### REGULAR ARTICLE

# Influence of potassium solubilizing microorganism (Bacillus mucilaginosus) and waste mica on potassium uptake dynamics by sudan grass (Sorghum vulgare Pers.) grown under two Alfisols

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**Abstract** The main aim of this research was to study the dynamics of K release from waste mica inoculated with potassium solubilizing microorganism (Bacillus mucilaginosus) and to investigate its effectiveness as potassic-fertilizer using sudan grass (Sorghum vulgare Pers.) var Sudanensis as test crop grown under two Alfisols. Results revealed that application of mica significantly enhanced biomass yield, uptake and per cent K recoveries by sudan grass than control (no-K). Biomass yield, uptake and per cent K recoveries increased further when mica was inoculated with bacterial strain in both the soils than uninoculated mica. Alfisol from Hazaribag recorded higher yield, uptake and K recoveries than Alfisol from Bhubaneswar. The dynamics of K in soils indicated that K was released from mica to water-soluble and exchangeable pools of K due to inoculation of mica with Bacillus mucilaginosus in both the soils. Significantly greater amounts of water-soluble, exchangeable and non-exchangeable K were maintained in Alfisol from Hazaribag than Bhubaneswar. Release kinetics of K showed significant release of K from mica treated with bacterial strain.

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Significant correlation between biomass yield, K uptake by sudan grass and different pools of K in soils were observed. X-ray diffraction analysis indicates greater dissolution of mica due to inoculation of *Bacillus mucilaginosus* strain in both the soils. Thus, bio-intervention of waste mica could be an alternative and viable technology to solubilize insoluble K into plant available pool and used efficiently as a source of K-fertilizer for sustaining crop production and maintaining soil potassium.

**Keywords** Waste mica  $\cdot$  Alfisol  $\cdot$  Sudan grass  $\cdot$  Bacillus mucilaginosus  $\cdot$  Pools of K  $\cdot$  X-ray diffraction analysis

## Introduction

Potassium (K) is the third major essential nutrient for plant growth. It plays an essential role for enzyme activation, protein synthesis and photosynthesis. The issue of sustainable management of potassium in soil has partly been ignored during the last decades when the focus was aimed with potent environmental impact on use of nitrogen and phosphorus. However, in recent years there is a growing awareness among all concerned regarding the importance of potassium in crop production in several parts of India. There are many reports in recent past that Indian soils do show K deficiency because available soil K levels have dropped due to rapid development of agriculture



without replenishing it and application of potassium fertilizer to those soils gives positive response.

Potassium in soil is present in water-soluble (solution K), exchangeable, non-exchangeable and structural or mineral forms. Potassium from watersoluble and exchangeable pools is directly available for plant uptake. At low levels of exchangeable K in certain soil types, non-exchangeable K can also contribute significantly to the plant uptake (Memon et al. 1988; Sharpley 1989). Exchangeable K or available K is held by negative charge clay minerals and organic matter in soils, while non-exchangeable K consists predominantly of interlayer K of nonexpanded clay minerals such as illite and lattice K in K-minerals such as K-feldspars. The bulk of total soil K is in the mineral fraction (Sparks and Huang 1985; Sparks 1987). There are dynamic equilibrium and kinetic reactions between the different forms of soil K that affect the level of soil solution K at any particular time, and thus, the amount of readily available K for plants. Levels of soil solution K are determined by the equilibria and kinetic reactions between the other forms of soil K (Sparks 1987). The rate and direction of reactions between solution and exchangeable forms of K determine whether applied K will be leached into lower horizons, taken up by plants, converted into unavailable forms, or released into available forms (Sparks 2000). The fate of applied K in soil is also governed by clay content and clay mineralogy of soil, and the nature of crops grown. Release of nonexchangeable K to the exchangeable form occurs when levels of exchangeable and solution K are decreased by crop removal and/or leaching and perhaps by large increases in microbial activity (Sparks 1987). Mineral K is generally assumed to be only slowly available to plants (Sparks and Huang 1985); however, the availability is dependent on a number of factors, including the level of other forms of K, namely, solution, exchangeable and nonexchangeable, and the degree of weathering of the K-bearing minerals like feldspar and micas (Sparks 1987).

Potassium is added to soil in the form of potassic fertilizers. India ranks fourth after USA, China, and Brazil as far as the total consumption of K-fertilizers in the World is concerned (FAI 2007). However, there is no reserve of K-bearing minerals in India for production of commercial K-fertilizers and the whole consumption of K-fertilizers are imported in the form

of muriate of potash (KCl) and sulphate of potash (K<sub>2</sub>SO<sub>4</sub>) which leads to a huge amount of foreign exchange. These necessitate to find an alternate indigenous source of K for plant needs and maintain K status in soils for sustaining crop production. In this respect, India is fortunate to have the world's largest deposit of mica mines distributed in Munger district of Bihar and Koderma and Giridih districts of Jharkhand. During the dressing of raw micas large quantities of waste mica are generated (about 75% of total mica mined) which are not used in agriculture as source of potassium though contains significant amount of K (8–12% K<sub>2</sub>O) and dumped near the mica mines (Nishanth and Biswas 2008). These are mostly white mica and categorized as muscovite mica. They have flake-like sheet structure, insoluble in water and hydrochloric acid (HCl), but can be solubilized by digesting with phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) at 300–350°C for about an hour. These materials can effectively be used as a source of potassium, if modified or altered by some suitable chemical or biological means. One of the possible means of utilizing waste mica is by mobilizing their K through composting technology where unavailable K is converted into plant available form because of the acidic environment prevailing during composting (Nishanth and Biswas 2008).

The results of the few experiments done elsewhere on the K-releasing capacity of a range of crushed rocks and minerals as source of K-fertilizer has been investigated (Hinsinger et al. 1996; Sanz Scovino and Rowell 1988; Bakken et al. 1997, 2000). Although it is concluded that a substantial part of the K bound in crushed rocks and mine tailings containing biotite, K-feldspar and nepheline is plant available, these rock/mineral products weathered too slowly to replenish the native pool of plant available K. Ground rock has been proposed as a slow release K-fertilizer for highly weathered soils and leaching environments where soluble fertilizers may be easily dissolved and leached (Coroneos et al. 1996; Hinsinger et al. 1996; Sanz Scovino and Rowell 1988). Nutrients from ground rock under leaching conditions may be released at a rate that allows them to remain in the top-soil and utilized by plants (Coroneos et al. 1996). Ground silicate rocks are also considered as slow release K-fertilizer in situations where leaching rates of conventional fertilizers are particularly high, e.g. in sandy soils under wet climatic regimes (Harley and Gilkes 2000). However, the effectiveness of silicate



rock fertilizers in agricultural practices has been found very poor because of low solubility of silicate rocks and the subsequent low availability of nutrients to plants as well as the practicality of applying large amounts of ground rock to agricultural land (Hinsinger et al. 1996; Bolland and Baker 2000; Harley and Gilkes 2000). Fused potassium silicate, which contains K<sub>2</sub>Ca<sub>2</sub>Si<sub>2</sub>O<sub>7</sub>, has been prepared and used as a slow-releasing potassium fertilizer (Yao et al. 2003). Such result suggests the involvement of 'K priming', a process by which the addition of K fertilizers enhances plant K uptake from the soil.

Some microorganisms in the soil are able to solubilize 'unavailable' forms of K-bearing minerals, such as micas, illite and orthoclase, by excreting organic acids which either directly dissolves rock K or chelating silicon ions to bring the K into solution (Bennett et al. 1998; Barker et al. 1998). These microorganisms are commonly known as potassium solubilizing bacteria (KSB) or potassium dissolving bacteria or silicate dissolving bacteria. Some research has been made about the use of potassium dissolving bacteria, known as "biological potassium biofertilizer (BPF)", particularly in China and South Korea to investigate the bio-activation of soil K-reserves so as to alleviate the shortage of K-fertilizer. It was shown that KSB increased K availability in soils and increased mineral uptake by plant (Sheng et al. 2002, 2003). Therefore, application of KSB holds a promising approach for increasing K availability in soils. However, information on mobilization of K in waste mica and their use as K-fertilizer for crop production is lacking. The objectives of this study were (i) to see the dynamics of potassium release from waste mica as influenced by potassium solubilizing microorganism (Bacillus mucilaginosus) and (ii) to investigate its effectiveness as K-fertilizer using sudan grass (Sorghum vulgare Pers.) var Sudanensis as test crop grown under two Alfisols.

