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ARTICLE

Distribution of Forms of Sulphur and Their Relationships with Soil Attributes in Tea Growing Soils under Different Agro-climatic Zones of Northeastern India

Ranjan Bera[1], Antara Seal[1], A.K. Chatterjee[2], Pradip Bhattacharyya[3]*

[1] Inhana Organic Research Foundation, 168 Jodhpur Park, Kolkata - 700068, India

[2] Department of Soil Science, Visva Bharati University, Santiniketan, West Bengal 731236, India

[3] Agricultural and Ecological Research Unit, Indian Statistical Institute, New Barganda, Giridih 815301, Jharkhand, India

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ABSTRACT

Distribution of different forms of sulphur (S) and carbon-nitrogen-sulphur relationships were studied in surface and subsurface soils of some tea growing areas of Northeastern India. The soils were strongly acidic in reaction (pH - 4.0 to 5.5), low to very high in organic carbon (4 to 54 g kg^{-1}), with cation exchange capacity (8.8 to 19.2 cmol(p^{+})kg^{-1}) and base saturation (50 to 77 %). Organic S mostly contributed to the total - sulphur (62 to 77 %) followed by Non sulphate S (28.8 to 37.2%) and sulphate S (0.7 to 1.4 %). Except sulphate S, other forms of sulphur showed significant positive correlation among themselves as well as with organic carbon and total nitrogen. The C:N, C:S, N:S and C:N:S ratio varied from 8.2 to 10.0, 6.18 to 71.57, 0.62 to 7.26 and 100:10.1:1.4 to 100:12.2:16.2 respectively. Wider C:N:S ratios in all the surface and sub-surface soils indicated that the major portion of nitrogen and sulphur in tea growing soils of Northeastern India is locked up in organic combination which might pose as a potential threat towards tea plantation if application of sulphur is continuously ignored .

1. Introduction

Tea (Camellia sinensis L.) is one of the most accepted beverages globally. Tea plants are perennial shrubs cultivated mainly in acidic soils of the subtropics. The Northeastern region of India produces the world's best quality tea and constitutes over 80 percent of total tea producing zones of India. The yields (1600 $kgha^{-1}$) are however, lower than that obtained in southern part of India (2100 $kgha^{-1}$) (Sharma and Sharma, 1992)[13]. One of the major reasons for the low yield is the low level of available - S in the soils of this region (Sharma and Sharma, 1992)[13]. It is estimated that a hectare of tea removes around 6 kg - S for yielding 30 ton of made tea (Gohain and Dutta, 1994)[7]. Due to heavy rainfall and low pH, leaching and volatization losses from these soils are very high. In addition, use of S free fertilizer further increases the negative balance of S (Reddy et al., 2001)[11] in these soils.

**Corresponding Author:*
Pradip Bhattacharyya;
Email: b_pradip@rediffmail.com

 DOI:

Soil S is present in both organic and inorganic forms. Most S is accumulated as organic S. The proportion of inorganic S to total S is usually less than 10% in surface soils (Freney, 1986; Nguyen and Goh, 1994)[6][10]. Although inorganic S constitutes a very small proportion of total soil S, it contains SO_4^{2-} ions which can be readily available for plant uptake (Yang et al., 2007)[19]. In contrast, virtually all organic S is unavailable to plants until it is mineralized to SO_4^{2-} (Freney, 1986)[6].

In this context, the knowledge of different forms of sulphur in soils together with their distribution and interrelationship with various soil attributes is essential for improving the sulphur nutrition of tea (Bandyopadhyay and Chattopadhyay, 2002)[1]. Therefore the present study was undertaken to evaluate the status and different fractions of sulphur and their interrelationship with various soil attributes in the tea growing soils of the Northeastern region of India.

2. Materials and Methods

2.1. Study Site

The agro-ecological zone is a homogenous land unit in terms of climate, with a length of growing period and soil-physiographic conditions. Based on the superimposition of these basic maps, viz. soil-physiography, bio-climate and length of growing period, agro-ecological sub regions (AESR) (Mandal et al., 1995)[9] have been generated. The study sites are situated at the Banaspaty (Cachar, 24°50′N and 92°51′E, AESR-15.5), Putharjhora (Jalpaiguri, 26°32′N and 88°46′E, AESR-15.3), and Samabeong Tea Estate (Darjeeling, 27°03′N and 88°18′E, AESR-16.2), India. Soils were collected from different garden cultivated with tea, which is the major land use in this region. The region is typically monsoonal, with three distinct seasons in a year: a warm and wet rainy season (June to September), a heavy winter (October to February), and a hot and relatively dry summer (March to May). The long-term (1983 to 2003) yearly average rainfall is 2845-3500 mm; the average monthly temperature ranges from 5-10°C (January) to 25-30°C (May). The average relative humidity reaches up to 97% during September, and shows the minimum (38%) in March.

2.2. Soil Collection and Analysis

During the summer season (May) of 2006, a total thirty soil samples each from surface (0 – 25 cm) and subsurface (25 – 50 cm) were collected from three major agro-ecological sub regions (AESR) in the tea growing zones of Northeastern India. Soil samples collected from three representative Tea Estates viz Banaspaty, Putharjhora and Samabeong Tea Estate were air dried, sieved and analyzed for different physicochemical properties following standards procedures (Black, 1965)[2]. Total and organic-S were determined as per methods outlined by Choudhary and Cornfield (1966)[5] and Bradsley and Lancaster (1960)[3] respectively. Sulphate-S was extracted with 0.15 percent $CaCl_2$ (Black, 1965)[2]. Sulphur in all the extracts was determined by the turbidimetric procedure of Chesnin and Yien (1951)[4]. The difference between organic-S plus sulphate-S contents and total-S was denoted as nonsulphate-S (Sharma et al., 2003)[15].

2.3. Statistical Analysis

Statistical analyses such as standard deviation, correlation and LSD analyses were carried out using SPSS 13.0.

3. Results and Discussion

3.1 Physico-chemical Properties

Fig. 1 shows the physico-chemical properties of the studied soils. The soils are light in texture and highly acidic (pH - 4.0 to 5.5) in reaction. The concentration of H^+ ions (2.6 to 4.5 cmol(p^+)kg^{-1}) in all the tea growing soils remained higher in comparison to the Al^{3+} ions (1.1 to 2.6 cmol(p^+) kg^{-1}). Cation exchange capacity was between 8.8 to 19.2 cmol (p+)/kg, where as base saturation of the soils was lower (50 to 77 %) than the other soils (Bandyopadhyay and Chattopadhyay, 2002)[1] because of heavy leaching due to high rainfall conditions.

Organic carbon and total N contents were higher in the soils of AESR 16.2 followed by that of AESR 15.3 and AESR 15.1. Higher organic carbon status in the soils of AESR16.2 might be attributed to lower temperature regime, which prevented faster microbial decomposition of organic matter (Saggar et al., 1998)[12]. Availability of N, P_2O_5 and K_2O revealed that the soils were moderately to well supply with nutrients (Sharma et al., 2003)[15].

3.2. Total Sulphur

Total S content was highest in Samabeong followed by Putharjhora, and Banaspati (Fig. 2). The S contents ranged from 512 to 1200 $mgkg^{-1}$. Total-S was higher in the surface horizons and decreased at the sub-surface levels in all the soils. This might be due to the fact that most of the sulphur present in these soils was organic in nature. Total-S status was reported to follow similar trend in the soils from different parts of India (Sharma et al., 2003, Bandyopadhyay and Chattopadhyay, 2002)[15][1]. Total-S showed significant and positive relation with organic carbon (r = 0.625**) and total–N (r = 0.626**) while negative correlation with clay (r = - 0.356**) (Table 1).

3.3. Organic Sulphur

Organic–S contents ranged from 222.9 to 940 mg kg^{-1}

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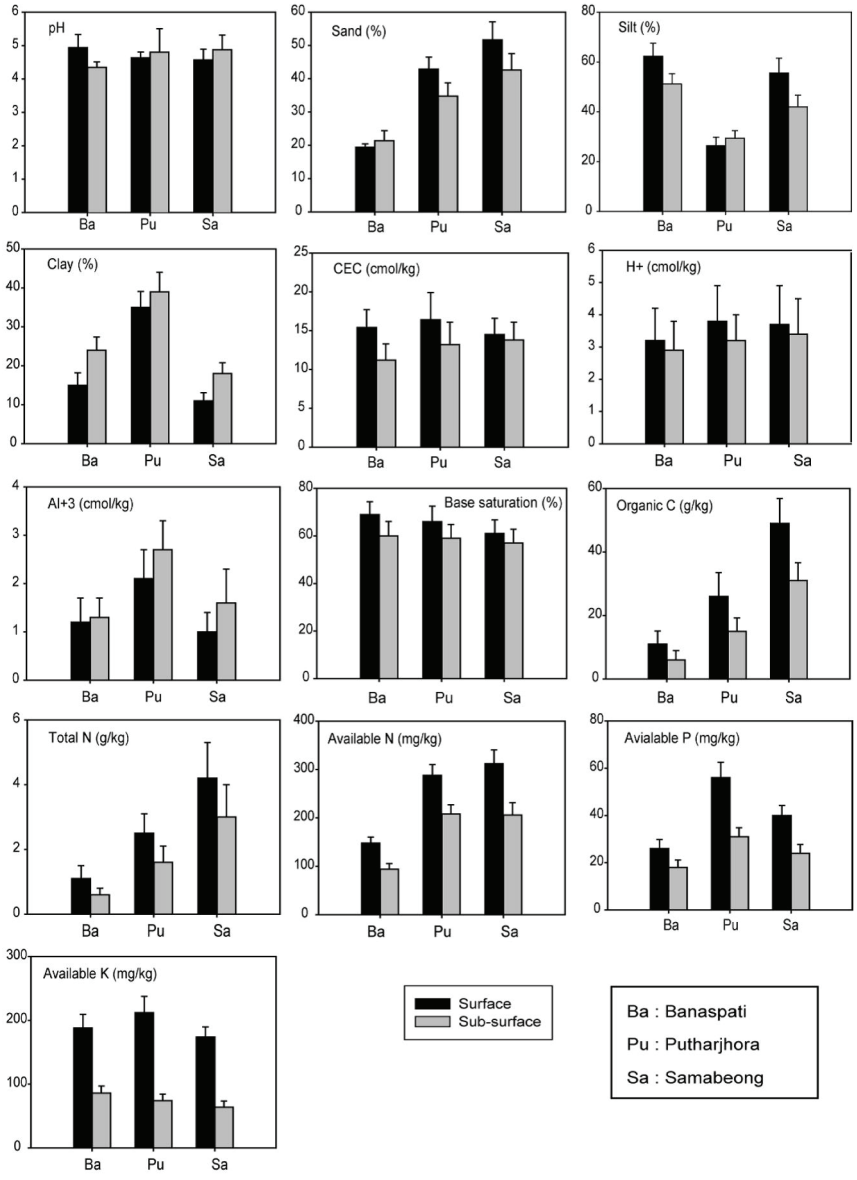

Figure 1. Physico-chemical properties of studied soils

and the value decreased in the sub-surface soils. The higher values of the organic – S in surface soils might be due to high content of organic matter (Srinivasarao et al., 2004)[16]. Organic–S constituted about 62 to 77 percent of total sulphur and this clearly indicated that major part of S in soils was locked up in organic matter and soil minerals, which might serve as a storehouse for tea nutrition following the mineralization process (Saggar et al., 1998)[12]. Kanwar and Takkar (1964)[8] found organic-S in the range of 46 to 91 percent of total–S in the tea growing soils of Kangra valley, India. In the soils of Samabeong and Putharjhora tea estate organic–S accounted for larger percent of total – S in comparison to the soils of Banaspaty tea estate, which may be attributed to the high organic carbon in these soils (Takkar, 1988)[17]. All the profiles showed the decreasing trend of organic S with the depth. The extent of decrease in organic S was in accordance with the decrease of organic carbon content of the soil. Intensive root activity, besides addition of considerable leaf litter during cropping, contributed to the higher organic carbon content in surface layers of the profiles, thus resulting in larger organic S in surface soils (Srinivasarao et al., 2004)[16]. Organic-S showed significant and positive correlation with Organic carbon (r = 0.629**) and total-N (r = 0.629**) and significant negative correlation with clay content (r = -0.389*) (Table 1). The positive relationships of organic-S with organic carbon and total-N suggested a simultaneous increase in the status of nitrogen and organic – S in these soils with increase in organic carbon content.

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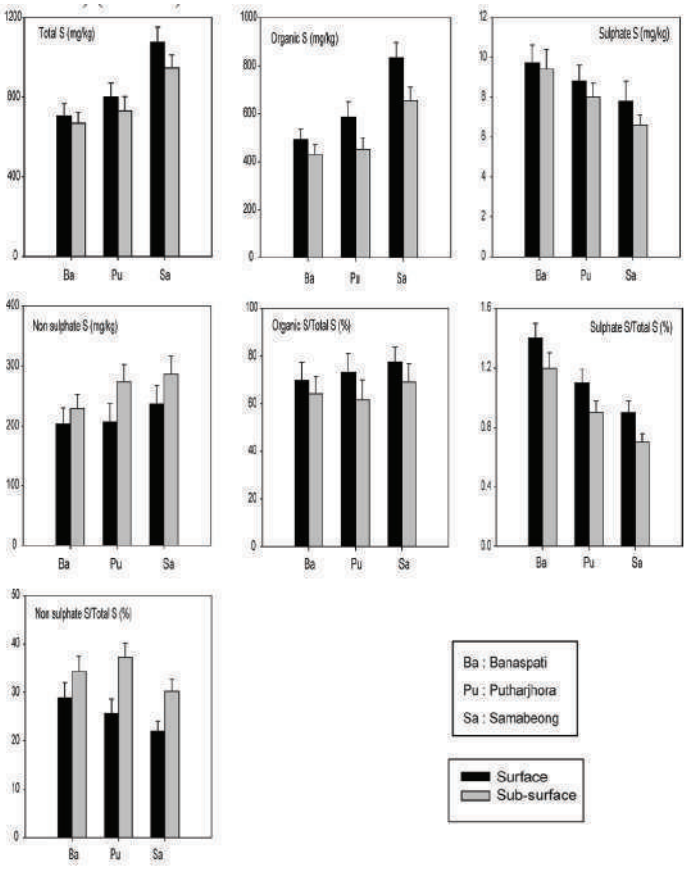

Figure 2. Distribution of different forms of sulphur in studied soils

Table 1. Correlation coefficient among soil attributes and forms of sulphur in studied soils

Parameter	pH	Org.C	Clay	CEC	Ex.Al^{3+}	Total-N	N:S	C:S	NS-S	SO_4-S	Org.-S
Total - S	0.191	0.625**	-0.356**	-0.158	-0.159	0.626**	0.251	0.264	0.492**	0.208	0.978**
Org - S	0.21	0.629**	-0.389**	-0.087	-0.155	0.629**	0.256	0.271	0.459**	0.352*	-
SO_4 - S	0.38**	-0.313*	0.132	-0.033	-0.369**	-0.319*	-0.531**	-0.525**	-0.022	-	-
NS - S	-0.018	0.468**	-0.017	-0.362**	-0.066	0.360*	0.106	0.107	-	-	-
C:S	0.09	0.900**	-0.056	0.503**	0.556**	0.903**	0.999**	-	-	-	-
N:S	0.073	0.889**	-0.05	0.499**	0.555**	0.894**	-	-	-	-	-

NS-S : Non sulphate sulphur ; *Significant at 5% level; **Significant at 1% level.

3.4. Sulphate Sulphur

Sulphate–S content in the soils varied from 4.0 to 10.9 and constituted merely 0.7 to 1.4 per cent of total sulphur. Available S content was found to be higher in surface layers and decreased with the depth in most of the profiles. Larger available S status in surface layers could be attributed to higher organic carbon content in those layers. Thus a sizeable chunk of total - S remained as unavailable form. This was in close agreement with the findings of Yang et al (2007)[19]. Considering the critical value of 10 ppm for sulphate-S (Kanwar and Takkar, 1964 and Banyopadhyay and Chattopadhyay, 2002)[8][1] in soils, most of the soils are deficient in available sulphur content and thus may contribute significantly to the lower productivity of tea with respect to southern part of India (Takkar, 1988) [17]. A positive and significant correlation (r = 0.380*) was found between pH and sulphate–S which indicates that availability of sulphate-S increases with increase in soil

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pH (Takkar, 1988)[17].

3.5. Non Sulphate Sulphur

Non sulphate-S content in the soils ranged from 189.7 to 300 mgkg^{-1} with a mean accounted 28.8 to 37.2 percent of total S. Non sulphate-S content in the soils increased in sub-surface soils indicating comparatively higher presence and highly reactive insoluble compounds of Fe and Al as well as low content of organic matter in these soils (Saggar et al., 1998)[12]. Non sulphate-S showed significant and positive correlation with organic carbon(r = 0.468**) and total-N (r = 0.360*) but a significant negative correlation was found with CEC (r = -0.362**) (Table 1).

3.6. Inter-relationship amongst Different forms of Sulphur

Since sulphur transformation and its availability in soils is dependent on its various forms, inter-relationship among them may be indicated from highly significant correlation of total-S with organic-S, sulphate-S and non sulphate-S. The existence of similar relationship was earlier reported by Srinivasarao et al. (2004)[16]. Organic–S showed a significant positive correlation with sulphate-S (r = 0.352*) and non sulphate-S (r = 0.459**) suggesting high linkage of these forms with organic fraction of the soils (Table 1).

3.7 Carbon – Nitrogen – Sulphur Interrelationships

The knowledge on the C:N, C:S, N:S and C:N:S ratios is helpful in understanding the mineralization, immobilization, stability and instability of the most important organically bound nutrients in soils such as nitrogen and sulphur (Saggar et al., 1998)[12]. The wider ratio (greater than the threshold values) indicates that a particular nutrient would exist in an immobilized form culminating into less mineralization which further indicates that it would be more stable in a given soil in its organic form (Sharma et al., 2003)[15]. However, reverse would be true if the ratios are narrower i.e. a narrow ratio would indicate that the organically bound nutrient is fairly amenable to mineralization and would likely exist to a greater extent, in an inorganic form (Sharma et al., 2003)[15].

The study of the relationship between S and other soil constitutes such as C and N showed that the C:N, C:S, N:S and C:N:S ratios of tea growing soils in surface and sub-surface layers were quite variable (Table 2). The C:N ratio ranged from 8.2: 1 to 10: 1 in surface layer and sub-surface layer with a grand mean of 9.5: 1 which resembles the values obtained by Sharma et al. (2003)[15] working with some tea growing soils of India. In general, higher values of C:N ratios were observed in lower depth, which might be due to anaerobic condition and low mineralization in lower depths as compared to upper depths (Sharma et al., 2000)[14]. In addition, nitrogen abundance may increase in topsoil during degradation because mineralized nitrogen is retained within microbial biomass (Saggar et al., 1998)[12], which is generally lowered with soil depth.

