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Effect of Brick kiln's emission on Plant growth promoting soil bacteria

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ABSTRACT

Plant growth promoting soil bacteria were isolated from the soil near brick kiln and half km away from brick kiln by culturing on Ashby's medium. Their phosphate solubilizing capacity, potassium solubilizing capacity and HCN production activity were measured. Soil was collected from 50m away, 100m away and 150m away from brick kiln at each side i.e. North, South, East and West. The CFU/gm value of plant growth promoting soil bacteria at a distance of 50m, 100m and 150m away from brick kiln was very poor in comparison to the CFU/gm value of these bacteria half km away from brick kiln. The lowest value of CFU/gm was observed in the soil 100m away from brick kiln in its East side ($15X10^6$) followed by 150m away ($20X10^6$) in East side and at 50m away the CFU/gm value was ($22X10^6$) in its East side while CFU/gm value of these bacteria in soil at a distance of half km away was measured as $32X10^6$ CFU/gm.

Key Words - Ashby's medium, Phosphate solubilizing capacity, Potassium solubilizing capacity, HCN, CFU/gm.

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INTRODUCTION

Rapid urbanization created a booming in construction industries. Bricks are the principle building material for construction. Due to heavy demand of bricks several brick kilns are opening in different parts of country. The brick kilns emission adversely affect the environment of surroundings (Biswas *et al.,* 2018). The emission of brick kiln contain Carbon dioxide, carbon monoxide, sulphur oxides, nitrogen oxides and heavy metals like Pb, Co, Ni, Cr and particulate matters. The particulate matters, heavy metals and dust deposits in surrounding area of brick kiln which adversely affect the soil fertility and soil microbial diversity. The brick kiln emissions significantly changes the physico-chemical properties and nutrient status of nearby soil (Sarkar *et al.,* 2016). The burning of enormous carbon and nitrogen in brick kiln degrade the soil and led to threat for atmospheric pollution and climate change (Khan *et al.,* 2007). The pollutants of brick kiln emission reduces crop yield, vegetational growth, microbial activity and nutrient cycle (Sharma-2000). Heavy metals generated from the brick furnace are areal pollutants which deposit shortly in surrounding area and distributed in soil. Enormous amount of heavy metals can be deposited in the biosphere which is 20 times higher than removal rate (Ravankhah *et al.,* 2017). Heavy metals as a soil pollutant remain persistent and causes toxicity (Li *et al.,* 2015). The concentration of Cadmium and Lead in agricultural soil near brick kiln area was outlined to be more than regulatory standards imposed by US environmental protection agency (Ismail *et al.,* 2012). Adrees *et al.,* (2016) reported that high concentration of Nickel, Lead

and Cobalt lowers concentration of Calcium, Magnesium and Sodium in soil as well as decreases organic matter content. Saha *et al.,*(2021) reported that the pH value in surrounding area of brick kiln remain acidic and the electrical conductivity becomes nearly double.

Brick kilns are considered harmful for environment as they produces greenhouse gas, black carbon, heavy metals, fluorides, PM_{10} etc as pollutant. Skinder *et al.,*(2014) reported that average emission of gases per thousand bricks were 6.35 to 12.3kg of CO, 0.52 to 5.9 kg of SO_2 and 0.64 to 1.4 kg of particulate matter.

MATERIAL & METHOD

The brick kiln in Mathahi village of Madhepura district was selected for the present study. Soil samples were collected from a distance of 50mt, 100mt and 150mt from North, South, East and West side of brick kiln and half km away from brick kiln. Soil samples were brought to the laboratory for microbial examination.

Isolation of Bacteria: For the isolation of bacteria, 1gm soil from each sample was dissolved in 10ml distilled water and diluted up to 10^{-5} dilution. Dilution was inoculated in culture media. Three culture media were used in the present study which are Nutrient agar, Ashby's medium and Cetrimide agar medium. The culture was incubated at 30°C temperature for 24 hours. Colonies were counted and CFU value in each medium was calculated by the formula:

$$CFU/_{gm} = \frac{No.of\ Colonies\ X\ Dilution\ factor}{Volume\ of\ inoculum}$$

The bacteria were identified on the basis of their morphology, Gram staining, biochemical test, sugar fermentation test and growth in selective media. For morphological study, slides were prepared from each culture and examined under microscope. Gram stain was used for the preparation of slide. Biochemical tests were performed for Catalase, Oxidase, Nitrate reductase, VP, and Methy red tests. For Sugar fermentation test culture was tested against Glucose, Fructose, Lactose, Mannitol, Raffinose and Arabinose.

For the identification and characterization of Plant growth promoting bacteria, bacterial culture of Ashby's medium was tested for Nitrogen fixation, HCN production, IAA production, Phosphate solubilization and Potash solubilization.

Estimation of Nitrogen fixation: All isolates were inoculated in Ashby's broth. One set of broth was kept without inoculation. Culture were incubated for 10 days at 37°C temperature. Percentage Nitrogen was determined by Kjeldahl method.