#### Materials and methods

## Waste mica

Waste mica, a K-bearing mineral, was obtained from the surroundings of mica mines located at Koderma district of Jharkhand, India. The waste mica is generated during the dressing of raw mica blocks, which is generally used as electrical insulator. This waste material is dumped near the mica mines and not used in agriculture as such. It belongs to muscovite mica, which has the theoretical composition of  $(OH)_4K_2(Si_6Al_2)Al_4O_{20}$ . The waste mica has a flake-like structure. It was ground in a Wiley mill and passed through 2-mm sieve before further use. The ground waste mica contained 10.0% total K and had 30.0 mg kg<sup>-1</sup> of water-soluble K. The amounts of exchangeable and non-exchangeable K in the ground waste mica were 157.5 mg kg<sup>-1</sup> and 260.0 mg kg<sup>-1</sup>, respectively.

#### Bacterial strain

Biological potassium fertilizer (BPF), a carrier based biofertilizer product containing Bacillus mucilaginosus strain was obtained from the Hebei Research Institute of Microbiology, Hebei Academy of Science, Baoding City, Hebei Province, P.R. China with due permission from the Government of India, Ministry of Agriculture, Department of Agriculture and Cooperation, Directorate of Plant Protection, Quarantine & Storage, Faridabad, Haryana, India. The BPF product is widely used as potassium solubilizing biofertilizers in China. The active bacterial strain was isolated from the BPF material using nutrient agar medium and subsequently multiplied for further use. The composition of the nutrient agar medium were: beef extract 3.0 g; peptone 5.0 g; agar 15.0 g; distilled water 1000 ml; pH 6.6-7.0. The isolated strain was maintained on potato-dextrose agar slants in a refrigerator at 4°C.

The Bacillus mucilaginosus strain was multiplied by standard technique. Broth culture medium of the bacteria was prepared according to the procedure suitable for potassium solubilizing bacteria (Sheng et al. 2002; Wu et al. 2005; Sheng and He 2006). The compositions of the broth culture medium used were: sucrose 5.0 g; sodium hydrogen phosphate (Na<sub>2</sub>HPO<sub>4</sub>) 2.0 g; magnesium sulphate (MgSO<sub>4</sub>.7H<sub>2</sub>O) 0.5 g; ferric chloride (FeCl<sub>3</sub>) 0.005 g; calcium carbonate (CaCO<sub>3</sub>) 0.1 g; waste mica (potassium source) 1.0 g; distilled water 1000 ml; pH of the medium was adjusted to 7.5 using dilute acid and/or alkali. All the ingredients except mica were dissolved in 1000 ml distilled water. The contents were transferred into four conical flasks of 500 ml capacity each and waste mica (2-mm size) was added. The flasks were plugged with cotton and sterilized at 120°C and 0.1 MPa for 20 min in an autoclave. Sterilized medium was kept at



ambient temperature (25±1°C) in a dust-free environment for another 7 days to produce sufficient number of bacterial cells in broth culture for further use.

#### Soils

Two surface soil samples (0–15 cm depth) were obtained from the research farm of (i) Orissa University of Agriculture and Technology (OUAT), Bhubaneswar, Orissa; and (ii) Central Rainfed Upland Rice Research Station, Hazaribagh, Jharkhand, India. These two soils were selected taking into account their variable amounts of different pools of K, particularly low in available potassium (K-deficient) as well as their varying mineralogical compositions. Both the soils are categorized as Alfisol (Typic Haplustalfs). The soil of Hazaribag is clay loam in texture with illite and montmorillonite as the dominant clay mineral, while soil of Bhubaneswar is sandy loam in texture with kaolinite and illite dominated clay.

# Soil analysis

Soil samples were air-dried and passed through a 2-mm sieve before laboratory analyses. The particle size analysis was determined by hydrometer method (Bouyoucos 1962) after dispersing the soil in sodium hexametaphosphate solution. Soil pH and electrical conductivity were measured in soil: water ratio of 1:2 (Richards 1954). The organic carbon content of soil was determined by rapid titration method (Walkley and Black 1934). Cation exchange capacity (CEC) of soil was determined as per the procedure outlined by Jackson (1973). Easily oxidizable N as an index of available N was determined by the alkaline permanganate method as outlined by Subbiah and Asija (1956). Available P was extracted following the method of Bray and Kurtz (1945), where soil was extracted by shaking with solution of 0.03 N NH<sub>4</sub>F + 0.025 N HCl for 30 min using a soil solution ratio of 1:20. Phosphorus in the extracts was analyzed spectrophotometrically using ascorbic acid as reductant (Watanabe and Olsen 1965). Water-soluble K was extracted by shaking soil samples with distilled water using a soil solution ratio of 1:5 (Page et al. 1982). The exchangeable K was extracted by shaking neutral 1 N NH<sub>4</sub>OAc solution for 5 min using a soil solution ratio of 1:5 (Hanway and Heidel 1952). Nonexchangeable K was extracted by adding 25 ml of  $1 N \text{ HNO}_3$  to 2.5 g of soil and boiling for 15 min (Page et al. 1982). The total K was determined by the method as described by Jackson (1973), which involved digesting soil (0.1 g) in a mixture of HF and HClO<sub>4</sub>. Potassium fixation was determined as per the procedure outlined by Jackson (1973). Potassium in the extracts was determined by a flame photometer. The important physicochemical properties of the soils are presented in Table 1.

## Crop

Sudan grass (Sorghum vulgare Pers.) var Sudanensis was selected as the test crop for greenhouse experiment. This crop was selected because of its quick growing nature. It is known as a K-exhausting crop as it requires high amount of K for its growth. Moreover, since it is a perennial grass, there is a possibility to accumulate K in excess of its own requirements depending upon the availability of K in soil and potassium added through fertilizers. Sudan grass has shown promise as a fodder crop particularly in northern India. During its growth multiple cuttings can be obtained to supply fodder for a longer period.

## Greenhouse experiment

The experiment was carried out in greenhouse in *rabi* season, November 2006 to April 2007 at the Indian Agricultural Research Institute (IARI), New Delhi, located at 28° 37′–28° 39′ N latitude and 77° 9′–77° 11′ E longitude, at an altitude of 220 m above sea level. The climate of the study area is semi-arid subtropical region showing hot summers and cold winters with a mean annual maximum and minimum temperature of 40.5°C and 6.5°C, respectively and total annual rainfall of 760 mm (approximately) occurring mostly during the months of July to September.

Sixteen treatments consisting of factorial combinations of four rates of waste mica ( $M_0$ ,  $M_1$ ,  $M_2$  and  $M_3$  corresponding to 0 mg K kg<sup>-1</sup>, 50 mg K kg<sup>-1</sup>, 100 mg K kg<sup>-1</sup> and 200 mg K kg<sup>-1</sup> soil), two bacterial cultures (without and with *Bacillus mucilaginosus*) and two Alfisols (Bhubaneswar and Hazaribag) were used. The experiment was laid out in a completely randomized design (CRD) with three replications for this study.