Likewise, the C:S ratio ranged from 12.00: 1 - 51.67: 1 in surface layers and 6.18: 1 – 71.57: 1 in the sub-surface layers. Similarly the N:S ratio in surface and sub-surface soil ranged from 1.30: 1 – 5.33: 1 and 0.62: 1 – 7.26: 1 respectively. The C:N:S ratio of these soils, varied widely from 100: 10.1: 1.4 to 100: 12.2: 16.2 with a mean value of 100: 10.55: 5.07. The variations in these soils may be attributed to the variation in agro-ecological zone which could be explained on the variety of factors such as type of soil, status and kind of organic matter vis-à-vis climatic

Table 2. Relationships among organic carbon, total nitrogen and sulphur in the studied soils

Soil depth (cm)	C : N	C : S	N : S	C : N : S
Banaspaty T.E. (Cacher)				
Surface (0 –25)	8.2 – 10.0	12.73 – 16.90	1.43 – 1.69	100:10.0:5.9 – 100:12.2:7.9
Subsurface (25 –50)	8.2 – 10.0	6.18 – 14.66	0.62 – 1.61	100:10.0:6.8 – 100:12.2:16.2
Putharjhora T.E. (Jalpaiguri)				
Surface (0 –25)	9.0 – 9.7	12.00 – 51.67	1.30 – 5.33	100:10.3:1.9 – 100:11.1:8.3
Subsurface (25 –50)	9.3 – 10.0	13.96 – 53.23	1.50 – 5.70	100:10.0:1.9 – 100:10.8:7.2
Samabeong T.E. (Darjeeling)				
Surface (0 –25)	9.5 – 9.8	38.14 – 51.82	3.90 – 5.27	100:10.2:1.9 – 100:10.6:2.7
Subsurface (25 –50)	9.4 – 9.9	36.76 – 71.57	3.92 – 7.26	100:10.1:1.4 – 100:10.7:2.7
Mean	9.50	29.83	3.12	100:10.55:5.07
LSD (p=0.05)	0.39	1.46	0.13	-

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situation-most importantly rainfall, temperature and elevation (Mondal et al., 1995)[9].

Further more a highly significant and positive correlation between C:N and C:S indicates that N and S are the important constituents of soil organic matter and that accumulation of one is accompanied by the simultaneous accumulation of all other components. This suggests that an increase in N content in soil also results in an increase in the S content (Takkar, 1988)[17]. In many of the previous studies documented by different workers (Sharma et al., 2003)[15], the accepted norms to categorise the soils into narrow and wider ratio suggests a C:N:S ratio < 100: 10: 1 as a narrow ratio and vice-versa. Based on these criteria, it was observed that all of the studied tea soils had wider C:N:S ratios in surface and sub-surface layers. Hence considering the magnitude of the ratio obtained it may be concluded that the major portion of nitrogen and sulphur in tea growing soils of Northeastern India is locked up in organic combination. This may serve as a store-house for tea nutrition following mineralization, for which there is a great possibility in a given tea soil ecosystem as this system operates probably through nutrient recycling similar to forest system (Vannier and Guillet, 1994; Sharma et al., 2003)[18][15].

4. Conclusion

The findings of the study revealed that the majority of soil sulphur exists as organic form with a wider C:N:S ratio. This indicates that the availability of sulphate - S in soil might be a major cause of concern with respect to balance nutrition in tea plantations. So long habits of sulphur free fertilization should be abandoned with emphasis on split doses of sulphur fertilization on the basis of soil fertility as well as soil adsorption characteristics.

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ARTICLE

System of Rice Intensification Verses Conventional Rice System: Off-farm Field Studies

Ravi Chandra Sharma[1], Nobuhiko Fuwa[2] and Pabitra Banik[3]*

[1] R esearch and Development Division, Rashtriya Chemicals and Fertilizer Limited, Mumbai 400 074; Email: ravi.iari@gmail.com

[2] University of Tokyo, Japan; Email: fuwa@pp.u-tokyo.ac.jp

[3] Agricultural and Ecological Research Unit, Indian Statistical Institute, Kolkata 700103; Email: pbanik@isical.ac.in

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ABSTRACT

With inevitable growth of demand for human and industrial needs, water available for agriculture will become scarcer in the future. India is a highly water-stressed country. Hence, India needs to invest in improving its water productivity, and any capacity to produce more rice with less water. System of Rice Intensification (SRI) has attracted much attention in increasing rice yield per unit area. For this study, fifteen farmers were selected those were practicing SRI technology by themselves during the Boro-cultivation season (January-April). The study was continued for three consecutive years 2012 to 14 on the same fields. In addition to the SRI plots, a similar size of non-SRI plot was maintained in conventional cultivation for comparison purpose. On an average, the non-SRI ight increased by 12%, number of tillers per square meter by 85%, number of reproductive tillers per hill by 286%, weight of panicle per hill by 139%, number of seeds per panicle by 41% and test weight by 26% due to SRI practice over the non-SRI practice. Average increment in straw and grain yield due to SRI over the non-SRI is 70% and 59% respectively. The physico-chemical and biological properties of soil improved due to SRI practice.

1. Introduction

Rice is life for millions of people in the world, particularly in developing countries. It is the main cereal for the majority of population in India. The global annual production of rice is 600-800 million tons (FAO, 2004)[3]. India has the largest area under rice in the world-about 44 million hectares (ha)—but its productivity is the way behind a dozen other countries. In contrast, China, the biggest producer of rice in the world, churns out 193 million tonnes of paddy on just 29.2 million ha, notching up yields of 6.61 tonnes per ha compared with 3.37 by India. Given the fact that there is negligible scope for area expansion, the growth rate of rice production must not only be sustained but even accelerated in order to meet the growing demand. Increasingly, water is becoming a single most constraint to produce more rice to meet increasing demand (Kunimitsu, 2006)[8].

**Corresponding Author:*
Pabitra Banik.
Agricultural and Ecological Research Unit, Indian Statistical Institute, Kolkata 700103*;*
Email: pbanik@isical.ac.in

 DOI:

There is a crisis in rice production-both for the farmer, battling unprecedented changes in weather and escalating costs of cultivation, and the government, which needs to ramp up rice production by two million tonnes annually to ensure the nation's food security. In spite of providing assured irrigation, use of pest-resistant, high-yielding varieties, and high inputs of fertilizers and pesticides, rice yields in India are plateauing. With inevitable growth of demand for human and industrial needs, water available for agriculture will become scarcer in the future (Kunimitsu, 2006)[8]. India is a highly water-stressed country. Hence, India needs to invest in improving its water productivity, and any capacity to produce more rice with less water (Shobarani et al, 2010; Satyanarayana et al. 2007)[16][13]. This will guide to sustainable water and food security. Moreover, every kilogram of rice requires 3000-5000 liters of water, making it an ecologically unsound crop; there is a question mark over the issue of increasing rice production. More than 70 percent of the country's ground and surface water is being used for agriculture, and out of this, 70 percent is allocated to rice cultivation.

Recently a new approach, widely known as System of Rice Intensification (SRI), has attracted much attention in the agricultural scientific community as well as among some farmers because of its report (by some) success in increasing rice yield per unit area without investing more for its inputs (with the possible exception of labor). SRI was conceptualized by Henri de Laularié, a French missionary priest, in Madagascar in the early 1980s as a complementary suite of rice management techniques. The SRI is (seen by some as) one of the most promising agricultural innovations that are claimed to be both more sustainable and more productive than conventional rice cultivation (Satyanarayana et al. 2007; Kunimitsu, 2006)[13][8]. SRI is proposed as more accessible to small landholders (Stoop et al. 2002)[17] and more favorable for the environment than is conventional transplanting, with its continuous flooding and heavy reliance on inorganic fertilization and agrochemical crop protection (Uphoff 2003)[22].

It has been claimed that SRI can increase rice yield substantially (Kabir and Uphoff, 2007; Lin and Zhu 2011)[6] whereas some agricultural scientists noted that it reduced input requirements such as seeds and water. It has been claimed by its proponents that using SRI technology rice yield can be increased up to 15 to 20 tons ha^{-1} (Uphoff and Randriamiharisoa, 2002)[22]. The relative scarcity of studies based on farmers' plots in a variety of conditions raises the question of the replicability of higher yields due to SRI practices, as obtained from(at least some) controlled experiments, under different conditions and by ordinary farmers.

This paper addresses those lacunas in the existing literature. First, it focuses on soil dynamics as a possible mechanism linking SRI practices and higher yields. Our data set contains information on chemical and biological compositions in the soil under SRI and non-SRI practices. Secondly, we set up farmers' trials in 15 villages with resident farmers operating SRI practices (as well as conventional/non-SRI) practices on their own farms, with technical assistance from the research team. While the use of farmers' plots, rather than of experimental stations, possibly introduces data Errors that may compromise, to some extent, scientific rigor in establishing the relationship between soil dynamics and yields, we believe that the stability of our empirical findings across a relatively large number of farmers' plots among different villages could provide a high level of confidence in the potential replicability of our SRI results in the hands of ordinary farmers who is rarely found in the existing literature.

2. Materials and Methods

2.1 Study Site

Table 1. Name of village with their geographical position

Name of the Village	Latitude	Longitude
Alampur	22°23'09"	87°35'07"
Alidadpur	22°21'31"	87°30'59"
Amodpur	22°23'59"	87°31'06"
Balabhadrapur	22°30'29"	87°35'59"
Banasda	22°22'06"	87°37'00"
Brindabanpur	22°25'05"	87°33'11"
Chaltageriya	22°25'48"	87°32'53"
Dingal	22°20'58"	87°38'09"
Galimpur	22°21'39"	87°30'31"
Kazichak	22°24'22"	87°29'31"
Khasbazar	22°23'27"	87°37'51"
Madhabpur	22°23'17"	87°32'44"
Nandeswar	22°30'06"	87°35'33"
Naraharipur	22°24'23"	87°31'04"
Paikpari	22°24'06"	87°38'40"

2.2 Crop Management Condition

Those farmers were selected for the studies that were practicing SRI technology by themselves own their farms

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during the Boro cultivation season (January-April). The study was continued for three consecutive years 2012-14 on the same fields. Farmers were provided necessary inputs, including seeds and fertilizer (but no labor) by the research team. In addition, technical know-how of SRI cultivation was also provided through regular visits and/or personal communication by the research team. In addition to the SRI plots, a similar size of the non-SRI plot was maintained for conventional cultivation in comparison purposes. Farmers were provided a sheet to keep the record on input uses as well as production throughout the crop growing period. Soil samples from each plot were collected before the start of the experiment as well as after harvesting of the crop.

SRI is an acronym for System of Rice Intensification, a new technique to grow rice more efficiently using much less water. In SRI, 8-12-day seedlings instead of the normal three-four-week-old seedlings are transplanted at wider spacing (25 cm x 25 cm). Only one seedling is planted per hill. Water is used sparingly to keep the soil moist (alternate wetting and drying) but not continuously flooded. Five times weeding was carried out mechanically through a rotary weeder (small hand-driven machine) at 10-day intervals, but instead of throwing out the weeds these are pushed through the soil for aeration and providing organic matter. Use of farmyard manure is encouraged because SRI cultivation responds better to organic fertilizer than chemical fertilizers. Seedlings are raised in unflooded nurseries, not planted densely and have to be well supplied with organic matter. There is an option of direct-seeding, but transplanting is common. Two cm irrigation water was applied after hairline cracks appeared in the soil surface up to panicle initiation (PI); then after PI, irrigation was given 1 day-after the disappearance of pond water. Inter-cultivation was done five times with a rotary weeder at a 10-day intervals. The same recommended fertilizer was applied as with conventional practice.

In conventional practice 21-24-day-old seedlings with the above plant density; plots were irrigated to a 5 cm depth 1 day-after the disappearance of pond water; hand weeding was done three times; recommended fertilizers were applied: 120 kg ha^{-1} N, 60 kg ha^{-1} P_2O_5, 60 kg ha^{-1} K_2O. The P was applied basally, while N was applied in four splits: 40% basal and 20% each at active tillering, panicle initiation and first flowering stages. The K was applied in three splits: 50% basal and 25% each at tillering and panicle initiation stages.

2.3 Soil Analysis

Soil samples from 0-20 cm depth were collected scientifically from each plot. These samples were air-dried under shed and sieved through 2 mm mesh sieve. Fresh soil samples were used for estimating of biological parameters, and results were expressed on the moisture-free basis. The moisture content was determined by the gravimetric method. Population densities of total bacteria and fungus were enumerated by using serial dilution plate technique. Data were log transformed and expressed as colony-forming units (CFU) log10 g^{-1} dry soil. Soil reaction, conductivity, organic carbon, available nitrogen, phosphorus and potassium are estimated by the standard methods advocated by Jackson, 1973.

Total plants in an area of 5 m× 5 m (25 m^2) for each replicate were harvested (excluding border rows) for determining of rice grain yield per unit area, and reported grain yield was adjusted to 14.5% seed moisture content. The Harvest Index was calculated by dividing the dry grain yield into the total weight of dry matter of aboveground parts. Plant height, effective tiller number, panicle length, grain weight, and dry matter were determined from the crop harvested from a representative square meter area from each replication.

4. Statistical Analysis

All the data were statistically analyzed using analysis of variance (ANOVA) as applied to a completely randomized block design (Gomez and Gomez 1984)[4]. The significance of the treatment effect was determined using F-Tests; and to determine the significance of the difference between the means of the treatments, least significant difference (LSD) was calculated at the 5% probability level.

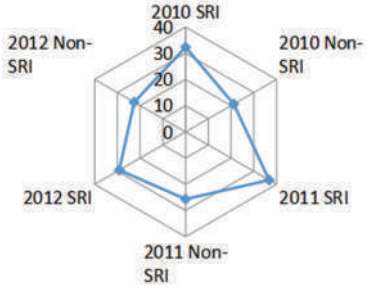

Figure 1. (a) Soil reaction (pH)

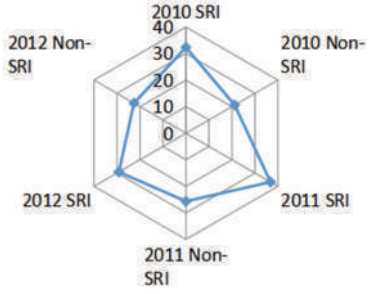

Figure 1. (b) Soil organic carbon (%)

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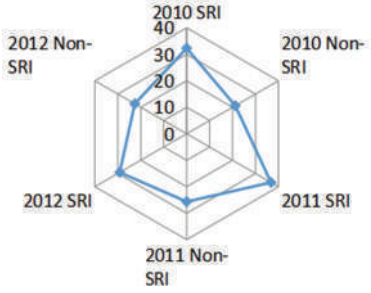

Figure 1. (c) Available Nitrogen (kg ha^{-1})

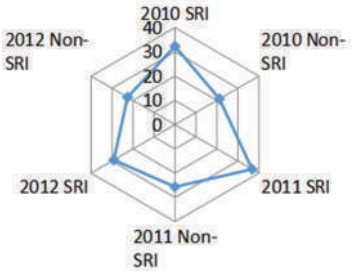

Figure 1. (d) Available Phosphorus (kg ha^{-1})

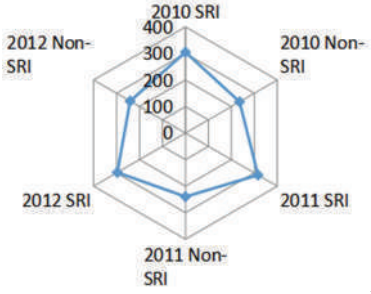

Figure 1. (e) Available Potassium (kg ha^{-1})

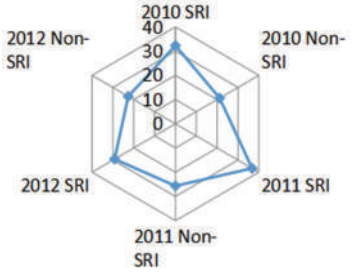

Figure 1. (f) Soil Fungal Population (CFU 10^{-4}/ml)

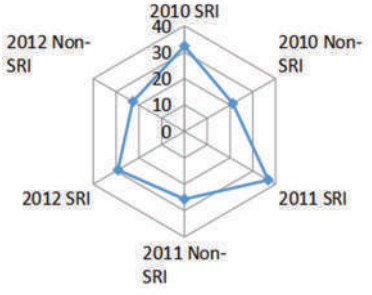

Figure 1. (g) Soil Bacterial Population (CFU 10^{-6}/ml)

Figure 1. Soil properties as influenced by SRI and Non-SRI practices

5. Results and Discussion

5.1 Rice growth and Yield Component

Rice growth and yield component such as plant height, number of tillers, panicle length and weight, number of seeds per panicle and test weight were recorded for three seasons. They were influenced remarkably under different crop management conditions (Table 2). Variation in the above parameters over the years was almost constant, but following the same trend. On an average, plant height increased by 12%, number of tillers per square meter by 85%, number of reproductive tillers per hill by 286%, weight of panicle per hill by 139%, number of seeds per panicle by 41% and test weight by 26% due to SRI practice over the non-SRI practice.

Tillering ability (panicle bearing tillers) in rice has a close relationship to the number of phyllochrons completed before entering the reproductive stage (Stoop et al. 2002; Thakur et al. 2009)[17][20]. In the SRI method of rice cultivation, individual plants with their more favorable growing conditions have shorter phyllochrons, which results in their having more productive tillers and larger root systems (Katayama 1951; Thakur et al. 2009)[20]. Rice plants grown under standing water encounter hypoxic (anoxic) soil conditions, and about three-fourths of their roots are degenerated by the flowering stage (Kar et al. 1974)[7]. Further, transplanting of young seedlings, as in SRI methods, has the tendency to improve their root characteristics such as root length, root density and root weight compared with older seedlings, as used in non-SRI (Mishra and Salokhe 2008)[9]. Other studies have also reported that SRI plants have deeper root systems and larger roots compared to those conventionally grown in flooded rice systems (Satyanarayana et al. 2007; Tao et al. 2002)[13][18]. Better root

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Table 2. Change in growth and yield parameters of rice as influenced by the crop management conditions

Parameter	2012			2013			2014			Mean		
	SRI	non-SRI	Percent Change	SRI	non-SRI	Percent Change	SRI	non-SRI	Per cent Change	SRI	non-SRI	Percent Change
Plant height (cm)	85.40	75.49	13.13	100.06	86.49	15.69	99.86	92.76	7.65	95.11	84.91	12.01
Number of tillers m^{-2}	1109.78	636.38	74.39	985.31	559.73	76.03	799.21	364.98	118.98	964.77	520.36	85.40
Number of tillers per hill	61.09	11.89	413.83	49.77	16.24	206.39	37.81	12.17	210.76	49.56	13.43	269.02
Reproductive tillers per hill	50.64	9.41	438.30	43.90	14.24	208.16	35.48	10.02	253.92	43.34	11.22	286.27
Panicle length (cm)	21.12	18.30	15.42	24.28	18.67	30.08	24.60	18.43	33.46	23.33	18.47	26.31
Panicle weight /hill	126.62	64.31	96.88	91.40	49.42	84.93	146.38	38.59	279.34	121.47	50.77	139.26
Seeds/ panicle	162.20	121.13	33.90	208.42	148.78	40.09	230.45	156.00	47.73	200.36	141.97	41.13
Grain weight (g)	23.64	18.60	27.12	24.83	19.82	25.29	21.71	17.11	26.91	23.39	18.51	26.36
Grain yield (kg/ha)	7148.13	3327.34	114.83	7219.37	5380.00	34.19	8619.79	5793.33	48.79	7662.43	4833.56	58.53
Straw yield (kg/ha)	13086	5524.19	136.89	8640.0	6311.02	36.90	12303	8153.28	50.90	11343	6662.83	70.24

development in the SRI system might have increased all growth and yield parameters (Randriamiharisoa & Uphoff, 2002)[11].

