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VARIETAL SCREENING FOR TOLERANCE AGAINST LEAF ROLLER OF MUNGBEAN UNDER FIELD CONDITION

MOHAMMAD MOHASIN HUSSAIN KHAN[1*] AND MD. HABIB ULLAH[2]
[1]Department of Entomology, Patuakhali Science and Technology University, Patuakhali-8602, Bangladesh.
[2]Research Officer (Agriculture), Bangladesh Water Development Board, Dhaka, Bangladesh.

Abstract

An experiment was carried out in the farmer's field at Sonakhali, Barguna sadar, Barguna of Bangladesh during January to April 2016 to evaluate different varieties of mungbean against leaf roller in order to find out tolerant/less susceptible varieties of mungbean. Results revealed that all varieties regarding leaf roller population the variety, local Mung, IPSA Mung-5, BU Mung-2 and BINA Mung-2 had highest population of leaf roller and were highly susceptible varieties to leaf roller. The varieties, BARI Mung-2, GK Mung-27, BU Mung-1 and IPSA Mung-12 had lowest population of leaf roller, which indicated that these varieties were least susceptible to leaf roller. Among all tested varieties, none showed complete resistance against leaf rollers but BARI Mung-5 and BARI Mung-6 showed comparatively better resistance against leaf roller. The highest population of leaf roller/m^2 (7.0) plants in the field was observed in the 2nd week of March and then declined gradually up to the 4th week of March in 2016. BARI Mung- 6, BINA Mung-2, BU Mung- 1 and local Mung varieties had the highest percent leaf area damage by leaf roller while IPSA Mung-5, GK Mung-27, BARI Mung-5 and BU Mung-2 varieties had the lowest percent leaf area damage by leaf roller. There was a strong negative correlation between number of leaf roller and yield.

Keywords: Damage, Leaf roller, Mungbean, Susceptible, Tolerance

Introduction

Mungbean [*Vigna radiata* (L.) Wilczek] belongs to Fabaceae family and usually it is known as green gram (Patel *et al*., 2014). It is one of the most important pulses in south-east Asia (Mondal et al., 2013). However, its yield is much lower than that of other legume crops such as grasspea, chickpea and lentil (FAO 2007). It is one of the leading legume crops widely grown in Bangladesh during Rabi season as well as in many tropical and subtropical countries of the world (Asante *et al.*, 2002). In Bangladesh, the area under pulse crops in Bangladesh is 0.372 million hectares with a production of 0.425 million tons but 0.041million hectares of land are under mung bean cultivation where its

*Corresponding author: mohasin1965@pstu.ac.bd

production is 0.044million tons (BBS, 2021) which is lower than any major vegetables. It is considered as a quality pulse in the country but production perunit area is very low (931 kg/ha) as compared to other countries of the world (BBS, 2021). It ranks fifth both in acreage and production and contributes 6.5% of the total pulse production in Bangladesh (Alam *et al.,* 2021). It is highly nutritious pulse crop contain 24 per cent protein, 59.9 per cent carbohydrate and 40-70 ppm iron contents, considered it an ultimate resort for balanced diets (Selvi *et al.,* 2006 and Vairam *et al.,* 2016). It is a good source of proteins, carbohydrates and vitamins for the human race all over the world. It contains 51% carbohydrate, 26% protein, 10% water, 4% minerals and 3% vitamins (Kaul, 1982) like other pulses widely used as 'Dal' in Bangladesh. Mungbean seed have higher percentage of protein (28.5%), fiber (0.95%), fats (0.65%) and ash (3.75%) (Monem *et al.*, 2012). It is protein rich and also contains amino acid lysine, which is generally deficiet in food grains (Elias *et al.,* 1986). After chickpea, it is called as poor people diet owing to its protein nature and is meeting the major protein demand of the people (Shafique *et al.,* 2009). Besides these, it has the ability to fix the atmospheric nitrogen in symbiotic association with Rhizobium bacteria (Ali and Ahmad, 1993). Mungbean has ability to endure water scarcity conditions because of its short life cycle it can be adjusted in cropping systems of spring and summer crops (Raina *et al.,* 2016). A number of insect pests adversely affect the production of mung bean. According to the report of Rahman *et al.* (2000), more than twelve species of insect pests were found to infest mung bean in Bangladesh among which stem fly, flea beetles, flower thrips and pod borers cause serious damage. These insect pests attack the crop from seedling to fruiting stage and are responsible to cause significant yield loss (Hossain, 2015). The infestation of these insect pests inflicts serious economic loss causing annual yield loss about 27.03 to 38.06% in India (Duraimurugan and Tyagi, 2014). The most damaging insect pests of mungbean recorded so far are stem fly (Rahman,1987), jassid (Baldev *et al*., 1988), whitefly (Rahman *et al*., 1981), thrips (Chhabra and Kooner, 1985), hairy caterpillar (Rahman *et al.,* 1981) and pod borer (Nair,1987). Out of several insect pests, leaf folder/roller is one of the important insect pest causing severe damage from seedling to vegetative stages of mungbean. In mungbean larvae of leaf folder fold the leaves and feed on green tissues (the mesophyll layer) of the leaf causing in the appearrance of linear, pale white stripe damage. Ultimately the damaged leaves dry up or even plant may die. At present day, management of insect pest has largely been relied on chemical control. However, the demands for clean and ecologically sound control envisages, careful planning for rationalizing the insecticides interventions. Variety plays an important role in producing high yield of mungbean because different varieties perform differently for their genotypic characters also vary from genotype to genotype.

Development of resistant varieties is an ideal component against buildup of pest population at no additional cost, compatible with other methods of pest control and free from control pollution. Various biophysical and biochemical characters of the plants play an important role by providing resistance against this pest. The exploitation of host plant resistance, an economically viable genotypes measure against insect pests has become imperative to find out resistance source with higher yield (Tamang *et al*., 2017). Keeping this in view, the present study was carried out to evaluate 10 varieties to find out tolerant/less susceptible varieties of mungbean against leaf roller.

Materials and Methods

Field experiment was carried out in the farmer's field at Sonakhali, Barguna sadar, Barguna of Bangladesh during January to April 2016 to find out tolerant/less susceptible varieties of mungbean against leaf roller. Randomized block design with three replications was used (Gomez and Gomez, 1984). The 10 varieties include BARI Mung-2, BARI Mung-5, BARI Mung-6, BU Mung-1, BU Mung-2, IPSA Mung-5, IPSA Mung-12, GK Mung-27, BINA Mung-2 and local Mung were used as study materials. The land was prepared at 'Joe' condition by 3 times deep ploughing and cross ploughing followed by laddering with a power tiller until the desired tilth during the last week of January, 2016. All the weeds, residues and stubbles of the previous crops were removed from the field. After leveling, the land was leveled and the experimental plot was partitioned into the unit plots in accordance with the experimental design. The whole field was divided into 3 unit blocks representing 3 replications. Each unit block was divided into 10 sub unit plots. The total number of plots was 30 and the size of the individual plot was 3.0m x 2.0m. The distance between unit plots was 0.75m and between blocks was 1.0m. The treatments were randomly assigned to the plots within a block. The seeds were sown on 1 February 2016 at the rate of 20 kg/ha. The seeds were placed in the line continuously at a depth of 4-5cm and covered by loose soil with the help of hand. The spacing was 15 cm between rows and 10 cm between plants. Intercultural operations were done as and when necessary to ensure normal growth and development of crops. Irrigation was applied as and when needed. Proper drainage system was also developed for draining out excess water. As the seeds were sown continuously into the line, there were so many seedlings which needed thinning. Emergence of seedling was completed within 10 days after sowing. Overcrowded seedlings were thinned out twice to keep plant to plant distance about 10 cm. First thinning was done after 15 days of sowing which was done to remove unhealthy and out of line seedlings. The second thinning was done at 10 days after the first thinning. There were some common weeds found in the mungbean field. First weeding was done at 30 DAS (days after sowing) followed by once a week to keep the plots free from weeds and to keep the soil loose and aerated for the whole period of the crop growth.

Data collection procedure:

Weekly data were collected and recorded by direct counting early in the afternoon (4.0 – 6.0 pm). The observations were made at 15 DAS and continued till maturity of the crop at weekly intervals on randomly selected 10 plants from each variety. The population of leaf roller was collected four times (23 DAS, 30 DAS, 37 DAS and 44 DAS) from seedling to flowering. Infested mungbean plants were recorded at vegetative and reproductive stage. The total number of infested plants was recorded from the selected 1 m^2 area of the center of each unit plot to determine the level of infestation by leaf roller.

The leaf area of 5 representative leaves from randomly selected 5 plants of each unit plot was recorded on leaf area meter (Model LI-3100C) and the mean leaf area was computed. Percentage of damaged leaves area/plant by leaf roller were determined by eye estimation. Infestation of mungbean leaves /plant was recorded at different periods of plant growth (at different DAS). The total damaged leaves area was counted and percentage of damaged leaves area was calculated from selected and taged plants of each plot. These include 3 healthy leaves and 2 infested leaves. Mean value of them was recorded as each plot wise and expressed in cm^2.

The percentage of damaged leaves area was calculated by the following formula:

Percentage of damaged leaves area = $\frac{B}{A} \times 100$

Where,

A= Total leaves area checked per plant

B= Damaged leaves area per plant

$$\% \text{ damage leaves area} = \frac{\text{Damage leaves area per plant}}{\text{Total leaves area checked per plant}} \times 100$$

Harvesting and Seed preservation

Mungbean plant produces flower over an extended period. For this reason, pod maturity of mungbean is not uniform which makes it difficult to the harvesting time of mungbean. Generally, harvest should begin when one half to two-thirds of the pods are mature. Pod should be picked after it become black in color. Mungbean was harvested thrice at 65-72 DAS. First only ripen pods were harvested at 65 DAS (4th April 2016) when about 80% of the pods became black in color. After first picking, flowering appeared and pod formation occurred second time. These pods were harvested at 72 DAS (second time on 11th April 2016) after ripening. The harvested crop of 1 m^2 area from each unit plot was bundled separately. Grains were recorded from 1 m^2 area per plot wise and the yields were expressed in kilogram (kg) per hectare. Mungbean was properly stored because seed is vulnerable to heavy bruchid damage. The seeds were cleaned, dried thoroughly in sun keeping moisture content below 8-9 %. Then seeds were cooled and kept either in sealed polythene bags along with naphthalene balls covered by jute bags, tin containers or air tight earthen pots in store rooms. Finally, seeds were stored in cool dry place above the ground.

Meteorological data

Meteorological data in respect of temperature, relative humidity and rainfall for experimental period were collected from Meteorological office, Kalapara, Patuakhali.

Statistical analysis

The collected data were analyzed following the analysis of variance (ANOVA) using WASP program and the mean differences were adjudged by CD (critical difference) values.

Results and Discussion

The results of the experiment are presented and discussed under the following subheadings:

*Population of leaf roller on different varieties of mungbean at different DAS***:** Mean number of leaf roller per square meter was recorded in different mungbean varieties at different days after sowing (DAS) and is presented in Table 1. At 23 DAS, the highest number of leaf roller/m^2 was observed in the variety BARI Mung-5 (1.96), which was statistically similar BARI Mung-2 (1.95). The second highest number was recorded in BINA Mung-2 (1.86) followed by IPSA Mung-5 (1.86), IPSA Mung-12 (1.86) and BU Mung-2 (1.76). IPSA Mung-5 was statistically identical with IPSA Mung- 12. However, the lowest number of leaf roller was observed in the variety BARI Mung- 6 (1.18) followed by local mung (1.47), BU Mung-1 (1.66) and GK Mung-27 (1.68).

At 30 DAS, the highest number of leaf roller/m^2 was observed in the variety BARI Mung-2 (6.67) which was statistically similar to local Mung (6.67). The second highest number was recorded in IPSA Mung-5 (6.33) followed by BU Mung-2 (6.0), BARI Mung-5 (5.67), BINA Mung-2 (5.33) and IPSA Mung-12 (5.0). Likewise, variety of BINA Mung-2 (5.33) was statistically similar with variety of IPSA Mung-12 (5.0) in respect of leaf roller population (Table 3). However, the lowest number of leaf roller was observed in the variety BARI Mung-6 (3.00) followed by BU Mung-1 (3.67) and GK Mung-27 (4.33).

At 37 DAS, significantly the highest population of leaf roller (7.67) was recorded in the variety BINA Mung-2. Local mung and IPSA Mung-5 were statistically identical with BINA Mung-2. The second highest number was recorded in IPSA Mung-5 (7.0) followed by local Mung (7.0), BU Mung-2 (6.67), BARI Mung-5 (6.0), IPSA Mung-12 (6.0) and BU Mung-1 (5.67). Likewise, variety of BARI Mung-2 (7.67) was statistically similar with variety of local Mung (7.0) in respect of leaf roller population (Table 1). BARI Mung-5 was statistically identical with IPSA Mung-12. However, the lowest number of leaf roller was found in the variety BARI Mung-6 (4.33) followed by BARI Mung-2 (4.67) and GK Mung-27(4.67). In addition, BARI Mung-2 was statistically identical with GK Mung-27.

At 44 DAS, the highest number of leaf roller/m^2 was observed in the variety local mung (2.04). The second highest number was recorded in BINA Mung-2 (1.86) which was statistically similar to BARI Mung-5 (1.86), IPSA Mung-12(1.86), GK Mung-27(1.86) followed by BARI Mung-2 (1.65) and IPSA Mung-5 (1.49). However, the lowest number of leaf roller was observed in the variety BU Mung-2 (1.18) followed by BARI Mung-6 (1.35) and BU Mung-1 (1.38) (Table 1).

From the mean of all varieties regarding leaf roller population it was evident that the variety, local Mung, IPSA Mung-5, BU Mung-2 and BINA Mung-2 had highest population of leaf roller, which indicated that these varieties were highly susceptible to leaf roller. On the other hand, the varieties, BARI Mung-2, GK Mung-27, BU Mung-1 and IPSA Mung-12 had lowest population of leaf roller, which indicated that these varieties were least susceptible to leaf roller. Among all tested varieties, none showed complete resistance against leaf rollers. However, BARI Mung-5 and BARI Mung-6 showed comparatively better resistance against leaf roller.

Table 1. Leaf roller population on different varieties of mungbean at different DAS.

Varieties name	Number of leaf roller/m^2 at days after sowing (DAS)				Mean
	23 DAS	30 DAS	37 DAS	44 DAS	
BARI Mung-2	1.95	6.67	4.67 cd	1.65	3.74 ab
GK Mung-27	1.68	4.33	4.67 cd	1.86	3.13 bc
BINA Mung-2	1.86	5.33	7.67 ab	1.86	4.17 ab
IPSA Mung-5	1.86	6.33	7.00 ab	1.49	4.17 ab
IPSA Mung-12	1.86	5.00	6.00 bcd	1.86	3.68 abc
BU Mung-1	1.66	3.67	5.67 bcd	1.38	3.09 bc
BU Mung-2	1.76	6.00	6.67 bc	1.18	3.90 ab
BARI Mung-5	1.96	5.67	6.00 bcd	1.86	3.87 ab
Local Mung	1.47	6.67	7.00 ab	2.04	4.30 ab
BARI Mung-6	1.18	3.00	4.33 d	1.35	2.46 c
Level of significance	NS	NS	**	NS	*
CV (%)	16.07	34.49	20.56	22.35	23.19

* Significant at 5 % level, ** Significant at 1 % level, NS- Non significant.

Means within column followed by the same letter are not significantly different from one another by CD (critical difference) values. Values are average of three replications.

Seasonal incidence of leaf roller on mungbean: The incidence of leaf roller varied significantly throughout the growing season which was shown in Figure 1. Leaf roller appeared in 16th February, 2016 (2nd week after germination) and population increasing gradually and reached its peak on fourth week (1st March, 2016) from declined with the progress of time. Local mungbean variety is most susceptible to leaf roller. Such incidence of leaf roller on mungbean varieties is agreement with the findngs of Nath *et al.* (1998), who reported that leaf roller was a major pest of mungbean and appeared at the vegetative stage and continued to infest the crop until the pos-reproductive stage.

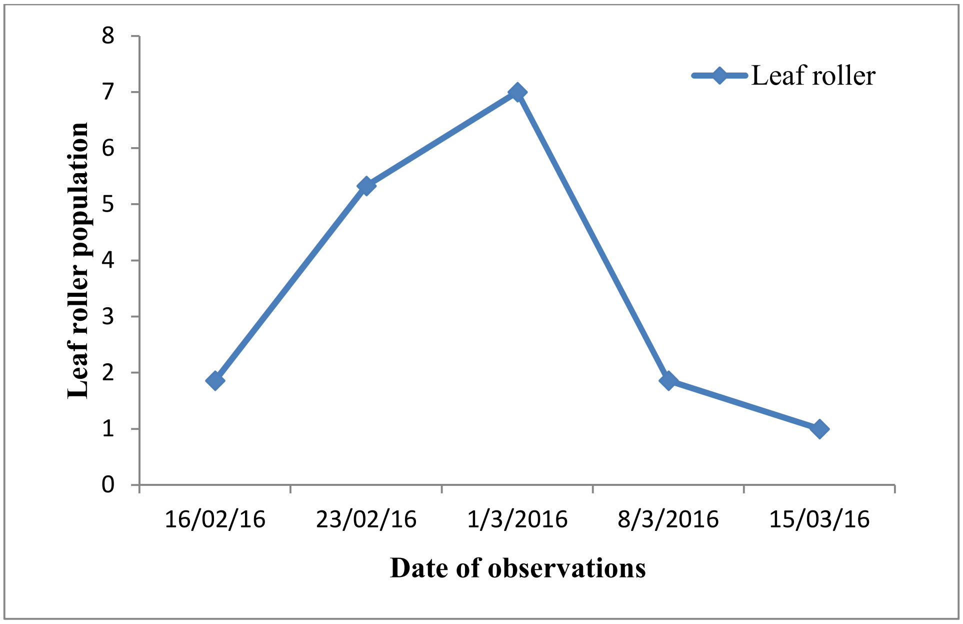

Fig 1. Seasonal abundance of leaf roller on mungbean at various dates of observation

Damage severity by leaf roller: Mean leaf area damaged by flea beetle on different mungbean varieties at different days after sowing is presented in Table 2. At 23 DAS, among the varieties, significantly the highest percent leaf area damage (10.33%) was recorded from the variety BARI Mung-6. The second highest percent leaf area damage was recorded in BU Mung-2 (9.67%) which was statistically similar with BINA Mung-2 (9.33%) followed by IPSA Mung-5 (8.00%), BU Mung-1 (8.0%), local Mung (6.00%) and IPSA Mung-5 (5.67%). IPSA Mung-5 (8.00%) was statistically similar with BU Mung-1 (8.0%). Also local Mung (6.00%) was statistically similar with IPSA Mung-5 (5.67%). However, the lowest percent leaf area damage (4.0%) was found in the variety of BARI Mung-5 followed by BARI Mung-2 (4.67%) and GK Mung-27 (4.67%).

At 30 DAS, among the varieties, significantly the highest percent leaf area damage (16.0%) was recorded from the variety BINA Mung-6. The second highest percent leaf area damage was recorded in IPSA Mung-12(15.67%) followed by IPSA Mung-5 (15.0%), BINA Mung-2 (14.00%), BARI Mung-2 (13.33%), GK Mung-27 (13.33%) and (13.33%). BARI Mung-2 and GK Mung-27 were statistically identical with local mung. However, the lowest percent leaf area damage (12.33%) was found in the variety of BU

Mung-1 followed by BU Mung-2 (10.0%) and BARI Mung-5 (12.33%) and BU Mung-2 (12.33%). BU Mung-2 and BARI Mung-5 were statistically identical with BU Mung-2.

At 37 DAS, among the varieties, significantly the highest percent leaf area damage (24.00%) was recorded from the variety of local Mung. The second highest percent leaf area damage was recorded in BINA Mung-2 (23.67%) followed by IPSA Mung-5 (22.67%), IPSA Mung-12 (22.67%), BARI Mung-6 (22.67%), GK Mung-27 (22.00%), BARI Mung-5 (22.00%). BINA Mung-2 (23.67%), IPSA Mung-5 (22.67%), IPSA Mung-12 (22.67%), BARI Mung-6 (22.67%), GK Mung-27 (22.00%) and BARI Mung-5 (22.00%) ware statistically similar with local Mung (24.00%). However, the lowest percent leaf area damage (14.00%) was found in the variety of BU Mung-1 followed by BU Mung-2 (18.00%) and BARI Mung-2 (18.00%).

At 44 DAS, among the varieties, significantly the highest percent leaf area damage (24.67%) was recorded from the variety BARI Mung-6. The second highest percent leaf area damage was recorded in local Mung-2 (24.33%), followed by BINA Mung-2 (24.00%), IPSA Mung-5 (23.33%), IPSA Mung-12 (23.00%), GK Mung-27 (22.33%) and BARI Mung-5 (22.00%). However, the lowest percent leaf area damage (19.67%) was found in the variety of BU Mung-2 followed by BARI Mung-2 (20.00%) and BU Mung-1 (21.00%) (Table 2).

From the average mean leaf area damage of all varieties, it was evident that the variety of BARI Mung-6, BINA Mung-2, BU Mung-1 and local Mung had highest damage leaf area by leaf roller, which indicated that these variety were highly leaf area damage by leaf roller. On the other hand, the varieties, IPSA Mung-5, GK Mung-27, BARI Mung-5 and BU Mung-2 had leaf area damage by leaf roller which indicated that these varieties had marginal leaf area damage by leaf roller. Among all tested varieties, none showed complete absence of leaf area damage by leaf roller. However, IPSA Mung-12 and BARI Mung-2 showed comparatively less leaf area damage by leaf roller. From the results of table 2, it was evident that the percent leaf area damage by leaf roller increasing due to the age of plant availability of maximum number of leaves in plant.

Damage of Leaf roller: Larvae fold the leaves longitudinally by stitching the leaf margins and eat the green surface. A band was formed due to fusion of individual spun threads and the desiccation of the band facilitates contraction of silk stitches, hence, the leaf rolls. Once protected, the larvae scrape and feed on the green tissues (the mesophyll layer) of the leaves, resulting in the appearance of linear, pale-white stripe damage. In severe infestations, damaged leaves dry up or even plant may die (Verma and Saxena, 1987).

Table 2. Damaged severity of leaf roller attacking mungbean in different dates of observation

Varieties Name	% Damaged leaf area by leaf roller/plant at days after sowing (DAS)				Mean
	23 DAS	30 DAS	37 DAS	44 DAS	
BARI Mung-2	4.67c	13.33cd	18.00b	20.00	14.00e
GK Mung-27	4.67c	13.33cd	22.00a	22.33	15.59bcde
BINA Mung-2	9.33a	14.00bcd	23.67a	24.00	17.75ab
IPSA Mung-5	5.67bc	15.00abc	22.67a	23.33	16.59abcd
IPSA Mung-12	8.00ab	15.67ab	22.67a	23.00	13.835e
BU Mung-1	8.00ab	12.33d	14.00c	21.00	17.34abc
BU Mung-2	9.67a	12.33d	18.00b	19.67	14.92cde
BARI Mung-5	4.000c	12.33d	22.00a	22.00	15.09bcde
Local Mung	6.00bc	13.33cd	24.00a	24.33	16.92abc
BARI Mung-6	10.33a	16.00a	22.67a	24.67	18.42a
Level of significance	**	**	**	NS	*
CV (%)	23.83	8.36	7.26	8.63	11.70

* Significant at 5 % level, ** Significant at 1 % level, NS- Non significant

Means within column followed by the same letter are not significantly different from one another by CD (critical difference) values. Values are average of three replications.

Fluctuation of leaf roller with weather parameters on IPSA Mung-5: Figure 2 illustrates the population of leaf roller insect pests was gradually increased with weather parameters on highly susceptible variety (IPSA Mung-5) of mungbean. The infestation of leaf roller started in the 4th week of February and it was reached maximum in the 2nd week of March at active vegetative stage. Then decreased gradually and reached to zero at 4th week of March. The prevailing temperature and relative humidity were 33-34^{0}C and 96-98% at the peak population period (Figure 2). The highest population of leaf roller (7.0) in per square meter plants in the field was observed in the 2nd week of March and then declined gradually up to the 4th week of March in 2016 (Figure 2).

Relationship between leaf roller population and yield: The result revealed that there was a strong negative correlation between number of leaf roller and yield. It indicated that with the increase of leaf roller there was progressive fall in the yield. A linear regression was fitted between leaf roller abundance and yield (Figure 3). The correlation coefficient (r) was 0.95 and the contribution of the regression (R^2 = 0.9206, when Y= -52.613x + 935.53) was 92.06%.

Plate 1. Damage symptom by leaf roller on mungbean leaf.

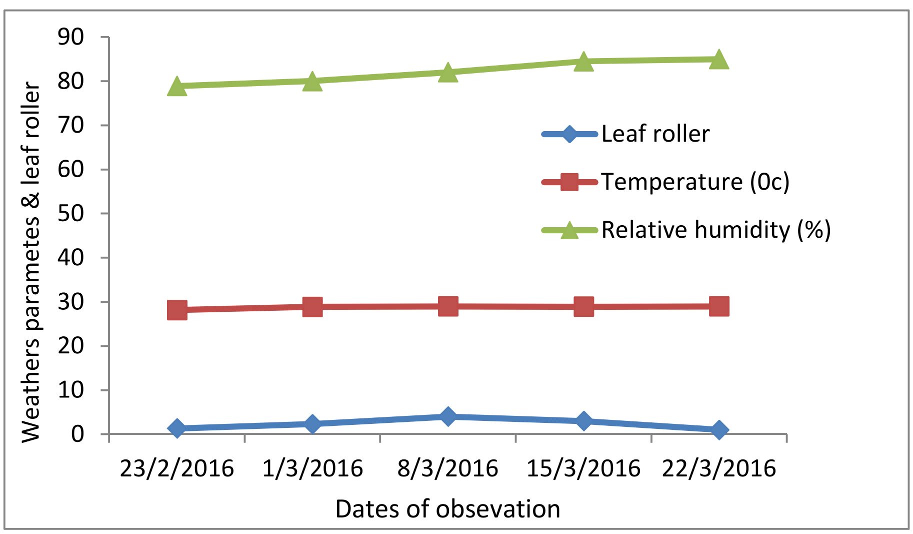

Fig 2. Trend in population fluctuation of leaf roller with mean temperature (°C), mean relative humidity (%) and rainfall (mm) on highly susceptible variety IPSA Mung- 5 of mungbean.

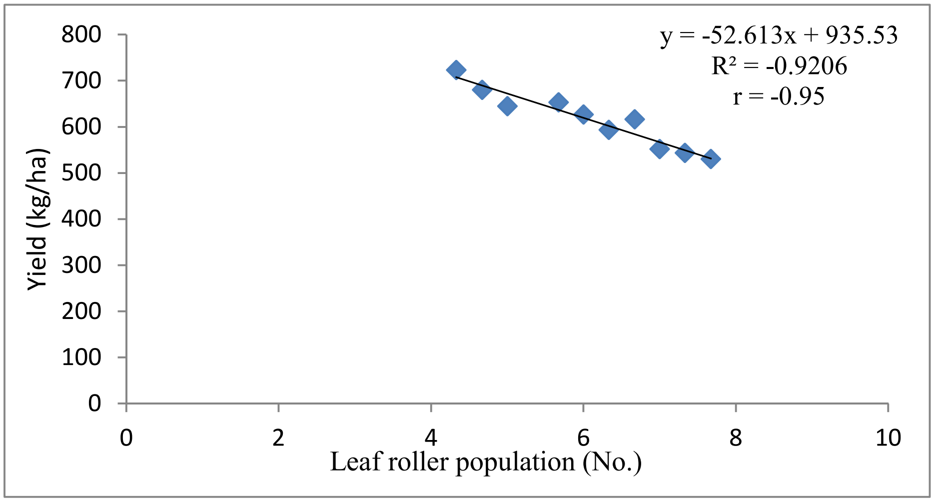

Fig 3. Relationship between leaf roller population and yield of different mungbean varieties.

The findings of the present study are in agreement with the results of Biswas and Islam (2012) who found that Leaf roller infestation was observed in the 3rd week of January at the vegetative and flowering stages (45-60 days after sowing=DAS) of the crop and continued up to pre-maturity period (80-85 DAS). The highest leaf roller population (0.9 and 1.00/plant in 2008 and 2009, respectively) and infestation (90% plant in 2008 and 95% plant in 2009) were recorded in the last week of February at the pod formation stage of the crop (65-70 DAS). Hunt *et al.* (1995) showed mean consumption of mungbean leaf by leaf roller was 0-31 cm^2 d^{-1} and final estimated tissue loss caused by one insects feeding at vegetative stage (14 days) was 13.93 cm^2. Mungbean leaf roller is a polyphagous insect (Alam *et al.* 1964, Correa *et al.* 1987, Hill 1987) but Gazzani (1983) found that the mungbean leaf roller is considered to a secondary pest. It damaged the leaves by eating the leaves without vein. Gowda and Kaul (1982) showed that the leaf roller/folder larvae caused damage by folding and rolling the leaves and web them together. A larva then feed inside on young leaves and buds. Newly hatched larvae weave a white web sticking two adjacent leaflets together or roll up a single or several adjacent leaflets to form cartridges within which they develop.

Conclusion

It is concluded that GK Mung- 27 and BARI Mung- 5 varieties are comparatively better tolerant against leaf roller of mungbean.

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RISK ASSESSMENT OF CARCINOGENIC METALS IN SOILS AND FOOD CROPS NEAR BRICK KILN CLUSTER

NASRIN CHOWDHURY[1*] AND MD. MAMUNUR RASID[2]
[1]Department of Soil Science, University of Chittagong, Chattogram 4331, Bangladesh
[2]Simple Approach Ltd. Dhaka, Bangladesh

Abstract

A study was conducted in the Charia area, Hathazari, Chattogram district, Bangladesh to determine the levels of carcinogenic metals Cd and Pb in soils and crop plants in the vicinity of brick kiln clusters. The health risk was evaluated by computing cancer risk using the US EPA model. The amounts of Cd and Pb in the soils and the parts of crop plants were determined by AAS. The soils of the study area were acidic. Metal concentrations indicating the anthropogenic input of Cd and Pd in the soils was in the range from 0.27 to 1.07 mg/kg dry soil and from 19.07 to 52.07 mg/kg dry soil respectively. Accumulation of Cd (0.00 to 0.27 mg/kg) and Pb (0.09 to 0.13 mg/kg) in the studied crops. A significantly high correlation (r = 0.89, p < 0.001) of the elements between soil and plant suggests that the source of contamination is the soil under study. 72% of all the studied edible plant parts exceeded the recommended threshold risk limit of the cumulative cancer risk (ΣILCR), which was higher in the edible part of fruit crops than in root crops. Cd was the dominant carcinogen in the study area.

Keywords: Agricultural soils, Brick kiln, Heavy metals, Incremental lifetime cancer risk, Transfer factor

Introduction

The environmental impact of brick production has become a significant concern in Bangladesh. The brick sector in Bangladesh is distinguished by its use of soil as a necessary raw material, highly energy-intensive burning technique, and reliance on human labor (Biswas *et al*., 2018). The incineration technique used in brick production significantly increases the mobility and bioavailability of heavy metals from burning components. Heavy metals produced in brick furnaces (Ravankhah *et al*., 2017; Ismail *et al.,* 2012) are released into the atmosphere, where they are then deposited and dispersed throughout soils and water sources. Because of their toxicity, bioaccumulation, and persistence, heavy metals as soil contaminants can be potential sources of pollution in soil (Chowdhury and Rasid, 2021).

[*]Corresponding author: nasrin@cu.ac.bd

Different plant species were found to accumulate Cd and Pb in edible plant parts near metal-contaminated sites (Hamoud *et al*., 2024; Sulaiman and Hamzah, 2018; Rashid *et al*., 2023; Gajbhiye *et al*., 2022; Siaka *et al*., 2014; Sikder *et al.*, 2016). Regular intake of Cd-contaminated vegetables can cause damage to the liver and kidney (Tuzen, 2009) cardiovascular system and bones (Fang *et al*., 2014). High Pb consumption through vegetables can cause pathological changes in organs and the central nervous system, which can affect intelligence quotients in children. Moreover, consumption of Cd and Pb even at very low doses can develop cancer in the human body (Michalczyk *et al*., 2023) therefore, they are categorized by the International Agency for Research on Cancer (IARC) as carcinogenic agents (Sultana *et al*., 2017).

Bangladesh, following China, India, and Pakistan, is the world's fourth-largest manufacturer of clay-fired bricks. Over 8,000 brick kilns are in operation, producing roughly 50 billion bricks a year (UNDP, 2011). According to Ferdausi *et al*. (2008), traditional kilns in Bangladesh use 20–22 tons of coal for every 100,000 bricks produced, and their particle emissions exceed 1,000 mg m^{-3}. Many studies (e.g., Mizan *et al*., 2023; Rahman *et al*., 2021; Shammi *et al*., 2021; Alam *et al*., 2020; Hasan *et al*., 2020; Proshad *et al*., 2019) have been conducted on heavy metal pollution in Bangladeshi agricultural soils. A few research (Islam *et al*., 2018; Tasrina *et al*., 2015; Zakir *et al*., 2014) concentrated on the heavy metal contamination of agricultural soils by metal-contaminated airborne deposits from high traffic. Nevertheless, no thorough investigation into the effects of brick kiln clusters on the buildup of carcinogenic heavy metals in crop plants and agricultural soil has yet been carried out. In Hathazari, Chattogram, there are numerous massive clusters of brick kilns. Fumes from these kilns can contaminate the surrounding vegetation and soil with carcinogens like Cd and Pb. There are potential health risks associated with crop consumption and metal exposure for the local population. With this background, the objectives of this research were to analyze the contamination level of Cd and Pb in soils and crop plants, subsequent plant transfer factor, estimate daily intake of Cd and Pb and the extent of potential carcinogenic health risk from the crops consumed that grow in the vicinity of brick kiln cluster in Hathazari, Bangladesh.

Materials and Methods

A cluster of brick kilns in the Charia area, Hathazari, Chattogram district, Bangladesh surrounded by crop fields were chosen. Since brick kilns are the main source of air pollution during the manufacturing season, which runs from October to March dependent on the monsoonal rains, the research area's sampling was done during the dry season. Six distinct locations for soil sample (Fig. 1, Table 1) were chosen from the area surrounding the brick kiln cluster, each at a varying distance. The agricultural soils 1.5 km away from the brick kiln cluster served as the reference sites (C). Soil samples were taken (0-30 cm) from the soil's surface down to the root zone. Plant samples (fruit crops, root crops and rice) growing in the sampled soils was collected. The direction of wind movement was taken into account when assessing how fume and fly ash deposition affected the soil.

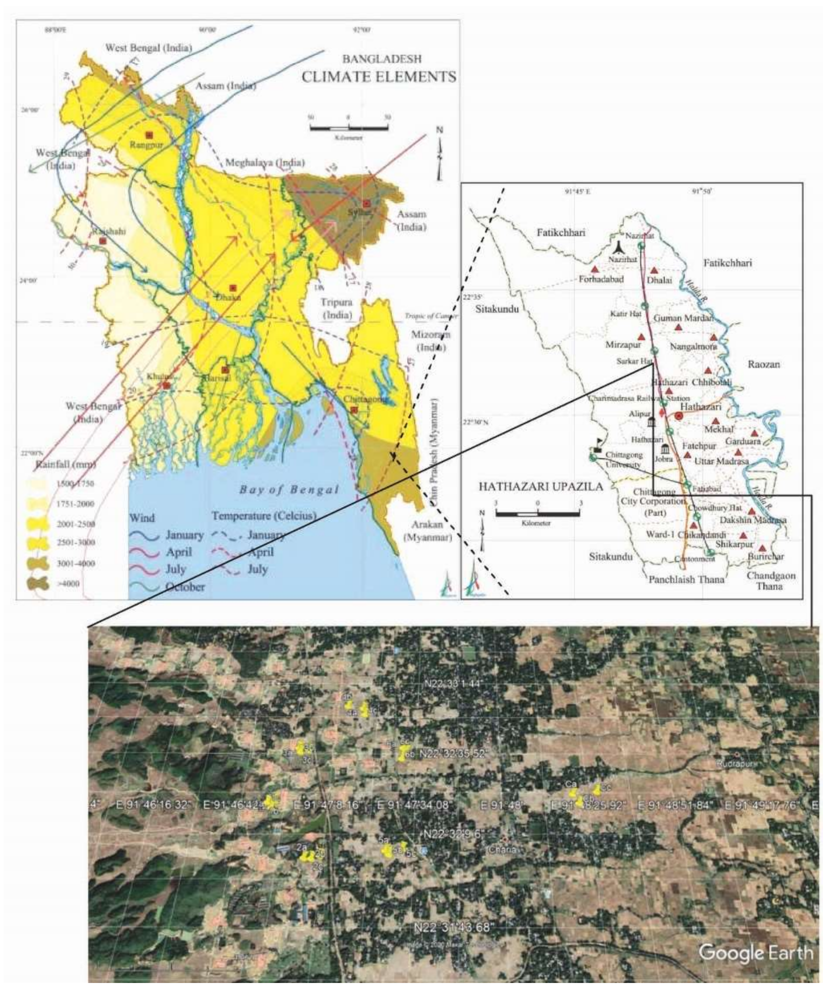

Fig. 1. Study sites in Hathazari, Chattogram, Bangladesh. In the satellite view, brick kilns are clearly visible. Wind flow as a component of the country's climate depicted on the map of Bangladesh (Banglapedia, 2012).

Table 1. Site legend, crop species and locations of agricultural soils adjacent to the brick kiln cluster.