Processed soil (<5-mm size, 4.5 kg) was placed on a clean polyethylene sheet. Waste mica and *Bacillus mucilaginosus* strain (20 ml of broth culture per pot



Table 1 Some physicochemical properties of the experimental soils

Characteristics	Values		Method/Reference	
	Bhubaneswar	Hazaribagh		
pH (soil: water:: 1:2)	5.64	6.13	Richards (1954)	
EC (soil:water::1:2) (dS m <sup>-1</sup> )	0.21	0.23	Jackson (1973)	
Mechanical analysis			Bouyoucos (1962)	
Sand (%)	67.7	63.4		
Silt (%)	12.4	11.7		
Clay (%)	17.9	24.9		
Textural class	Sandy loam	Clay loam		
Organic carbon (g kg <sup>-1</sup> soil)	4.1	4.7	Walkley and Black (1934)	
CEC [cmol(p <sup>+</sup> ) kg <sup>-1</sup> soil]	8.93	9.45	Jackson (1973)	
Available N (mg kg <sup>-1</sup> soil)	86.3	91.1	Subbiah and Asija (1956)	
Available P (mg kg <sup>-1</sup> soil)	4.87	5.54	Bray and Kurtz (1945)	
Different pools of K			• • • • • • • • • • • • • • • • • • • •	
Water-soluble K (mg kg <sup>-1</sup> soil)	8.44	14.1	Page et al. (1982)	
Exchangeable K (mg kg <sup>-1</sup> soil)	19.7	47.3	Hanway and Heidel (1952)	
Non-exchangeable K (mg kg <sup>-1</sup> soil)	51.2	112.7	Page et al. (1982)	
Total K (g kg <sup>-1</sup> soil)	4.50	11.25	Page et al. (1982)	
Potassium fixing capacity (%)	68.8	74.5	Jackson (1973)	

containing about  $1.0 \times 10^7 - 1.5 \times 10^8$  cfu) were added to soil as per the treatment combinations and mixed thoroughly. A basal dose of nitrogen (50 mg N kg<sup>-1</sup> soil) and phosphorus (50 mg P kg<sup>-1</sup> soil) through urea and sodium di-hydrogen orthophosphate (NaH<sub>2</sub>PO<sub>4</sub>), respectively were applied as in solution form to each pot and mixed thoroughly with the soil. Adequate amount of water was added so as to raise the moisture content of soil to field capacity. The soil treated with fertilizer materials were finally placed in the polyethylenelined earthen pots having 25 cm upper diameter and 30 cm depth.

Twenty seeds of sudan grass were sown in each pot on 21st November 2006, which after germination (7 d) was thinned to retain eight healthy plants per pot to ensure enough dry matter production and nutrient removal from the soil. The pots were kept weed-free and maintained in an optimum soil moisture regime, approximately at 60% water holding capacity of the soil throughout the experiment by irrigating the crop on a regular basis to ensure that water was not a limiting factor. The crop was harvested by cutting sample at 8-10 cm above soil surface in each cutting so as to regenerate the biomass for next cutting. Altogether five cuttings of biomass were obtained with an interval of 1 month each (30 day, 60 day, 90 day, 120 day and 150 day after sowing) and recorded the respective biomass yield after drying at 65±1°C. The oven-dried plant samples were mixed well, ground by a Wiley mill (5-mm size) and digested (0.5 g) with di-acid mixture (8 ml) containing HNO<sub>3</sub>:HClO<sub>4</sub>:: 9:4 on an electric hot plate (Piper 1967). Potassium content in the acid digest was determined by a flame photometer and uptake of K was computed. Per cent K recovery by crop was computed by the relationship as given below:

Per cent K recovery = 
$$100 \times (UK_t - UK_c)/A$$

where, UK<sub>t</sub>=Uptake of K in fertilizer treated pot (mg K pot<sup>-1</sup>); UK<sub>c</sub>=Uptake of K in control pot (mg K pot<sup>-1</sup>); A=Amount of K applied (mg K pot<sup>-1</sup>).

Measurement of the different pools of K in soils was carried out after drawing sub-sample of soil from each pot with the help of soil auger (0–15 cm depth) after each cutting of the crop to investigate the K dynamics of the soil. Air-dried soil samples were passed through 2-mm sieve and analyzed for water-soluble K (Page et al. 1982), exchangeable K (1 N NH<sub>4</sub>OAc extractable K) (Hanway and Heidel 1952) and non-exchangeable K (1 N boiling HNO<sub>3</sub> extractable K) (Page et al. 1982). Potassium in the extracts was determined by a flame photometer.

## Kinetics of K release

Kinetics of K release from waste mica as influenced by *Bacillus mucilaginosus* strain was carried out and



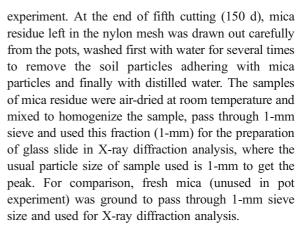
the rate of release of K was computed. To compute the rate of release of K, the data generated in pot experiment were fitted into the kinetics equation. There are many equations employed by different workers including first-order, Elovich, parabolic diffusion, zero-order and power function equation to describe the kinetics of K release pattern in soils (Sparks et al. 1980; Feigenbaum et al. 1981; Martin and Sparks 1983; Jardine and Sparks 1984; Havlin et al. 1985; Sparks 1987; Dhillon and Dhillon 1990). These equations have been used to determine reaction order and rate coefficients between the different pools of soil K. In this study we used the best-fitted equation as tested by larger correlation (r) value and least square regression analysis to determine which equation is best described the K release from waste mica. Based on this, we employed the best-fitted simple first-order equation of Jardine and Sparks (1984) as given below:

$$\ln(a-p) = \ln a - kt$$

where,  $\ln = \text{natural logarithm}$ ; a = exchangeable K (mg pot<sup>-1</sup>) present initially in soil; p = K (mg pot<sup>-1</sup>) released at a particular time 't' (d) plus mean K uptake by plant in that stage; (a-p) = nutrient present finally in soil; and k = rate constant.

### X-ray diffraction analysis

In the present study to see the structural changes of mica, if any, as affected by potassium solubilizing microorganism (Bacillus mucilaginosus), another set of treatments were selected for conducting pot experiment using same soils used in earlier experiment. To achieve this objective one rate of mica (100 mg K kg<sup>-1</sup> soil) was used with and without inoculation of bacterial broth culture (Bacillus mucilaginosus) and applied in two soils. There were four treatments consisting of two mica (without bacterial culture and with bacterial culture) and two soils (Hazaribag and Bhubaneswar) for this pot study, which were replicated three times. The experiment was laid out in a completely randomized design. Processed soil (<5-mm size, 4.5 kg) was placed in each pot. Inoculated and uninoculated waste mica (2-mm size) were first kept within a nylon cloth (fine mesh) and then placed into soils (10 cm depth) as per the treatments. Sudan grass was grown in these pots by sowing the crops and five cuttings were performed, but no soil samples were drawn from these pots after each cutting of crop as in case of previous



For X-ray diffraction analysis, a small portion of sample of mica residue was mounted on a glass slide (2.5 cm×4 cm size). The concentrated gum acacia solution was first sprayed on the half portion of the glass slide on level surface and air-dried. Powder (1-mm size) sample of mica residue was then sprinkled over the glass slide containing concentrated gum acacia solution so as to adhere mica particles strongly on it. The slides were then subjected to X-ray diffraction analysis by using Cu-K $\alpha$  radiation and Ni filter in a X-ray diffractometer (Model: Philips PW 1710) for measurement of integral width and full width at half maximum (FWHM) at 10 Å peak. Fresh mica (unused, 1-mm size) sample was also run for X-ray diffraction analysis as standard.

## Statistical analysis

Data obtained in the greenhouse experiments were subjected to analysis of variance (ANOVA) appropriate to the experimental design. F-test was carried out to test the significance of the treatment differences and the least significant difference (LSD at P=0.05) was computed. For statistical analysis of data Microsoft Excel (Microsoft Corporation, USA) and MSTATC packages were used. Pearson's correlation matrix between biomass yield, K uptake by crop and different pools of potassium in soils was computed by the SPSS window version 10 (SPSS Inc., Chicago, USA).