5.2 Rice Grain and Straw Yield

Rice grain and straw yield were significantly affected by soil conditions. In all three-year straw and grain yield was higher in SRI (Table 3). Average increment in straw and grain yield due to SRI over the non-SRI is 70% and 59% respectively. Plants grown in SRI had more open architecture, with the wide spread of tillers, covering more ground area, and more erect the leaves (data not shown), which avoided mutual shading of leaves (Seshu & Cady, 1984; Senthilkumar et al, 2008)[15][14]. With higher light interception, this would lead to more photosynthesis and higher grain yield in SRI compared to non-SRI. A number of previously published reports on SRI have shown enhancement in rice yield with these methods (Namara et al. 2008; Satyanarayana et al. 2007; Sato and Uphoff 2007; Thakur et al. 2009)[10][13][12][20]. The higher number of days taken to maturity in SRI practice was directly correlated to higher rice yield over the Non-SRI practice (Table 3).

5.3 Soil Reaction and Organic Carbon

SRI had a positive impact on soil reaction and organic matter content. Before starting the experiments, soil reaction of all the fields was acidic. The soil became more acidic in non-SRI practices, whereas some positive corrections were observed in SRI practices (Table 4 & 8 & Figure 1). Similarly, organic carbon was built up in SRI practices because of higher root volume and biomass (Carpenter-Boggs et al, 2000; Chapagain et al, 2010)[1][2].

5.4 Residual Soil Fertility

Residual soil fertility was measured in terms of available nitrogen, phosphorus (P) and potassium (K). SRI practices had a positive impact on residual soil fertility owing to higher microbial and biological activity guides to better soil fertility (Shobarani et al, 2010; Thakur et al, 2010; Thiyagarajan et al, 2002)[16][19][21]. However, reduction in soil fertility was observed in non-SRI practices (Table 5,6 & 8 & Figure 1).

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Table 3. Number of days taken to maturity by the rice varieties under different crop management condition

Name of village	Crop management condition	Variety			Days to maturity		
		2012	2013	2014	2012	2013	2014
Alampur	SRI	IET-4786	IET-1010	Shyamasri	91	111	98
	Non-SRI	IET-4786	IET-1010	Shyamasri	88	--	111
Alidadpur	SRI	Saru lalat	IET-4786	Saru lalat	100	99	97
	Non-SRI	Saru lalat	IET-4786	Saru lalat	86	88	112
Amodpur	SRI	IET-4786	IET-4786	IET-4786	87	110	98
	Non-SRI	IET-4786	IET-4786	IET-4786	61	115	98
Balabhadrapur	Non-SRI	IET-4786	IET-4786	IET-4786	49	94	94
	SRI	IET-4786	IET-4786	IET-4786	93	118	112
Bansda	SRI	Sankar	Sankar	Shyamasri	91	110	102
	Non-SRI	Sankar	Sankar	Shyamasri	83	85	110
Brindabanpur	SRI	Supar sankar	IET-1010	Shyamasri	68	122	105
	Non-SRI	Supar sankar	IET-1010	Shyamasri	110	108	110
Chaltagerya	SRI	IET-4786	IET-4786	5152	92	101	112
	Non-SRI	IET-4786	IET-4786	5152	83	83	109
Dingal	SRI	IET-4786	IET-4786	IET-4786	84	84	85
	Non-SRI	IET-4786	IET-4786	IET-4786	97	107	106
Galimpur	SRI	IET-4786	IET-4786	Natia	96	102	109
	Non-SRI	IET-4786	IET-4786	Natia	87	89	92
Kazi Chak	SRI	IET-1010	Ananya	Ananya	86	81	89
	Non-SRI	IET-1010	Ananya	Ananya	72	117	94
Khasbazar	SRI	IET-4786	IET-4786	IET-4786	104	113	88
	Non-SRI	IET-4786	IET-4786	IET-4786	44	123	111
Madhabpur	SRI	IET-4786	IET-4786	IET-4786	96	95	101
	Non-SRI	IET-4786	IET-4786	IET-4786	85	88	96
Nandeswar	SRI	IET-4786	IET-4786	IET-4786	95	120	113
	Non-SRI	IET-4786	IET-4786	IET-4786	71	96	91
Naraharipur	SRI	IET-4786	IET-4786	IET-4786	79	112	106
	Non-SRI	IET-4786	IET-4786	IET-4786	--	113	91
Paiakpari	SRI	IET-4786	IET-4786	IET-4786	105	112	105
	Non-SRI	IET-4786	IET-4786	IET-4786	79	100	94

DOI:

Table 4. Soil reaction and organic carbon as influenced by the crop management conditions

Name of villages	Soil reaction								Soil organic carbon (%)							
	2012		2013		2014		Mean		2012		2013		2014		Mean	
	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI
Alampur	6.35	6.71	6.36	6.63	6.45	6.73	6.39	6.69	0.97	1.19	1.01	1.01	0.92	0.98	0.97	1.06
Alidadpur	6.42	6.88	6.11	6.91	5.93	6.95	6.15	6.91	1.01	1.31	1.01	1.29	0.99	1.21	1.00	1.27
Amodpur	6.40	6.55	6.48	6.71	6.38	6.69	6.42	6.65	1.01	1.37	1.12	1.23	0.98	1.24	1.04	1.28
Balabhadrapur	6.02	6.45	6.02	6.95	6.12	6.68	6.05	6.69	1.01	1.12	0.9	1.03	0.88	1.19	0.93	1.11
Bansda	5.75	6.52	5.97	6.12	5.9	6.84	5.87	6.49	0.79	0.95	0.76	0.86	0.79	0.91	0.78	0.91
Brindabanpur	5.65	6.59	5.81	6.72	5.62	6.82	5.69	6.71	1.18	1.47	0.94	1.51	1.01	1.43	1.04	1.47
Chaltageriya	5.21	5.73	5.55	5.98	5.94	6.02	5.57	5.91	0.98	1.23	0.96	1.19	0.94	1.11	0.96	1.18
Dingal	6.69	6.78	6.72	7.08	6.75	7.03	6.72	6.96	0.91	0.98	0.86	0.99	0.87	1.01	0.88	0.99
Galimpur	5.50	6.18	5.81	5.98	5.32	6.01	5.54	6.06	0.91	0.91	0.71	0.89	0.84	0.88	0.82	0.89
Kajichak	5.35	5.75	5.64	5.97	5.54	6.03	5.51	5.92	0.97	0.98	0.87	1.06	0.84	1.02	0.89	1.02
Khasbazar	4.75	6.55	4.94	6.32	5.59	6.64	5.09	6.50	0.98	1.04	0.89	1.06	0.91	0.99	0.93	1.03
Madhabpur	5.75	6.75	5.84	6.9	5.54	6.72	5.71	6.79	0.95	1.10	0.94	1.26	0.89	1.32	0.93	1.23
Nandeswar	5.40	6.35	5.89	6.51	5.82	6.34	5.70	6.40	0.79	0.98	0.87	0.95	0.82	1.01	0.83	0.98
Naraharipur	6.51	7.01	6.74	7.12	6.84	7.24	6.70	7.12	0.89	1.16	0.96	0.97	0.9	1.09	0.92	1.07
Paikpari	6.50	6.71	6.78	6.64	6.68	6.76	6.65	6.70	0.93	0.88	0.89	0.95	0.87	0.91	0.90	0.91
Mean	5.88	6.50	6.04	6.57	6.03	6.63	5.99	6.57	0.95	1.11	0.91	1.08	0.90	1.09	0.92	1.09
SEm(+/-)	0.020		0.019		0.018		0.017		0.006		0.007		0.006		0.005	
CD (0.05%)	0.043		0.040		0.038		0.036		0.012		0.014		0.012		0.010	

Table 5(a). Available nitrogen and phosphorus as influenced by the crop management conditions

Name of villages	Available Nitrogen (kg ha^{-1})							
	2012		2013		2014		Mean	
	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI
Alampur	251.11	278.75	278.51	294.78	306.07	316.98	278.56	296.84
Alidadpur	250.21	275.97	266.9	285.16	280.99	316.11	266.03	292.41
Amodpur	200.7	246.38	188.51	269.69	210.74	263.42	199.98	259.83
Balabhadrapur	197.25	225.79	136.87	196.32	316.11	386.36	216.74	269.49
Bansda	207.98	266.61	328.33	343.42	280.99	316.11	272.43	308.71
Brindabanpur	267.39	318.34	244.92	301.05	245.86	333.67	252.72	317.69
Chaltageriya	300.76	327.74	267.98	308.41	351.23	386.36	306.66	340.84
Dingal	268.93	281.47	316.78	343.5	289.34	323.48	291.68	316.15
Galimpur	216.38	235.2	218.15	256.97	263.42	280.99	232.65	257.72
Kajichak	201.83	241.47	200.52	284.6	234.76	258.78	212.37	261.62
Khasbazar	319.87	322.83	257.85	325.13	263.42	298.55	280.38	315.50
Madhabpur	284.8	297.92	225.79	296.97	280.99	316.11	263.86	303.67
Nandeswar	201.25	227.42	225.79	275.26	298.55	386.36	241.86	296.35
Naraharipur	294.43	301.66	281.88	294.43	368.79	333.67	315.03	309.92
Paikpari	144.26	172.48	184.23	206.14	280.99	351.23	203.16	243.28
Mean	240.48	268.00	241.53	285.46	284.82	324.55	255.61	292.67
SEm(+/-)	0.758		1.180		1.489		0.852	
CD (0.05%)	1.551		2.447		3.088		1.767	

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Table 5(b). Available phosphorus as influenced by the crop management conditions

Name of villages	Available Phosphorus (kg ha^{-1})							
	2012		2013		2014		Mean	
	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI
Alampur	49.18	73.92	61.18	90.89	59.35	62.27	56.57	75.69
Alidadpur	62.84	72.19	55.35	80.25	79.84	83.3	66.01	78.58
Amodpur	60.27	87.46	60.15	95.65	70.72	91.28	63.71	91.46
Balabhadrapur	51.39	66.43	67.53	74.54	60.34	75.8	59.75	72.26
Bansda	41.83	62.08	47.26	66.2	50.21	55.69	46.43	61.32
Brindabanpur	59.00	81.47	84.85	97.55	65.38	80.25	69.74	86.42
Chaltageriya	64.57	69.86	66.45	88.22	70.35	82.41	67.12	80.16
Dingal	70.10	80.10	80.76	94.22	73.32	86.97	74.73	87.10
Galimpur	56.64	65.28	73.94	87.41	82.47	93.41	71.02	82.03
Kajichak	47.28	72.31	58.3	92.82	53.65	87.89	53.08	84.34
Khasbazar	65.23	99.03	73.78	89.71	80.15	95.39	73.05	94.71
Madhabpur	51.20	57.77	47.65	63.10	51.47	72.53	50.11	64.47
Nandeswar	38.25	46.96	52.26	61.37	43.60	56.55	44.70	54.96
Naraharipur	12.22	35.93	16.34	45.82	60.49	80.25	29.68	54.00
Paikpari	66.25	74.57	71.1	81.18	60.38	75.3	65.91	77.02
Mean	53.08	69.69	61.13	80.60	64.11	78.62	59.44	76.30
SEm(+/-)	0.430		0.044		0.369		0.309	
CD (0.05%)	0.892		0.913		0.765		0.642	

Table 6. Residual soil available potassium as influenced by the crop management conditions

Name of villages	Available Potassium (kg ha-1)							
	2012		2013		2014		Mean	
	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI
Alampur	180.32	267.52	168.80	248.95	240.50	279.25	196.54	265.24
Alidadpur	214.27	242.24	208.15	289.70	120.15	163.55	180.86	231.83
Amodpur	181.12	280.56	188.15	317.20	162.10	166.10	177.12	254.62
Balabhadrapur	256.21	313.60	219.50	296.00	196.85	300.40	224.19	303.33
Bansda	363.68	461.44	323.70	452.50	279.25	393.95	322.21	435.96
Brindabanpur	289.48	354.80	222.90	267.68	226.80	278.45	246.39	300.31
Chaltageriya	205.36	216.44	235.62	301.34	292.05	304.45	244.34	274.08
Dingal	165.44	174.48	180.25	231.67	173.67	225.54	173.12	210.56
Galimpur	234.92	270.40	255.30	283.50	209.80	251.50	233.34	268.47
Kajichak	141.20	213.92	134.10	284.00	154.87	243.45	143.39	247.12
Khasbazar	413.28	576.00	416.80	536.20	454.85	478.00	428.31	530.07
Madhabpur	171.75	235.84	264.50	304.65	291.50	328.00	242.58	289.50
Nandeswar	220.12	293.28	263.58	318.50	250.65	353.00	244.78	321.59
Naraharipur	297.60	359.44	280.00	322.90	297.85	382.50	291.82	354.95
Paikpari	201.20	313.92	234.10	284.20	264.15	301.00	233.15	299.71
Mean	235.73	304.93	239.70	315.93	241.00	296.61	238.81	305.82
SEm(+/-)	1.902		1.802		1.636		1.210	
CD (0.05%)	3.945		3.738		3.393		2.510	

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Table 7. Microbial populations as influenced by the crop management conditions

Name of villages	Soil Fungal Population (Colony Forming Units 10^{-4}/ml)								Soil Bacterial Population (Colony Forming Units 10^{-6}/ml)							
	2012		2013		2014		Mean		2012		2013		2014		Mean	
	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI	Non-SRI	SRI
Alampur	12.02	15.68	13.90	14.90	27.50	41.00	17.81	23.86	56.52	82.40	25.60	41.30	30.40	33.80	37.51	52.50
Alidadpur	12.10	14.32	13.20	14.60	10.70	12.00	12.00	13.64	16.40	36.20	22.20	27.30	18.50	20.80	19.03	28.10
Amodpur	11.48	14.85	13.80	10.20	9.90	11.00	11.73	12.02	12.52	13.90	32.60	48.60	15.60	26.00	20.24	29.50
Balabhadrapur	12.60	16.00	15.20	16.70	12.30	15.10	13.37	15.93	14.70	20.00	17.50	32.80	27.20	36.30	19.80	29.70
Bansda	10.27	15.41	11.10	12.50	21.00	25.00	14.12	17.64	24.15	50.20	24.10	48.12	25.50	32.00	24.58	43.44
Brindabanpur	11.20	18.40	12.20	16.90	16.50	17.20	13.30	17.50	6.27	15.20	18.90	24.81	10.10	15.60	11.76	18.54
Chaltageriya	12.20	10.85	13.75	11.10	12.20	12.50	12.72	11.48	6.50	13.80	15.50	16.50	18.00	19.20	13.33	16.50
Dingal	14.25	17.34	13.60	19.20	12.98	18.34	13.61	18.29	25.80	38.82	12.40	28.00	19.76	32.48	19.32	33.10
Galimpur	12.50	16.20	13.50	16.20	11.85	12.00	12.62	14.80	59.20	72.80	69.50	81.20	13.00	25.00	47.23	59.67
Kajichak	13.97	14.39	14.20	15.80	14.87	16.87	14.35	15.69	14.30	25.20	28.50	36.30	21.45	30.67	21.42	30.72
Khasbazar	10.55	15.08	12.20	15.00	14.30	14.70	12.35	14.93	19.60	29.10	35.50	41.20	28.70	34.10	27.93	34.80
Madhabpur	13.20	11.80	11.80	12.60	10.60	11.50	11.87	11.97	21.20	23.40	27.50	48.30	30.80	42.00	26.50	37.90
Nandeswar	12.10	11.60	12.50	14.00	13.40	18.90	12.67	14.83	16.20	32.50	29.90	32.80	35.90	40.06	27.33	35.12
Naraharipur	13.13	13.48	14.30	13.90	11.00	12.00	12.81	13.13	11.95	14.02	11.65	28.10	17.50	19.00	13.70	20.37
Paikpari	12.00	13.46	12.50	15.10	12.40	18.90	12.30	15.82	12.76	17.10	13.40	16.30	26.90	30.60	17.69	21.33
Mean	12.24	14.59	13.18	14.58	14.10	17.13	13.17	15.43	21.20	32.31	25.65	36.78	22.62	29.17	23.16	32.75
SEm(+/-)	0.117		0.112		0.168		0.092		0.380		0.336		0.185		0.198	
CD (0.05%)	0.243		0.232		0.349		0.190		0.789		0.697		0.384		0.412	

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5.5 Soil Microbial Population

Microbial population measured in terms of fungal and bacterial plate count was significantly influenced by the rice management condition. Microbial population was consistently higher in the SRI system (Table 7 & 8& Figure 1). Quantification of microbial population through plate-count techniques estimates probably less than 10% of the total microflora in the soil. Therefore, molecular quantification (a more reliable method) needs to be done in future studies. The presence of more microbial and biological activity leads to beneficial functions for crops such as plant growth promotion, nitrogen fixation, phosphate solubilization, induced systemic resistance, and protection against pathogens (Carpenter-Boggs et al, 2000)[1]

Table 8. Change in soil properties as influenced by the crop management conditions

Parameter	2012		2013		2014	
	non-SRI	SRI	non-SRI	SRI	non-SRI	SRI
pH	(0.09)	0.16	(0.11)	0.08	(0.14)	0.10
Organic carbon (%)	(0.07)	0.12	(0.08)	0.10	(0.08)	0.12
Available Nitrogen (kg ha^{-1})	(18.52)	21.84	(14.29)	28.19	(12.49)	19.28
Available Phosphorus (kg ha^{-1})	(4.17)	7.98	(4.30)	3.89	(3.43)	5.98
Available Potassium (kg ha^{-1})	(37.85)	35.38	(23.40)	23.08	(34.20)	28.13
Soil Fungal Population (Colony Forming Units 10^{-4}/ml)	(1.37)	2.20	0.26	1.61	(0.34)	1.96
Soil Bacterial Population (Colony Forming Units 10^{-6}/ml)	(2.13)	15.65	(1.20)	15.67	(1.79)	16.86

*Figures in parenthesis are negative

6. Conclusions

During the present three-year investigation, fifteen farmers were selected those were practicing SRI technology. On an average, the study noted that plant height increased by 12%, number of tillers per square meter by 85%, number of reproductive tillers per hill by 286%, weight of panicle per hill by 139%, number of seeds per panicle by 41% and test weight by 26% due to SRI practice over the non-SRI practice. Average increment in straw and grain yield due to SRI over the non-SRI is 70% and 59% respectively. The physico-chemical and biological properties of the soil improved due to SRI practice. The water saved for rice can be effectively used for increasing the area under rice or for other irrigated dry crops in the cropping sequence, thereby, enhancing the rice productivity.