Site no.	Crop species name		Geographical Coordinate	
	Scientific	Local	Latitude	Longitude
1A	*Solanum tuberosum*	Potato	22°32ʹ15.33ʺN	91°46ʹ51.10ʺE
1B	*Artemisia vulgaris*	Mugwort	22°32ʹ17.58ʺN	91°46ʹ51.98ʺE
1C	*Ipomoea batatas*	Sweet potato	22°32ʹ17.16ʺN	91°46ʹ49.66ʺE
2A	*Capsicum species*	Chilli	22°32ʹ0.36ʺN	91°47ʹ2.47ʺE
2B	*Oryza sativa*	Rice	22°32ʹ0.37ʺN	91°47ʹ4.71ʺE
2C	*Solanum tuberosum*	Potato	22°32ʹ1.02ʺN	91°47ʹ7.08ʺE
3A	*Oryza sativa*	Rice	22°32ʹ35.02ʺN	91°46ʹ56.82ʺE
3B	*Trichosanthes anguina*	Snake gourd	22°32ʹ33.76ʺN	91°46ʹ57.44ʺE
3C	*Raphanus sativus*	Radish	22°32ʹ34.28ʺN	91°46ʹ59.85ʺE
4A	*Oryza sativa*	Rice	22°32ʹ49.48ʺN	91°47ʹ15.71ʺE
4B	*Brassica nigra*	Mustard	22°32ʹ50.25ʺN	91°47ʹ10.83ʺE
4C	*Capsicum species*	Chilli	22°32ʹ47.26ʺN	91°47ʹ16.43ʺE
5A	*Capsicum species*	Chilli	22°32ʹ2.60ʺN	91°47ʹ25.55ʺE
5B	*Artemisia vulgaris*	Mugwort	22°32ʹ1.44ʺN	91°47ʹ26.56ʺE
5C	*Raphanus sativus*	Raddish	22°32ʹ3.06ʺN	91°47ʹ30.38ʺE
6A	*Artemisia vulgaris*	Mugwort	22°32ʹ35.20ʺN	91°47ʹ27.24ʺE
6B	*Solanum lycopersicum*	Tomato	22°32ʹ31.54ʺN	91°47ʹ28.95ʺE
6C	*Brassica nigra*	Mustard	22°32ʹ34.08ʺN	91°47ʹ30.32ʺE
CA	*Oryza sativa*	Rice	22°32ʹ19.90ʺN	91°48ʹ20.71ʺE
CB	*Solanum tuberosum*	Potato	22°32ʹ17.01ʺN	91°48ʹ22.38ʺE
CC	*Vigna mungo*	Faba Bean	22°32ʹ20.62ʺN	91°48ʹ27.99ʺE

All of the samples that were collected by a stainless steel spade (alcohol cleansed before every sample) were wrapped in polythene bags and brought to the laboratory on the day of sampling. To prepare the composite soil samples for physical and chemical examination, they were homogenized, sieved through a 2 mm sieve, and air-dried. General soil characteristics (pH, textural classes, organic matter) were determined following the standard procedures (Huq and Alam, 2005). After strong acid-digestion (1:1 mixture of concentrated nitric and perchloric acids) of the soil samples, the total amounts of Cd and Pb were measured using an Atomic Absorption Spectrophotometer (AAS) (Agilent 240). Following Ure (1990), the digested samples were filtered and collected in 5 milliliters of 2.0 M HCL. After washing off any remaining soils using running tap water, the plant samples were oven-dried for 48 hours at 80°C. Using a laboratory stainless steel grinder, each sample of the dried plant materials was ground into a fine powder and passed through a screen with a 1 mm hole. Aqua regia was used to extract the metals from the plant samples before the AAS analysis.

Health risk levels of Cd and Pb in the plant samples were evaluated using risk indices, such as transfer factor (TF), estimated daily intake (EDI), and target carcinogenic risk factor (lifetime cancer risk, ILCR) which are widely used to assess the risk levels of heavy metals in the crops (Islam *et al*., 2016).Transfer factor (TF), one of the primary factors regulating human exposure to metals through the food chain, can be used to assess a plant's capacity to transfer metals from soil to edible tissues. The following formula was used to compute TF:

$$TF = \frac{C_{Plant}}{C_{Soil}}$$

where, TF = transfer factor, C_{Plant} = heavy metal content in the edible parts of the crop plant, C_{Soil}= total heavy metals concentration in soils on a dry weight basis.

The formula utilized to calculate the estimated daily intake (EDI) of metal through the consumption of food crops is as follows:

$$EDI = \frac{FIR \times C}{BW}$$

where EDI = estimated daily intake, FIR = food crop ingestion rate (g/person/d), C = metal concentration in crop samples [mg/kg, fresh weight (FW)], BW = body weight assuming 60 kg for adult residents in the present study (FAO, 2006). On a fresh weight basis, the average daily food crop consumption rate for adult dwellers is 170.04 g.

The following equation (US EPA, 2002) was used to estimate the target carcinogenic risk factor (lifetime cancer risk):

$$ILCR = \frac{EFr \times ED \times FIR \times C \times CSFo}{BW \times AT}$$

where $ILCR$ = incremental lifetime cancer risk, EFr = 365 d/year of exposure frequency, ED = 70 years of exposure, FIR = rate of food ingestion (g/person/d), C = food metal concentration on the fresh weight (FW) basis [mg/kg], $CSFo$ =According to US EPA (2002) database oral carcinogenic slope factor [15 and 8.5×10^{-3} (mg/kg/d) for Cd and Pb, respectively]. BW = body weight in the current study, with adult residents assuming 60 kg (FAO, 2006) AT = the average duration of carcinogens (365 days'/year × ED).

The Microsoft Excel 2016 application was used to determine the standard deviation and correlations between the selected parameters. One-way analysis of variance (ANOVA) was used to determine the effects of Cd and Pb, and the multiple range test with the least significant difference at $p < 0.05$ was used to test the significance of the parameters. IBM SPSS was used to perform the dendrogram grouping for the cluster analysis.

Results and Discussion

Cd and Pb distribution in agricultural soil: The results of the Cd and Pb concentrations in the soil sample are shown in Fig. 2. The agricultural soils had Cd concentrations ranging from 0.25 to 1.05 mg/kg dry soil and Pb concentrations ranging from 19.05 to 52.10 mg/kg dry soil. The average concentrations of Cd and Pb, which were higher than the reference background level, were 0.64 and 29.97 mg/kg, respectively. The values for Cd and Pb in reference soils were 0.15 mg/kg dry soil and 8.06 mg/kg dry soil, respectively. Specifically, the mean concentrations of Cd and Pd in the study area were 3.7 and 4.3 fold higher than those of the reference sites. Thus, the Cd and Pb contamination in the agricultural soils may be caused by anthropogenic activities associated with brick kiln operations. The Chinese Environmental Quality Standards for Soil (Act No. 220/2004 Coll. of Laws) provide guidelines for Pb and Cd levels in dry agricultural soil at pH < 6.5. The heavy metal concentrations in the study area were also compared to these values (Chen *et al*., 2018). The average metal concentration showed a diverse variation among the sites. There was an uneven distribution of Cd and Pb in the area as the fumes from the brick kiln cluster spread out over the agricultural fields in the wind and settled on the crop plants and soils. Soil Cd concentration showed strong significant correlations with soil Pb ($r = 0.89$, $p < 0.001$) suggesting that the two may have a common origin in the soil.

Concentrations of Cd and Pb in selected crop plants: There were significant differences in the Cd and Pb concentrations of different food crop species in different sites. The result presented in Fig. 2 showed that the Cd and Pb status of food crops in the study area ranged from 0.00 to 0.22 mg/kg and 0.00 to 1.41 mg/kg dry wt respectively in agricultural soils near brick kiln cluster. No Cd and Pb were detected in the food crops growing in reference soil. The average maximum concentrations of Cd and Pb were recorded as 0.09 mg/kg and 0.02 mg/kg fresh wt respectively in *Solanum tuberosum* at site 2C. *Solanum tuberosum* in site 1A was also higher in Cd (0.02 mg/kg FW) and Pb (0.05 mg/kg FW) concentrations than the other agricultural sites. In other plants, Cd and Pb content were negligible or not detected. There was a significant ($p < 0.001$) high positive correlation between soil Cd and Pb contents to the corresponding Cd (r = 0.80) and Pb (r = 0.51) contents in crop plants, which proved metal uptake by crop plants. The concentration levels of Cd and Pb in our study were lower than the allowable levels as set by the Commission of the European Communities (EC) (EC, 2006) and World Health Organization (WHO) (FAO/WHO, 2005). EC set the Pb as 0.3 mg/kg fresh weights for brassica and leaf vegetables, and 0.1 mg/kg fresh weights for all remaining food crops. EC and WHO set the allowable level of Cd as 0.2 mg/kg fresh weights for leafy vegetables and fresh herbs, 0.1 mg/kg for stem and root vegetables (Naser *et al*., 2009). Although the mean carcinogenic metal levels were low, the detectable level of the metals was a concern as it may be magnified if the brick kiln operation continued.

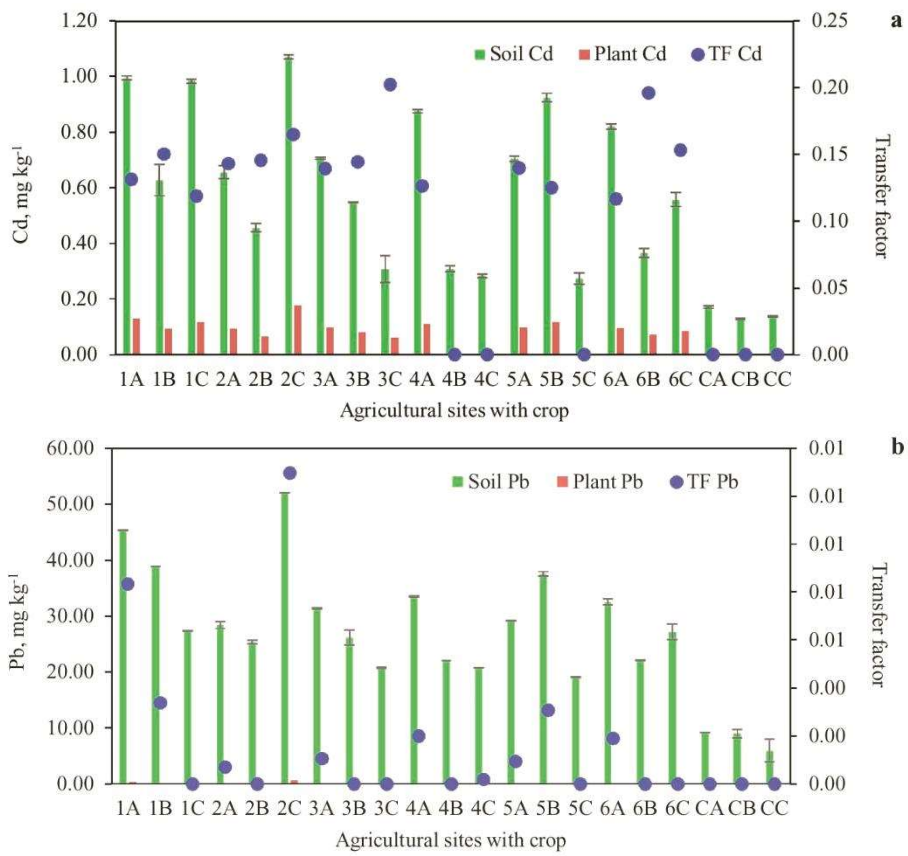

Fig. 2. Concentration (mg/kg) of Cd (a) and Pb (b) in agricultural soil adjacent to the brick kilns and selected food crop species and their subsequent transfer factor. Legends for plant names are detailed in Table 1.

There were significant differences in the sampling sites for soil Cd contents of the same sampling location, but in the case of a plant, it was not, except for *Solanum tuberosum* at 1A and 2C sites (Fig. 2a). The findings of this study showed that the concentrations of Cd and Pb were substantially lower than the reference soils. This may be ascribed to the root system which acts as a barrier to the upward movement of metals. The average concentration of Cd and Pb was observed to be higher in the edible part of fruit crops than in root crops (Fig. 3). Fumes containing different contaminants from brick chimneys are distributed to the surrounding area, after deposition some Pb may be absorbed in the plant through areal parts. Although both Cd and Pb are deposited in soil, Pb uptake through plant roots is not common in contaminated soil as Pb is immobile, whereas Cd is easily translocated to the aerial parts (Radovanovic *et al.*, 2020). Detected Pb concentration varied from 0.01 to 0.06 mg/kg in the edible parts of *Solanum tuberosum* (1A, 2C), *Capsicum species* (2A), *Artemisia vulgaris* (5B, 6A). Cd was detected in the edible part of *Artemisia vulgaris* as 0.02 mg/kg (5B) and 0.01 mg/kg in *Solanum tuberosum* (1A, 2C), *Artemisia vulgaris* (1B, 6A), *Capsicum species* (2A, 5A), *Trichosanthes anguina* (3B), *Solanum lycopersicum* (6B) and *Brassica nigra* (6C).

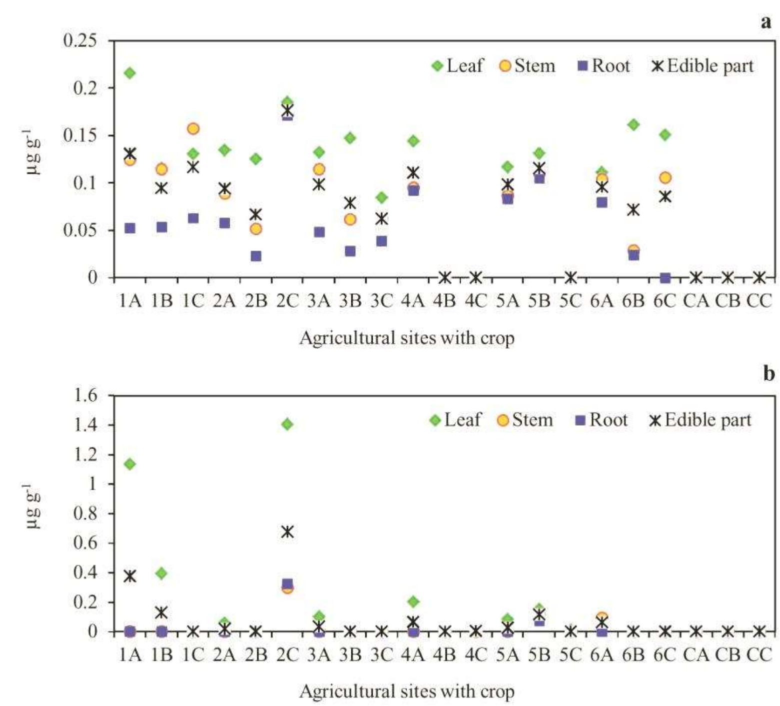

Fig. 3. Distribution of Cd (a) and Pb (b) in different plant parts. Legends for plant names are detailed in Table 1.

Heavy metal transfer from soil to food crops: The capability of plants that allow them to transfer metals to edible tissues can be evaluated by the metal's transfer factor (TF). Metals with high TF are easily transferrable compared to ones with low TF. The TF values for Cd and Pb varied from 0.000 to 0.002 and 0.000 to 0.020, respectively (Fig. 2). The TF values of Pb were lower than Cd. Transfer of both Cd and Pb were detected in *Capsicum species* (2A), *Artemisia vulgaris* (5B, 6A). No transfer of Cd and Pb from soil was detected for the vegetable *Ipomoea batatas* (1C), *Oryza sativa* (3A, 4A), *Capsicum species* (4C) and *Raphanus sativus* (5C). The difference may be due to variations in metal concentration in the soil and divergent capacities of heavy metal uptake by different crops (Sultana *et al*., 2017). Generally, the TF of heavy metals is controlled by the soil properties, such as pH and salinity and plant species (Nasircilar *et al*., 2024; Thien *et al*., 2021; Alengebawy *et al*., 2021; Islam *et al*., 2015). In the plant system, the root cell wall mainly controls the transfer of metal ions, the ion transmembrane transport occurs in the endoderm cytoplasm membrane and the water transports through the xylem vessel.

Estimated daily intake from contaminated crops: A reliable method for examining a population's diet in terms of metal intake levels is the dietary exposure approach to food crop consumption, which offers crucial details regarding the potential exposure to food contaminants (WHO, 2004). The estimated daily intake (EDI) of carcinogenic metal Cd and Pb for the local people close to the brick kiln cluster area is shown in Table 2. The calculated EDI for Cd and Pb for the food crops were compared with R_fD value established by USEPA (2002) [R_fD is the chronic oral reference dose for the metals (mg/kg of body weight per day), which does not cause a lifetime deleterious effect]. EDI for Pb and Cd was above the R_fD value in the edible part of *Solanum tuberosum* (1A, 2C), *Capsicum species* (2A) and *Artemisia vulgaris* (5B, 6A) which corresponded to high soil metal contents. On the other hand, the higher EDI of Cd was also associated with *Oryza sativa* (2B), *Trichosanthes anguina* (3B), *Raphanus sativus* (3C), *Brassica nigra* (4B, 6C) and *Solanum lycopersicum* (6B).The result suggests that residents near the brick kiln cluster were most likely exposed to some possible health concerns due to consuming locally cultivated crops containing Cd. Food crops did not provide a substantial concern for Pb consumption. The possible health concerns associated with consuming local crop consumption should not be disregarded when the total amount of metals ingested through diet is considered.

Carcinogenic health risk assessment: The acceptable carcinogenic risk level for these heavy metals was set to be lower than 1×10^{-5}, as defined in the Polish Regulation of the Minister of the Environment (Polish Regulation of the Minister of the Environment, 2016). The safe limit for carcinogenic risks recommended by the US EPA is below 10^{-4}

Table 2. Estimated daily intake (EDI) of Cd and Pb for the study area's inhabitants through crop intake (mg/kg/day) and subsequent incremental lifetime cancer risk (ILCR) and cumulative cancer risks (ΣILCR) for the region's adult inhabitants.

Site no.	Vegetable species	Estimated daily intake (EDI)		Incremental Lifetime Cancer Risk (ILCR)		
		Cd	Pb	$ILCR_{Cd}$	$ILCR_{Pb}$	ΣILCR
1A	*Solanum tuberosum*	5.46E-03	4.71E-03	5.35E-03	2.61E-04	5.61E-03
1B	*Artemisia vulgaris*	4.23E-03	0.00E+00	4.14E-03	0.00E+00	4.14E-03
1C	*Ipomoea batatas*	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
2A	*Capsicum species*	3.23E-03	5.76E-03	3.16E-03	3.19E-04	3.48E-03
2B	*Oryza sativa*	4.30E-04	0.00E+00	4.21E-04	0.00E+00	4.21E-04
2C	*Solanum tuberosum*	3.65E-03	8.30E-03	3.57E-03	4.61E-04	4.03E-03
3A	*Oryza sativa*	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
3B	*Trichosanthes anguina*	3.14E-03	0.00E+00	3.08E-03	0.00E+00	3.08E-03
3C	*Raphanus sativus*	1.24E-03	0.00E+00	1.22E-03	0.00E+00	1.22E-03
4A	*Oryza sativa*	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
4B	*Brassica nigra*	1.22E-03	0.00E+00	1.19E-03	0.00E+00	1.19E-03
4C	*Capsicum species*	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
5A	*Capsicum species*	2.96E-03	0.00E+00	2.90E-03	0.00E+00	2.90E-03
5B	*Artemisia vulgaris*	7.20E-03	1.41E-02	7.05E-03	7.85E-04	7.83E-03
5C	*Raphanus sativus*	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
6A	*Artemisia vulgaris*	2.31E-03	2.36E-02	2.27E-03	1.31E-03	3.58E-03
6B	*Solanum lycopersicum*	2.77E-03	0.00E+00	2.72E-03	0.00E+00	2.72E-03
6C	*Brassica nigra*	2.37E-03	0.00E+00	2.32E-03	0.00E+00	2.32E-03
CA	*Oryza sativa*	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
CB	*Solanum tuberosum*	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
CC	*Vigna mungo*	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Threshhold risk limit	4.60E-02[a]	2.10E-01[a]		1.00E-04[b]	
		1.00E-03[b]	3.50E-04[b]		1.00E-05[c]	

Note: [a] JECFA (2003); [b] US EPA (2002); [c] Polish Regulation of the Minister of the Environment (2016).

(Table 2) The calculated incremental lifetime cancer risk (ILCR) for Cd and Pb and cumulative cancer risks (ΣILCR) of the studied plants are shown in Table 2. ILCR was higher than the risk level in all the edible parts of the studied plants where Cd and Pb were detected. Cd tends to accumulate more at the toxic level in edible parts of plants than Pb. A high ILCR level of Cd was detected in *Artemisia vulgaris* in site 5B and *Solanum tuberosum* in site 1A. No Pb contamination at the carcinogenic level was detected in rice. Cd has crossed the threshold risk limit in 72% of food crops and for Pb, the value was 28% of the studied crops. In the study area, cadmium was the dominant carcinogen. The trend of the risk of acquiring cancer due to exposure to Cd and Pb from consuming the studied edible plant parts was in the order: fruit crop > root crop > paddy.

Plants detected with the carcinogenic threat having the highest ΣILCR was *Artemisia vulgaris* in site 5B and the lowest was *Oryza sativa* in site 2B (Table 2). According to US EPA recommendation, the risk values show that ingestion of beans from *Artemisia vulgaris* (5B) would produce an overabundance of 78 cancer cases per 10,000 people exposed while ingestion of rice (2B) would produce an overabundance of 42 cancer case per 100,000 people exposure (Sultana *et al*., 2017). *Ipomoea batatas* (1C), *Oryza sativa* (3A, 4A), *Capsicum species* (4C) and*Raphanus sativus* (5C), did not accumulate Pb or Cd to impact human health. The recommended threshold risk limit ($>10^{-4}$) of ΣILCR of the studied edible plant parts exceeded 60%, 38% and 2% respectively for fruit crops, root crops and rice (Fig. 4). The potential health risks due to exposure to Cd must therefore be apprehended.

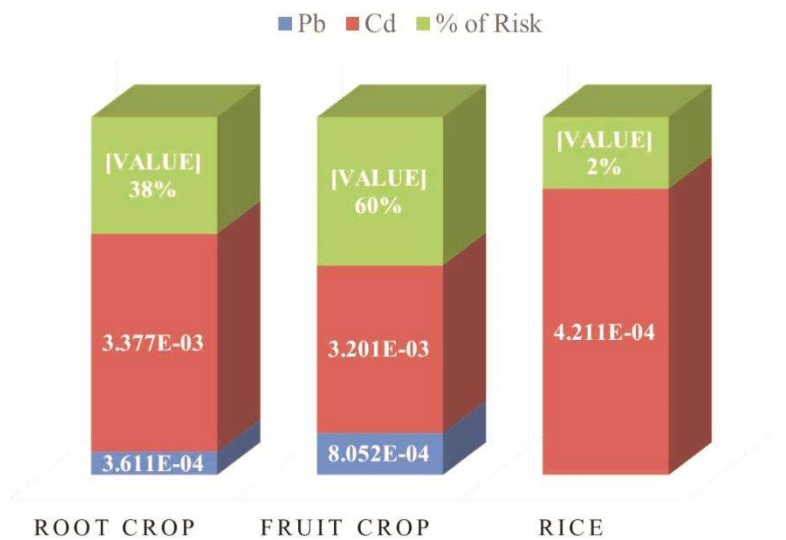

Fig. 4. Cumulative cancer risks (ΣILCR,**%**) associated with carcinogenic heavy metals in the studied crops.

Characterization of heavy metals in soils and crops:Dendrogram grouping of contaminated soils by carcinogenic heavy metals Cd and Pb, were characterized by similar responses of crop plants by their heavy metal concentration, toxicity index (*TF*, *EDI* and *ILCR*) to soil heavy metal concentration along with soil physicochemical properties were performed (Fig. 5). Hierarchical cluster analysis has shown that 75% of

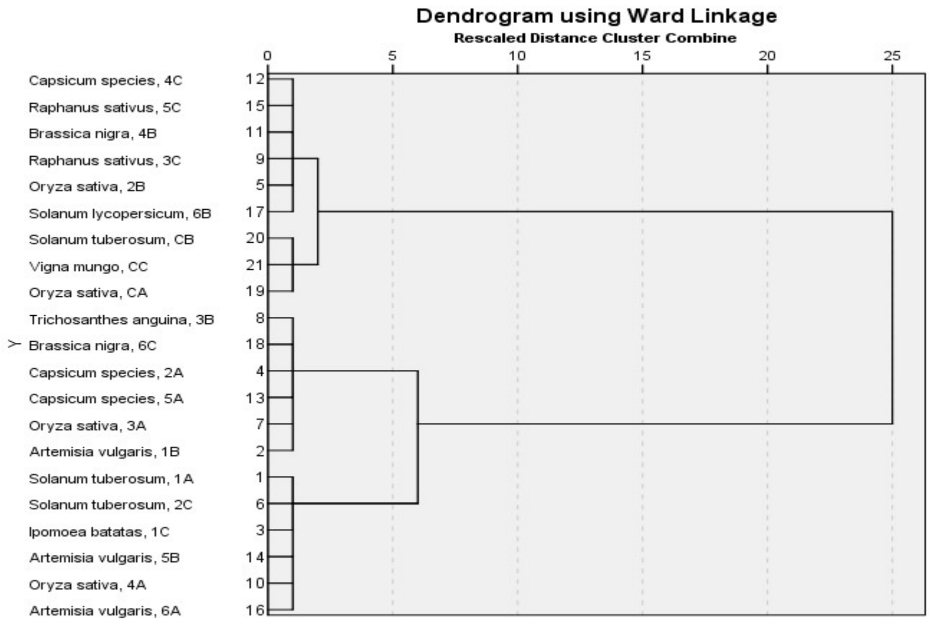

Fig. 5. Hierarchical cluster analysis of the Cd and Pb concentrations at sample sites and their effects on related crop species are displayed in a dendrogram.

agricultural soils of the brick kiln area were contaminated with carcinogenic heavy metals Cd and Pb. Two major clusters of similarities in the soils contaminated with heavy metals were identified in the dendrogram. In the sites (4C, 5C, 4B, 3C, 2B and 6B) that are clustered together with reference sites (CA, CB and CC), the average plant ecology in these soils was not under any stressed condition. Among the contaminated sites, the cluster difference shows that Cd and Pb stress on plants were in the sequence as

1A=2C=1C=5B=4A=6A>3B=6C=2A=5A=3A=1B>4C=5C=4B=3C=2B=6B.

The present study manifested that extensive, uncontrolled brick kiln operation is a possible source of carcinogenic trace metals (Pb and Cd) in the surrounding environment. Cd was the dominant carcinogen in the study area. Some of the crops in the area were at the carcinogenic risk level. Small accumulations of Cd in agricultural soils should be taken into consideration. Although the Cd concentrations were in the acceptable range provided by the standard agency, the computed carcinogenic data however, showed potential carcinogenic effects on human consumption. It is recommended from the study that growing specific metal-excluding crop species can help lower the possible health concerns related to metal toxicity.

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ASSESSING THE SPATIO-TEMPORAL VARIABILITY IN THE FREQUENCY AND MAGNITUDE OF FLASH FLOODS AND THEIR DRIVING MECHANISMS: EVIDENCE FROM HAOR REGION OF BANGLADESH

RIAZ HOSSAIN KHAN[1], M. SAIFUL ISLAM[2*] AND FARHANA ZAMAN[3]
[1]BRAC James P Grant School of Public Health, BRAC University, Bir Uttom A K Khandakar Road, Dhaka-1213, Bangladesh
[2]Department of Geology, University of Dhaka, 1000, Bangladesh
[3]Department of Biotechnology and Genetic Engineering, University of Development Alternative, Dhaka, 1209, Bangladesh

Abstract

There are a limited number of studies addressing the spatiotemporal variability of pre-monsoon flash floods and their driving forces in Bangladesh. This study examines long-term trends in temperature, rainfall, and the frequency and magnitude of flash floods in the five most vulnerable *haor* districts of northeastern Bangladesh. Temperature, rainfall, and surface water level datasets, up to 2018, were collected from the Bangladesh Meteorological Department (BMD) and the Bangladesh Water Development Board (BWDB). Based on the normal distribution of these datasets observed in quantile-quantile (Q-Q) plots, regression models were used to analyze the long-term temperature trends. The models predict a gradual rise in maximum temperature, ranging from 1.06% to 1.94% over decadal periods. Additionally, an average annual rainfall increase of 4.1% at Sreemangal and 2.28% at Sylhet stations are forecasted. Analysis of historical data from the past sixty years shows a relatively lower peak river stage in tidal rivers compared to non-tidal river stations during pre-monsoon months. The frequency of peak surface water levels at six non-tidal and ten tidal river monitoring stations was estimated using Gumbel's probability estimation method. Frequency analysis suggests a high probability of flash floods across most floodplain areas, with a return period of five to ten years, based on flood danger levels established by government agencies. Furthermore, MODIS satellite imagery (with cloud cover <10%) from the peak flood months (March to May) between 2004 and 2017 was analyzed to assess the extent of flash floods in the study area. Geospatial analysis revealed temporal variations in peak flood extents across different locations. While no clear trends were observed in the frequency of flash floods, their magnitude has significantly increased in recent decades, potentially leading to greater losses in agriculture and property. The increased vulnerability to flash floods in the region can be attributed to several factors, including a rise in pre-monsoon heavy rainfall in the upstream hilly regions of Assam and Meghalaya, high sediment loads in Transboundary Rivers, drainage congestion, poorly designed and maintained flood control structures, and the absence of a reliable flash flood warning system.

Keywords: Climate change, Flash flood, Frequency analysis, Haor, Hazards, Tidal River.

* Corresponding author: msaiful@du.ac.bd

Introduction

Flash flood generally occurs in low-lying areas shortly after a heavy rainfall event which can also occur in the downstream areas from the source of the precipitation. Earlier studies reported the occurrence of frequent flash floods due to changes in rainfall patterns and intensity in peninsular, east, and northeast India and Myanmar, creating food insecurity and flood risk (Boori *et al.,* 2017; Guhathakurta *et al.,* 2011). High drainage densities, topographic relief, along with heavy rainfall have increased the frequency of flash floods and their risk potential in Western Nepal (Pangali Sharma *et al.,* 2021; Pokharel *et al.,* 2020). Along with climate change effects, encroachment and excessive interventions in the flood plains and flood-prone areas, destruction of forests and hill slopes development and other manmade activities has significantly increased the frequency of flood and therefore increased risks to humans, property, and the economy in different parts of Malaysia and the Brahmaputra basin of India (Bhattacharyya and Bora, 1997; Maqtan *et al.,* 2022; Mohamad *et al.,* 2012; Muhammed *et al.,* 2022; Weng, 1997). To combat the flood-induced losses, the local and regional agencies undertook various conventional approaches to controlling floods, including structural interventions, many of which were found less effective or entirely ineffective in the long run. For instance, the authorities in the Brahmaputra basin adopted various flood control measures since the early fifties, which became non-operational over time (Bhattacharyya and Bora, 1997). Another example is the breach of the Kosi embankment in Nepal resulted from the high sediment content of the Kosi River, which proved ineffective in controlling floods in Bihar (Dixit, 2009).

Bangladesh is considered one of the most vulnerable countries to climate-induced hazards and disasters (Ali *et al.,* 2013; Khan and Islam, 2018; Sammonds, 2021). Lack of adequate institutional capacities, infrastructural development, and climate variability results in significant damage to agriculture and fish production in the north-eastern *haor* region of Bangladesh (Sammonds, 2021; Hossain, 2013; Hossain *et al.,* 2017; Kamruzzaman and Shaw, 2018; Suvra, 2021). Pre-monsoon (March to May) flash floods often result from excess rainfall in the upstream hilly areas of Asam and Meghalaya in India and enter the Haor areas through the transboundary rivers within 5-6 hrs (Dey *et al.,* 2021; Roy *et al.,* 2017; Sarker and Rashid, 2013). Therefore, the emergency response system was often unsuccessful in reducing the adverse impacts of the sudden intense flood (Rahman *et al.,* 2011). These climate impacts significantly hinder the economic growth, livelihood improvement, environment, and public health of the *haor* communities (Suvra, 2021; Sadeque, 2018). Bangladesh Water Development Board took the initiative to erect low-height submersible embankments, drainage channels, and sluices and regulators to delay pre-monsoon floods and to reduce the damage to agriculture crop productions since 1960 (Suvra, 2021; Khan, 2010; Rahman and Salehin, 2013).

In Bangladesh, no comprehensive studies were conducted regarding how the magnitude and frequency of flash floods vary spatially over different temporal scales in the NE *haor* districts; tectonically, *haor* is a bowl-shaped large depression (Banglapedia, 2021). There is also a significant gap in understanding the influences of the key driving forces and their characteristics which is a significant constraint for the early preparedness and emergency response to reduce the adverse impacts in flash flood vulnerable areas. Earlier, the flood danger levels at and nearby the river stations were set by the Flood Forecasting and Warning Center (FFWC) of the Bangladesh Water Development Board (BWDB), based on the riverine peak floods during the monsoon period (June to October) (Roy *et al.,* 2019). The flood danger level indicates the threshold above which flood may potentially overflow the floodplain areas and affect the lives, properties, and crop production (Rahman *et al.,* 2011). Although pre-monsoon flash flood causes significant damage to crop cultivation and properties, there are neither any scientifically well-defined danger levels, particularly for flash floods, nor any reliable early warning system considering the flash flood magnitudes, elevation of the submersible embankments, and floodplains. There is also a lack of regional collaboration to establish hydro-meteorological networks to strengthen the flash flood early warning system. Therefore, this study is aimed at assessing the long-term variability of the frequency and magnitude of flash floods spatially in the *haor* districts of Bangladesh. A further aim was to explore how the variations in climate conditions and human interventions contribute to the vulnerability of flash floods. Finally, this study attempted to elucidate any technical gaps that need to be addressed to improve the flash flood warning system. That information will be beneficial to identify flash flood danger levels and to adopt efficient warning systems in vulnerable areas.

A study by Center for Environment and Geographic Information Services (CEGIS) shows that about 373 Haors cut across Kishorenganj, Netrokona, Sunamganj, Sylhet, Habiganj, Mymensingh, and Brahmanbaria districts of Bangladesh (CEGIS, 2012). As many as 28 Upazilas under the five most vulnerable Hoar districts were considered for this study. Those include eleven Upazilas from Sunamganj, three from Habiganj, four from Netrokona, four from Kishoreganj, and six from Brahmanbaria (Table 1; Fig. 1).

Table 1. Locations of the study area.

Name of the districts	Name of the upazilas
Sunamganj	Sunamganj Sadar, Tahirpur, South Sunamganj, Bishwambapur, Jamalganj, Derai, Sulla, Chahtak, Dowarabazar, Dharmapasha and Jagannathpur
Habiganj	Azmiriganj, Lakhail, Baniachong
Netrokona	Khaliajuri, Komlakanda, Modon, Mohanganj
Kishoreganj	Itna, Mithamoin, Astagram, Nikli
Brahmanbaria	Nasirnagar, Nobinagar, Sarail, Ashuganj, Brahmanbaria Sadar, Bancharampur

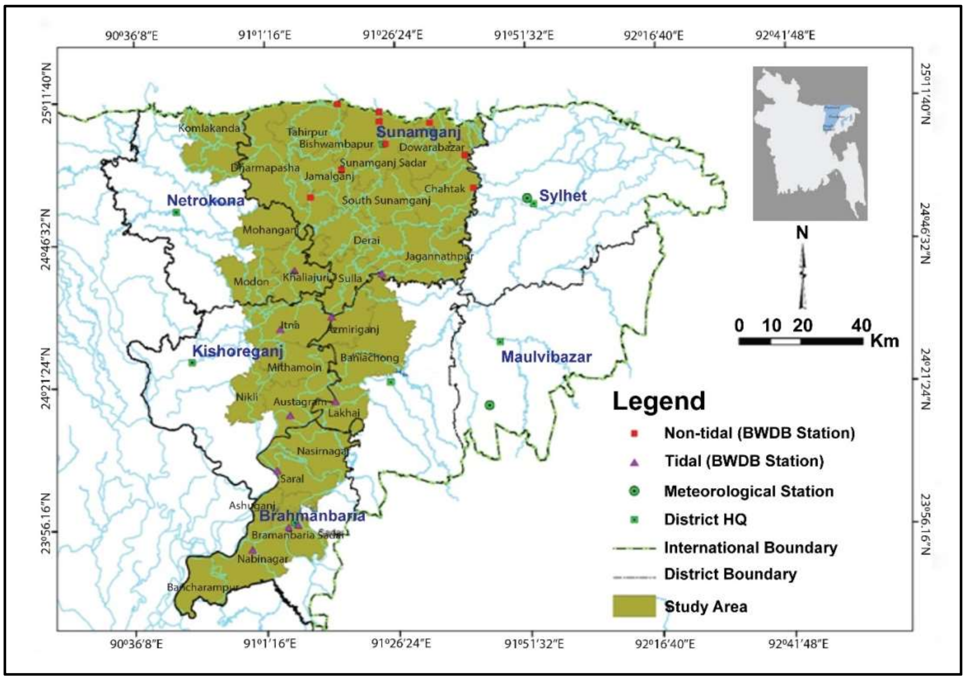


Fig. 1. Location map of the study area.

Materials and Methods

Available temperature datasets of up to 2018 were collected from the Bangladesh Meteorological Department (BMD). Trend analyses were conducted at nearest station Id 10704 (Sreemangal) and station Id 10705 (Sylhet) to see the annual trend of the maximum and minimum temperature values over decadal-scale periods. The mean annual maximum and minimum temperature datasets distribution was normal in the quantile-

quantile (Q-Q) plots. So linear regression models were run to examine the long-term trends of temperature. Future temperature projections were performed using the Statistical Downscaling Model (SDSM) based on observed data from 1991 to 2015. Climate projections were performed for three-time slices: 2023-37, 2043-57, and 2073-87, and for two Representative Concentration Pathways (RCP) scenarios: RCP 4.5 and RCP 8.5 (Wikipedia, 2024).