## **Results**

## Biomass yield

Biomass yield (sum of five cuttings) of sudan grass (Table 2) enhanced significantly with addition of waste



Table 2 Effect of waste mica as influenced by *Bacillus mucilaginosus* strain on biomass yield (g pot<sup>-1</sup>) of sudan grass (sum of five cuttings) grown in two Alfisols

Treatment	Microbial culture		Soils		Mean
	Without Bacillus mucilaginosus	With Bacillus mucilaginosus	Alfisol from Bhubaneswar	Alfisol from Hazaribag	
Rate of mica (mg K kg $^{-1}$ soil)					
$M_0 (0 \text{ mg K kg}^{-1})$	14.1	20.7	12.2	22.6	17.4
$M_1 (50 \text{ mg K kg}^{-1})$	17.6	22.8	14.9	25.5	20.2
M <sub>2</sub> (100 mg K kg <sup>-1</sup> )	18.8	25.3	15.9	28.3	22.1
M <sub>3</sub> (200 mg K kg <sup>-1</sup> )	18.1	25.4	15.2	28.3	21.8
Soil					
Alfisol from Bhubaneswar	12.6	16.5			
Alfisol from Hazaribag	21.8	30.6			
Mean	17.2	23.5	14.6	26.2	
$LSD \ (P=0.05)$					
Rate of mica	1.9				
Bacterial culture	3.6				
Soil	3.6				
Rate of mica×Bacterial culture	NS				
Rate of mica×Soil	NS				
Bacterial culture×Soil	5.0				
Rate of mica×Bacterial culture×Soil	NS				

mica compared to control (17.4 g pot<sup>-1</sup>) in both the soils. The biomass yield increased (P < 0.05) with increase in rates of mica up to 100 mg K kg<sup>-1</sup> soil (22.1 g pot<sup>-1</sup>), thereafter it was at par with increase in rate of mica (200 mg K kg<sup>-1</sup> soil). Inoculation of Bacillus mucilaginosus strain had also shown increase (P < 0.05) in biomass yield (23.5 g pot<sup>-1</sup>) than the treatment without bacterial inoculated pots (17.2 g pot<sup>-1</sup>) irrespective of rates of mica application and soils. Inoculated treatment recorded 36.6% higher biomass yield over uninoculated treatment. No synergetic effect between waste mica and bacterial inoculation on biomass yield (P < 0.05) was noticed than the yield obtained by them individually. However, application of waste mica @ 100 mg K kg<sup>-1</sup> soil when inoculated with Bacillus mucilaginosus recorded the highest biomass yield (25.3 g pot<sup>-1</sup>) of sudan grass. With regard to the experimental soils, significant increase (P < 0.05) in the biomass production was noticed in Alfisol from Hazaribag (26.2 g pot<sup>-1</sup>) than the Alfisol from Bhubaneswar (14.6 g pot<sup>-1</sup>) irrespective of rates of mica and bacterial culture.

## Potassium uptake

Application of mica resulted in increase (P < 0.05) in K uptake by sudan grass (Table 3) over control. The

uptake of K enhanced significantly with increase in rates of mica application up to 100 mg K kg<sup>-1</sup> soil (433.7 mg K pot<sup>-1</sup>), which was 36.0 and 13.1 per cent higher over control and application of mica @ 50 mg K kg<sup>-1</sup> soil, respectively. However, further increase in rate of mica application to the tune of 200 mg kg<sup>-1</sup> soil did not increase K uptake significantly. Inoculation of mica with bacterial strain Bacillus mucilaginosus attributed significantly (P < 0.05) higher K uptake (486.6 mg pot<sup>-1</sup>), which was 59.6 per cent higher over uninoculated mica (304.9 mg pot<sup>-1</sup>). Results revealed that bio-intervention of waste mica with Bacillus mucilaginosus performed significantly in enhancing the K uptake by sudan grass in both the soils. Significant synergetic effect on potassium uptake (P < 0.05) was also noticed due to combined application of waste mica and bacterial inoculation than the K uptake obtained by them individually. Application of waste mica @ 100 mg K kg<sup>-1</sup> soil when inoculated with Bacillus mucilaginosus recorded the highest K uptake (531.2 mg pot<sup>-1</sup>) by sudan grass. Alfisol from Hazaribag exhibited an increase of 3.56 times higher K uptake (618.2) over Alfisol from Bhubaneswar (173.4).

Data revealed that cumulative K uptake by sudan grass increased significantly with increase in rate of



**Table 3** Effect of waste mica as influenced by *Bacillus mucilaginosus* strain on potassium uptake (mg pot<sup>-1</sup>) by sudan grass (sum of five cuttings) grown in two Alfisols

Treatment	Microbial culture		Soils		Mean
	Without Bacillus mucilaginosus	With Bacillus mucilaginosus	Alfisol from Bhubaneswar	Alfisol from Hazaribag	
Rate of mica (mg $K kg^{-1}$ soil)					
$M_0 (0 \text{ mg K kg}^{-1})$	240.0	397.7	137.8	499.9	318.8
$M_1 (50 \text{ mg K kg}^{-1})$	308.5	458.4	178.7	588.2	383.5
M <sub>2</sub> (100 mg K kg <sup>-1</sup> )	336.1	531.2	192.2	675.2	433.7
$M_3 (200 \text{ mg K kg}^{-1})$	335.0	559.1	184.8	709.4	447.1
Soils					
Alfisol from Bhubaneswar	134.4	212.4			
Alfisol from Hazaribag	475.5	760.9			
Mean	304.9	486.6	173.4	618.2	
$LSD \ (P=0.05)$					
Rate of mica	38.3				
Bacterial culture	73.0				
Soil	73.0				
Rate of mica×Bacterial culture	54.2				
Rate of mica×Soil	54.2				
Bacterial culture×Soil	103.0				
Rate of mica×Bacterial culture×Soil	NS				

mica application in both the soils (Fig. 1). Both the soils exhibited a similar trend in increasing cumulative K uptake in each cutting. In general, the trend in cumulative uptake was linear up to fourth cutting (120 d) and then there was a steep increase in the fifth cutting (150 d), irrespective of treatments. Inoculation with *Bacillus mucilaginosus* resulted in further increase in cumulative K uptake by sudan grass. This is true for both the Alfisols (Fig. 1). The cumulative K uptake was much higher in Alfisol from Hazaribag than that of Alfisol from Bhubaneswar.

### Per cent K recovery

Per cent K recoveries by sudan grass grown in two Alfisols (Table 4) showed that treatment receiving mica @ 50 mg K kg<sup>-1</sup> soil recorded the highest recoveries in both the soils, while the lowest K recoveries were obtained with treatment receiving mica @ 200 mg K kg<sup>-1</sup> soil. Thus, over all K recoveries decreased with the increase in rates of mica applied either alone or inoculated with *Bacillus mucilaginosus* strain. It was observed that without bacterial strain, application of mica @ 50 mg K kg<sup>-1</sup> soil recorded 20.5 and 40.5 per cent K recoveries in Alfisol from Bhubaneswar and Hazaribag, respectively.

On the other hand with bacterial inoculation, these values were 48.3 and 90.4 per cent, respectively. In general, the K recoveries in Alfisol from Hazaribag were much higher than Alfisol from Bhubaneswar at a particular rate of mica application.

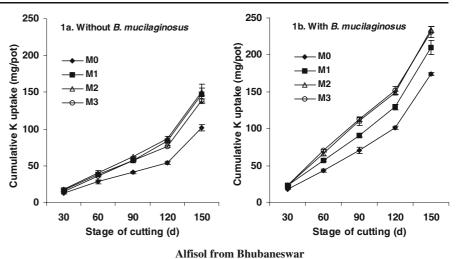
Dynamics of K in soils

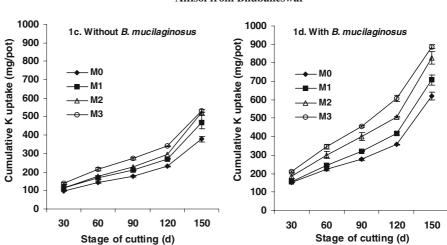
# Water-soluble K

There was a general increase in water-soluble K (Fig. 2) in soils up to second cutting (60 d), thereafter it decreased gradually during the remaining period of greenhouse experiment. Lowest amount of water-soluble K was found in control where no K was added compared to treatments receiving mica as source of K after each cutting of crop, irrespective of bacterial inoculation and soils. Significantly greater amount of water-soluble K was obtained with increase in rate of mica up to 100 mg K kg<sup>-1</sup> soil application, thereafter it was at par. Alfisol from Hazaribag recorded significantly greater amount of water-soluble K than Alfisol from Bhubaneswar across the rates of mica application, inoculation of bacterial strain and stages of cuttings. Treatments receiving mica along with bacterial strain Bacillus mucilaginosus enhanced water-soluble K in both the soils.