After 10 days incubation, 250ml isolate containing broth was transferred in Kjeldahl microflask. Digestion salt mixture and 3ml Conc. Sulphuric acid was added and digested on sand bath. After digestion, 10ml distilled water was added and cooled (Digestion salt mixture contain Potassium sulphate, Copper sulphate and metallic selenium in a ratio of 50:10:01).

Digested sample was added in Kjeldahl distillation apparatus. 10ml 40% NaOH was added in distillation flask. 10ml 40% Boric acid and 3 to 4 drops Bromo-cresol reagent was taken in a conical flask. The conical flask was connected to the condenser of Kjeldahl apparatus. After distillation, the content of conical flask was titrated against HCl. Appearance of Pink color showed end point. Similar experiment was conducted with Ashby's broth without isolate which was the blank. Percentage Nitrogen was estimated by the formula:-

$$\%\ Nitrogen = \frac{Sample\ titre - Blank\ titre}{Volume\ of\ sample} X\ Normality\ of\ HCl\ X\ 14X100$$

HCN Production: HCN production was tested on HCN induction medium.

The preparation of Chromazurol-s reagent is mentioned as follows:

Preparation of Chromazurol-s reagent:

i). All glassware were cleaned with 6M HCl and rinse with de-ionized water.

ii). Solution 1: 0.06 g of CAS (Fluka Chemicals) was dissolved in 50 ml of ddH_2O.

iii). Solution 2: 0.0027 g of $FeCl_3$-6 H_2O was dissolved in 10 ml 10 mM HCl.

iv). Solution 3: 0.073 g of HDTMA was dissolved in 40 ml of ddH_2O.

v). Solution 1 was mixed with 9 ml of Solution 2 and then Solution 3 was added to prepare Chromazurol-s reagent.

IAA production: For estimation of IAA production 2 days old culture was inoculated in N2-free liquid culture medium supplemented with L-Tryptophan and incubated for 48 hrs. at 30°C. DMACA reagent was mixed and the intensity of developed color was measured at 530nm in spectrophotometer.

Isolation of Potassium solubilizing bacteria: 1 gm of soil sample was mixed thoroughly in 10 ML autoclaved distilled water. Serial dilution was made up to 10^{-6}. Diluted soil samples were plated on Aleksandrow medium. The plates were incubated at 30°C for three days. Colonies exhibiting clear zones were selected as potassium solubilizer. Selected colonies were plated on nutrient agar medium for obtaining pure culture. The pure culture was again inoculated in Aleksandrow medium to confirm their K solubilizing efficiency.

Estimation of Solubilization index and Solubilizing efficiency: Qualitative analysis of K solubilizing activity of various isolates was calculated by measuring halozone and colony diameter on Aleksandrow medium. Halozone and colony diameter was measured using Calipers.

Calculation of Solubilization efficiency (SE) and Solubilization index (SI) were calculated by following formula:

$$SE = \frac{\text{Halozone} - \text{Colony diameter}}{2}$$

$$SI = \frac{\text{Halozone}}{\text{Colony diameter}}$$

Isolation of Phosphorus solubilizing bacteria: 1 gm of soil sample was mixed thoroughly in 10 ML autoclaved distilled water. Serial dilution was made up to 10-6. Diluted soil samples were plated on Pikovskaya medium and NBRIP medium. The plates were incubated at 30°C for three days. Colonies exhibiting clear zones were selected as phosphorus solubilizer. Selected colonies were plated on nutrient agar medium for obtaining pure culture. The pure culture was again inoculated in Pikovskaya medium to confirm their P solubilizing efficiency.

Estimation of Solubilization index and Solubilizing efficiency: Qualitative analysis of P solubilizing activity of various isolates was calculated by measuring halozone and colony diameter on Pikovskaya medium. Halozone and colony diameter was measured using Calipers.

Calculation of Solubilization efficiency (SE) and Solubilization index (SI) were calculated by following formula:

$$SE = \frac{\text{Halozone} - \text{Colony diameter}}{2}$$

$$SI = \frac{\text{Halozone}}{\text{Colony diameter}}$$

RESULT

Soil samples were collected from 50m, 100m and 150m away from the brick kiln of the Mathahi village (Madhepura) in all directions (North, East, South, West) and half km away from brick kiln. Bacteria were isolated from each sample. Soil samples were inoculated in Ashby's medium and bacterial colonies were identified on the basis of their morphological, biological and sugar fermentation tests. Altogether, 5 isolates of *Azotobacter* were isolated. CFU/gm value was observed in each soil sample. The phosphorus solubilizing efficiency, potassium solubilizing efficiency, HCN production, IAA production and Nitrogen estimation of each isolate were performed.

The CFU/gm value of plant growth promoting soil bacteria at a distance of 50m, 100m and 150m away from brick kiln was very poor in comparison to the CFU/gm value of these bacteria half km away from brick kiln. The lowest value of CFU/gm was observed in the soil 100m away from brick kiln in its East side ($15X10^6$) followed by 150m away ($20X10^6$) in East side and at 50m away the CFU/gm value was ($22X10^6$) in its East side while CFU/gm

value of these bacteria in soil at a distance of half km away was measured as $32X10^6$ CFU/gm.