Available rainfall data for stations 10704 (Sreemangal) and 10705 (Sylhet) were collected from the BMD. Cumulative annual rainfall data from 1991 to 2018 were examined for trend analysis for these locations.

Available surface water level data (1950-2018) were collected from BWDB. The Gumbel and generalized extreme value (GEV) distributions provided the most reliable estimates among the available methods tested to assess the frequency analysis of pre-monsoon flash floods. Therefore, the frequency of peak surface water levels of different monitoring stations was estimated using Gumbel's probability estimation method up to a one-hundred years return period (Smail and Myrtene, 2004). A total of six non-tidal and ten tidal river stations were considered for frequency analyses. Log-normal distributions of surface water level frequency analysis results showed that the distributions were normal enough in the quantile-quantile (Q-Q) plots. Although slight curviness was noticed at stations 268, 131.5, and 33, no significant heteroscedasticity influenced the outcomes.

Available Moderate Resolution Imaging Spectroradiometer (MODIS) satellite images for the peak flood months (March to May) from 2005 to 2017 were studied to evaluate the flash flood extents in those areas. Bangladesh transverse Mercator (BTM) projection was used to transfer locations from 3D to 2D space. Finally, unsupervised classification was carried out using the isodata clustering techniques to classify pixels that represent water bodies. Flash flood extents were determined based on the areas inundated by water. Finally, trend analyses were conducted to study the long-term trends of flash floods in those *haor* regions.

Results and discussions

Long-term variations of meteorological condition: Halder *et al.* (2023) examined the temperature and rainfall patterns in the Guwahati City of Assam using data from 1970 to 2019, which showed an increase in the seasonal and annual min-max temperature over the years. Results showed that the monsoon and winter rainfall decreased, but the pre-monsoon rainfall has increased over the past 50 years. Marak *et al.* (2020) studied the spatial and temporal rainfall variations in the Umiam and Umtru watersheds of Meghalaya using gridded rainfall data from 1901 to 2018. Results showed no definite patterns of the annual minimum and maximum rainfall spatially. However, the high-intensity rainfall is increasing at the seasonal and annual scales. Overall, the increases of the pre-monsoon rainfall in Assam and the ratio of high-intensity rainfall in Meghalaya increase the likelihood of frequent and high-magnitude flash floods due to the rapid movement of the flood water through the transboundary rivers in the study area.

Temperature trends: The mean maximum annual temperature varied between 31.62°C and 29.88°C in Sreemangal, whereas values ranged between 31.47°C and 29.12°C in Sylhet (Fig. 2). A comparatively higher mean annual maximum and a lower mean annual minimum temperature were found at Sreemangal compared to Sylhet station. However, both maximum and minimum temperature datasets at Sylhet show relatively higher variability than those at the Sreemangal station. Although a strong correlation (R = 0.88) was noticed between the mean annual maximum temperatures at both locations, the correlation was relatively weaker between the minimum temperature datasets at a 95% confidence interval.

The given models in Fig. 2 predict an average increase of the mean maximum and minimum temperature of 1.06% and 0.92%, respectively in Sreemangal at every decadal-scale time. On the other hand, a comparatively higher increase in mean annual maximum (1.88%) and minimum (1.94%) values were found in Sylhet within the same time frame. In addition to the trend analysis, monthly mean maximum and minimum temperatures were studied to predict the mean monthly temperature fluctuations in the study area. In every case, higher-order polynomial functions produced the best-fit models with explanatory power of at least 89% with respect to the observed data points (Equations 1-4). The maximum temperature was predicted to be highest in July and August. In contrast, the lowest temperature was predicted to be in January at both stations.

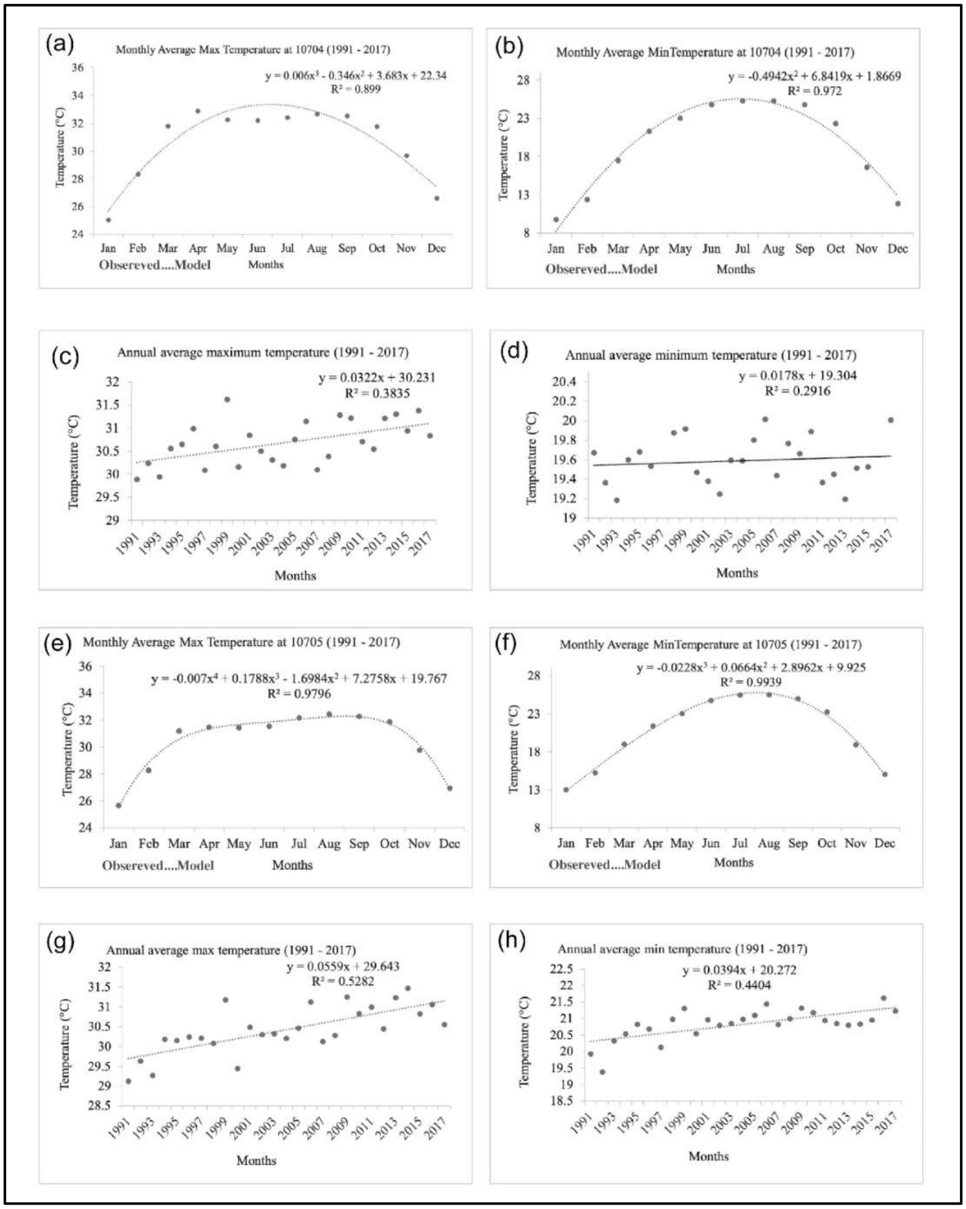
The following models were chosen for the given locations.

Model for the mean monthly maximum temperature at station 10704:

$$Y = 0.0062x^3 - 0.346x^2 + 3.6839x + 22.348;\ R^2 = 0.8997 \qquad \text{(Equation 1)}$$

Model for the mean monthly minimum temperature at station 10704:

$$Y = -0.4942x^2 + 6.8419x + 1.8669;\ R^2 = 0.972 \qquad \text{(Equation 2)}$$



Source: Temperature data from Bangladesh Meteorological Department (BMD).

Fig. 2. Monthly and annual average maximum and minimum temperature from 1991 to 2017 at the Sreemangal station 10704 (a to d) and Sylhet station10705 (e to h).

Model for the mean monthly maximum temperature at station 10705:

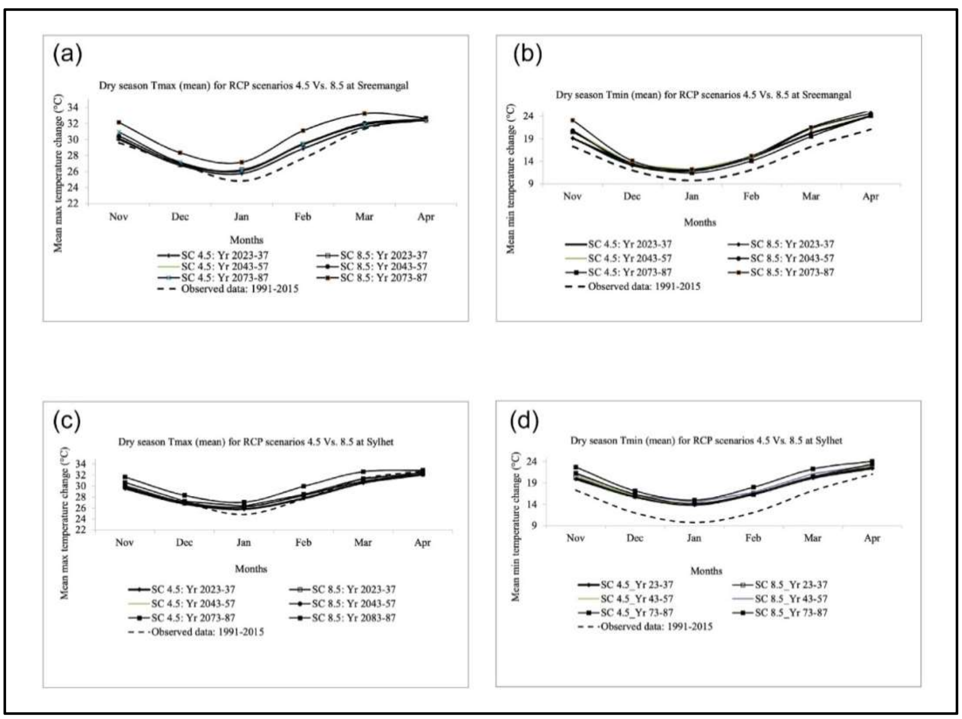
$$Y = -0.007x^4 + 0.1788x^3 - 1.6984x^2 + 7.2758x + 19.767;\ R^2 = 0.9796 \qquad \text{(Equation 3)}$$

Model for the mean monthly minimum temperature at station 10705:

$$Y = -0.0228x^3 + 0.0664x^2 + 2.8962x + 9.925;\ R^2 = 0.9939 \qquad \text{(Equation 4)}$$

Where x and y are the time (in years) and mean monthly temperature (in °C), respectively, the value of R^2 represents the model's explanatory power.

Both RCP scenarios 4.5 and 8.5 at both stations predicted a higher temperature during the winter months (Fig. 3). Therefore, a gradual rise of temperature over decadal scale time may pose significant threats to agriculture and fisheries production over longer time intervals.



Source: Temperature data from BMD.

Fig. 3. Representative Concentration Pathways (RCP) scenarios of dry season temperature forecast using Statistical DownScaling Model (SDSM) at the Sreemangal station 10704 (a to b) and Sylhet station10705 (c to d).

Rainfall condition: Annual cumulative rainfall at Sylhet station (Id: 10705) shows higher variability than that at the Sreemangal station (Id: 10704). The minimum and maximum rainfall varied between 1741mm and 3813mm in stations 10704 and 3100 mm and 5944 mm in station 10705. The median annual rainfall of 3891 mm at station 10705 was considerably higher than that at station 10704 which is 2266 mm. A moderately strong positive correlation was noticed (correlation coefficient 0.62) between the cumulative annual rainfalls at those two locations.

No noticeable differences were observed in the intensity of pre-monsoon rainfall at any of those stations. However, a slight increase in annual rainfall was noticed in both locations. At station 10704, the following regression model showed an average of 4.1% increase in the annual rainfall over a decadal scale period.

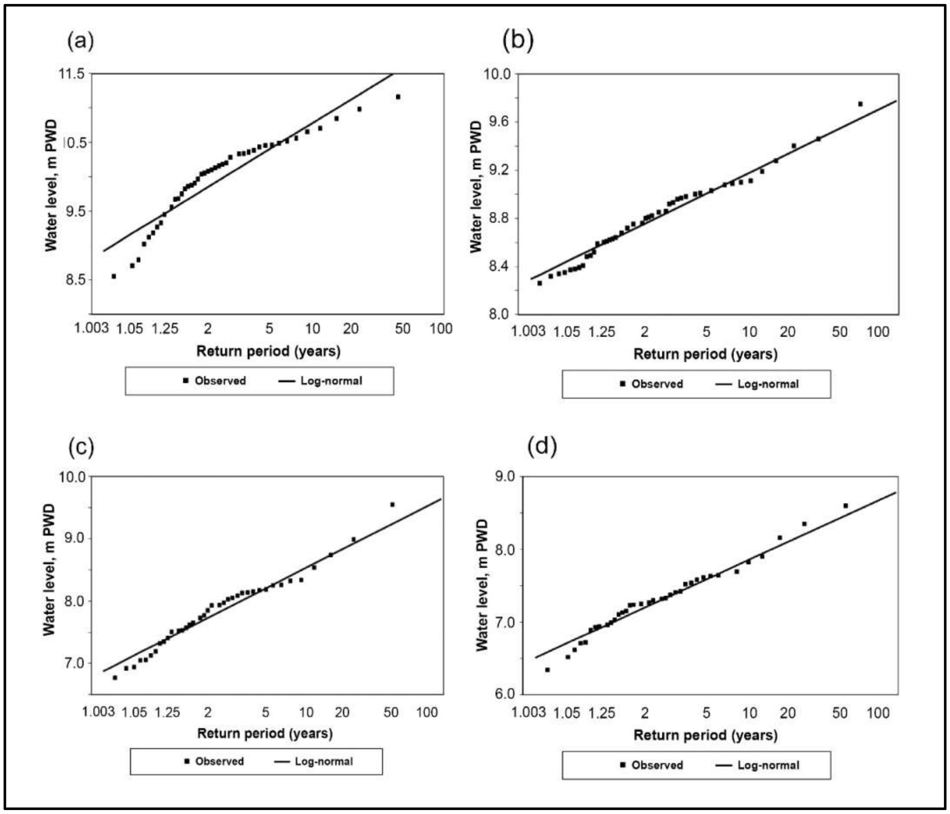
$$\text{Rainfall (in mm)} = f\,(\text{Number of years}) \times 9.27 + 2261 \qquad \text{(Equation 5)}$$

On the other hand, an approximately 2.28% increase in the annual rainfall was predicted for similar time frames at station 10705. The linear regression model is given below-

$$\text{Rainfall (in mm)} = f\,(\text{Number of years}) \times 8.89 + 3876.8 \qquad \text{(Equation 6)}$$

Pre-monsoon Flood Frequency analyses: Locations of the BWDB tidal and non-tidal river stations are shown in Fig. 1. Among the six non-tidal river stations considered, the highest and lowest peaks of surface water levels were predicted as 13.46 m PWD at station 131.5 and 8.72 m PWD at station 72B, respectively over a one-hundred-year return period (Table 2 and Fig. 4).

A total of ten tidal river stations located in the southern part of the study area were considered for frequency analysis (Fig. 1). Relatively lower peak river stages were predicted for the tidal rivers compared to the non-tidal rivers within a one-hundred-year return cycle (Table 2 and Fig. 4). In addition, the variability of the probable higher river stages is statistically higher in the non-tidal river stations. The possible highest and the lowest peaks of tidal river stages were predicted for station 72 and station 298, respectively, up to a return period of 50 years. However, a noticeably higher positive slope for stations 271 and 272.1 was resulted from a significant rise in water level at the Upper Meghna River. The maximum surface water peaks were predicted in station 271 at and beyond fifty-year time intervals. Finally, frequency analysis results indicate high chances of very high magnitude flash flood events at most river stations, especially at Jadukata (Id: 131.5) and upper Meghna River (Ids: 271 and 272.1) within a fifty-year return period.



Source: Surface water level data from Bangladesh Water Development Board (BWDB).

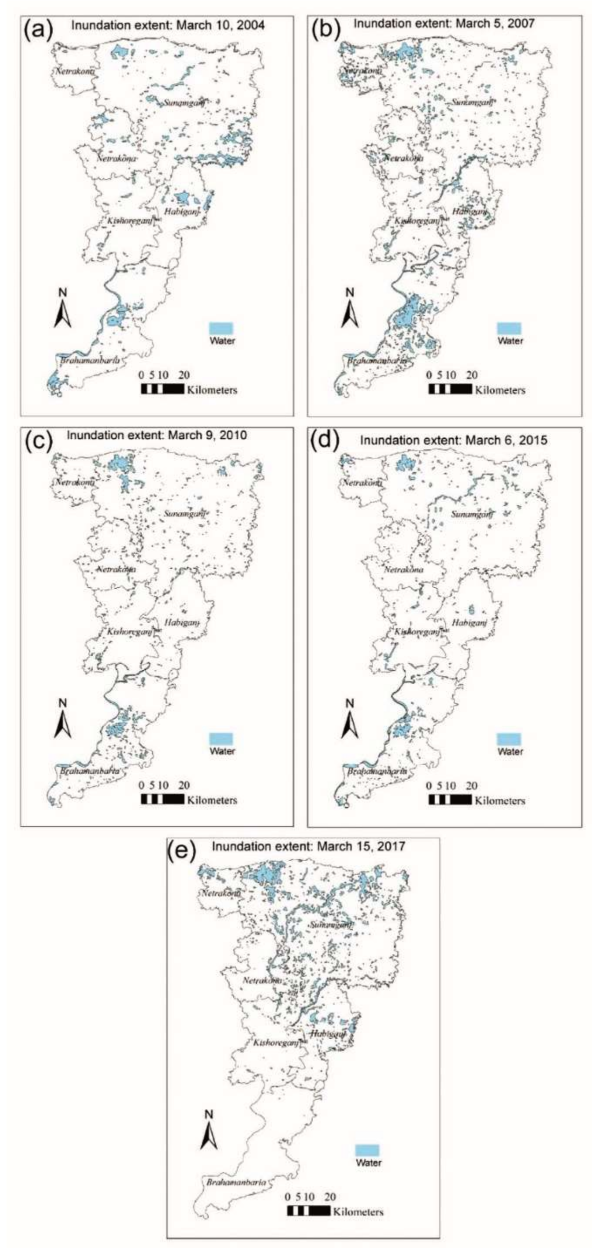
Fig. 4. Frequency analyses of pre-monsoon non-tidal surface water level at stations (a) 268 and (b) 269, and tidal surface water level at stations c) 72 and d) 271.

Results show that the surface water danger levels currently being practiced by the FFWC for monsoon flood lie below two years pre-monsoon flood return period at the surface water stations in Dhanu-Boulai Ghorutrautra and Jadukata rivers and Anderson Khal (canal). The danger level lies below the 5-10 years flood return period at several stations in Surma-Meghna and Lower Meghna rivers (Table 2). Therefore, heavy rainfall in the upstream hilly areas results in frequent flash floods in the floodplain communities, ultimately affecting the agriculture production of the study area.

Table 2. Frequency analyses results during the pre-monsoon period at the non-tidal and tidal river stations in the study area.

Rivers' Name	Station ID	Water level (m PWD) in different Return periods (years)						Available data (Years)	Existing DL (in m PWD)
		2	5	10	20	50	100		
Non-tidal rivers									
Bhattakhal	33	9.96	10.60	11.03	11.43	11.96	12.35	1958-1981	-
Dhanu-Boulai	72B	7.23	7.63	7.89	8.15	8.47	8.72	1982-2018	5.31
Jadukata	131.5	10.62	11.38	11.88	12.36	12.99	13.46	1993-2009	8.53
Julakhali	333	9.62	9.89	10.07	10.25	10.46	10.64	1988-2009	-
Surma-Meghna (SM)	268	9.85	10.41	10.78	11.13	11.59	11.93	1960-2018	9.5
SM	269	8.75	9.03	9.21	9.38	9.60	9.77	1960-2018	8.25
Tidal rivers									
Anderson Khal	3A	6.31	6.78	7.09	7.38	7.77	8.06	1960-2009	5.5
Dhanu-Boulai Ghorutrautra (DBG)	72	7.73	8.24	8.58	8.90	9.32	9.63	1960-2009	6.31
DBG	73	7.47	7.94	8.25	8.54	8.93	9.21	1960-2009	-
Lower Meghna (LM)	270	7.48	7.90	8.17	8.43	8.77	9.03	1969-2009	8.5
Upper Meghna (UM)	271	7.24	7.93	8.38	8.82	9.38	9.8	1968-2018	-
UM	272.1	6.36	7.10	7.59	8.07	8.67	9.13	1965-2009	-
LM	272	6.81	7.36	7.73	8.08	8.54	8.88	1960-2009	-
Titas	295	6.52	7.12	7.52	7.90	8.40	8.77	1960-2009	-
Titas	297	6.16	6.70	7.06	7.40	7.85	8.18	1960-2009	-
Titas	298	6.06	6.54	6.86	7.17	7.57	7.87	1960-2018	-

Source: BWDB; DL=Danger Level; PWD=Power Works Datum



Source: MODIS satellite imageries.

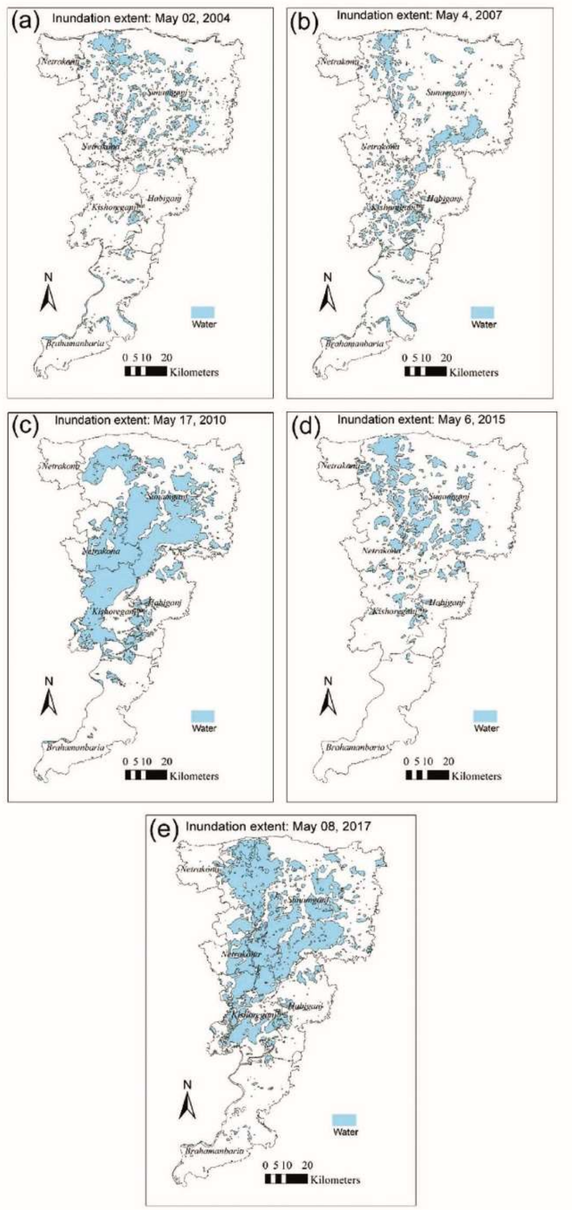
Fig. 5. Flash flood extents (% area inundated) in five *haor* districts in March of a) 2004, b) 2007, c) 2010, d) 2015, and e) 2017. Authors analyzed MODIS satellite images to generate the flood extent maps

Spatio-temporal trends of Flash flood inundation: The *haor* areas experienced significant floods in 1974, 1988, 1998, 2004, 2007, 2010, and 2017 (Rahman et al. 2011). Analysis of available satellite imageries for the pre-monsoon (March to May) from 2005 to 2017 reveals that the largest land area was inundated in the late of April to May. However, time-dependent variations of peak flood extents were noticed spatially from the same flash flood event (Fig. 5 and Fig. 6). In March, Brahamanbaria and Habiganj districts were found to be the most and the least inundated among the five Haor districts. On the other hand, Sunamganj areas became highly flooded in April. In general, the majority of the extreme flood events were found to take place in May. In May of 2006, 2007, 2010, and 2015, more than 15% of the land was flooded with water in several haor districts (Fig. 6). In May 2010, about 60%, 39%, 35%, and 15% of the land area of Kishorganj, Sunamganj, Netrokona, and Habiganj districs were flooded, respectively. Results showed about 58%, 23%, 19%, 10% and 7% areas in Sunamganj, Kishoreganj, Netrakona, Habiganj, and Brahamanbaria districts, respectively were inundated in 2017.

A slight annual decreasing trend of flash flood extents (i.e., the percentage of the area inundated) was observed in Brahamanbaria, Netrokona, and Sunamganj. However, no definite trends are observable in Habiganj and Kishoreganj in March. In April, flood extents slightly decreased in Brahamanbaria and slightly increased at Habiganj and Sunamganj districts. No definite patterns of changes were noticed in other two districts over the years. A gradual declination in flood extents was observed in all of the *haor* districts except Netrokona, where the trends of the flood extents appear to be relatively constant.

Flash flood magnitude at different return intervals: Fig. 7 shows the intensity and frequency of high-magnitude pre-monsoon flash floods in April against different return periods in the selected Haor districts in north-eastern Bangladesh. Results indicate that the frequency of flash floods has not increased in the past 50 years, but the magnitude of the flood level has noticeably increased in those areas. Therefore, the earlier flood control structures, including the existing low-elevation embankments, would be unsuccessful in protecting agriculture, life, and properties from the possible upcoming floods with much higher magnitudes.

The submersible embankments' age dates from the early sixties to the present, and therefore the structural design varies significantly. The 2004, 2010, and 2017 flash floods noticeably damaged human life and property. During those years, the flood water overtopped most of the submersible embankments designed for floods with 1-10 year



Source: MODIS satellite imageries.

Fig. 6. Flash flood extents (% area inundated) in five Haor districts in May of a) 2004, b) 2007, c) 2010, d) 2015, and e) 2017. Authors analyzed MODIS satellite images to generate the flood extent maps.

return periods. Those infrastructural interventions were not very successful because of noticeable rise in pre-monsoon rainfall intensity during the recent decades in the upstream areas in Assam and Meghalaya, backdated design for the constructions of the earlier embankments including adequacy of design crest level, improper drainage systems, river sedimentation, erosion and breaching of the embankments during the flood flow, public cuts and breaches of the embankments, and the lack of regular operation and maintenance (Suvra, 2021; Mahtab *et al.,* 2018).

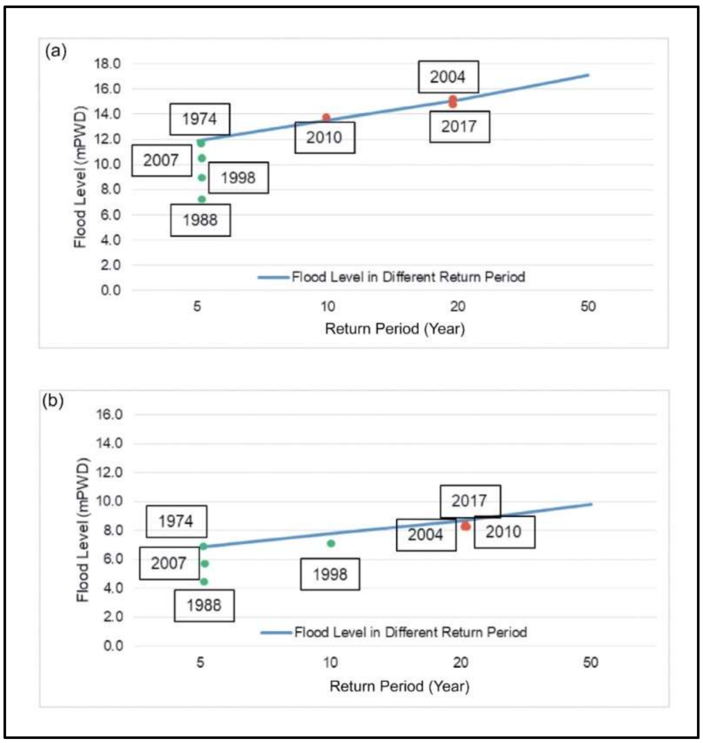


Fig. 7. Peak flood level versus different return periods in the month of April: (a) Sylhet-Sunamganj and (b) Sunamganj-Habiganj stations.

Conclusion

During recent years, an increase in the pre-monsoon heavy rainfall in upstream hilly areas in Assam and Meghalaya has substantially increased the likelihood of high-magnitude flash floods and hence the vulnerability of the losses of agriculture and properties in the northeastern *haor* region of Bangladesh. Results show that the frequency of flash floods did not show any specific trends of rise or fall in the past fifty years. However, the flash flood's magnitude has increased, ultimately inundating a significant portion of the *haor* region in recent decades. A variety of factors simultaneously influence the effectiveness of the flash flood management systems ranging from climate variability, siltation on the river beds, backdated design of the earlier embankments, the elevation of the flood control structures, including behavioral and institutional components such as public cuts, breaches, improper operation, and lack of proper maintenance of the flash flood embankments. The monsoon flood danger levels currently being practiced were found inadequate to implement the pre-monsoon flash flood warning system. Therefore, it is inevitable to adopt an efficient flash flood warning system incorporating an appropriate flash flood danger level and the redesigned elevations of the flood control structures through a rigorous cost-benefit analysis. There is also a great need to develop collaboration with the upstream regional authorities in Assam and Meghalaya to establish an effective hydro-meteorological networks with the view to strengthening the flash flood early warning system further.

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ECOLOGICAL IMPACT ON THE PREVALENCE OF GIANT SCALE INSECT (HOMOPTERA : MONOPHLEBIDAE) IN JAHANGIRNAGAR UNIVERSITY CAMPUS, BANGLADESH

IBNA ZAYED CHOWDHURY[1*], G. M. SAIFUR RAHMAN[2]
[1]*Department of Zoology, Tongi Government College, Gazipur-1711, Bangladesh*
[2]*Department of Zoology, National University, Gazipur-1704, Bangladesh*

Abstract

Most monophlebid insects are highly polyphagous, dimorphic, and well-known sap-sucking plant pests throughout the world. To determine their abundances, distribution, incidences, seasonal dynamics, feeding nature and the effects of ecological changes on their populations, an initial study (September 2004–August 2005) and a review study (March 2023–February 2024) were carried out in Jahangirnagar University Campus (JUC), Savar, Dhaka, Bangladesh. The host plants (trees and shrubs) were examined through biweekly visual counts. In the initial investigation, 14,802 monophlebid insects from three species (Biodiversity Index or BI = 0.002, H = 1, D = 0.387, 1-D = 0.613, 1/D = 2.58, and E_H = 0.91) were documented from 10 plant species. In the review study, a total of 423 insects under five species (two previously identified and three new) (BI = 0.141, H = 0.22, D = 0.917, 1-D = 0.083, 1/D = 1.1, and E_H = 0.14) detected from five plant species, including one new species. Within around 20 years from the first study, the percentage of total plants and infested plants decreased significantly by 47.14% and 92.96%, respectively. The amount of insects decreased by 2.86%, while 87.67% of shrubs decreased (F = 5.8, df = 1, P = 0.07). The pest insects from trees and shrubs reduced by 3.67% and 0.45%, respectively. The identified most suitable host plant was *Ficus benghalensis* for sheltering maximum scale insect in both studies. The only insect pest, *Icerya aegyptiaca* was consistently abundant throughout the period in both studies. The insect, *I. aegyptiaca* was found available on every part of the plant, though the majority of them survived on leaves. The present findings might contribute understanding ecological imbalances and helpful for developing effective management strategies against these insect pests.

Keywords: Prevalence, Ecology, Scale insects, Plant pests, Bangladesh.

Introduction

A major share of insects often poses a threat by destroying and damaging our economically important plants as well as injuring humans and animals (Allison *et al*., 2023). The giant scale insect is a notable soft-bodied commercial pest that causes significant damage to a variety of host plants, primarily woody ones. It is dimorphic, polyphagous, extremely fecund, and cryptic. Giant scales live in protected habitats such

[*]Corresponding author: t05075@nu.ac.bd

as bark cracks and crevices, at the base of leaf petioles, on the underside of leaves, within fruit bunches, in the plant crown, in branch crotches, on stems near soil, and they infest all parts of host plants. Their wax-coated bodies shield them from contact insecticides and other mortality factors. Despite their apparent monophyletic grouping, the giant scales exhibit diversity in both morphology and biology (Mani *et al*., 2011; Morales *et al*., 2016).

Certain scale insects provide benefits to humanity by producing valuable products like wax and dyes (Kondo and Gullan, 2022). But the majority is significant plant pests including transmission of fungal, bacterial and viral pathogens (Allison *et al*., 2023). Bangladesh has few records on its total scale insect species and their pest association with different host plant species (Chowdhury *et al*., 2023). In addition, our flora and fauna is continuously decreasing due to our environmental changes by gradual deforestation to meet the need of over burden human population (Reza and Hasan, 2020; Brandão *et al.*, 2022). So, continuous study is essential to update the status of our pest population.

As Jahangirnagar University Campus (JUC) is a protected area with rich biodiversity (Khan *et al*., 2021), any kind of changes on its environment and plant pest association can be understood easily. Therefore, it was selected to conduct the present study to estimate the population abundance of giant scale, their seasonal richness, feeding habit and ecological impacts on their population fluctuation and feeding nature. To assess the above-mentioned factors including influence of major climatic factors on monophlebid population also studied to determine the ideal time for operating pest management activities.

Materials and Methods

Study area: The study was conducted in Jahangirnagar University Campus (JUC), Savar, Dhaka. The campus is located on an area of 282.29 hectares. It is situated on the western side of the Dhaka-Aricha Highway (Fig. 1) of 32 km northwest of Dhaka City (23.8671°–23.8977° E and 90.2588°–90.2731° N) having iron rich fertile soil (Khan *et al*., 2021).

Methods: Study sites were visited in an interval of twice in a month for both studies of September 2004 to August 2005 and March 2023 to February 2024 to monitor the occurrence and field adaption of giant scales. The last study was conducted mainly to compare the changes in the habitat, abundance and diversity of test insects. At the start of the study, ten major plant species in 2004 and five plant species in 2023 were identified and labeled as host plants with the aid of a plant taxonomist from the same university's

Botany Department. The experimental plant species included both trees and shrubs. The trees were *Albizia procera* (Roxb.) Benth., *Albizia lebbeck* (L.) Benth., *Mangifera indica* L., *Artocarpus heterophyllus* Lam., *Ficus benghalensis* L., *Achras sapota* L., *Alstonia scholaris* (L.) R. Br., and *Cassia javanica* L., while the shrubs were *Citrus medica* L., *Citrus grandis* (L.) Osbeck, and *Psidium guajava* L.

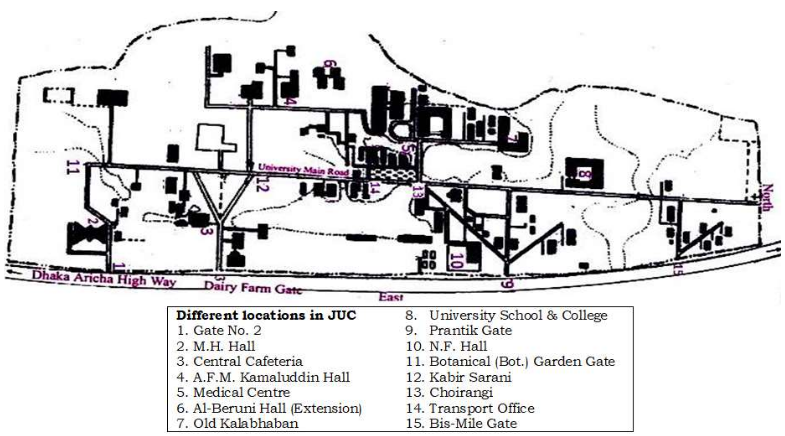

Fig. 1. Study area.

Using a 4–10x magnification lens, the extent of insect infestations on plants was evaluated visually, and data were recorded using standard procedures (Ullah, 1987; Prasanna and Balikai, 2015; Akter *et al*., 2017). Scale nymphs and adults were found on the surfaces of leaves, bark, and in the crevices of stems, branches, and rootstock of every plant, extending up to an average height of two meters from the ground. In the field, a few scales were preserved in plastic containers with 70% alcohol and appropriately labeled with information on the plant species, date, and other details. They were then transported to the laboratory. The samples were counted and then sent to Pampel's fluid for further research. The samples were picked out to the species level using standard keys (Ullah, 1987; Morales *et al*., 2016; Tian *et al*., 2018).

Monthly weather data, including average maximum temperature (T_{max}), minimum temperature (T_{min}), average temperature (T_{avg}) (°C), average relative humidity (RH%), and average monthly rainfall (RF) (mm), were obtained for the JU area from the Geography and Environment Department weather station of JU, located behind the Central Cafeteria of JUC. Bangladesh has three distinct seasons: the scorching summer season (March – May), the cool, dry winter season (November – February), and the rainy monsoon season (June – October).

Data analysis: Using IBM SPSS Statistics 28.0.0.0, the data were organized in an Excel spreadsheet and subjected to a one-way ANOVA analysis. To determine significant differences in pest occurrence on various plants, across different months, and in the combined effect of climatic conditions on giant scales in hosts, Least Significant Differences (LSD) tests at a 0.05 probability level were employed. Simple correlation (r) values were calculated to examine the relationship between scale occurrence and the number of individual trees, as well as the mean records of the three investigated weather parameters, using the statistical program JASP 0.16. The Duncan Multiple Range Test (DMRT) was used to differentiate the means at the 0.05 significance level.

