Materials and Methods

Vegetation analysis

Vegetation analysis was carried out in five brickfields of North Dinajpur and South Dinajpur districts (Table 2).

Table 2 : Brick fields where vegetation analysis were carried out

Sl. No	Name & Address of Brick Industry	Abbreviation	Sub Division	Production / Year
1	Bikash Chandra Kundu Vill: Haripur, P.O. Beltala Park Dist: South (Dakshin) Dinajpur	BCK	Balurghat	8 lakh
2	Balurghat Bricks P.O. Balurghat Vill: Raghunathpur Dist: South (Dakshin) Dijanpur	BB	Balurghat	10 lakh
3	Saraswati Bricks Industry P.O. Dhankoil (Laxmipur) P.S. Kaliyaganj Dist:: North (Uttar) Dinajpur	SBI	Raiganj	10 lakh
4	Raja Brick Industry Vill: Hatkaliyaganj P.O. Tarangapur Dist: North (Uttar) Dinajpur	RBI	Raiganj	12 lakh
5	Shaikh Kasim Ali Choudhury P.O. & Vill : Chopra Dist: North (Uttar) Dinajpur)	SKC	Islampur	15 lakh

In the present investigation grass species were not separated since in comparison to other herbaceous taxa grasses grow, horizontally and root out from the nodes. The presence and absence of grasses were indicated by '+' and '–' sign respectively.

For vegetation analysis quadrat sampling method was followed (Mishra, 1966). A list counts quadrat measuring 1 sq.m. was used. In the present study mainly herbaceous plants were recorded.

Different distances e.g. 50 m, 100 m, 200 m and 500 m towards south and south-west from the chimney were selected in each brick field. Thirty observations were taken at each radial distance. The presence of herbaceous plants was recorded and density was calculated.

Density was calculated by the following formula and expressed as number of individuals per unit area sq.cm.

$$\text{Density} = \frac{\text{Total no. of individuals of the species in all units}}{\text{Total no. of sampling units studied}}$$

Site of vegetation analysis

The direction of airflow at the time of brick kiln operation (October-March) was from north / north-east towards south / south-west. In the present investigation vegetation analysis was carried out at different distances towards south and south west direction from the chimney in each brick kiln.

Soil analysis

Two brick fields at South Dinajpur District were selected for soil analysis. These fields are

1. Bikash Chandra Kundu Brick Industry, (BCK) P.O. Balurghat, District: South Dinajpur

2. Balurghat Brick, (BB), P.O. Balurghat, Dist: South Dinajpur

Both the kilns are operating more than ten years.

Methods for soil analysis

Soil collection

Soil samples were collected from two brickfields of South Dinajpur in the month of May before the rainy season i.e. before June-July. Four sites each situated at a distance of 50 m, 100 m, 200 m and 500 m from the chimney were selected. The sites were situated on the south and south-west sides of the chimney. Ten soil samples were collected for a particular distance i.e. ten samples were collected in and around 5 m radius of a central place of each site (50 m, 100 m, 200 m and 500 m). Soil samples (control) were also collected similarly from a site beyond 500 m from the chimney.

Sampling method and soil preparation

After removal of weeds and undesirable materials from the top of the ground layer, soil samples were collected from ten randomly selected place of each site situated 50 m, 100 m, 200 m and 500 m away from the kiln chimney using soil auger or by giving a 'V' shaped cut up to depth of 15 cm with a spade. 1.5-2.0 cm thick uniform slice of soil was collected and kept in a dry container.

Soil samples from 10 spots were mixed thoroughly and spread over a clean paper and dried under shade and then pulverized. The entire soil was then again thoroughly mixed and divided into four equal parts in a tray. Two parts were rejected and other two were mixed thoroughly. The process of elimination was continued till the sample was reduced to 500 g. Separate label was used for each site.

Preparation of soil sample for chemical analysis

Soil sample were crushed with a wooden pestle in a heavy porcelain morter. Pebbles, roots and any unwanted material were discarded. The crushed soil was then passed through a 2 mm sieve. The soil sample were kept for further analysis.

Estimation of Soil Organic Carbon

Soil organic carbon was estimated following the method of Walkley and Black, 1934 and Baruah and Barthakur, 1998.

Chemicals required

1. Potassium dichromate ($K_2Cr_2O_7$) solution 1.00 N:49.04g dry potassium dichromate was dissolved in distilled water to prepare 1 L of solution.
2. Sulphuric acid (concentrated) (H_2SO_4)
3. Phosphoric acid (concentrated) (H_3PO_4)

Ferrous ammonium sulphate; 5N:196.076 g. Fe $(NH_4)_2(SO_4)_2$, $6H_2O$ was dissolved in distilled water, adding 15 ml concentrated H_2SO_4 to prepare 1 L of solution.

