

Project ID:798

**Competitive Research Grant**  
**Sub-Project Completion Report**

**on**

**Heavy Metal in the Industrial Polluted Area: Spatial  
Distribution, Risk Assessment and Bacterial  
Biogeography of Contaminated Soils**

**Project Duration**

**May 2017 to September 2018**

**Department of Soil Science**

**Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU)**

**Gazipur-1706**



**Submitted to**  
**Project Implementation Unit-BARC, NATP 2**  
**Bangladesh Agricultural Research Council**  
**Farmgate, Dhaka-1215**



**September 2018**

**Competitive Research Grant (CRG)**

## **Sub-Project Completion Report**

**on**

### **Heavy Metal in the Industrial Polluted Area: Spatial Distribution, Risk Assessment and Bacterial Biogeography of Contaminated Soils**

**Project Duration**

**May 2017 to September 2018**

**Department of Soil Science**

**Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU)  
Gazipur-1706**

**Submitted to**

**Project Implementation Unit-BARC, NATP 2  
Bangladesh Agricultural Research Council  
Farmgate, Dhaka-1215**



**September 2018**

## Citation

**M. S. Alam and G. K. M. M. Rahman. 2018. Heavy Metal in the Industrial Polluted Area: Spatial Distribution, Risk Assessment and Bacterial Biogeography of Contaminated Soils.** A report of Competitive Research Grant Sub-Project under National Agricultural Technology Program-Phase II Project (NATP-2), Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka, Bangladesh.

Project Implementation Unit  
National Agricultural Technology Program-Phase II Project (NATP-2)  
Bangladesh Agricultural Research Council (BARC)  
New Airport Road, Farmgate, Dhaka - 1215  
Bangladesh.

Edited and Published by:

Project Implementation Unit  
National Agricultural Technology Program-Phase II Project (NATP-2)  
Bangladesh Agricultural Research Council (BARC)  
New Airport Road, Farmgate, Dhaka-- 1215  
Bangladesh.

### ***Acknowledgement***

The execution of CRG sub-project has successfully been completed by Bangabandhu Sheikh Mujibur Rahman Agricultural University using the research grant of USAID Trust Fund and GoB through Ministry of Agriculture. We would like to thank to the World Bank for arranging the grant fund and supervising the CRGs by BARC. It is worthwhile to mention the cooperation and quick responses of PIU-BARC, NATP 2, in respect of field implementation of the sub-project in multiple sites. Preparing the project completion report required to contact a number of persons for collection of information and processing of research data. Without the help of those persons, the preparation of this document could not be made possible. All of them, who made it possible, deserve thanks. Our thanks are due to the Director PIU-BARC, NATP 2 and his team who given their whole hearted support to prepare this document. We hope this publication would be helpful to the agricultural scientists of the country for designing their future research projects in order to generate technology as well as increasing production and productivity for sustainable food and nutrition security in Bangladesh. It would also assist the policy makers of the agricultural sub-sectors for setting their future research directions.

Published in: September 2018

Printed By:

## Acronyms

---

| <b>Acronym</b> | <b>Meaning</b>  |
|----------------|---|
| BBS            | Bangladesh Bureau of Statistics                           |
| BLAST          | Basic Local Alignment Search Tool                         |
| BSMRAU         | Bangabandhu Sheikh Mujibur Rahman Agricultural University |
| CF             | Conversion Factor   |
| DIM            | Daily intakes of metals                                   |
| FAO            | Food and Agriculture Organization                         |
| GIS            | geographic information system                             |
| HRI            | Health Risk Index   |
| IPPS           | Industrial Pollution Projection System                    |
| NCBI           | National Centre of Biotechnology Information              |
| PCR            | Polymerase Chain Reaction                                 |
| PTE            | Potential Toxic Element                                   |
| SEPA           | State Environmental Protection Administration             |
| US EPA         | United States Environmental Protection Agency             |
| WHO            | World Health Organization                                 |

---

## Table of Contents

| Sl. No.   | Subject   | Page No. |
|-----------|---|----------|
|           | <b>Cover Page</b>   | i        |
|           | <b>Citation</b>   | ii       |
|           | <b>Acronyms</b>   | iii      |
|           | <b>Table of Contents</b>  | iv       |
|           | <b>Executive Summary</b>  | v        |
| <b>A.</b> | <b>Sub-project Description</b>                                      | 02       |
| 1.        | Title of the CRG sub-project  | 02       |
| 2.        | Implementing organization   | 02       |
| 3.        | Name and full address with phone, cell and E-mail of PI/Co-PI (s)   | 02       |
| 4.        | Sub-project budget  | 02       |
| 5.        | Duration of the sub-project   | 02       |
| 6.        | Justification of undertaking the sub-project                        | 02       |
| 7.        | Sub-project goal  | 03       |
| 8.        | Sub-project objectives  | 03       |
| 9.        | Implementing locations  | 03       |
| 10.       | Methodology in brief  | 03-08    |
| 11.       | Results and discussion  | 09-31    |
|           | References  | 31       |
| 12.       | Research highlight/findings   | 32       |
| <b>B.</b> | <b>Implementation Position</b>                                      | 32-33    |
| <b>C.</b> | <b>Financial and physical progress</b>                              | 33       |
| <b>D.</b> | <b>Achievement of Sub-project by objectives</b>                     | 33-34    |
| <b>E.</b> | <b>Materials Development/Publication made under the Sub-project</b> | 34       |
| <b>F.</b> | <b>Technology/Knowledge generation/Policy Support</b>               | 34-35    |
| <b>G.</b> | <b>Information regarding Desk and Field Monitoring</b>              | 35       |
| <b>H.</b> | <b>Lesson Learned/Challenges</b>                                    | 35       |
| <b>I.</b> | <b>Challenges</b>   | 35       |

## Executive Summary

The increasing trend of potential toxic element (PTE) concentrations in the environment is a great concern worldwide. The PTEs in the environment may arise from natural or anthropogenic routes and their concentrations are elevated due to industrial solid-waste disposal and industrial wastewater irrigation on land. The rapid growth and haphazard industrialization in Gazipur and Mymensingh areas have led to an increase in waste water generation and environmental pollution. Bacteria constitute a major portion of the biodiversity in soils, and play an essential role in maintaining soil processes and fertility. An understanding of the factors that influence the biodiversity of soil bacterial communities and heavy metal toxicity in contaminated areas is urgently needed. However, qualitative information of heavy metal and bacterial biodiversity in contaminated soils in the vicinity of industrial areas of Bangladesh are not sufficient to draw any conclusive recommendation. Therefore, the present study was done to investigate the accumulation, spatial distribution, and risk assessment of heavy metals and bacterial biodiversity in the contaminated areas. To conduct the study, four upazilas of Gazipur (Sadar and Sreepur) and Mymensingh (Bhaluka and Trisal) districts were selected. Projection of digital map (shape file) of the above mentioned upazilas and using Arc. GIS software, nearly equally spaced grid map of each upazila was generated. Based on the map, probable sampling points were identified. Collected soil samples were analyzed for different soil properties with special consideration of heavy metals concentration. Based on analyzed data spatial distribution map was prepared for different parameters including soil pH, organic carbon, Pb, Cd, Ni, Cu, Zn, Fe and bacterial population. The spatial distribution maps provide clear understanding of the soil parameters of the upazila. Study results clearly demonstrate that soil pH, organic carbon content and heavy metal content increased in the contaminated soils as compared to the uncontaminated soils. Pb and Ni contents in the contaminated soil are very close to the WHO/FAO standard. The soil Cd content has exceeded the SEPA (State Environmental Protection Administration, China) standard. Translocation factor (AF), Daily intakes of metals (DIM) ( $\text{mg kg}^{-1} \text{ person}^{-1} \text{ d}^{-1}$ ) and the Health Risk Index (HRI) for heavy metals in food crops were calculated. Study results reveal that heavy metal translocation was higher in vegetables as compared to rice, but DIM was higher in rice as compared to vegetables due to higher consumption of rice as compared to vegetables. Considering the Health Risk Index (HRI) for heavy metals, still the consumption of rice and vegetables are safe as the HRI value is less than 1. Nevertheless, HRI value in the contaminated soil is high as compared to the uncontaminated soil. Bacterial population decreased due to the addition of untreated industrial waste water to the agricultural soil. The bacterial community structure in contaminated soil also differed from that of uncontaminated soil. Stress tolerance species of bacteria were isolated from the contaminated soil which might be utilized effectively for the bioremediation of the contaminated soils.

## CRG Sub-Project Completion Report (PCR)

### A. Sub-project Description

1. Title of the CRG sub-project: Heavy Metal in the Industrial Polluted Area: Spatial Distribution, Risk Assessment and Bacterial Biogeography of Contaminated Soils
2. Implementing organization: Department of Soil Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University
3. Name and full address with phone, cell and E-mail of PI/Co-PI (s):

Principal Investigator: Dr. M. Saiful Alam  
Professor  
Department of Soil Science, BSMRAU  
Gazipur-1706, Phone: 02-9205310-14 Ext. 2189  
Mobile: 01724720655  
E-mail: saiful@bsmrau.edu.bd

Co-Principal Investigator: Dr. GKM Mustafizur Rahman  
Professor  
Department of Soil Science, BSMRAU  
Gazipur-1706, Phone: 02-9205310-14  
Ext. 2283, Mobile: 01718186642  
E-mail: mustafiz@bsmrau.edu.bd

4. Sub-project budget (Tk):
  - 4.1 Total: 3436655.00
  - 4.2 Revised (if any): Not applicable
5. Duration of the sub-project:
  - 5.1 Start date (based on LoA signed): 11 May 2017
  - 5.2 End date: 30 September 2018

6. Justification of undertaking the sub-project:

The increasing trend of potential toxic element (PTE) concentrations in the environment has caused great concern worldwide. The PTEs in the environment may arise from natural or anthropogenic routes and their concentrations are elevated due to industrial solid-waste disposal and industrial wastewater irrigation on land (Zheng *et al.*, 2007). The rapid growth and unplanned industrialization in Gazipur and Mymensingh areas have led to an increase in waste water generation and environmental pollution. The industrial pollution problems are faced by the stakeholders of the industrial zone in Bangladesh because of haphazard industrialization without environmental protection. Pollution abatement technologies are largely absent and the consequence is a gross pollution of natural resources and environmental media. Since effective

environmental protection cannot take place in a data vacuum, Industrial Pollution Projection System (IPPS), which is a rapid environmental management tool for pollution load and health risk assessment, has been employed in this study to estimate industrial pollution loads and conventional effluent analysis. Bacteria constitute a major portion of the biodiversity in soils (Fulthorpe *et al.*, 2008), and play an essential role in maintaining soil processes and fertility (Bardgett *et al.*, 2008). An understanding of the factors that influence the biodiversity of soil bacterial communities and heavy metal toxicity in contaminated areas are urgently needed. However, qualitative information of heavy metal and bacterial biodiversity in contaminated soils in the vicinity of industrial areas of Bangladesh are not sufficient to draw any conclusive recommendation. Therefore, the present study was proposed to investigate the accumulation, spatial distribution, risk assessment of heavy metals and bacterial biodiversity in the contaminated areas

7. Sub-project goal: Pollution load assessment in the soil-plant system of the industrial areas for evaluating health risk index (HRI) and generate the bacterial biogeography.
8. Sub-project objective (s):
  - a. Investigate accumulation and spatial distribution of heavy metals in industrial contaminated soils using GIS tools and its consequences on crops grown in contaminated areas.
  - b. Assess the risk of heavy metal pollution from spatial and analytical data.
  - c. Make a broad-scale map of patterns of bacterial biodiversity and determine the health risk index (HRI) of different heavy metals.
9. Implementing location (s): BSMRAU, Sadar and Sreepur upazila under Gazipur district, and Bhaluka and Trishal upazilas under Mymensingh district.
10. Methodology in brief:

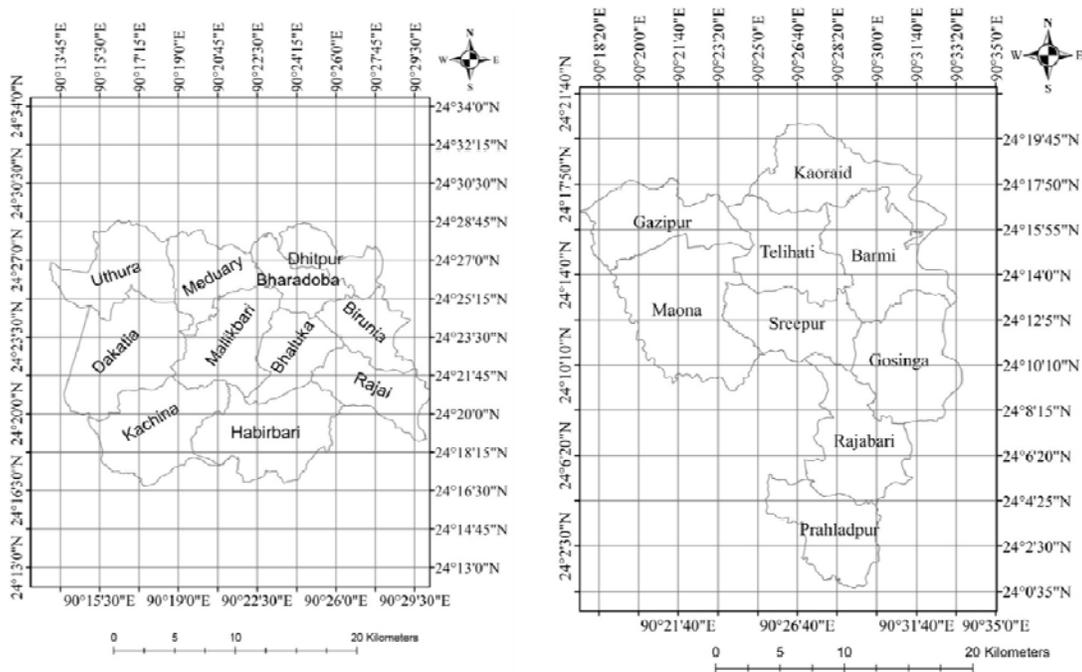
**Activity I:** Spatial Distribution and Contamination of Heavy Metal in Gazipur and Mymensingh Industrial Zone