To explain the diversity of species, the Biodiversity Index (BI) was computed by dividing the number of species present by the total number of individuals in the area (AMNH, 2020). The Shannon and Simpson diversity indices were computed as described by Shannon and Weaver (1949) and Simpson (1949). The Shannon Index (H) quantifies species diversity by accounting for both the number of species and the distribution of individuals among them. Simpson's Index (D) evaluates diversity by considering species richness and evenness. The Simpson's diversity index (or Gini-Simpson index) = 1-*D*, and Simpson's reciprocal index is 1/D. Shannon's equitability (Evenness Index) (E_H) (Magurran, 2013) is used to measure degree in abundance of species. Sorenson's Coefficient (CC) was also calculated (Sorensen, 1948) using to gauge the similarity of two study years. Statistical analysis was done by R, a statistical programming language.

Results and Discussion

Monophlebids insect infestation in 2004-2005: A total of 14,802 giant scale insects, representing three types of monophlebid insects, were reported from 142 afflicted plants out of 490 woody plants under ten host plant species during a year-long study conducted at JUC starting in September 2004 (Table 1, Fig. 2). The mean number of different insects in different months did not differ significantly (F=0.871, df = 2, P=0.428). *Icerya aegyptiaca* (52.14%) was the most prevalent among the monophlebids, followed by *Crypticerya jacobsoni* (25.76%) and *Icerya minor* (22.1%). For scale insect occurrence in various months, *F. benghalensis*, *P. guajava*, *A. procera*, *A. heterophyllus*, and *M. indica* were shown to be more suitable hosts than other plants (F=2.599, df = 9, P=0.009). Among the ten plant species, there was no discernible variation in the mean infestation of giant scales (F=0.9, df = 9, P=0.543). Insect frequency on trees was greater than on shrubs (F=3.422, df = 1, P=0.138) (Table 1, Figs. 3-5, and Plate 1).

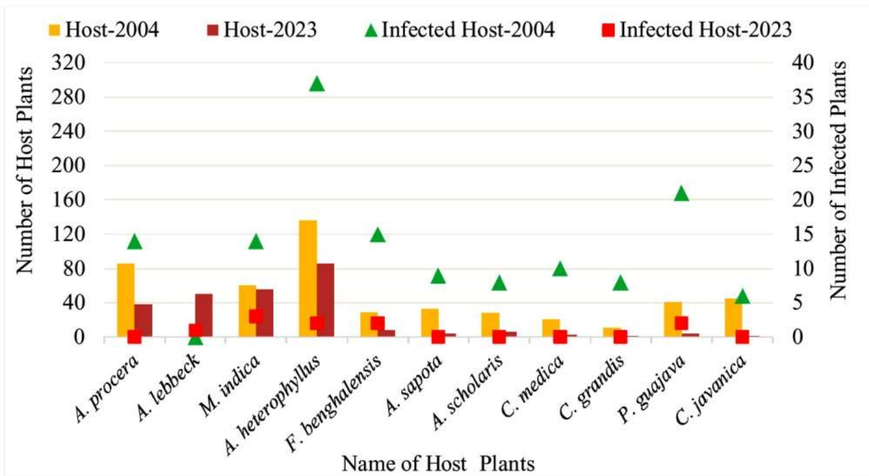

Fig. 2. Comparing the host and infected host in 2004 and 2023.

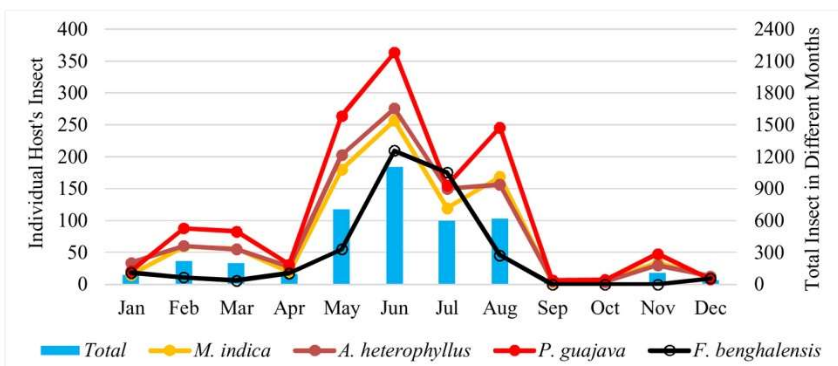

Fig. 3. Incidence of *C. jacobsoni* in various months during 2004-05.

C. jacobsoni: A total of 3,813 *C. jacobsoni* individuals were found on 40 plants out of 265 plants belonging to 4 species in JUC over a single year (Fig. 2, 3). June showed a substantially higher pest population (F=17.07, df = 47, P < 0.001) compared to other months. The frequency of *C. jacobsoni* on various plants did not differ significantly (F=0.975, df = 47, P=0.413), with each plant species hosting an average of 46–110 insects per month (Table 1 and Fig. 3).

I. aegyptiaca: A total of 7,717 *I. aegyptiaca* individuals were recorded on 57 plants out of 188 plants across 4 species in JUC over a single year (Fig. 2, 4). No specific plant species was particularly preferred by these insects (F=0.742, df = 47, P=0.533). The mean number of pest insects per month for each plant species ranged from 63 to 234. The insects were absent from October through January, with April and May producing the significantly highest populations (F=12.46, df = 47, P<0.001). Outside these months, the population was generally low (Table 1, Fig. 4).

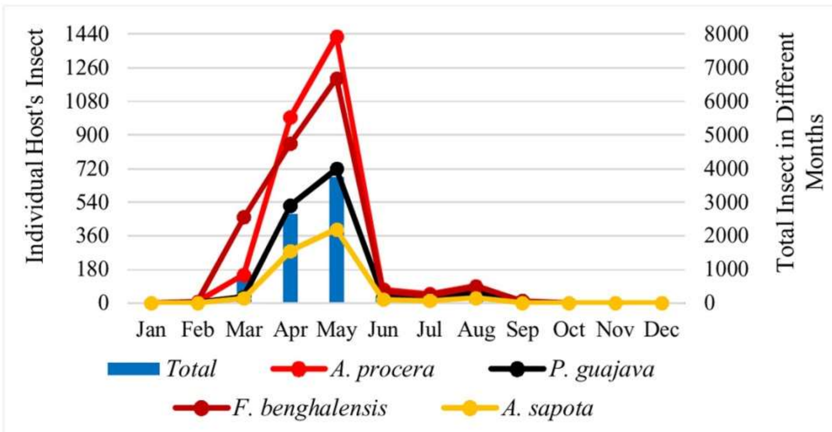

Fig. 4. Incidence of *I. aegyptiaca* in various months during 2004-05.

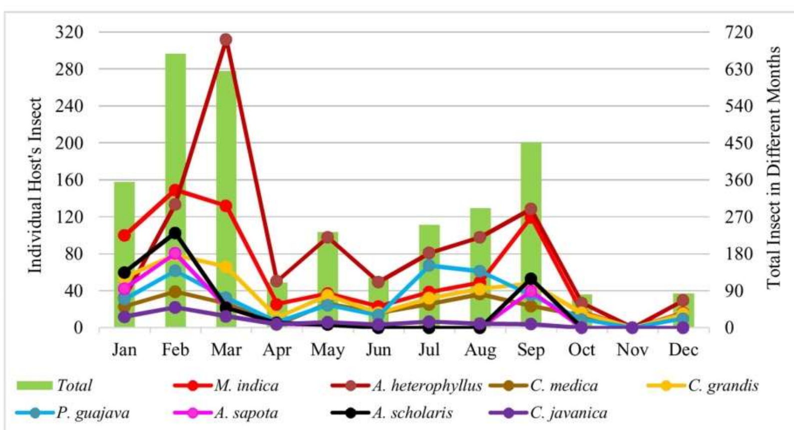

Fig. 5 Incidence of *I. minor* in various months during 2004-05.

I. minor: A total of 3,272 *I. minor* individuals were recorded in a year from 107 plants out of 375 plants across 8 species in JUC (Fig. 2, 5). This species was absent in November. Its monthly population was generally moderate to low, with February and March having the highest populations ($F=3.723$, $df = 95$, $P=0.000$). Each plant species hosted an average of 6–87 insects per month, with *A. heterophyllus* significantly harboring the highest quantity of insects ($F=5.314$, $df = 95$, $P<0.001$) (Table 1, Fig. 5).

Monophlebids insect infestation in 2023-2024: In a review conducted in the same study region during 2023–2024, 423 insects from 5 different Monophlebid species were discovered over a 12-month period. Of the 259 plants across 11 species that were examined, the pest insects were found on 10 plants belonging to 5 plant species (Table 2, Fig. 2). The mean number of different insects varied significantly across months ($F=4.51$, $df = 4$, $P=0.003$). The highest mean density was observed for *I. aegyptiaca* (95.74%), followed by *I. minor* (2.84%), *Drosicha corpulenta* (0.71%), *Icerya seychellarum* (0.47%), and *Drosicha mangiferae* (0.24%). For scale incidence across different months, *F. benghalensis* and *M. indica* were identified as the most suitable hosts ($F=3.496$, $df = 4$, $P=0.013$) compared to other hosts. Five plant species had similar average levels of scale insect contamination ($F=0.778$, $df = 4$, $P=0.552$). The incidence of various insects was significantly greater on trees compared to shrubs ($F=1.014$, $df = 1$, $P=0.343$) (Table 2, Plate 1).

I. aegyptiaca: A total of 405 *I. aegyptiaca* individuals were detected on 4 infected plants out of 68 host plants belonging to 3 species in JUC over a year (Table 2, Fig. 2). Among the plant species, *F. benghalensis* was preferred ($F=2.729$, $df = 35$, $P=0.08$) by these insects. The mean number of pest insects per plant species per month ranged from 1 to 24. Generally, its monthly population was low or absent, except in January and February ($F=2.166$, $df = 35$, $P=0.0551$) (Table 2).

The incidence of other scale species was extremely low during 2023–2024 (Table 2).

Comparative analysis of two studies: In the research area, the number of host plants decreased from 2004 to 2023 ($F=2.093$, $df = 1$, $P=0.163$, 47.14%), and the number of infested plants also decreased ($F=16.64$, $df = 1$, $P=0.001$, 92.96%). The reduction rate of shrubs ($F=5.8$, $df = 1$, $P=0.07$, 87.67%) was significantly higher ($F=5.826$, $df = 1$, $P=0.0733$) compared to that of trees ($F=1.266$, $df = 1$, $P=0.279$, 40%). Additionally, one species of host plant was recently introduced (Table 1-2, Fig. 2). Due to the declining number of host plants, pest insects decreased to 2.86% ($F=9.582$, $df = 1$, $P=0.005$).

Table 1. Incidences of monophlebid fauna on host plant species at JUC in 2004 - 2005.

	Tree							Shrub			
	A. procera	*M. indica*	*A. heterophyllus*	*F. benghalensis*	*A. sapota*	*A. scholaris*	*C. javanica*	*C. medica*	*C. grandis*	*P. guajava*	Total
Host/ Insect	86 (14)	60 (14)	136 (37)	29 (15)	33 (9)	28 (8)	45 (6)	21 (10)	11 (8)	41 (21)	490 (142=28.98%)
C. jacobsoni	0	928	1014.5	548	0	0	0	0	0	1322	3813
I.aegyptiaca	2808	0	0	2734.5	766	0	0	0	0	1408.5	7717
I. minor	0	706.5	1040	0	192.5	245.5	75	245.5	414.5	352	3272
Total	2808	1634.5	2054.5	3282.5	958.5	245.5	75	245.5	414.5	3082.5	14802
	11058.5 (74.71%) [BI=3/(11058.5/12)=0.003]							3742.5 (25.29%) [BI=0.009]			[BI=0.002]

*Data in the parenthesis show the number of host plants with scales.

Table 2. Incidences of monophlebid fauna on host plant species at JUC, 2023 - 24.

Host/ Month	*I. aegyptiaca*				*I. minor*			*I. seychellarum*	*D. corpulenta*	*D. mangiferae*	Total	Total (2004-05)
	M. indica 56 (1)	*F. benghalensis* 8 (2)	*P. guajava* 4 (!)	Total 68 (4)	*M. indica* (1)	*A. heterophyllus* 86 (2)	*P. guajava* (1)	*M. indica* (1)	*A. heterophyllus* (1)	*A. lebbeck* 50 (1)		
Jan	27	94	12	133	0	0	0	0	0	0	133	444
Feb	44	110	0	154	0	0	0	0	0	0	154	910
Mar	0	0	0	0	2	6	0	2	0	0	10	1496
Apr	4	6	0	10	0	2	0	0	3	1	16	2859
May	2	5	0	7	0	0	0	0	0	0	7	4682
Jun	12	28	0	40	0	0	0	0	0	0	40	1425
Jul	0	0	0	0	0	0	0	0	0	0	0	980
Aug	0	0	0	0	0	0	0	0	0	0	0	1171
Sep	0	0	0	0	0	0	0	0	0	0	0	499
Oct	0	0	0	0	0	0	0	0	0	0	0	98
Nov	2	4	0	6	0	0	0	0	0	0	6	112
Dec	16	36	3	55	0	0	2	0	0	0	57	126
Total		405				12		2	3	1	423	14802

*Data in the parenthesis show the number of host plants with scales

Specifically, pests were reduced to 3.67% (F=8.473, df = 1, P=0.008) in trees and 0.45% (F=13.15, df = 1, P=0.001) in shrubs. Between 2004–05 and 2023–24, *I. aegyptiaca* and *I. minor* decreased to 5.25% (F=2.932, df = 1, P=0.101) and 0.367% (F=18.87, df = 1, P=0.000), respectively. One insect species disappeared completely, while three new insect species were introduced, resulting in a Sorenson's Index or Community Coefficient (CC) value of 0.444. The diversity indices of insects (from 2004–05: Insect Biodiversity Index or BI = 0.002, H = 1, D = 0.387, 1-D = 0.613, 1/D = 2.58, E_H = 0.91; to 2023–24: BI = 0.141, H = 0.22, D = 0.917, 1-D = 0.083, 1/D = 1.1, E_H = 0.14) reflect this deteriorating trend (Tables 1-3, Fig. 2).

Table 3. Different insect diversity indices are shown in both studies.

Diversity Indices	2004-05	2023-24	Comments
Total Number of Individuals (N)	14802	423	Number of individual insect decreases
Number of Species	3	5	Number of species increases
H (Shannon Index)	1	0.22	Insect diversity decreases
D (Simpson Index)	0.387	0.917	Insect diversity decreases
1-D (Simpson's diversity index)	0.613	0.083	The community diversity decreases
1/D (Simpson's reciprocal index)	2.58	1.1	Species diversity decreases
E_H (The Shannon Equitability Index)	0.91	0.14	The insect abundances far differed
CC (Sorenson's Coefficient)	0.444		The communities overlapped slightly

Plant specific interaction: Between 2004 and 2005, each insect pest survived on four to eight host plants. Furthermore, *P. guajava* served as the host plant for the highest number of three Monophlebid species and did not show any additional preference for competing pest insects (F=1.184, df = 2, P=0.319). This was followed by *M. indica* (F=0.42, df = 1, P=0.524), *A. heterophyllus* (F=2.387, df = 1, P=0.137), *F. benghalensis* (F=2.387, df = 1, P=0.137), and *A. sapota* (F=1.55, df = 1, P=0.226), which were hosts for two species each. The next five plant species hosted a single insect species each. The variety of host plants explains the abundance of specific insect species (r = 0.2) (Table 1, Fig. 3-5). In 2023–24, many host plants lost their previous insect biota and attracted new insect species. For example, in *M. indica*, *I. aegyptiaca* was significantly more abundant compared to the other two species (F=4.718, df = 2, P=0.0158), followed by *A. heterophyllus* and *P. guajava* for two insect species each, while each of the remaining plant species hosted only one insect species (Table 2).

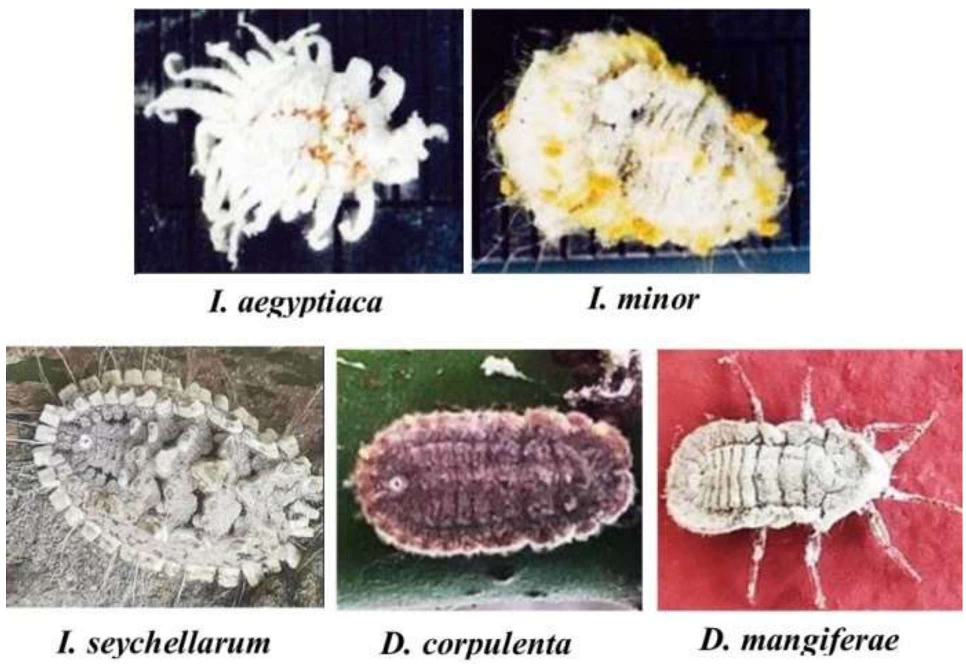

Plate 1. Different giant scales found in JUC.

Plant parts infestation: In 2004–2005, almost all Monophlebid species significantly took refuge on plant leaves (F=4.899, df = 3, P=0.0322; 66.96%). Although *I. aegyptiaca* and *I. minor* also favored other regions, *C. jacobsoni* only consumed the leaves of plants. In our study from 2023–24, the largest number of insects was also found on leaves (F=0.962, df = 3, P=0.434; 43.01%), and *I. aegyptiaca* predominantly favored leaves. *I. minor* was absent from stems in both studies (Table 4).

Table 4. Nature of monophlebid insect infestations in the study area over the two study years.

Insect	Stem	Branch	Twig	Leaf	Total	Insect	Stem	Branch	Twig	Leaf	Total
C. jacobsoni	0	0	0	3813	3813	*I. minor* (2023-24)	0	2	3	7	12
I. aegyptiaca (2004-05)	2808	219	326	4364	7717	*I. seychellarum*	0	2	0	0	2
I. aegyptiaca (2023-24)	0	12	36	357	405	*D. corpulenta*	0	0	0	3	3
I. minor (2004-05)	0	686	851	1735	3272	*D. mangiferae*	1	0	0	0	1

Weather effect on monophlebids: The weather had a significant influence on the population fluctuations of Monophlebid insects. The insect populations were negatively affected by both exceptionally high and low temperatures. During the 2004–05 study, the insect population was influenced by mean temperature, humidity, and rainfall, with impacts of up to 54.31% ($F=3.17$, $df = 3$, $P=0.085$). Among these factors, temperature played the most important role, followed by humidity and rainfall. However, due to the brief winter season, the average minimum temperature was a key factor in the increase of these insects. Among the three individual pests, maximum and minimum temperatures had the least impact on *I. minor* and *I. aegyptiaca*, respectively. For *C. jacobsoni*, average humidity had the least effect, while for *I. aegyptiaca*, average rainfall had the minimal impact. In 2023–24, the average temperature, humidity, and rainfall negatively impacted the insect population by up to 67.2% ($F=5.463$, $df = 3$, $P=0.02445$), with rainfall and humidity being the next most significant factors, in order of importance, after temperature (Table 5).

Table 5. Correlation ('r' value) of abiotic factors with monophlebid in different months.

Weather Factors	Correlation	Total (2004-05)	Total (2023-24)	*I. aegyptiaca* (2004-05)	*I. aegyptiaca* (2023-24)	*I. minor* (2004-05)	*I. minor* (2023-24)	*C. jacobsoni*	*D. corpulenta*	*D. mangiferae*	*I. seychellarum*
T_{max}	r	0.5	-0.6	0.5	-0.6	-0.0	0.2	0.4	0.5	0.5	0.2
T_{min}	r	0.5	-0.8	0.3	-0.8	0.2	-0.2	0.6	0.1	0.1	-0.1
T_{avg}	r	0.5	-0.8	0.4	-0.8	0.2	0.0	0.5	0.3	0.3	0.0
RH	r	-0.4	-0.4	-0.3	-0.4	-0.4	-0.6	-0.2	-0.4	-0.4	-0.5
RF	r	-0.0	-0.6	-0.1	-0.6	0.1	-0.2	0.2	-0.1	-0.1	-0.1

Bionomic impact on Monophlebids: Between 2004 and 2023, the insect and plant biota in the study region steadily declined 97.14% and 47.14%, respectively and old plant biota's declined rate 57.35% (Table 1-2, Fig. 2). The plants that were present in early study were between five and twenty years old, although their typical lifespan is about 30 years. Insects were predominantly observed at heights of less than 25 feet, the study focused on populations up to head level. The remaining old trees have grown significantly taller and larger over the past twenty years, making it difficult for the scale insect population to be maintained. This factor led to the migration or decline of the Monophlebid population (Ullah, 1987; Jackson, 2024).

Additionally, biotic factors such as long distances between host plants and intraspecific and interspecific competition contribute to the decline of insect species (Allison *et al*., 2023). In this study, anthropogenic activities, including tree cutting, fires, fuel wood collection, pollution (air, water, light, and soil pollution), application of lime to plants, plant death, lack of new plantings and awareness, and improper maintenance, were primarily responsible for increasing distances between host plants. This situation, characterized by rapid population growth such as the expansion in staff, student list, and faculty activities; enhanced intervention efforts, and the impact of faulty urbanization, building temporary shops and global warming, is not unique to the current study area but is observed nationwide and globally. Authorities need to address this trend by planting trees, conducting workshops and seminars on forest management, implementing effective policies and regulations, allocating funds and resources for conservation, and enforcing penalties for rule violations. A healthy atmosphere and protection of natural habitats are essential for the survival of all living things that is essential for stable ecosystem (Allison *et al*., 2023; Malanson and Alftine, 2023).

Infestation Nature of Monophlebids: D. corpulenta, *D. mangiferae*, and *I. seychellarum*, three of the six detected polyphagous monophlebid insect species (Morales *et al*., 2016) in the current study region, were observed feeding on a single plant species. In contrast, the other three species attacked at least four to eight plant species (Tables 1, 2). Prior research has revealed that a larger percentage of economically significant scale insect species are associated with a broad range of host plants, and many pests can be raised or survive on alternative hosts that they had not previously colonized. For instance, among the identified species, *I. aegyptiaca* infested the highest number of host plant genera—at least 129—across 65 families, while *I. minor* invaded the fewest, with 3 host genera from 3 families (Morales *et al*., 2016). In addition to their frequent consumption of leaves, many of these insects (Table 4) also commonly reside on stems and other plant parts (Morales *et al*., 2016). *I. aegyptiaca* was observed feeding on nearly all surface plant components in the current study, demonstrating its greater flexibility, higher reproductive capacity, and abundance in Bangladesh (Bragard *et al*., 2023).

Most insects documented on leaves are likely found in the soft tissues, where they can easily infiltrate and feed on the sap, which is preferred by scales (Hsu, 2019). Additionally, since practically all Monophlebids are highly polyphagous, the fluctuation in the number of scales on different portions of the host plants is related to their polyphagous and phytophagous nature (Friedrichs *et al*., 2022). The resistance and diverse morphological, physiological, and chemical characteristics of different plants are key determinants of their polyphagous nature. Certain plants have up to four times higher concentrations of chemical components (Awmack and Leather, 2002). According to He *et al*. (2015), the concentration of nitrogen, proteins, minerals, and water is greatest in leaves and lowest in stems and branches. Elevated levels of nitrogen, minerals, and water result in the development of succulent, juicy, and relatively smooth leaves. Furthermore, the area and quantity of leaves attracts sucking insects, such as scale insects (Huang *et al*., 2021; Huntley, 2023).

Weather Effect on Monophlebids: Climate variables affecting giant scale infestation include temperature, relative humidity, and rainfall. These variables can be interconnected and have positive, negative, or negligible impacts on infestation rates (Bashir *et al*., 2022). Giant scales primarily inhabit regions with tropical and subtropical climates (Bragard *et al*., 2023). The current study region is characterized by a tropical climate with both dry and rainy seasons and significant humidity during certain periods (Mondol *et al*., 2019). Giant scales respond differently to these meteorological conditions, which may explain the variation in insect population density observed across plants. *C. jacobsoni* was present year-round among the Monophlebid species, while other species were only present during specific seasons. The seasonal climate was conducive to the growth of host plants, and in most cases, giant scale incidences were positively associated with various seasons (Chrysantus, 2012; Nandi and Chakraborty, 2015) (Table 5).

C. jacobsoni peaked in June. Yukawa (1984) observed that a large number of leaves and twigs from different plant species positively affected *C. jacobsoni* during the monsoon season in Indonesia. *I. minor* reached its peak in February 2005 during the cooler months. The highest incidence for *I. aegyptiaca* occurred in May 2004 and February 2024. These findings align with those of Awadalla and Ghanim (2016) and Helmy (2021), who also recorded peaks for *I. aegyptiaca* in summer and winter. The study’s conclusions indicate that the Monophlebid population on host plants was either nonexistent or very low depending on the season. In such cases, the insects may have taken refuge underground, under bark, or inside the trunk for pupation, thereby protecting themselves from predators, unfavorable climate conditions, and other threats. Downward migration also depends on mutualistic relationships with ants and the availability of food. The population is influenced by seasonal, biotic, abiotic, and anthropogenic factors, as well as the substrates they feed on and their movement (Allison *et al*., 2023).

Conclusion

To sum up, plants are fundamental elements of the planet and the living world. They are connected to specific insect species, either directly or indirectly. Insects provide valuable services to mankind and the environment (Allison *et al*., 2023). They become pests only when their population exceeds a certain threshold. Monophlebids are widespread pests affecting many species of trees and shrubs, as they feed on plant parts and carry various bacteria and viruses that cause diseases. For instance, *I. purchasi* is a pest affecting 65 families of woody plants (Morales *et al*., 2016). Regular and thorough research is required to understand their dispersal, distribution, involvement in various habitats, and ecosystem roles, areas that remain underexplored in Bangladesh.

Because JUC is a small, isolated ecosystem, it provides a unique opportunity to understand the roles of different pest insects in various host plants and seasons, as well as the effects of ecological changes. A preliminary investigation into the population dynamics of monophlebids was conducted earlier (Chowdhury *et al*., 2022). The current study, involving a year-long investigation from 2004–05 and a twelve-month review from 2023–24 in the same region, has revealed the feeding patterns and the impact of human activities on Monophlebid habitats and survival.

In the previous study, ten plant types, including both trees and shrubs, were found to be attacked by three monophlebid species. In the current investigation, however, only *I. aegyptiaca* and *I. minor*, along with three new insect species, were detected on five host plant species, including one new plant species. This shift is attributed to a significant reduction in the number of host plants and insect biota. According to this study, *I. aegyptiaca* can persist longer in an unstable environment due to its varied feeding habits. Furthermore, the rapid loss of flora and fauna is causing environmental changes and deterioration. A diverse biotic flora is essential for sustaining a rich biotic fauna. Therefore, taking preventative measures is crucial for maintaining a healthy ecology and environment.

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ASSESSING THE FLOOD AND RIVERBANK EROSION IMPACTS AND COPING STRATEGIES IN HATIA UNION OF ULIPUR, KURIGRAM, BANGLADESH

MD. ABRAR TAZWAR RIJON[1] AND MD. FARUK HOSSAIN[2]*

[1]*Department of Geography and Environment, Pabna University of Science and Technology, Pabna, Bangladesh*

[2]*Departments of Geography and Environment, University of Dhaka, Dhaka, Bangladesh*

Abstract

The present paper aims to focus on the flood and riverbank erosion impacts and coping strategies in Hatia Union of Ulipur, Kurigram of Bangladesh. Due to the prevailing geographical location and topographical features, Bangladesh is considered as one of the most disaster-prone countries in the world. Kurigram district, located in the northern part of Bangladesh, is particularly flood and riverbank erosion prone because of its extensive river networks and its location within the Teesta-Brahmaputra River basin. Hatia Union of Ulipur Upazila was chosen as the study area due to its susceptibility to these disasters. In order to identify the flood and riverbank erosion impacts and coping strategies, field level data and information were collected through field survey and interviewing the respondents. Using ArcGIS Pro software, Sentinel-1 (SAR) and LANDSAT images with a resolution of 30 meters, the flood and riverbank erosion maps were produced. Floods and riverbank erosion both have significant impacts on local communication, food, shelter, health, employment, security, and people's movement. Local people suffer a lot and their lives and livelihoods are affected severely by these disaster events. The affected communities undertake various coping strategies with the support of governmental and non-governmental organizations to overcome the incumbent impacts of flood and riverbank erosion. Policy level development and effective implementation of various projects and programs prioritizing the most vulnerable areas, focusing on socio-economic advancement, and resolving relevant issues can limit the sufferings of local people.

Keywords: Flood, Riverbank erosion, Impacts, Coping strategies, Ulipur upazila, Kurigram district, Bangladesh

Introduction

Bangladesh is one of the most disaster-prone countries in the world and situated at the lower part of the Ganges-Brahmaputra-Meghna River basin (Islam *et al.,* 2013; Roy *et al.*, 2017; Shahriar, 2020). Natural disasters occur on a regular basis and disrupt people's

*Corresponding author: faruk.geoenv@du.ac.bd

livelihoods in various parts of the country (Hossain and Fahad, 2023). Bangladesh is vulnerable to both disasters and climate change, ranking seventh in the world for extreme disaster risk, according to the Global Climate Risk Index 2021 assessment. This country is ranked 9th most disaster-risk-prone country among 193 countries (Atwii *et al.,* 2022), 27th among the 191 multi-hazard-prone countries in 2022 (IASC and EC, 2022), and 7th among the 180 long-term disaster-affected countries of the world in between 2000 and 2019 (Eckstein *et al*., 2021). From 1980 to 2008, Bangladesh was affected by 219 natural disasters and caused damages of approximately US$16 billion (Uddin *et al.,* 2019). Bangladesh's geographical location close to the Bay of Bengal, near the foothills of the Himalayan Mountains, landscape characteristics, the abundance of rivers, and the monsoon climate- all contribute to frequent extreme weather events such as cyclones, droughts, floods, storm surge, river erosion, and salinity intrusion (Faisal *et al*., 2021).

In recent decades, there has been an exponential growth in the frequency and huge socioeconomic impacts of natural disasters (Harrison and Williams, 2016). Various natural disasters have become more common nowadays (Thomas *et al.,* 2014). Among these disasters, floods have shown an increasing trend in both frequency and intensity. Floods can happen slowly or suddenly, and are usually caused by a river overflowing its natural bed (Tîncu *et al.,* 2018). Every year, floods affect approximately 215 million people worldwide, with Asia accounting for 95% of all victims (Saulnier *et al.,* 2018). Riverbank erosion is another notable geomorphological issue primarily observed in alluvial river floodplains (Bordoloi *et al.,* 2020), but the impacts of riverbank erosion stretch across all continents, varying in intensity (Das *et al*., 2014). This erosion problem is widespread, affecting many countries in both the North and South (Bhuiyan *et al*., 2017), and has far-reaching consequences, affecting millions of people worldwide every year (Naher and Soron, 2019). Being a riverine country at the confluence of the Ganges-Brahmaputra-Meghna (GBM) river system, Bangladesh experiences substantial annual challenges with flooding and riverbank erosion.

Flood and riverbank erosion are the major natural events in Bangladesh, and these are responsible for making thousands of people homeless and landless every year (Hossain and Fahad, 2023). Flooding and riverbank erosion affect people's way of life and change their livelihood patterns (Islam and Uddin, 2020). Floods frequently disrupt people's lives and livelihoods, as they account for almost 50 percent of all natural disasters (Ali *et al.,* 2019). Every year, flooding affects approximately 20% of Bangladesh's geographical area (Islam *et al*., 2018) and causes severe property damages and significant loss of lives. In Bangladesh, six major floods occurred in the nineteenth century, with years of occurrence in 1842, 1858, 1871, 1875, 1885, and 1892 (FFWC, 2020). The 20th century witnessed 18 significant floods, including disastrous ones in 1951, 1987, 1988, and 1998. Between 1990 and 2021, Bangladesh experienced 90 floods and floods of 1998, 2007, 2017, 2019, and 2020 are mentionable (FFWC, 2020). The 1998 flood inundated 68 percent of the country (FFWC, 2020). Floods have many adverse consequences since the water stays on the land for a long time, making all forms of communication obsolete. Infrastructure, development activities, domesticated animals, homesteads, crops, food storage and human life are all seriously affected (Roy *et al.,* 2015).

Riverbank erosion poses a serious and direct threat to Bangladesh's riverine districts. Around 20 out of the 64 districts in Bangladesh are vulnerable to riverbank erosion, with a staggering amount of approximately 8700 hectares of land being lost each year, affecting approximately 200,000 people by eroding their homes and agricultural lands (Alam *et al.,* 2017). Every year, riverbank erosion directly affects one million people and an estimated four million become homeless or are forced to live in constant uncertainty (Islam and Rashid, 2011). In Bangladesh, both frequency and intensity of riverbank erosion has significant consequences (Billah *et al*., 2023). It affects socio-economic structure and quality of life of the people also (Bhuiyan *et al.,* 2017). It is a local and recurring natural event that has a huge impact on riverbank residents' livelihoods (Islam *et al.,* 2016; Ahmed, 2016; Alam *et al.,* 2017).

Kurigram district, located in the Northern part of Bangladesh, is particularly flood and riverbank erosion prone because of its extensive river networks and strategic location within the Teesta-Brahmaputra River basin. At least 16 large and small rivers flow through Kurigram district. This region faces recurring challenges in its livelihood sector due to floods and riverbank erosion. Of particular concern is the occurrence of a massive flood every 5-6 years in this area (Nahar *et al.,* 2014). Almost all of the major rivers among the 16 have flood and riverbank erosion problems. At least 30 points along the Brahmaputra, Dharla, Teesta, Dudhkumar, and Gangadhar rivers are currently vulnerable to riverbank erosion and flooding (BWDB, 2022). Flooding is a significant factor in riverbank erosion in this area. The Brahmaputra River enters Bangladesh from the north in Kurigram district. Riverbank erosion took place in this region due to a number of natural and human - induced factors such as changes in the river's course, variations in the shear strength, geomorphology, features of the bed and bank materials, sudden drawdown and pressure imbalance at the bank face, low vegetation cover, obstructions in the channel flow, presence of structures, wakes from boats and exposure to wind and waves (Islam and Rashid, 2011; Rahman *et al.,* 2015).

The study site is in Hatia Union, Ulipur Upazila (sub-district) of Kurigram District, on the bank of the Brahmaputra River. Local people are facing difficulties in adjusting with unexpected flooding. Heavy rainfall in this region as well as in the upper stream countries causes high magnitude flooding. Any major fluctuation in river water level causes substantial riverbank erosion (Chowdhury, 2012). During the monsoon season, this area receives considerable rainfall, which enhances the probability of flood and riverbank erosion (Uddin and Basak, 2012). In recent times, severe floods have been observed in the present study area in 2020 due to heavy rainfall and huge water discharge of the Brahmaputra River (FFWC, 2020; Islam and Uddin, 2020). Brahmaputra River tends to lose its depths and bank erosion is escalating (Islam and Uddin, 2020). The shifting course of the river within this area results in the loss of houses, agricultural lands and urban centers (Pahlowan and Hossain, 2015). Moreover, the situation became worse due to the climate change induced problems (Shiferaw *et al.,* 2023). The livelihoods of local communities are severely affected by flood and riverbank erosion. Local people have little choice but to cope with disasters because they have a long history of experiencing them. Coping refers to the immediate response to the disaster which includes strategies, measures, and actions taken to address the specific hazard impacts during or after a disaster, and relies on existing institutional frameworks (Birkmann, 2011). Following a disaster, locals use their available resources to restore their livelihood so that they can deal with the catastrophe (Tanvir *et al*., 2015). They traditionally developed some strategies to survive during and after disasters.

Several studies have looked at different aspects of floods and erosion in different parts of Ulipur Upazila, but their cumulative impact on the local population of Hatia Union has not been studied. This research addresses this gap by examining the diverse challenges faced by locals and exploring coping strategies in the selected study area. The objectives of this research are to present flood and riverbank erosion scenarios in the study area by assessing the impacts of floods and riverbank erosion on local communities. In addition to this, this study explores how local people are coping with flood and riverbank erosion.

Materials and Methods

Selection of the study area: Ulipur Upazila is situated in Kurigram district and its geographical coordinates ranging between approximately 25°33' to 25°49' north latitudes and 89°29' to 89°51' east longitudes. The upazila shares borders with Kurigram Sadar and Rajarhat to the north, Pirgachha to the south, Assam (India) to the east, and Raumari and Chilmari to the south. Three major rivers: the Tista, Brahmaputra and Dharla, are flowing through this upazila. Hatia and Burabari Unions of Ulipur Upazila under Kurigram district situated along the Brahmaputra River, are particularly vulnerable to natural disasters like flood and riverbank erosion (BWDB, 2022). Moreover, according to the surveyed Key Informants, Hatia union was more affected by flood and riverbank erosion in the previous period. That is why this area was suitable for the present study.