4. Diphenylamine indicator, 0.5g. diphynyl amine was dissolve in a mixture 20 ml distilled water and 100 ml concentrated sulphuric acid.

Procedure

a) About 0.5 g ground soil was taken in a 250 ml conical flask.

b) First 5 ml $K_2Cr_2O_7$ solution was added and then 10 ml of concentrated H_2SO_4 was added. It was mixed by gentle swirling.

c) The mixture was kept for 30 minutes to complete the reaction.

d) Then the mixture was diluted with 100 ml distilled water and 5 ml phosphoric acid was added to it followed by 0.5 ml diphenylamine indicator.

e) The sample was tritrated with 0.5 N Ferrous ammonium sulphate.

f) A blank was run with same quality of chemicals without soil.

Calculation:

Volume of 1N $K_2Cr_2O_7$ used for oxidation of C = 0.5 x (B – S) ml

[1 ml of 1N $K_2Cr_2O_7$ (= 3 (= 12/4) mg of org. C = 0.003 g of org. C]

Walkley averaged a 77% recovery of org. C by this method. Thus, the correction factor is 100/77 = 1.3

Now,

% of org. C in the soil (uncorrected) = 0.5 x (B – S) x *1X 0.003 x (100/W)

= Q

% or org. C in the soil (corrected) = Q x 1.3 = R

Hence, % of org. matter in the soil = R x 1.724

W g = Weight of the Soil

B ml for the blank titration = Volume of 0.5N $Fe(NH_4)_2 (SO_4)_2$ solution used

S ml for the sample titration = Volume of 0.5N Fe $(NH_4)_2 (SO_4)$ solution used

* = Normality (N) of $K_2Cr_2O_7$ solution

Determination of available Potassium (K) in Soil

Available potassium was estimated by the method of Bray and Kurtz, 1945 and Humphrey, 1957.

Chemicals Required

1) Normal ammonium acetate (pH 7.0): 82.0 g, of solid CH_3COONH_4 was dissolved in distilled water and the volume was made up to one liter. The pH was adjusted at 7.0 by adding either dilute NH_4OH or dilute Acetic acid.

2) Standard potassium solution: 1.5851 g. of extra pure KCl was dissolved in distilled water and the volume was made up to one litre. This solution is equivalent to 1 g available potassium / litre. After quantitative dilution of standard potassium solution 5, 10,20, 30, 40 and 50 ppm solutions were prepared.

Procedure

1) 5 g. dry soil was weighed in a 200 ml conical flask.

2) 25 ml of neutral normal ammonium acetate solution was added.

3) Contents of the flask was shaken thoroughly and filtered through a dry filter paper (W. 1).

4) The filtrate was feed to the atomizer of the flame photometer after its scale (0-100) had been adjusted to zero with blank and to 100 with 50 ppm standard solution of potassium. The reading was noted and amount of potassium was calculated from the standard curve.

Preparation of the Standard Curve

The 100 value of the Galvanometer, attached to the flame photometer was adjusted using 50 ppm potassium solution. Then different readings were taken using different concentrations of potassium 5, 10, 20, 30, 40 and 50 ppm and standard curve was plotted showing relationship between concentrations of K^+ against galvanometer readings.

Calculations:

- Weight of the Sample = 5 g
- ppm as read from the standard curve = B
- ppm of available potassium / g of soil = B x total dilution = A

$= B \times 5 = A$

[μ g of available potassium (K) / g of soil]

Available K in Kg / ha = A x 2.24

Then Kg of available potassium per hectare of soil was calculated as follows:

1 acre of soil = 20,000,00 lbs

= 20,000,00 x 453.6 g [1 lb = 453.6 g]

Available potassium in μ g / acre = A x 20,000,00 x 453.6

$$\text{Available potassium in mg / acre} = \frac{A \times 20{,}000{,}00 \times 453.6}{1000}$$

$$\text{Available potassium g / acre} = \frac{A \times 20{,}000{,}00 \times 453.6}{1000 \times 1000}$$

$$\text{Availabe potassium Kg / acre} = \frac{A \times 20{,}000{,}00 \times 453.6}{1000 \times 1000 \times 1000}$$

$$\text{Available potassium in Kg / hectare} = \frac{A \times 20{,}000{,}00 \times 453.6 \times 2.47}{1000 \times 1000 \times 1000}$$

[1 hectare = 2.47 acre]

= A x 2.24

Estimation of available Phosphorous (P) in Soil

Available phosphorous in the soil was measured following the method of Bray & Kurtz 1945 and Humphrey, 1957.