#### **Sampling strategy**

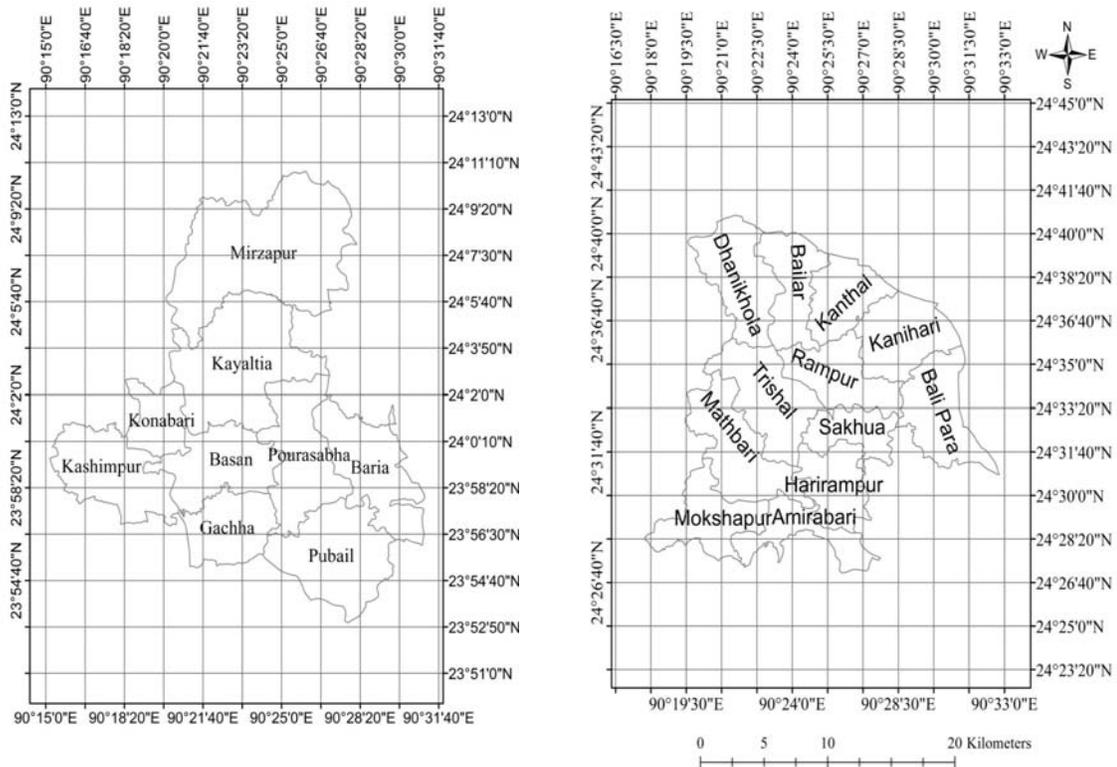
- The industrial contaminated upazilas of Gazipur and Mymensingh districts were selected. Contaminated and non-contaminated soil samples (0-30 cm depth) were collected by 3 x 3 km grid from the Sreepur and Bhaluka upazilas. Each sample is the composite of five sub- samples of 5 x 5 = 25 m<sup>2</sup> area with GPS value.

- Crop samples were also collected from the corresponding geo-referenced soil sampling points accordingly.

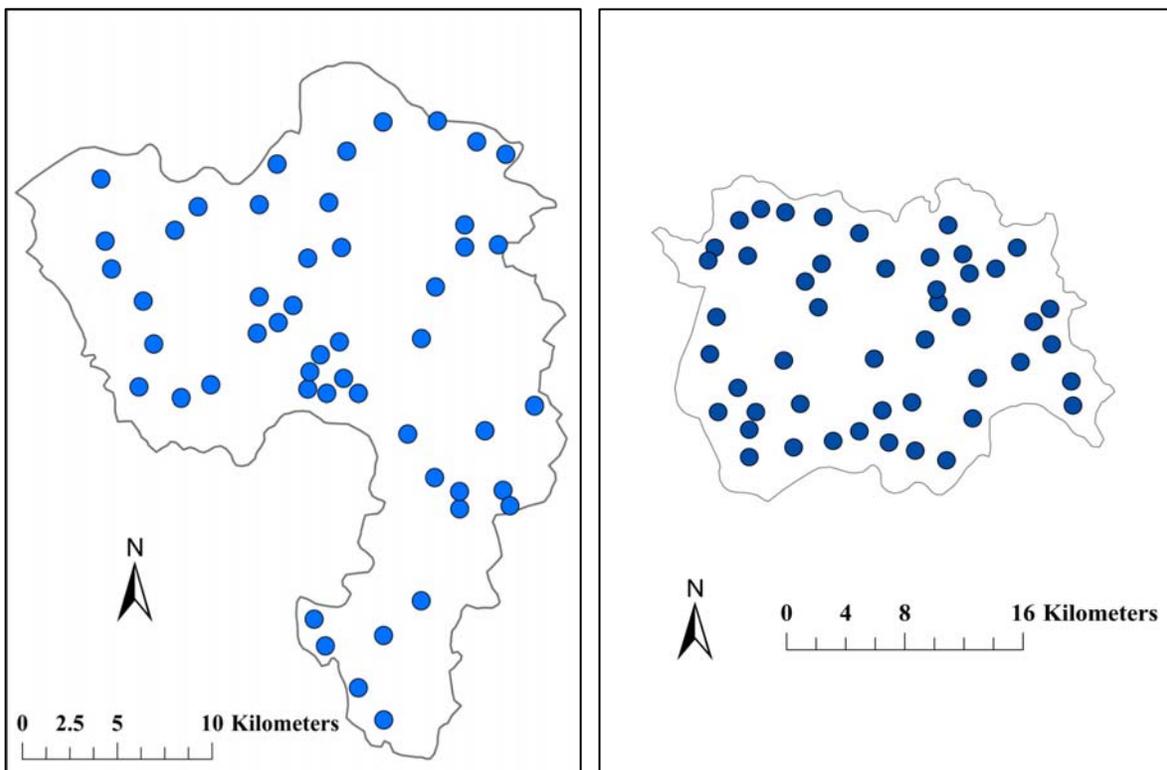
Projection of digital map and Arc GIS software, probable sampling points were identified; four maps for 4 upazilas have been generated and shown in Figure 1 and 2. GIS records were kept from all of the sampling points. For example data collection based on soil, plant and waste water samples of Sreepur upazila are presented in Table 1. Based on the GIS values of the actual soil sampling points, maps of actual soil sampling points were also generated (Figure 3).



**Fig 1. Soil sampling points of Bhaluka (left side) and Sreepur (right side) upazila. Points intercept the longitude and latitude line inside the map is the probable sampling points**



**Fig 2. Probable Soil sampling points of Gazipur Sadar (left side) and Trishal (right side) upazila. Points intercept the longitude and latitude line inside the map is the probable sampling points**



**Fig 3. Actual soil sampling points of Sreepur (left side) and Bhaluka upazila (right side)**

**Table 1. Soil sampling points of Sreepur, Gazipur**

| <b>SL. No.</b> | <b>Sample ID</b> | <b>Location</b>                           | <b>GPS Reading</b>         |
|----------------|------------------|---|----------------------------|
| 01             | SR-1             | Vill: Joynarayonpur<br>Union: Rajabari    | N: 24.13142<br>E: 90.50201 |
| 02             | SR-2             | Vill: Doaibari<br>Union: Rajabari         | N: 24.16630<br>E: 90.47740 |
| 03             | SR-3             | Vill: Doaibari<br>Union: Rajabari         | N: 24.14601<br>E: 90.49013 |
| 04             | SR-4             | Vill: Raja rampur<br>Union: Rajabari      | N: 24.08854<br>E: 90.48376 |
| 05             | SG-1             | Vill: Dossonarayonpur<br>Union: Goshingha | N: 24.14010<br>E: 90.52256 |
| 06             | SG-2             | Vill: berabari<br>Union: Goshingha        | N: 24.13287<br>E: 90.52583 |
| 07             | SG-3             | Vill: Goshingha<br>Union: Goshingha       | N: 24.17937<br>E: 90.53749 |
| 08             | SG-4             | Vill: Kaichabari<br>Union: Goshingha      | N: 24.16785<br>E: 90.51397 |
| 09             | SG-5             | Vill: Chaobon<br>Union: Goshingha         | N: 24.13952<br>E: 90.50195 |
| 10             | SK-1             | Vill: Jogir Chit<br>Union: Kaoride        | N: 24.29192<br>E: 90.41543 |
| 11             | SK-2             | Vill: Nayapara<br>Union: Kaoride          | N: 24.29787<br>E: 90.44852 |
| 12             | SK-3             | Vill: Damloi<br>Union: Kaoride            | N: 24.31153<br>E: 90.46571 |
| 13             | SK-4             | Vill: purbosona<br>Union: Kaoride         | N: 24.31201<br>E: 90.49145 |
| 14             | SK-5             | Vill: Kaoride<br>Union: Kaoride           | N: 24.30232<br>E: 90.51009 |
| 15             | SK-6             | Vill: Beldiya<br>Union: Kaoride           | N: 24.29648<br>E: 90.52388 |

### **Processing of Soil Samples**

- The collected soil samples were processed like air drying, removal of unwanted materials, grinding, sieving and storing for further chemical analysis.
- A portion of all soil samples was preserved in the refrigerator for bacterial diversity study.

### **Soil and plant sample analysis:**

The collected soil samples were analyzed for pH, OC, EC, T N, TC, P and heavy metals (Cu, Pb, Cd, Ni, Zn) etc.

The plant samples were also analyzed for macronutrients (N, P, K, Ca, Mg), micronutrients (Fe, Zn, Mn, Cu), and toxic elements (Ni, Cd, Pb).

**Spatial distribution GIS map:** Heavy metal distribution maps of contaminated and uncontaminated soils for different parameters were produced for different upazilas.

### **Activity II: The Bacterial Biogeography of Industrial Contaminated Soils**

- Soil samples were collected from the corresponding geo-referenced soil sampling points of the industrial contaminated areas. Soil samples were also collecting from the nearest uncontaminated areas to compare the bacterial communities between contaminated and uncontaminated soils.
- Bacterial population was enumerated using serial dilution method and their diversity was determined following standard techniques.
- Isolation of bacteria was done from different samples using the standard protocols.
- Biochemical and molecular characterization of bacterial isolates were performed such as Colony characteristics, Gram staining, BTB test, Congo red test, catalase test, IAA production test, phosphate solubilization test and limited sequencing of selected isolates to assess the biogeography of soil bacteria.
- Identification of bacteria through DNA sequencing: The following steps were followed
  - Isolation of DNA
  - Quantification of Isolated DNA by NanoDrop2000
  - PCR
  - Agarose Gel Electrophoresis
  - PCR Product Purification
  - Sanger Sequencing by Apical Scientific-Malaysia
  - BLAST (Basic Local Alignment Search Tool) analysis
  - Identify the bacteria
  - Prepare phylogenetic tree

### Activity III: Heavy Metal Risk Assessment of food crops

#### **Translocation factor calculation:**

Heavy metals have the capability to translocate from the soil to the edible parts of the food crop and were determined by the accumulation factor (AF) (Balkhair and Ashraf, 2016).

$$AF = \frac{\text{Heavy metal concentration in the food crops edible parts}}{\text{Heavy metal concentration in the soil}}$$

#### **Daily intake of metals (DIM):**

The daily intake of metals was calculated using the following equation (Balkhair and Ashraf, 2016):

$$IM = \frac{C_{\text{metal}} \times C_{\text{factor}} \times D_{\text{intake}}}{B_{\text{weight}}}$$

Where,  $C_{\text{metal}}$ ,  $C_{\text{factor}}$ ,  $D_{\text{intake}}$  and  $B_{\text{weight}}$  represent the heavy metal concentrations in the food crops, the conversion factor, the daily intake of the food crops and the average body weight, respectively. The conversion factor (CF) of 0.085 was used for the conversion of fresh vegetables to dry weights and 0.86 for rice. The average daily intake of rice was 0.367 kg person<sup>-1</sup> d<sup>-1</sup> (BBS, 2018), and the average body weight for the adult population of 55.5 kg was used for the calculation. The DIM value was used for the calculation of HRI as well.

#### **Health risk index (HRI):**

The HRI refers to the ratio of the daily intake of metals in the food crops to the oral reference dose (RfD) and was calculated using the following equation (Balkhair and Ashraf, 2016):

$$HRI = \frac{DIM}{RfD}$$

The oral reference dose values used in this study for Cd, Cu, Ni and Pb were 0.001, 0.003, 0.02 and 0.0035 (mg /kg body weight /day) respectively (USEPA, 2005). An HRI > 1 for any metal in food crops indicates that the consumer faces a health risk. If the health risk index value was less than 1, then the exposed population was considered to be safe.