Hatia Union is located to the east of Ulipur Upazila headquarters and has an area of approximately 7571 acres (30.64 square kilometers) (Fig. 1). The population is approximately 27,552, with 12,937 females and 14,615 males, and there are 4,610 total households (BBS, 2022). The average literacy rate of Hatia Union is 37.7 percent. Hatia Union contains 38 villages, each of which contributes to the region's vibrant rural landscape.

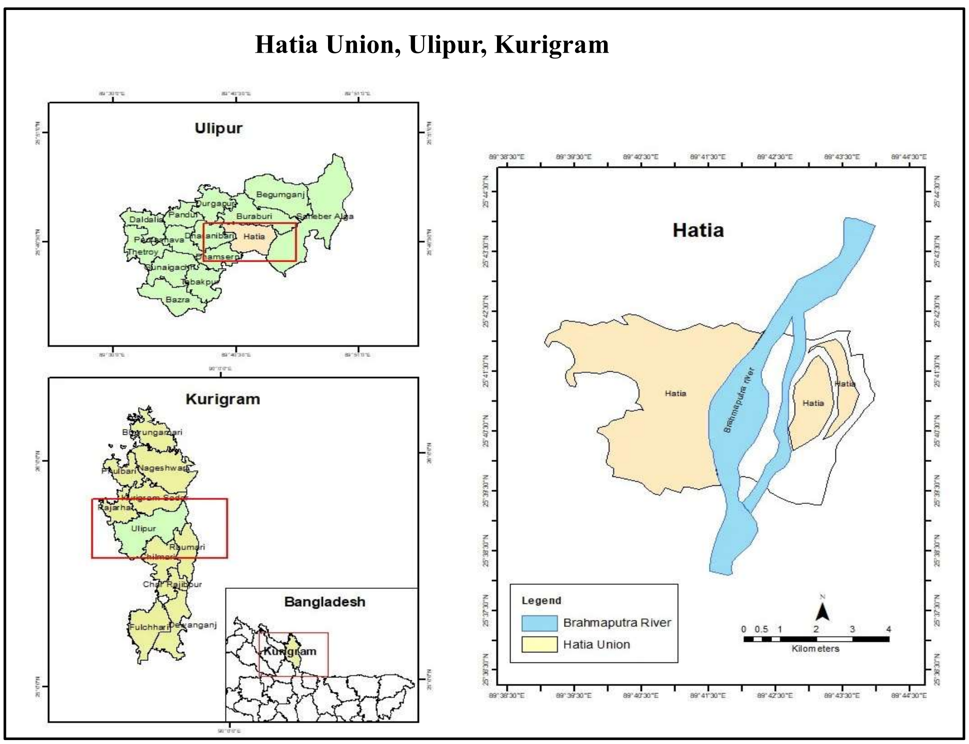


Fig. 1. Location of the study area.

Sample size selection

The sample size for this study was calculated by using Yamane's formula (Yamane, 1967), considering the number of households in the study villages. The formula is given as:

$$n = N / (1 + N \times e^2)$$

Here, n represents the sample size (number of households chosen for interviews),

N stands for the total households in the study areas, and e indicates the level of error.

To ensure a reliable sample size determination, a confidence level of 95 percent and a precision level of 7 percent were used. Rounding to the nearest whole number, the sample size (n) would be approximately 160.

Sampling method: The surveyed households were selected based on the ratio of total households in the respective villages. Field data relating to household survey was collected using a simple random sampling method (Table 1).

Table 1. Number of respondents from different villages in the study area.

Village name	Total number of households	Surveyed households
Bagua Anantapur (Paler Hat)	160	34
Majhi Para	65	14
Hatiar Gram	120	26
Nilkontho	35	9
Kamar Tari	220	43
Noya Dara	90	21
Jelengar Kuthi	60	13
Total	750	160

Source: Field Survey, 2023

Data collection techniques: The field survey for the current study was carried out in two phases: the pre-field survey on May 5, 2023, and the main phase from June 11 to June 18, 2023. The study incorporated both qualitative and quantitative approaches to meet the objectives of the research. Secondary data was collected through various sources such as journal articles, books, book chapters, documents of various organizations and data and information from various online sources. A semi-structured questionnaire was designed for this study to collect data from the surveyed households. To get in depth information from the respondents, six Key Informant Interviews (KIIs) were conducted in different parts of the study area.

Data analysis techniques: The collected primary and secondary data were analyzed by using ArcGIS, Google Earth Pro software, SPSS and Microsoft Excel. The ArcGIS Pro software was used to create the map of the study area (Fig. 1). The flood and riverbank erosion maps (Fig. 2 and Fig. 4) were created using Sentinel-1(SAR) and LANDSAT images with a resolution of 30 meters and ArcGIS Pro software. NDWI processed data were obtained from LANDSAT 5, 8, and 9 to detect the water bodies for creating the erosion map (Fig. 6) and ArcGIS was used to calculate the total area (sq.km) for flood inundation and bank erosion of the study area.

For further analysis and interpretation of data, Weighted Average Index (WAI), Perception Index and Satisfaction Index were used. WAI was calculated for each indicator using the Ha & Thang (2017) formula:

$$WAI = \Sigma (Si \times Fi) / N$$

In order to evaluate the households' perceptions and comprehension of the various indicators pertaining to the impacts and coping strategies of flooding and riverbank erosion in the study area, a perception index was employed (Table 2).

Table 2. Perception Index Levels.

Categories	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
Scale	0.01-0.2	0.21-0.4	0.41-0.6	0.61-0.8	0.81-1

The satisfaction index (WAI) is calculated using the following formula:

$$WAI = (0.2\times f1 + 0.4\times f2 + 0.6\times f3 + 0.8\times f4 + 1\times f5) / N$$

Results and Discussion

The surveyed respondents were selected from the study area to know their views regarding the flood and riverbank erosion induced impacts and coping strategies (Table 3).

Table 3. Demographic Characteristics of the Surveyed Respondents.

Factors	Classes	Percentage
Gender	Male	77
	Female	23
Age range	21-30	11
	31-40	25
	41-50	31
	51-60	18
	>60	15
Level of education	Illiterate	76
	Class 1-3	7
	Class 4-5	8
	Class 6-8	3
	Class 9-10	3
	HSC	2
	Bachelor	1
Occupation	Unemployed	3
	Farming	35
	Animal husbandry	3
	Day labour	23
	Fishing	9
	Service	4
	Business	12
	Others	11
House type	Huts	33
	Kutcha/ Tin shed	62
	Semi-pucca	3
	Pucca	2

Source: Field Survey, 2023

Flood vulnerability level of the study area: During the monsoon season of 2020, the Brahmaputra basin experienced significant and prolonged flooding; with floods having a serious impact on both people and property in the Hatia Union. Most of the areas of this

Union were submerged several times at regular intervals. The Brahmaputra River began to rise in June 2020, causing massive flooding in the area by the first week of July (Fig. 2).

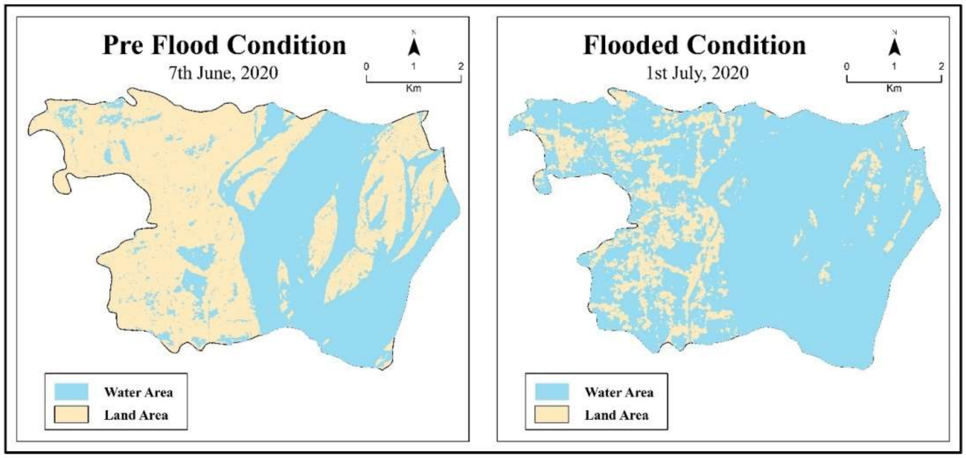


Fig. 2. Flooding in the Study Area.

Table 4. Before and After Flood Damage in Hatia Union, 2020.

Key Term	Area (Sq. km)	Percentage
Total area	30.81	100
Pre-flood waterbodies	13.59	44
Waterbodies during flood	25.63	83
Only land area flooded	12.04 out of 17.22	70

Source: Remote Sensing (RS) Data, 2020.

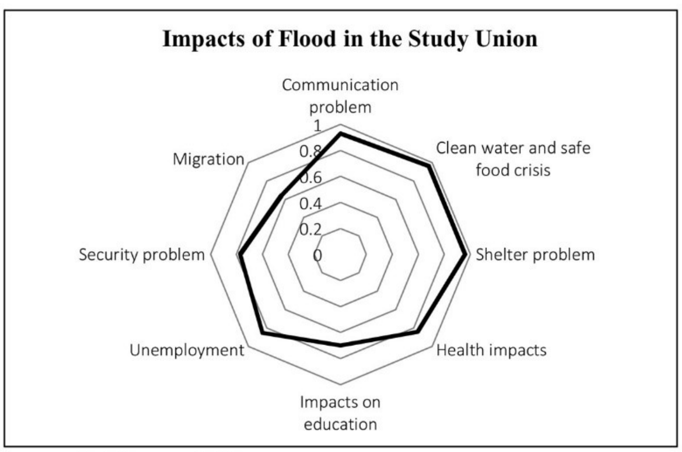
Prior to the flood, when the Brahmaputra River water began to increase from the normal level, and it covered an area of 13.59 square kilometers (44 percent) of the total area. During the flood, water covered 25.63 square kilometers of land, accounting for 83 percent of the entire area. The total flooded area was 12.04 square kilometers, accounting for 70 percent of the total land area (Table 4). This flood caused huge impacts on the people and the neighborhoods.

Perceived response on impacts of flood: The overall Weighted Average Index was calculated in order to fully comprehend the impacts of floods in the study area. The respondents' perceptions are represented on a scale of 0 to 1, with 0 denoting strongly disagree, indicating no impact on any given sector, and 1 denoting strongly agree, indicating a very high impact on anything. These values were used to calculate the overall severity of flood effects.

Table 5. Relative Grading of Impacts of Flood by Respondents as Reflected in Survey.

Impacts	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree	WAI	OA
Communication problem	0	0	5	44	111	0.93	VH
Shortage of safe water and food	0	0	0	36	124	0.96	VH
Shelter problem	0	0	1	34	125	0.96	VH
Health problem	0	1	12	106	41	0.84	H
Impacts on education	0	4	89	53	14	0.70	H
Unemployment problem	0	2	30	53	75	0.85	H
Security problem	0	6	51	62	41	0.77	H
Migration	3	43	43	60	11	0.64	M

Note: Strongly disagree (SD): 0.01-0.2; Disagree (D): 0.21-0.4; Neutral (N): 0.41-0.6; Agree (A): 0.61-0.8; Strongly agree (SA):0.81-1. WAI: weighted average index, OA: overall assessment.



Source: Field Survey, 2023

Fig. 3. Impacts of flood in the study area Hatia union (WAI).

Flooding impacts, according to respondents, are more severe in some cases and occur more frequently than others. For example, shortage of safe water and food, and shelter problem both have the highest WAI of 0.96 and occur frequently, contributing to their greater impact (Fig. 5). Another serious issue with the WAI value of 0.93 is communication problem (Table 5). Inundation of roads and walkways causes serious mobility and communication challenges. The Brahmaputra River inundates a large area during high floods, and its width is around 12-15 kilometers, covering a large portion of the land. As a result, residents need to use small fishing boats or construct rafts out of banana or bamboo trees to travel from one location to another. Those who do not have these options must walk in the water at their own risk. Floodwater damages the structures of houses and roads because it remains in the ground for several days and takes time to retreat.

Floodwater damages the structures of houses and roads because it remains in the ground for several days and takes time to retreat. Another significant issue in this area is security, which has a WAI value of 0.77. Robberies are common during floods, and a gang of robber raid villages with large boats to seize cattle, goats, and other livestock. So, social violence becomes common problem during the flood period.

Security issues for women and girls become prevalent issue during flood. Girls sometimes find themselves at risk of eve-teasing or sexual harassment in the flood shelter or other locations where numerous people seek shelter during floods. Flood-induced migration has the lowest WAI of 0.64, resulting in less impact. However, during a flood, the majority of the affected families leave their homes and seek shelter in high ground nearby.

Riverbank erosion in the study area: From 2003 to 2023, the study area experienced both erosion and accretion events, occurring frequently throughout this period (Table 6). The timeline has been divided into four periods, each comprising five years of data on erosion and accretion occurrences. Typically, accretion events were observed to happen following severe erosion incidents in the recent past (Roy and Sarker, 2016). The provided table presents the erosion and accretion scenarios in the study area between 2003 and 2023.

The map presented below encompasses data from 2003 to 2023, providing an overview of the erosion pattern of the study area (Fig. 6). The terrain of this area was formed by processes of accretion and erosion during this time. The map enables a comprehensive understanding of the spatial patterns and temporal changes of Brahmaputra riverbank erosion in the study area's land area over the two decades. The map's susceptible areas highlighted the dynamic nature of the riverbank erosion.

Table 6. Erosion and accretion scenarios between 2003 and 2023.

Period	Net erosion (sq. km)	Net accretion (sq. km)
2003-2008	2.25	2.72
2008-2013	7.73	1.47
2013-2018	2.26	6.13
2018-2023	3.53	2.92

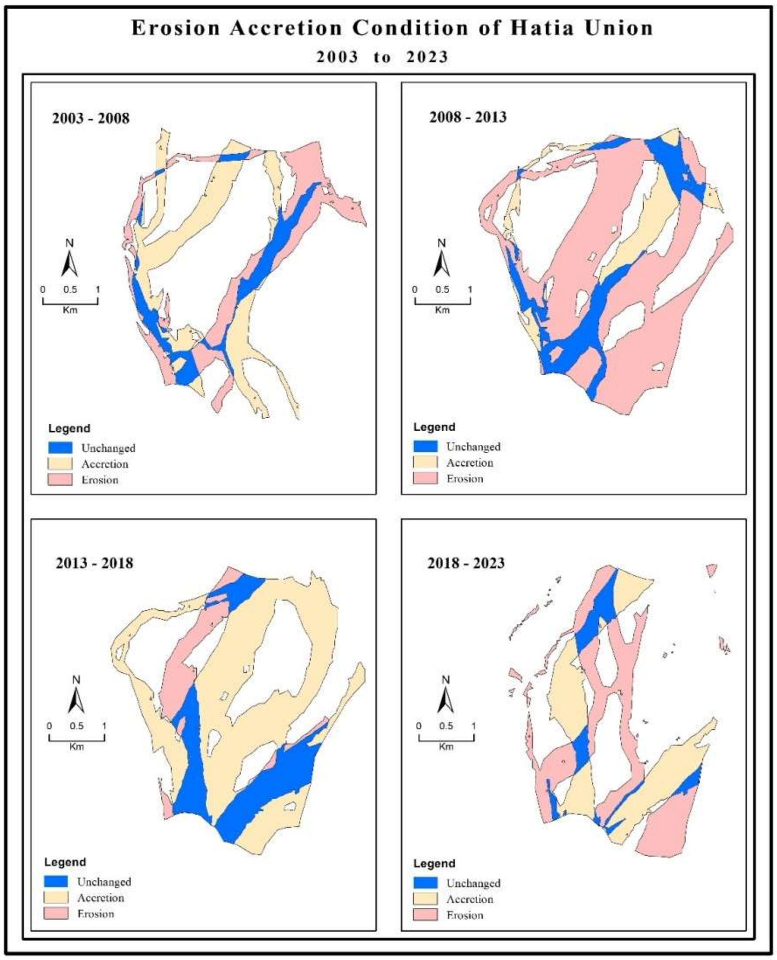


Fig. 4. Spatial Variations in Erosion Accretion Extent at Hatia Union in Ulipur Upazila, between 2003 and 2023.

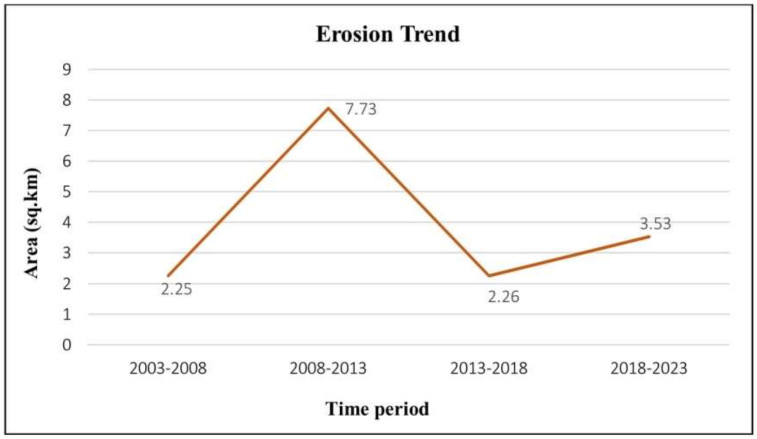


Fig. 5. Erosion trend in last two decades.

The erosion trend over the last two decades reveals as an oscillation pattern. There was less erosion at the beginning, then a significant increase, later a minor slowdown, and followed by a slow increase of erosion rates (Fig. 7).

Perceived response on impacts of riverbank erosion: Population displacement from erosion-prone areas is a common phenomenon (Bhuiyan et al., 2017). Since 1988, people have been facing riverbank erosion and the resulted impacts are huge land loss and displacement of several communities in this Union. Erosion has caused a substantial change in the land area of the Union in last two decades. The study suggests that, about 93% of the surveyed respondents have lost their land or property as a result of river erosion. Most of the affected land is agricultural, with residential property accounting for approximately 49%. Among those who have lost residential property, around 75% reported losing their homes more than three times. In terms of agricultural property, 45% of the respondents have lost more than 5 bighas of agricultural and business land. The impacts of the erosion have been severe, leading to many villages being completely eroded and lost to the river. It resulted in displacement; and those who were affected, endured increasing poverty and severe economic loss.

According to the locals, the Brahmaputra River used to run roughly half a kilometer away from the place from where it was few years back. However, due to riverbank erosion, river came to closer and made the area more vulnerable. For example, a village named Noya Dara eroded and was completely lost in the river only three to four years ago. At this point, Paler Hat Bazar and the surrounding areas are highly vulnerable due to the erosion. Moreover, flood induced impacts increased the area's vulnerability to more erosion, perhaps leading to more losses and displacements for the local communities living nearby.

Table 7. Relative Grading of Impacts of Riverbank Erosion as Perceived by Respondents Reflected in Survey.

Impacts	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree	WAI	OA
Loss of land property	0	0	0	39	121	0.95	VH
Homelessness	0	0	0	46	114	0.94	VH
Increase of poverty	0	0	5	25	130	0.96	VH
Loss of jobs	0	6	19	39	96	0.88	VH
Disruption of child education	2	14	63	59	22	0.71	H
Decline of family bonding	18	37	64	17	24	0.59	M
Migration	0	2	8	49	101	0.91	VH
Increase of social violence	4	13	56	51	36	0.73	H

Note: Strongly disagree (SD): 0.01-0.2; Disagree (D): 0.21-0.4; Neutral (N): 0.41-0.6; Agree (A): 0.61-0.8; Strongly agree (SA):0.81-1. WAI: weighted average index, OA: overall assessment.

Based on the data and information, there are serious consequences of Brahmaputra riverbank erosion on a number of aspects of the lives of the respondents, including land property loss, homelessness, poverty, loss of employment, disruption of education, social violence, and migration (Fig. 8). The WAI suggested a very high overall impact (VH-Very High) on most aspects, while some factors showed a high (H-High) and even a moderate (M-Moderate) impact.

With the WAI of 0.96 increase of poverty is the dominant effect here following by loss of land property and become homelessness accounting for WAI 0.95 and 0.94 respectively. Migration due to erosion contained WAI 0.91 which is also very high (Table 7). Affected people have no choice but relocate to other areas. Poverty causes various consequences, such as disruption in child education or a decline in family bonding over time. Even social violence, such as theft or conflicts were observed in the study. Another important finding was that poverty leads to child marriage of the females in the neighborhood. Early marriage occurs when the head of the family becomes landless or homeless, or when he/she faces the possibility of becoming impoverished. Another crucial fact is that dowry is a fairly common practice in every case of early marriage or marriage, and this dowry system is practiced in this area for decades.

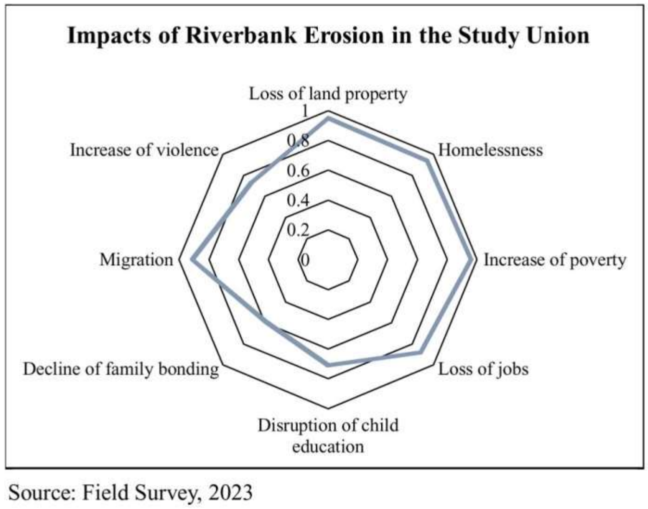
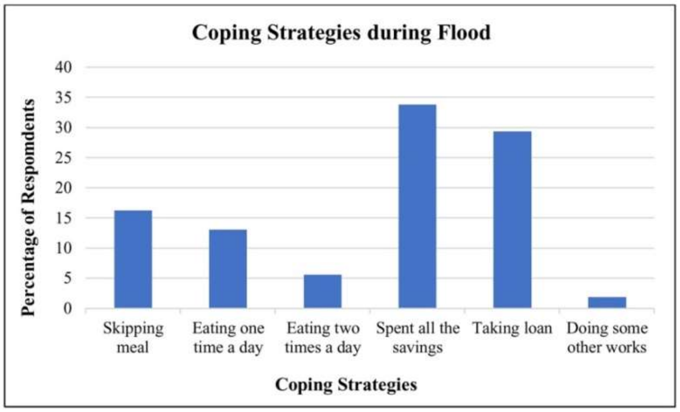


Fig. 6. Impacts of riverbank erosion in the study union.

Coping strategies with flood and riverbank erosion: Despite receiving minimal assistances from different sources, the surveyed respondents have undertaken few coping strategies with the disasters they faced. It includes skipping meal, eating less meal or less nutritious foods, not spending all the money or taking out loans, and so on. Around one-third of the respondents (33.8 percent) choose to spend all of their savings or to take out loans by 29.4 percent (Fig. 9). The respondents save for months each year, and during a flood, they are compelled to spend all of their savings. The affected people had to spend all of their savings and also take loans from various sources to rebuild or relocate their houses due to floods and riverbank erosion.

According to the respondents, the majority of them took loans from local NGOs. However, some of them reported that the NGOs or banks sometimes refused to provide loans because they might be failed to return the money in the schedule time. As a result, they were forced to seek personal loans. Some people lend money at a high interest rate. One must pay at least 100 taka or sometimes even more interest on a loan of 1000 taka per month. The loan receivers must pay the interest every month on time. As a consequence, loan receivers pay double or even more than that amount of money with the interest they took.

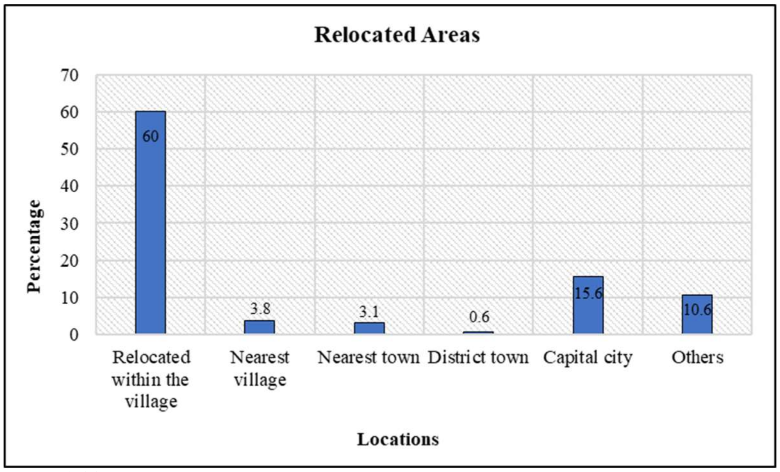


Source: Field Survey, 2023

Fig. 7. Variation of coping strategies adopted by the respondents during flood in Hatia union.

From the field survey it has been observed that more than half (60 percent) of the respondents relocated inside the same village (Fig. 10). This presumably reflecting an attempt to maintain ties to their origin and properties. Furthermore, the locals had several land properties in their villages, after losing one land, they moved into another. Interestingly, 15.6 percent of the respondents mentioned that their family members moved to the capital city for better jobs and living conditions. It also revealed that if a family lost all of their lands, they had to relocate to other people's land or take lease a nearby land. Usually, the day laborers moved from the study area to district level towns.

Some of the respondents fall into the category of migrating to nearest villages and others. Due to severe erosion problem in some villages such as Noyadara, Dagarkuti, Hatiargram etc., the residents of these villages were forced to relocate to nearby villages like Kamartari, Majhipara, Nilkontho and others. The displaced people usually live in a dense settlement. For instance, four or five families purchase a land jointly. Each family usually build one or two rooms and they live together. They have to stay in this way since they share nearly identical mental and financial conditions as a result of their displacement. Those who cannot afford to buy own land, they need to stay in rented house. Many well-off people who reside away from the river and setup Tin shed kutcha houses close to the river. For example, respondents need to pay taka 20,000 per decimal of land over the course of four or five years. Thus, a dense village area was built by the locals in the Hatiar gram under Kamartai where almost every house is used for rent purposes. Since 1988, the displaced people have been residing in these areas.



Source: Field Survey, 2023

Fig. 8. Variations in resettlement options explored by the respondents during riverbank erosion in hatia union.

Conclusion

Hatia Union, located in Ulipur Upazila, Kurigram District, is one of the most disaster-prone areas of Kurigram. Flooding and bank erosion have severe adverse effects on individual and the community. The objectives of the study were to assess the cumulative impacts of flooding and riverbank erosion on the local people of Hatia Union. Moreover, it aimed to explore how they cope with these disaster events and the strategies they adopt during and after disasters like flooding and riverbank erosion. The study reveals that people in this area endure constant hardship due to these disasters. Floods have a considerable detrimental impact, causing extensive damage to the houses of the respondents, crops, livestock, economic activities, educational institutions, and healthcare facilities. All modes of communication are seriously disrupted. Residents have to leave their houses and seek shelter during flood events. They rely on relief as flooding damages all forms of food security. Furthermore, floods create a major unemployment problem, leading to severe economic hardship in this area. The situation worsens when riverbank erosion occurs alongside floods. Riverbank erosion leads to severe land loss to the inhabitants. Over time, acres of valuable land including residents and prominent villages have been eroded and lost completely to the Brahmaputra River. Many affected people had no choice but to migrate to other places, where they often live miserable lives. They were forced to relocate their houses, and many previously well-off landowners have been reduced to extreme poverty due to erosion.

Various measures were undertaken to combat floods and riverbank erosion in this area are inadequate, and the necessary facilities have not reached to the underprivileged people. There is a lack of co-ordination too. In response to these disasters, more active policies must be implemented as soon as possible. If this area is neglected, the whole Kurigram District, and by extension the country, will face significant setbacks due to the negative impacts. Policies and plans should not only focus on minimizing the immediate impacts of floods and riverbank erosion but also prioritize building long-term resilience. Ensuring the safety, prosperity, well-being, and cultural heritage of the local population is crucial. Co-ordination is essential to focus on improving the livelihoods of local people. Engaging local communities in disaster risk reduction planning and decision-making processes can result in more sustainable and community-centered initiatives.

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CYTOLOGY AND YIELDS ANALYSIS OF ONIONS (*ALLIUM CEPA* L.) UNDER COMPOST APPLICATION IN COASTAL SALINE SOIL

TASLIM SADIA[1], DIBAKAR DUTTA[1], SUBROTO KUMAR DAS[1], AFROJA NASRIN[2], MAHIN AFROZ[1] AND MD. UZZAL HOSSAIN[1*]
[1]*Department of Botany, University of Barishal, Barishal-8254, Bangladesh*
[2]*Department of Soil and Environmental Sciences, University of Barishal, Barishal-8254, Bangladesh*

Abstract

Onion (*Allium cepa* L.) is a valuable crop in Bangladesh for its diversified usage and availability of enriched germplasms including local and exotic ones. However, the cultivation of onions in the coastal regions of Bangladesh has become a major challenge because of salinity and soil fertility. Therefore, a pot experiment of coastal saline soil amendment with compost was carried out with mostly cultivated varieties i.e. Taherpuri and King. The treatments T_1 (2.5 t/ha) and T_2 (5.0 t/ha) significantly improved the plant height for the Taherpuri variety and revealed an escalation to the no. of the leaflet, root length and bulb diameter for the King variety. Compost application on saline soil didn't increase the mitotic index (MI%) for all the treatments for both of the experimented varieties but increased the mitotic inhibition index (MII%) for the treatments T_3 (7.5 t/ha) and T_4 (10.0 t/ha) treatment for the Taherpuri and King varieties respectively. The correlation coefficient between the treatment and different yields of onion was found better for the Taherpuri than the King variety.

Keywords: Saline soil**,** Compost, Coastal, Cytology, Yields, *Allium cepa*

Introduction

Onion (*Allium cepa* L.) is a species from the family Amaryllidaceae and is widely cultivated in almost every country of the world though its center of origin is middle Asiatic countries (Peters, 1990). The species is highly demandable because of its medicinal, culinary and personal care potentiality. Onion is a rich source of different nutrients and metabolites. These nutrient and metabolic profiles may vary for different factors like maturity stage, storage duration, species cultivated and locality of cultivation (Kumar *et al*., 2010). In addition, Onion (2n=16) is a model species for cytological

*Corresponding author: muhossain@bu.ac.bd

studies because of having healthy root tip, quick sensitivity to stress, clear mitotic phases, stable chromosome number (2n=16) and diversity in the chromosome morphology (Firbas and Amon, 2014). Bangladesh is one of the top most onion (*Allium cepa*) yielding countries of the world (FAO, 2016), where different exotic and local cultivars are available (Pinky *et al*., 2017). These cultivars could be differentiated by bulb size and color of scale leaves. It is ranked first among the spices crops concerning production amount and second regarding acreage cultivated in Bangladesh (BBS, 2015). Madaripur, Faridpur, Rajbari, Kushtia, Pabna, Rangpur, Rajshahi, Manikganj, Magura and Jhenaidah are the major commercial onion-producing districts in Bangladesh that cover 79% of total production (BBS, 2018). Despite the increasing production, the yield rate per unit area of onion isn't capable of meeting the country's total demand. So there is an acute shortage of onion in Bangladesh. The best solution to meet the demand is to bring arable land as well as coastal saline land under onion cultivation.

Plant growth and yields result from the coordinated interaction of cell cycle and cell expansion. Soil salinity hampers the crops in different ways like affecting crop quality, growth, yield and cytology (Dong *et al*., 2008). Additionally, salinity stress has adverse effects on cell cycle and overall growth of plants by altering cell proliferation (Teerarak *et al*., 2009) and affecting the root zone of crops (Qi and Zhang, 2020) respectively. On the contrary, soaring salinity in the coastal areas of Bangladesh decreases the availability of suitable arable lands day by day. In this situation, soil amendments with various organic substances such as farmyard manure, poultry manure and compost can be the best practices to bring the coastal soil under cultivation (Zaki, 2011). The amendment of compost in soil releases essential nutrients like Nitrogen (N), Potassium (K) and Phosphorus (P). These three nutrients have an immense effects on growth and development of onions. For example, N is required for cell division, plant growth, boost leaf rate and bulb diameter extension (Garcia, 1980); P promotes early root formation, cell enlargement and growth (Diriba-Shiferaw *et al.*, 2015); K is an essential nutrient for improving onion yields and efficiency (Sharma *et al*., 2003). Several studies have been carried out by different researchers in the coastal region of Bangladesh (Hossen *et al.,* 2021) including Cox's Bazar Sadar Upazila (Hoque and Hossain, 2018) on suitable land identification for onion cultivation. There was hardly any evidence of a comprehensive study that can correlate the cytology and yield of onion for compost amendment in coastal saline soil. Therefore, the present study aims to analyze the changes in the mitotic proportion and yields. Cytological indices for root tips cell division and yield attributes of onions are used to analyze the effects compost application in coastal saline soil of Bangladesh.

Materials and Methods

Coastal saline soil collection and preparation: For this experiment, the coastal soil was collected from the Satkhira district under the Khulna division. The soil was air dried for 4-5 days by spreading on a clean piece of paper. All sorts of debris including plant roots and plastics were discarded from the soil samples. The soil samples were exposed to sunlight to hasten the drying process. For further processing, a portion of the larger and more massive aggregates were broken by crushing them gently with a wooden hammer after drying. Ground samples were passed through 2mm stainless steel sieve. Finally, the different amount of composed fertilizer was mixed separately with this soil. This ready soil was kept for seven days to plant the seedlings.

Physico-chemical properties of soil and compost: Different established protocols were applied to estimate the physico-chemical properties of soil and compost. For the measurement of pH, electrical conductivity (EC) and salinity, a multimeter analyzer was used. Different nutrient profiles were determined by following earlier reports viz Nitrogen and organic carbon (Huq and Alam, 2005), Phosphorus according to Olsen and Sommer (1982) with slight modification, Sulfur by the calcium di-hydrogen phosphate extraction method, Potassium, Calcium and Magnesium were measured by the N-NH_4OAc method (Warncke and Brown 1998) Zinc & Iron by DTPA extraction method described by Lindsay and Norvell (1978).

Germplasm collection and plantation: The seedlings of two different varieties of onion namely King and Taherpuri were collected from the local market of Faridpur. Those seedlings were planted and maintained in the net house of the University of Barishal. The seedlings of onions were cultivated in different pots containing different dosages of compost viz. T_0 (control), T_1 (2.5 t/ha), T_2 (5.0 t/ha), T_3 (7.5 t/ha) and T_4 (10.0 t/ha) with three replications. Other intercultural operations like gap filling & thinning, soil loosening, weeding, irrigation and required plant protection were meticulously performed.

Cytological study: For the cytogenetical study, healthy roots were collected from two varieties of each treatment followed by pre-treatment with 8-hydroxyquinoline (0.02 M) for 1.5 hrs. Then the roots were fixed in 45% acetic acid at 4ºC followed by hydrolyzation in a mixture of 1 N HCl and 45% acetic acid (2:1) at 60ºC for 30 sec. The young healthy roots were cut 0.5 cm away from the tip by a clean blade. Then the root tips were stained and squashed in 1% aceto-orcein. The slides were observed in the electric microscope and photographs were taken by using the Euromex camera. Then the mitotic index and mitotic inhibition index were calculated by the following formula:

$$\text{Mitotic index (MI) \%} = \frac{\text{Number of dividing cell}}{\text{Total number of cell}} \times 100$$

$$\text{Mitotic inhibition index (MII) \%} = \frac{\text{MI in control - MI in treatment}}{\text{MI in control}} \times 100$$

Yield attributes collection and analysis: Yielding attributes like individual plant height, number of leaflets per plant and root length were recorded at 30 days after transplant (DAT) and bulb diameter was measured at 50 DAT. The plant height and root length were recorded by using measuring tape at centimeter scale and bulb diameter was measured by using vernier calipers at millimeter scale. The mean for all the treatments was calculated and statistical analysis was performed with Microsoft Excel 2010 and Statistics 10.

Results and Discussion

Physico-chemical properties of soil and compost: The physico-chemical properties of soil are prime indicators of soil quality and productivity. The comparative physical and chemical qualities of soil samples are presented in Table 1. Soil pH is the sign of soil microbial activity, plant growth, biochemical breakdown, solubility and absorption of nutrients (Brady and Weil, 2004). According to the Bangladesh Agricultural Research Council (BARC, 2018), the optimum pH range for sufficient nutrient availability in most of the soil is 6.0-7.5. The pH of the experimental soil and compost was found 7.2 and 7.9 respectively (Table 1) and which indicated that, this soil was slightly saline according to BARC (2018). The pH of the experimental soil sample is consistent with the result of Bhadha *et al.* (2010). Another attribute, Electrical conductivity (EC) measures the extent of salts in the soil. In saturated soil extracts the range of salinity for most of the vegetable crops is 1.0-2.5 dS/m (Haque, 2021). In the experiment, the EC of soil and compost was found 5.34 dS/m and 4.08 dS/m subsequently (Table 1). The EC of the experimental coastal soil sample is alike the soil of Kalapara upazila of Patuakhali district (Khanam *et al*., 2020).

The most importantly soil organic matter (SOM) is known to play a significant role in the biological profile, soil fertility and productivity. The SOM (%) of experimental coastal soil and compost were 2.02 and 2.47 accordingly (Table 1). The SOM (%) of the experimental soil sample is higher than that of the Kalapra upzilla of Patuakhali (Hossin *et al*., 2022). Moreover, N, P, K, Ca, Mg and S are known as essential nutrient elements since all plants require these nutrient elements (Samuel and Ebenezer, 2014) for their growth and development.

Table 1. Physico-chemical properties of soil and compost.

