0.1*)
Grain	$22,6^{bc}$ (*1.7*)	$20,7^{ab}$ (*1.0*)	$20,1^{ab}$ (*1.7*)	$19,2^{a}$ (*2.6*)	$21,1^{ab}$ (*0.2*)	$22,5^{bc}$ (*0.3*)	$18,5^{a}$ (*1.4*)	$24,4^{c}$ (*1.2*)
Root	$13,2^{b}$ (*2.0*)	$9,6^{ab}$ (*0.8*)	$11,9^{ab}$ (*0.5*)	$8,6^{a}$ (*3.2*)	$12,9^{ab}$ (*3.6*)	$10,6^{ab}$ (*1.0*)	$9,7^{ab}$ (*2.0*)	$9,8^{ab}$ (*1.6*)
Sum of biomass	$62,9^{b}$ (*6.1*)	$56,0^{ab}$ (*1.8*)	$56,3^{a}$ (*3.6*)	$48,4^{ab}$ (*6.9*)	$56,4^{ab}$ (*3.8*)	$56,5^{ab}$ (*0.9*)	$49,7^{ab}$ (*3.7*)	$58,9^{ab}$ (*1.8*)

Table 3 - Total and individual yield of wheat biomass (g/pot). All values represent means (n=3). Standard deviations are listed in the brackets in italics and different letters in the superscript indicate significant difference (LSD, $p < 0.05$) between means.

The highest and the lowest total biomass produced by wheat were 62.9 g per pot and 48.4 g per pot in straw ash treatment which is inoculated with BE3 (SA-BE3) and in WA treatment which is inoculated with BE3 (WA-BE3) respectively. Total biomass was slightly higher in both SA and WA treatments as compared to Control (Table 3).

The highest and the lowest yield of grain produced by wheat were 24.4 g per pot and 18.5 gram per pot in Control-PK and Control respectively. Mixing with both ashes resulted in slightly higher grain production as compared to Control. All SA treatments were significantly higher in leaf yield compared to Control while no significant increase was observed in the case of WA

treatments. There was significantly high yield of grain, stem and leaves in SA-BE3 treatment as compared to Control. There were no significant differences in dry weight of root between all treatments and Control. Generally inoculation of plants by both BEs did not cause any significant difference over treatment with no inoculation (NoBE) (Table 3).

5.1.2. Maize

Cambisol								
Ash	**Straw ash (SA)**			**Wood ash (WA)**			**Control**	**Control-PK**
PGPR	**BE3**	**NoBe**	**BE4**	**BE3**	**NoBe**	**BE4**	**0**	**0**
Stem	$67,0^{b}$ (*3.7*)	$63,4^{b}$ (*1.8*)	$65,9^{b}$ (*3.2*)	$55,5^{a}$ (*2.5*)	$54,6^{a}$ (*4.4*)	$53,4^{ac}$ (*4.7*)	$54,9^{ab}$ (*2.7*)	$47,3^{c}$ (*1.0*)
Leaves	$27,8^{abc}$ (*2.9*)	$29,4^{c}$ (*0.5*)	$29,1^{bc}$ (*0.7*)	$27,2^{abc}$ (*0.3*)	$25,9^{abd}$ (*1.3*)	$25,0^{ad}$ (*1.8*)	$26,7^{abc}$ (*1.8*)	$22,9^{d}$ (*1.7*)
Grain	$9,7^{ac}$ (*0.4*)	$12,1^{ab}$ (*0.6*)	$9,5^{c}$ (*2.4*)	$12,0^{ab}$ (*1.6*)	$9,8^{ac}$ (*0.5*)	$13,7^{b}$ (*0.5*)	$11,9^{ab}$ (*0.2*)	$12,6^{b}$ (*0.8*)
Root	$21,8^{ab}$ (*0.9*)	$21,9^{b}$ (*1.1*)	$20,5^{ab}$ (*0.8*)	$19,3^{ab}$ (*2.1*)	$20,3^{ab}$ (*0.8*)	$18,9^{ab}$ (*3.1*)	$21,1^{ab}$ (*1.2*)	$18,5^{a}$ (*1.3*)
Sum of biomass	$126,2^{b}$ (*3.4*)	$126,7^{b}$ (*2.0*)	$125,0^{b}$ (*2.0*)	$113,9^{a}$ (*2.0*)	$110,5^{ac}$ (*5.2*)	$111,0^{a}$ (*8.0*)	$114,6^{a}$ (*5.0*)	$101,3^{c}$ (*4.2*)

Table 4 - Total and individual yield of maize biomass (g/pot). All values represent means (n=3). Standard deviations are listed in the brackets in italics and different letters in the superscript indicate significant difference (LSD, $p < 0.05$) between means.

The highest and the lowest biomass produced by maize was 126.7 g per pot and 101.3 g per pot in straw ash treatment with no BE (SA-NoBE) and Control-PK respectively. Total biomass production was higher in all SA treatment as compared to control and also higher than Control-PK, but it was lower than control in all WA treatments. Biomass production was significantly higher in SA treatments than that of WA treatments (Table 4).

The highest and the lowest yield of grain produced by maize was 13.7 g per pot and 9.5 g per pot in WA treatment which is inoculated with BE4 (WA-BE4) and SA-BE3 respectively. Straw ash treatments which is inoculated with BE4 (SA-BE4) gained significantly high amount of grain dry weight than SA-NoBE. SA-NoBE treatment produced significantly high amount of stem dry weight as compared to WA treatments with no BE (WA-NoBE). All treatments did not acquire any significant difference in stem, leaf and root over control. BE4 significantly decreased grain yield in SA and significantly increased grain yield in WA (Table 4).

5.2. Content of major nutrients in plant parts

5.2.1. Phosphorous

Wheat								
Ash	**Straw ash (SA)**			**Wood ash (WA)**			**Control**	**Control-PK**
PGPR	**BE3**	**NoBe**	**BE4**	**BE3**	**NoBe**	**BE4**	**0**	**0**
Stems	360.2^{c} (*34*)	361.9^{c} (*37*)	279.3^{ac} (*66*)	225.0^{ab} (*11*)	220.4^{ab} (*60*)	196.2^{ab} (*43*)	162.8^{b} (*27*)	259.3^{a} (*29*)
Leaves	801.5^{b} (*60*)	970.1^{c} (*49*)	944.2^{c} (*74*)	531.6^{a} (*31*)	500.4^{a} (*34*)	461.9^{a} (*39*)	288.6^{d} (*41*)	786.0^{b} (*81*)
Grains	2609.8^{bc} (*36*)	2582.5^{bc} (*48*)	2658.6^{c} (*84*)	2327.1^{a} (*82*)	2346.5^{a} (*39*)	2483.7^{b} (*80*)	2108.5^{d} (*75*)	2321.5^{a} (*21*)

Table 5 - P content in wheat biomass (mg/kg). All values represent means (n=3). Standard deviations are listed in the brackets in italics and different letters in the superscript indicate significant difference (LSD, $p < 0.05$) between means.

The highest and the lowest content of P in wheat grain were 2658.6 mg/kg and 2108.5 mg/kg in SA-BE3 and Control respectively. Grain P content was significantly higher in all treatments than Control (Table 5).

The highest and the lowest content of P in wheat stem were 361.9 mg/kg and 162.8 mg/kg in SA-NoBE and Control respectively. And stem P content in both SA-BE3 and SA-BE4 treatments were significantly higher than control (Table 5).

The highest and the lowest content of P in wheat leaves were 970.1 mg/kg and 288 mg/kg in SA-NoBE and Control respectively. Wheat leaf P content in all treatments were higher than Control. Leaves of SA-BE3 contained significantly lower P than SA-NoBE (Table 5).

Maize								
Ash	**Straw ash (SA)**			**Wood ash (WA)**			**Control**	**Control-PK**
PGPR	**BE3**	**NoBe**	**BE4**	**BE3**	**NoBe**	**BE4**		
Stems	1180.7^{c} (*94*)	1117.5^{ce} (*19*)	1177.4^{c} (*67*)	816.1^{bd} (*80*)	681.3^{ab} (*139*)	682.7^{ab} (*148*)	584.6^{a} (*77*)	977.1^{de} (*49*)
Leaves	581.3^{bcd} (*64*)	662.4^{d} (*31*)	623.9^{cd} (*44*)	533.2^{abc} (*11*)	451.3^{a} (*47*)	457.1^{a} (*57*)	434.6^{a} (*58*)	522.6^{ab} (*42*)
Grains	2544.0^{ab} (*109*)	2411.6^{ab} (*95*)	2555.3^{ab} (*305*)	2957.1^{b} (*502*)	2445.7^{ab} (*51*)	2362.8^{a} (*379*)	2334.6^{a} (*86*)	2431.0^{ab} (*295*)

Table 6 - P content in maize biomass (mg/kg). All values represent means (n=3). Standard deviations are listed in the brackets in italics and different letters in the superscript indicate significant difference (LSD, $p < 0.05$) between means.