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ARTICLE

Effects of Green Silver Nanoparticles on Soil Quality and Induced Germination: A Future Alternative Fertilizer or Environmental Toxicant?

Pallabi Das[1], Chaitali Roy Chowdhuri[1], Soma Barman[1]*

[1] Soil and Agro Bio-engineering Lab, Department of Environmental Science, Tezpur University, Tezpur-784 028, India

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ABSTRACT

Silver nanoparticles (AgNP) synthesized from It influenced the inherent soil properties like bulk density (BD), water holding capacity (WHC), available N, P, K, urease, phosphatase activity and TOC. The apparent increment WHC, N, P, K, urease, and phosphatase in soil were observed whereas reduction of BD was noticed. Due to application of nanosolutions the pH of the soil shifted towards neutrality from 0 to 60 days. Moreover, they also did not have any toxicity upon plant as well as earthworm ecosystem.

1. Introduction

Silver nanoparticles (AgNP) are among the most widely utilized engineered nanoparticles (Anjum et al., 2013)[2]. Since long, silver is known to show severe toxicity to numerous types of microorganisms; and, silver in nano form has been reported to be more lethal to microorganisms as compared to other forms (Morones et al., 2005). As a result, potential antimicrobial activities of AgNPs against all classes of microorganisms (even at very low concentrations) endorse their notable applicability in biomedical research. In recent past, few workers have observed promising role of AgNPs as effective and alternative pesticides to the conventionally applied synthetic organic compounds (Jagtap et al., 2013; Babu et al., 2014)[12]. In view of using AgNPs as agrochemicals, increment in accumulation of these materials in soil is an eventual fate; which should results into alteration of soil physico-chemical properties (dispersibility, dissolution rate, surface area, surface chemistry, size, agglomeration, transformation, ionic strength, and charge etc). This in turn may determine the stability and transportation of nanoparticles in soil (Tourinho et al., 2012; Anjum et al., 2013)[20][2]. Moreover, extensive application of AgNPs in the consumer based industries leads them to dispose as a waste material in the soil environment (Ben-Moshe et al., 2013)[6].

A differential retention of silver nanoparticle in suspension of natural soil with respect to the contents of clay particles has been reported earlier (Cornelis et al. 2012)[9]. However, presence or absence of organic matter (surfactants or humic acid) may also greatly influence the mobility of silver nanoparticle (Anjum et al., 2010)[3]. Moreover, considerable shift in soil pH was observed in both acid and alkaline soils due to high exposure of silver nanoparticles (Benoit et al., 2013)[7]. On the other hand, few

**Corresponding Author:*
Dr. Soma Barman,
Department of Environmental Science, Tezpur University, Assam 784028*, India;*
Email: vb.somabarman@gmail.com

DOI:

workers claimed that AgNPs have no detrimental effects on physico-chemical properties of soil .Therefore, considerable amount of research gaps can be identified in regard to time and concentration of exposure and the interaction of AgNPs with soil properties. Moreover, the effects of AgNPs on soil macro-animals like earthworm are yet to be studied thoroughly.

Here in this study we applied various concentrations of a *Mentha arvensis* leaf extract mediated green silver nanoparticles (AgNP) to soil. The changes in physico-chemical properties of soil were assessed along with solubility study of ions in the respective treated soil. Moreover, the toxicity of AgNP was assessed with two earthworm species: *Eisenia fetida* and *Metaphire postheuma*. Seed germination assay was also performed to determine the efficacy of AgNP on probable plant growth.

2. Materials and methods

2.1 Source of AgNP and treatment combinations

Mentha arvensis mediated nanoparticles were procured from the department of Chemical Sciences, Tezpur University for the purpose of this experiment. Required concentrations of the nanoparticle (10, 25, 50, 75, 100 mg kg^{-1}) were obtained through serial dilution method from the stock solution. Treatment combinations were listed below:

Control-Soil without AgNP

$AgNP_{10}$-10 mg kg^{-1} concentration of AgNP, $AgNP_{25}$-25 mg kg^{-1} concentration of AgNP

$AgNP_{50}$-50 mg kg^{-1} concentration of AgNP, $AgNP_{75}$-75 mg kg^{-1} concentration of AgNP

$AgNP_{100}$-100 mg kg^{-1} concentration of AgNP

2.2 The experimental setup

2.2.1 Lab scale soil incubation study

Typical alluvial soil samples were collected from nearby vicinity (order: typic endoaquept) (Napaam, Tezpur, Assam). Consequently the collected soil samples were air dried, removing the plant parts and breaking the clods. Sieving was debarred to maintain the natural condition of the composite soil. Afterwards the prepared soil samples were poured into cone shaped porous earthen vessels with a volume of 2L and dimension 0.45m × 0.25m (height × diameter). Required concentrations of nanosolutions were applied in each vessel as listed above. Each treatment combination was replicated thrice and the study was continued for 60 days. Proper ambience temperature was maintained within 25-30°C. Sprinkling of water was done at 2-3 days interval to ensure the natural condition of the treated soil. Periodically samples were collected at 0, 30 and 60 days and analysis of various physico-chemical properties was done.

2.2.2 Physico-chemical changes of the experimental soil samples

Water holding capacity (WHC), bulk density (BD), pH, available nitrogen (Avl N), available phosphorus (Avl P), exchangeable potassium (Avl K), total organic carbon (TOC) were analyzed following Page et al., 1982 . Additionally the efficiency of nanoparticles on some vital soil enzymes (urease and phosphatase) was analyzed through standard protocoals (Tabatabai and Bremner, 1969; 1972) [18][19].

2.2.3 Effect of AgNPs on periodical solubility of ions

Soil samples from the lab scale soil incubation study were collected and utilized to assess the potential influence of AgNPs on periodical solubility of ions. Composite soil samples were prepared for experimental setup following the steps: breaking the clods, removing plant parts etc. as mentioned in earlier experiment. Afterwards 10g of the prepared soil samples were treated with required concentrations of AgNPs i.e., 10, 25, 50, 75 and 100 mg kg^{-1} and poured into erlenmeyer flasks of 250 ml capacity. Each flask containing the treated samples were replicated thrice to maintain complete randomization process. Subsequently, distilled deionized water was mixed in each flasks at 1:10 ratio with the substrate (10 g soil substrate: 100 ml deionized water) and reacted at 120 rpm for 7, 14 and 21 days in a mechanical shaker at room temperature (25-30° C). Filtration was done at each sampling period with Whatmann No. 1 filter paper. Samples are analyzed for PO_4^{3-}, NO_3^-, SO_4^{2-}, Cl^-, total alkalinity, pH by following the standard methodologies (Page et al., 1982)[16].

2.2.4 Phytotoxicity: Seed germination assay

10 number of seeds of *Vigna radiata* were placed in tissue papers per petriplates. 5 ml of nano solutions of required concentrations (100, 75, 50, 25 and 10 mg kg^{-1}) were added in each plate. Subsequently the inoculated seeds were maintained at 25°C in dark condition. The number of germinated seeds, length of plumule and radical were measured after 48 hours of incubation. The measurement of relative root growth (RRG), relative seed germination (RSG) and germination index (GI) were done following Karak et al., 2014.

$$RSG\,(\%) = \frac{Number\ of\ seeds\ germinated\ with\ AgNP\ nanosolutions}{Number\ of\ seeds\ germinated\ with\ distilled\ water} \times 100$$

$$RRG\,(\%) = \frac{Mean\ root\ length\ of\ seeds\ receiving\ AgNP\ nanosolutions}{Mean\ root\ length\ of\ seeds\ receiving\ distilled\ water} \times 100$$

$$GI\,(\%) = \frac{RSG \times RRG}{100}$$

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2.2.5 Earthworm population and health analysis

The toxicity of nanoparticles was tested upon earthworm species *Eisenia fetida* and *Metaphire postheuma*. Urine free cowdung collected from a nearby farm were used as a substrate material. Juvenile, non-clitellated specimens of earthworms with an average length of 2–3.5cm and weighing about 200-250mg were collected from the vermiculture unit of the department of Environmental science, Tezpur University, Assam (India). Afterwards, the earthworms were cleaned with water and kept in a moist filter paper for overnight @ 25°C for gut evacuation; afterwards used for incubation study. A 50 ml stock solution of different concentrations of the nanoparticle (100, 75, 50, 25 and 10mg kg^{-1}) was mixed with the substrate. 20 worms per kg of substrate were added in each earthen vessel.

Efficacy of AgNP nanosolutions on earthworm population and health were enumerated by taking earthworm count, body weight and length measurement at 10 days interval and continued up to 60 days.

2.2.6 Statistical analysis and graphical representation

One way ANOVA was conducted in SPSS 16.0 software and analyzed the variations between different treatment combinations. However, least significance difference (LSD) test was also performed to understand the efficiency of nanoparticles. Graphical representation was done with the help of Sigma plot 10 and MS excel.

3. Results and discussion

3.1 Changes in BD, WHC, pH, available N, available P, available K, urease and phosphatase activity, total organic carbon (TOC) of lab scale soil study

The inherent soil properties are represented in Table 1. Data on changes in BD, WHC, available N, P, K, urease, phosphatase activity and TOC are presented in Figure 1. Bulk density of the soil particles temporally decreased in the lower concentrations of nanosolutions (10, 25 and 50mg kg^{-1}). However in higher doses of nanosolutions (100 and 75 mg kg^{-1}) slight increment was observed. Incorporation of nanosolutions in soil @ 10 mg kg^{-1} followed by 25 and 50 mg kg^{-1} elevated the WHC of the substrate after 60 days of incubation (Figure 1) ($P_{0.05}$=0.000, LSD=0.54). We recorded significant reduction in BD under lower concentrations of AgNP (50, 25 and 10 mg kg^{-1}). This may be due to increase in soil porosity caused by AgNP by forming stable aggregates in the soil. Depending on the ionic strength of the media AgNPs can form stable aggregates (Zhang and Zhang, 2014)[22]. Simultaneously, WHC of the soil also increased significantly under these concentrations. This is interesting, because WHC is directly related to the porosity of the soil. This could be due to the higher surface area created by the nanosized particles in soil, which in turn considerably improved soil structural aggregation.

Table 1. Inherent properties of the test soil

Parameters	mean±stdev
pH	5.08±0.1
BD (g /cc)	0.64±0.05
WHC (%)	49.5±0.87
TOC (%)	0.18±0.03
Available K (mg kg^{-1})	150±1.74
Available P(mg kg^{-1})	40.5±1.25
Phosphatase (µ g g^{-1} h^{-1})	156.7±0.57
Available N (mg kg^{-1})	305.4±2.5
Urease (µ g g^{-1} h^{-1})	12.86±0.58

Inherently the soil pH was acidic in nature (Table 1). However, due to application of nanosolutions the pH shifted towards neutrality from 0 to 60 days ($P_{0.05}$=0.000, LSD=0.095). Maximum increment in pH was depicted from 10 mg kg^{-1} followed by 25 and 50 mg kg^{-1} in the later period of the study, this may be due to the neutral characteristics of AgNP (Barua et al., 2013)[4]. N availability remarkably increased under lower concentrations of the AgNP treatments during the study period. N augmentation was recorded from 10 mg kg^{-1} followed by 25 and 50 mg kg^{-1} ($P_{0.05}$=0.000). However, 100 and 75 mg kg^{-1} depicted slight increment decrement in terms of N availability compared to the lower concentrations of the study. Similarly, considerable increase in soil urease activity was observed in 10 mg kg^{-1} followed by 50 and 25 mg kg^{-1}. However, such improvement in urease activity was not observed in 100 and 75 mg kg^{-1} of AgNP application ($P_{0.05}$= 0.000, LSD=0.39). Urease is one of the most important soil enzymes that regulate N mineralization in arable soils. Interestingly, in the present study significantly high urease activity under lower doses of AgNP treatments were recorded. Improvement in soil pH, enhanced urease activity coupled with favourable physical environment (additional porosity) probably increased N availability in soil.

Temporal augmentation was observed in available K content in AgNP treated soils during the study ($P_{0.05}$=0.000, LSD=1.74). At later period of the study the status of available K was in the order: 10 mg kg^{-1}>25 mg kg^{-1}>50 mg kg^{-1}>100 mg kg^{-1}>75 mg kg^{-1}. However in the present study, higher K mineralization in nanoparticle treated soil could be due to the improvement in particle size distribution and granular stability (evidenced from the data on bulk density) provided by the added nanomaterials.

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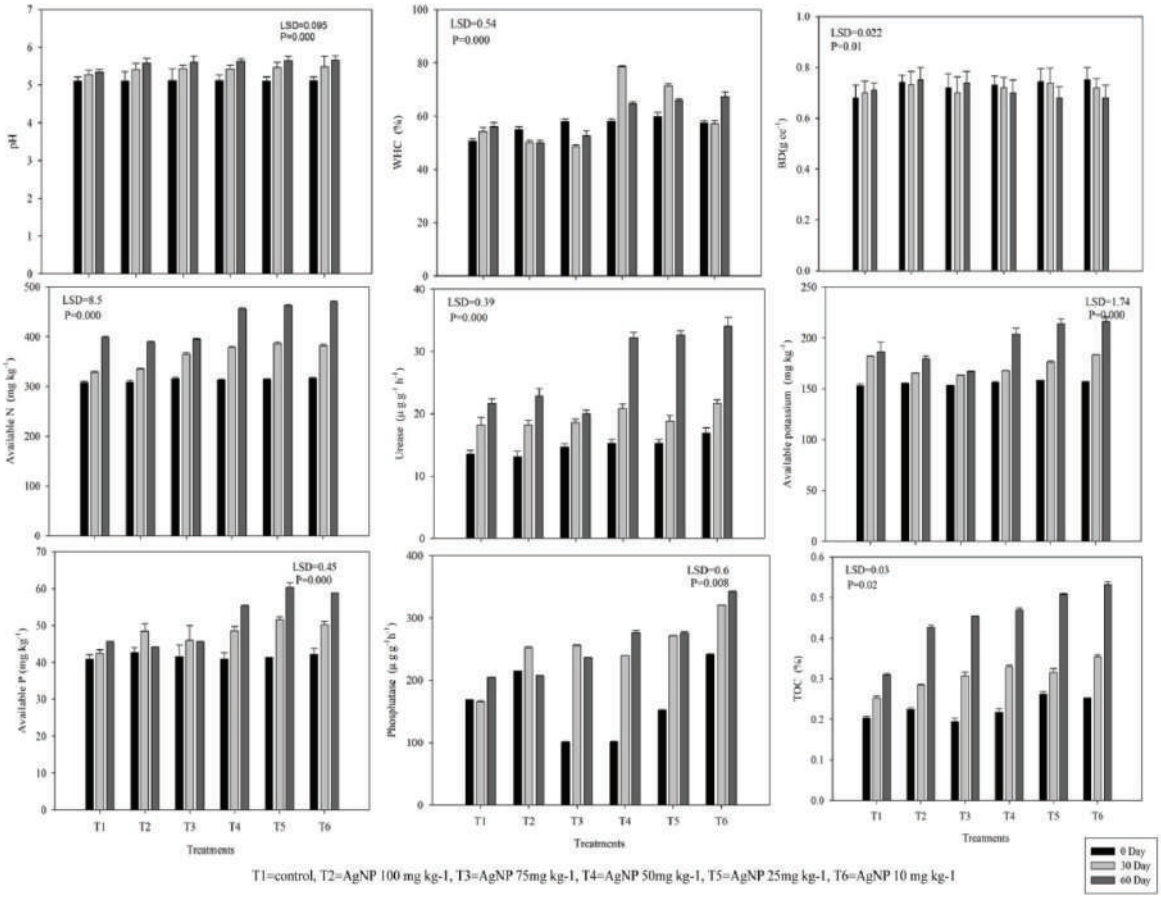

Figure 1. Changes in pH, WHC, BD, available N, available P, available K, urease and phosphatase activity, total organic carbon (TOC) of soil incubation study due to application of different combinations of AgNP nanosolutions

P availability dramatically enhanced in lower concentrations of AgNP treatments i.e., 25 mg kg^{-1} followed by 10 and 50 mg kg^{-1}. Whereas in case of 100 and 75 mg kg^{-1} P availability was increased upto 30 days, after that reduced P availability was observed at 60 days of incubation period. Enzyme phosphatase has a significant role in enhancement of phosphorus availability in soil solutions. Here in this study phosphatase availability was observed in the order: 10 mg kg^{-1}>50 mg kg^{-1}>25 mg kg^{-1}>75 mg kg^{-1}>100 mg kg^{-1}. Positive effect of AgNPs on soil phosphatase activity may be one of the factors that enhances available P content in soil solution.

The TOC content of the experimental soil was initially very low in nature. Overall due to application of AgNP, TOC content gradually increases @ 10, 25 and 50 mg kg^{-1} from 0 to 60 days. However, TOC change was very marginal in case of 100 and 75 mg kg^{-1} during the study. At the end of the study period TOC level of soil treated with AgNP was in this order: 10 mg kg^{-1}> 25 mg kg^{-1}>50 mg kg^{-1}>75 mg kg^{-1}>100 mg kg^{-1} ($P_{0.05}$=0.02, LSD=0.03). Similar impacts of nanomaterials on stability of soil organic matter content have been reported earlier (Xie et al., 2008; Johnson et al., 2009)[21][13]. Ag has been reported to have a high affinity for reduced S group (thiol) of soil organic matter and can form S-Ag-S bonds (Bell and Kramer, 1999)[5]. These types of organo-metallic complexation might have decreased organic matter degradation or mineralization and thus enhanced sequestration of C in soil.

3.2 Changes in pH, alkalinity, chloride, sulphate, phosphate, nitrate ions in solubility study

Figure 2 depicted the changes of pH, alkalinity, chloride, sulphate, phosphate, nitrate ions in solubility study. A temporal escalation was observed in the pH from 10, 25 and 50mg kg^{-1} (0 and 21 days) ($P_{0.05}$=0.000, LSD=0.262), yet a sudden drop was found to occur in the 14th day. pH varied significantly in our experiment although there was an overall rise in pH up to 50 mg kg^{-1}concentration of AgNP inoculated soils. Such changes in pH significantly influence mobility of trace elements in soil (Reddy et al., 1994; Bhattacharyya et al., 2011)[17][8]. Much escalation in alkalinity was comprehended in 50mg kg^{-1} in 7 and 14 days with inconsequential drop in the 21th day. The alkalinity was found to be constant in case of 25, 10mg kg^{-1} of AgNP. An upsurge was observed in chloride concentration

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in 10 and 25 and 50mg kg^{-1} at the 21st day (LSD=2.47, P=0.000).