Physico-chemical parameters	Soil	Compost	BARC (2018) soil category
pH	7.2	7.9	Neutral (6.6-7.3)
EC (ds/m)	5.34	4.08	Slight saline (4.1-8.0)
Soil organic matter (SOM %)	2.02	2.47	Medium (1.8-3.4)
Available N (%)	0.100	0.124	Low (<0.180%)
P (µg/g)	70.0	45.5	Very high (>37.5)
K (meq/100g)	1.28	0.47	Very high (>0.45)
Fe (µg/g)	3.80	7.83	Low (3.1-6.0)
Ca (meq/100g)	4.11	5.38	Medium (3.1-4.5)
Mg (meq/100g)	2.05	2.56	Very high (>1.875)
Zn (µg/g)	0.43	0.66	Very low (<0.45)
S (µg/g)	11.3	8.0	Low (7.51-15.0)

The available N (%) contents of the soil and compost were 0.10 and 0.124 respectively (Table 1) which is close to the low categories of BARC (2018) and supportive of that of Rahman *et al.,* (2014). N availability of soil is influenced by several environmental factors such as soil profile, salinity, temperature, water tables and logging frequency (Bai *et al*., 2012). P is essential for plant growth and health. The application of fertilizers for agricultural practices determines the availability and solubility of P (Hossain *et al*., 2015). The P content was 70µg/g and 45.5µg/g for experimental soil and compost respectively and according to BARC (2018) the study area is very high category (Table 1).

K is another important nutrient for early growth of plants, the efficiency of water and resistance to diseases and pests (Hasanuzzaman *et al*., 2018). The K content was found 1.28 (meq/100 g) in soil and 0.47 (meq/100 g) in compost (Table 1). The Fe content of the experimental soil and compost was 3.80 and 7.83 µg/g, respectively. According to Lindsay, 1974 Fe activity is inversely proportional to soil pH. Moreover, the Ca and Mg content of the experimental coastal soil and compost were 4.11 meq/100 g and 2.05

meq/100 g, respectively (Table 1). This result categorizes the both type of soil as medium (Ca) and very high category (Mg) in comparison with BARC (2018) The Zn and S content of soil were 0.43 and 11.3 µg/g, respectively and according to BARC (2018) the soil is in very low and low categories on the basis of Zn and S availability, respectively (Table 1).

Cytology of compost amendment in saline soil: Cell division rate, mitotic phase rate, mitotic index (MI%) and mitotic inhibition index (MII%) are the major indices of cytology any plant. Firbas and Amon (2014) found different aberrations in response of salt stress in onion. In this study mitotic index (MI%) result was not consistent for both Taherpuri and King varieties. The MI% was reduced abruptly for T_2 and T_3 treatments with respect to T_0 and T_1 treatments and increased suddenly at T_4 treatment in contrast to T_3 treatment for the Taherpuri variety. In the King variety the MI% was reduced suddenly at T_1 with respect to control, but gradual reduction of MI% was found at all the treatments except T_3 treatment (Fig. 1). The mitotic inhibition index (MII%) showed abrupt increased tendency for all the treatments in contrast to control (T_0) in the Taherpuri variety while fluctuate tendency was found for all the treatments in compare to control (T_0) in the King variety (Fig. 2). Kiełkowska (2017) studied the effect of artificial salt stress on mitotic index by applying NaCl and KCl and found reduction in root growth along with reduction in mitotic activity of onion root tip cells.

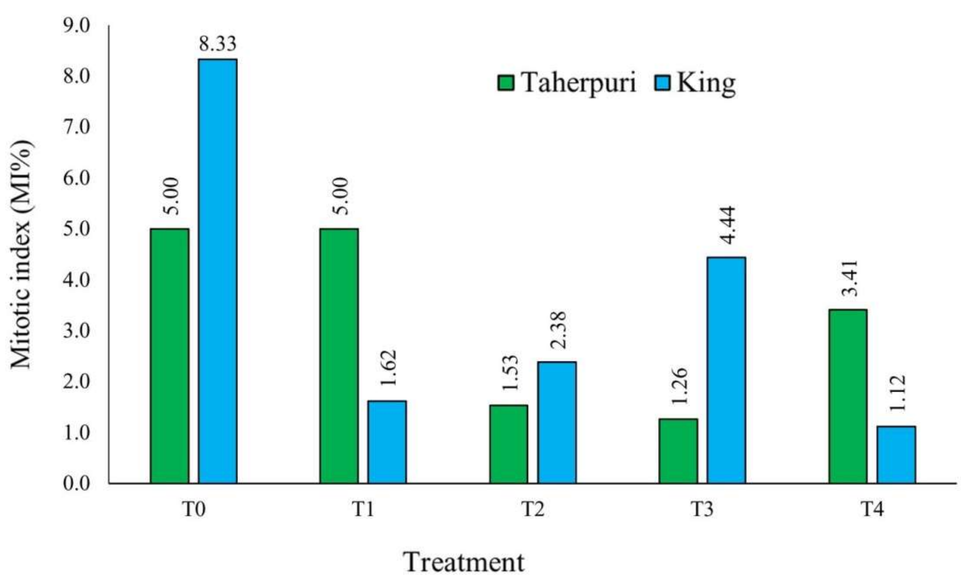

Fig. 1. Mitotic index (MI%) for different doses of treatment.

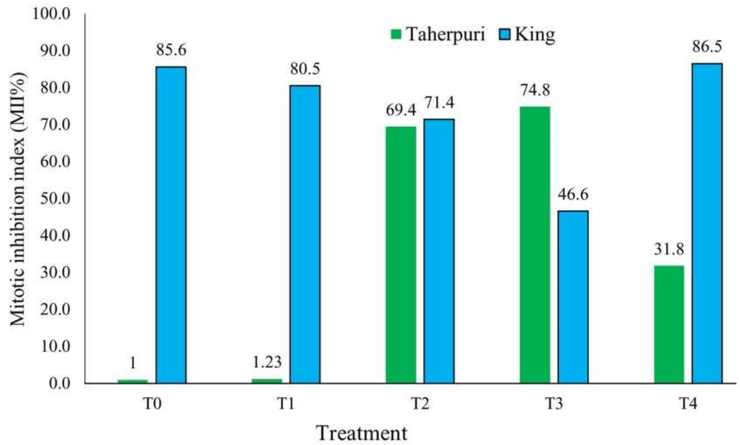

Fig. 2. Mitotic inhibition index (MII%) of different doses of treatment.

Table 2. Effects of compost on yields of experimental varieties of onion.

Treatment	Variety	Yields			
		Plant height (cm)	No. of leaflet	Root length (cm)	Bulb diameter (mm)
		30 DAT			50 DAT
T_0	Taherpuri	31.67**abc**	4.33**a**	5.50**bc**	5.73**bcd**
	King	23.33**d**	3.00**cd**	6.00**abc**	7.00**ab**
T_1	Taherpuri	35.00**a**	3.67**abc**	5.50**bc**	5.16**d**
	King	26.16**cd**	2.67**d**	6.37**abc**	7.33**a**
T_2	Taherpuri	33.67**ab**	3.67**abc**	6.33**abc**	6.83**ab**
	King	24.33**d**	4.00**ab**	7.00**a**	6.17**abcd**
	Taherpuri	27.00**cd**	3.00**cd**	5.33**c**	5.33**cd**
T_3	King	26.00**cd**	3.33**bcd**	6.67**ab**	6.87**ab**
	Taherpuri	27.33**bcd**	3.33**bcd**	5.67**bc**	5.67**bcd**
T_4	King	29.33**abcd**	4.00**ab**	6.00**abc**	6.70**abc**
Mean	Taherpuri	30.93	6.0	5.66	5.74
	King	25.83	3.4	6.40	6.81
SEM	Taherpuri	2.3369	0.3073	0.5028	0.3656
	King	0.4441	13.69	0.3469	0.3607
CV (%)	Taherpuri	13.09	14.79	15.37	11.02
	King	2.98	13.69	9.38	9.17
LSD	Taherpuri	7.2611	1.0022	1.6396	1.1923
	King	1.4483	0.8764	1.1313	1.1763
Level of significance	Taherpuri	0.1332	0.1176	0.6706	0.773
	King	0.0001	0.0268	0.2736	0.33112

Similar letters in a column are not significantly different at the 5% level by DMRT. DAT- Day after transplantation

Yield response and correlation to compost application: The treatments T_1 and T_3 showed the highest and lowest response i.e. 35.0 and 27.0 cm, respectively in term of plant height for Taherpuri variety onion at 30 DAT while the T_4 and T_2 performed the highest and lowest effects by resulting 29.33 and 24.33 cm plant height, respectively in King variety at 30 DAT (Table 2). The result regarding no. of leaflets for all the treatments was more or less similar to control (T_0) for both Taherpuri and King varieties at 30 DAT. The treatment T_3 showed the highest result i.e. 6.33 and 7.0 cm for root length of both Taherpuri and King varieties respectively at 30 DAT (Table 2). The treatments T_1 and T_2 showed the largest bulb diameter i.e. 7.33 and 6.83 mm for King and Taherpuri varieties, respectively (Table 2) at 50 DAT.

Table 3. Correlation coefficient (r) matrix of among treatment, plant yield and cytology.

		Treatment	Plant height	No. of leaflet	Root length	Bulb diameter	MI	MII
Treatment	Taherpuri	1						
	King	1						
Plant height	Taherpuri	-0.724	1					
	King	0.819	1					
No. of leaflet	Taherpuri	-0.855	0.60*	1				
	King	0.706*	0.35*	1				
Root length	Taherpuri	0.069*	0.40*	0.14*	1			
	King	0.109*	-0.319	0.27*	1			
Bulb diameter	Taherpuri	0.012*	0.26*	0.22*	0.943	1		
	King	-0.392	0.07*	-0.893	-0.559	1		
MI	Taherpuri	-0.605*	0.41*	0.68*	-0.374	-0.446	1	
	King	-0.623	-0.713	-0.389	-0.263	0.193*	1	
MII	Taherpuri	0.600*	-0.411	-0.679	0.373*	0.445*	-0.999	1
	King	-0.307	-0.106	-0.037	-0.654	0.147*	-0.046	1

* Significant at 5% probability level.

Furthermore, treatment revealed a significant positive correlation for the Taherpuri variety for root length, bulb diameter and MII% while it was for no. of leaflet and root length for the King variety at 5% probability level (Table 3). The MI% exhibited a significant positive correlation for Taherpuri variety for plant height and no. of leaflet yield of the Taherpuri variety while it was for the bulb diameter of the King variety at 5% probability level (Table 3). The MII% manifested a significant positive correlation for root length and bulb diameter yields of the Taherpuri variety while it was only for bulb diameter of the King variety (Table 3).

Conclusion

Soil salinization decreases soil fertility. Soil fertility is inseparably interconnected with plant growth and productivity. In addition, plant growth and yields also depend on cytology. But soil salinity impedes the cytology of plants. Amendment of saline soil with compost application is one of the major methods. The present study revealed that compost amendment has a positive contribution to onion (*Allium cepa*) yield increment. The comprehensive analysis of treatments, cytology and yield unveiled that the application of compost in saline soil has a reduction tendency for mitotic index and mitotic inhibition index, but increasing tendency for some yields at different doses of treatments. So further extensive study is needed to confirm exact dose of compost for saline soil amendment to bring under onion cultivation in Bangladesh.

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MICROBIOLOGICAL QUALITY OF DRIED BOMBAY DUCK (*HARPODON NEHEREUS*) SAMPLES OBTAINED FROM NORTH-EASTERN BANGLADESH (SYLHET DISTRICT)

SHARIF FARLIHA MAHIYAT, MD. JAKIUL ISLAM, MOHAMMAD ABU JAFOR BAPARY, MD. ASHRAF HUSSAIN AND MD. MOTAHER HOSSAIN*

Department of Fisheries Technology and Quality Control, Sylhet Agricultural University, Sylhet, Bangladesh

Abstract

This research was selected to assess the microbial and sensory quality of dried Bombay duck (loitta) in Sylhet district. Fourty eight (48) samples of dried Bombay duck (loitta) were collected from eight Upazila dried fish markets in Sylhet district in each of the eight upazilas during September 2022 to February 2023, the study collected 48 samples from two markets in each of the eight upazilas. Microbiological analyses included Aerobic Plate Count (APC), Total Coliform Count (TCC), presence of *Escherichia coli* & *Salmonella* spp., and sensory evaluation. Results revealed that aerobic plate counts showed the highest (log 5.69 ± 0.2) in Gowainghat upazila markets and the lowest (log 3.453 ± 0.2) in Sylhet sadar market. Total Coliform counts (ranged from 21 ± 1 to 80 ± 3 MPN/g) of the samples analyzed remained within permissible limits. The prevalence of *Escherichia coli* was found the highest in Gowainghat (67%) and the lowest in Sylhet Sadar and South Surma (17%), and the prevalence of *Salmonella spp*. was found the highest in Companiganj and Fenchuganj (67%) and the lowest in Sylhet Sadar (17%). Sensory examination favored samples from South Surma. Presence of pathogenic bacteria suggests unsanitary processing conditions. Dried fish quality varied significantly among upazilas, emphasizing the need for improved hygiene and sanitation practices to ensure safe, high-quality dried loitta production.

Key words: Upazila, Dried loitta, *Salmonella* spp., *E. coli*, Health risk

Introduction

Dried fish is popular in Bangladesh coastal and riverine communities, and gradually it becomes popular in other areas in Bangladesh. Dried fish holds cultural significance, being the most consumed fish category in Bangladesh, providing access to year-round nutrition. It is an excellent source of high-quality proteins, and are a unique source of important nutrients & minerals such as iodine, zinc, copper, selenium, and also contains healthful fatty acids, including long-chain omega-3 fatty acids like eicosapentaenoic acid

*Corresponding author: motaher03@yahoo.com

(EPA) and docosahexaenoic acid (DHA) (Siddhnath *et al*. 2022). This category of dried fish is particularl important for people who cannot afford fresh fish or meat (Banna *et al*. 2022), and in addition to dietary mainstay dried fish contributes significantly to Bangladesh's economy.

It ranks second globally in dried fish production, exporting around 2339.36 metric tons in 2018-19, earning nearly USD 4 million (FRSS, 2019). This industry is a major employer, particularly for women, boosting foreign exchange reserves through exports.

One prominent fish species is Bombay duck (*Harpodon nehereus*), or "loitta," vital to Bangladesh's fishing sector. Comprising over 10% of marine catches in the Bay of Bengal, it's a favorite among coastal populations and commands strong export value. "Loitta shutki," is widely enjoyed and has gained international acceptance for using in various culinary applications. Dried Bombay duck dried fish (Loitta shutki) contributes significantly to Bangladesh's economy, with an annual export of around 7000 metric tons generating approximately USD 14 million (Mandal, 2021).

However, inadequate sanitation and poor hygiene in Bangladesh's drying processing and practices (i.e. improper handling and storage of dried fish in general) pose risk for microbial contamination (Rana *et al*., 2020) and resulting in compromised food safety, driven by *Escherichia coli* and *Salmonella* spp. (Beuchat and Mann 2011) and becomes public health concern (Rasul *et al*., 2020).

The consequences of bacterial contamination are terrible, affecting both health and economics. Deterioration of dried fish quality due to bacterial growth results in spoiled product with reduced shelf life, off-flavors, and altered texture. Pathogenic bacteria like *Vibrio parahaemolyticus, Escherichia coli, and Salmonella* spp. can cause foodborne illnesses, especially, *Salmonella* spp. can cause a range of diseases in humans, collectively referred to as salmonellosis (symptoms like diarrhea, abdominal cramps, fever & vomiting). These typically emerge 12 to 72 hours after bacterial exposure and can persist for about a week. In severe case Salmonellosis can result in high fever, headache, and abdominal pain. It is caused by the *Salmonella enterica* serovar Typhi and is typically contracted through having contaminated food or water (Coburn *et al.* 2007). *Escherichia coli,* is commonly found in - human and animal guts (Bélanger *et al*. 2011). It can be transmitted through contaminated food or water, as well as with infected individuals or animals. Some strains of *E. coli* are associated with outbreaks of foodborne illness and can cause symptoms like diarrhea, abdominal cramps, and fever. In acute cases, *E. coli* infection can lead to complications as hemolytic uremic syndrome (HUS), which can cause kidney failure and other complications can be life-threatening (Bélanger *et al*. 2011).

This situation underscores the importance of proper handling and storage. Ensuring the correct temperature, adequate drying, appropriate hygienic packaging, and preventing cross-contamination with other foods are essential. Regular testing and monitoring further enhance product safety. Hygiene measures are to be taken for reducing potential loss of nutritional value and health hazards due to microbial contamination.

Despite some existing research on dried fish safety and quality, this study aims to fill the gap of knowledge, evaluating microbial safety specifically for dried Bombay duck. The present research was undertaken to assess bacterial load, total coliform, *E. coli*, and *Salmonella* spp. in dried Bombay duck and to analyze the presence of pathogenic bacteria to evaluate potential health risks associated with consumption. As dried Bombay duck holds cultural, economic, and culinary importance, this study has far-reaching implications for public health, food safety, and local economies.

Materials and Method

Study Area: The study cover eight upazila dried fish markets of the Sylhet district. Two dried fish markets were taken to collect samples randomly from each upazila.

Study period: September 2022 to February 2023

Study place: Laboratory of the Department of Fisheries Technology and Quality Control under the Faculty Fisheries, Sylhet Agricultural University.

Sample size: 48 dried Bombay duck, (*Harpodon nehereus*) samples were collected from the eight Upazila dried fish markets of Sylhet district.

Preparation of Sample: Using the sequential decimal dilution method with pour plates, standard plate counts are computed and represented as Colony Forming Units per gram (CFU/g) of material. A gram of the sample was taken and aseptically homogenized in a sterile mortar. The sample was homogenized with 10 mL peptone water and then transferred to a clean container. Then the samples were diluted with sterile peptone water in test tubes upto 10^{-6}, and so forth.

Procedure of Aerobic Plate Count: The each of diluted sample was transferred onto plate count agar (PCA) plate, spread uniformly and were kept in an incubator for 24 hours at 37°C. After 24 hours of incubation, plates only containing 30 to 300 colonies were counted. The formula used to compute the number of bacteria per gram of the sample (CFU/g) is as follows:

$$\text{CFU/g} = \frac{\text{No. of colonies on petridish} \times 10 \times \text{dilution factor} \times \text{Volume of total sample solution}}{\text{Wt. of fish sample (g)}}$$

Procedure of Total Coliform Count: The total number of coliforms was counted by the MPN (Most Probable Number) method, and the results were recorded.

Isolation & Identification of Escherichia coli: Escherichia coli was isolated from the samples using the MacConkey Ager and Violet Red Bile agar (VRB) where pink colour and red coloured colonies of *E. coli* developed, respectively.

Presumtive identification of the *E. coli* isolates was done by growing isolates on EMB agar, where colonies showed metalic sheen colour. EMB, MacConkey, and BGA agar was used as subculture medium for the VRB agar colonies. The *Escherichia coli* strains tested positive for the tests for indole, catalase, motility, and MR, but not for VP.

Isolation & Identification of Salmonella spp.: *Salmonella* sp. was isolated from the samples by using *Salmonella-Shigella* (SS) agar, where colonies of *Salmonella* sp. had a dark foundation and were black on SS agar. Nutrient agar, MacConkey (MC) agar, Brilliant Green agar, Salmonella-Shigella (SS) agar, and Violet Red Bile agar are all used to grow *Salmonella* spp. *Salmonella* colonies were smooth, clear, and opaque on nutrient agar. Colonies on (MC) agar are light or colorless. The colonies on Brilliant Green agar had a cream color. Colonies had a dark foundation and were black on SS agar. Colonies on VRB agar had a light cream color. On TSI (Triple Sugar Iron) slant and SS-agar, *Salmonella* created black hydrogen sulfide (H_2S), which is a product of the bacterial process. *Salmonella* tested positive for the MR, motility, catalase, and TSI tests, while it tested negative for the Indole and VP tests.

Analysis of data: For the initial processing of the unprocessed raw data for dried loitta used in this investigation, the mean and standard deviation were established. These data were used for additional statistical analysis and interpretations using software like Microsoft Excel**.** SPSS (IBM 2020 and Version 16) was used for the statistical analysis. To determine the significance difference at the 5% level of confidence, a one-way ANOVA was conducted. The Chi-square test was applied for group comparison.

Sensory analysis: Dry fish products are evaluated based on sensory qualities that indicate their freshness and acceptability. In this study, sensory analysis was conducted using the Quality Index method, considering seven sensory attributes: color, odor, texture, general appearance, fungus, broken pieces, and insect infestation.

Results and Discussion

This investigation examined the Aerobic Plate Count (APC) of dried loitta fish from different Upazilas shown in Figure 1. The highest bacterial load was found in Gowainghat Upazila, (5.695±0.2), and next Companiganj upazila (5.24±0.1). Conversely, Sylhet Sadar exhibited the lowest bacterial load (3.453±0.2), which is deemed acceptable and complied with the ICMSF (1986). The bacterial load in the remaining samples from other Upazilas also complied with the ICMSF (1986). The study noted a variation in APC among different

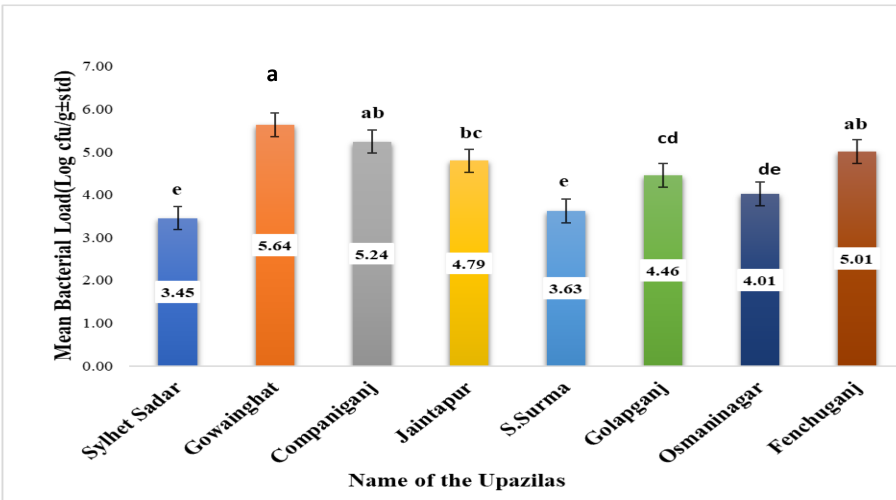

Fig 1. Bacterial load of dried loitta in different Upazilas (Different letters indicates significant differences).

Upazila markets. Statistical analysis confirmed highly significant different Upazila markets. Furthermore, statistical analysis confirmed highly significant results with a p-value <0.05. According to ICMSF, 1986 it may be noted that the aerobic Plate Count of the collected samples of the markets of Gowainghat upazila and Companiganj upazila little bit exceeded the standards. It probably happened due to the unhygienic condition of the markets and poor handling of dried fish. From this statement it may be concluded that due to the partial contamination by the bacterial load, might have some risks of human health through consumption. On the other hand, the samples of all other upazilas were quite ok. Ultimately no health risks through consumption of the said dried loitta. The result is related to the study of Mukilan *et al*. (2022), where they conducted a study in

Parangipettai, Tamil Nadu, focusing on the pathogenic bacterial contamination and quality of various dried fishes obtained from different fish markets.

According to ICMSF (1986) standards, plate counts below 5.69 log CFU/g indicated good, counts between 5.69 and 7 log CFU/g indicated marginally acceptable quality, and counts at or above 7 log CFU/g are considered unacceptable in terms of quality. Coliform bacteria are a type of microorganism that serves as an indicator of the presence of diseases, parasites, viruses & sanitary bacteria *(E. coli)* in a given sample. The present study showed that the highest value of the Total Coliform Count (TCC) was 80±3 MPN/g in the samples of Companiganj upazila. On the other hand, the lowest value of TCC was 21±1 MPN/g was in the samples of Sylhet Sadar (Figure 2). But all the value of TCC from the samples of all upazila markets were within the limit. Statistically it was observed that coliform counts vary significantly in different markets of different upazilas

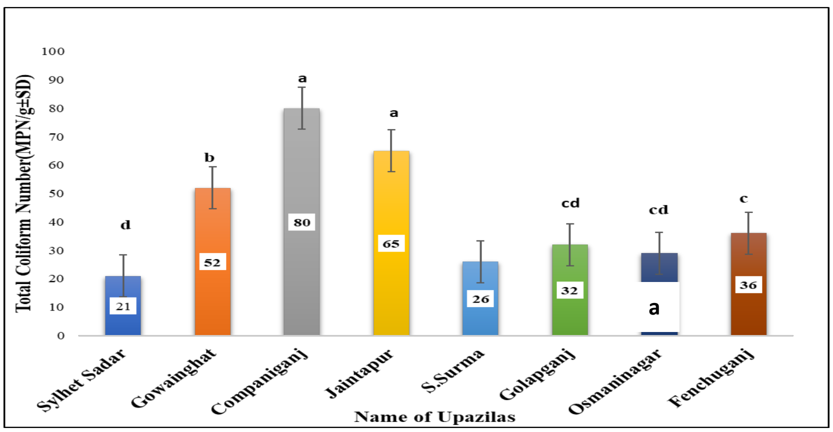

Fig 2. Total Coliform Count of dried loitta in different Upazilas (Different letters indicates significant differences).

($P<0.05$). The probable cause of the presence of coliform is poor hygiene throughout the drying process, which may have involved using dirty processing equipment and handling the dried fish carelessly. These led to the presence of coliforms in such levels. According to ICMSF (1982) guidelines, the acceptable limit for total coliform in dried fish is< 100 MPN/g. The findings of this study are comparable to the findings of Belgische (2012) who reported the highest total coliform count (132 MPN/g) in several samples from local markets, while the lowest count (68 MPN/g) was observed in Adjuevan, a traditional Ivorian fermented fish. This study identified pathogenic bacterial species, including *E.*

coli and *Salmonella sp*., in dried loitta fish, were also found almost in all upazilas which pose risks to human health. The presence of *E. coli* and *Salmonella spp.* in this dried fish samples indicated that poor hygienic conditions were adopted during the processing of dried fish. Factors such as the use of low-quality raw fish, inadequate hygiene practices during processing, improper packaging and storage, moisture absorption during drying from the environment, and mishandling during different stages of the marketing chain can contribute to high microbial counts (Nayeem and Pervin 2010). The highest occurrence of *E. coli* was found in Gowainghat Upazila, (67%) and the lowest in Sylhet sadar (17%), and South Surma (17%) of the analyzed samples of dried loitta (Table 1). The presence of *E. coli* in most of the examined samples indicated poor hygiene and sanitary conditions, which aligns with the findings of Fratamico *et al*., 2016.

Table 1. Upazila wise prevalence of *E. coli* in samples studied.

Name of the Upazila	No of total tested samples	No of positive samples	% of the *E. coli*	Chi square ($\chi 2$)	P value
Sylhet Sadar	6	1	17		
Gowainghat	6	4	67		
Companiganj	6	3	50		
Jaintapur	6	3	50	5.48	.601
Golapganj	6	2	33		
South Surma	6	1	17		
Osmaninanagar	6	2	33		
Fenchuganj	**6**	**2**	33		
Overall			37.5		

Salmonella, another pathogenic bacterium, was also isolated and identified from the dried fish samples. In the case of *Salmonella spp*., the highest occurrence was found was observed in both Companiganj and Fenchuganj Upazilas (67%), and the lowest in Sylhet sadar (17%) of the analyzed samples of dried loitta (Table 2). The presence of *Salmonella* in fish was also reported by Hatha and Lakshmanaperumalsamy (1997). The overall prevalence rates were 37.5% for *E. coli* (Table 1) and 43.75% for *Salmonella* (Table 2). Statistical analysis did not reveal any significant difference. Several factors may contribute to these statistical findings, but one possible explanation is that the source of the dried fish did not have a significant impact on the samples. Instead, the prevalence of

Salmonella and *E. coli* can be attributed to unhygienic market conditions, poor storage facilities, lack of handling knowledge, and failure to wear gloves, among other factors.

Table 2. Upazila wise prevalence of *Salmonella* spp. in samples studied

Name of the Upazila	No of total tested samples	No of positive samples	% of the *Salmonella* spp.	Chi square ($\chi2$)	P value
Sylhet Sadar	6	1	17		
Gowainghat	6	3	50		
Companiganj	6	4	67		
Jaintapur	6	2	33	5.33	0.619
Golapganj	6	3	50		
South Surma	6	2	33		
Osmaninagar	6	2	33		
Fenchuganj	6	4	67		
Overall			43.75		

A lower sensory score indicated higher freshness and acceptability of dried loitta fish is shown in Table 3.

Table 3. Grading of dried fish acceptance according to sensory score (Bremner 1985).

Sl. No	Grade	Average defect point	Degree of acceptance
1	A	<2	Excellent, Highly acceptable
2	B	2 to <5	Good/ acceptable
3	C	5	Rejected

The dried loitta from South Surma received the most acceptable score of 1.11±0.247, indicating its high quality. On the other hand, the highest score was found in Gowainghat Upazila, which was 2.8±0.712, considered good and acceptable (Figure 3). However, no significant differences were observed among the samples. These findings are in line with a study conducted by Mithun *et al*. 2021 where they assessed the quality of dried Bombay Duck and found the highest score to be 2.42.The sources of the collected samples from Gowinghat, Companiganj & Fenchuganj upazila indicated that the quality of these samples were not quite good i.e, moderately good this occurred due processing or handling in a less hygienic condition.

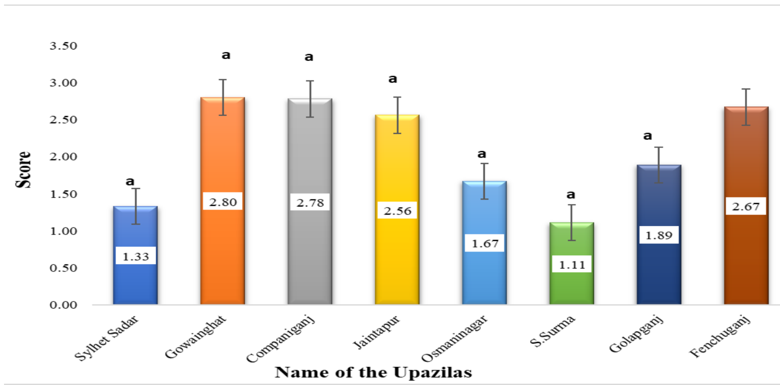

Fig. 3. Sensory scores of dried loitta in different Upazila markets

Conclusions

Dried Bombay duck, known for its high nutrition, is a favorite among Sylhet's people due to its protein-rich content. Also called marine lizard fish, it's abundant in calcium too. However, research highlights concerning microbial conditions, including Total coliform, *E. coli*, and *Salmonella*. Unhygienic handling, inadequate storage, and processing methods contribute to this issue, urging a focus on safety standards. While the sensory scores of studied dried fish were acceptable, enhancing processing, hygiene, and storage is vital to meet growing demand and maintain market reputation. Authorities must implement prevention measures such as training producers, modern drying tech, quality materials, proper packaging, and consumer education on food safety. These actions can ensure safe, nutritional dried Bombay duck in Sylhet's markets and beyond, reducing health risks. Although Sylhet's residents prefer dried loitta so they should have them as cooked not in raw because raw loitta have the presence of pathogenic bacteria. The presence of harmful bacteria raises concerns, urging further study on handling, transport, and processing methods.

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EFFECTS OF GA_3 ON GROWTH, YIELD AND PIGMENTS CONTENT OF MUNGBEAN (*VIGNA RADIATA* L. WILCZEK)

A.M.M. GOLAM ADAM*, SABINA ESMIN AND HASNA HENA BEGUM
Department of Botany, Jagannath University, Dhaka-1100, Bangladesh

Abstract

A field trial was carried to explore the effect of GA_3 (foliar, seed soaking or both) on growth, yield and photosynthetic pigments content of BARI Mung-6. The trial comprised of ten treatments *viz.* T_0 = Control (Distilled water), T_1, T_2 and T_3 = seed soaking with 25, 50 and 100 ppm GA_3 respectively; T_4, T_5 and T_6 = foliar spray of 25, 50 and 100 ppm GA_3 respectively; T_7 = 12.5 ppm GA_3 as seed soaking + 12.5 ppm GA_3 as foliar spray, T_8 = 25 ppm GA_3 as seed soaking + 25 ppm GA_3 as foliar spray and T_9 = 50 ppm GA_3 as seed soaking + 50 ppm GA_3 as foliar spray. Results revealed that foliar spray of 50 ppm GA_3 produced significantly taller plant (48.0 cm) than all other treatments although statistically identical to 25 ppm foliar treatment. The maximum number of branches (5.17) and leaves per plant (17.33), highest dry weight of leaves (0.73 g) and roots were noted from 25 ppm foliar application. Foliar application of 25 ppm GA_3 also produced higher shoot-root ratio, biomass duration, absolute growth rate and relative growth rate than control. Outcome indicated that the highest number of pods per plant (5.50), longest pod (8.01cm), highest fresh (3.60 g) and dry weight (2.99 g) of pods, maximum number of seeds per plant (49.00), 1000-seed weight (47.72 g), yield per plant (2.34 g), yield per hectare (0.78 ton) and harvest index (62.46%) were obtained from 25 ppm foliar GA_3 treatment. Increase in yield per plant due to foliar application of 25 ppm GA_3 was 42.88% higher than control followed by seed soaking with 50 ppm (18.29%), 50 ppm foliar + 50 ppm seed soaking (13.42%) and seed soaking with 25 ppm (10.37%) treatments, respectively. Pigments content of leaves was remarkably influenced by GA_3 treatments but varies depending on the methods application, concentration and plant growth. Out of ten treatments, foliar spray of 25 ppm GA_3 produced better stimulation.

Keywords: Growth, Yield, Pigments, BARI Mung-6, GA_3

Introduction

Mungbean (*Vigna radiata* L. Wilczek) is one of the most important pulse crops and an excellent source of vegetable protein of Bangladesh. Among the pulse crops, it is the only crop that can be grown in three seasons (BARI, 2008) and can contribute a lot to meet up the demand of pulses. Seeds contains 1-3 % fat, 50.4 % carbohydrates, 4 % minerals, 3 % vitamins, 10 % moisture, 3.5-4.5 % fibers and 4.5-5.5 % ash with significant amount of

*Corresponding author: adamammg@bot.jnu.ac.bd

calcium and phosphorus (Frauque *et al*., 2000). In addition, the mature seeds supply an invaluable source of digestible protein for humans where meat is deficient (AVRDC, 2012). Mungbean is mostly grown in alternation with cereal crops having its short duration, least care, nominal input, and drought liberal nature. In cereal cropping system it can also boost sustainability of agriculture by increasing farm income and saving irrigation, water (Hussain *et al.,* 2012).

The yield of mungbean is very low than the other legumes *viz*. grasspea, chickpea and lentil (FAO, 2007). Bangladesh faces an acute shortage of pulses and yield of pluses should be improved immediately to meet up the national demand, avoid import as well as to save foreign currency. This demand needs urgent boost up in the crop yield from existing area where, use of plant growth regulators plays dramatic role. Plant growth regulators (PGRs) are magic substances that can modify various physiological processes of plants in minute concentration (Tan *et al.*, 2012). However, the success of these chemicals depends on concentration, timing and different modes of application (Sachin-Kumar *et al.,* 2018).

Gibberellic acid (GA_3) at appropriate concentrations has inducing effect in promoting growth and yield of a number of plants throughout the world *viz.* strawberry (Asadi *et al.,* 2013), cowpea (Nabi *et al.,* 2014), okra (Mohammadi *et al*., 2014), eastern black walnut (Parvin *et al.,* 2015), chickpea (Jahan *et al.,* 2018) etc. In Bangladesh, limited study has been done regarding the application of GA_3 on mungbean varieties (Hoque and Haque, 2002 and Tasnim *et al.*, 2019) and there is no recommended dose for the application of this hormone at farmer level. Therefore, the current investigation was taken to assess the effect of GA_3 on growth, yield and photosynthetic pigments of BARI Mung-6.

Materials and Methods

A field experiment was carried out at the research field of the botanical garden of the Jagannath University, Dhaka during Kharif-1 (April-June) season. The trial was laid out in a RBD with three replications. Seed of a high yielding, photoinsensitive and mungbean yellow mosaic virus tolerent variety of mungbean var. BARI Mung-6 was collected from BARI (Bangladesh Agricultural Research Institute), Joydebpur, Gazipur. The soil of the experimental land was prepared conventionally. The area of the experimental field was 33 m^2 area having foot paths of 50 cm in between. The size of the individual plot was approximately 0.366 m^2. The inter row spacing was 30cm with an inter plant spacing of 10 cm. Recommended amount of cow-dung (7,790 kg/ha) and chemicals fertilizer *viz.* urea (44 kg/ha), TSP (100 kg/ha), MP (40 kg/ha) and boric acid (7.5 kg/ha) were applied as described in FRG (2012). Seeds were surface sterilized with 0.05 % calcium hypochlorite solution to avoid fungal infection. Gibberellic acid (GA_3) was used as seed soaking, foliar spray alone and in combination in this investigation. Seeds were soaked with water (control) and different concentrations (25, 50 and 100 ppm) of GA_3 for 24 hours. The trial comprised of ten treatments *viz.* T_0 = Control (Distilled water), T_1, T_2 and T_3 = seed soaking with 25, 50 and 100 ppm GA_3 respectively; T_4, T_5 and T_6 = foliar spray of 25, 50 and 100 ppm GA_3 respectively; T_7 = 12.5 ppm GA_3 as seed soaking + 12.5 ppm GA_3 as foliar spray, T_8 = 25 ppm GA_3 as seed soaking + 25 ppm GA_3 as foliar spray and T_9 = 50 ppm GA_3 as seed soaking + 50 ppm GA_3 as foliar spray. Foliar spray was given at the age of 21 days in sunny morning. Cultural practices and fertilizer application were maintained as described by (Chowdhury and Hassan, 2013).