The highest and the lowest content of P in grain of maize were 2957.1 mg/kg and 2334.6 mg/kg in WA-BE3 treatment and Control respectively. There was significantly higher amount of P in grain of WA-BE3 compared to both WA-BE4 and Control. Inoculation with BE doesn't show any significant difference over NoBE treatments (Table 6).

The highest content of P in stem of maize was 1180.7 mg/kg in SA-BE3 treatment and the lowest was 584.6 mg/kg in Control. Stem P content was significantly higher in all SA treatments than Control. BE treatments did not cause any significant difference in stem P content compared to No-BE treatments (Table 6).

The highest and the lowest P content in leaves of maize were 662.4 mg/kg and 434.6 mg/kg in SA-NoBE and control respectively. Leaf P content was significantly higher in SA

treatments as compared to Control but that of WA treatments did not cause any significant difference over Control (Table 6).

5.2.2. Potassium

Wheat								
Ash	**Straw ash (SA)**			**Wood ash (WA)**			**Control**	**Control-PK**
PGPR	**BE3**	**NoBe**	**BE4**	**BE3**	**NoBe**	**BE4**	**0**	**0**
Stems	14343^{d} (*896*)	14942d (*383*)	12094^{c} (*1739*)	9994^{abc} (*75*)	10877^{bc} (*1877*)	8580a (*456*)	9204^{ab} (*530*)	9082^{ab} (*626*)
Leaves	27624^{ab} (*1391*)	29583a (*2695*)	28662^{a} (*1984*)	28289^{ab} (*1955*)	24097^{bc} (*259*)	27572^{ab} (*849*)	22745^{c} (*3463*)	26027^{abc} (*1598*)
Grains	3035^{cde} (*125*)	3137de (*128*)	3212^{e} (*43*)	2840^{abc} (*136*)	2785^{abc} (*106*)	2892^{bcd} (*249*)	2579^{a} (*65*)	2706^{ab} (*140*)

Table 7 - K content in wheat biomass (mg/kg). All values represent means (n=3). Standard deviations are listed in the brackets in italics and different letters in the superscript indicate significant difference (LSD, $p < 0.05$) between means.

The highest and the lowest content of K in wheat grain were 3212 mg/kg and 2579 mg/kg in SA-BE3 and Control respectively. K content in grain of all SA treatments was significantly higher than Control. And in WA-BE4 there was significantly higher amount of grain P content as compared to Control (Table 7).

The highest and lowest Content of K in stem of wheat was 14942 mg/kg and 8580 mg/kg in SA-NoBE and WA-BE4. There was significantly low content of K in stem of BE4 treatment as compared to SA-NoBE4 and WA-NoBE4. Stem K content was significantly higher in SA-BE3 than SA-BE4 (Table 7).

The highest and the lowest Content of K in leaves of wheat were 29583 mg/kg and 22745 mg/kg in SA-NoBE and Control respectively. There was significantly high content of K in leaves of SA treatments than Control (Table 7).

Maize								
Ash	**Straw ash (SA)**			**Wood ash (WA)**			**Control**	**Control-PK**
PGPR	**BE3**	**NoBe**	**BE4**	**BE3**	**NoBe**	**BE4**	**0**	**0**
Stems	17534^{b} (*659*)	17492^{b} (*282*)	17427^{b} (*837*)	7757^{a} (*513*)	7707^{a} (*454*)	7462^{a} (*571*)	4769^{c} (*1507*)	9341^{d} (*79*)
Leaves	17113^{b} (*906*)	18121^{b} (*989*)	18234^{b} (*934*)	12068^{a} (*423*)	11384^{a} (*575*)	11955^{a} (*275*)	7949^{c} (*555*)	9700^{d} (*924*)
Grains	2828^{ab} (*113*)	2832^{ab} (*79*)	2806^{ab} (*313*)	3257^{b} (*466*)	2769^{ab} (*103*)	2706^{ab} (*393*)	2700^{ab} (*120*)	2677^{a} (*266*)

Table 8 - K content in maize biomass (mg/kg). All values represent means (n=3). Standard deviations are listed in the brackets in italics and different letters in the superscript indicate significant difference (LSD, $p < 0.05$) between means.

The highest and the lowest content of K in grain of maize were 3257 mg/kg and 2700 mg/kg in WA-BE3 and Control respectively. There was no any significant difference in grain K content between all treatments (Table 8).

The highest and lowest content of K in stem of maize was 17534 mg/kg in SA-BE3 and 4769 mg/kg in Control respectively. All treatments significantly incresed K content of stem as compared to Control and also all SA treatments caused significantly high amount of stem K content than WA treatments (Table 8).

The highest content of leaf K was 18234 mg/kg in SA-BE3 and the lowest was 7948 mg/kg in Control. All treatments gained significantly high amount of K in leaves as compared to Control. And all SA treatments had significantly high amount of K in leaves than WA treatments (Table 8).

5.2.3. Calcium

Wheat								
Ash	**Straw ash (SA)**			**Wood ash (WA)**			**Control**	**Control-PK**
PGPR	**BE3**	**NoBe**	**BE4**	**BE3**	**NoBe**	**BE4**	**0**	**0**
Stems	1114 (*228*)	1296 (*100*)	1078 (*68*)	984 (*33*)	1099 (*154*)	1457 (*769*)	1108 (*44*)	1018 (*108*)
Leaves	7939^{b} (*99*)	$9350b^{c}$ (*938*)	8878^{b} (*914*)	11287^{ac} (*326*)	12129^{a} (*659*)	11608^{a} (*467*)	11644^{a} (*2491*)	9945^{abc} (*212*)
Grains	314^{ab} (*18*)	327^{b} (*12*)	306^{ab} (*11*)	304^{ab} (*24*)	302^{ab} (*7,7*)	300^{ab} (*15*)	259^{c} (*7,4*)	285^{ac} (*18*)

Table 9 - Ca content in wheat biomass (mg/kg). All values represent means (n=3). Standard deviations are listed in the brackets in italics and different letters in the superscript indicate significant difference (LSD, $p < 0.05$) between means.

The highest and lowest content of Ca in grain of wheat was 327 mg/kg and 259 mg/kg in SA and Control respectively. In all treatments there was significantly higher content of grain Ca compared to Control. But both bacteria were not effective in causing significant difference (Table 9).

The highest and the lowest stem Ca content were 1457 mg/kg and 984 mg/kg in WA-BE4 and WA-BE3 respectively. There was no significant difference in stem Ca content between all treatments (Table 9).

The highest Ca content in leaves of wheat was 12129 mg/kg in WA-NoBE and the lowest was 7939 mg/kg in SA-BE3. Treating with both BE3 and BE4 significantly decreased leaf Ca content on straw ashes (Table 9).

Maize								
Ash	**Straw ash (SA)**			**Wood ash (WA)**			**Control**	**Control-PK**
PGPR	**BE3**	**NoBe**	**BE4**	**BE3**	**NoBe**	**BE4**	**0**	**0**
Stems	694^{ab} (*46*)	658^{a} (*32*)	681^{ab} (*44*)	879^{c} (*40*)	837^{bc} (*55*)	788^{abc} (*112*)	790^{abc} (*180*)	758^{abc} (*26*)
Leaves	3348^{a} (*283*)	3153^{a} (*174*)	3392^{ab} (*60*)	3425^{ab} (*133*)	3936^{cd} (*137*)	4131^{d} (*194*)	3498^{ab} (*162*)	3734^{bc} (*33*)
Grains	106^{c} (*19*)	100^{ac} (*10*)	106^{c} (*34*)	98^{ac} (*4,0*)	72^{ab} (*5,3*)	70^{ab} (*9,4*)	70^{ab} (*6,0*)	57^{b} (*8,9*)

Table 10 - Ca content in maize biomass (mg/kg). All values represent means (n=3). Standard deviations are listed in the brackets in italics and different letters in the superscript indicate significant difference (LSD, $p < 0.05$) between means.