## 11. Results and discussion:

### Spatial Distribution and Contamination of Heavy Metal in Gazipur and Mymensingh Industrial Zone

#### Soil pH and Organic carbon:

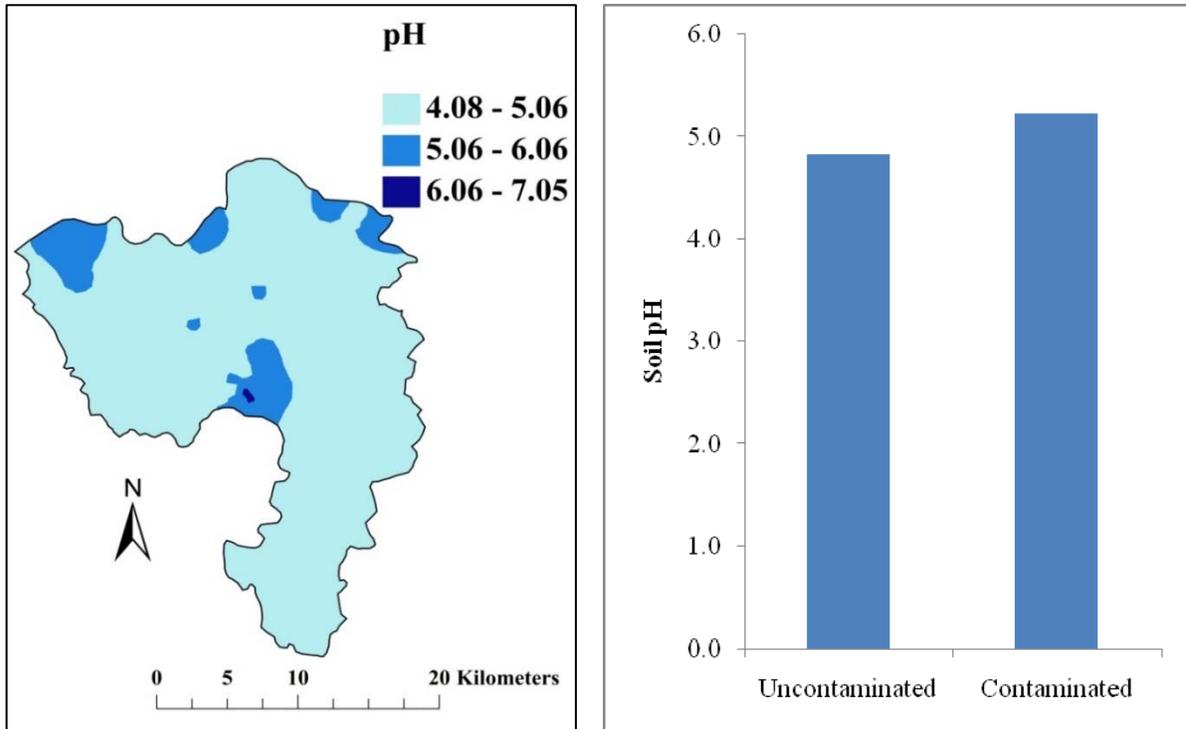
Soil pH value was influenced by the application of untreated industrial waste water to the agricultural soil. Soil pH value increased in the contaminated soil as compared to the uncontaminated control soil for all the study sites (Fig. 4-5, Table 2-3). PH value ranges from 4.15 to 5.66 with a mean value of 4.83 for uncontaminated soil in Sreepur upazila, whereas for contaminated soil the pH value ranges from 4.08 to 7.05 having the mean value 5.23. Similar increasing trend of soil pH was estimated in the industrial effluent contaminated soil of Bhaluka upazila as compared to the uncontaminated soil. The increased soil pH in the industrial effluent contaminated soils might be due to the effluents contain any strong salt which on dissociation release the basic cations from the industrial waste water.

Soil organic carbon content also increased in the contaminated soils in comparison with the uncontaminated control soils (Fig. 6-7, Table 2-3). In Sreepur upazila, soil organic carbon content varies from 0.42 to 1.38% with the mean value of 0.84% in uncontaminated soil, while it ranges from 1.22 to 1.61% having average value of 1.39% in the industrial waste water contaminated soil. In Bhaluka upazila, mean value of soil organic carbon in contaminated and control soil was 1.07 and 0.64% respectively. An increase in the organic carbon was observed with effluent contamination in the soil which might be ascribed due to the high load of organic matter in industrial effluents. The variation of soil organic carbon content in Sreepur and Bhaluka upazila were clearly shown in the spatial distribution maps of figure 6 and 7.

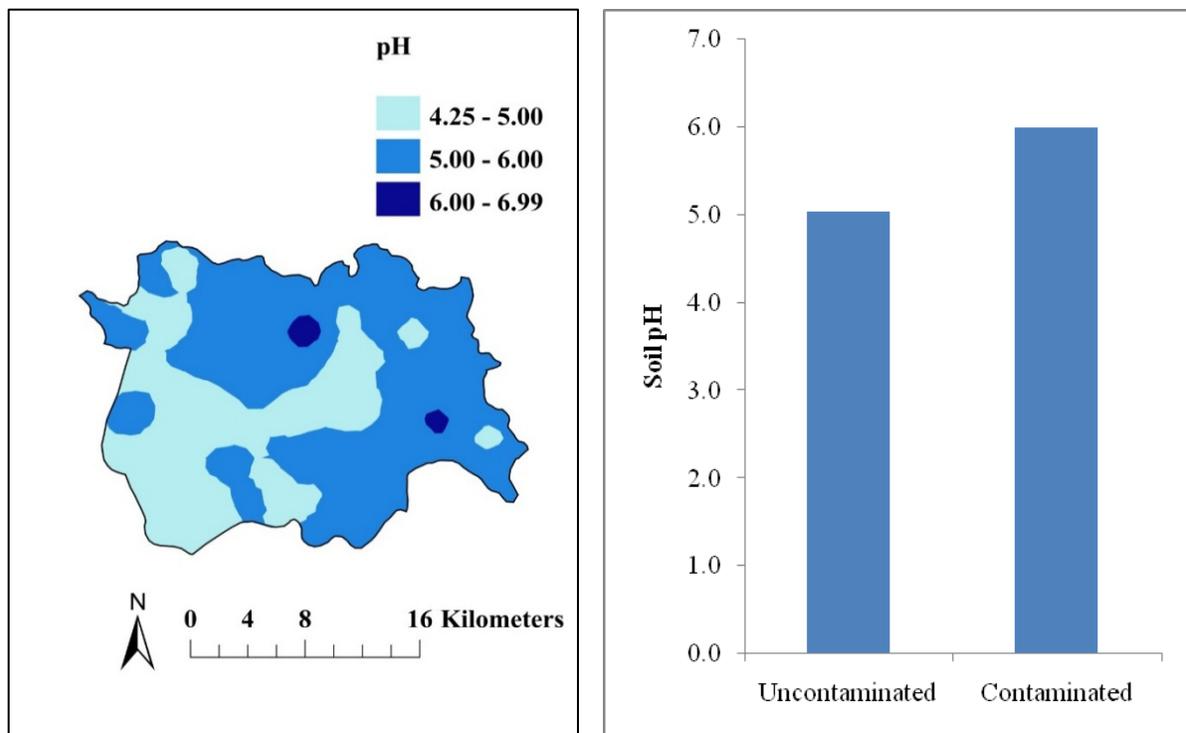
#### Heavy metals:

With the growth of industry, there has been a considerable increase in the discharge of industrial waste to the environment which has led to the accumulation of heavy metals in soil. The indiscriminate release of heavy metals into the soil and waters is a major health concern worldwide, as they cannot be broken down to non-toxic forms and therefore have long-lasting effects on the ecosystem. We have collected soil samples from the contaminated and nearby uncontaminated soils of the industrial areas of Gazipur and Mymensingh districts to find out the contamination levels of heavy metals as well as to prepare the spatial distribution maps.

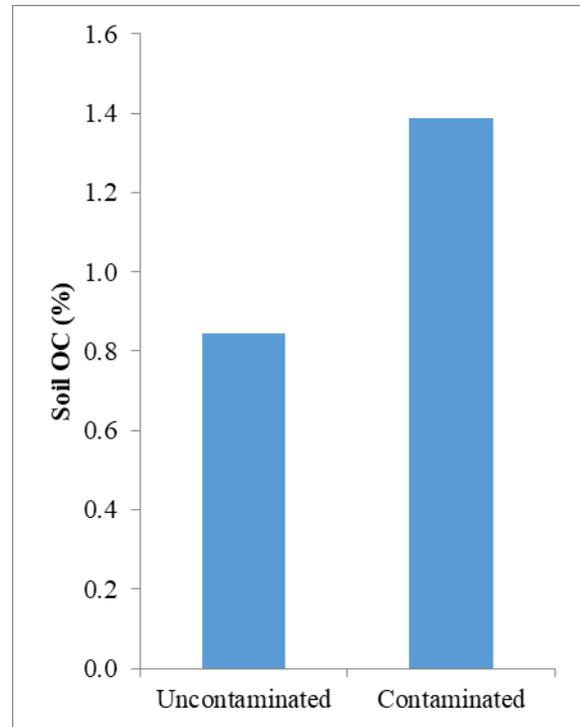
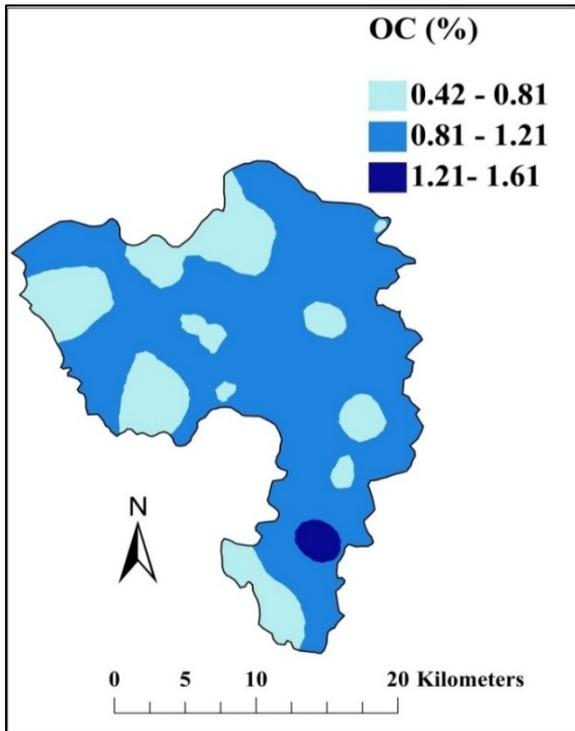
**Lead (Pb):** Pb contamination in soils has been seriously emphasized in recent years since this metal is very toxic for humans and animals which enters human or animal metabolism via the food chain. Lead (Pb) content in Sreepur upazila ranges from 8.0 to 29.7 mg/kg in uncontaminated soil having the mean value of 17.63 mg/kg but it ranges from 32.4 to 38 mg/kg with the average value of 35.7 mg/kg in the contaminated soil (Figure 8, Table 2). Pb content was almost double in the contaminated soils as compared to the uncontaminated soils. Similar trend was observed in the Bhaluka upazila (Fig. 9, Table 3). It is notable that Pb contents in the contaminated soils were within the maximum permissible limit but very close to the WHO/FAO standard of 50 mg/kg (Fig. 8 -9).



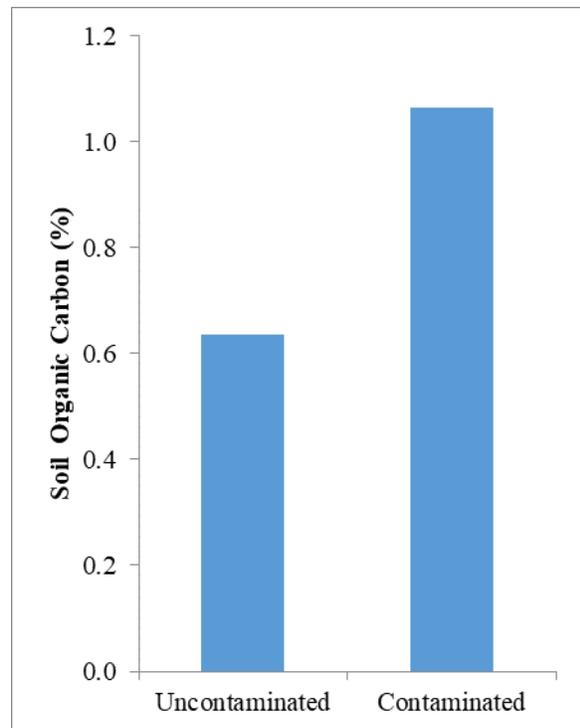
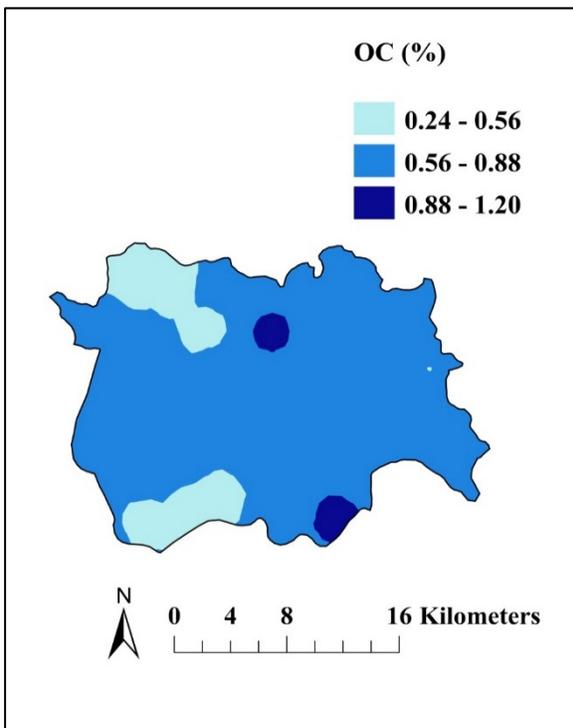
**Fig. 4. Soil pH of Sreepur upazila**



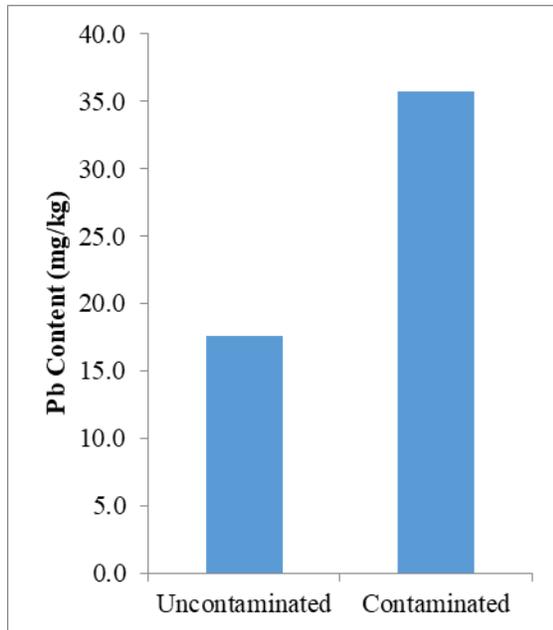
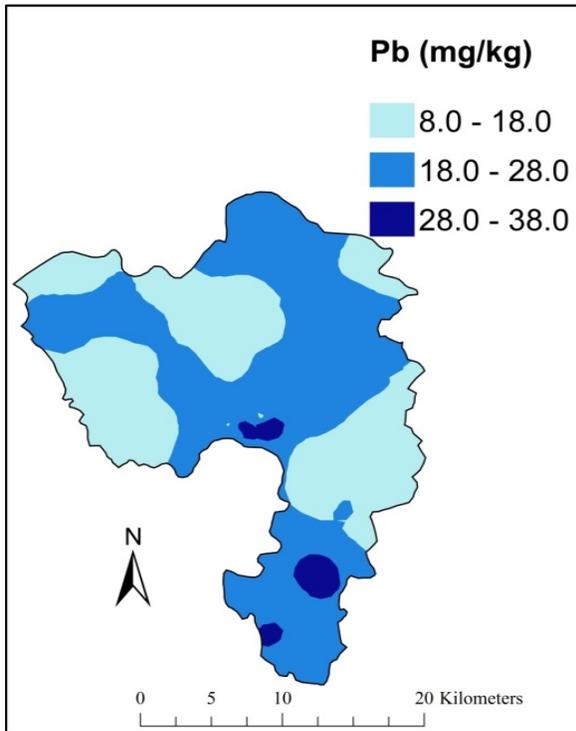
**Fig. 5. Soil pH of Bhaluka upazila**