Chemicals required

1) 0.5 M $NaHCO_3$, pH adjusted to 8.5

2) 10 (N) HCl

3) Stannous chloride

4) Ammonium molybdate powder

5) Activated Charcoal (Daico G-60)

6) Chloromolybdic acid reagent (1.5%)

7) Pottassium dihydrogen phosphate (KH_2PO_4)

8) Stannous Chloride (Stock Solution)

Procedure

a) 2.5 g of soil sample was taken in a 100 ml conical flask

b) A pinch of activated charcoal (Daico G-60) and 50 ml. of 0.5 M sodium bicarbonate solution (pH 8.5) was added.

c) The mixture was shaken thoroughly and filtered through dry filter paper.

d) First few ml. was discarded.

e) 5 ml of the filtrate was taken in a 25 ml. volumetric flask and

f) 5 ml of chloromolybdic acid reagent was added slowly and carefully. by shaking with hand until the liberation of CO_2 stopped.

g) A blank solution without soil was prepared similarly

h) 1 ml of freshly prepared stannous chloride reagent was added (From stock solution)

i) The colour developed was read at 660 nm against the blank solution and amount of phosphorous was calculated from the standard curve.

Preparation of the Standard Curve

A standard solution of Phosphorous was prepared by dissolving 0.439 g of KH_2PO_4 at 50^0 C in 500 ml distilled water. 25 ml of 7(N)H_2SO_4 was added and volume was made upto one litre with distilled water.(This stock solution contains 100 ppm. phosphorous) From this stock solution 5 ml was taken into a 250 ml volumetric flask and was made upto mark with distilled

water. This is 5 ppm working solution. 1, 2, 3, 4, and 5 ml of this solution was pipetted out in 5 different 25 ml. volumetric flasks. Then 5 ml chloromolybdic acid reagent and 1 ml freshly prepared stannous chloride reagents were added. A blank was prepared without any phosphorous. The developed colour of each solution was read at 660 nm against the blank. The standard curve was ploted.

Calculation

Weight of Sample = 2.5 g.

Volume of 0.5(N) $NaHCO_3$ Soln. added = 50 ml.

Volume of filtrate = 5 ml.

Final volume after colour development = 25 ml.

(dilution= 20 x 5 = 100 times)

Conc. of available phosphorous from the standard curve = C ppm

ppm of available P = C x 100

Then Kg of available phosphorous / hectare of soil was calculated as per calculation for available potassium.

Kg of available P / ha = ppm P x 2.24

Determination of Soil pH

In this study pH was determined by an electronic pH meter (Systronics).

Procedure

20 g of air-dried soil was taken in a 100 ml beaker and 40 ml of distilled water was added and stirred gently for 30 minutes.

The P^H of the soil solution was read using an electronic pH meter (Systronics)

Seed Germination

Seeds of ladies finger, jute and radish were collected from local seed market. These seeds were suitably disinfected.

The varieties,viabilities and sources of the seeds are shown in Table 4.

Table 4 : Varieties and sources of different seeds

Material	Variety	Company	Viability
Jute	JRO 524	Maharastra State, Seed Corporation Ltd., Mahabees Bhawan, Kashinagar, AKOLA, 444104,India	82%
Ladies Finger	OKRA NAJUKA F_1	Seminis Vegetable Seeds (India) Ltd., 24, Chitegaon TQ, Parthan, Aurongabad, 431105, India	85%
Radish	L. W. MINONG	Seminis Korea Operation Center, 244-3 Sanharj Wongok-Mycon, Arjung City, S.Korea.	91%

Preparation of Soil Extract

Soil samples, were collected and prepared as described earlier 100 gm of each soil sample was sterilized in an autoclave at 115 ^{0}C for 20 minutes. Then it was taken in a beaker and 100 ml of distilled water was added and shaken vigorously, filtered and kept for further use.

Forty seeds of each variety were spread over a Whatman blotting paper soaked in the soil extract in two separate Petriplates (six inches diameter).

Soil extracts prepared from soil collected from the 500 m away from brick kiln following usual method described earlier (l.c) were used as control soil extract.

The experimental and control sets of Petriplates with seeds were kept in germinating chamber at 27°C ± 2°C at 1400 Lux of light.

The growing of roots and shoots were measured subsequently.

First reading was taken after three days of germination. Afterwards four measurements were taken with a gap of forty eight hours i.e. second

reading after five days, third reading after seven days, fourth reading after nine days and fifth reading after eleven days of germination.

Statistical analysis of the growth of root and shoots were done and graphs were prepared using Microsoft Excel software on Windows XP operating system in a computer.

Health Problems

The workers, male and female, of 15 brick kilns of North and South Dinajpur were given a questionnaire to respond and it included questions related to present occupational history. In some cases labourers spontaneously reported about their health problems, i.e. self-reported method was also applied. Double blind prospective method was also considered in this study.