The highest content of Ca in grain of maize was 106 mg/kg in SA-BE3 and the lowest was 70 mg/kg in WA-NoBE and Control. Treating by both BE3 and BE4 did not caused any significant differences in Ca content of grain in both SA and WA treatments. But Ca content in grain of SA-BE3 and SA-BE4 treatments were significantly higher than Control (Table 10).

The highest and the lowest content of Ca in stem of maize were 879 mg/kg and 658 mg/kg in WA-BE3 and SA-NoBE respectively. There was significantly high content of Ca in SA-NoBE than WA-NoBE treatment. There was no significant difference in stem Ca content between all treatments and Control (Table 10).

The highest and the lowest amount of Ca in leaves of maize were 4131 mg/kg and 3153 mg/kg in Control and in WA-NoBE respectively (Table 10).

5.2.4. Magnesium

Wheat								
Ash	**Straw ash (SA)**			**Wood ash (WA)**			**Control**	**Control-PK**
PGPR	**BE3**	**NoBe**	**BE4**	**BE3**	**NoBe**	**BE4**	**0**	**0**
Stems	320^{ab} (*36*)	369^{a} (*21*)	365^{a} (*46*)	361^{a} (*14*)	371^{a} (*71*)	304^{ab} (*34*)	344^{ab} (*7,2*)	285^{b} (*27*)
Leaves	1100^{b} (*21*)	1257^{ab} (*63*)	1354^{ab} (*344*)	1599^{ac} (*53*)	1711^{c} (*39*)	1549^{ac} (*39*)	1520^{ac} (*274*)	1145^{b} (*83*)
Grains	868^{ac} (*34*)	833^{ab} (*13*)	894^{c} (*20*)	818^{ab} (*17*)	844^{abc} (*12*)	858^{ac} (*31*)	802^{b} (*13*)	827^{ab} (*36*)

Table 11 - Mg content in wheat biomass (mg/kg). All values represent means (n=3). Standard deviations are listed in the brackets in italics and different letters in the superscript indicate significant difference (LSD, $p < 0.05$) between means.

The highest and the lowest wheat grain Mg contents were 894 mg/kg and 802 mg/kg in SA-BE3 treatment and Control respectively. There was significantly high content of grain Mg content in SA-BE3 and SA-BE4 treatments as compared to Control (Table 11).

The highest and the lowest Mg contents in stem of wheat were 369 mg/kg and 304 mg/kg in SA-NoBE and WA-BE4. There was no any significant difference in stem Mg content between all treatments and Control (Table 11).

The highest content of Mg in leaves of wheat was 1711 mg/kg in WA-NoBE and the lowest one was 1100 mg/kg in SA-BE3. And it was significantly higher in SA-BE3 than Control. There was no any significant difference between BE treatments and NoBE treatments (Table 11).

Maize								
Ash	**Straw ash (SA)**			**Wood ash (WA)**			**Control**	**Control-PK**
PGPR	**BE3**	**NoBe**	**BE4**	**BE3**	**NoBe**	**BE4**	**0**	**0**
Stems	376^{a} (*18*)	377^{a} (*7,9*)	412^{a} (*15*)	615^{d} (*9,7*)	597^{cd} (*34*)	547^{c} (*21*)	489^{b} (*51*)	490^{b} (*7,1*)
Leaves	1012^{a} (*27*)	1079^{a} (*30*)	1126^{a} (*65*)	1607^{b} (*44*)	1628^{b} (*73*)	1684^{b} (*38*)	1411^{c} (*60*)	1297^{c} (*74*)
Grains	1093^{a} (*40*)	1075^{a} (*56*)	1103^{a} (*147*)	1363^{b} (*183*)	1115^{ab} (*32*)	1067^{a} (*168*)	1101^{a} (*49*)	1111^{ab} (*156*)

Table 12 - Mg content in maize biomass (mg/kg). All values represent means (n=3). Standard deviations are listed in the brackets in italics and different letters in the superscript indicate significant difference (LSD, $p < 0.05$) between means.

The highest and the lowest Mg contents in grain of maize were 1363 mg/kg and 1067 mg/kg in WA-BE3 and WA-BE4 respectively. There was significantly higher content of Mg in WA-BE3 than Control. Both BE3 and BE4 were not effective in causing any significant effect (Table 12).

The highest and the lowest Mg contents in stem of maize were 615 mg/kg and 376 mg/kg in WA-BE3 and SA-BE3 respectively. Content of Mg in stem of all SA treatments was lower than Control. But in that of WA treatments there was significantly high content of Mg in stem of maize as compared to Control. There was no any significant difference between BE treatments and NoBE treatments (Table 12).

The highest and lowest Mg content in leaves of maize were 1684 mg/kg and 1012 mg/kg in WA-BE4 and SA-BE3 respectively. All WA treatments caused significant increment in leaf Mg content than Control but in that of all SA treatments there was lower amount of leaf Mg content than Control (Table 12).

5.3. Availability of major nutrient in soil solution

5.3.1. Phosphorus

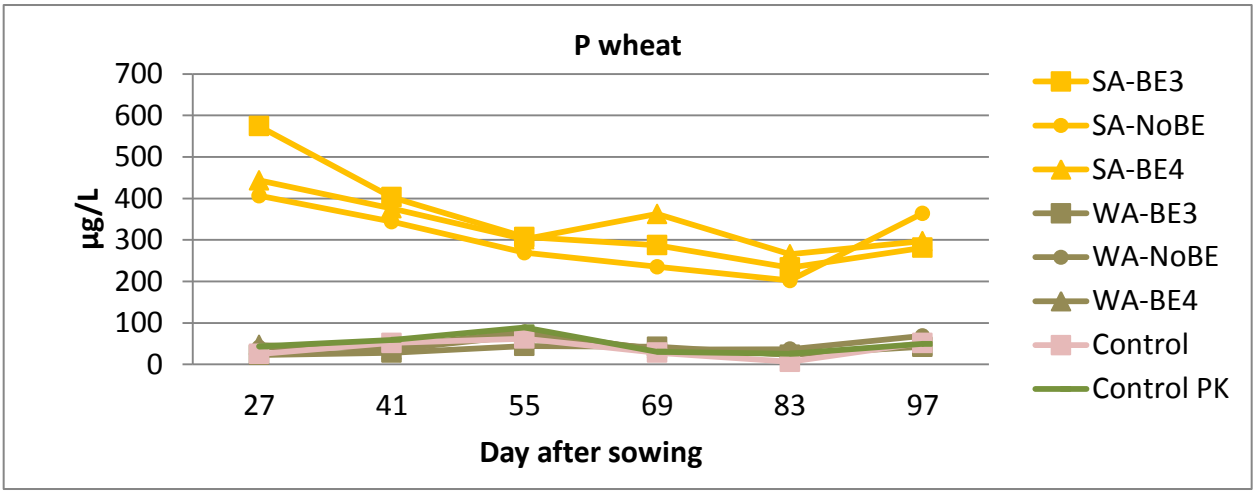


Figure 1 - P concentration in wheat soil solution (µg/L).

Concentration of P was significantly higher in treatment of SA soil solution as compared to WA treatments. And it was decreased dramatically within two months and remained almost constant for the rest of collection times. But in that of WA treatment concentration of P was almost constant through the whole growing season. Effect of BEs on both SA and WA was not significant (Figure 1).