**Fig. 6. Soil OC of Sreepur upazila**



**Fig. 7. Soil OC of Bhaluka upazila**

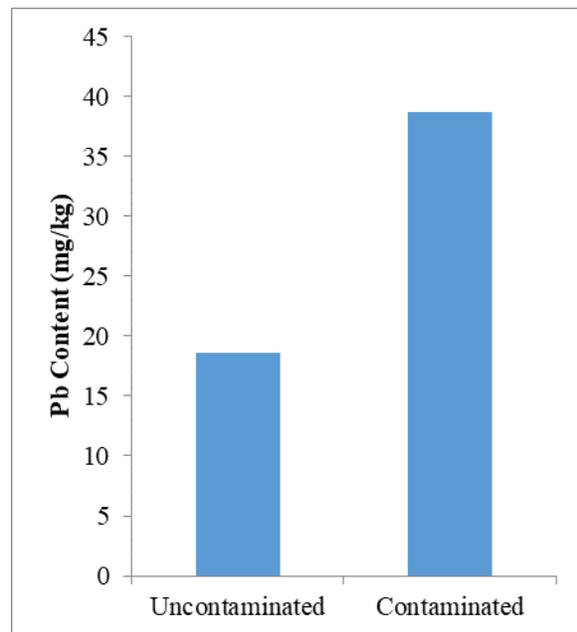
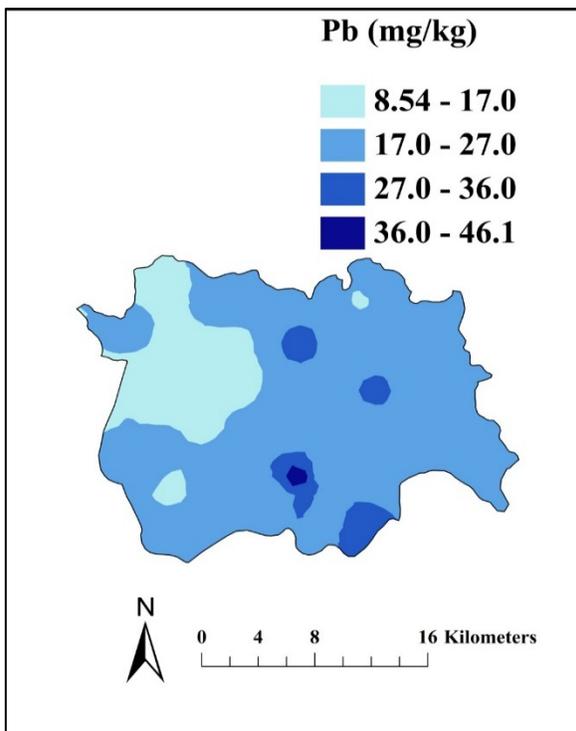


**Maximum permissible limit of Pb in soil**

|                |             |
|----------------|-------------|
| WHO/FAO (2001) | SEPA (1995) |
| 50 mg/kg       | 350 mg/kg   |

SEPA- State Environmental Protection Administration, China

**Fig. 8. Status of Pb in Sreepur upazila soil**



**Maximum permissible limit of Pb in soil**

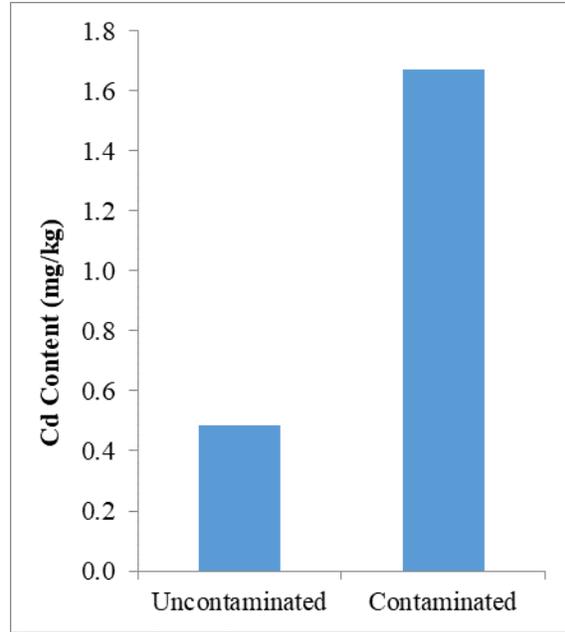
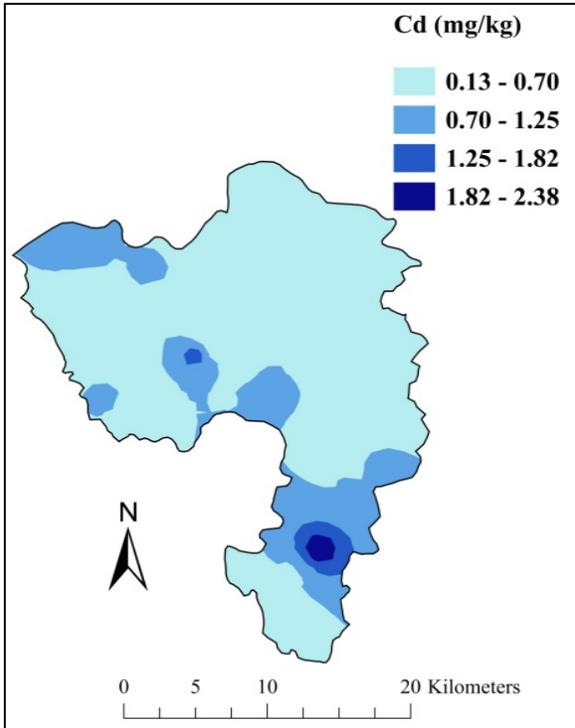
|                |             |
|----------------|-------------|
| WHO/FAO (2001) | SEPA (1995) |
| 50 mg/kg       | 350 mg/kg   |

**Fig. 9. Status of Pb in Bhaluka upazila soil**

**Cadmium (Cd):** There is a growing environmental concern about Cd being one of the most eco-toxic metals, exhibiting highly adverse effects on soil health, biological activity, plant metabolism, and the health of humans and animals. Cadmium content in all of the study sites were increased in the contaminated soils than those of the uncontaminated control soils. Cadmium content in the contaminated soil was more than double than that of the control soil (Table 2-3). Cadmium content in the control soil of Sreepur upazila was 0.13 to 1.07 mg/kg with the mean value of 0.49mg/kg, whereas, in Bhaluka upazila the value ranges from 0.23 to 1.19 mg/kg having the mean value 0.60 mg/kg. In the contaminated soils of Sreepur upazila, Cd content ranges from 1.16 to 2.38 mg/kg with average Cd content 1.67, while the value ranges from 0.98 to 2.1 mg/kg having the mean value of 1.62 mg/kg in the contaminated soils of Bhaluka upazila. It is alarming that Cd content in the contaminated soils exceeds the SEPA (State Environmental Protection Administration, China) standard (Fig. 10 -11), though the values were within the WHO/FAO limit. Therefore, it is urgent to take necessary steps against the contamination of agricultural soil through the waste water generated from the industries.

**Nickel (Ni):** Nickel is other important heavy metals that has deleterious consequences on plants, animal and human health. The total concentration of nickel in the uncontaminated soils of Sreepur upazila ranges from 1.02 to 22.4 mg/kg with the mean value of 13.27 mg/kg, while the value ranges from 9.8 to 22.3 mg/kg with the average value of 16.16 mg/kg in Bhaluka upazila (Table 2 -3). Study results further illustrate that nickel content increased dramatically in the contaminated soils of all the study areas. In Sreepur upazila, nickel content varied from 29.4 to 40.2 mg/kg having the mean value of 32.25 mg/kg due to the untreated industrial waste water. In Bhaluka upazila, almost similar trend was estimated where the average nickel content in the contaminated soil was 30 mg/kg. Though the Ni content in the contaminated soils is below the permissible limit (Fig. 12-13) but if the situation continues for several years, the Ni content may exceed the maximum permissible limit.

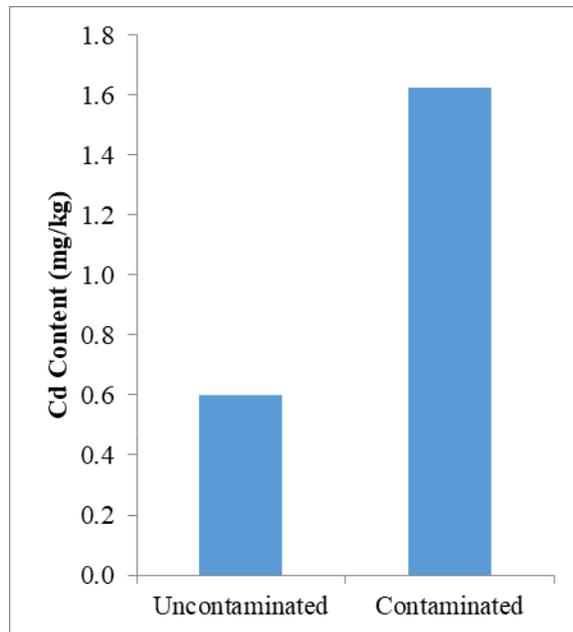
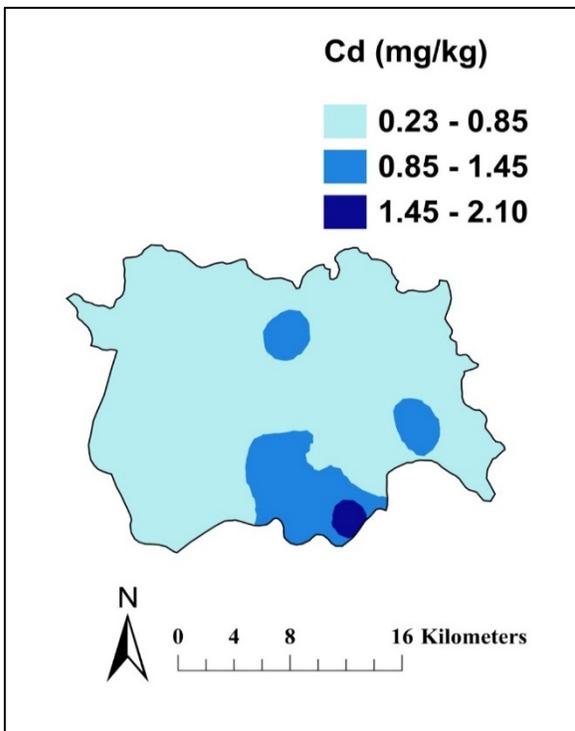
**Copper (Cu):** There was a sharp variation in average Cu concentration in the industrial waste water contaminated soils from that of the uncontaminated soils (Fig. 14-15, Table 2-3). Cu content of soils in the Sreepur upazila was between 1.0 and 5.2 mg/kg with an average of 2.88 mg/kg for uncontaminated soil and ranging between 6.0 to 9.4 mg/kg with an average of 7.93 mg/kg for contaminated soil. In Bhaluka upazila, Cu content varies from 1.2 to 6.2 mg/kg with an average of 3.13 mg/kg for uncontaminated soil and ranging between 5.9 to 8.9 mg/kg with the mean value of 7.42 mg/kg for contaminated soil. Though Cu content in the contaminated soil is high as compared to the uncontaminated soil but the values are still far behind the maximum allowable limits suggested by SEPA and WHO/FAO (Fig. 14-15).