All isolates were Gram negative, catalase, oxidase and citrate positive. Phosphate solubilizing index of isolate No. A1, A2 and A3 were recorded as 5.113, 3.055 and 4.234 respectively. Potassium solubilizing index of isolate No. A1, A2 and A3 were recorded as 4.503, 3.805 and 3.588 respectively.

All isolates were able to produce Siderophore and HCN. IAA production was observed at concentration of 0, 2.5 and 5mM L-tryptophan. The maximum IAA production was observed in 5mM L-tryptophan concentration. At this concentration, the IAA production of isolate No. A1, A2 and A3 were recorded as 31.56 µg/ml, 31.24 µg/ml and 40.06 µg/ml respectively.

Percentage nitrogen content of isolate No. A1, A2 and A3 were recorded as 6.46, 5.50 and 8.14 respectively.

Table 1- CFU/gm value of isolates near Brick kiln and half km away from Brick kiln

Isolate No.	Directions	CFU/gm value near Brick Kiln			CFU/gm value (Reference)
		50m	100m	150m	Half Km away
A1	North	24	18	23	$32X10^6$
	South	23	17	24	
	East	22	15	20	
	West	24	19	20	
A2	North	23	16	22	$34X10^6$
	South	24	17	23	
	East	23	16	21	
	West	26	19	25	
A3	North	24	17	26	$33X10^6$
	South	23	17	25	
	East	22	16	21	
	West	25	19	26	

Table 2- P-Solubilization efficiency and P-solubilization index of isolates

Isolate	Colony Size (mm)	Holozone (mm)	Efficiency (mm)	Solubilization Index
A1	5.28	27.00	10.86	5.113
A2	7.2	22.00	7.40	3.055
A3	6.14	26.00	9.93	4.234

Table 3- K-Solubilization efficiency and K-solubilization index of isolates

Isolate	Colony Size (mm)	Holozone (mm)	Efficiency (mm)	Solubilization Index
A1	6.24	28.10	10.93	4.503
A2	7.20	27.40	10.10	3.805
A3	7.05	25.30	09.12	3.588

Table 4- Screening of PGP properties of isolates

Isolates	P. Sol.		Siderophore	HCN	IAA production(µg/ml)		
					mM L-tryptophan		
	PKV	NBRIP			0	2.5	5
A1	+	+	+	+	3.56	20.05	31.56
A2	+	+	+	+	3.10	20.35	31.24
A3	+	+	+	+	4.5	30.26	40.06

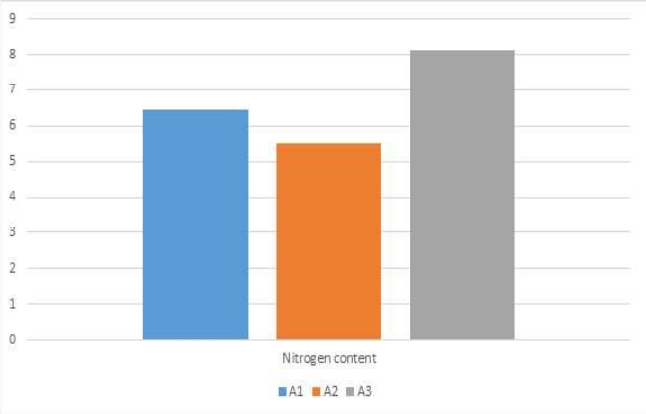

Fig. 1- Percentage Nitrogen content in different isolates

CONCLUSION

Soil samples were collected from 50m, 100m and 150m away from the brick kiln of the Mathahi village (Madhepura) in all directions (North, East, South, West) and half km away from brick kiln. Bacteria were isolated from each sample. Soil samples were inoculated in Ashby's medium and bacterial colonies were identified on the basis of their morphological, biological and sugar fermentation tests. Altogether, 5 isolates of *Azotobacter* were isolated. CFU/gm value was observed in each soil sample. The phosphorus solubilizing efficiency, potassium solubilizing efficiency, HCN production, IAA production and Nitrogen estimation of each isolate were performed. It was observed that the CFU/gm value of all isolates was minimum at a distance of 100m from brick kiln and maximum CFU/gm was observed at half km away from the brick kiln.

All isolates were Gram negative, catalase, oxidase and citrate positive. Phosphate solubilizing index of isolate No. A1, A2 and A3 were recorded as 5.113, 3.055 and 4.234 respectively. Potassium solubilizing index of isolate No. A1, A2 and A3 were recorded as 4.503, 3.805 and 3.588 respectively.

All isolates were able to produce Siderophore and HCN. IAA production was observed at concentration of 0, 2.5 and 5mM L-tryptophan. The maximum IAA production was observed in 5mM L-tryptophan concentration. At this concentration, the IAA production of isolate No. A1, A2 and A3 were recorded as 31.56 µg/ml, 31.24 µg/ml and 40.06 µg/ml respectively.

Percentage nitrogen content of isolate No. A1, A2 and A3 were recorded as 6.46, 5.50 and 8.14 respectively.

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