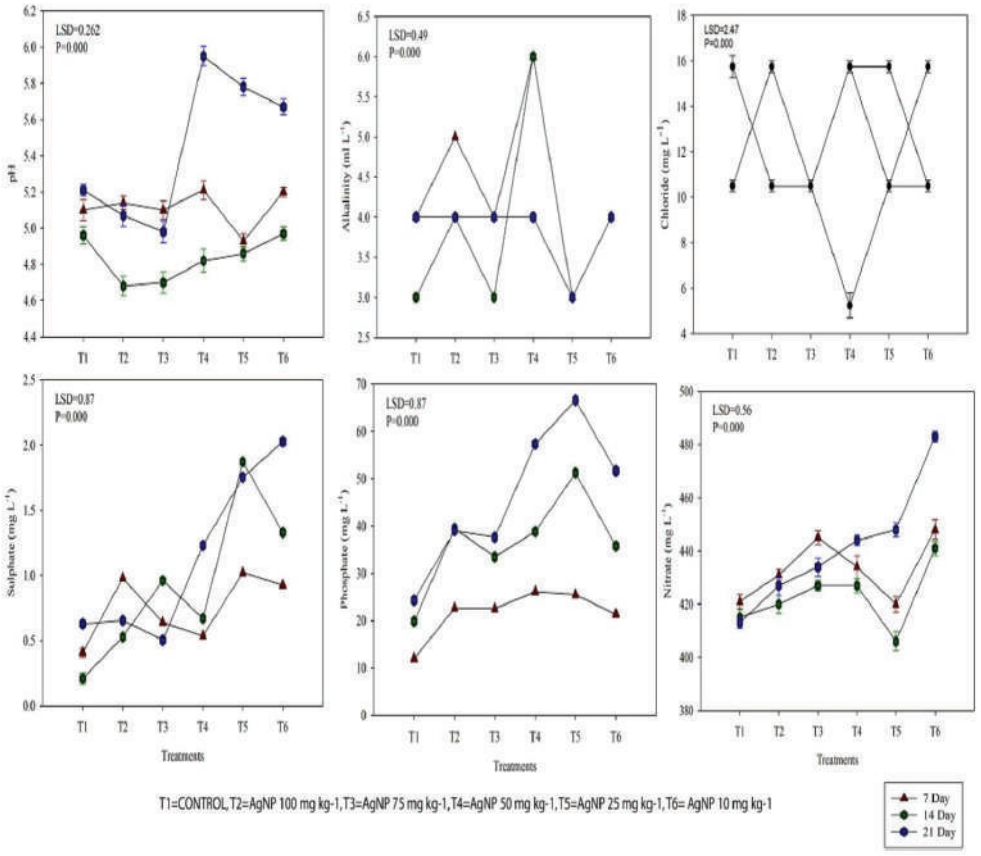

Figure 2. Changes in pH, alkalinity, chloride, sulphate, phosphate, nitrate ions in solubility study due to application of AgNP

Substantial augmentation was observed in phosphate solubility in 10, 25 and 50mg kg^{-1} AgNP treatments from 7th to 21th day; nevertheless a slight increment in phosphate solubility was observed in 100 & 75mg kg^{-1} in the later period of the study ($P_{0.05}$=0.000, LSD=0.87).

Abundant increase in sulphate solubility was found to occur in 10mg kg^{-1} AgNP followed by 25 and 50mg kg^{-1} at 21 days ($P_{0.05}$=0.000, LSD=0.87). Nitrate solubility was higher in 10, 25 and 50mg kg^{-1} of AgNP treatments at 21 days; unlike 100 and 75mg kg^{-1} which have lesser nitrate N compared to initial value ($P_{0.05}$=0.000, LSD=0.56).

3.3 Phytotoxicity: Effect of AgNP on seed germination

AgNP provided minimal inhibition of seed germination and growth compared to the control one (Figure 3). The germination index followed the order: 10 mg kg^{-1}>25mg kg^{-1}>75mg kg^{-1}>50 mg kg^{-1}>100 mg kg^{-1} (P=0.000, LSD=0.487). Whereas, the relative seed germination of *V. radiata* of AgNP treated seeds was in the order: 10 mg kg^{-1}>25 mg kg^{-1}=75 mg kg^{-1}=50 mg kg^{-1}=100mg kg^{-1} (P=0.000, LSD=0.826).This figure present a probable effect of nanoparticles on agricultural crops. From the figure it is comprehensible that lower concentrations of the nanoparticles are providing better environment in germination of the seeds. Primarily 10 mg kg^{-1} of AgNP depicted most significant effect in RSG (111%) than the rest of the treatments. It was conspicuous that high doses of AgNP showed equal amount of RSG (100%). In case of RRG the efficacy was in the order: 10 mg kg^{-1}> 25mg kg^{-1}>75 mg kg^{-1}> 50mg kg^{-1}> 100mg kg^{-1}. Here also lower doses provide better germination condition for *Vigna radiata*. It is noteworthy to describe that 10 mg kg^{-1} AgNP provide most significant effect followed by 25 mg kg^{-1}. In some previous studies positive effect of AgNP on seed germination was reported by some researchers (Abdel-Azeem et al., 2013; Najafi et al., 2013)[1][15]. Nanoparticles have a general tendency to form complex in solutions and thus remain in dispersed state. In addition, the seed coats are selectively permeable to AgNPs and possess good antimicrobial property. All these factors together might have indirectly created a favorable condition for the plant seeds.

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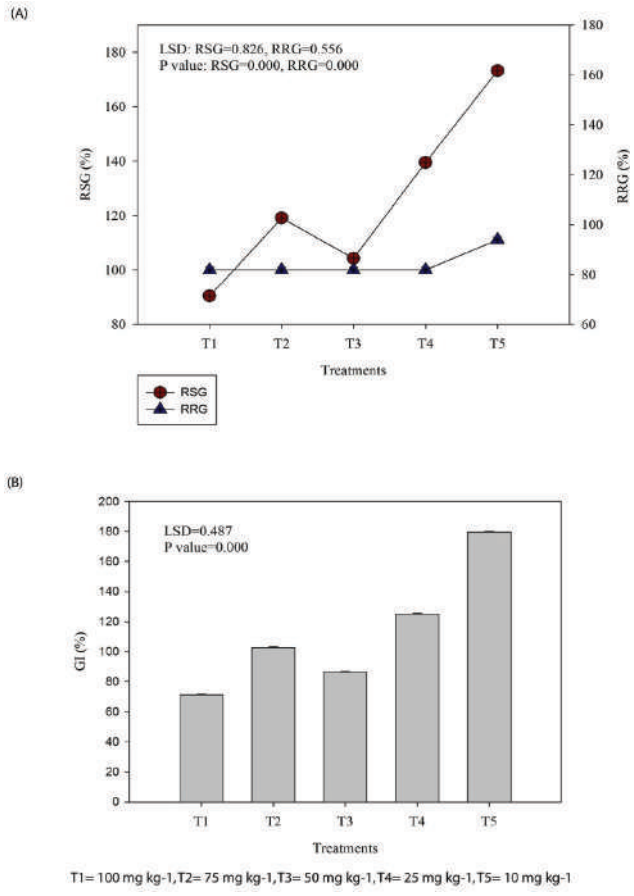

Figure 3. Effect of AgNP on RSG, RRG and GI of V. radiate seeds

3.4 Effect of AgNP on earthworm proliferation and changes in morphology

Table 2 and 3 represented the data of earthworm count, body weight and length measurement. Drastic mortality was observed in the population of *P. excavates* after 10 day of the incubation and total reproductive failure was examined in both higher and lower concentrations of AgNP. The growth and fecundity of *E. fetida* and *M. postheuma* substantially reduced with interemittent mortality when exposed to higher than 75 mg kg^{-1} of AgNP during the period of incubation.However, reduction in proliferation rate and body weight of these two species was not evidenced under low concentration treatments of AgNP (10, 25 and 50 mg kg^{-1}) till 60 days. The survivality rate of *E. Fetida* was more prominent than *M. Postheuma* in AgNP treated substrate. 10 mg kg^{-1} treated feedstock depicted the most positive outcome in terms of earthworm count in *E. fetida*. However, in *M. Postheuma* 25 mg kg^{-1} feedstock provided the most viable environment for earthworm propagation.

Body weight and length enumeration was considered to observe the feasibility of feedstock mixture for earthworm growth. Body weight and length for *E. Fetida* prominently enhances @ 10 mg kg^{-1} treated substrate. Moreover for *M. Postheuma* 25 mg kg^{-1} was the most suitable substrate for earthworm growth.

The general idea about nanoparticle is that they have harmful effect on organisms and ecosystem. Previous results reported that AgNP had adverse effects on growth and proliferation of earthworm species *E. fetida* (Heckman et al., 2011)[11]. However, this research revealed that low dose AgNP exposure may not cause severe helath hazard to earthworms if the duration of exposure is not too long. This may be due to the fact that incorporation of nanomaterials causes increase in porosity in the substrate (Barua et al., 2013)[4]. As earthworms can absorb oxygen from their surrounding environment through their moist skin and they are mostly susceptible to anaerobic environment (Barua et al., 2013)[4]. Hence, enhancement in porosity probably ensured adequate circulation air to sustain normal growth and proliferation of the earthworm speices. Our findings are in good agreement with previous report (Barua et al., 2013)[4].

4. Conclusion

The present study revealed that silver nanoparticles prepared through the leaf extract of Mentha arvensis cause not rigorous detrimental effect to the soil environment. This also showed that lower concentrations of AgNPs (10, 25 and 50 mg kg^{-1}) did not hamper the growth and proliferation of earthworms, the nature's chemical managers. Seed germination assay also depicted feasibility of lower concentrations in *V. radiata* seeds growth. The solubility experiment demonstrated enhancement of plant essential PO_4^{3-} and NO_3^- in the lower concentrations of the AgNPs; this indicated the probability of better growth of plants, which we can assume through seed germination assay data. Moreover, the results proved that the soil quality improved substantially without any detectable hindrance from lower range of AgNPs.

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Table 2. Earthworm count, body weight and length measurement of *Eisenia fetida incubated* in AgNP treated substrate.

	Earthworm count						Body weight (g)						Body length (cm)					
Conc	10 D	20 D	30 D	40 D	50 D	60 D	10 D	20 D	30 D	40 D	50 D	60 D	10 D	20 D	30 D	40 D	50 D	60 D
$AgNP_{100}$	16±1	25±1	15±1	20±1	25±1	28±1	0.3±0.2	0.4±0.1	0.8±0.3	1.3±0.2	1.1±0.3	1±0.3	3.5±0.7	4.5±0.5	6.5±0.5	8.1±0.9	8.5±0.5	8.8±0.5
$AgNP_{75}$	25±1	21±1	19±1	34±1	26±1	24±1	0.6±0.1	0.9±0	1.2±0.3	1±0.5	0.8±0.1	0.9 ±0.2	3.8±0.5	4.2±0.7	5.8±0.9	6.5±0.5	7.8±1	9.3±0.7
$AgNP_{50}$	20±1	20±1	19±1	54±1	87±2.6	77±1	0.3±0.1	1.2±0.2	1.3±0.2	1.2±0.2	1.4±0.1	1.72±0.3	3.7±0.7	5.2±0.7	6.7±0.1	8.5±0.5	9.4±0.9	10.9±0.9
$AgNP_{25}$	20±1.7	21±1	36±1	53±1	75±1	89±1	0.4±0.1	1±0.5	1.2±0.4	1.1±0.4	1.54±0.1	1.72±0.1	3.3±0.7	5.3±0.7	7.8±0.9	8.7±0.7	9.8±0.5	11.5±0.5
$AgNP_{10}$	20±1	20±1	45±1	58±1	101±1	95±1	0.1±0.1	1.5±0.5	1.1±0.4	1.3±0.2	1.64±0.5	1.8±0.2	3.5±0.7	6.1±0.8	8.5±0.5	9.5±0.5	11.5±0.9	12.5±0.5
Control	20±1	14±1	18±1	45±1	25±1	28±1	0.2±0.1	1.1±0.4	1.0±0.4	1.2±0.2	1.2±0.1	1.08±0.2	3.3±0.2	5.8±0.7	7.3±0.7	8.5±0.5	9.2±0.7	9.8±0.9
L.S.D at P <0.01	0.97	0.82	0.82	0.82	1.03	0.89	0.08	0.25	0.25	0.21	0.19	0.17	0.52	0.56	0.59	0.54	0.59	0.52

Table 3. Earthworm count, body weight and length measurement of *Metaphire postheuma* due to application of AgNP

	Earthworm count						Body weight (g)						Body length (cm)					
Conc.	10 D	20 D	30 D	40 D	50 D	60 D	10 D	20 D	30 D	40 D	50 D	60 D	10 D	20 D	30 D	40 D	50 D	60 D
$AgNP_{100}$	20±1	11±1	14±1	18±1	23±1	19±1	1.2±0.3	1.1±0.3	1±0.4	0.8±0.2	1±0.5	1±0.4	3.05±0.7	4.5±0.5	6.8±0.5	7.7±0.9	9.1±0.5	8.5±0.7
$AgNP_{75}$	21±1	13±1	17±1	22±1	26±1	18±1	0.4±0.1	1.3±0.2	1.1±0.2	0.9±0.1	1.1±0.3	1.1±0.3	3.1±0.5	4.6±0.7	5.1±0.9	5.9±0.5	6.4±1	8.3±0.7
$AgNP_{50}$	21±1	19±1	23±1	40±1	35±1	45±1	1.2±0.3	1.1±0.2	0.8±0.2	0.9±0.1	1.4±0.2	1.2±0.3	3.8±0.7	4.5±0.7	5.8±1.1	6.5±0.5	7.1±0.9	9.4±0.5
$AgNP_{25}$	21±1	21±1	29±1	48±1	70±1	58±1	1.2±0.2	1.4±0.1	1.1±0.3	1.1±0.3	0.8±0.2	1.29±0.2	3.3±0.7	5.9±0.7	7.9±0.9	9.3±0.7	9.7±0.5	10.4±0.7
$AgNP_{10}$	22±1	20±1	24±1	41±1	44±1	51±1	0.3±0.2	0.9±0.1	1.5±0.3	1.6±0.2	1±0.5	1.3±0.2	3.8±0.9	5.5±0.7	8.5±0.5	9.7±0.5	10.5±0.9	11.8±0.5
Control	20±1	23±1	20±1	37±1	15±1	21±1	0.3±0.2	1.7±0.1	0.8±0.2	0.8±0.2	1±0.5	0.9±0.1	3.3±0.3	4.8±0.7	7.3±0.2	8.8±0.5	9.2±0.7	9.5±0.7
L.S.D at P <0.01	0.82	0.89	0.82	0.97	1.03	0.82	0.18	0.15	0.20	0.26	0.34	0.20	0.55	0.59	0.53	0.52	0.50	0.48

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ARTICLE

Isolation and Characterization of Bacterium isolated from Bantala Tannery Solid Wastes

Anannya Banerjee[1] and Sudipta Tripathi[2]*

[1] IRDM Faculty centre, School of Agriculture and Rural Development, Ramakrishna Mission Vivekananda University, Narendrapur, Kolkata -700103

[2] Institute of Agricultural Science, University of Calcutta, 51/2 Hazra Road, Kolkata-700019

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ABSTRACT

Leather industries that uses the conventional chrome tanning process are subjected to high risk of contamination due to the emission of toxic Cr(VI) that poses a serious threat to the environment and human's wellbeing. The present study were made to isolate and characterize chromium tolerant bacteria in the samples collected from four different plots of Bantala Tannery, Kolkata, West Bengal, India. Pure chromium tolerant bacterial strains were isolated from the tannery sludge samples and their relative MIC (Minimum Inhibitory Concentration) were recorded at different concentrations of Cr (VI) salts to select the highest chromium tolerant bacterium. The selected bacterium was further taken for their growth studies followed by different cultural, morphological and molecular analysis (16S rDNA). The bacterial strain was further studied through SEM (Scanning Electron microscopy) and EDX (Energy Dispersive X-ray) spectroscopy which revealed that TW4 was a gram positive, rod shaped, endospore forming, pleomorphic bacterium with phylogenetic similarities with *Isoptericola sp.* and genebank accession number SUB1732465 TW4 KX640927.

1. Introduction

The process of production of leather has been a very important process since ancient times due to its high demand in daily life. The process involves several mechanical and chemical stages. Most of the tanneries release the untreated wastes in the environment. This leads to deposition of huge amount of solid wastes and wastewater containing toxic chromium salts used during tanning (Alam and Malik, 2008; Familec *et al*., 2011)[1][7]. The presence of high concentration of chromium leads to the adaptation of microorganisms that can develop the mechanism to withstand the metal and sustain in the hostile environment.

The leather complex which is 20 Kms from Kolkata, West Bengal, India on its south-eastern periphery, is a living hell.

The smell of the chemicals used to treat the leather

**Corresponding Author:*
Sudipta Tripathi.
Institute of Agricultural Science, University of Calcutta, 51/2 *Hazra Road, Kolkata*-700019.
E-mail:sudipta_t@yahoo.com

DOI:

often sickens one to nausea. The water canal gets choked with rotting animal hair, fat and the omnipresent plastic. The visible canal water often gets coloured with blood, dyes or chromium and even shines in grimy bubbles. Most of the tanneries are devoid of the infrastructure which may suitably treat the tannery plant effluents. The burning and boiling of shaving dust, flesh linings and trimmings are often used to serve as fertilizers and fish feeds. The chromium content in these can pollute surface water and also can leach down to contaminate ground water. The supply of water laden with chemicals and salts in the surrounding farming lands has devastatingly reduced paddy yield. (Bera, 2013; Banerjee *et al*, 2018)[4][3]There had been very scarce studies available regarding the microbial population of the area and their potential in improving the metal toxicity of the environment. It is of immense need to start a study on the potential bacteria of the place that can thrive in the hostile environment so that it may help in remediation of the wastes in near future. Thus the present study was carried out to elucidate the isolation and characterization of potential chromium tolerant bacteria from tannery effluent (Alam and Malik, 2008)[1].

2. Materials and methods

2.1 Isolation of bacterial strains

Bacterial strains resistant to Cr (VI) were isolated from solid tannery sludge sample collected fromCalcutta Leather Complex, Bantala, East Kolkata, West Bengal, Indiausing nutrient agar

(NA), (Hi Media, Mumbai, India) plates supplemented with Cr (VI) salt ($K_2Cr_2O_7$). Ten grams of soil sample was suspended in 90 ml sterile water and shaken vigorously for 10 min. A 0.1 ml aliquots of appropriate dilutions were plated on NA plates and incubated at 30 °C for 24 h. Individual bacterial colonies on NA plates which varied in shape and colour were picked up and purified by repeated sub culturing on the same medium. Sludge samples were also analyzed for the total chromium concentrations by atomic absorption spectrophotometer (Instrument: Varian AA240FS; Flame atomizer. Software: Work sheet Oriented AA software; Version 5.1 pro).