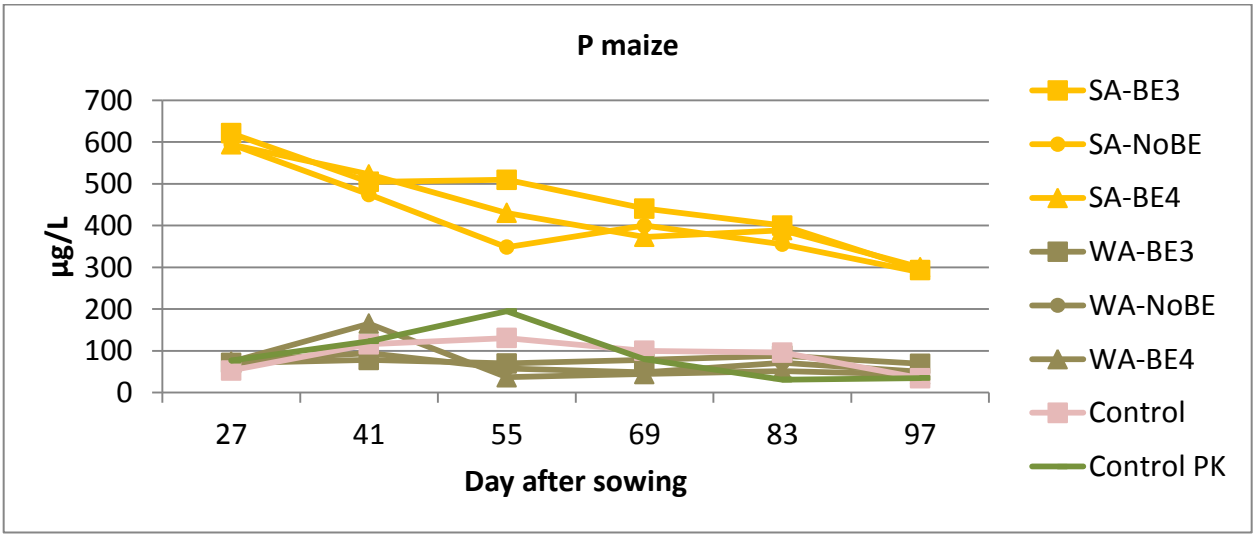


Figure 2 - P concentration in maize soil solution (µg/L).

Concentration of P in soil solution of SA treatment of maize was higher as compared to WA treatments. P was almost constant in soil solution of WA treatments. In SA treatments, there were observed linearly decreasing trend. Effect of BEs was not significant on both ashes (Figure 2).

5.3.2. Potassium

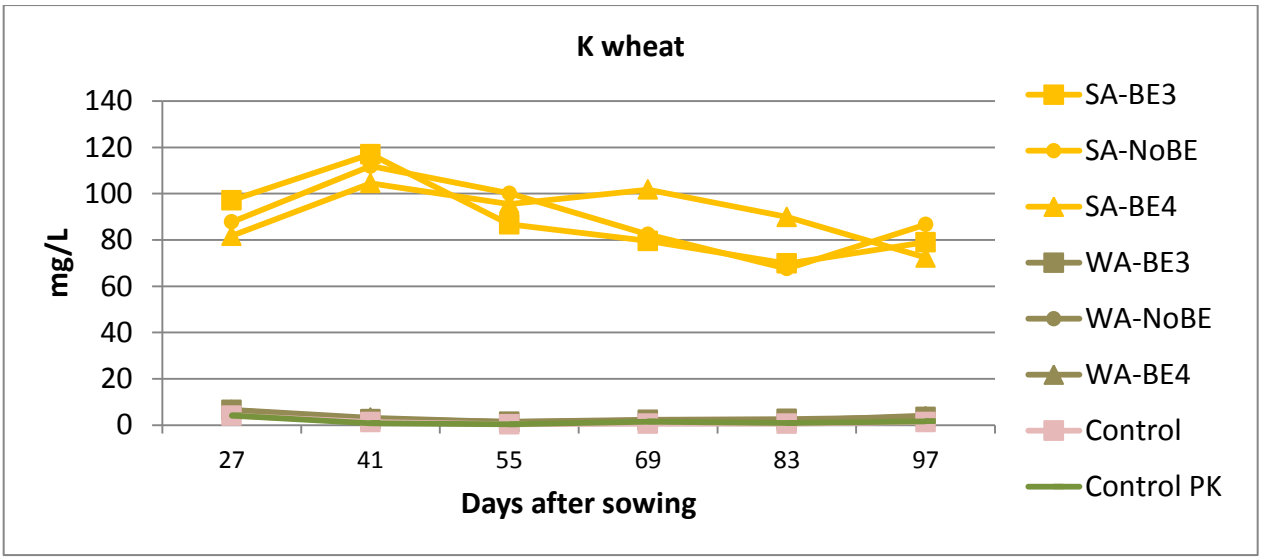


Figure 3 - K concentration in wheat soil solution (mg/L).

Wheat soil solution which was collected from SA treatments exhibited higher K as compared to WA treatments. P concentration was constant in that of WA and for SA there was slight swinging during the growing season. BEs were not affecting concentration of K in soil solution (Figure 3).

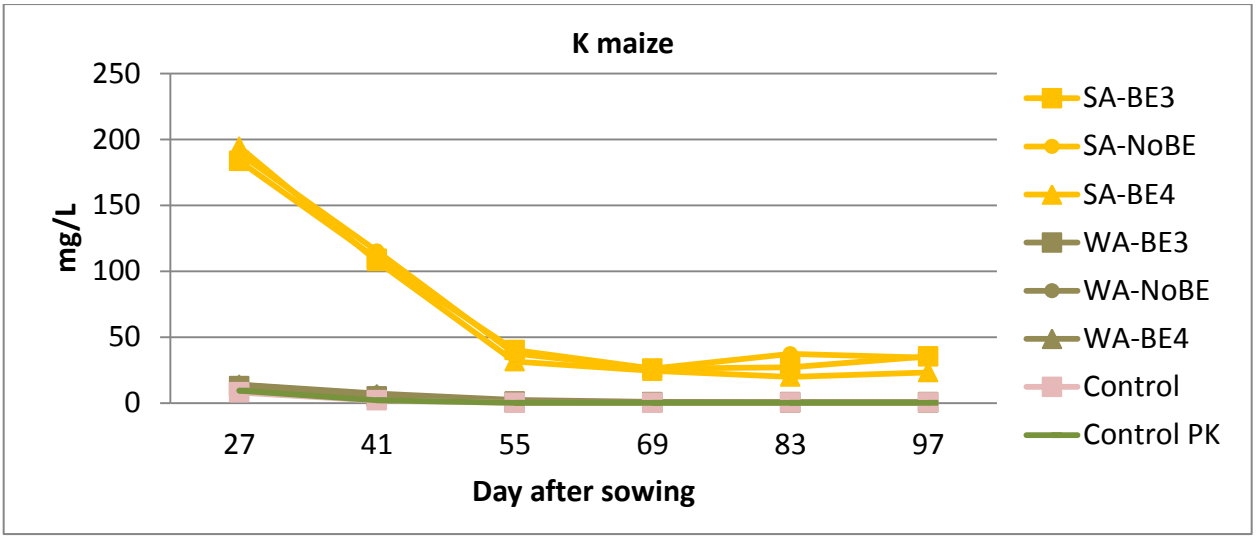


Figure 4 - K concentration in maize soil solution (mg/L).

Concentration of K in SA solution of maize was higher as compared to WA treatments. K was higher for the first collection time and then exhibited a radical decrease at the second and third collection time and then remained constant for the remaining growing season. Inoculation with both BEs was not effective in causing significant difference (Figure 4).

5.3.3. Calcium

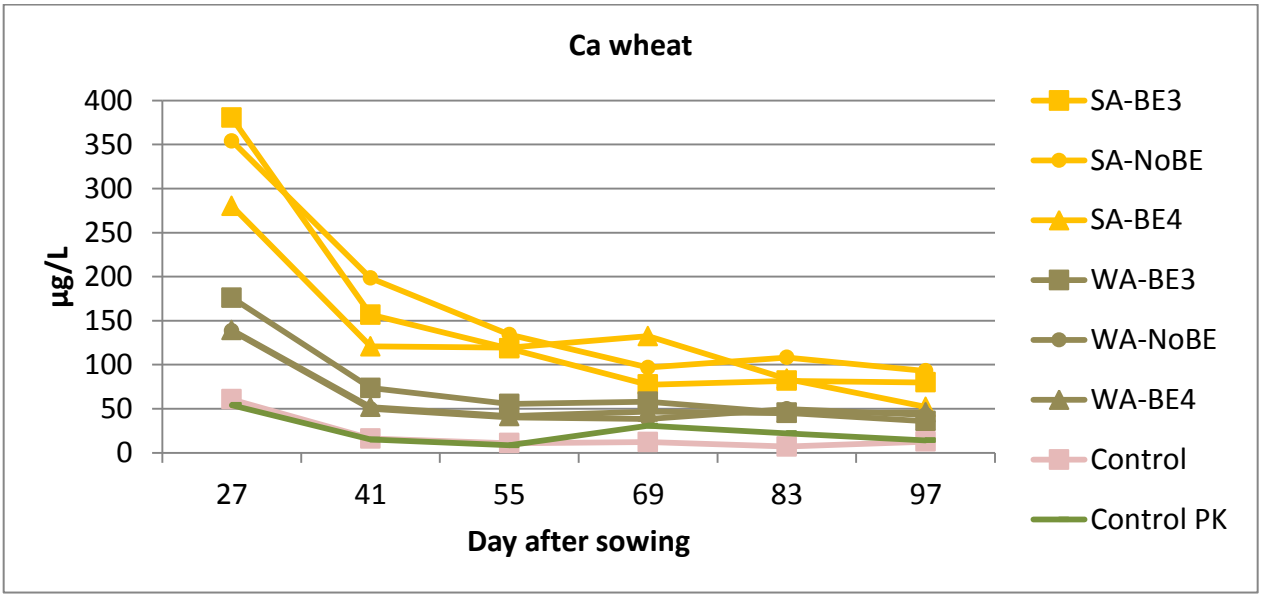


Figure 5 - Ca concentration in wheat soil solution (mg/L).