Growth parameters and yield parameters were recorded at harvest. Biomass duration, absolute growth rate and relative growth rate were analyzed following standard methods (Sestak *et al.,* 1971 and Gardner *et al.,* 1985). Photosynthetic pigment content of leaves were estimated at flowering, pod filling and harvest stages with the help of approved procedures (Mckinney, 1940; von Wettstein, 1957; Maclachalan and Zalik, 1963). Six plants (2 from each replication) were collected separately to record data on various parameters. Collected data were subjected for statistical analysis and treatment means were compared by LSD test at 5% level of significance (Steel *et al.*, 1997).

Results and Discussion

Results presented in Table 1 revealed that plant height of BARI Mung-6 was positively influenced following different GA_3 treatments where significantly taller plants were noted from foliar application of 50 and 100 ppm treatments although identical to each other. Similar results of increases due to GA_3 treatments have been found in various plants by different researchers (Houque and Hauque, 2002; Mohammadi *et al*., 2014; Nabi *et al*., 2014, Rahman *et al.*, 2015, Jahan *et al.*, 2018 and Tasnim *et al.*, 2019).

Number of branches of BARI Mung-6 responded differently by different GA_3 treatments although, seed soaking treatments produced better stimulations than foliar spray alone and in combination with an exception (Table 1). However, the highest number of branches per plant (5.17) was noted from 25 ppm foliar GA_3 treatment but statistically identical to all other treatments. Positive responses in number of branches per plant due to GA_3 treatments have also been observed in cowpea (Nabi *et al*., 2014), chickpea (Jahan *et al.*, 2018) and mungbean (Tasnim *et al.,* 2019). Although, Asadi *et al*., (2013) did not find any significant response of GA_3 application on strawberry. Thus, the current results are in harmony with the results of earlier works.

Application of GA_3 had inducing effects on the number of leaves per plant of BARI Mung-6 except 100 ppm foliar GA_3 alone and 50 ppm GA_3 as seed soaking + 50 ppm GA_3 as foliar spray treatment but with insignificant variations (Table 1). Nabi *et al*., (2014) recorded increased number of leaves due to GA_3 application on cowpea. Findings indicated that biomass of leaves, stem and root per plant were positively stimulated following GA_3 treatments but with few exceptions.

Foliar application of 100 ppm GA_3 produced maximum biomass of leaves and root where the number of leaves per plant was significantly higher than the control. But in case of dry weight of stem, foliar spray of 100 ppm GA_3 produced significantly higher weight of stem than control. Outcome of the present investigation are fairly supported to the results of Jahan *et al.,* 2018 on chickpea. Shoot-root ratio of BARI Mung-6 was both positively and negatively influenced by GA_3 treatments with non-significant variations. By applying IAA as growth promoting substance, Sen (2015) also recorded similar results in BARI mung-6.

Results obtained from Table 1 indicated that foliar spray of GA_3 had inducing responses on biomass duration of BARI Mung-6 where 25 ppm resulted significantly higher biomass duration than control. Although, the maximum BMD (11.26) was noted from combination of 12.5 ppm foliar and 12.5 ppm seed soaking treatments but statistically identical to the seed soaking with 100 ppm, foliar spray of 25 and 50 ppm treatments. Present results are in accord with the outcome of Sen (2015) in BARI Mung-6. Outcome revealed that absolute growth rate and relative growth rate were positively stimulated following GA_3 application except due to seed soaking treatment with 100 ppm in both the cases and foliar spray of 50 ppm in case of relative growth rate. Current findinmgs are in agreement with report of Nabi *et al*., (2014) on cowpea.

Yield parameters of BARI Mung-6 were both positively and negatively influenced depending on the concentrations and methods of GA_3 application (Table 2).

Foliar application of 25 ppm GA_3 and seed soaking with 25 ppm GA_3 and 50 ppm foliar+50 ppm seed soaking treatments resulted higher number of pods per plant but statistically identical to the rest of the treatments. Increase in number of pod per plant due to this GA_3 treatments has been obtained by Nabi *et al.*, (2014) and Tasnim *et al.,* (2019). However, Rahman *et al., (*2015) recorded both increase and decrease in number of fruits per plant in tomato due to GA_3 treatments. Thus, the present results are in agreement with the findings of previous results. Results revealed that length of pods were increased following most of the GA_3 treatments and the maximum length (8.01 cm) was obtained from foliar application of 25 ppm treatment. The stimulative effect of GA_3 on pod length was observed by Nabi *et al*., (2014) on cowpea. However, Mohammadi *et al.,* (2014) found non-significant effect on pod length in okra.

Findings showed that increases in fresh and dry weight of pods per plant were noted from seed soaking with 25, 50 ppm, foliar spray of 25 ppm and 25 ppm foliar+25 ppm seed soaking treatments only with similar statistical valuesr. Application of 25 ppm foliar GA_3 had resulted significantly higher fresh and dry weight of pods than control. Significant increase in fresh and dry weight of pods were also noted from the experiment of Hoque and Haque, (2002), Nabi *et al*., (2014) and Jahan *et al.*, (2018). Thus, the outcome are in accord with the findings of previous research. Number of seeds per pod was found to increase following all GA_3 application except 100 ppm foliar application but affected non-significantly. Similar results of increases in the number of seeds per pod following GA_3 were found by Mohammadi *et al*., (2014), Jahan *et al.,* (2018) and Tasnim *et al.,* (2019). However, Nabi *et al*., (2014) recorded both positive and negative responses following GA_3 treatments in cowpea. Application of 25 ppm GA_3, seed soaking with 25 ppm GA_3 and combination of 50 ppm foliar+50 ppm seed soaking treatments produced higher number of pods per plant although statistically similar to each other. This result is very much resembled to the findings of Hoque and Haque (2002) and Tasnim *et al.,* (2019). Weight of 1000-seed was both significantly and non-significantly affected by different GA_3 treatments (Table 2). Foliar application of 25 ppm foliar treatment resulted maximum 1000-seeds weight (47.72 g) which was significantly higher than control but statistically identical to 25 (T_1) and 50 ppm (T_2) seed soaking treatment, 12.5 ppm foliar+12.5 ppm seed soaking treatment (T_7) and 50 ppm foliar+50 ppm seed soaking treatment (T_7). These results are in fully agreement with the reports of Nabi *et al*., (2014) on cowpea, Jahan *et al.,* (2018) on chickpea and Tasnim *et al,* (2019) on mungbean. However, Mohammadi *et al*., (2014) reported that 100-seeds weight of okra was not affected by GA_3 application. Thus, the findings of current study are in concurrence with the outcome of previous workers.

Yield per plant and yield per hectare were both positively and negatively influenced following GA_3 application (Table 2). Seed soaking with 25, 50 ppm GA_3, foliar application of 25 ppm GA_3 and 50 ppm foliar + 50 ppm seed soaking treatments resulted higher yield per plant and yield per hectare although statistically identical to each other but significantly different from rest of the treatments. Foliar application of 25 ppm GA_3 as foliar treatment produced 42.88% higher yield than control followed by seed soaking with 50 ppm (18.29%), 50 ppm foliar + 50 ppm seed soaking (13.42%) and seed soaking

Table 1. Effects of GA_3 on growth parameters of BARI Mung-6 at harvest.

Treatments	Height (cm)	No.of branches/ plant	No. of leaves/ plant	Dry weight of leaves/ plant (g)	Dry weight of stem/ plant (g)	Dry weight of root/ plant (g)	Shoot-root ratio	Biomass duration	Absolute growth rate (g/day)	Relative growth rate (g/g/day)
T_0	39.55c	4.33	15.00	0.46bc	0.54bc	0.20	5.49	8.51bc	-0.02c	0.00cd
T_1	40.45bc	4.67	16.00	0.53a-c	0.53c	0.26	3.99	8.33bc	0.04a-c	0.03a-d
T_2	42.18bc	4.67	16.00	0.45bc	0.69a-c	0.19	6.69	7.83bc	0.08ab	0.07a-c
T_3	43.45bc	5.00	17.00	0.52bc	0.54bc	0.23	4.81	9.23ab	-0.03c	-0.02d
T_4	43.23bc	5.17	17.33	0.73a	0.77a-c	0.27	6.52	11.12a	0.05ab	0.04a-d
T_5	48.00a	5.00	17.00	0.56a-c	0.52c	0.25	4.63	9.52ab	-0.01bc	-0.01cd
T_6	44.58ab	4.17	14.50	0.46bc	0.80a	0.25	5.09	8.66bc	0.11a	0.09ab
T_7	42.07bc	4.23	16.50	0.64ab	0.79ab	0.20	9.05	11.26a	0.01bc	0.01b-d
T_8	43.38bc	5.00	17.00	0.52bc	0.65a-c	0.18	6.07	7.62bc	0.11a	0.11a
T_9	41.8bc	3.83	13.50	0.43c	0.53c	0.20	5.30	7.14c	0.05a-c	0.05a-d
CV (%)	9.26	23.06	20.14	29.51	31.07	30.62	48.07	20.99	193.20	166.05
LSD (0.05)	4.14	NS	15.00	0.20	0.25	NS	NS	2.26	0.09	0.08

Mean followed by same letter (vertically) or without letter are statistically similar at 5 % level.
[T_0 = Control (Distilled water), T_1, T_2 and T_3 = seed soaking with 25, 50 and 100 ppm GA_3 respectively; T_4, T_5 and T_6 = foliar spray of 25, 50 and 100 ppm GA_3 respectively; T_7 = 12.5 ppm GA_3 as seed soaking + 12.5 ppm GA_3 as foliar spray, T_8 = 25 ppm GA_3 as seed soaking + 25 ppm GA_3 as foliar spray and T_9 = 50 ppm GA_3 as seed soaking + 50 ppm GA_3 as foliar spray]

Table 2. Effects of GA_3 on yield parameters of BARI Mung-6 at harvest.

Treatments	No. of pods/plant	Length of pod (cm)	Fresh weight of pods/plant (g)	Dry weight of pods/ plant (g)	No. of seeds/ pod	No. of seeds/ plant	1000-seeds weight (g)	Yield/ plant (g)	Yield / hectare (ton)	Harvest index (%)
T_0	4.50a-c	7.25	2.32b-d	1.85b-d	8.46	37.83a-d	43.18cd	1.64a-d	0.55a-d	60.33ab
T_1	4.50a-c	7.54	2.69a-c	2.20a-c	9.28	39.83a-d	45.47a-c	1.81a-c	0.60a-c	60.33ab
T_2	4.17a-c	7.62	2.84ab	2.40ab	9.30	41. 83a-c	46.35ab	1.94ab	0.65ab	61.55ab
T_3	3.00cd	7.19	1.76cd	1.31d	8.36	25.50cd	43.32cd	1.11cd	0.37cd	49.99de
T_4	5.50a	8.01	3.60a	2.99a	9.88	49.00a	47.72a	2.34a	0.78a	62.46a
T_5	3.67b-d	7.25	1.98b-d	1.63b-d	8.56	30.83b-d	42.68cd	1.32b-d	0.44b-d	52.95cd
T_6	3.00cd	7.62	1.82cd	1.43cd	9.54	28.50b-d	45.08b-d	1.29b-d	0.43b-d	47.64e
T_7	2.17d	7.75	1.43d	1.23d	9.95	20.83d	46.42ab	0.97d	0.32d	40.71f
T_8	4.83ab	7.40	2.66a-c	2.29a	8.95	43.17ab	43.00d	1.86a-c	0.62ab	61.26ab
T_9	3.50b-d	7.75	2.16b-d	1.80b-d	9.75	33.00a-d	45.98ab	1.49b-d	0.50b-d	56.53bc
CV (%)	49.06	8.15	54.31	49.42	12.36	49.74	5.96	50.79	50.67	22.14
LSD (0.05)	1.81	NS	0.97	0.81	NS	17.18	2.36	0.78	0.26	5.28

Mean followed by same letter (vertically) or without letter are statistically similar at 5 % level.
[T_0 = Control (Distilled water), T_1, T_2 and T_3 = seed soaking with 25, 50 and 100 ppm GA_3 respectively; T_4, T_5 and T_6 = foliar spray of 25, 50 and 100 ppm GA_3 respectively; T_7 = 12.5 ppm GA_3 as seed soaking + 12.5 ppm GA_3 as foliar spray, T_8 = 25 ppm GA_3 as seed soaking + 25 ppm GA_3 as foliar spray and T_9 = 50 ppm GA_3 as seed soaking + 50 ppm GA_3 as foliar spray]

Table 3. Effects of GA_3 on photosynthetic pigment of BARI Mung-6 at three different stages.

Treatments	Flowering stage			Pod filling stage			At harvest		
	Chl.a	Chl.b	Carotenoids	Chl.a	Chl.b	Carotenoids	Chl.a	Chl.b	Carotenoids
T_0	0.39bc	0.12bc	2.16bc	0.25bc	0.10	1.87	0.45bc	0.17bc	3.42cd
T_1	0.37bc	0.14bc	2.61bc	0.46bc	0.22	3.38	0.86a	0.25ab	5.19ab
T_2	0.15c	0.06c	0.99c	0.61ab	0.33	4.07	0.77a	0.26ab	5.40a
T_3	0.31c	0.15bc	2.14bc	0.31bc	0.15	3.15	0.61ab	0.22ab	4.57a-c
T_4	0.22c	0.13bc	2.05bc	0.61ab	0.40	2.57	0.29c	0.18a	3.37cd
T_5	0.67ab	0.25ab	3.45ab	0.08c	0.07	1.86	0.36bc	0.27a	4.66a-c
T_6	0.74a	0.27ab	2.59bc	0.34bc	0.16	2.02	0.23cd	0.18ab	4.16a-d
T_7	0.82a	0.32a	2.69bc	0.09c	0.09	2.45	0.08d	0.08c	2.65d
T_8	0.65ab	0.24ab	4.91a	0.87a	0.43	5.41	0.40bc	0.19ab	3.79b-d
T_9	0.24c	0.15bc	1.96bc	0.89a	0.44	4.31	0.43bc	0.22ab	4.23a-c
CV (%)	61.61	53.21	54.41	77.39	71.88	50.71	73.10	44.11	35.36
LSD (0.05)	0.33	0.16	1.77	0.39	NS	NS	0.25	0.09	1.53

Mean followed by same letter (vertically) or without letter are statistically similar at 5 % level.
[T_0 = Control (Distilled water), T_1, T_2 and T_3 = seed soaking with 25, 50 and 100 ppm GA_3 respectively; T_4, T_5 and T_6 = foliar spray of 25, 50 and 100 ppm GA_3 respectively; T_7 = 12.5 ppm GA_3 as seed soaking + 12.5 ppm GA_3 as foliar spray, T_8 = 25 ppm GA_3 as seed soaking + 25 ppm GA_3 as foliar spray and T_9 = 50 ppm GA_3 as seed soaking + 50 ppm GA_3 as foliar spray]

with 25 ppm (10.37%) treatments respectively. Outcome revealed that treatments *viz.* seed soaking with 25 ppm GA_3, foliar application of 25 ppm GA_3 and 50 ppm foliar + 50 ppm seed soaking produced higher harvest index than control although statistically similar to each other. Results obtained in case of yield per plant and harvest index are in agreement with the results of many workers on various plants *viz.* mungbean (Hoque and Haque, 2002, Tasnim *et al.,* 2019), cowpea (Nabi *et al*., 2014) and chickpea (Jahan *et al.,* 2018).

Photosynthetic pigment content was positively affected by most of the GA_3 treatments at three stages *viz.* flowering, pod filling and at harvest with both significant and non-significant variations (Table 3). At flowering stage, chlorophyll a content was recorded higher due to foliar application of 50 and 100 ppm, 25 ppm foliar +25 ppm seed soaking and 50 ppm foliar +50 ppm seed soaking treatments only where foliar application of 100 ppm and 12.5 ppm foliar +12.5 ppm seed soaking treatments resulted significantly superior than control. Results raveled that chlorophyll a contents of leaves at pod filling stage were noted maximum from 50 ppm foliar +50 ppm seed soaking treatment followed by and 25 ppm foliar +25 ppm seed soaking treatments where both the treatments produced significantly higher chlorophyll a content than the control treatment. In case of harvest, higher amount of Chl.a has been estimated from seed soaking of 25, 50 and 100 ppm treatments where 25 and 50 ppm GA_3 produced significantly higher amount than the control. Results of the present exploration also show that different concentration of GA_3 as foliar or seed soaking and in combination had positive effects on chlorophyll b and carotenoids content of leaves at three stages with few exceptions (Table 3). The affirmative effects of GA_3 on the photosynthetic pigments was reported by Rahman *et al.,* (2015) on tomato and Jahan *et al.,* (2018) on chickpea. Thus, the results are in compliance with the conclusion of other workers.

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DIVERSITY AND MITOCHONDRIAL CO1-BASED BARCODING OF ORTHOPTERAN SPECIES OF BANGLADESH

SANTOSH MAZUMDAR[1]*, A.S.M. SHARIFUR RAHMAN[2], MATHEW SEYMOUR[3], MOHAMMAD AL-BARUNI CHOWDHURY[4]

[1]*Department of Zoology, University of Chittagong, Chittagong-4331, Bangladesh.*
[2]*Department of Zoology, Noakhali Science and Technology University, Noakhali-3814, Bangladesh.*
[3]*School of Biological Sciences, University of Hong Kong.*
[4]*Department of Genetic Engineering and Biotechnology, University of Chittagong, Chittagong-4331, Bangladesh.*

Abstract

Recognizing the ecological and economic significance of Orthoptera species in Bangladesh, maintaining an up-to-date inventory of the species within the country becomes crucial. Building upon previous records, the current investigation places a central focus on documenting the taxonomic identity, species distribution, and evolutionary relationships of Orthoptera species in Bangladesh, employing the mitochondrial COI-based barcoding technique. The findings reveal a total of 13 orthopteran species across 13 genera and 9 families, with three species identified through barcoding. Notably, *Phlaeoba sikkimensis* is recorded as a first for the Bangladeshi entomofauna, highlighting its importance in local biodiversity. The comprehensive genetic data presented in this study significantly enhance our understanding of orthopteran diversity, facilitating the development of effective measures for their conservation and sustainable management, thus fostering a harmonious coexistence with these ecologically and economically valuable species.

Keywords: Orthopterans, DNA barcoding, Phylogenetic relationships, Haplotype diversity, Bangladesh.

Introduction

Orthopterans (Orthoptera: Insecta), encompassing grasshoppers, locusts, and crickets, constitute a vast and diverse group with over 28,000 species worldwide (Tan and Wahab, 2018). The global and regional ecosystems and economies are significantly influenced by this diversity, as highlighted by studies (Naskrecki, 2013; Sun *et al*., 2015; Paul *et al*., 2016). Their impact is twofold, as they can cause extensive crop damage, potentially leading to widespread famine (Appert and Deuse, 1982; Resh and Cardé, 2009; Sun *et al*., 2015; Le Gall *et al*., 2019), and concurrently serve as crucial bio-indicators for

*Corresponding author: mazumdarsantosh@gmail.com

various ecological habitats, including forests (Armstrong and van Hensbergen, 1997). However, the rapid changes in species distribution and habitat degradation due to globalization and climate change pose significant challenges to their conservation (Kumar and Usmani, 2015). Noteworthy, in the Chittagong Hill Tracts of Bangladesh, large acridids among orthopterans play a vital role in sustaining indigenous communities, as documented by Mazumdar in 2019a. Moreover, Bangladesh offers abundant opportunities for biofuel research, utilizing phytophagous pests like acridids through cellulose extraction and application technology, as emphasized by Mazumdar in 2019b. Alarmingly, certain acridid species in Bangladesh are at risk of being classified as near-threatened, underscoring the urgency of conservation efforts (Mazumdar, 2020). As Bangladesh proactively prepares to repel a potential locust invasion, the government has undertaken measures to raise awareness, formulate a robust long-term strategy, and alleviate panic, all while addressing the associated risks of climate change (CABI, 2020). To effectively manage and respond to potential outbreaks, it is imperative to comprehend the current distribution and identity of local and regional orthopteran species.

The application of accurate, rapid, and cost-effective identification methods, such as mitochondrial DNA barcoding, is gaining prominence for enhancing biodiversity assessment, understanding species distributions, and formulating conservation plans (Hebert *et al*., 2003; Li *et al*., 2017, 2020). Taxonomic studies on acridids have been ongoing since the mid-sixties, with researchers like Alam (1962, 1967, 1970) pioneering efforts to compile information on agro-arthropod pests, including acridids. Subsequent contributions from Gapud (1992), Das (2004), Ahmad *et al*. (2009), and Aktar *et al*. (2018) have significantly enriched our understanding of orthopteran diversity and their interactions with crops and grasslands. In 2022, Mazumdar *et al*. reported on orthopteran species found on the Chittagong University Campus, contributing valuable insights to the ongoing exploration of these insects in various ecosystems.

The presentation of DNA barcoding data, comprehensive references, and discussions on Orthopterans in Bangladesh, including ecologically and economically important species records, aims to establish a baseline for future monitoring and management efforts. The current study adopts a comprehensive approach to compile an updated list of Orthopteran species within Bangladesh. Utilizing the Mitochondrial CO1-based barcoding technique, the research provides valuable insights into phylogenetic relationships, haplotype diversity, and distribution patterns of Orthopterans in both Bangladesh and its surrounding region. This holistic understanding is pivotal for informing conservation strategies, enabling the development of well-informed and effective measures to ensure the preservation and sustainable management of these species and their ecosystems.

Materials and Methods

Sample Collections and Preparation: Townes Malaise trap deployed for a two-week duration between an agriculture and grassland field adjacent to the Noakhali Science and Technology University (NSTU) campus (22°47′31″N; 91°06′07″E). The collected samples, consisting of 23 orthopterans, underwent initial screening for subsequent taxonomic identification, and seven candidates were chosen for molecular analysis. The taxonomic identification process drew upon references including Bhowmik (1985), Mandal *et al.* (2007), Shishodia *et al.* (2010), Srinivasan and Prabakar (2013), Kundu *et al.* (2020) and BIP (2022) to ensure accuracy in identifying *Phlaeoba sikkimensis*, which emerged as a first country record in this study. Furthermore, articles containing descriptions of the original collections of orthopterans were scrutinized, and species were identified through DNA barcoding. Notably, only articles presenting firsthand collections were included. The reference lists of collected articles were thoroughly searched to identify additional relevant articles, ensuring a comprehensive and rigorous approach in documenting the orthopteran species in the specified location.

Molecular analysis: Samples were prepared for DNA barcoding by following the guidelines of Standard Operation Procedures provided by BIO (2017) (www.dnabarcoding.ca). Specifically, the hind femoral muscles of each specimen (somatic tissue rich in mitochondria) were selected for DNA extraction, and the rest of the body of each specimen was stored as a voucher specimen at the department museum of the Zoology Department, NSTU.

All genomic steps from DNA extractions to PCR product generation were done at a molecular lab in the Poultry Research and Training Centre, Chattogram Veterinary and Animal Sciences University, Chattogram-4225. Tissue lysis, DNA extraction, and polymerase chain reaction (PCR) amplification for DNA Barcoding were conducted following the Monarch Genomic DNA Purification Kit Protocol (NEB#T3010, New England BioLabs Inc.). Vouchers were recovered after DNA extraction for imaging and curation. PCR amplification of the target region of cytochrome oxidase 1 (COI) using *Forward Primer*: LCO1490: 5'-ggtcaacaaatcataaagatattgg-3' and *Reverse Primer*: HC02198: 5'-taaacttcagggtgaccaaaaaatca-3'. The thermo cycle protocol included and initial denaturing stage of 95°C (5 min) followed by 30 cycles of 94°C (45 s), 48°C (45 s) and 70°C (1 min), and a final extension stage of 72°C (10 minutes). Finally, PCR products were checked via electrophoresis using a 1% agarose gels and visualized a gel visualized using a gel recording system (BioDoc Analyzer, Biometra, Germany). PCR products were then purified and quality checked prior to submission to Macrogen Inc. for sequencing. Once sequenced, the resulting partial mitochondrial cytochrome oxidase 1 DNA sequence chromatograms were assessed for quality using Bio Edit v.7.0.5 software. Each sequence was examined through the *Basic Local Alignment Search Tool* (*BLAST)* (https://blast.ncbi.nlm.nih.gov) and *Open Reading Frame Finder* (ORF finder) (https://www.ncbi.nlm.nih.gov/orffinder/) to diminish the indels, mismatches, and start-stop codons. The Obtained COI sequences were analyzed using sequence scanner version 2 of the applied biosystem. A total of 7 DNA barcode sequences of 2 subfamilies were submitted to the *National Center for Biotechnology Information* (NCBI) after finalization, and the accession numbers were obtained from the GenBank for every species we uploaded (Table 1).

Phylogenetic Network Constructions: A total of 56 Orthopteran sequences distributed in Bangladesh were obtained from BOLD database (Bold Systems v4) for overall analysis. The final dataset was aligned using the Clustal X program (Thompson *et al*., 1997), and the genetic divergences were estimated by Kimura 2 parameter (K2P) in Molecular Evolutionary Genetics Analysis (MEGA) programming 10 or MEGA10 (Kumar *et al*., 2016, 2018).

Haplotype network constructions: For constructing a haplotype network, the sequences of orthopterans were obtained from the BOLD database which includes 56 sequences of Bangladesh, each 10 sequences from India, Pakistan, China, Spain, Germany, Canada, etc. used for haplotype network construction purpose include MEGA, DNA SP, Popart, and TCS network. Besides, the Haplotype of each contributed species including *P. sikkimensis*, *A. crenulate,* and *O. fuscuvittata* along with their BOLD repository of available locations analyzed for a proper understanding of the distribution patterns of these species.

Results and Discussion

In the examination of 23 specimens, we partially sequenced the COI gene of 7 samples. These sequences were identified as any one of the three orthopteran species – *Atractomorpha crenulata* (one sequence: OQ842338, 644bp), *Oxya fuscovittata* (four sequences: OQ842271, 564bp; OQ842336, 651bp; OQ845424, 598bp; OQ842339, 652bp), and *Phlaeoba fuscovittata* (four sequences: OQ842271, 564bp; OQ842336, 651bp; OQ845424, 598bp; OQ842339, 652bp), and *Phlaeoba sikkimensis* (two sequences: OQ844086, 657bp; OQ845423, 631bp). A total of 17 of the specimens were morphologically identified as *Aulacobothrus luteipes, Chondracris rosea*, *Choroedocus violaceipes*, *Oxya hyla, Phlaeoba tenebrosa, Trilophidia annulata* (Acrididae), *Atractomorpha lata* (Pyrgomorphidae), *Euparatettix nigritibis* (Tetrigidae), *Conocephalus exemptus*, *Ducetia japonica*, and three up to genus such as *Furcilarnaca* sp. (Gryllacrididae), *Tetrix* sp. (Tetrigidae) and *Homoeoxipha* sp. (Trigonidiidae). The remaining three specimens included unidentified species.

Of the barcoded samples, our *O. fuscovittata* sequences exhibit a strong association with previously documented records from Bangladesh, including GMBCD3305, GMBCC1761, and GBMNB59901 (Fig. 1). Phylogenetic analysis reveals a noteworthy connection between the Indian isolate GBMND81283-21 and our newly submitted *O. fuscovittata* sequences, providing enhanced clarity in understanding the species distribution. Additionally, at a divergence level of 0.06, neighboring species such as *O. hyla*, identified by accession numbers GMBCD3304, GMBCI3080, and GMBCN749, contribute to the intricate genetic landscape. Notably, *A. crenulata* (OQ842338) exhibited

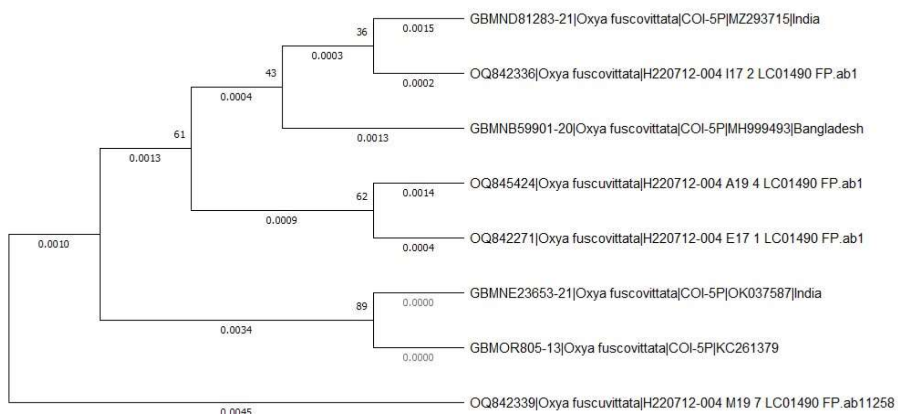

Fig. 1. Phylogenetic tree showing genetic relationships of *Oxya fuscuvittata*, rooted at the midpoint.

a close association with other Bangladeshi specimens of *Atractomorpha* (GMBCH3829-15, GMBCC1789-15, GMBCI3081-15, and GBMOR6888-19) in the BOLD repository specimen displaying slight divergence and a moderate to high confidence level. In our current investigation, the newly submitted Bangladeshi sequence OQ842338 demonstrates a close relationship with Pakistani isolates MTINS062-18, as illustrated in Fig. 2. Similarly, our contributed *P. sikkimensis* specimens (OQ844086 and OQ845423) exhibit associations with accession numbers GBMOR7400-19, GMBCB1681-15, GMBCC1764-15, GMBCJ2773-15, and GMBCN748-15, boasting a high confidence level and a divergence level of approximately 0.04 (Fig. 3). Notably, although *P.*

sikkimensis isolates from Bangladesh are absent in the BOLD database, our recent submission demonstrates a close relationship (CI = 99) with other isolates from India. The Bangladeshi isolates' closest neighbors are identified as GBMND7038-21 and GBMND7036-21, both originating from Indian isolates. These findings collectively contribute valuable insights into the genetic relationships and distribution patterns of the studied orthopteran species in the South Asian region.

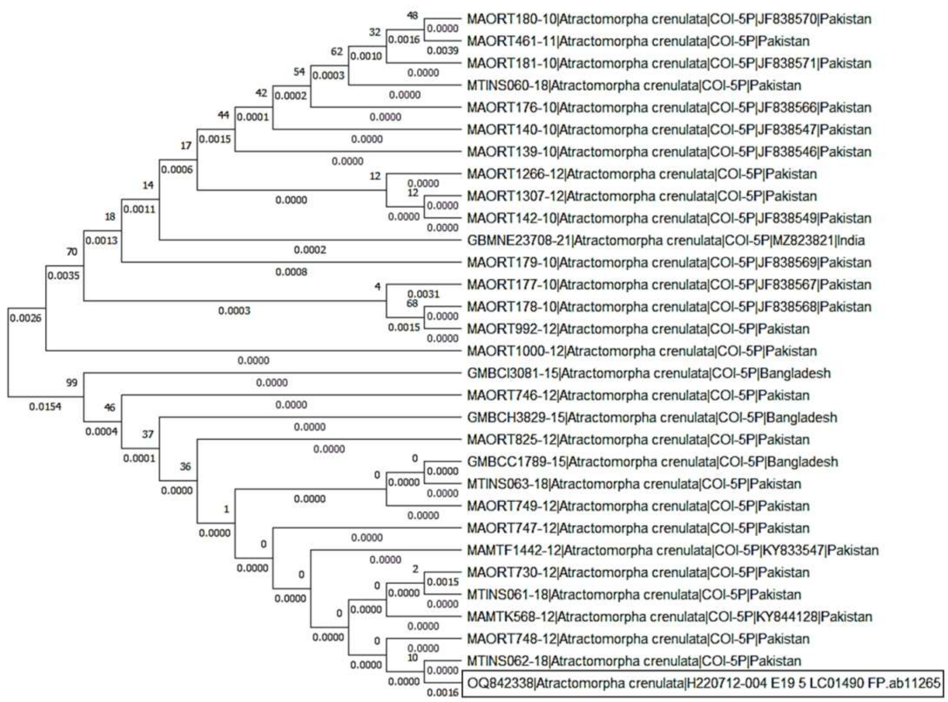

Fig. 2. Phylogenetic tree showing genetic relationships of *Atractomorpha crenulata*, rooted at the midpoint.

The haplotype analysis of Orthopteran species reveals intriguing patterns of interconnectedness among global populations. Australian haplotypes exhibit links with various Bangladeshi counterparts, marked by linker nodes with a divergence level of 18 nucleotide substitutions in single haplotype cases. Canadian haplotypes show connections with intermediate nodes, forming associations with German sequences. The Chinese haplotype is associated with both Pakistani and Spanish haplotypes through interconnected nodes. German haplotypes display associations with Bangladeshi counterparts, featuring a relatively minor divergence level and interconnected nodes. Notably, they show a slight connection with Australian sequences after three interconnected nodes. Spain's haplotypes exhibit substantial diversity, with some linked to Indian, Australian, Canadian, Chinese, and Bangladeshi interconnected nodes (Fig. 4). Furthermore, the individual haplotype analysis of *P. sikkimensis* reveals eight haplotype nodes, with Hap_8 containing two Bangladeshi sequences contributed in this study. Other sequences from Indian isolates and the Bangladeshi haplotype closely relate to Indian

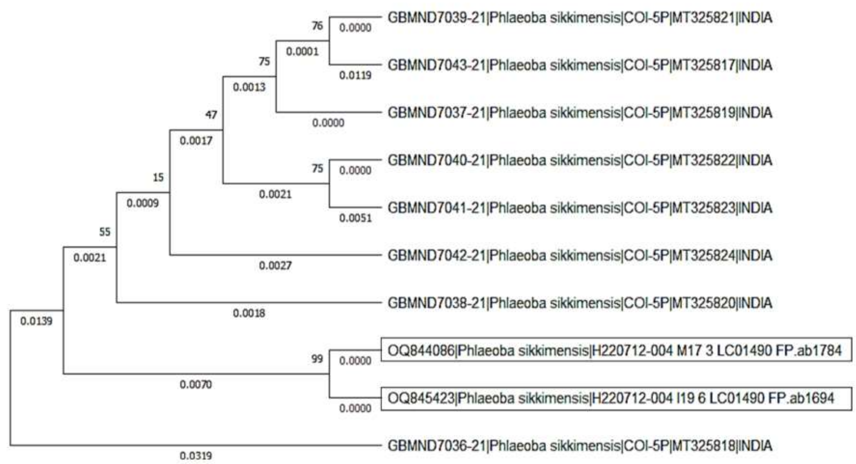

Fig. 3. Phylogenetic tree showing genetic relationships of *Phlaeoba sikkimensis*, rooted at the midpoint.

Hap_6 (Accession no of GBMND7042-21) with an interconnected node and a six-mutational divergence level, consistent with the phylogenetic analysis of this species (Fig. 5A). In the case of *O. fuscovittata*, one overlapping haplotype is identified with Indian isolates, suggesting a potential migration of the Indian species (GBMNE23653-21) to Bangladesh and close association with BD isolates of GBMOR805-13 (Fig. 5B). This finding is corroborated by phylogenetic analysis, highlighting a strong correlation between the two sequences from Indian GBMNE23653-21 and Bangladeshi isolates GBMOR805-13 (Fig. 1). In the case of *A. crenulata*, haplotype analysis indicates that Indian, Bangladeshi, and Pakistani species share only two haplotypes, suggesting a wide distribution with low divergence and de novo mutations. Bangladeshi sequences are closely associated with partial sequences of Pakistani nodes, showcasing a minor divergence level between them (Fig. 5C). These results offer valuable insights into the genetic connectivity and migration patterns of Orthopteran species across different geographical regions.

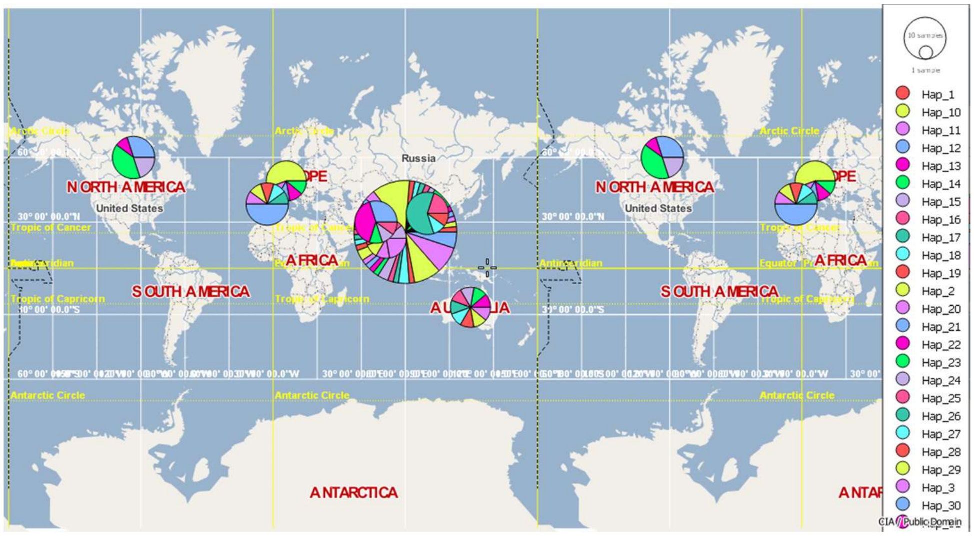

Fig. 4.A map displaying the haplotype distribution of Orthoptera species across various geographical regions

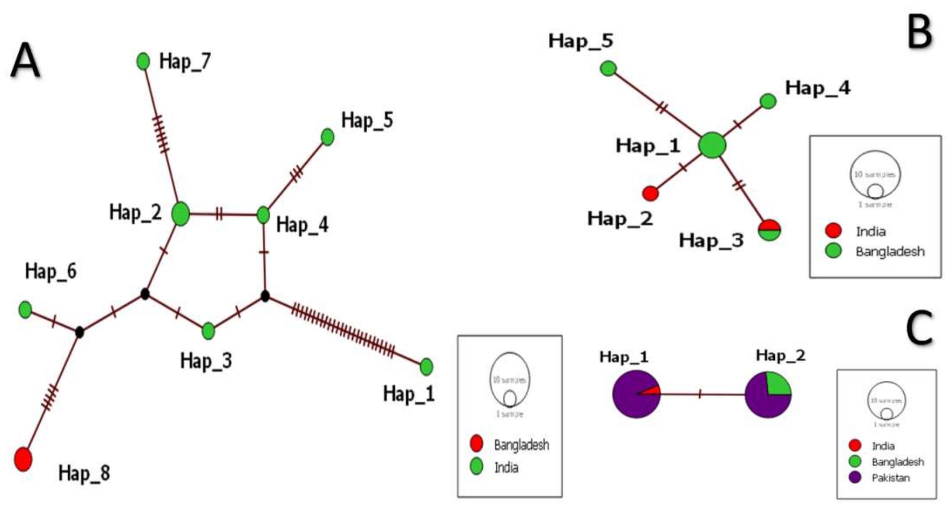

Fig. 5 (A, B, C). Haplotype analysis of the present studied orthopterans *P. sikkimensis, O. fuscuvittata, A. crenulate.*

However, in the case of *A. crenulate* species haplotypes of Indian, Bangladeshi, and Pakistani species are found to be distributed in just two haplotypes. This indicates the wide distribution of this species with a low level of divergence and de novo mutations. Our Bangladeshi sequences are found associated with the partial sequences of the Pakistani nodes (Fig. 5C). Phylogenetic relationships further confirmed this finding as Pakistani isolates MTINSO62 are closely related to our contributed species with a minor divergence level (Fig. 2).