2.2 Identification of isolate

In the present study, a total of 16 bacterial isolates were isolated from the chromium contaminated solid sludge. Bacterial isolates were identified on the basis ofcultural characterization of the bacterium following the *Bergey's Manual of Systematic Bacteriology* and morphological characteristicsthat included negative staining, gram staining and spore staining following the established methods (Aneja,2003; Ghori *et al*., 2011)[2][8].Out of the 16 isolates, the isolate TW4 was specially chosen due to its simultaneous high resistance towards chromium. This isolate was further identified by 16S rDNA analysis using the primers 8F: (5′AGA GTT TGA TCC TGG CTC AG 3′) and 1492r (5′-GGT TAC CTT GTT ACG ACTT-3′) as *Isoptericola* sp. TW4. The Gen Bank accession number of 16S rDNA sequence of the isolate is SUB1732465 TW4 KX640927 (Shakoori *et al*., 2010; Shekhar*et al*., 2014)[10][11].

2.3 Minimum Inhibitory Concentrations of Chromium

Minimum inhibitory concentration (MIC) of chromium against the test isolates weredetermined by the plate dilution method. The metal Cr^{6+}were used as $K_2Cr_2O_7$ (Hi Media, Mumbai, India), respectively, in increasing concentrations ranging from 50ppm to 250ppm were added to sterilized NA and poured into plates which were then spot (10 µl) inoculated aseptically with exponentially growing culture of *Isoptericola* sp. TW4. The plates were incubated at 37 °C for 24 hrs. The lowest concentration of the metal at which no growth occurred was considered as MIC.

2.4 Growth Studies

Growth of the *Isoptericola* sp. was determined by taking about 5ml of 24hrsbroth culture which was aseptically transferred to a fresh broth culture amended with 50ppm-$K_2Cr_2O_7$.Initial optical density (OD) at 600nm wavelength was recorded and the inoculated culture flask was placed in the shaker (120rpm) at 30ºC for 12hrs. After 1h of incubation, 5ml of the culture was aseptically transferred to a cuvette and the optical density of the sample was recorded at 600nm. A control was studied simultaneously with the sample culture. OD values were recorded at 1h interval and a growth curve was obtained using the observed OD of the control culture and the sample culture (Chaturvedi, 2011)[5].

2.5 Scanning Electron Microscopy and Energy Dispersive X-ray Analysis

The sample for the SEM and EDX study was prepared by taking 1mL of bacterial broth which was centrifuged at 12,000rpm. The pellet was treated with 4% gluteraldehyde (in Na- phosphate, pH - 7.2) after buffer wash and kept overnight. Dehydrolysis of sample was followed by different volumes of ethanol starting from 50%, 70%, 90%, 100%.SEM and EDX stub was prepared by applying the adhesive tape and fixing of the glass slides smeared with the treated bacterial cultures. Slides were then screened under a Scanning Electron Microscope and images were used to generate the EDX report (Narayani,2012)[9].

 DOI:

3. Results and Discussion

The total chromium concentration was found to be 21.43 mg/gm of sludge sample. The *Isoptericola* sp. TW4 was isolated from solid tannery sludge heavily contaminated with chromium. The bacterial colony had white colouration. The different staining analysis showed the bacterium as rod shaped (Plate 2), gram positive (Plate 3) and endospore forming bacterium (Plate 4).The partial 16S rDNA sequence of the bacterial isolate was compared with the sequences in GenBank database by BLAST-N algorithm to identify sequences with high degree of similarity. The gel, when UV transilluminated, revealed a very bright, thick band of PCR product which was about 1.5 kb in size (Plate 5).Amplification of 16s rDNA yielded a 1475 bp product. The isolate TW4 showed 99% sequence similarity with *Isoptericola sp.* (Fig. 1). So, from 16s rDNA partial sequence data along with cultural features and morphological characteristics, the TW4 isolate could be identified as belonging to the genus *Isoptericola*. The bacterium is classified as belonging to the phylum Bacteroidetes, the class Flavobacteria, the order Flavobacteriale and the family Flavobacteriaceae. This genus was first described by .

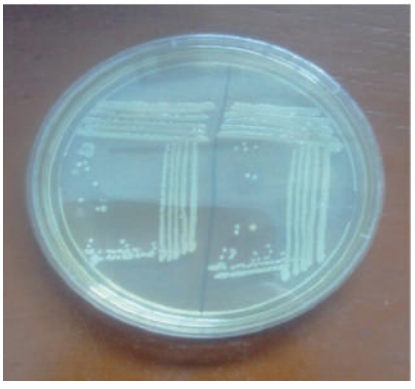

Plate 1. Establishment of pure cultures on NA plates amended with 50ppm $K_2Cr_2O_7$

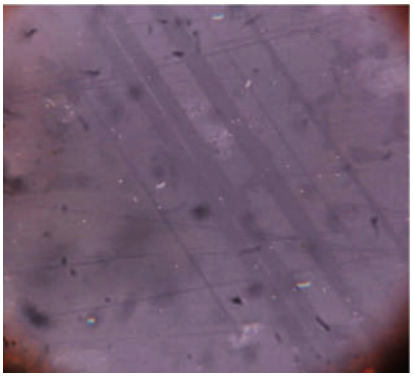

Plate2. Negative staining (1000X)

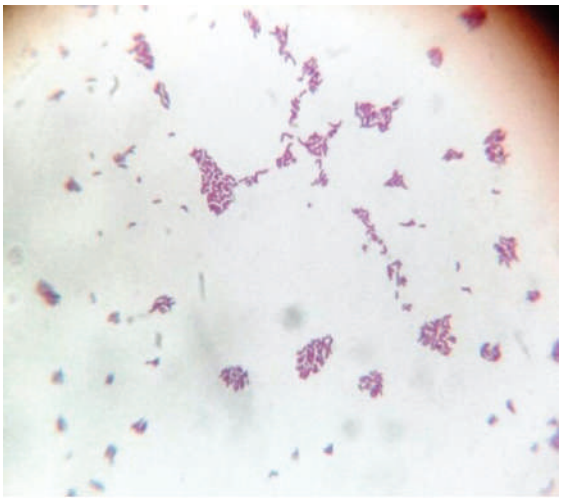

Plate 3. Gram staining (1000X)

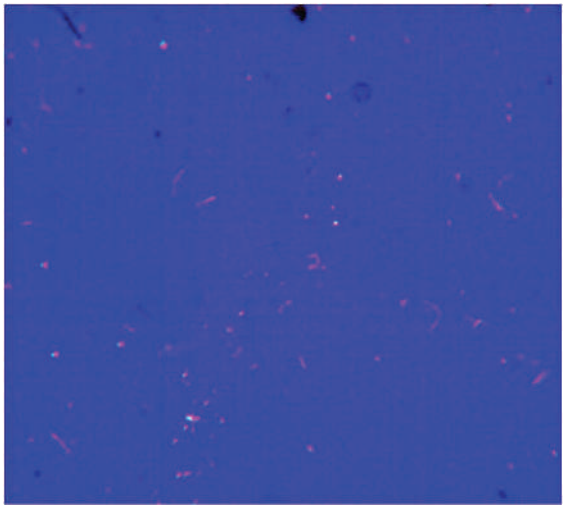

Plate 4. Endospore staining(1000X)

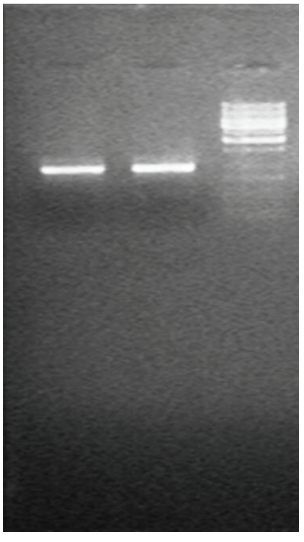

Plate 5. 16S rDNA PCR amplified product

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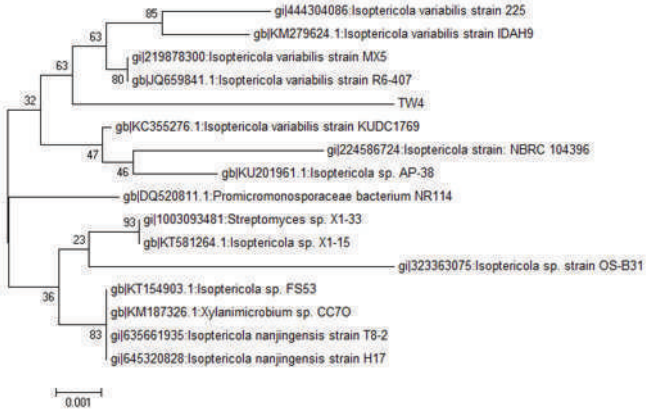

Figure 1. Phylogenetic analysis of TW4

Isoptericola sp. TW4 was found to show highest resistance against 250ppm of $K_2Cr_2O_7$. High level of chromium resistance in different bacteria was reported previously by several workers (Alam and Malik, 2008)[1]. Reports on chromium resistance by the genera *Isoptericola* are very rare. The bacterial growth curve clearly showed a distinctive change in growth between the bacteria under control condition and chromium stress condition (Fig. 2). The control bacteria showed a steep rise in its growth while the bacteria treated with $K_2Cr_2O_7$ showed a drastic reduction in growth.

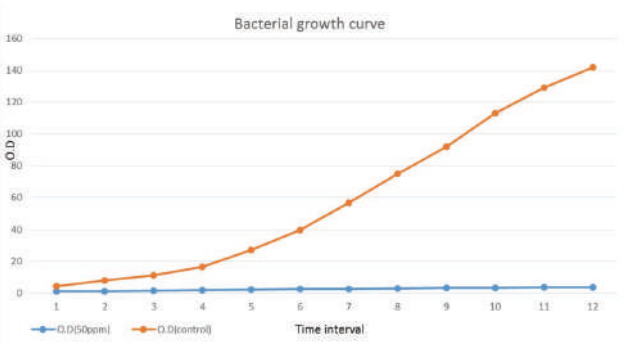

Figure 2. Growth studies of TW4

The SEM report of the isolated organism TW4 clearly revealed a drastic morphological change (from rods to spherical) when the organism was treated with 100ppm Chromium(VI)salts which is a typical characteristic of pleomorphic bacteria (Plate6; Plate7). Comparative EDX analysis between the control and the 100ppm chromium(VI) treated organisms clearly depicted that there were trace amounts of stress accumulation of chromium in the treated organisms (Plate8;Plate9) (Das *et al*.,2014)[6].

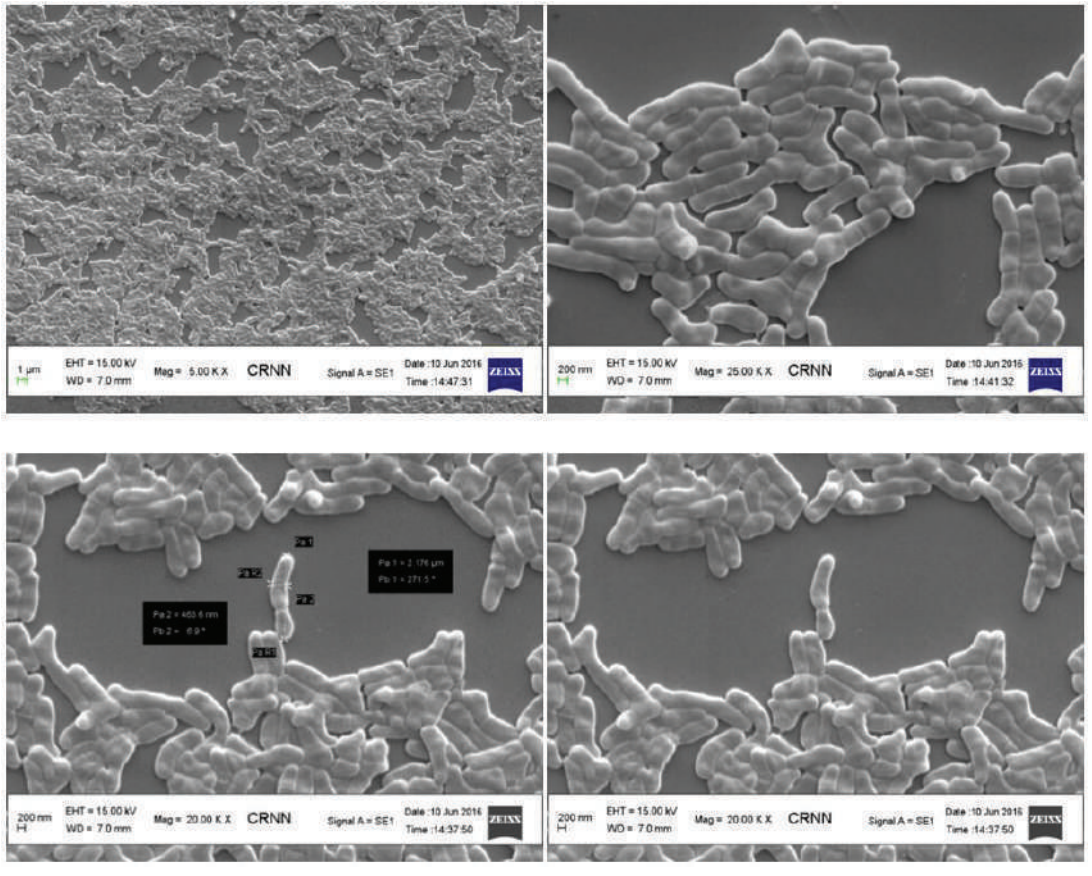

Plate 6. Control images of TW4 under SEM

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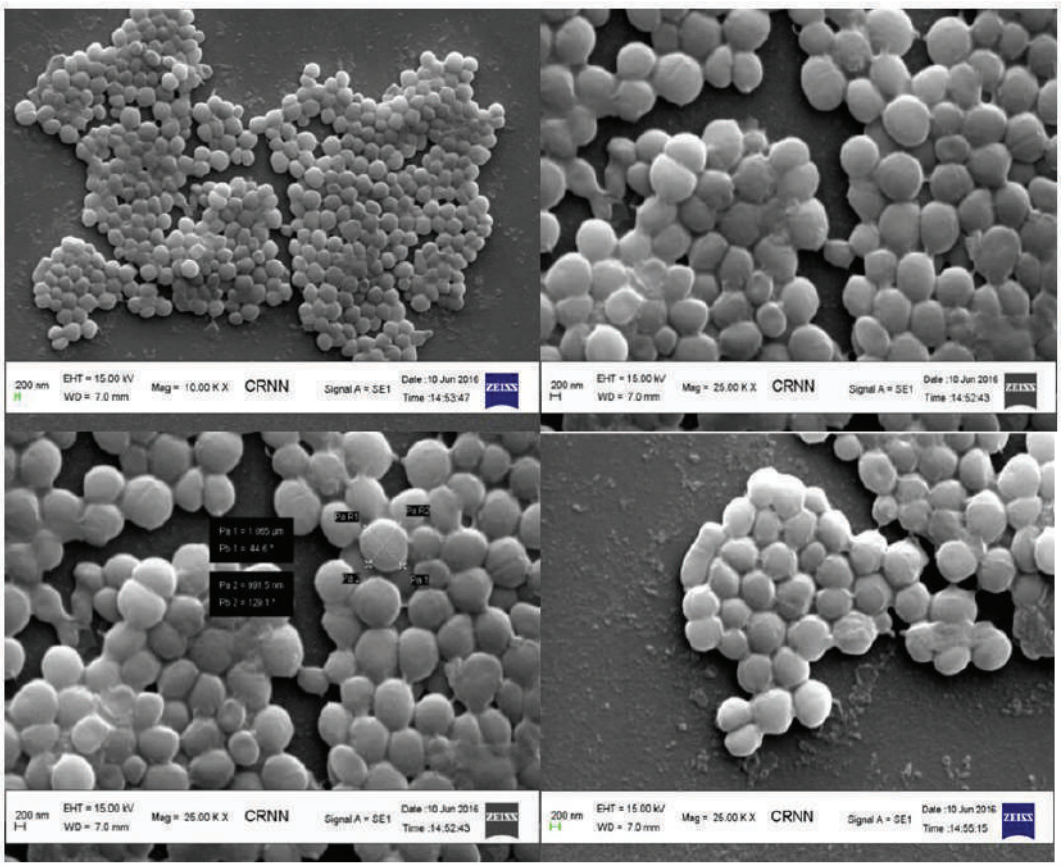
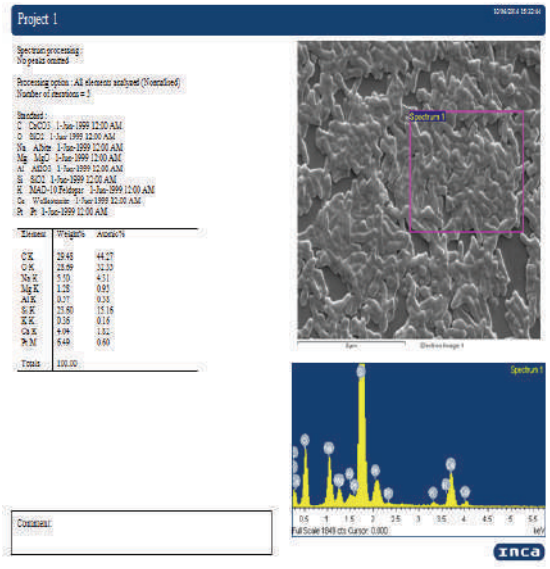


Plate 7. SEM images of TW4 at 100ppm of Cr (VI)



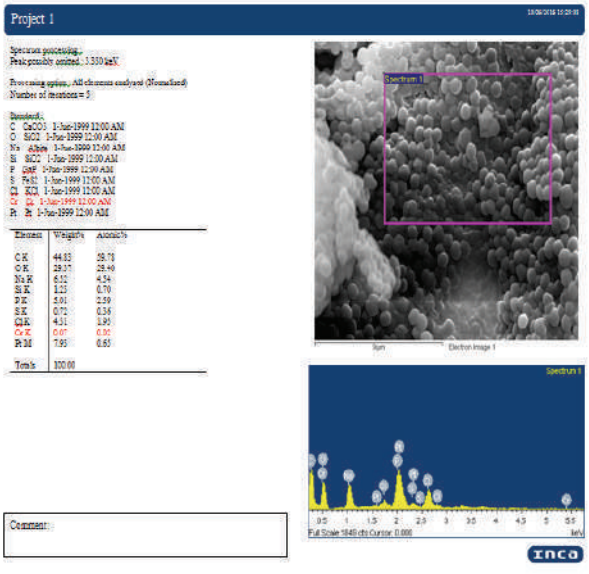


Plate 8. EDX analysis of control sample of TW4

Plate 9. EDX analysis of sample TW4 treated with 100ppm of Cr (VI)

4. Conclusion

Till date, not much is known about the Isoptericola genus in general. Therefore, these results add a clue as to their function in the environment especially with respect to

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their morphological changes related to chromium stress. These results demonstrate that *Isoptericola* sp. TW4 was resistant to high level of chromiumand showed a rapid morphological change (from rod to coccus) under high stress of chromium. The bacterium also showed stress accumulation of trace amount of chromium. Therefore, the *Isoptericola* sp. TW4 used in this study could be exploited for remediation of soil and waste streams contaminated with chromium.