Maximum permissible limit of Cd in soil

| WHO/FAO (2001) | SEPA (1995) |
|----------------|-------------|
| 3 mg/kg        | 0.6 mg/kg   |

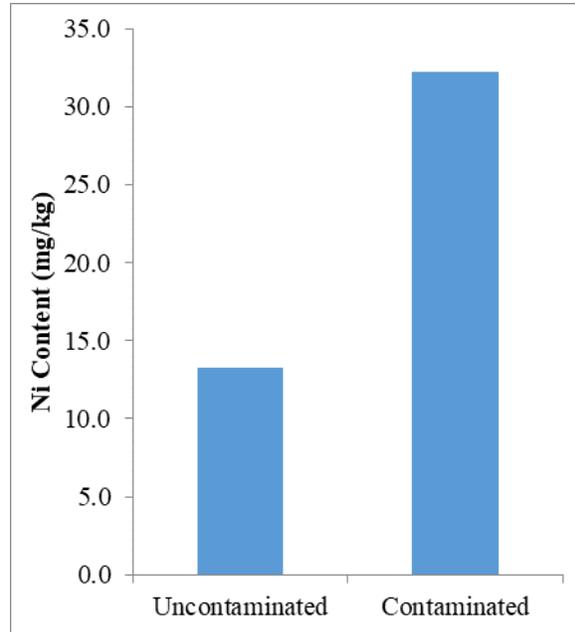
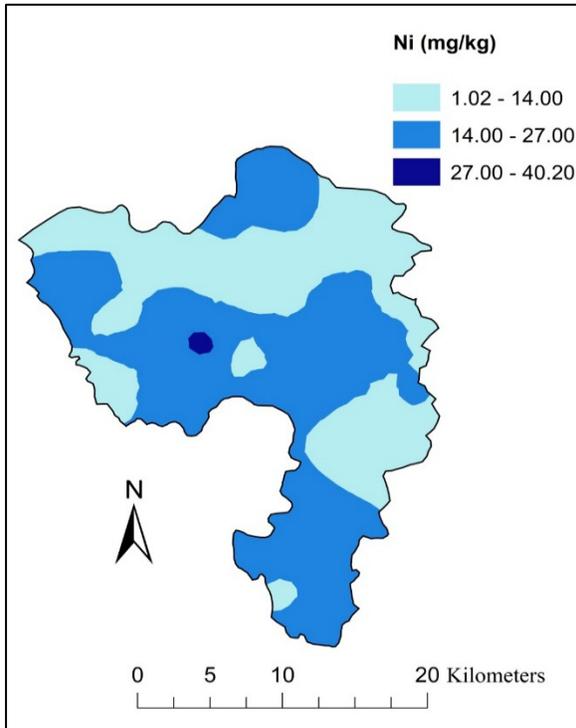
Fig. 10. Status of Cd in Sreepur upazila soil



Maximum permissible limit of Cd in soil

| WHO/FAO (2001) | SEPA (1995) |
|----------------|-------------|
| 3 mg/kg        | 0.6 mg/kg   |

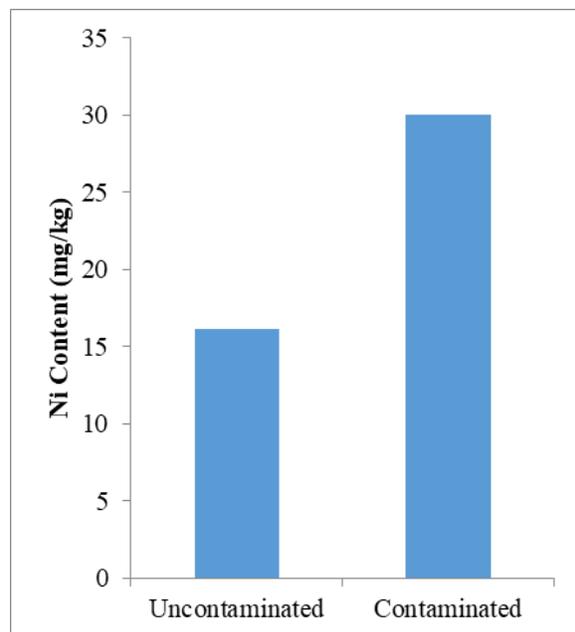
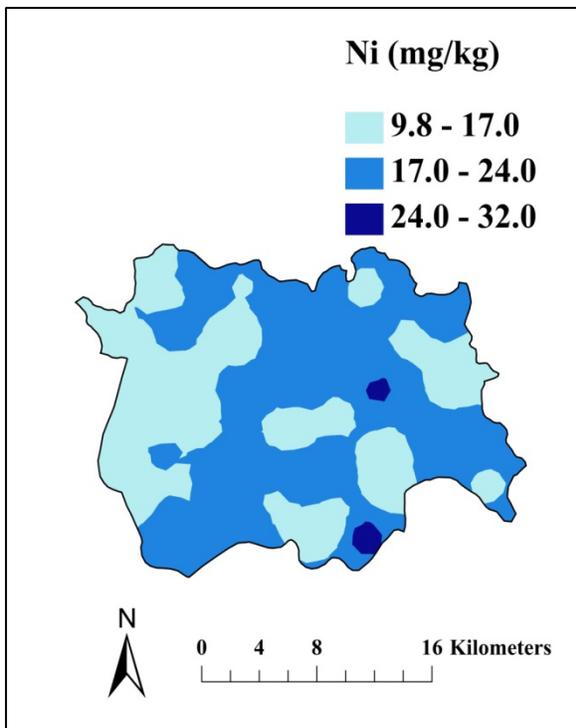
Fig. 11. Status of Cd in Bhaluka upazila soil



**Maximum permissible limit of Ni in soil**

| WHO/FAO (2001) | SEPA (1995) |
|----------------|-------------|
| 50 mg/kg       | 60 mg/kg    |

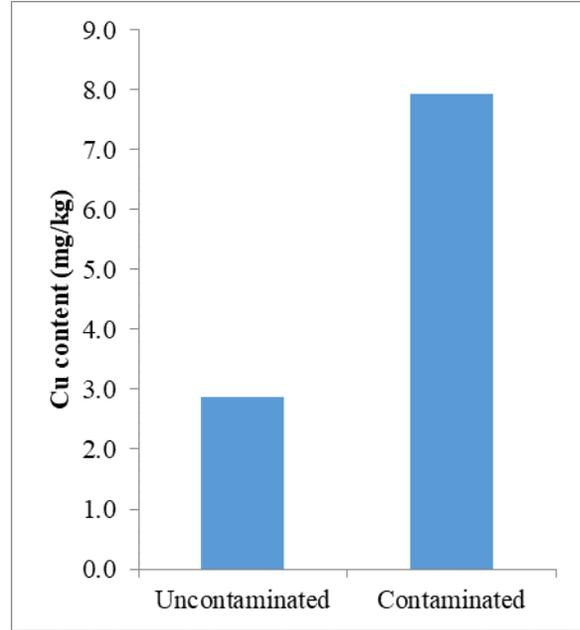
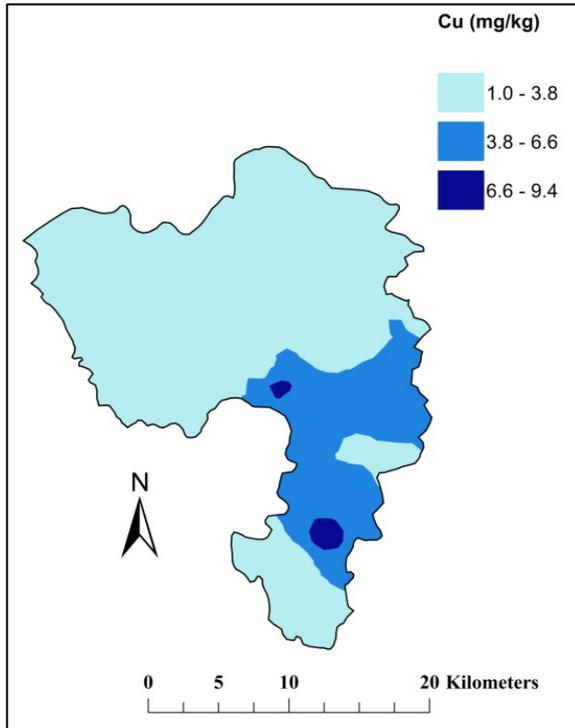
**Fig. 12. Status of Ni in Sreepur upazila soil**



**Maximum permissible limit of Ni in soil**

| WHO/FAO (2001) | SEPA (1995) |
|----------------|-------------|
| 50 mg/kg       | 60 mg/kg    |

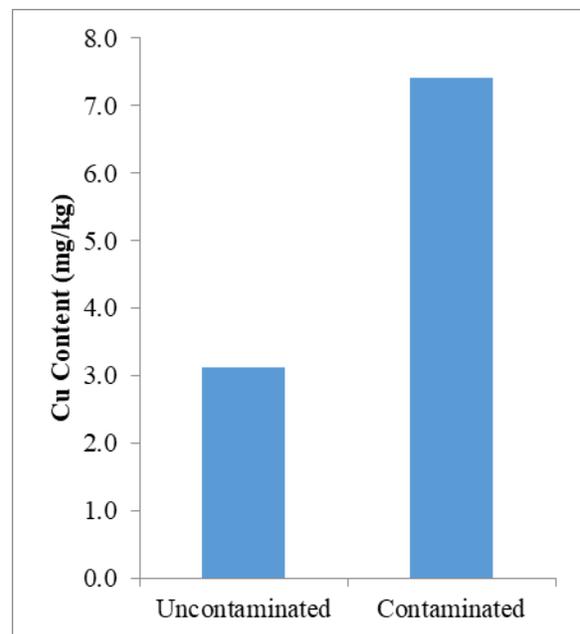
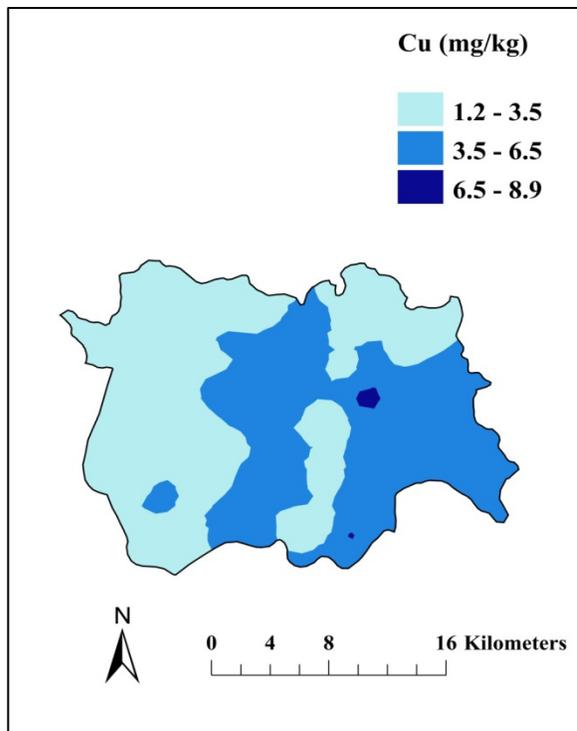
**Fig. 13. Status of Ni in Bhaluka upazila soil**



**Maximum permissible limit of Cu in soil**

| WHO/FAO (2001) | SEPA (1995) |
|----------------|-------------|
| 100 mg/kg      | 100 mg/kg   |

**Fig. 14. Status of Cu in Sreepur upazila soil**



**Maximum permissible limit of Cu in soil**

| WHO/FAO (2001) | SEPA (1995) |
|----------------|-------------|
| 100 mg/kg      | 100 mg/kg   |

**Fig. 15. Status of Cu in Bhaluka upazila soil**

**Zinc (Zn):** Zinc is an essential microelement which plays a very essential catalytic role in enzyme reactions but high concentration of Zn can, however, pose health threats to humans. Zn content in the uncontaminated control soil of Sreepur upazila ranged from 1.08 to 4.5 mg/kg having a mean value of 2.17 mg/kg, but Zn content in the contaminated soil was between 6.8 and 9.18 mg/kg with an average of 7.6 mg/kg (Table 2). In case of Bhaluka upazila, Zn content varied from 0.42 to 4.6 mg/kg with a mean value of 2.03 mg/kg for uncontaminated soil, whereas it ranged between 3.9 to 8.7 mg/kg illustrating the mean value of 6.01 mg/kg for contaminated soil (Table 3). Zinc content in both contaminated and uncontaminated soil was very low as compared to the maximum allowable limits of 300 mg/kg Zn in soil as prescribed by SEPA and WHO/FAO (Fig. 16-17).

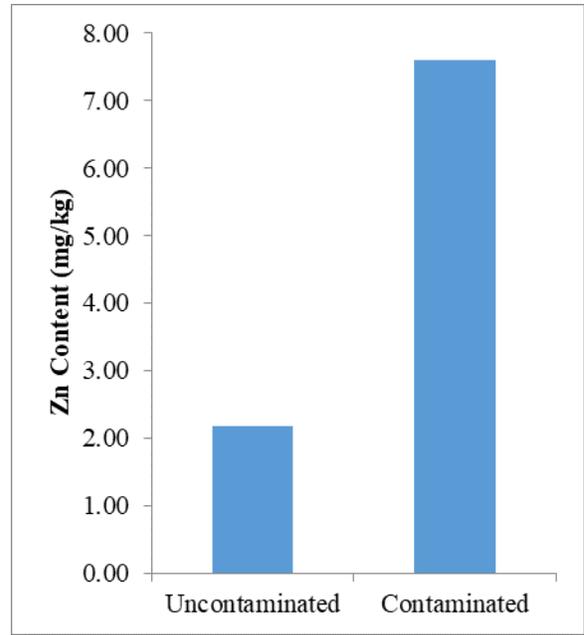
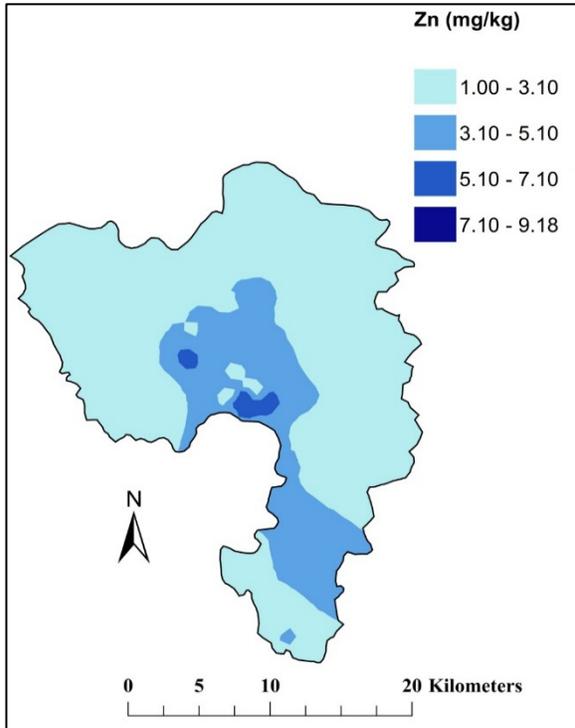
**Iron (Fe):** Results presented in table 2-3 clearly indicate that iron content in the contaminated soils of the study sites is higher as compared to the uncontaminated soils (Fig. 18-19). Fe content in the uncontaminated soils of Sreepur upazila varied from 44 to 88 mg/kg with a mean value of 64.63 mg/kg and it ranged from 78 to 102 mg/kg with the average value of 93 mg/kg for contaminated soil (Table 2). In Bhaluka upazila, iron content was comparatively higher as compared to the Sreepur upazila. Iron content in the uncontaminated control soils of Bhaluka upazila ranged from 34 to 104 mg/kg with a mean value of 74.83 mg/kg but in the contaminated soil the value of Fe content was from 127.5 to 160.5 mg/kg having a mean value of 149.3 mg/kg (Table 3). The Fe content in the contaminated soil is almost same with the safe limit of India which is 75-150 mg/kg (Awashthi, 2000). The highest deposition of Fe in soil might be due to its long-term use in the production of paints, pigments, and alloying in various industries of the study area that may result in contamination of the soil and a change to the soil structure thus making it risky for use in cultivation.