**Fig. 1** Effect of waste mica as influenced by *Bacillus mucilaginosus* strain on cumulative potassium uptake by sudan grass (mg pot<sup>-1</sup>) grown in two Alfisols





\*  $M_0 = \text{Mica} @ 0 \text{ mg K kg}^{-1} \text{ soil};$   $M_1 = \text{Mica} @ 50 \text{ mg K kg}^{-1} \text{ soil};$   $M_2 = \text{Mica} @ 100 \text{ mg K kg}^{-1} \text{ soil};$   $M_3 = \text{Mica} @ 200 \text{ mg K kg}^{-1} \text{ soil}.$ 

Alfisol from Hazaribag

# Exchangeable K

Data in Fig. 3 showed that after first cutting of sudan grass, exchangeable K increased significantly with

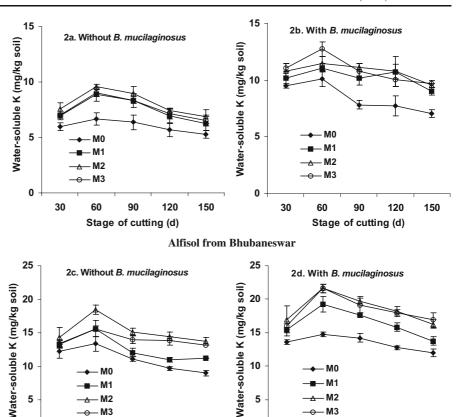
addition of mica compared to control. In case of Alfisol from Bhubaneswar, the exchangeable K increased up to third cutting (90 d), then decreased drastically at the fourth cutting (120 d) and then

Table 4 Per cent recoveries of potassium by sudan grass in two Alfisols as affected by application of different rates of mica inoculated with *Bacillus mucilaginosus* strain

Rate of mica (mg K kg <sup>-1</sup> soil)	Without Bacillus m	Without Bacillus mucilaginosus		With Bacillus mucilaginosus	
	Alfisol from Bhubaneswar	Alfisol from Hazaribag	Alfisol from Bhubaneswar	Alfisol from Hazaribag	
M <sub>0</sub> (0 mg K kg <sup>-1</sup> )	-	-	-		
M <sub>1</sub> (50 mg K kg <sup>-1</sup> )	20.5	40.5	48.3	90.4	
M <sub>2</sub> (100 mg K kg <sup>-1</sup> )	10.9	31.9	29.5	73.2	
M <sub>3</sub> (200 mg K kg <sup>-1</sup> )	4.1	17.0	14.4	56.5	



Fig. 2 Changes in watersoluble K (mg kg<sup>-1</sup>) in soils after each cutting of sudan grass grown in two Alfisols as affected by different rate of mica inoculated with Bacillus mucilaginosus strain



Alfisol from Hazaribag

10

5

0

30

 $M_0 = Mica @ 0 mg K kg^{-1} soil;$  $M_1 = Mica @ 50 mg K kg^{-1} soil;$  $M_2 = Mica @ 100 \text{ mg K kg}^{-1} \text{ soil}; M_3 = Mica @ 200 \text{ mg K kg}^{-1} \text{ soil}.$ 

150

Initial water-soluble K: 8.44 and 14.1 mg K kg<sup>-1</sup> soil in Bhubaneswar and Hazaribag,

slowly after fifth cutting (150 d), irrespective of rates of mica application and inoculation of bacterial strain, which resulted in less K being available for plants. In case of Hazaribag soil, the exchangeable K increased up to second cutting (60 d), and then decreased in third cutting (90 d), thereafter no significant changes in exchangeable K were noticed till fifth cutting (150 d), which was reflected well in higher K uptake by the crop because of effective absorption of K by plants. The exchangeable K was much lower in Bhubaneswar soil than Hazaribag soil across the rates of mica application, inoculation of bacterial strain, and stages of cuttings. Application of mica increased exchangeable K up to 100 mg kg<sup>-1</sup> soil throughout the crop stages. Treatments receiving mica along with bacterial

10

5

0

30

**M2** 

90

Stage of cutting (d)

120

60

strain Bacillus mucilaginosus consistently maintained the higher exchangeable K than uninoculated mica in both the soils and after each cutting of crop.

MO M1 M2

- M3

90

Stage of cutting (d)

120

150

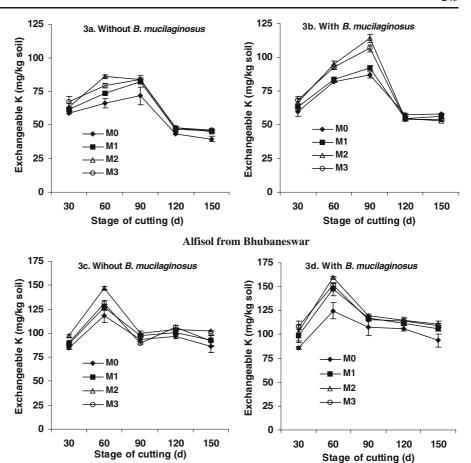
60

#### Non-exchangeable K

The non-exchangeable K decreased gradually from first to fifth cuttings of the crop in Alfisol from Bhubaneswar (Fig. 4). On the other hand, in case of Alfisol from Hazaribag, the non-exchangeable K decreased gradually up to third cutting (90 d). The non-exchangeable K, thereafter, increased gradually till the end of fifth cutting (150 d) across the rate of K application and inoculation of bacterial strain. Lowest amount of non-exchangeable K was observed in control



Fig. 3 Changes in exchangeable K (mg kg<sup>-1</sup>) in soils after each cutting of sudan grass grown in two Alfisols as affected by different rate of mica inoculated with *Bacillus mucilaginosus* strain



Alfisol from Hazaribag

- \*  $M_0 = Mica @ 0 mg K kg^{-1} soil;$   $M_1 = Mica @ 50 mg K kg^{-1} soil;$   $M_2 = Mica @ 100 mg K kg^{-1} soil;$   $M_3 = Mica @ 200 mg K kg^{-1} soil.$
- \*\* Initial exchangeable K: 19.7 and 47.3 mg K kg<sup>-1</sup> soil in Bhubaneswar and Hazaribag, respectively.

treatment where no mica was added, and it increased with increase in rates of mica up to 100 mg K kg<sup>-1</sup> soil application in both the soils. Increase in non-exchangeable K in soils was observed due to inoculation of bacterial strain *Bacillus mucilaginosus*. In general, the non-exchangeable K pool was two to three times higher in Alfisol of Hazaribag than Bhubaneswar.

#### Correlation matrix

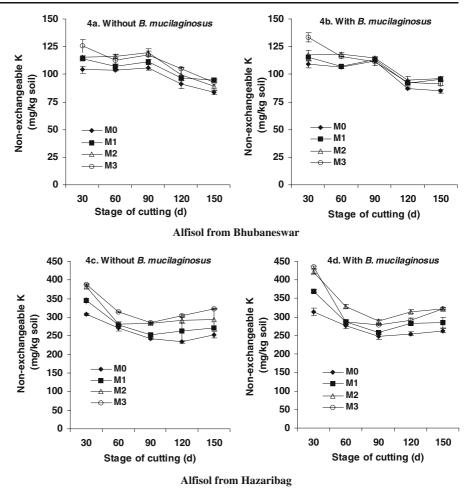
Data on Pearson's correlation matrix in Alfisol from Bhubaneswar (Table 5) showed that biomass yield of sudan grass was significantly and positively correlated (P < 0.01) with K uptake (r=0.953\*\*) by the crop, but negatively correlated with water-soluble K (r=-0.280\*\*), exchangeable K (r=-0.562\*\*) and non-exchangeable K

 $(r=-0.502^{**})$ , indicating that these pools of potassium might have contributed towards higher biomass production. Potassium uptake was significantly but negatively correlated with exchangeable K  $(r=-0.355^{**})$  and nonexchangeable K  $(r=-0.483^{**})$ , suggesting that the uptake of K by crop was mainly influenced by exchangeable pool of K in soil. Significantly and positively correlation between water-soluble and exchangeable K  $(r=0.447^{**})$  was noticed. Further, exchangeable K was significantly and positively correlated with non-exchangeable K  $(r=0.315^{**})$ .

In case of Alfisol from Hazaribag (Table 5), biomass yield was significantly and positively correlated with K uptake (r=0.931\*\*) by the crop. However, it was significantly but negatively correlated with exchangeable K (r=-0.289\*\*). Potassium uptake was signifi-



Fig. 4 Changes in non-exchangeable K (mg kg<sup>-1</sup>) in soils after each cutting of sudan grass grown in two Alfisols as affected by different rate of mica inoculated with *Bacillus mucilaginosus* strain



- \*  $M_0 = Mica @ 0 mg K kg^{-1} soil;$   $M_1 = Mica @ 50 mg K kg^{-1} soil;$   $M_2 = Mica @ 100 mg K kg^{-1} soil;$   $M_3 = Mica @ 200 mg K kg^{-1} soil.$
- \*\* Initial non-exchangeable K: 51.2 and 112.7 mg K kg<sup>-1</sup> soil in Bhubaneswar and Hazaribag, respectively.

cantly but negatively correlated with exchangeable  $(r=-0.315^{**})$  as well as non-exchangeable K  $(r=-0.370^{**})$ . Water-soluble K was significantly and positively correlated with exchangeable K  $(r=0.373^{**})$  and non-exchangeable K  $(r=0.287^{**})$ , while exchangeable K was significantly and negatively correlated with non-exchangeable K  $(r=-0.219^{*})$ .