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ARTICLE

Effect of Different Levels of Water Soluble Phosphorus in Complex Fertilizers on Crop Productivity and Soil Health

D. V. Bhagat[1], S. N. Gawade[1], R. C. Sharma[1]*, A. P. Kale[1], J. A. Shaikh[1], P Banik[2]

[1] Research & Development Dept., Bio-Agro Research, RCF Ltd., Chembur, Mumbai-400074

[2] Agriculture and Ecological Research Unit, Indian Statistical Institute, Kolkata 700 108 (WB)

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ABSTRACT

Field experiments were undertaken on sandy soils with three cropping systems at Giridih, Jharkhand, India for two years during 2012-2014. The experiments were executed in split plot design by assigning water soluble phosphorus (WSP) fertilizers in main-plot and recommended dose of phosphorus (RDP) in sub-plot with three replications. The maximum economical yield of rice (4705 kg/ha), baby corn (842 kg/ha) and Chickpea (920 kg/ha) were recorded with the application of 30% WSP. The maximum economical yield of successive crops - wheat (3185 kg/ha), mustard (1720 kg/ha) and groundnut (1578 kg/ha) were recorded with the application of 30% WSP and 100% RDP treatment. Almost similar trends were noticed in terms of by-product yield, nutrient uptake and residual soil fertility status. All the levels of WSP (30% - 89%) in complex fertilizers were found to be equally effective for grain yield, straw yield, nutrient uptake, and residual soil fertility.

1. Introduction

Phosphorus is the second most deficient nutrient in agriculture production systems around the world next to nitrogen (Balemi and Negisho, 2012)[3]. It is an essential element for plant growth. It plays an important role in photosynthesis, energy transfer and storage. Plant growth is restricted unless the soil contains adequate level of phosphorus or it is supplied to soil from external source (Tekchand and Tomar, 1993; Tomar, 2000; Setia and Sharma, 2007)[28][29][26]. The fraction of soil phosphorus utilized for crop growth is called as 'Available Phosphorus'. Phosphorus is absorbed by plants mostly the primary and secondary orthophosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) which are present in soil solution. The amount of each form present depends primarily on soil pH. At pH 7.22 there are approximately equal amount of $H_2PO_4^-$ and HPO_4^{2-}. Plant uptake of HPO_4^{2-} is much slower than $H_2PO_4^-$. The amount of P held in the cycling pools of soil P (i.e. plant-available P) is the working capital of the soil that determines crop productivity.

**Corresponding Author:*
R. C. Sharma.
Research & Development Dept., Bio-Agro Research, RCF Ltd., Chembur, Mumbai-400074.
E-mail: rcsharma@rcfltd.com

DOI:

High quality rock phosphate is a finite source and there is an on-going debate about the longevity of global P resources (Cordell *et al*. 2009; Van Kauwenbergh 2010)[7] [14]. Phosphorus is important for sustainable agricultural production and global food security. To ensure equitable use of scarce P resources, inefficiencies in P use in agriculture needs to be addressed. Diammonium phosphate (DAP) is the most commonly used P-fertilizer throughout South-East Asia. However, raw materials required for its production are being imported resulting in drain of foreign exchange. Keeping this and the anticipated short supply of sulphur in the world market, fertilizer manufacturers have introduced nitrophosphates as an alternative (Khurana *et al*. 2003)[15].

Nitrophosphates with a combination of water soluble phosphate (30–60%) and citrate-soluble phosphate offer an optimal solution to increase P-fertilizer use efficiency for majority of crops on varied soils (Wichmann 1977) [32]. Energy requirements of nitrophosphate production are 20% lower than sulphur-based P-fertilizers. From environmental point of view, nitrophosphate manufacturing does not lead to the generation of sulfur dioxide, large volumes of solid wastes and waste waters (Anonymous 1988)[1]. On the other hand, phosphoric acid- and sulphuric acid-based P-fertilizers have a major problem of phosphogypsum disposal (Reuvers and Lee 1994)[22]. Carbon dioxide produced during nitrophosphate manufacturing is consumed in N fertilizer production (Anonymous 1994)[2]. In addition, nitrophosphate production process has the ability to accommodate low-grade phosphate rock by removing inerts (Bonekamp 1984)[5].

There has an interest in the research that how much water soluble P should be present in different kind of fertilizer for getting higher P-use efficiency and optimum crop yield. With this background, experiments were designed to study the effect of different levels of water soluble P in complex fertilizers in different cropping systems at Giridih, Jharkhand, India.

2. Methodology

2.1 Experimental Site

The three experiments on different cropping systems were conducted at Giridih, Jharkhand, India (Table). The mean maximum temperature is generally recorded in the month of June (40–45 °C) and minimum temperature in January (2–5 °C). The average annual rainfall is 1349 mm of which 82% occurs within the monsoon period (June–September). Relative humidity ranges from 78% to 95%. Annual potential evapo-transpiration (PET) is 1293 mm. The mean daily evaporation reaches a maximum of 12–15 mm per day in June and a minimum of 0.5–0.7 mm per day in January. The mean wind velocity varies from 3.5 km hr^{-1} during October to 6.4 km hr^{-1} during April. The physico-chemcial parameters of study soils are given in Table 1.

Table 1. The physico-chemcial parameters of study soils.

Parameter	Rice-wheat	Baby corn-Mustard	Chick-pea-Groundnut
pH	6.10	5.80	5.60
Conductivity (dSm^{-1})	0.12	0.17	0.14
Organic carbon (%)	0.33	0.29	0.25
Available N	135	169	226
Available P_2O_5	9.30	11	7.0
Available K_2O	225	244	258

2.2 Experimental Design

A split plot design with three replications was used in the study by allocating levels of water soluble Phosphorus (WSP) in complex P-fertilizers in main-plots and three recommended dose of phosphate treatments in sub-plots. Four levels of water soluble Phosphorus (WSP) in complex fertilizers viz T_1: 30 % WSP, T_2: 60 % WSP, T_3: 80 % WSP, T_4: 100 % WSP along with a T_0: Absolute Control was taken in main-plot. To observe the residual effect, three levels of recommended doses of phosphate (RDP) viz P_1: 50 % RD of phosphorus, P_2: 100 % RD of phosphorus along with P_0: Control was accommodated in sub-plot. Source of P-fertilizers and their properties are mentioned in Table 2. Overnight water-soaked seeds of each different crop were sown at a depth of 3–5 cm below the soil surface. The full dose of P and K, and 50% N was applied at the time of sowing. The remaining 50% N was applied in two equal doses as band application at 20 and 40 days after sowing. Weeding was done twice to keep the field weed free.

2.3 Soil Sampling and Analysis

Soil samples from 0 to 20 cm depth were collected after each crop harvest, air dried, and sieved (2 mm mesh). Soil organic carbon (SOC) was analyzed by the wet oxidation method (Walkley and Black 1934)[31]. Soil available nitrogen was estimated by alkaline potassium permanganate (Subbiah and Asija 1956)[27], phosphorus by sodium bicarbonate (Olsen *et al*. 1954)[20] and potassium by ammonium acetate (Hanway and Heidel 1952)[9] method. A suspension of soil and water 1:2.5 and 1:5 was used to determine the pH and electrical conductivity (EC), respectively (Jackson 1973)[13].

2.4 Sstatistical Analysis

Collected data were subjected to statistical analysis in a split-plot design (Gomez and Gomez 1984)[8]. Least sig-

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Table 2. Source of fertilizer and their properties

SPECIFICATION	Nitrophosphate with potash "Suphala" (15:15:15)	Nitrophosphate "Suphala" (20:20:0)	Urea Ammonium Phosphate (20:20:0)	Diammonium phosphate (18:46:0)
Total nitrogen (%)	15.0	20	20	18.0
Ammonical nitrogen (%)	7.5	10	6.4	15.5
Nitrate nitrogen (%)	7.5	10	0.0	0.0
Urea nitrogen (%)	0.0	0.0	13.6	2.5
Neutral ammonium citrate soluble P_2O_5(%)	15.0	20	20	46.0
Water soluble P_2O_5 (%)	4.0 (27%)*	12 (60%)*	17 (85%)*	41.0 (89%)*
Water soluble K_2O (%)	15	0.0	0	0
Fertilizer treatment	T_1	T_2	T_3	T_4

**Figures in parenthesis are percent water soluble phosphorus.*

nificant difference (LSD) was worked out where variance ratio (*F* test) was significant and presented/tested at 5% level of significance.

3. Results

3.1 Grain and Straw Yield

Rice and baby corn grown in *Kharif* season and chickpea in *Rabi* with four levels of WSP- water soluble phosphorus in complex fertilizers along with control plots to note their effects on grain and straw yields. A minor variation was recorded in the yield data over the years may be due to the environmental conditions. The maximum economical yield of rice (4705 kg/ha), baby corn (842 kg/ha) and Chickpea (920 kg/ha) were recorded with the application of 30% WSP. Similar trend was noticed in terms by-product yield of rice (6675 kg/ha), baby corn (3264 kg/ha) and Chickpea (1694 kg/ha). Although there was no significant difference observed among the fertilizer treatments with respect to grain and straw yield. The minimum yields were noted where no phosphatic fertilizer was applied (Table 3-5).

Wheat and mustard in *Rabi* season and groundnut in *Kharif* were grown on residual soil fertility of previous phosphorus treatments and three levels of recommended dose of phosphorus (RDP) viz 0%, 50% and 100%. A minor variation was recorded in the yield data over the years may be due to the environmental conditions. Irrespective of crop, economic yield and by-product yield were increased with increasing the levels of RDP. The maximum economical yield of wheat (3185 kg/ha), mustard (1720 kg/ha) and groundnut (1578 kg/ha) were recorded with the application of 30% WSP and 100% RDP treatment. Similar trend was noticed in terms by-product yield of wheat (4496 kg/ha), mustard (2365 kg/ha) and groundnut (2667 kg/ha). Although there was no significant difference observed amongst the fertilizer treatments and RDP in terms of economic and by-product yields. The minimum yields were observed where no P-fertilizer was applied (Table 3-5).

3.2 Nutrient uptake

Direct and residual effect of different levels of WSP in complex fertilizers on nutrients uptake (i.e. N, P & K) by crops are presented in Table 6-8. The maximum nutrients (NPK) up taken by rice (87.3 N, 14.4 P and 105.9 K kg/ha), baby corn (70.9 N, 8.67 P & 111.9 K kg/ha) and Chickpea (66.7 N, 15.4 P & 84.1 K kg/ha) were recorded with the application of 30% WSP. Although there was no significant difference observed amongst the fertilizer treatments. The minimum nutrients uptake was noted where no phosphatic fertilizer was applied (Table 6-8).

Follow-up crops; wheat, mustard and groundnut were grown on residual soil fertility of previous phosphorus treatments and with three levels of RDP viz 0%, 50% and 100%. Irrespective of crop, nutrients uptake was increased with increasing the levels of RDP. The maximum uptake of nutrient by wheat (50.9 N, 12.1 P & 96.4 K kg/ha), by mustard (72.8 N, 8.2 P & 101.3 K kg/ha) and by groundnut (91.5 N, 16.3 P & 93.4 K kg/ha) were recorded with the application of 30% WSP and 100% RDP treatment. However, there was no significant difference observed amongst the fertilizer treatments. The minimum nutrients uptake was observed where no P-fertilizer was applied (Table 6-8).

3.3 Residual soil fertility

Residual soil fertility in terms of N, P & K was remarkably influenced by direct and residual effect of different levels of WSP in complex fertilizers Table 9-11. The max-

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Table 3. Direct effect of different levels of water soluble phosphorus in complex fertilizer on rice yield and residual effect on wheat yield and system productivity.

Treatment	Rice				Wheat			
	Grain yield (kg/ha)		Straw yield (kg/ha)		Grain yield (kg/ha)		Straw yield (kg/ha)	
	2012	2013	2013	2014	2012	2013	2013	2014
P_0T_0	3034	4020	4339	5668	2220	2620	3130	3713
P_0T_1	4090	5320	5849	7501	2430	3030	3426	4294
P_0T_2	4050	5259	5792	7416	2400	2970	3384	4208
P_0T_3	4000	4950	5720	6980	2370	2760	3342	3911
P_0T_4	3940	4674	5634	6590	2280	2710	3215	3840
P_1T_0	--	--	--	--	2340	2937	3311	4317
P_1T_1	--	--	--	--	2650	3143	3750	4620
P_1T_2	--	--	--	--	2610	2841	3693	4177
P_1T_3	--	--	--	--	2580	2790	3651	4101
P_1T_4	--	--	--	--	2550	2770	3608	4072
P_2T_0	--	--	--	--	2440	3095	3418	4333
P_2T_1	--	--	--	--	2870	3550	4021	4970
P_2T_2	--	--	--	--	2820	3470	3951	4858
P_2T_3	--	--	--	--	2790	3390	3909	4746
P_2T_4	--	--	--	--	2800	3410	3923	4774
Sem± (Main)	34.71	32.88	52.07	54.91	27.33	23.71	36.22	32.40
Sem± (Sub)					12.56	12.21	20.74	19.50
CD (p= 0.05) Main	80.05	75.83	120.08	126.64	63.03	54.68	83.52	74.71
CD (p= 0.05) Sub					26.20	25.48	43.27	40.67

imum residual soil fertility after harvesting of rice (187.4 N, 25.4 P and 280.9 K kg/ha), baby corn (231.3 N, 17.1 P & 288.6 K kg/ha) and Chickpea (304.2 N, 9.2 P & 354.6 K kg/ha) were recorded with the application of 30% WSP. Although there was no significant difference observed amongst the fertilizer treatments. The minimum nutrients uptake was noted where no phosphatic fertilizer was applied.

Follow-up crops; wheat, mustard and groundnut were grown on residual soil fertility of previous phosphorus treatments and with three levels of RDP viz 0%, 50% and 100%. Irrespective of crop, residual soil nutrients were increased with increasing the levels of RDP. The residual soil fertility was recorded the highest with the application of 30% WSP and 100% RDP treatment for wheat (218.6 N, 19.2 P & 323.9 K kg/ha), mustard (261.7 N, 20.1 P & 299.6 K kg/ha) and groundnut (287.8 N, 10.2 P & 322.9 K kg/ha). However, there was no significant difference observed amongst the fertilizer treatments. The minimum residual soil nutrients were observed where no P-fertilizer was applied (Table 6-8).

4. Discussions

4.1 Grain and Straw Yield

Irrespective of crops or/and cropping system, crop grain and straw yield were statically at par with the application P-fertilizers having different WSP (30-89%). Similar results were also observed by Saha *et al*., 2014[25]; Khurana *et al*., 2003 & 2004[16]. The phosphorus nutrition of plants is predominantly controlled by P dynamics in the soil/ rhi-

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Table 4. Direct effect of different levels of water soluble phosphorus in complex fertilizer on baby corn and residual effect on mustard and system productivity.

Treatment	Baby corn				Mustard			
	Cob yield (kg/ha)		Straw yield (kg/ha)		Grain yield (kg/ha)		Straw yield (kg/ha)	
	2012	2013	2013	2014	2012	2013	2013	2014
P_0T_0	436	510	1683	1984	1010	1105	1374	1503
P_0T_1	810	874	3127	3400	1252	1471	1703	2001
P_0T_2	783	843	3022	3279	1195	1411	1625	1919
P_0T_3	757	844	2922	3283	1180	1389	1605	1889
P_0T_4	717	840	2768	3268	1167	1376	1587	1871
P_1T_0	--	--	--	--	1181	1230	1618	1685
P_1T_1	--	--	--	--	1398	1555	1915	2130
P_1T_2	--	--	--	--	1384	1505	1896	2062
P_1T_3	--	--	--	--	1329	1478	1821	2025
P_1T_4	--	--	--	--	1294	1448	1773	1984
P_2T_0	--	--	--	--	1248	1335	1716	1836
P_2T_1	--	--	--	--	1667	1773	2292	2438
P_2T_2	--	--	--	--	1599	1737	2199	2388
P_2T_3	--	--	--	--	1514	1703	2082	2342
P_2T_4	--	--	--	--	1501	1698	2064	2335
Sem± (Main)	23.07	31.05	89.01	121.34	24.12	24.77	29.66	32.95
Sem± (Sub)					14.88	12.68	18.30	16.86
CD (p= 0.05) Main	53.21	86.25	205.21	336.16	55.61	57.12	68.40	75.97
CD (p= 0.05) Sub					31.03	26.45	38.17	35.18

zosphere- plant continuum. The concentration of available soil phosphorus seldom exceeds 10 mM (Bieleski, 1973)[4], which is much lower than that in plant tissues where the concentration is approximately 5 to 20 mM phosphorus (Raghothama, 1999)[21]. Because of the low concentration and poor mobility of plant-available phosphorus in soils, applications of P-fertilizers are needed to improve crop growth and yield. The chemical and biological processes in the rhizosphere determine mobilization and acquisition of soil nutrients as well as microbial dynamics. These processes also control nutrient use efficiency of crops, and thus profoundly influence crop productivity (Hinsinger *et al*., 2009; Richardson *et al*., 2009; Wissuwa *et al*., 2009; Zhang *et al*., 2010)[11][23][33][34]. In a long-term study on calcareous soils of Haryana, Meelu *et al*. (1977)[19] and Chaudhary *et al*. (1979)[6] did not found significant difference in grain yield @ 120 kg P_2O_5 ha^{-1} application through water soluble sources SSP, DAP, UAP and nitrophosphate of 30% WSP. Similarly, Saha *et al*., 2013[24] and Khuran *et al*., 2004[16] reported effectiveness of nitrophosphate at par with SSP, DAP and UAP in terms of crop yield.

4.2 Nutrient Uptake

Irrespective of crop or/and cropping system, data recorded on nutrients uptake in terms of NPK did not show significant differences among the WSP treatments. Because of the unique properties of P in soil such as low solubility, low mobility, and high fixation by the soil matrix, the availability of P to plants is dominantly controlled by two key processes: (1) spatial availability and acquisition of P in terms of plant root architecture and (2) bioavailability and acquisition of P based on the rhizosphere

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Table 5. Direct effect of different levels of water soluble phosphorus in complex fertilizer on chickpea and residual effect on groundnut and system productivity.