Clinical investigation

Physical examination of the workers was conducted by two qualified physician of Government District Hospital, Balurghat on obtaining consent from the subjects in presence of investigator (Sri Arup Kamal Guha) Blood samples were collected by a well trained technical assistant. Some workers were advised for further investigation. X-ray analysis and special clinical tests were done at the District Hospital.

Respiratory disease was diagnosed clinically. In one labourer lung cancer was confirmed by FNAC test following x-ray diagnosis.

The dermatological problems were clinically diagnosed. The medical team advised the labourer for skin smear test. In case of leprosy the labourer were asked for leprosy test.

Hematological disorders were at first diagnosed clinically and finally by pathological test.

The gastrointestinal diseases were clinically diagnosed by the medical experts. In some cases like hepatities and enteric fever labourers were advised for pathological test and liver function test (LFT)

The ophthalmological disorders were diagnosed initially by the medical team and finally they were referred to ophthalmological check-up.

The urinogenital problems were at first diagnosed clinically. In case of urinary tract infection the labourer were asked for microbiological test.

The musculoskeletal problems were diagnosed through clinical investigation and x-ray.

The neurological disorder diagnosed clinically. After behaviour study and counseling the physician confirmed their diagnosis.

In case of auditory problem the audiometric instruments were used to diagnose the problem

Venereal diseases were first clinically diagnosed and then confirmed through microbiological tests, swab test and VDRL test for chlamydia, gonorrhea and syphilis respectively.

Sample questionnaire

Sl. No	Name	Sex	Age	Length of service	Working hours	Nature of job	Health problems if any

I am ready to undergo health checkup.

Signature / Thumb impression

Hematological study

Blood sample collected by vein puncture and according to the requirement (Fig. 15)

Haemoglobin content

Haemoglobin of blood was converted into acid haematin with addition of N/10 HCl and the colour was matched against the standard glass tube of Haemoglobinometer.

Total count and differential count

Materials required

(i) Improved Neubauer haemocytometer, (ii) a thick square cover glass, (iii) two pipettes for drawing of blood-RBC pipette and WBC pipette, (iv) RBC diluting fluid (v) WBC diluting fluid, (vi) lancet or needle, (vii) cotton (non-absorbent), (viii) rectified spirit (90% ethanol) and (ix) compound microscope.

Preparation of solutions / reagents

RBC diluting fluid (Hayem's diluting fluid)

It is an isotonic solution which prevents coagulation, haemolysis, rouleaux formation, and bacterial growth:

Sodium sulfate (NaS_2O_4)	-	2.5 g
Sodium chloride (NaCl)	-	1.0 g
Mercuric chloride ($HgCl_2$)	-	0.25 g
Distilled water	-	100 ml

Method

Total count of RBC

1. Fingertip was punctured aseptically by a lancet or needle. First drop of blood was wiped off by cotton and blood was allowed to flow freely.

2. Blood was drawn into the RBC pipette up to 0.5 or 1 mark.


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3. Extra blood was wiped off from the outer surface of the pipette and immediately RBC fluid was drawn up to 101 mark.

4. The contents of the bulb was thoroughly mixed by using the red coloured bead present inside the bulb of the pipette.

5. Quickly a drop of diluted blood was put at the edge of coverglass placed over the Naubauer haemocytometer, and allowed the very drop to flow under the cover glass by capillary action.

6. The cells were allowed to settle at the bottom of the chamber for 2-3 minutes.

7. RBC was counted at the five groups of 16 smallest squares of the central square in ruled area.

Calculation

Number of RBC /cc

$$\frac{X}{80} \times 4000 \times \text{dilution factor}$$

X = number of RBC present in 80 smallest squars.

WBC diluting fluid

It contains a weak acid to lyse RBC and a stain for staining the nucleus of white blood cells glacial acetic acid (1.5 to 3 ml), distilled water (97 to 98.5. ml), and a few drops of an aqueous solution of methylene blue or gentian.

Total count of WBC

1. Following the same procedure as described for TC of RBC blood was drawn into the WBC pipette up to 0.5 or 1 mark, and then quickly Hayem's diluting fluid was drawn up to 11 mark.

2. WBC was counted in the four corner squares of the ruled area of Naubauer haemocytometer each of these four squares in subdivided into 16 smaller squares.

Calculation

Number of WBC/cc = x/64x160x dilution factor

X = Number of WBC in 64 small squares.

Statistical analysis was carried out with the help of systat package,

Systat Software Asia Pacific Ltd., Shankar Narayan Building, Block-1, 5th Floor, 25, M.G. Road, Bangalore-560 001.