**Table 2. Maximum, minimum and mean value of soil parameters in Sreepur upazila**

| Parameter                         | Uncontaminated Soil |                   |                   | Contaminated Soil |                   |                   |
|-----------------------------------|---------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                                   | Maximum             | Minimum           | Mean              | Maximum           | Minimum           | Mean              |
| Soil pH                           | 5.66                | 4.15              | 4.83              | 7.05              | 4.08              | 5.23              |
| Soil organic carbon (%)           | 1.38                | 0.42              | 0.84              | 1.61              | 1.22              | 1.39              |
| Pb content (mg/kg)                | 29.7                | 8.0               | 17.63             | 38                | 32.4              | 35.7              |
| Cd content (mg/kg)                | 1.07                | 0.13              | 0.49              | 2.38              | 1.16              | 1.67              |
| Ni content (mg/kg)                | 22.4                | 1.02              | 13.3              | 40.2              | 29.4              | 32.3              |
| Cu content (mg/kg)                | 5.2                 | 1.0               | 2.88              | 9.4               | 6.0               | 7.93              |
| Zn content (mg/kg)                | 4.5                 | 1.08              | 2.17              | 9.18              | 6.8               | 7.6               |
| Fe content (mg/kg)                | 88                  | 44                | 64.6              | 102               | 78                | 93.0              |
| Bacterial population (cfu/g soil) | $6.0 \times 10^5$   | $2.2 \times 10^5$ | $3.4 \times 10^5$ | $2.4 \times 10^5$ | $1.4 \times 10^5$ | $1.8 \times 10^5$ |

**Table 3. Maximum, minimum and mean value of soil parameters in Bhaluka upazila**

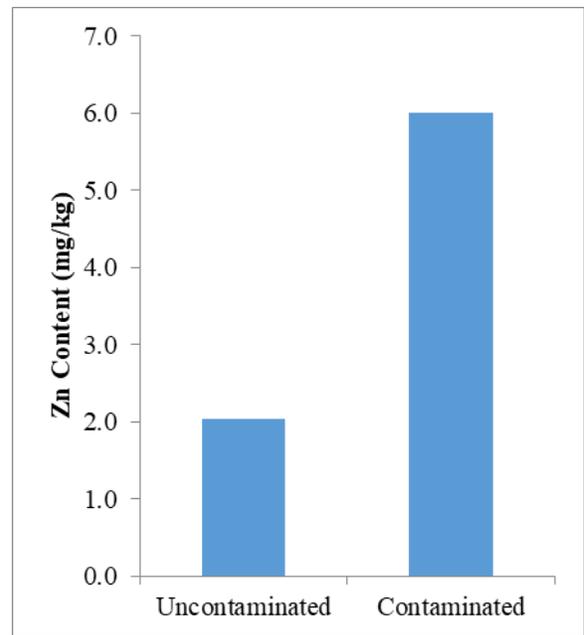
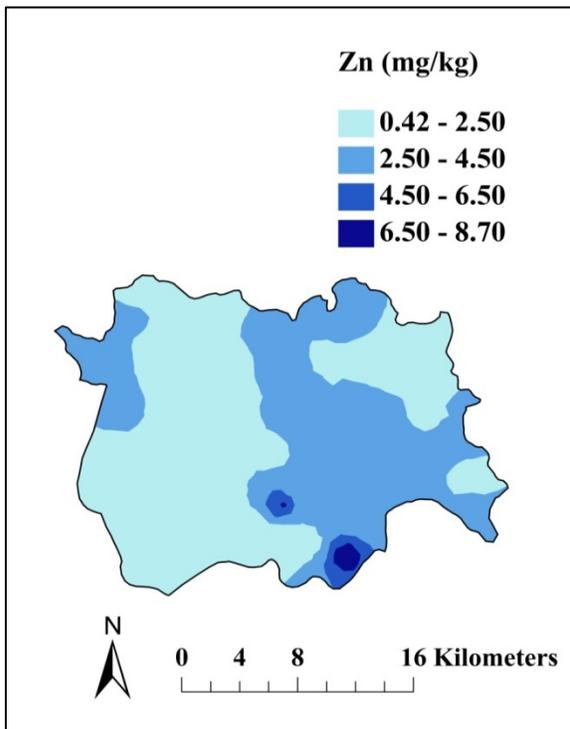
| Parameter                         | Uncontaminated Soil |                   |                   | Contaminated Soil |                   |                    |
|-----------------------------------|---------------------|-------------------|-------------------|-------------------|-------------------|--------------------|
|                                   | Maximum             | Minimum           | Mean              | Maximum           | Minimum           | Mean               |
| Soil pH                           | 5.95                | 4.25              | 5.03              | 6.99              | 5.1               | 6.0                |
| Soil organic carbon (%)           | 0.85                | 0.24              | 0.64              | 1.2               | 0.87              | 1.07               |
| Pb content (mg/kg)                | 28.7                | 8.54              | 18.57             | 46.1              | 35.4              | 38.7               |
| Cd content (mg/kg)                | 1.19                | 0.23              | 0.60              | 2.1               | 0.98              | 1.62               |
| Ni content (mg/kg)                | 22.3                | 9.8               | 16.16             | 32                | 28.7              | 30                 |
| Cu content (mg/kg)                | 6.2                 | 1.2               | 3.13              | 8.9               | 5.9               | 7.42               |
| Zn content (mg/kg)                | 4.6                 | 0.42              | 2.03              | 8.7               | 3.9               | 6.01               |
| Fe content (mg/kg)                | 104                 | 34                | 74.83             | 160.5             | 127.5             | 149.3              |
| Bacterial population (cfu/g soil) | $3.6 \times 10^6$   | $2.3 \times 10^5$ | $5.2 \times 10^5$ | $2.4 \times 10^5$ | $1.7 \times 10^5$ | $2.12 \times 10^5$ |



**Maximum permissible limit of Zn in soil**

| WHO/FAO (2001) | SEPA (1995) |
|----------------|-------------|
| 300 mg/kg      | 300 mg/kg   |

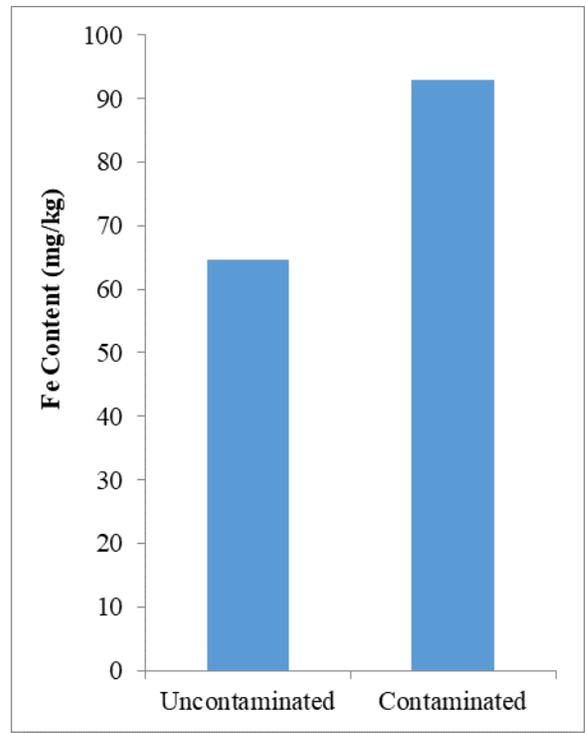
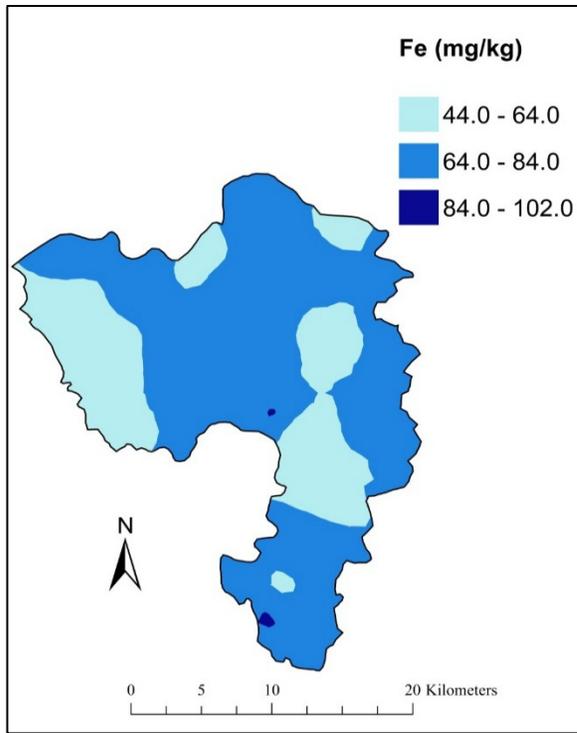
**Fig. 16. Status of Zn in Sreepur upazila soil**



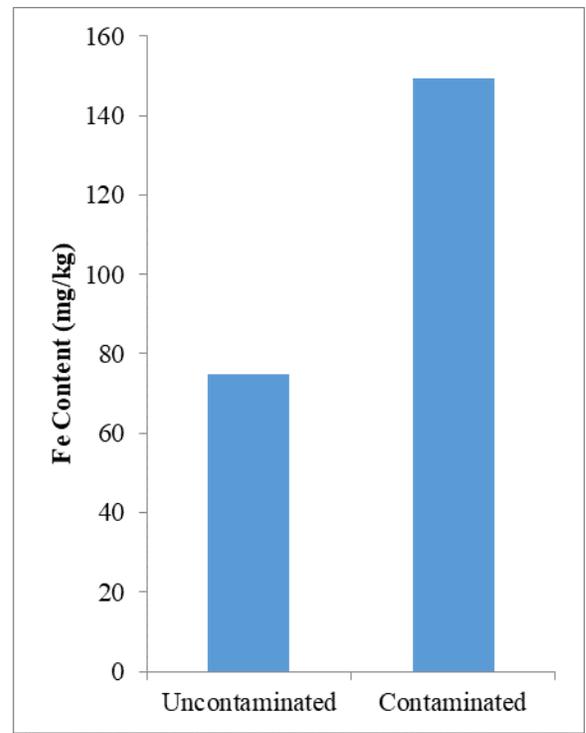
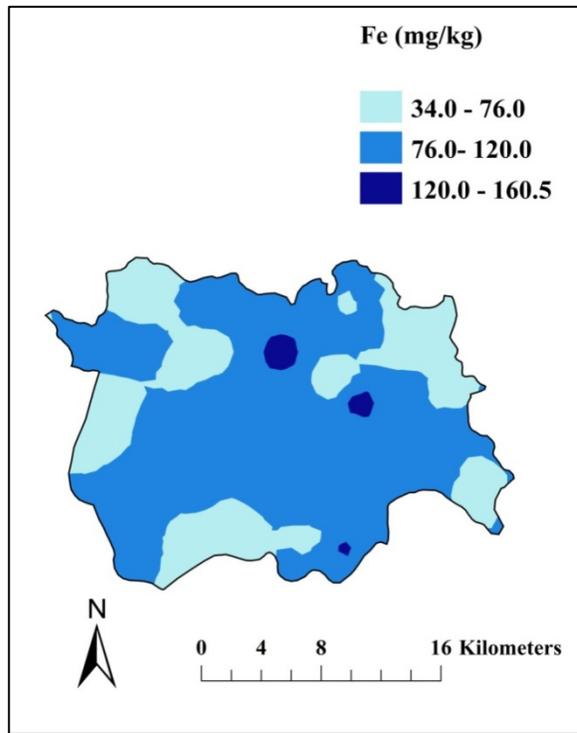
**Maximum permissible limit of Zn in soil**

| WHO/FAO (2001) | SEPA (1995) |
|----------------|-------------|
| 300 mg/kg      | 300 mg/kg   |

**Fig. 17. Status of Zn in Bhaluka upazila soil**



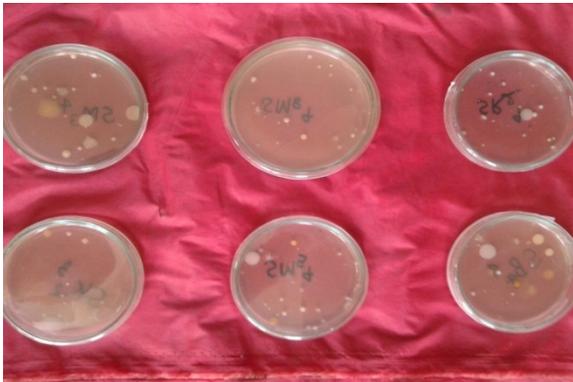
**Fig. 18. Status of Fe in Sreepur upazila soil**



**Fig. 19. Status of Fe in Bhaluka upazila soil**

### The Bacterial Biogeography of Industrial Contaminated Soils and Uncontaminated Soils:

Soil microorganisms specially the soil bacteria play important role in maintaining soil fertility. Heavy metals from untreated industrial effluents at elevated concentrations are known to effect the composition of soil microbial population and their associated activities qualitatively and quantitatively, which may directly influence the soil fertility. To study the bacterial diversity of contaminated and uncontaminated soils of the study sites, isolation and characterization of bacteria was done using the standard protocols (Fig. 20). Metals influence microorganisms by harmfully affecting their growth, morphology, and biochemical activities, resulting in decreased biomass and diversity (Malik and Ahmed, 2002). Several researchers using isolation-based techniques have demonstrated that heavy metal contamination can cause shifts in microbial populations (Roane and Kellogg, 1996). Our results also demonstrate that bacterial population in contaminated soil decreased dramatically as compared to the uncontaminated soil (Table 2-3, Fig. 21-22). In Sreepur upazila, the mean bacterial population in the control soil was  $3.4 \times 10^5$  whereas, in the contaminated soil the population decreased to  $1.8 \times 10^5$ . Similarly in Bhaluka upazila the bacterial population was  $5.2 \times 10^5$  and  $2.12 \times 10^5$  for uncontaminated and contaminated soils respectively.