### Potassium release kinetics

Data generated in the present experiments were fitted into a kinetics equation and rate of release kinetics of K from waste mica as affected by bacterial inoculation in soils were computed. Accordingly, a release curve of 'ln (a-p)' versus

'time' was plotted for different treatments. In case of Alfisol from Bhubaneswar (Fig. 5), no significant release of K from mica was found up to third cutting (90 d), thereafter released K at a faster rate up to fifth cuttings, irrespective of treatments. On the other hand the release kinetics of K in Alfisol from Hazaribag showed that irrespective of treatments, significant amount of release of K from mica was noticed up to third cutting (90 d) of the crop; the amount of release of K decreased thereafter up to fourth cutting (120 d). The release of K increased substantially till the end of last cutting. Treatments receiving mica along with *Bacillus mucilaginosus* released significantly higher amounts of K over uninoculated mica.

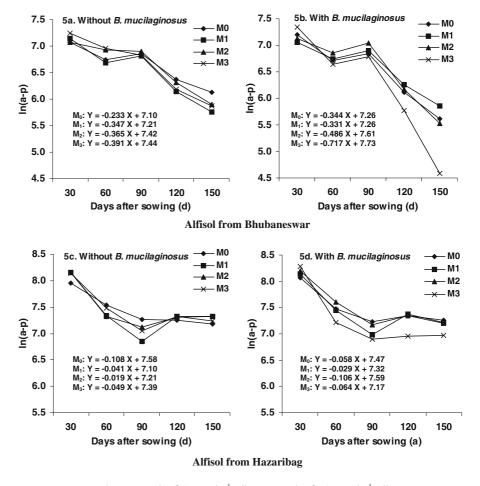


Table 5 Pearson's correlations matrix between biomass yield, K uptake by sudan grass and different pools of K after each cutting of crop grown in Alfisol from Bhubaneswar and Hazaribag

Parameter	Biomass yield	Potassium uptake	Water-soluble K	Exchangeable K	Non-exchangeable K
Alfisol from Bhubanesw	var				
Biomass yield	1.00	0.953 <sup>b</sup>	$-0.280^{b}$	$-0.562^{b}$	$-0.502^{b}$
Potassium uptake		1.00	NS	-0.355 <sup>b</sup>	$-0.483^{b}$
Water-soluble K			1.00	0.447 <sup>b</sup>	NS
Exchangeable K				1.00	0.315 <sup>b</sup>
Non-exchangeable K					1.00
Alfisol from Hazaribag					
Biomass yield	1.00	0.931 <sup>b</sup>	NS	-0.289 <sup>b</sup>	NS
Potassium uptake		1.00	NS	$-0.315^{b}$	$-0.370^{b}$
Water-soluble K			1.00	0.373 <sup>b</sup>	$0.287^{\rm b}$
Exchangeable K				1.00	$-0.219^{a}$
Non-exchangeable K					1.00

<sup>&</sup>lt;sup>a</sup> Correlation is significant at P = 0.05 level (2-tailed), <sup>b</sup> Correlation is significant at P = 0.01 level (2-tailed)

**Fig. 5** Release kinetics of potassium from waste mica by sudan grass grown in two Alfisols as affected by *Bacillus mucilaginosus* strain



 $M_0 = Mica @ 0 mg K kg^{-1} soil;$   $M_1 = Mica @ 50 mg K kg^{-1} soil;$   $M_2 = Mica @ 100 mg K kg^{-1} soil;$   $M_3 = Mica @ 200 mg K kg^{-1} soil.$ 



## X-ray diffraction analysis

X-ray diffraction patterns of the residue of mica mineral left after the crop harvest showed noticeable structural changes due to uninoculated and inoculated treatment from those of the original unused fresh mica (Table 6). The values of Pk position ( $^{\circ}2\theta$ ) due to uninoculated and inoculated treatments were statistically non-significant (P < 0.05). On the contrary, the values of full width at half maximum (FWHM) were significant (P < 0.05) due to bacterial inoculation. The X-ray diffraction pattern revealed that all the treatments resulted in lower FWHM values in both the soils as compared to unused fresh mica (0.266 °2θ). Application of mica inoculated with Bacillus mucilaginosus recorded lower FWHM values in both the soils than uninoculated mica. Between the two soils, Alfisol from Bhubaneswar resulted in lower FWHM value (0.134 °2θ) than Hazaribag soil (0.180 °2θ) when mica was applied along with Bacillus mucilaginosus. The results indicate that dissolution of some portion of mica at the edges might have taken place by the action of Bacillus mucilaginosus strain during the growing period of sudan grass.

#### Discussion

Bacillus mucilaginosus strain influences on biomass accumulation

Increased biomass yield of sudan grass was observed with addition of waste mica compared to control. The poor biomass production in potassium-unfertilized soil (control) may be due to low in available K (water-soluble and exchangeable K) in the experimental soils. This was observed by having an additional treatment of soluble K fertilizer like muriate of potash that recorded significantly higher biomass yield than treatment receiving no-K (control) (data not shown). The biomass yield increased significantly with increase in rates of mica up to 100 mg K kg<sup>-1</sup> soil beyond which it was at par, indicating that this much quantity of potassium is sufficient for optimum crop growth. Inoculation of Bacillus mucilaginosus strain had shown significant increase in biomass yield in the present work than uninoculated pots. This suggests that the bacterial inoculant enhanced biomass yield either directly by solubilizing soil K reserve, or indirectly by causing increased growth of the plant (for example by producing plant growth hormones—a phenomenon well documented in the case of Rhizobium inoculants). The biomass yield further increased when mica was applied along with bacterial inoculant. This may be due to mobilization of potassium from waste mica because of secretion of organic acids by the bacterial strain, which in turn increased the biomass yield. It is reported that potassium solubilizing microorganism are capable of solubilizing the unavailable forms of K in K-bearing minerals such as micas, illite and orthoclase through production and excretion of organic acids like citric, oxalic, and tartaric acids. Organic acids produced can facilitate the weathering of minerals by directly dissolving K from rocks or through the formation of metal-organic complexes by forming chelate with silicon ions to bring the K into solution (Song and Huang 1988; Friedrich et al. 1991;

**Table 6** Structural changes of mica after the harvest of sudan grass grown in two Alfisols as affected by *Bacillus mucilaginosus* strain using X-ray diffraction technique

Treatments	Pk position <sup>b</sup> (°2θ)	FWHM <sup>a</sup> (°2θ)	
Fresh mica (unused mica)	8.852	0.266	
Alfisol from Bhubaneswar			
Mica (100 mg K kg <sup>-1</sup> ) (without bacterial culture)	8.850	0.251	
Mica (100 mg K kg $^{-1}$ ) + Bacillus mucilaginosus	8.907	0.134	
Alfisol from Hazaribag			
Mica (100 mg K kg <sup>-1</sup> ) (without bacterial culture)	8.854	0.239	
Mica (100 mg K kg $^{-1}$ ) + Bacillus mucilaginosus	8.847	0.180	
SEm (±)	0.077	0.014	
LSD (P=0.05)	NS	0.030	

<sup>&</sup>lt;sup>a</sup> FWHM=Full width at half maximum (°20), <sup>b</sup> Pk=Peak position (°20)



Bennett et al. 1998). Many workers have reported that production of carboxylic acids and capsular polysaccharide is associated with solubilization of feldspar by application of K solubilizing microorganisms viz., Bacillus mucilaginosus and Bacillus edaphicus (Lin et al. 2002; Sheng and Huang 2002). Malinovskaya et al. (1990) opined that a mixture of polymers and low molecular weight ligands had a synergistic effect on mineral weathering by Bacillus mucilaginosus. Bennett et al. (2001) reported that microorganisms produced various organic ligands during their metabolism. These included metabolic byproducts, extracellular enzymes, chelates and both simple and complex organic acids, which helped in dissolution of feldspar by decreasing the pH of the environment. Increase in biomass yield may also be attributed to plant growth promoting activity of Bacillus mucilaginosus strain, which has an ability to convert potassium from unavailable to available form through biological processes. The present results corroborate the finding of other workers (Sheng 2005; Wu et al. 2005).