Treatment	Chickpea				Groundnut			
	Cob yield (kg/ha)		Straw yield (kg/ha)		Grain yield (kg/ha)		Straw yield (kg/ha)	
	2012	2013	2013	2014	2012	2013	2013	2014
P_0T_0	680	739	1251	1375	1050	1082	1733	1785
P_0T_1	862	969	1586	1802	1312	1402	2165	2313
P_0T_2	823	963	1514	1791	1297	1374	2140	2267
P_0T_3	787	957	1448	1780	1250	1286	2063	2122
P_0T_4	762	956	1402	1778	1275	1310	2104	2162
P_1T_0	--	--	--	--	1221	1355	2039	2263
P_1T_1	--	--	--	--	1498	1592	2502	2659
P_1T_2	--	--	--	--	1344	1491	2244	2490
P_1T_3	--	--	--	--	1302	1432	2174	2391
P_1T_4	--	--	--	--	1294	1438	2161	2401
P_2T_0	--	--	--	--	1325	1372	2239	2319
P_2T_1	--	--	--	--	1410	1746	2383	2951
P_2T_2	--	--	--	--	1325	1685	2239	2848
P_2T_3	--	--	--	--	1403	1644	2371	2778
P_2T_4	--	--	--	--	1365	1583	2307	2675
					159	115	80	93
Sem± (Main)	41	47	89	90	59	33	30	27
Sem± (Sub)					368	265	184	214
CD (p= 0.05) Main	95	98	205	206	124	69	62	56
CD (p= 0.05) Sub					1050	1082	1733	1785

chemical and biological processes. Plants are able to respond to P starvation by changing their root architecture, including root morphology, topology, and distribution patterns (Vance et al., 2003)[30]. Root-induced chemical and biological changes in the rhizosphere play a vital role in enhancing the bioavailability of soil P (Hinsinger, 2001)[10]. Root-induced acidification can decrease rhizosphere pH by 2 to 3 units relative to the bulk soil, resulting in substantial dissolution of sparingly available soil P (Marschner, 1995)[18]. The pH change in the rhizosphere is mainly affected by cation/anion uptake ratios and nitrogen assimilation. Organic acid excretion and function in increasing P mobilization is well documented (Raghothama, 1999; Vance et al., 2003; Hinsinger *et al*., 2005)[21][30][11]. The total P uptake was maximum with water soluble sources (SSP, DAP and UAP) followed by partially water soluble nitrophosphate. The trend of yield with respect to P sources also reflected in the total P uptake by wheat. Such a trend might be explained on the basis of availability of P and solubility of fertilizer in the soil system. Effectiveness of nitrophosphate was statistically at par with other WSP fertilizers (Saha *et al*., 2014; Khurana *et al*., 2003 & 2004)[25][17].

4.3 Residual soil fertility

Irrespective of crops or/and cropping system, residual soil fertility in terms of NPK was recorded higher due to application of nitrophosphates (30% WSP) followed by UAP and DAP. Under P deficiency, plants can develop adaptive responses to facilitate efficient P acquisition and translocation. The phosphorus utilize efficiently by ad-

 DOI:

Table 6. Direct effect and residual effect of different levels of water soluble phosphorus in complex fertilizer on nutrient uptake by rice and wheat.

Treatment	Nutrient uptake by rice (Kg/ha)						Nutrient uptake by wheat (Kg/ha)					
	Nitrogen		Phosphorus		Potassium		Nitrogen		Phosphorus		Potassium	
	2012	2013	2013	2014	2012	2013	2013	2014	2013	2014	2013	2014
P_0T_0	62.71	57.88	11.22	10.13	83.74	64.65	44.95	43.36	8.68	8.22	54.97	53.26
P_0T_1	93.90	80.71	15.32	13.55	116.68	95.13	67.68	52.96	9.80	8.20	81.77	71.03
P_0T_2	89.90	76.87	15.03	13.15	110.23	91.56	62.23	53.02	9.19	7.63	79.59	74.09
P_0T_3	87.97	73.04	14.80	12.95	104.78	89.46	50.62	50.84	8.14	7.34	77.32	71.19
P_0T_4	86.64	75.78	14.44	13.05	103.56	86.98	50.44	47.38	9.04	8.75	62.94	61.15
P_1T_0	--	--	--	--	--	--	45.59	33.27	9.72	7.93	68.84	69.01
P_1T_1	--	--	--	--	--	--	80.58	56.30	10.91	10.21	91.67	84.44
P_1T_2	--	--	--	--	--	--	58.88	49.01	10.62	10.27	90.60	84.67
P_1T_3	--	--	--	--	--	--	41.43	39.07	10.30	10.12	96.36	89.82
P_1T_4	--	--	--	--	--	--	46.87	42.88	10.33	10.02	94.54	82.29
P_2T_0	--	--	--	--	--	--	48.17	47.37	10.91	10.35	73.57	76.97
P_2T_1	--	--	--	--	--	--	62.38	39.56	12.33	11.84	99.38	93.44
P_2T_2	--	--	--	--	--	--	59.27	46.55	12.29	11.59	96.71	94.12
P_2T_3	--	--	--	--	--	--	59.06	40.15	12.41	11.80	95.28	94.08
P_2T_4	--	--	--	--	--	--	51.01	44.47	12.45	11.76	97.04	95.17
Sem± (Main)	9.84	9.29	1.36	2.82	8.69	9.77	6.08	5.24	0.48	0.64	3.95	4.36
Sem± (Sub)							2.75	3.75	0.51	0.44	3.16	3.78
CD (p= 0.05) Main	22.73	21.43	3.15	5.26	19.85	20.93	14.05	11.58	1.19	1.39	9.18	10.70
CD (p= 0.05) Sub							5.75	6.62	1.71	0.94	6.59	6.42

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Table 7. Direct and residual effect of different levels of water soluble phosphorus in complex fertilizer on nutrient uptake by baby corn and mustard.

Treatment	Nutrient uptake by baby corn (Kg/ha)						Nutrient uptake by mustard (Kg/ha)					
	Nitrogen		Phosphorus		Potassium		Nitrogen		Phosphorus		Potassium	
	2012	2013	2013	2014	2012	2013	2013	2014	2013	2014	2013	2014
P_0T_0	56.22	63.42	6.15	6.30	83.16	93.21	45.61	53.54	4.13	4.29	70.20	74.27
P_0T_1	65.54	68.72	8.35	8.99	101.46	108.87	55.86	63.54	5.70	5.53	83.11	87.08
P_0T_2	68.50	66.23	8.10	9.02	104.46	115.44	58.53	67.69	5.97	5.74	87.72	88.72
P_0T_3	69.80	67.39	8.50	8.75	109.73	114.25	59.44	65.76	5.40	5.66	90.90	92.56
P_0T_4	71.69	70.04	8.28	8.59	108.01	113.51	61.73	64.16	5.76	5.45	90.79	96.23
P_1T_0	--	--	--	--	--	--	50.97	56.90	5.68	5.38	77.93	78.39
P_1T_1	--	--	--	--	--	--	61.73	67.35	6.68	6.75	93.87	92.98
P_1T_2	--	--	--	--	--	--	63.44	68.03	7.00	6.05	97.51	91.09
P_1T_3	--	--	--	--	--	--	65.87	67.72	6.51	6.85	97.93	98.82
P_1T_4	--	--	--	--	--	--	64.00	70.33	6.31	6.19	98.04	97.05
P_2T_0	--	--	--	--	--	--	51.70	57.28	6.31	6.39	81.16	83.06
P_2T_1	--	--	--	--	--	--	68.53	76.76	7.70	8.10	97.68	97.68
P_2T_2	--	--	--	--	--	--	66.37	76.68	7.33	8.02	98.16	101.52
P_2T_3	--	--	--	--	--	--	69.08	76.54	7.93	8.52	103.67	99.07
P_2T_4	--	--	--	--	--	--	67.11	69.65	7.11	8.59	101.04	102.27
Sem± (Main)	5.89	6.80	2.53	3.34	6.57	7.00	4.28	7.66	0.78	1.50	2.56	5.04
Sem± (Sub)							2.30	2.34	0.40	0.39	1.70	1.42
CD (p= 0.05) Main	13.44	15.68	5.85	7.71	15.16	16.15	9.86	17.66	1.79	3.45	5.90	11.62
CD (p= 0.05) Sub							4.79	4.88	0.83	0.81	3.54	2.96

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Table 8. Direct and residual effect of different levels of water soluble phosphorus in complex fertilizer on nutrient uptake by chickpea and groundnut.

Treatment	Nutrient uptake by Chickpea (Kg/ha)						Nutrient uptake by Groundnut (Kg/ha)					
	Nitrogen		Phosphorus		Potassium		Nitrogen		Phosphorus		Potassium	
	2012	2013	2013	2014	2012	2013	2013	2014	2013	2014	2013	2014
P_0T_0	54.12	59.55	13.35	3.81	67.24	68.54	60.14	62.18	11.46	12.29	67.01	69.07
P_0T_1	65.05	68.33	16.00	6.48	81.49	86.66	73.39	79.45	13.50	14.90	82.79	89.04
P_0T_2	62.95	67.95	15.53	6.62	81.16	85.37	72.00	78.20	13.29	14.56	80.79	87.29
P_0T_3	62.45	66.45	15.53	6.79	80.80	84.06	68.25	75.71	12.93	14.21	79.35	87.49
P_0T_4	61.38	64.38	15.73	7.51	80.80	82.93	71.05	76.45	12.39	13.84	77.78	86.91
P_1T_0	--	--	--	--	--	--	65.42	68.48	12.68	13.98	72.74	79.29
P_1T_1	--	--	--	--	--	--	82.98	86.71	14.52	15.95	89.25	92.84
P_1T_2	--	--	--	--	--	--	81.66	82.58	14.76	15.30	87.56	90.86
P_1T_3	--	--	--	--	--	--	78.11	79.81	13.76	15.13	85.54	88.08
P_1T_4	--	--	--	--	--	--	79.72	78.65	13.86	14.87	82.24	89.16
P_2T_0	--	--	--	--	--	--	72.96	72.03	13.65	14.35	76.66	82.05
P_2T_1	--	--	--	--	--	--	89.12	93.83	15.35	17.18	92.73	94.03
P_2T_2	--	--	--	--	--	--	87.09	91.50	14.87	16.93	87.35	92.80
P_2T_3	--	--	--	--	--	--	85.76	89.62	14.94	16.76	86.31	91.00
P_2T_4	--	--	--	--	--	--	86.30	88.96	14.99	16.81	85.64	90.68
Sem± (Main)	4.265	3.583	0.563	0.773	1.103	1.236	10.23	7.13	3.08	2.92	4.46	5.60
Sem± (Sub)							5.14	4.66	1.01	1.29	2.79	3.25
CD (p= 0.05) Main	9.834	8.262	1.297	1.783	2.544	2.852	23.62	16.44	7.15	6.73	10.28	8.95
CD (p= 0.05) Sub							10.73	9.73	2.10	2.56	5.83	2.54

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Table 9. Direct and residual effect of different levels of water soluble phosphorus in complex fertilizer on residual soil fertility after harvest of rice and wheat.

Treatment	Residual soil fertility after rice (Kg/ha)						Residual soil fertility after wheat (Kg/ha)					
	Nitrogen		Phosphorus		Potassium		Nitrogen		Phosphorus		Potassium	
	2012	2013	2013	2014	2012	2013	2013	2014	2013	2014	2013	2014
P_0T_0	158.16	167.37	12.76	13.81	235.35	247.45	162.26	170.01	10.98	12.32	270.50	277.39
P_0T_1	185.69	189.06	23.93	26.80	275.58	286.15	201.02	198.61	15.52	15.78	284.38	291.57
P_0T_2	182.96	183.81	21.76	24.45	270.62	283.77	197.03	193.85	15.12	15.67	285.65	287.81
P_0T_3	178.16	179.38	21.64	23.39	265.88	276.11	187.34	192.26	14.87	15.34	280.58	282.53
P_0T_4	174.15	183.13	20.74	23.06	266.45	274.83	171.42	177.96	14.91	15.12	281.42	281.20
P_1T_0	--	--	--	--	--	--	189.10	193.85	12.28	14.22	275.34	284.48
P_1T_1	--	--	--	--	--	--	211.35	201.79	18.12	19.12	300.44	308.09
P_1T_2	--	--	--	--	--	--	201.12	206.56	17.97	18.89	295.84	305.63
P_1T_3	--	--	--	--	--	--	179.34	176.37	17.52	18.57	300.44	307.47
P_1T_4	--	--	--	--	--	--	162.32	177.96	17.5	17.84	302.38	304.48
P_2T_0	--	--	--	--	--	--	170.24	174.78	13.38	16.72	280.65	293.81
P_2T_1	--	--	--	--	--	--	232.27	204.97	19.54	18.94	305.48	342.35
P_2T_2	--	--	--	--	--	--	209.11	198.61	18.98	19.08	300.65	323.43
P_2T_3	--	--	--	--	--	--	196.15	196.42	18.43	18.87	297.77	328.48
P_2T_4	--	--	--	--	--	--	194.95	190.67	18.03	18.67	300.86	326.24
Sem± (Main)	14.59	16.62	9.07	3.98	13.41	15.98	9.55	34.47	8.57	6.16	31.30	22.69
Sem± (Sub)							6.14	22.44	6.49	4.86	24.18	18.28
CD (p= 0.05) Main	33.57	38.33	20.86	9.19	30.95	36.86	21.96	79.49	19.76	14.15	72.17	52.29
CD (p= 0.05) Sub							12.79	46.81	13.54	10.14	50.44	38.06

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Table 10. Direct and residual effect of different levels of water soluble phosphorus in complex fertilizer on residual soil fertility after harvest of baby corn and mustard.

Treatment	Residual soil fertility after baby corn (Kg/ha)						Residual soil fertility after mustard (Kg/ha)					
	Nitrogen		Phosphorus		Potassium		Nitrogen		Phosphorus		Potassium	
	2012	2013	2013	2014	2012	2013	2013	2014	2013	2014	2013	2014
P_0T_0	204.15	206.44	13.42	12.77	263.35	271.01	184.43	199.00	11.80	12.45	253.55	268.99
P_0T_1	225.24	231.38	16.59	17.41	281.59	295.55	198.61	239.23	15.36	16.40	270.55	277.33
P_0T_2	227.43	235.12	15.99	17.95	278.56	297.39	202.52	242.06	14.88	16.77	276.85	279.20
P_0T_3	226.98	233.45	16.70	17.58	282.99	290.26	210.33	236.42	15.35	17.09	275.38	283.36
P_0T_4	229.52	235.77	16.47	17.82	284.38	293.34	212.34	241.12	14.96	16.15	278.36	280.44
P_1T_0	--	--	--	--	--	--	204.89	208.64	12.89	15.67	262.45	272.69
P_1T_1	--	--	--	--	--	--	214.89	242.08	17.38	18.94	290.37	280.00
P_1T_2	--	--	--	--	--	--	219.52	249.56	16.88	18.71	288.12	283.40
P_1T_3	--	--	--	--	--	--	217.88	247.09	17.66	19.10	282.36	285.87
P_1T_4	--	--	--	--	--	--	219.07	241.20	16.94	18.97	286.60	279.68
P_2T_0	--	--	--	--	--	--	216.33	216.78	14.56	16.24	275.38	283.49
P_2T_1	--	--	--	--	--	--	247.15	269.42	20.36	19.90	298.80	292.88
P_2T_2	--	--	--	--	--	--	250.53	259.00	19.44	19.85	302.88	293.40
P_2T_3	--	--	--	--	--	--	254.70	257.50	19.65	19.80	300.85	298.37
P_2T_4	--	--	--	--	--	--	259.43	263.98	19.84	19.21	305.38	293.75
Sem± (Main)	17.85	17.29	5.10	3.22	8.29	23.75	6.46	20.67	11.56	3.88	4.59	12.37
Sem± (Sub)							6.40	8.76	5.60	2.14	7.54	12.46
CD (p= 0.05) Main	41.16	39.86	11.75	7.42	19.12	54.76	14.90	47.67	26.67	8.94	10.58	28.53
CD (p= 0.05) Sub							13.35	18.28	11.68	4.46	15.72	25.98

 DOI:

Table 11. Direct and residual effect of different levels of water soluble phosphorus in complex fertilizer on residual soil fertility after harvest of Chickpea and Groundnut.

Treatment	Residual soil fertility after Chickpea (Kg/ha)						Residual soil fertility after Groundnut (Kg/ha)					
	Nitrogen		Phosphorus		Potassium		Nitrogen		Phosphorus		Potassium	
	2012	2013	2013	2014	2012	2013	2013	2014	2013	2014	2013	2014
P_0T_0	257.46	268.46	7.81	8.24	303.33	324.41	228.83	239.16	7.02	7.51	273.33	279.89
P_0T_1	299.22	309.23	8.82	9.51	335.50	373.65	260.95	269.43	8.44	8.92	298.91	308.75
P_0T_2	294.56	304.97	8.00	9.37	329.83	365.71	259.92	267.85	8.83	8.87	293.75	302.17
P_0T_3	291.97	301.56	7.84	9.11	322.67	357.41	257.95	261.33	7.96	8.77	291.28	297.33
P_0T_4	289.23	299.22	7.67	8.92	316.17	356.29	253.37	259.36	8.07	8.59	187.41	296.68
P_1T_0	--	--	--	--	--	--	249.40	255.35	7.62	7.73	286.07	293.71
P_1T_1	--	--	--	--	--	--	267.77	281.71	8.87	9.97	313.67	319.87
P_1T_2	--	--	--	--	--	--	266.44	278.54	8.97	9.87	308.88	314.61
P_1T_3	--	--	--	--	--	--	265.20	275.18	8.78	9.86	304.98	310.02
P_1T_4	--	--	--	--	--	--	262.26	274.03	8.59	8.69	302.92	308.37
P_2T_0	--	--	--	--	--	--	253.45	256.94	7.93	8.11	291.06	302.35
P_2T_1	--	--	--	--	--	--	281.54	294.14	9.87	10.50	319.22	326.54
P_2T_2	--	--	--	--	--	--	278.86	289.66	9.55	10.10	312.60	321.87
P_2T_3	--	--	--	--	--	--	279.04	286.59	8.95	9.87	309.88	314.62
P_2T_4	--	--	--	--	--	--	274.86	284.06	8.69	9.65	306.97	311.77
Sem± (Main)	23.83	22.98	5.40	8.38	14.72	24.12	23.67	20.48	5.85	7.96	21.49	82.61
Sem± (Sub)							14.67	19.48	3.78	4.57	13.14	43.16
CD (p= 0.05) Main	54.96	52.99	12.45	19.32	33.95	55.62	54.59	47.23	13.48	18.36	49.55	42.10
CD (p= 0.05) Sub							30.61	40.63	7.88	9.54	27.42	29.62

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justing P recycling internally, limiting P consumption, and reallocating P from old tissues to young and/or actively growing tissues (Marschner, 1995)[18]. Taken together, plants have developed a series of adaptive responses to take up and utilize P efficiently, including morphological, physiological, and biochemical responses.

5. Conclusion

Study data showed that all four levels of WSP in P-fertilizers (i.e. 30 %, 60%, 85% and 89%) and three cropping systems namely Rice-wheat, Baby corn-Mustard and Chickpea-Groundnut at Giridih, Jharkhand, India were found to be equally effective for crop yield, nutrient uptake, and soil fertility. However, all studied parameters were increased with increasing the levels of phosphorus (i.e. 0%, 50% RDF and 100% RDF).

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