Concentration of Ca was slightly higher in SA treatments soil solution of wheat and it was higher during the first soil solution collection time which is then exhibited a decrease path till third collection time and remained almost constant for the remaining growing season (Figure 5).

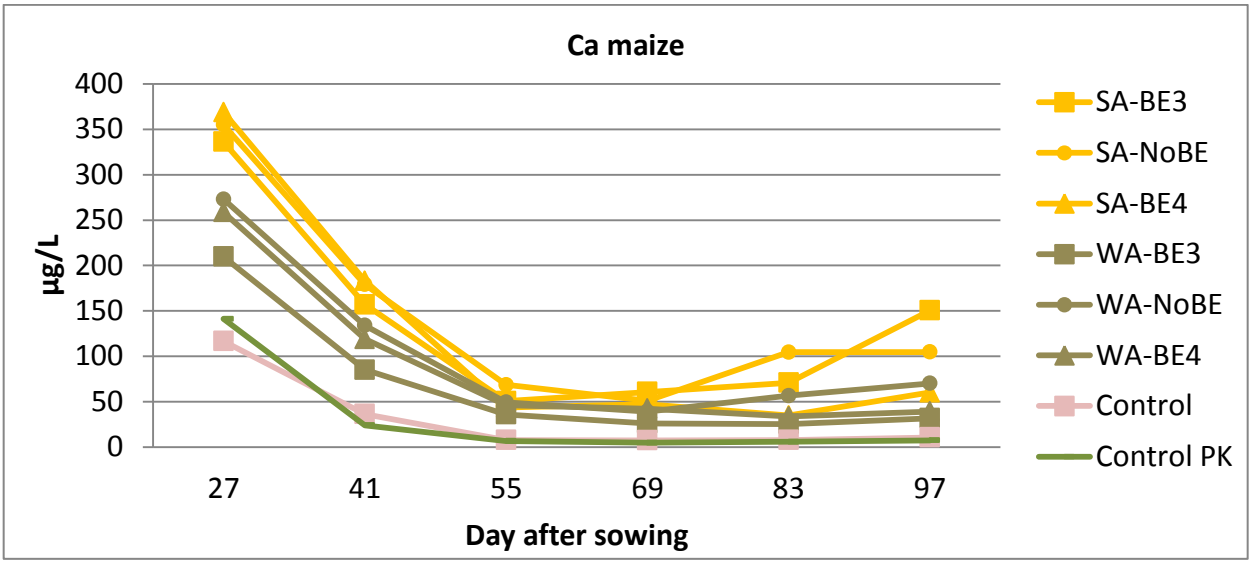


Figure 6 - Ca concentration in maize soil solution (mg/L).

Concentration of Ca was almost comparative in both ashes with slightly higher amount in SA soil solution. Ca concentration then showed a decrease path in the first two month of growing season and then stayed constant in both ashes soil solution. Effect of BEs was negligibly small, insignificant and inconsistent (Figure 6).

5.3.4. Magnesium

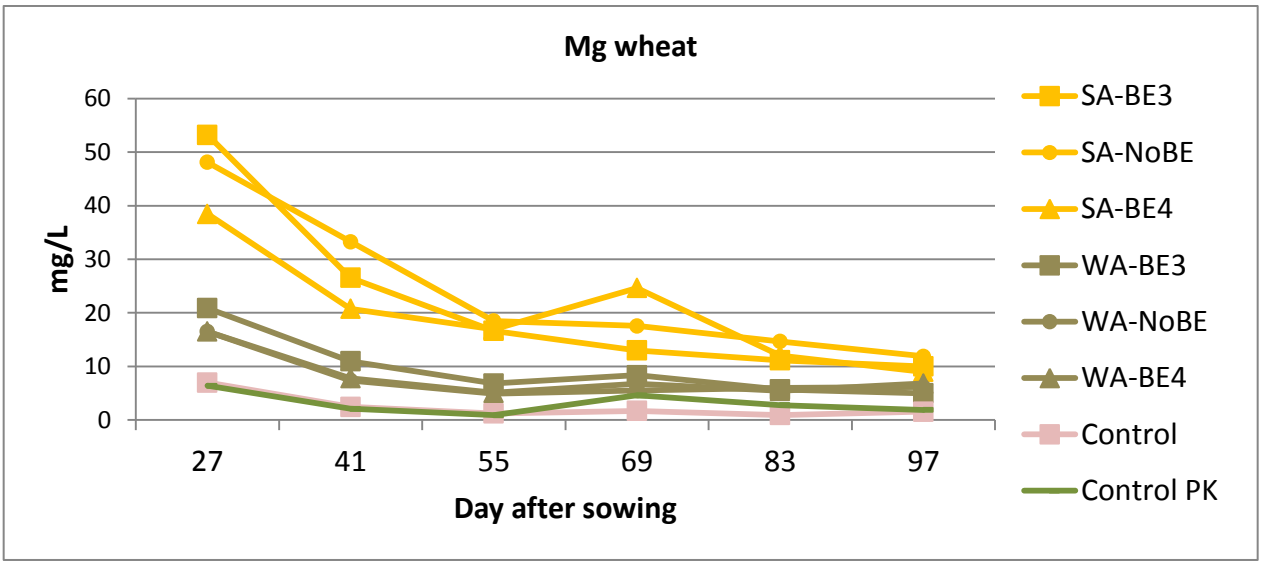
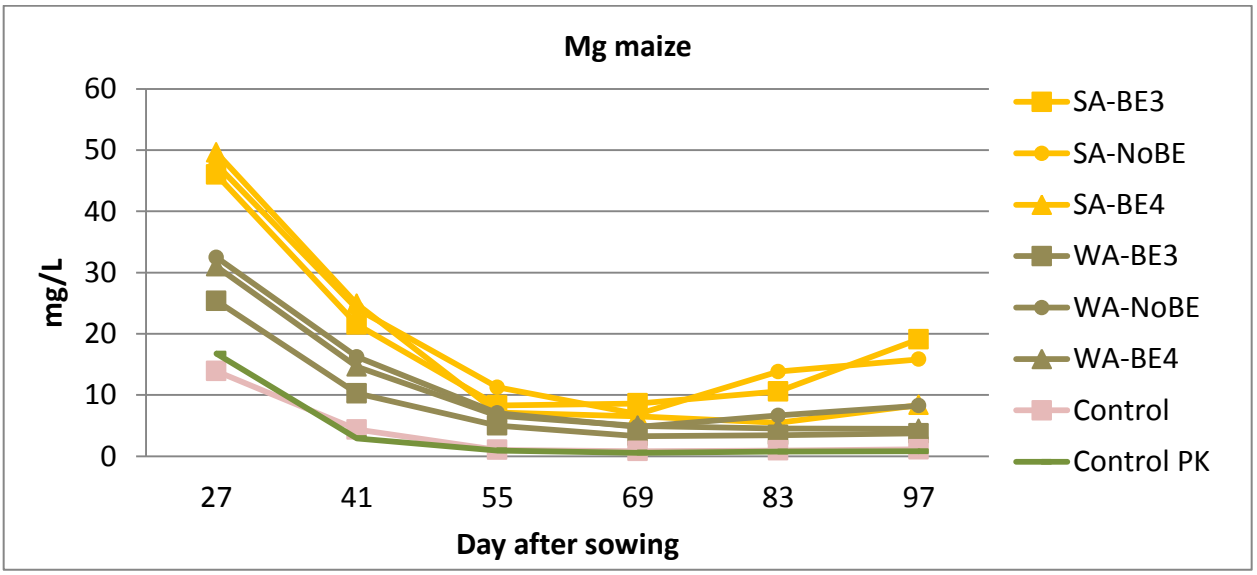


Figure 7 - Mg concentration in wheat soil solution (mg/L).