The other possible hypotheses/mechanisms to mobilize soil K reserve are due to biofilms formation on the rhizospheric mineral surfaces by certain bacterial strains (Balogh-Brunstad et al. 2008). Biofilms are known to protect the microbial community from various environmental effects and even from antibiotics, regulate transport of heavy metals and possibly other cations and nutrients to the microbes, and isolate mineral weathering and nutrient uptake by bacteria from bulk soil processes. They reported that ectomycorrhizal hyphal networks and root hairs of non-ectomycorrhizal trees, embedded in biofilms (microorganisms surrounded by extracellular polymers), transferred nutrients to the host. These results suggest that biofilms help to accelerate weathering of minerals like biotite and anorthite, thereby increase plant nutrient uptake.

Role of root derived organic acids in the mobilization of nutrients from the rhizosphere has been evaluated (Jones and Darrah 1994; Jones et al. 1996; Jones 1998). Jones et al. (2003) reported that organic acids have been hypothesized to perform many functions in soil including root nutrient acquisition, mineral weathering, microbial chemotaxis and metal detoxification. Calvaruso et al. (2006) reported the significant impact of plant root hairs on mineral weathering and demonstrated that a bacterial isolate, *Burkholderia glathei* PML1(12), significantly improved biotite

weathering by a factor of 1.3 for Mg and 1.7 for K and promoted plant growth, mainly because of its effect on mineral weathering. In addition, they observed a significant positive effect of different bacterial strains on pine growth and on root morphology like number of lateral roots and root hairs. Similarly, the impact of Douglas-fir and Scots pine seedlings on plagioclase weathering in a laboratory experiment under acidic conditions was reported by Bakker et al. (2004). Their results thus underline the importance of taking into account biotic and abiotic environments, like the composition of root exudates, the presence of ectomycorrhizal fungi, and soil properties (pH, aeration, and physicochemical characteristics), when characterizing the weathering effect of a given bacterial strain because these parameters may influence the expression of the weathering ability of the bacteria. Another possibility of increase in weathering is due to a synergistic effect, which could result from three hypothetical processes: (i) the fragmentation of the mineral caused by root activity increases the direct positive effect of the bacteria on mineral weathering by increasing the reactive surfaces; (ii) the root exudates indirectly provide the substrates required for the production of weathering metabolites by the bacteria, or (iii) the production of growth phytohormones by the bacteria, in addition to weathering agents, stimulates root development and modifies root physiology and root exudation, which improves mineral weathering and nutrient uptake (Gahoonia et al. 1997). Leyval and Berthelin (1989) showed that ectomycorrhizal fungi were able to solubilize K, Fe, Mg and Al from phlogopite within the rhizosphere of beech. The potential for dissolution of silicate rock powders is enhanced through the removal of nutrients and the addition of acids by mycorrhiza. It is also reported that some rock-eating fungi (ectomycorrhizal fungi) have the capacity to exude low molecular weight organic anions (LMWOA) to an extent that forms microscopic tunnels through exuded at hyphal tips within minerals like feldspar and hornblende grains in soils thereby weathering rates are significantly increased (van Schöll et al. 2008).

In the case of nutrients such as P, the primary mechanisms of solubilization of rock phosphate attributed are H<sup>+</sup> excretion and organic acid production. Excretion of H<sup>+</sup> by plant roots into the rhizosphere is caused by a higher uptake of cations relative to anions (Hinsinger et al. 2003). Phosphate solubilizing micro-



organisms are known to produce organic acids, namely citric, oxalic, tartaric, acetic, lactic, gluconic,  $\alpha$ -ketogluconic, etc. (Babana and Antoun 2006; Vassilev et al. 2006). These acids are sources of  $H^+$  ions able to dissolve the mineral phosphate and to make it available for the plant. In addition to pH reduction, organic acid anions can solubilize rock phosphate through chelation reactions (Reyes et al. 2006).

With regard to the experimental soils, the biomass production was much higher in Alfisol from Hazaribag than that of Bhubaneswar, irrespective of rates of mica and inoculation of bacterial strain. The poor performance in Alfisol from Bhubaneswar may be attributed to higher acidity (pH 5.64) as against lower acidity (pH 6.13 in Hazaribag soil) as well as lower initial fertility status (low available N, P and K) than Hazaribag soil.

Bacillus mucilaginosus influences on K uptake and recoveries

Bio-intervention of waste mica with Bacillus mucilaginosus performed significantly in enhancing K uptake by sudan grass in both the soils. The results confirm the findings of earlier workers where they reported greater total uptake of K by crop when Kbearing minerals were inoculated with potassium solubilizing bacteria (Sheng and Huang 2002). Sheng (2005) also reported significant increase in shoot and root dry yield as well as greater uptake of K by cotton and rape due to application of K-bearing mineral (illite) inoculated with potassium releasing strain Bacillus edaphicus NBT. It leads to generalization that the potassium dissolving bacteria play an important role in plant nutrition through the increase in K uptake by the plant. Significantly higher uptake of K by wheat grown in a K-deficient yellow-brown soil in Nanjing, China were reported by a wildtype bacterial strain Bacillus edaphicus NBT and their mutants MPs (+) and MPs(+)1 (Sheng and He 2006). Han et al. (2006) also reported the beneficial effect of Bacillus mucilaginosus on mobilization K from potassium mineral, and nutrient uptake and growth of pepper and cucumber on an Inceptisol from Korea. Han and Lee (2005) reported the synergistic effects of soil fertilization with rock P and K materials and co-inoculation with phosphate solubilizing bacteria (PSB) Bacillus megatherium and potassium solubilizing bacteria (KSB) Bacillus mucilaginosus KCTC 3870 on the improvement of P and K uptake by eggplant grown under limited P and K soil in greenhouse.

The higher mobilization of K from mica and its subsequent uptake by sudan grass due to inoculation with Bacillus mucilaginosus could be attributed to increase population of bacteria in the root and rhizosphere soil. Lin et al. (2002) and Egamberdiyeva and Hoflich (2003) demonstrated that bacterial inoculation resulted in growth promotion and higher K contents of plant components. As successful plant growth promoting inoculants, bacteria must be able to rapidly colonize the root system during the growing season (Defreitas and Germida 1992). Sheng (2005) demonstrated that Bacillus edaphicus NBT was able to colonize the rhizosphere soil and root of cotton and rape. The inoculated bacterium was able to establish larger population on the root and the rhizosphere soil of cotton and rape up to 5-weeks after sowing.

Per cent K recoveries by sudan grass showed that treatment receiving mica at the lowest level recorded the highest recoveries of K in both the soils, while the lowest K recoveries were obtained with the highest level of K applied either alone or inoculated with bacterial strain *Bacillus mucilaginosus*. In general, the recoveries of K in Hazaribag soil were much higher than Bhubaneswar. This may be attributed to higher biomass production due to greater amount of different pools of K (water-soluble, exchangeable, non-exchangeable and total K content) as well as better physicochemical properties like higher organic carbon, pH, CEC, and available N and P status in the initial soil of the former.

# K dynamics

There was a general trend in increase in water-soluble K in soils up to second cutting (60 d) then decrease in this pool till the end of the greenhouse experiment. The initial increase in this pool of K may be due to release of K from exchangeable and non-exchangeable pools of K, which was utilized by the crop during their growth period resulting in decrease in this pool towards the end of the crop. The treatment in which waste mica was incorporated was found to increase water-soluble, exchangeable and non-exchangeable K content in soils than control receiving no mica. This is obvious because of low available potassium (water-soluble and exchangeable K) in both the soils. It is reported that the effectiveness of silicate rock powder may increase when initial nutrient levels in soils are



low (Hinsinger et al. 1996; Sanz Scovino and Rowell 1988). Further, water-soluble K as well as exchangeable K increased significantly when mica was inoculated with Bacillus mucilaginosus strain in both the soils, which can be attributed to solubilization of nonexchangeable and structural K by the microbe through production of organic acids like oxalic and citric acids. The results of the present study corroborate the findings of other workers (Barker et al. 1998). There is much evidence showing that mineral K can be directly utilized by crops. Frequently, in continuous cropping systems, the removal of soil K by crops is incomparable to depletion in the soil available and the non-exchangeable K pool, which indicates that the release of mineral K contributes some proportion of K taken up by crops (Wang et al. 2000). The work by Coroneos et al. (1996) indicates that water soluble KCl is much more effective for immediate plant growth, but they stress that the non-exchangeable fraction of K from granite may be beneficial for plant growth in leaching environments.