Again, in the case of *P. sikkimensis* most of the BD sequence is placed on the group of Hap_1 and the center nodes that interconnect with all Indian and Bangladeshi sequences. Here, Hap_1, Hap_3, Hap_4, Hap_5, Hap_6, Hap_7 contain individual sequence of GBMND7036-21, GBMND7038-21, GBMND7040-21, GBMND7041-21, GBMND7042-21, GBMND7043-21 respectively. Where Hap_2 includes two sequences of GBMND7037-21 and GBMND7039-21. Similarly, Hap_8 contains two sequences of H220712-004_M17_3_LC01490_FP_*P. sikkimensis* and H220712-004_I19_6_LC01490 _FP_*P. sikkimensis*. Among the 8 haplotype variants, Hap_1 to Hap_7 are of Indian origin and Hap_1 only is of Bangladeshi origin. In addition, Hap_2 is the ancestor type, and Hap_1, Hap_5, Hap_7, Hap_6 & Hap_8 are recently evolved types. Hap_3 differs from Hap_2 with a few mutations, whereas Hap_4 is distinct by two times more mutations. Hap_7 differs from Hap_2 with 7-time mutations than Hap_3 and Hap_1 with a maximum of 26 times more mutations. The two Bangladeshi sequences incorporated in Hap_8 evolved from Hap_2 or Hap_3 with two unknown internodes and total mutations of 7 times. Hap_6 and Hap_8 are connected with a common internode and their complete divergence is about a few mutations for Hap_6 and 5 times for Hap_6 from the common unknown internodes

Most specifically, *P. sikkimensis* Ramme, 1941, a member of the Acrididae family, Acridinae subfamily, and Phlaeobini tribe, was confirmed first country record through DNA barcoding in the present study, was documented in various faunal surveys across South Asia. Described by Kirby in 1914 and Ramme in 1941, this species is characterized by straight and continuous lateral carinae of the pronotum, with greenish basally tinted hind wings. The distribution of *P. sikkimensis* spans Bangladesh, where it is also recorded in India, Nepal, and Bhutan according to Bhowmik (1985), Shishodia *et al*. (2010) and BIP (2022). This species is still restricted in South Asia, eight COI gene sequences of *P. sikkimensis* are present in the global DNA barcode database (BIP 2022). Kundu *et al.* (2020) had previously confirmed the species through DNA barcoding in India. This short note highlights the importance of molecular techniques in confirming the identity and distribution of species, adding valuable data to the understanding of South Asian biodiversity.

Conclusions

The present study builds upon the foundations laid by Das *et al.* (2022) and Mazumdar *et al.* (2022) in the field of DNA barcoding, contributing to an updated catalog of orthopteran species in Bangladesh. The orthopteran fauna in Bangladesh remains incompletely explored, with numerous species yet to be discovered. There is a pressing need for advanced studies to unravel the intricacies of their biology and potential applications. The present study sheds light on the evolutionary relationships within various Orthoptera species documented in Bangladesh, offering valuable insights through phylogenetic analyses. Noteworthy outcomes include the identification of unique haplotypes, elucidation of distribution patterns, and documentation of first country records. This comprehensive update enhances our understanding of the orthopteran diversity in Bangladesh, shedding light on the intricate ecological dynamics within the region. These findings collectively contribute to a deeper understanding of the distribution and identity of this understudied yet ecologically significant species group. The gaps filled by this research provide a foundation for further investigations, emphasizing the importance of continued exploration and study of orthopteran biodiversity in the context of Bangladesh.

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SOIL ORGANIC CARBON AND NITROGEN STORAGE AND DISTRIBUTION IN THE AGRICULTURAL SOILS AS AFFECTED BY SOIL DEPTHS AND INUNDATION LAND TYPES

M. J. UDDIN* AND A.S.M. MOHIUDDIN
Department of Soil, Water and Environment, University of Dhaka, Dhaka-1000, Bangladesh

Abstract

A study was initiated on soil organic carbon (SOC) and total nitrogen (TN) storage and their distribution as affected by soil depths and inundation land types selecting ideal two catena across eight soil profiles up to the depth of 120 cm in the Brahmaputra and the Ganges alluviums. Soil organic carbon and TN storage is higher in the surface soil depth than the other soil depths. The contents and distribution of SOC and TN in all the soil depths varies significantly. Moreover, inundation land types and soil depths exhibited a significant effect ($p<0.001$) on SOC and TN storage. Soil organic carbon and TN storage were higher in the lowland (LL) and medium lowland (MLL) sites than that in the highland (HL) and medium highland (MHL) sites across the alluviums, which indicates that the topographic variability as well as their water recession conditions which ultimately focuses on SOC loss or sequestration. The Brahmaputra alluvium possesses higher SOC and TN storage than the Ganges alluvium which may be due to the variability of their land use and local management practices. Proper emphasis should be given on sub soil depths and inundation levels in formulating any agricultural policy planning.

*Keywords***:** Soil organic carbon and nitrogen, Storage and distribution, Soil depths and inundation land.

Introduction

The biogeochemical cycles of carbon (C) and nitrogen (N) in terrestrial ecosystems have received increasing attention worldwide over the past few decades because the emission of their oxides contributes greatly to global warming (Fu *et. al.,* 2010; Canadel *et al.,* 2021)). As soils contain significantly more carbon than is present as CO_2 in the atmosphere, the stability of this soil store, particularly under changing temperature and other climatic factors, is a major source of uncertainty in future climate change predictions (Knorrs *et. al.,* 2005; Davidson and Janssen, 2006). Soil is a major pool of carbon and nitrogen and plays an important role in their global cycles (Batjes, 1996). The loss of C and N via the emissions of greenhouse gases, (GHGs) (CO_2, CH_4 and N_2O)

*Corresponding author: juswe@du.ac.bd

from soil to the atmosphere by natural or anthropogenic processes is a contributory factor to global warming. Consequently, sequestration of soil organic carbon (SOC) and conservation of total nitrogen (TN) are of increasingly scientific and political interests worldwide. Soil organic carbon and TN, often tightly coupled, are controlled by a number of natural and anthropogenic factors, including climate, vegetation, topography, parent material, intrinsic soil properties, land use and management practices (Homann *et. al.,* 1995; Ajami *et. al.,* 2016)). A better understanding of SOC and TN contents and their relationships with these controlling factors is critical to evaluate soil C and N pools as well as potentials for C sequestration and N conservation to offset anthropogenic greenhouse gas emissions. Soil organic carbon is one of the main factors affecting soil quality and agricultural productivity.

Being a source as well as storage of plant nutrients, SOC plays an important role in terrestrial C cycle (Freixo *et al.,* 2002; Elbasiouny *et al.,* 2022)). Land use has a significant effect on SOC storage, since it affects the amount and quality of litter input, litter decomposition rate, and stabilization of SOC (Bronson *et al.,* 2004). Information on global and regional SOC pool in topsoil is generally available for a variety of land use and climatic conditions (Batjes, 1996). However, study on SOC and TN storage in soils as affected by inundation land is very scanty, particularly in Bangladesh. It is widely accepted that SOC is largely concentrated in the top 30 cm of the soil, but there is a growing evidence that deeper soil horizons have the capacity to sequester high amounts of SOC (Jobbagy and Jackson, 2000; Swift, 2001) and that this should be considered for SOC emission-storage analysis. The importance of SOC sequestration in sub-soils mitigating the greenhouse effect is related to the fact that subsoil SOC occurs in fairly stable and highly recalcitrant forms to biodegradation (Batjes, 1996; Nierop and Verstraten, 2003; Lorenz and Lal, 2005). SOC surveys usually consider a fixed soil depth, typically 1 meter. Global surveys based on vegetation units (Post *et al.,* 1982) and soil taxonomic units (Eswaran *et al.,* 1993; Batjes, 1996) indicate that soil store 1500-1600 Pg C in the top one meter. However, soil carbon can be underestimated in its global budgets by fixing a lower boundary at 1m depending on the vertical distribution of SOC. Soil organic carbon content exhibits considerable variable regarding spatially, both horizontally according to land use and vertically within the soil profile (Dhakal *et al.,* 2010). The SOC diminishes with depth regardless of vegetation, soil texture, and clay size fraction (Trujilo *et al.,* 1997). Soils of the world are potentially viable sinks for atmospheric carbon and may significantly contribute to mitigate the global climate change (Lal *et al.,* 1998). However, the assessment of potential carbon sequestration in soil requires estimating carbon pools under existing land uses and its depth wise distribution in the soil profile. The objectives of this study were: (i) to estimate the SOC and TN distribution and storage across the study sites; and (ii) to assess the impact of land types and soil depths on SOC and TN distribution and storage.

Materials and Methods

Two ideal catena were selected across the two alluviums: the Brahmaputra and the Ganges, based on the land types for the current study. Forty eight soil samples from the eight profiles of the two catena at different soil depths (0-20, 20-40, 40-60, 60-80, 80-100 and 100-120 cm) were collected. Prior to analysis, the soil samples were air-dried and gently disaggregated. The soil samples were then gently ground using a mortar and pestle and passed through 2 mm sieve and mixed thoroughly. The samples were then preserved in sealed plastic containers for laboratory analysis. An outline of the site characteristics of the land types of the eight profiles is presented in Table 1.

The highland (HL) means the land which is above normal flood level. The medium highland (MHL) indicates the land which normally is flooded up to about 90 cm deep during the flood season. The medium lowland (MLL) denotes the land which normally is flooded up to between 90 cm and 180 cm deep during the flood season. Lowland (LL) states the land which normally is flooded up to between 180 cm and 300 cm deep during the flood season (FAO-UNDP, 1988).

Soil organic carbon was determined by following the method of Walkley and Black (Nelson and Sommers, 1982) and the Kjeldahl method (Bremner and Mulvaney, 1982) was used for total soil nitrogen (TSN) determination. The particle size analysis of soils was carried out by the hydrometer method as described by (Gee and Bauder, 1986). Soil bulk density was determined by using the core method as described by Blake and Hartge (1986). It may be noted that the bulk density and SOC concentration (%) are the two prerequisites for estimating SOC stock or storage. Thus, the SOC and TN storage were calculated using the following equations (Batjes, 1996; Chen *et al.,* 2007; Zhang *et al.,* 2013). Data is reported as mean± standard deviation. Two-way analysis of variance (ANOVA) was employed to assess the effects of land types and soil depths on SOC and TN storage or concentrations. One-way ANOVA was used to examine the effect of soil depths on SOC and TN storage. Regression analyses were used to test the relationships between SOC and TN storage at 0-20 cm depths, 0-60 cm depths, and 0-120 cm depths. All statistical analyses were conducted using SPSS, version 20.0.

Table 1. Information on the inundation land types of the eight profiles of the Brahmaputra and the Ganges alluvium.

Land types	Information/ Characteristics	Brahmaputra alluvium	Ganges alluvium
HL	Location	24˚08' N and 89˚ 58' E	23˚ 49' N and 89˚ 00' E
	Topographic position	Upper part of the ridges of a catena under the Brahmaputra alluvium	Upper part of the ridges of a catena under the Ganges alluvium
	Land use	Banana -Fallow	Banana/orchards-Fallow
	Soil series	Sonatala series (Aeric Endoaquepts)	Sara series (Aeric Endoaquepts
MHL	Location	24˚ 06' N and 89˚ 56' E	23˚ 51' N and 89˚ 01' E
	Topographic position	Middle part of the ridges	Middle part of the ridges
	Land use	Boro rice – Transplanted Aman rice	Boro rice –Tobacco/Transplanted Aman/Pulses
	Soil series	Silmandi series (Aeric Endoaquepts)	Ishurdi series (Aeric Endoaquepts)
MLL	Location	25˚00' N and 89˚ 45' E	23˚56' 88˚59'
	Topographic position	Moderately lower part of the ridges	Moderately gentle lower part of the ridges
	Land use	Boro rice- Fallow	Boro rice-Transplanted Aman/Fallow
	Soil series	Ghatail series (Typic Endoaquepts)	Gheor soil series (Typic Endoaquepts)
LL	Location	24˚08'89˚55'	23˚56' 88˚59'
	Topographic position	Lower part of the ridges of the Brahmaputra alluvium	Lower part of the ridges of the Ganges alluvium
	Land use	Boro- Fallow/Grazing grass	Transplanted Aman/ Rabi vegetables- Fallow
	Soil series	Balina series (Typic Endoaquepts)	Garuri series (Vertic Endoaquepts)

Results and Discussion

Soil organic carbon contents at different soil depths across the inundation land types: The highest SOC concentration was found in the topsoil (0-20 cm) across the eight land types of the two alluviums (Table 2). Soil organic carbon contents depend on the balance between organic carbon (OC) input and loss from soils (Zhuang *et al.,* 2007). Topsoil layer (0-20 cm) is tilled and receives greater residue inputs which are subsequently mineralized. Thus this layer possesses higher SOC than the other soil layers (20-120cm). Chaplot *et al.* (2010) reported that the topsoil layer may be able to sequester atmospheric CO_2 and thus mitigate climate change where more biophysical activities take place. Xiao-

Wei *et al.* (2012) noted that surface soils are rich in SOC due to being covered by highly productive vegetation or subject to long-term use of organic fertilizers or flooding conditions. Soil organic carbon in the top soil layer (0-20 cm) varies significantly ($P<0.001$) when tested using Tukey's Honestly Significant Difference (HSD). Besides, SOC concentration showed a decreasing trend from the top soil layer to the bottom layer for all land types of the Brahmaputra and the Ganges alluviums (Table 2).

The mean SOC concentration across the Brahmaputra alluvium (BA) varies from 0.41% (4.15 g/kg) to 1.15% (11.56 g/kg). Lowland sites of BA show the highest SOC concentration than the HL and MHL sites. The mean SOC concentration across the Ganges alluvium (GA) varies from 0.36% (3.61 g/kg) to 0.74% (7.48 g/kg) where low land sites show the highest SOC concentration than the HL and MHL sites (Table 2). Among the two alluviums, the Brahmaputra alluvium (BA) contains more SOC than the Ganges alluvium (GA). Low land (LL) sites contain a higher SOC concentration in both the alluviums than the other land types (HL and MHL). Thus, lowland (LL) and even medium lowland (MLL) types of the both alluviums contain higher SOC due to the nature of inundation depths. On the other hand, the HL and MHL types lose their SOC due to the increased decomposition being not inundated, erosion, and more intensive tillage (Ritchie *et al.,* 2007). Roose and Barthes (2001) noted that SOC is lost in the higher topography, through erosion, runoff and leaching where erosion and runoff contribute a large portion of carbon losses and these are highly accelerated in cultivated land as compared to undisturbed land.

Table 2. Soil organic carbon (SOC) distribution (%) at different soil depths across the eight land types of the alluviums.

Depths (cm)	Brahmaputra Floodplains				Ganges Floodplains			
	HL	MHL	MLL	LL	HL	MHL	MLL	LL
0-20	0.71	0.88	1.05	1.82	0.80	1.0	1.4	1.81
20-40	0.46	0.63	0.68	1.60	0.38	0.51	0.61	0.79
40-60	0.40	0.54	0.60	1.0	0.30	0.40	0.50	0.61
60-80	0.36	0.40	0.51	0.91	0.28	0.31	0.38	0.50
80-100	0.30	0.25	0.41	0.90	0.21	0.20	0.21	0.39
100-120	0.26	0.18	0.33	0.71	0.20	0.14	0.20	0.39
Mean± SD	0.41± 0.16	0.48± 0.25	0.59± 0.25	1.15± 0.44	0.36± 0.22	0.42± 0.31	0.55± 0.44	0.74± 0.54

TN contents at different soil depths across the inundation land types: The highest TN concentration was found in the topsoil (0-20 cm) across the eight land types like SOC. TN concentration across the four land types of Brahmaputra alluvium (BA) varied from 0.03 to 0.18% (0.30 to 1.8 g/kg) where the MLL/LL types contains the highest TN concentration and the HL and MHL contains the lowest TN concentration. TN concentration across the land types of the Ganges alluvium (GA) varied from 0.02 to 0.20% (0.22 g/kg to 2.0 g/kg) where MLL and LL sites contains highest TN concentration and the HL and MHL sites contains the lowest TN concentrations which are consistent with their SOC levels. MLL and LL types contain higher TN concentrations than the HL and MHL land types across the alluviums (Table 3). Among the two alluviums, BA contains more TN than the GA as reported for SOC. TN in the top layer varies significantly (P<0.001) when tested using Tukey's Honestly Significant Difference (HSD). TN concentration showed a decreasing trend downward from the top soil layer (0-20 cm) across the land types of the two alluviums.

Table 3. Total nitrogen (TN) distribution (%) at different soil depths across the eight land types of the alluviums.

Depths (cm)	Brahmaputra alluvium				Ganges alluvium			
	HL	MHL	MLL	LL	HL	MHL	MLL	LL
0-20	0.10	0.09	0.12	0.18	0.08	0.12	0.14	0.20
20-40	0.07	0.08	0.08	0.17	0.04	0.07	0.07	0.09
40-60	0.07	0.07	0.08	0.14	0.04	0.05	0.07	0.08
60-80	0.07	0.06	0.07	0.13	0.04	0.05	0.06	0.08
80-100	0.05	0.03	0.06	0.12	0.03	0.03	0.03	0.06
100-120	0.05	0.03	0.06	0.11	0.03	0.02	0.03	0.06
Mean± SD	0.07 ± 0.01	0.06 ± 0.02	0.07 ± 0.02	0.14 ± 0.02	0.04 ± 0.01	0.05 ± 0.03	0.06 ± 0.04	0.09 ± 0.05

The above result showed that the effect of land types and soil depths across the study sites on SOC is significant indicating both land types and soil depths are important factors influencing the SOC distribution across the inundation land types. A similar observation of the effect of topographic land condition and soil depths on SOC have been made by other authors (Chen *et al.,* 2007; Fang *et al.,* 2012; Fu *et al.,* 2010; Uddin *et al.,* 2019). Land types and soil depths exhibited a significant effect on SOC and TN concentration as tested by two-way ANOVA. The SOC and TN contents varied significantly (P<0.001) across the land types as well as soil depths. Land types and soil depths showed a significant effect on TN concentration (P<0.001) (Table 4), and the distribution of TN in soil was similar to SOC.

Table 4. Two-way ANOVA for the effect of land types and soil depths on SOC and TN.

Parameters	df	Soil Organic carbon (SOC)		Total nitrogen (TN)	
		F	P	F	P
Land types	7	34.949	<0.001	31.710	<0.001
Soil depths	5	18.865	<0.001	27.808	<0.001

The current study revealed that the highest SOC and TN concentration were found in the top soil layer (0-20 cm) in all the profiles across the alluviums. This layer is the most important part of the profile where maximum pedogenic activities take place. The high residue inputs in the surface soils may contribute to the increased SOC and TN distribution (Wu *et al.,* 2004; Liu *et al.,* 2005). SOC and TN is less variable in the deeper soil layers (60-120 cm) across the land types, than the 0-60 cm layer, which suggests that SOC and TN remained relatively stable in the soil depths between 60-120 cm. The study also showed that SOC and TN were found variable within 0-60 cm depths across the land types where most physical and chemical activities taken place. The SOC and TN contents across the land types decreased with increasing depths. On the other hand, the lowest SOC and TN were found in the HL and MHL sites and the highest SOC and TN were found in the LL sites across the two alluviums. A moderate level of SOC and TN was found in the MLL types (Tables 2 and 3).

The above results agreed with other findings (Chen *et al.,* 2007), indicating that both topographic nature and land use influence the SOC as well as TN contents. The lower SOC in the HL and MHL sites may be attributed to the reduced residue input in the soil and extensive soil erosion because of their higher elevation in the landscape and also due to intensive tillage, which is common in such land types. Guo and Gifford (2002) reported that plant roots also play an essential role in influencing SOC and TN distribution. Wei *et al.* (2009) revealed that distributions of fine roots are lower in higher topographic level than lower topographic level due to differences in vegetation. Similarly, LL and MLL types provide fine root system under anaerobic rice-rice cultivation with even residue decomposition which may also be responsible for higher SOC and TN contents in the MLL and LL types. The SOC and TN contents of the MLL and LL sites were higher than those of other land types, which may be attributed due to their inundation nature as well as their nature of farming. The topographic nature and anaerobic farming systems in the MLL and LL types may have greatly reduced the nutrients losses from reduced soil erosion. Erosion and leaching are more prevalent in the HL and MHL types because their drainage. SOC and TN losses are more prevalent in the HL and MHL sites due to the processes of erosion and runoff. Roose and Barthes (2001) noted that erosion and runoff contribute a large portion of C losses and these are highly accelerated in the cultivated land than the uncultivated soils.

Soil organic carbon and total nitrogen storage at different soil depths for different inundation land types: The average amounts of SOC storage varied from 1.70 kg/m^2 to 4.52 kg/m^2 in the 0-20 cm layer, 0.97 kg/m^2 to 2.52 kg/m^2 in the 20-60 cm layer and 0.33 kg/m^2 to 2.40 kg/m^2 in the 60-120 cm layer across the two alluviums of the eight profiles respectively. On the other hand, SOC storage across the inundation land types of the BA varied from 6.03 to 17.46 kg/m^2. SOC storage across the inundation land types of the GA varied from 5.20 to11.37 kg/m^2 (Table 5). Similar observations have been reported by several studies regarding the SOC storage. Tarnocai (1997) and Uddin *et al.* (2022) reported that average SOC content in the surface soils ranged from 4.9 to 18.7 kg/m^2. Sakin (2012) also reported that SOC content varies from 3.57 kg/m^2 to 6.47 kg/m^2 in the Harran plain soils in Southeastern Turkey. In the present study, compared with the HL and MHL sites, the SOC storage in the MLL and LL sites was higher across the two alluviums. The SOC storage decreases with increasing depths across the different land types.

Table 5. Soil organic carbon storage (kg/m^2) at different soil depths across the land types of the two alluviums.

Depths (cm)	Brahmaputra alluvium				Ganges alluvium			
	HL	MHL	MLL	LL	HL	MHL	MLL	LL
0-20	1.70	2.14	2.60	4.44	1.92	2.40	3.41	4.52
20-40	1.12	1.56	1.74	4.03	0.91	1.25	1.52	2.02
40-60	0.97	1.33	1.53	2.52	0.73	0.99	1.28	1.62
60-80	0.89	0.99	1.32	2.40	0.68	0.77	0.97	1.33
80-100	0.74	0.60	1.06	2.37	0.50	0.48	0.53	0.94
100-120	0.61	0.43	0.79	1.70	0.46	0.33	0.48	0.94
Total	6.03	7.05	9.04	17.46	5.20	6.22	8.19	11.37
Mean ± SD	1.00± 0.38	1.17± 0.63	1.50± 0.63	2.91± 1.07	0.86± 0.54	1.03± 0.74	1.36± 1.08	2.91± 1.07

TN storage in the soils was similar to SOC. The average amounts of TN storage varied from 0.20 to 0.50 kg/m^2 in the 0-20 cm layer, 0.10 to 0.42 kg/m^2 in the 20-60 cm layer and 0.07kg/m^2 to 0.34 kg/m^2 in the 60-120 cm layer across the alluviums of the eight profiles respectively. The TN storage across the inundation land types of the BA ranged from 0.85 to 2.12 kg/m^2. The TN storage across the inundation land types of the GA varied from 0.65 to1.44 kg/m^2 (Table 6). Similar observations have been reported by several studies. Carter *et al.* (1998) reported that TN in Canada farming soils ranged from 0.36 to 1.05 kg/m^2 and the TN storage in the MLL and LL sites were higher than those HL and MHL soils. They also noted that TN storage also varied with the increasing depths across different land types. Liu *et al.* (2012) also reported that the average densities of SOC and TN at a depth of 1m were about 7.72 and 0.93 kg/m^2, respectively, in the northeastern margin of the Qinghai-Tibetan Plateau. The above situation regarding SOC and TN contents are consistent with Bangladesh situation because plateau margin occupies alluvial characteristics similar to the alluvial soils of Bangladesh.

Table 6. Total nitrogen storage (kg/m^2) at different soil depths across the land types of the two alluviums.

Depths (cm)	Brahmaputra alluvium				Ganges alluvium			
	HL	MHL	MLL	LL	HL	MHL	MLL	LL
0-20	0.24	0.21	0.30	0.44	0.20	0.28	0.34	0.50
20-40	0.19	0.19	0.21	0.42	0.11	0.17	0.17	0.24
40-60	0.19	0.17	0.20	0.35	0.10	0.12	0.17	0.21
60-80	0.17	0.14	0.18	0.34	0.10	0.12	0.15	0.21
80-100	0.12	0.07	0.15	0.31	0.07	0.07	0.07	0.14
100-120	0.11	0.07	0.14	0.26	0.07	0.07	0.07	0.14
Total	1.02	0.85	1.18	2.12	0.65	0.83	0.97	1.44
Mean ± SD	0.17± 0.04	0.14± 0.06	0.19± 0.05	0.35± 0.06	0.10± 0.04	0.13± 0.07	0.16± 0.09	0.23± 0.13

The effect of soil depths on SOC and TN storage in soils are presented in Table 7. Soil depths had significant influence ($P<0.05$) on SOC and TN storage as assessed by a one-way ANOVA study.

Table 7. One-way ANOVA for the effect of soil depths on SOC and TN storage in soils.

Depths (cm)	df	Soil Organic carbon (SOC)		Total nitrogen (TN)	
		F	P	F	P
0-20	8	28.034	<0.05	17.308	<0.05
20-60	8	6.281	<0.05	6.179	<0.05
60-120	8	8.446	<0.05	8.560	<0.05

F and P values, from one-way ANOVA; df is degrees of freedom; All values show significant at P<0.05

The relationships between SOC and TN storage among the topsoil (0-20 cm) and deeper layers (0-60 cm), and (0-120 cm) are shown in Figs. 1-4. All the changes in SOC and TN storage with increasing depths were evaluated using regression equations. The relationships of SOC storage between the soil depths 0-20 cm and 0-60 cm (Fig. 1), and 0-20 cm and 0-120 cm (Fig. 2) show strong correlations (r = 0.92 and 0.85 respectively). Likewise, the relationship of TN storage between the soil 0-20 cm and 0-60 cm depths (Fig. 3) and 0-20 cm and 0-120 cm depths (Fig. 4) show strong correlations (r = 0.86 and 0.80 respectively). In this study, mean SOC and TN storage calculations also showed that SOC was higher in the surface soil (0-20 cm depth) than that in the deeper layers (Tables 1-2). This is consistent with the findings of Zhang *et al.,* (2011). On the other hand, SOC and TN storage was higher in the LL and MLL sites than that in the HL and MHL sites (Tables 1-2) across the alluviums, which indicates that the topographic variability as well as their water recession conditions are related to carbon loss or sequestration. Ritchie *et al.,* (2007) reported that topographic patterns and processes involved in SOC redistribution across agricultural landscapes are the key to understanding the potential for SOC dynamics. In the present study, SOC and TN storage was higher in the surface level (0-20 cm) than the deep layers (60-120 cm) across the study sites.

On the other hand, mean SOC and TN storage was higher in the Brahmaputra alluvium (BA) than the Ganges alluvium (GA). Soil organic carbon storage increases as it progresses from HL towards LL across the land types of BA and GA (Fig. 5); similarly, TN storage increases from HL to towards LL across the land types of BA and GA (Fig. 6). The low SOC in the soils of HL and MHL sites is linked to the removal of crop residues, deterioration of soil aggregation due to intensive tillage (Stoate *et al.,* 2001; Hamza and Anderson, 2005). The highest SOC densities were found in MLL and LL sites in each alluvium where these lands are utilized by irrigated paddy cultivation. Higher SOC densities in flooded paddy soils agrees well with previous studies (Jia-Guo *et al.,* 2010) and is explained by natural fertility of wetlands and other lowlands and by the long-term use of organic fertilizers and flooding, which provide a strong supply of organic carbon (OC) with lower decomposition rates (Wang *et al.,* 2003).

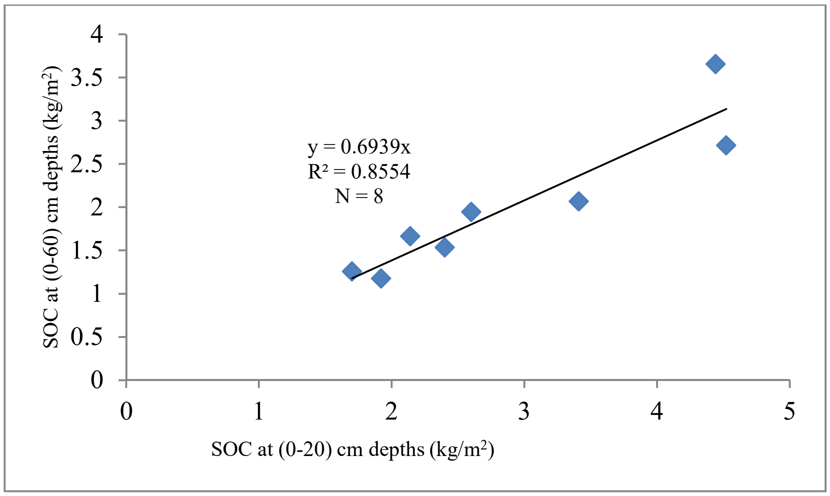

Fig. 1. Relationship of SOC storage between the soil depths 0-20 cm and 0-60 cm in the eight profiles of the two alluviums.

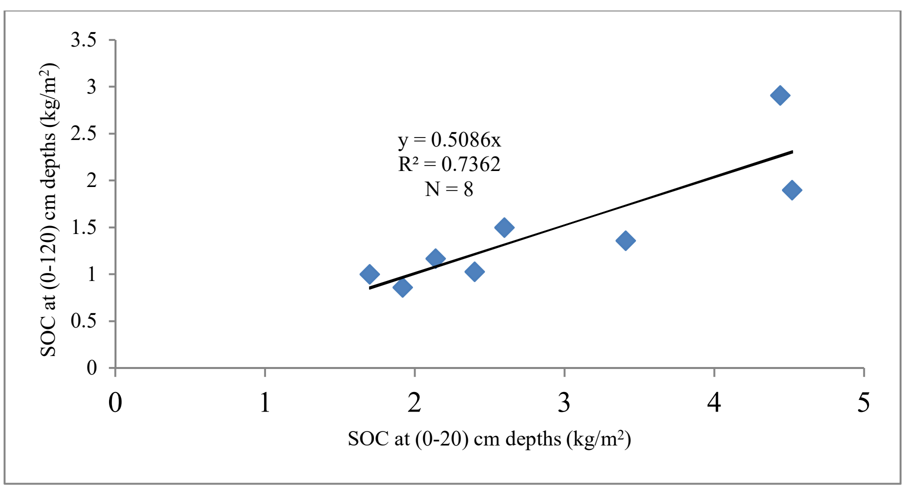

Fig. 2. Relationship of SOC storage between the soil depths 0-20 cm and 0-120 cm in the eight profiles of the two alluviums.

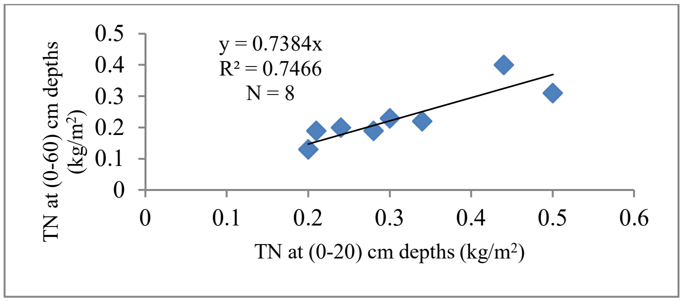

Fig. 3. Relationship of TN storage between the soil depths 0-20 cm and 0-60 cm in the eight profiles of the two alluviums.

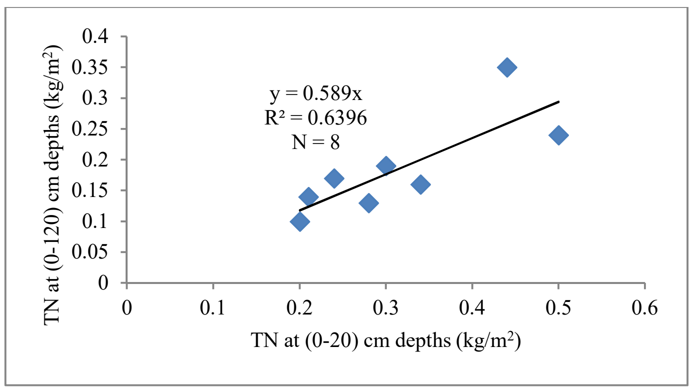

Fig. 4. Relationship of TN storage between the soil depths 0-20 cm and 0-120 cm in the eight profiles of the two alluviums.

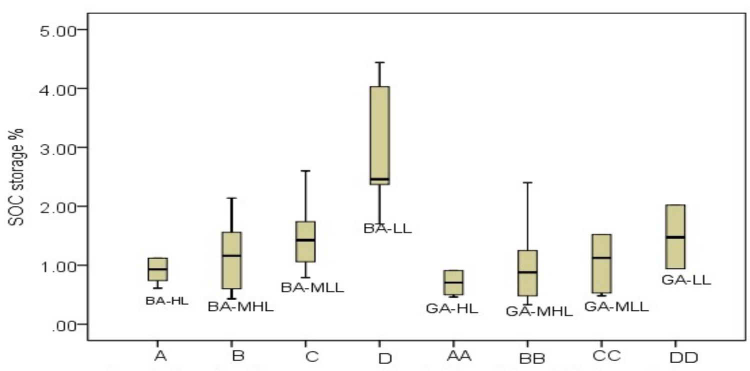

Fig. 5. Boxplots showing SOC storage (%) across the eight profiles of the two alluviums (A, B, C, and D: SOC storage (%) at HL, MHL, MHL and LL respectively across the Brahmaputra alluvium; AA, BB, CC, and DD: SOC storage (%) at HL, MHL, MLL and LL respectively across the Ganges alluvium).

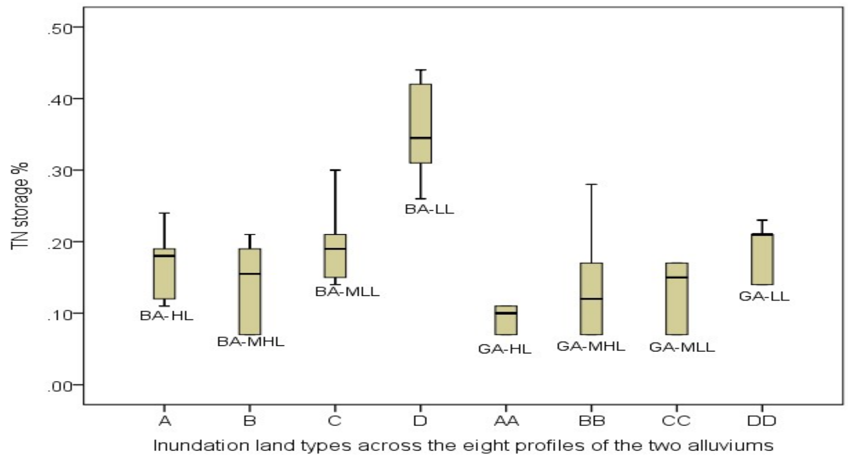

Fig. 6. Boxplots showing total nitrogen (TN) storage (%) across the eight profiles of the two alluviums (A, B, C, and D: TN storage (%) at HL, MHL, MLL and LL respectively across the Brahmaputra alluvium; AA, BB, CC, and DD: SOC storage (%) at HL, MHL, MLL and LL respectively across the Ganges alluvium).

Conclusion

The results showed that land types and soil depths significantly affect SOC and TN distribution, as well as their storages in soils. The SOC and TN contents in the surface layer are higher than those in the deeper layers due to the high residue inputs. Lower land elevation has higher SOC and TN than the higher land elevation across the Brahmaputra and the Ganges alluviums. The variation in SOC and TN distribution and storage is related to land variability and the inundation nature of the land across the study sites.

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