Behaviour of Mg concentration was almost similar to that of Ca in which it was slightly higher in SA soil solution. At the time of first soil solution collection concentration of Ca was higher and then decreased in a rhythmic path up to third collection and stayed constant. No treatment was significantly affected by application of BEs (Figure 7).



Graph 8 - Mg concentration in maize soil solution (mg/L).

Behaviour of Mg concentration in soil solution was similar with that of Ca in which it was almost comparative in both ashes with slightly higher amount in straw ash soil solution. Its amount then appeared to decrease in the first two month of growing time and then remained constant in both ashes soil solution. Effect of BEs was not significantly effective (Figure 8).

5.4. Low molecular weight organic acids in soil solution

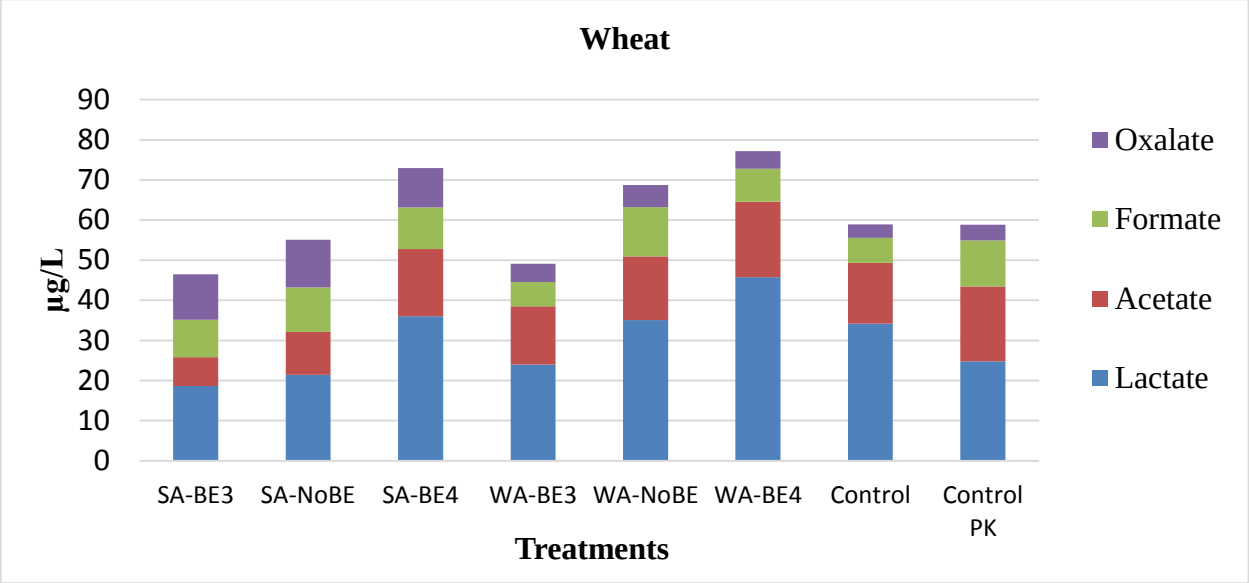


Figure 9 - Total accumulation of low molecular weight organic acids in soil solution of wheat (µg/L).

Accumulation of LMWOA was almost not affected by application of WA, SA and PK fertilizer, but slightly affected by BEs. BE3 relatively reduced concentration of LMWOA than NoBE treatments and BE4 relatively increased concentration of LMWOA than NoBE treatments in both ashes (Figure 9).

There was variation in percentage distribution of LMWOA in soil solution of individual treatments. The predominant LMWOA was lactate in all treatments followed by acetate, formate and oxalate 48%, 24%, 16% and 12% on average respectively. And when we see their relative distribution between the two ashes proportion of lactate and acetate accounted slight increment in SA treatments but that of formate and oxalate slightly increased in WA treatments (Figure 9).

The highest and the lowest proportion of lactate from LMWOA was 59.3% in WA-BE4 and 40% in SA-NoBE respectively. Lactate was declined in all treatments as compared to Control except WA-BE4. Acetate was the second most abundant LMWOA and varied in percentage between 25.7% and 15.4% in Control and in SA-BE3 respectively. Its amount was declined by both ashes. That of formate varied between SA-NoBE (20.1%) and Control (10.7%). Its proportion was slightly increased in SA-NoBE compared to WA-NoBE and Control but it was lower in WA-NoBE than in Control. Share of oxalate from LMWOA was the least and varied between 24.4% SA-BE3 and 5.6% WA-BE4. It was lower in all treatments as compared to Control (Figure 9).

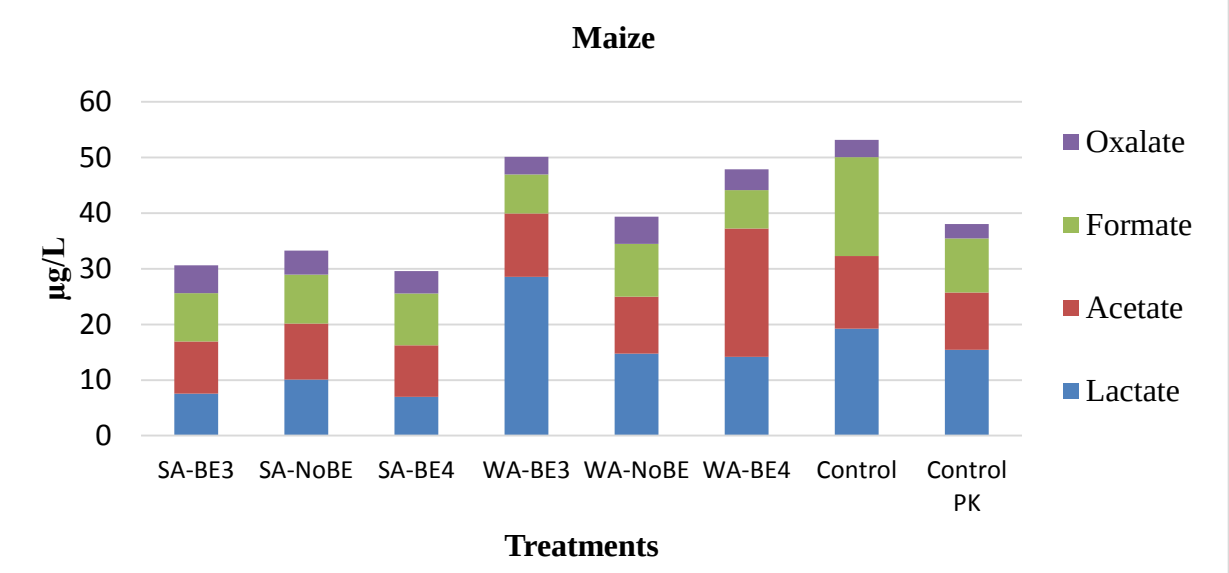


Figure 10 - Total accumulation of low molecular weight organic acids in soil solution of maize (µg/L).

There was slight variation in accumulation of LMWOA between treatments of SA, WA, Control and Control-PK. Accumulation of LMWOA was relatively reduced by application of both SA and PK fertilizer. In SA treatments their accumulation was in relatively lesser extent than Control and in WA treatments it was comparable with Control. It is also observable that WA treatments accumulated larger quantity of LMWOA than SA treatments. There was no observable

effect of BEs in SA treatments and in WA treatments both BEs was relatively increasing accumulation of LMWOA (Figure 10).

As that of wheat, percentage distribution of LMWOA in maize soil solution varied between treatments. Soil solution of maize was dominated by lactate and followed by acetate, formate and oxalate 35%, 30%, 25% and 10% on average respectively. Share of lactate and acetate from LMWOA was slightly increased in SA treatments but that of formate and oxalate were slightly higher in WA treatments (Figure 10).