Greater water-soluble and exchangeable K was observed in Hazaribag soil than Bhubaneswar in this study, which can be attributed to inherently higher K status in the former soil. It can also be due to higher solubilization of non-exchangeable pool to exchangeable pool of K. In general, the exchangeable K increased up to 90 days, then decreased drastically in Alfisol from Bhubaneswar, while in Hazaribag soil, the exchangeable K increased up to second cutting (60 d), then decreased till third cutting (90 d), and thereafter no significant changes were noticed till the end of the experiment (150 d), which was reflected well in higher K uptake because of efficient absorption of K by the crop.

With the progress of crop growth, non-exchangeable K in Alfisol from Bhubaneswar decreased gradually. It may be due to crop uptake of K from available pool, because as stressed by the crop demand some parts of non-exchangeable K might have been converted to exchangeable form. While in case of Alfisol from Hazaribag, the non-exchangeable K decreased gradually up to third cutting (90 d), because of either crop uptake or due to conversion into exchangeable K. The non-exchangeable K, thereafter, increased gradually till the end of fifth cutting (150 d) across the rate of K application and inoculation of bacterial strain, which can be attributed to fixation of water-soluble and exchangeable K to unavailable K *i.e.* fixed or structural K.

In general, the values of exchangeable and non-exchangeable K for  $M_0$  treatment were greater than the initial values of both the soils. This is because some amount of mineral K or structural K might have been converted first into non-exchangeable K and then exchangeable K during the crop growth. This is reflected well by the fact that the amount of K taken by plant is largely higher than the sum of the modification of exchangeable and non-exchangeable K in soils.

Non-exchangeable K reserves were in general high in Alfisol from Hazaribag, while Alfisol from Bhubaneswar maintained low levels. The abundance of non-exchangeable K in the former soil than latter broadly followed the same trend as their total K. Larger non-exchangeable K reserves in Alfisol from Hazaribag could be due to the presence of larger amounts of illite/mica in this soil. Lower nonexchangeable K in Alfisol from Bhubaneswar could be due to the presence of the major portion of total K in the form of K feldspars, which are highly resistant; thus, a major portion of total K is not dissolved in boiling 1 N HNO<sub>3</sub> (Subba Rao et al. 1988). Variation in amounts of total K and non-exchangeable K could be attributed to amounts of clay, silt, and sand and variations in mineralogical composition of the three particles (Sekhon et al. 1992).

Hinsinger et al. (1992, 1993) reported that dissolution of phlogopite (a trioctahedral mica) structure occurred in the rhizosphere of ryegrass and rape probably due to proton excretion by roots and vermiculitized after root induced release of interlayer K, which implies K in solid framework forms could be a source of K for plants. This verifies that some rocks containing K-bearing minerals have some potential for being used as K fertilizer, as demonstrated for granite by Coroneos et al. (1996) and Hinsinger et al. (1996). The release of non-exchangeable K from feldspar has also been attributed to exudation of acids, especially citric and oxalic acids, from roots (Song and Huang 1988; Wang et al. 2000; Moritsuka et al. 2004).

Soil 2:1 clay minerals play an important role for soil K availability. Indeed, several studies showed that the presence of such clay minerals, even in subsidiary quantities, increases effective soil K availability. It is reported that illitic 2:1 clay minerals behave as a renewable K reservoir (Barré et al. 2008). The filling or emptying of this reservoir could be followed efficiently through X-ray measurements of materials formed under field conditions. This reservoir is likely



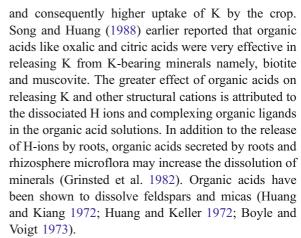
to have a key role for K cycle in soils. The most evident implications are that it could obviously supply short term K for plant needs and preserve long-term ecosystem productivity by reducing K leaching. The particular importance of such clay layers relies on their ability to adsorb and release K<sup>+</sup> ions from 2:1 clay mineral interlayer sites (Hinsinger 2002).

#### Correlation matrix

Significant correlation (P<0.01) between biomass yield and potassium uptake with water-soluble, exchangeable and non-exchangeable K indicate a dynamic relationship of different pools of K in both the soils. The results indicate that when water-soluble K is depleted in soil due to crop removal it is replenished by the exchangeable fraction of K, while non-exchangeable fraction of K is transformed into exchangeable K when the latter form is depleted in the soil solution to maintain the dynamic equilibrium of different pools of K. It may be due to crop uptake of K from available pool, because as stressed by the crop demand some parts of non-exchangeable K might have been converted to exchangeable form. Non-exchangeable K showed a significant positive correlation with exchangeable K in both the Alfisols, indicating the dynamic equilibrium between nonexchangeable and exchangeable K fraction. It is reported that complex interactions of soil mineralogical factors, and biological processes, determine how readily structural K in soil minerals, or fixed K may become available for crop uptake (Hinsinger and Jaillard 1993; Hinsinger et al. 1993; Wang et al. 2000). Benipal and Pasricha (2002) reported that cumulative K uptake was significantly related to both exchangeable and nonexchangeable K released from soils.

## K release kinetics

Release kinetics of K revealed that treatments receiving mica along with *Bacillus mucilaginosus* strain released significantly higher amounts of K over uninoculated mica throughout the growth of sudan grass grown under two Alfisols. This may be attributed to production of higher amount of organic acids in treatment receiving mica plus *Bacillus mucilaginosus*, which in turn solubilized more K from mica, resulting in higher release of K from mica



In Alfisol from Hazaribag, significant release of K from mica was noticed up to third cutting (90 days), thereafter decreased up to fourth cutting (120 days), which may be attributed to fixation of K. The release of K increased substantially till the end of last cutting (150 days).

## Structural changes of waste mica

Addition of mica in soils inoculated with Bacillus mucilaginosus indicates that dissolution of some portion of mica at the edges, as evident in the X-ray diffraction analyses, might have taken place by the action of bacterial strain during the growing period of sudan grass. It was expected as microorganism released some organic acids, particularly citric, oxalic, tartaric acid (Sheng et al. 2002) that helped in solubilization of waste mica in the present study. In a comparative study on the structural change of micaceous clay mineral, it was found that the integral width (full length at half maximum) of 10 Å peak decreased considerably due to dissolution of mica when mineral acid (1 N HNO<sub>3</sub>) was used for extraction (Datta and Sastry 1995), which supports the result obtained in the present study where integral width decreased possibly due to production of organic acids by intervention of Bacillus mucilaginosus. In the present study we observed the lower mica peak FWHM due to the effect of bacteria. While working with X-ray diffraction and the identification and analysis of clay minerals, Moore and Reynolds (1997) reported that higher the number of coherent diffracting clay layers smaller is the mica peak FWHM.



## Conclusion

We conclude that application of waste mica inoculated with potassium solubilizing microorganism (Bacillus mucilaginosus) has a significant effect on biomass yield, potassium uptake and recoveries by sudan grass grown under two Alfisols due to higher solubilization of K. Similarly, bacterial intervention of mica improves the water-soluble, exchangeable and non-exchangeable K pools in soils, thereby influences the K dynamics in soils into those pools which are relatively more available to plant. X-ray diffraction analysis indicates that dissolution of some portion of mica at the edges occurred due to the action of microbial culture during the growing period of sudan grass, resulting more releases of the structural K from mica. Thus, biointervention of waste mica could be an alternative and viable technology to solubilize insoluble K into soluble form and could be used efficiently as a source of Kfertilizer for sustaining crop production and maintaining soil potassium. Further studies are needed to see the effect of the new fertilization method tested is promising for big scale field application.

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