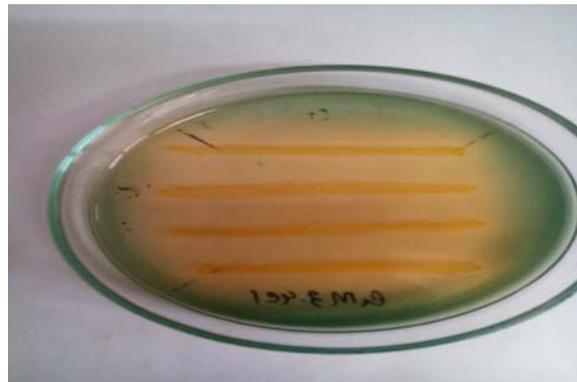
Colony formation of isolates on NA medium



Pure culture of isolates

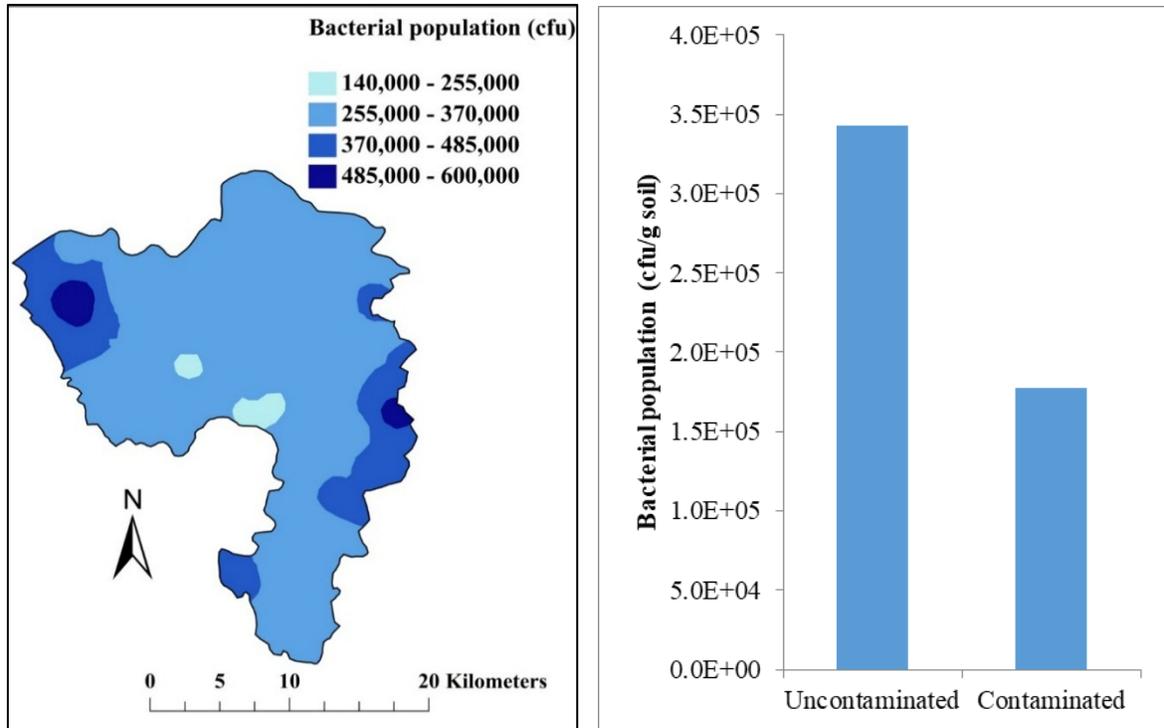


Growth of isolate on NA medium containing congo red

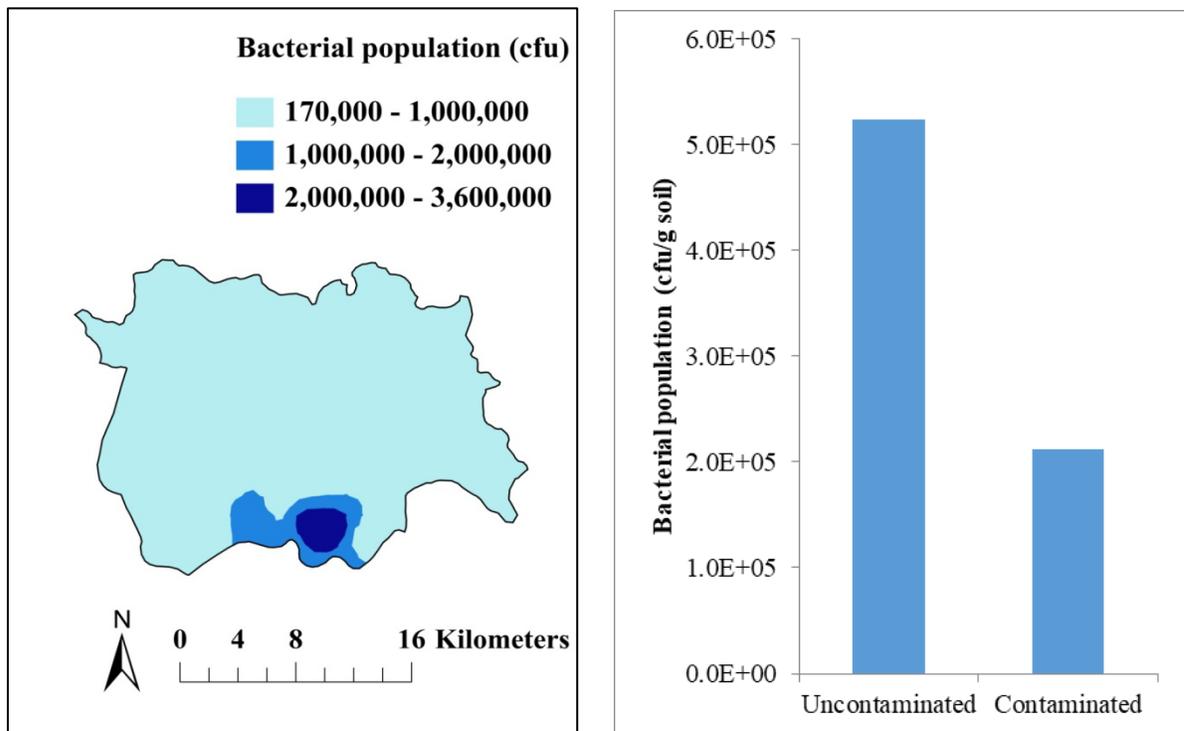


Growth of isolate on NA medium containing congo red

Fig. 20. Isolation and characterization of bacterial isolates



**Fig. 21. Bacterial population in Sreepur upazila soil**



**Fig. 22. Bacterial population in Bhaluka upazila soil**

As it is mostly recognized that the agricultural soils receiving untreated industrial waste water are contaminated by many of heavy metals that may affect the bacterial community structure. Therefore, current experiment has done to give a sight on bacterial diversity in contaminated soil as compared to the nearby uncontaminated soil. To fulfill the objective, bacteria was isolated from both contaminated and uncontaminated soil from different upazila of Gazipur and Mymensingh districts. In order to identify the isolates based on sequence comparison, initially DNA was extracted from the isolates and the Quantification of Isolated DNA was by NanoDrop2000. After the Polymerase Chain Reaction (PCR), Agarose Gel Electrophoresis was carried out to confirm the presence of PCR products. The purified PCR product was sent to Apical Scientific Company, Malaysia for sequencing. Gene sequence of some isolates are shown in Figure 23 .The sequence generated from automated sequencing of PCR amplified DNA was analyzed and compared with all accessible sequences in databases through NCBI(National Centre of Biotechnology Information) BLAST (<http://blast.ncbi.nlm.nih.gov/>) program to find out possible maximum similar organism through alignment of homologous sequences of known bacterial DNA (Fig. 24-25).Phylogenetic tree was prepared to show the closest relatives of the isolates and to support the BLAST analysis results (Figure 26).

```

>VVDd.1
GTGCGAGTGTGCATGAGCTTCTCTGAGCCATGATCAAACCTCTCNAGTGGGGGACAAACATTCCGAAAGGAATGCTAATACCG
CATACGCCCTACGGGGGAAAGCAGGGGATCTTCGGACCTTCGCTAATAGATGAGCCTAAGTCAGATTAGCTAGTTGGTG
GGGTAAAGGCCCTACCAAGGCCGACGATCTGTAGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGAGACACGGCC
AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGTGTGAAG
AAGGCCTTTTGGTTGTAAGCACTTTAAGCGAGGAGGAGGCTACTGAGATTAATACTCTTGGATAGTGGACGTTACTCGC
AGAATAAGCACCCGGCTAACTCTGTGCCAGCAGCCGGTAATACAGAGGGTGCAGCGTTAATCGGATTTACTGGCGT
AAAGCGTGCGTAGGCCGGCTTTTAAAGTCGGATGTGAAATCCCTGAGCTTAACTTAGGAATTGCATTGATACTGGGAAGC
TAGAGTATGGGAGAGGATGTTAGAATTCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGATGGCGAAG
GCAGCCATCTGGCCTAATACTGACGCTGAGGTACGAAAGCATGGGGAGCAACAGGATTAGATACCCTGGTAGTCCATGC
CGTAAACGATGTCTACTAGCCGTTGGGGCCTTTGAGGCTTTAGTGGCGCAGCTAACGCGATAAGTAGACCCGCTGGGGA
GTACGGTCCGAAGACTAAAACCTCAAATGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGTTTAAATCGATGCA
ACGCGGAAGAACCTTACCTGGTNTTGACATAGTAAGAACCTTCCAGAGATGGATGGGTGCCTTCGGGAACCTTACCTACAG
GGGCTGCATGGCTGTCGTGAGCTCGTGTCCGGAGATGTTGGGTTAAGTCCCGCAACGAGGGCAACCCCTTTCCTTATTT
GCCATCGGGTTAAGCCGGGA

>VVDd.2
TAACACGTGAGTAACCTACCCCTTGAAGCTCTGGGATAAGCCCGGGAAACTGGGTCTAATACCGGATAGGAACGTCCACC
GCATGGTGGTTGTTGGAAAGATTATCGGTGCATGGATGGACTCGCGGCCATCAGCTTGTGGTGGAGGTAATGGCTC
ACCAAGGCCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCAGACTCCT
ACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGGGGATGACGG
CCTTCGGGTTGTAACCTCTTTCAGTAGGGGAAGAAGCGAAAGTACGGTACCTGCAGAAGAAGCACCGGCTAACTA
CGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCCGT
TTGTCCGCTGTGTCGTGAAAGTCCGGGGCTTAAACCCGGATCTGCGGTGGGTACGGGCGAGACTAGAGTGCAGTAG
GGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCTC
TGGGCTGTAACCTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTA
ACGTTGGGCACTAGGTGTGGGGACCATCCACGGTTTCCGCGCCGACGCTAACGCATTAAGTGCCTCCGCTGGGG
AGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGGCCCGCACAAAGCGGCGGAGCATGCCGATTAATTCG
ATGCAACGCGAAGAACCTTACCAAGGCTTGACATGTTCTCGATCGCGTAGAGATACGGTTTCCCTTTGGGGCGG
GATCACAGGTGGTGCATGGTTGTGTCGTGAGCTCGTGTCTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC
TCGTTCCATGTT

```

**Fig. 23. Gene sequence of some isolates**



Study results demonstrate that bacterial community changed in industrial contaminated soil from that of the uncontaminated agricultural soils and beneficial bacteria affected due to industrial effluent contamination (Table 4-9). In most cases it was observed that beneficial soil bacteria are present in uncontaminated soils, while enzyme, antibiotics and disease causing bacteria are dominant in industrial waste water contaminated soils. This finding is very likely that in industrial contaminated sites, bacteria are continuously exposed to different heavy metals and other pollutants, thus giving rise to survival of stress tolerant strains. From this study it was observed that some bacterial species especially *Bacillus* was found in both contaminated and uncontaminated agricultural soils. Similar findings were showed by Tanu and Hoque (2013). It indicates that same bacterial species can survive in both polluted and unpolluted environment leading to increased incidence of stress tolerant species. The abundance of *Bacillus* in both contaminated and uncontaminated soils was probably due to the spore structure in the *Bacilli* group which increases its power of uptake and resistance against the stresses. However, other results have also reported that the diversity of *Bacilli* was greatest in contaminated soil (Sagardoy and Salerno, 1983). Shifting of the bacterial community in agricultural soil due to the heavy metal contaminated waste water may alter the cycling of soil nutrients and thus affect the soil quality.

Soil is one of the most important habitats for bacteria and is easily exposed to many pollutants from different sources including untreated industrial waste water. Isolation and identification of bacterial species present in contaminated sites is firstly essential to study on bacterial resistance in stress condition. It is also suggested that the resistance ability of the isolates could be exploited in considering the isolates as possible candidates for the bioremediation of contaminated soils.