Lactate which comprised the largest proportion of LMWOA slightly increased in WA treatments than SA treatments and Control. And its share was bigger in WA-BE3 with 56.96% and smaller in SA-BE4 with 23.69%. Proportion of acetate was low in all treatments than control except WA-BE3 treatment. Maximum percentage of acetate was found in WA-BE4 (48.20%) and the minimum was in WA-BE3 (22.65%). Formate comprised the biggest share in Control with 33.37% and the lowest in WA-BE3 with 14.44%. Share of formate was higher in the Control soil solution as compared to all other treatments. It was also higher in SA treatments as compared to WA treatments. In that of WA treatment its proportion was negatively affected by both BEs. And when we see oxalate, its proportion varied between 16.39% in SA-BE3 and 5.88% in Control which indicates its lesser percentage in Control than in other treatments. In SA, share of oxalate slightly increased due to effect of both BEs. But in WA it was negatively affected by both BEs (Figure 10).

6. Discussion

6.1. Biomass yield

Application of both wood and straw ash increased yield of total biomass and grain of wheat and straw ash increased leaf dry weight in all treatments of wheat. Increase in biomass yield of plants after ash application was reported by Nkana et al. (1998) as biomass yield of rye grass was affected by type of soil and round of plant harvest. Nkana et al. (1998) also found higher biomass yield increment due to ash amendment in clay soil compared to sandy clay soil. Dry matter yield was also increased by the second harvest and then decreased by the third harvest. There was no significant effect on P content but there was an increase in K and Ca content of the plant but trend of Mg was not consistent between the two soil types (clay and sandy soil) in which it was higher at first harvest and decreased as rate of WA application increased. Kikamagi et al. (2013) also found an increase in biomass yield of silver birch after application of WA as compared to control. Based on their report, there was also improvement in annual height increment of the plants after WA application. As it is described by Nkana et al. (1998) positive effect of WA on the biomass yield of crops is preferably caused by Ca, K supplementation, changes in soil chemistry (increasing pH of soil) and thus reducing Al and Mn toxicity. In our study biomass yield increment due to supplementation of Ca and K probably come into an account. Effects of ashes in maize treatments were slightly different from wheat in which that of SA was more effective than that of WA. Based on our finding, SA increased biomass yield of maize but WA showed even negative effect on the yield of maize biomass. This may be due to availability of P in raw SA which was higher than raw WA and resulted in increased uptake of P by both cultivated crops. Based on the report of Schiemenz and Löbermann (2010) availability of P from SA was even comparable with that of highly soluble P fertilizer like triple superphosphate. Application of ash did not cause any significant differences in grain yield, stem and root dry weight of maize. Generally difference in effectiveness of ashes in these two crops is in agreement with report of Schiemenz and Löbermann (2010) in which fertilizing effect of ash was different depending on the crop species which is fertilized by the ash. Inoculation of maize with both BEs was not effective even the yield of grain in SA treatments was significantly decreased after inoculation with BE4. Effect of BE3 is in agreement with Talboys et al. (2014) in which root uptake ability from soils with a low inorganic P concentration was reduced due to

Bacillus amyloliquefacizens FZB42 auxin production. Even if Idriss et al. (2002) reported positive effect by which BE3 increased nutrient and water uptake in *Triticum aestivum* due to its ability to stimulate early growth of both seminal and lateral root systems in high nutrient environment.

6.2. Content of major nutrients in plant parts

Even if several research works reported low solubility, low availability and low uptake of P from ashes, in our study grain, stem and leaf P content of wheat were significantly increased after application of both ashes. Only stem P increment in WA treated soil was not significant. But in the case of maize it was only SA which significantly increased stem and leaf P content. This difference in effectiveness of ashes is in agreement with chemical characterization of wood ash by Erich (1991) which confirmed that P from wood ash is very weekly available and also the dissolved P is probably immobilized in soil. In wheat application of SA also increased grain, stem and leaf K content but that of wood ash did not cause any significant increment. In the case of maize, application of both WA and SA increased K content in stem and leaf but their effect on grain K was not significant. It was SA which was slightly effective in increasing Ca content of wheat as compared to WA. Both SA and WA significantly increased Ca content of wheat grain. Ca content in maize grain and stem was not affected by both ashes. But WA increased Ca content in leaves but SA decreased Ca content in leaves of maize. Our finding is supported by work of Ohno and Erich (1990) in which they found an increased Ca and K contents in plant tissues. This is due to the reason that K and Ca are more available for plant uptake and even based on finding of Ohno (1992) availability of K from wood ash was comparable with that of K fertilizers. Wood ash K was highly soluble and also available for plant (Ohno, 1992). But Park et al. (2005) reported insignificant effect of wood ash application on nutrient uptake of plant even if application of wood ash increased available P, K, Ca and Mg. This insignificant effect in nutrient uptake after WA application indicates presence of other limiting factors for uptake of nutrients from ash by plants in addition to their availability like crop uptake efficiency (Schiemenz and Löbermann, 2010). Application of both ashes was not effective in increasing Mg content in wheat plant parts, even application of SA significantly decreased amount of stem and leaf Mg content of maize. This may be due to high availability of Ca and K in our soil solution as compared to Mg.

Thus reduced uptake of Mg by plants can be due to competition of these ions. This is in agreement with some reports (Gransee and Fuhrs, 2013; Grimme et al., 1974) where reduction in uptake of Mg by plants was observed when there is coincidence in increased concentration of Mg with both Ca and K in soil solution.

Even if several studies reported effectiveness of our PGPR (Idriss et al., 2002; Talboys et al., 2014; Broadbent, 1977; Castro, 2008) in our case generally they were not causing any significant effect on nutrient content of both crops. Even they were generating slight negative effects in some cases. For example BE3 decreased wheat leaf P content in SA treatment, BE4 decreased wheat stem K content in both ashes, and also both BE3 and BE4 decreased wheat leaf Ca content in SA and in case of maize BE3 decreased leaf Ca content WA treatment. It was only BE4 which increased wheat grain P content in WA. They did not cause any significant difference in maize P content, in maize K content and totally they did not cause any significant difference over plant Mg content. These low and even negative effects of PGPRs can be due to the different factors. Researchers suggested some reasons for low activities of plant growth promoting rhizobacteria. These include environmental factors such as soil types in which type of soil adversely affects diversity and effectiveness of PGPR (Latour et al., 1996). Availability of nutrients in soil also affects effectiveness of PGPR. Egamberdiyeva (2007) reported their effectiveness in nutrient poor soils as compared to nutrient rich soils. Based on this finding bacterial inoculation stimulated nutrient uptake and plant growth on nutrient deficient Calcisols and relatively reduced uptake of nutrient on relatively nutrient rich loamy sand soil. Low activities of PGPR may be also due to application of nitrogen fertilizer, in which N fertilizers decreased population of *Bacillus polymyxa* (Gouzou et al., 1995).

6.3. Availability of major nutrients in soil solution

Generally concentrations of P, Ca, K and Mg in SA soil solution were clearly higher than Control and WA. In soil solution of WA treatments concentrations of P and K were almost similar with that of Control and contents of Ca and Mg were negligibly higher which generally indicates low availability of nutrients from wood ash. And also both PGPR were not effective in modifying nutrient concentration of soil solution. However, many researchers found increased availability of nutrients from application of wood ash. For example Sano et al. (2013) reported

increased availability of P, K, Ca and Mg in soil solution even if response of P availability was not immediate. At the same time, week solubility of P from wood ash and the immobilization of large portion of the dissolved P in the soil is reported (Erich and Ohno, 1992; Erich, 1991).

In WA soil solution, P content was constant through all collection time and in that of SA decreased gradually with in the first three collection times then after remained almost constant. Ca content in soil solution of SA treatments was higher than WA treatments but Ca behaved similarly in soil solution of both ashes in which it was decreasing during the first three collection times and remained almost constant for the remaining growing season. It was easy to observe higher K content in SA soil solution than WA in both wheat and maize. K from WA treatments was almost constant in both maize and wheat. In SA treatments K behaviour were different based on crops in which its amount was swinging in wheat between the range of 68 and 119 mg/L and in maize it exhibited radical decrease while the first three collection and then remained constant. Mg was behaving similarly as Ca. Mg concentrations were slightly higher in SA soil solution than in WA treatments on both crops and during the first three collections, it was decreasing then it remained almost constant for the remaining growing season. But some researcher reported increased availability of Ca and Mg through time after application of ash. The difference between WA and SA is reported by Olander and Steenar (1995), where amount of K was higher in SA treated soil as compared to WA. But Park et al. (2005) found increased available P, K, Ca and Mg concentrations in soil solution after application of WA. Based on their findings K was the most elevated nutrient after application of WA. Due to its higher mobility of K concentration were mainly increasing at the greater depth, but that of Mg and Ca concentration increased at the shallower depth.

Generally researchers agree with low availability of nutrients from wood ash. For example Ohno and Erich (1990) reported very low availability of P from WA. Park et al. (2005) recommended wood ash as a fertilizer, especially in the acidic field conditions. Based on Naylor and Schmidt (1986) it was only 51% of K from WA, which were available for plants. P availability in wood ash is also reported from 28 to 70% lower as compared to commercial fertilizer (Erich and Ohno, 1992). This low availability of nutrients in soil solution after application of ashes may be due to several reasons. For example based on Park et al. (2004) and Mozaffari et al. (2002), application rate of ash were affecting availability of nutrients as their availability increased depending on application rate of ash. And also they reported greater

leaching of K, Ca and Mg in wood ash amended soil as compared to controls which can possibly decrease nutrient availability in the rhizosphere.

6.4. Low molecular weight organic acid

There was no any significant difference caused by PK fertilizer in accumulation of LMWOA in soil solution of wheat. In maize application of PK fertilizer reduced total accumulation of LMWOA in soil solution. Percentage and distribution of low molecular weight organic acids in soil solution were affected by the type of ash. Accumulation of lactate and acetate was slightly higher in WA treatments and accumulation of oxalate in SA treatments was two times higher compared to WA treatments. Acetate production was lower in all ash treatments as compared to Control. It was difficult to predict effect of BE on LMWOA as their effect was varying from treatment to treatment. Generally relative occurrence of low molecular weight organic acid lactate, acetate, formate and oxalate was 48%, 24%, 16%, 12% in soil solution of wheat and 35%, 30%, 25%, 10% in maize respectively. Which implies proportion of lactate was the highest and oxalate was the lowest in soil solution of both crops. Behaviour of oxalate in soil solution was related to the nutrients released from SA as soil solution of SA treatments contained the highest amount of oxalate and at the same time there was comparably high content of available P, K, Ca and Mg than in WA treatments. But that of lactate and acetate was high in low nutrient environment of WA treatments. Based on Jones (1998) oxalate release efficiency of P from inorganic P is greater than acetate. And also based on this review they generalized that releasing P in soil solution is extremely soil dependent, in some soils no P was mobilised after addition of organic acids and also they stated requirement of greater than 1mM oxalate to solubilize significant amount of P, but when we see amount of oxalic acid in our soil solution it was even below 100 µM. Some other findings also support release of nutrients by low molecular organic acids even if they did not describe the amount of organic acids in soil solution. For example based on Dakora and Phillips (2002), Khademi et al. (2010) or Hens (2003), low molecular weight organic acids (oxalic and citric acids) solubilized unavailable soil P, Ca and Fe.

Based on finding of Dotaniya et al. (2013) increment in concentration of P (in modified nutrient solution) where *Triticum aestivum* resulted in the reduction of oxalate exudation. In our case we found more oxalate in SA treatments where there was already high amount of available P

as compared to the WA treatments or Control. We can correlate this relative increment of oxalate in soil solution of SA treatments to different factors. May be it is related to high solubility of SA which could change anion equilibrium in soil. This phenomenon might cause the desorption of oxalate anions from soil particles to the soil solution. Dotaniya et al. (2014) also reported the increment in exudation of oxalate by plant root after addition of organic residue (mixture of press mud, bagasse and chopped rice straw) to the soil. This relative increment of oxalate in SA treatments as compared to WA treatments may be related to presence of high amount of oxalate in raw SA. However, these all are our possible reasons for slight increment in accumulation of oxalate in straw ash soil solution than wood ash and further work is required to confirm or disprove these assumptions.

7. Conclusion

In this experimental study abilities of wheat and maize plants to take up macronutrients from biomass ashes and effect of two PGPRs on the yield of plant biomass and their effect on accumulation of low molecular organic acid in soil solution were investigated. The results revealed increment in yield of both wheat and maize after application of straw and wood ash. After application of straw and wood ash, wheat total biomass increased by 12.7%, 13.5% and wheat grain yield increased by 11.9%, 14.1% respectively. In addition straw ash increased leaves dry weight of wheat by 21.9%. In maize straw ash increased biomass yield by 10.6%. Amount of available P, K, Ca and Mg in straw ash treated soil solution was twice higher than wood ash. SA increased P and K content of grain, leaf and stem of wheat and also it increased Ca content of grain. And also in maize it increased stem and leaf P and K content. SA is more efficient in causing high amount of available P, K, Ca and Mg in soil solution as compared to wood ash. Oxalate may be important in releasing nutrients from SA specially Ca and Mg, as these organic acids were higher in SA soil solution where raw straw ash have 3.9 time less Ca and 3.1 time less Mg content than raw wood ash. Then concentration of Ca and Mg in straw ash soil solution was almost two fold higher than wood ash. And when we see our PGPR both *Bacillus amyloliquefaciens* FZB42 and Bacto_PROF are not efficient in making change over available nutrients in soil solution and plant nutrient content.

Generally we can conclude that both wood and straw ashes are good source of nutrients for crop cultivation. Biomass ashes are different in their fertilizing capacity based on source of ash. It is also evident that effect of biomass ashes on yield of crops depends on nutrient uptake efficiency of crop species.

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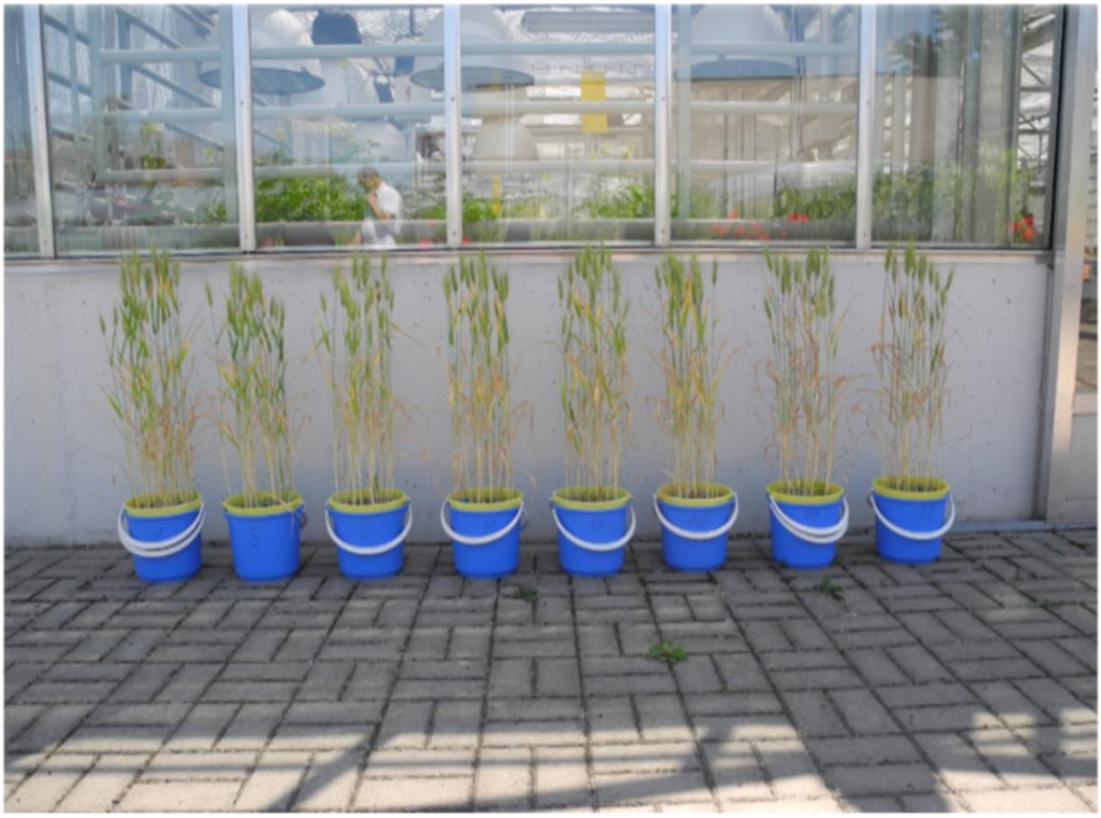
9. List of Appendices

Appendix A

Collection of soil solution samples

Appendix B

Comparative photographs of wheat plants 83 days after sowing.



Appendix C

Comparative photograph of maize plants 69 days after sowing.