**Table 4. Identified Bacteria for Bhaluka (Spot: Dhakatia)**

| Uncontaminated Soil |                                |   | Contaminated Soil |                                   |                   |
|---------------------|--------------------------------|---|-------------------|-----------------------------------|-------------------|
| Sample              | Bacteria                       | Function                                  | Sample            | Bacteria                          | Function          |
| VVDcl.1             | 1. <i>Acinetobacter sp.</i>    | Mineralization                            | VVDcd1            | 1. <i>Arthrobacter sp.</i>        | Enzyme inolase    |
| VVDcl.2             | 2. <i>Micrococcus luteus</i>   | Nitrate reduction                         | VVDcd2            | 2. <i>Bacillus sp.</i>            | Industrial enzyme |
| VVDcl.3             | 3. <i>Bacillus aryabhatai</i>  | Phytohormoneproduction                    | VVDcd3            | 3. <i>Arenimonas taoyuanensis</i> | Soil bacteria     |
| VVDcl.4             | 4. <i>Pseudomonas sp</i>       | Beneficial soil bacteria                  | VVDcd4            | 4. <i>Proteus mirabilis</i>       | Human disease     |
| VVDcl.5             | 5. <i>Fictibacillus sp</i>     | Beneficial soil bacteria                  |                   |                                   |                   |
| VVDcl.6             | 6. <i>Bacillus megaterium</i>  | Biocontrol agent, N <sub>2</sub> fixation |                   |                                   |                   |
| VVDcl.7             | 7. <i>Bacillus marisflavi</i>  | Tolerate high temp+ salt                  |                   |                                   |                   |
| VVDcl.8             | 8. <i>Bacillus haikouensis</i> | Halotolerant                              |                   |                                   |                   |

**Table 5. Identified Bacteria for Bhaluka (Spot: Habirbari)**

| Uncontaminated Soil |  |                               | Contaminated Soil |  |                                |
|---------------------|--|-------------------------------|-------------------|--|--------------------------------|
| Sample              | Bacteria                                 | Function                      | Sample            | Bacteria                                 | Function                       |
| VVDcl.9             | <b>1. <i>Bacillus cereus</i></b>         | cause foodborne illness       | VVDcd.5           | <b>1. <i>Bacillus marisflavi</i></b>     | Tolerate high temp+ salt       |
| VVDcl.10            | <b>2. <i>Acinetobacter lwoffii</i></b>   | Disease                       | VVDcd.6           | <b>2. <i>Fictibacillus arsenicus</i></b> | an arsenic-resistant bacterium |
| VVDcl.11            | <b>3. <i>Variovorax guangxiensis</i></b> | deaminase producing bacterium |                   |  |                                |

**Table 6. Identified Bacteria for Gazipur sadar (Spot: Mirzapur)**

| Uncontaminated Soil |  |   | Contaminated Soil |  |                                 |
|---------------------|--|---|-------------------|--|---------------------------------|
| Sample              | Bacteria                               | Function                                  | Sample            | Bacteria                                 | Function                        |
| GMcl.1              | <b>1. <i>Bacillus megaterium</i></b>   | Biocontrol agent, N <sub>2</sub> fixation | GMcd.1            | <b>1. <i>Bacillus pumilus</i></b>        | high salt tolerance             |
| GMcl.2              | <b>2. <i>Bacillus cereus</i></b>       | cause foodborne illness                   | GMcd.2            | <b>2. <i>Lysobacter daejeonensis</i></b> | production of antibiotics       |
| GMcl.3              | <b>3. <i>Brevundimonas olei</i></b>    | Resistance to ionizing Radiation          | GMcd.3            | <b>3. <i>Bacillus cereus</i></b>         | cause foodborne illness         |
| GMcl.4              | <b>4. <i>Pantoea ananatis</i></b>      | Plant pathogen                            | GMcd.4            | <b>4. <i>Lysobacter caeni</i></b>        | Plant disease bio control agent |
| GMcl.5              | <b>5. <i>Lysobacter caeni</i></b>      | Plant disease bio control agent           |                   |  |                                 |
| GMcl.6              | <b>6. <i>Aeromonas hydrophila</i></b>  | fish and amphibian pathogen               |                   |  |                                 |
| GMcl.7              | <b>7. <i>Bacillus ginsengisoli</i></b> | Nitrate reduction                         |                   |  |                                 |
| GMcl.8              | <b>8. <i>Bacillus aquimaris</i></b>    | Salt tolerant , carotenoid production     |                   |  |                                 |

**Table 7. Identified Bacteria for Gazipur sadar (Spot: Kaultia)**

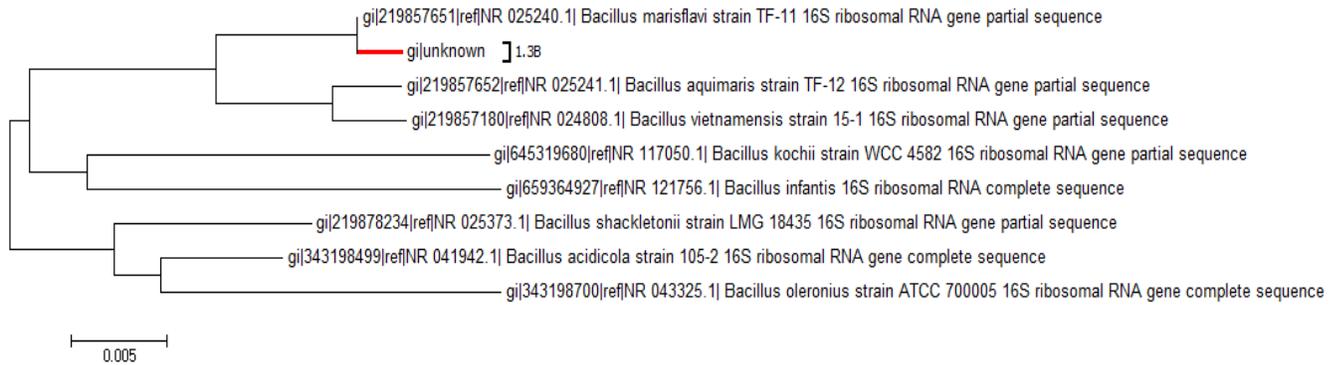
| Uncontaminated Soil |   |  | Contaminated Soil |  |   |
|---------------------|---|--|-------------------|--|---|
| Sample              | Bacteria                                | Function                                   | Sample            | Bacteria                                   | Function  |
| GMcl.9              | <b>1. <i>Acinetobacter lwoffii</i></b>  | cause of gastroenteritis                   | GMcd.5            | <b>1. <i>Bacillus cereus</i></b>           | cause foodborne illness                         |
| GMcl.10             | <b>2. <i>Bacillus megaterium</i></b>    | Biocontrol agent, N <sub>2</sub> fixation. | GMcd.6            | <b>2. <i>Bacillus pumilus</i></b>          | high salt tolerance                             |
| GMcl.11             | <b>3. <i>Comamonas testosteroni</i></b> | biodegradation in aerobic sewage           | GMcd.7            | <b>3. <i>Lysinibacillus fusiformis</i></b> | can hydrolyze urea, reduce chromate to chromium |

**Table 8. Identified Bacteria for Sreepur (Spot: Maona)**

| Uncontaminated Soil |   |  | Contaminated Soil |  |  |
|---------------------|---|--|-------------------|--|--|
| Sample              | Bacteria                                    | Function                                   | Sample            | Bacteria                                     | Function   |
| SMcl.1              | <b>1. <i>Bacillus megaterium</i></b>        | Biocontrol agent, N <sub>2</sub> fixation. | SMcd.1            | <b>1. <i>Pseudoxanthomonas mexicana</i></b>  | ability to reduce nitrite                        |
| SMcl.2              | <b>2. <i>Bacillus megaterium</i></b>        | Biocontrol agent, N <sub>2</sub> fixation. | SMcd.2            | <b>2. <i>Bacillus cereus</i></b>             | cause foodborne illness                          |
| SMcl.3              | <b>3. <i>Pseudoxanthomonas mexicana</i></b> | ability to reduce nitrite                  | SMcd.3            | <b>3. <i>Fictibacillus nanhaiensis</i></b>   | slightly halophilic, facultatively alkali philic |
| SMcl.4              | <b>4. <i>Staphylococcus equorum</i></b>     | biofilm formation                          | SMcd.4            | <b>4. <i>Bacillus halosaccharovorans</i></b> | Salt tolerant                                    |
| SMcl.5              | <b>5. <i>Bacillus mycoides</i></b>          | Inhibit the harmful bacteria and fungi.    | SMcd.8            | <b>5. <i>Bacillus megaterium</i></b>         | Biocontrol agent, N <sub>2</sub> fixation.       |
| SMcl.6              | <b>6. <i>Bacillus depressus</i></b>         | Endophytic                                 |                   |  |  |
| SMcl.7              | <b>7. <i>Staphylococcus arlettae</i></b>    | able to degrade azo dyes                   |                   |  |  |
| SMcl.8              | <b>8. <i>Bacillus aquimaris</i></b>         | Salt tolerant, carotenoid production       |                   |  |  |

**Table 9. Identified Bacteria for Sreepur (Spot: Sreepur)**

| Uncontaminated Soil |  |   | Contaminated Soil |  |   |
|---------------------|--|---|-------------------|--|---|
| Sample              | Bacteria                                   | Function                                    | Sample            | Bacteria                                 | Function                                      |
| SMcl.9              | <b>1. <i>Bacillus aquimaris</i></b>        | Salt tolerant ,<br>carotenoid<br>production | SMcd.5            | <b>1. <i>Bacillus oryzaecorticis</i></b> | Hallophytic                                   |
| SMcl.10             | <b>2. <i>Agrococcus jejuensis</i></b>      | mineralization                              | SMcd.6            | <b>2. <i>Bacillus megaterium</i></b>     | Biocontrol agent,<br>N <sub>2</sub> fixation. |
| SMcl.11             | <b>3. <i>Pseudomonas nitroreducens</i></b> | Plant Growth<br>Promotion                   | SMcd.7            | <b>3. <i>Bacillus aquimaris</i></b>      | Salt tolerant ,<br>carotenoid<br>production   |



**Fig. 26. Phylogenetic tree of *Bacillus marisflavi***

**Heavy Metal Risk Assessment of food crops grown in industrial areas of Gazipur and Mymensingh districts:**

**Translocation factor (AF):**

Principally, the food chain (soil–plant–human) pathway is recognized as one of major pathways for human exposure to soil contamination. Soil-to-plant transfer is one of the key components of human exposure to metals through the food chain. The metal translocation factor (AF) in plants is used to describe the extent of accumulation of a compound in an identified biological system. Tables 10-12 present the AF values of the metals in the consumed parts of the studied plants. Study results reveal that heavy metal translocation was higher in vegetables as compared to the rice for all the study sites. That means vegetables could accumulate more heavy metals as compared to the rice crop. The accumulation factor values of metals in the rice showed a trend in the order of Cu>Cd >Pb≥ Ni, whereas the trend was in the order of Cd > Cu >Pb≥ Ni for vegetables.

**Daily intakes of metals (DIM):**

The estimated daily intakes of metals (DIM) for adults in study sites via consumption of vegetable and rice are presented in Table 10-12. Study results indicate that though the heavy metal translocation was higher in vegetables as compared to the rice but DIM was higher through rice as compared to the vegetables for all metals which might be due to the higher consumption of rice as compared to the vegetables. Study results further illustrate that daily intake of metals from rice and vegetables grown in industrial effluent contaminated soils were much greater than from food crops grown in uncontaminated soils. The biggest contribution to the intake of heavy metals came from rice, compared to the intake through vegetables, as shown in tables 10-12. Thus, perennial intake of these contaminated food crops is likely to induce adverse health effects arising largely from Pb, Cu and Cd exposure.

**Health Risk Index (HRI):**

To observe the health risk of each pollutant, it is important to estimate the level of exposure by detecting the routes of exposure to target organisms. There are several possible pathways of exposure to humans, but among them the food chain is the most important pathway. In our study, the HRI for selected heavy metals (Cd, Pb, Ni and Cu) through consumption of rice and vegetables were calculated. The HRI value  $> 1$  indicates a possible future human health risk via the intake of food crops. Study results reveal that HRI value for rice is higher in all the sampling points as compared to the vegetables (Table 10-12). HRI values for the food crops grown in contaminated soils are considerably higher from that of the uncontaminated soils. Considering the Health Risk Index (HRI) for heavy metals, still the consumption of rice and vegetables are safe as the HRI value is less than 1 except the HRI value of Pb through the consumption of rice grown in the contaminated areas of Gazipur sadar upazila. But it is alarming that HRI value in the contaminated soil is very high as compared to the uncontaminated soil which is very close to the maximum allowable value. If the trend of discharging untreated industrial waste water to the agricultural soil continues for several years, it would create a severe health risk through the consumption of food crops grown in the vicinity of industries. Therefore, the policy makers should take immediate action against the discharge of untreated industrial waste water to the agricultural soil as well as to the water bodies.

**Table 10. Translocation factor (AF), Daily intakes of metals (DIM) ( $\text{mg kg}^{-1} \text{ person}^{-1} \text{ d}^{-1}$ ) and the Health Risk Index (HRI) for heavy metals in food crops grown in Sreepur upazila**

|    | Metal             | AF    |            | DIM      |            | HRI   |            |
|----|-------------------|-------|------------|----------|------------|-------|------------|
|    |                   | Rice  | Vegetables | Rice     | Vegetables | Rice  | Vegetables |
| Cd | Control Soil      | 0.063 | 0.250      | 2.84E-04 | 7.16E-05   | 0.284 | 0.072      |
|    | Contaminated Soil | 0.065 | 0.7        | 6.82E-04 | 1.28E-04   | 0.682 | 0.128      |
| Pb | Control Soil      | 0.006 | 0.034      | 6.82E-04 | 1.99E-04   | 0.195 | 0.057      |
|    | Contaminated Soil | 0.006 | 0.032      | 1.02E-03 | 3.07E-04   | 0.292 | 0.088      |
| Ni | Control Soil      | 0.012 | 0.027      | 6.82E-04 | 5.12E-05   | 0.034 | 0.003      |
|    | Contaminated Soil | 0.006 | 0.015      | 1.42E-03 | 2.05E-04   | 0.071 | 0.010      |
| Cu | Control Soil      | 0.24  | 0.32       | 6.82E-03 | 2.05E-04   | 0.171 | 0.005      |
|    | Contaminated Soil | 0.33  | 0.18       | 1.42E-02 | 5.63E-04   | 0.355 | 0.014      |

**Table 11. Translocation factor (AF), Daily intakes of metals (DIM) ( $\text{mg kg}^{-1} \text{ person}^{-1} \text{ d}^{-1}$ ) and the Health Risk Index (HRI) for heavy metals in food crops grown in Bhaluka upazila**

|    | Metal             | AF    |            | DIM      |            | HRI   |            |
|----|-------------------|-------|------------|----------|------------|-------|------------|
|    |                   | Rice  | Vegetables | Rice     | Vegetables | Rice  | Vegetables |
| Cd | Control Soil      | 0.1   | 0.4        | 2.27E-04 | 5.12E-05   | 0.227 | 0.051      |
|    | Contaminated Soil | 0.08  | 0.22       | 9.10E-04 | 8.70E-05   | 0.910 | 0.087      |
| Pb | Control Soil      | 0.004 | 0.023      | 5.69E-04 | 1.53E-04   | 0.162 | 0.044      |
|    | Contaminated Soil | 0.007 | 0.029      | 1.42E-03 | 2.92E-04   | 0.406 | 0.083      |
| Ni | Control Soil      | 0.007 | 0.011      | 5.12E-04 | 4.60E-05   | 0.026 | 0.002      |
|    | Contaminated Soil | 0.006 | 0.028      | 1.08E-03 | 2.30E-04   | 0.054 | 0.012      |
| Cu | Control Soil      | 0.289 | .35        | 7.39E-03 | 1.79E-04   | 0.185 | 0.004      |
|    | Contaminated Soil | 0.279 | .27        | 1.08E-02 | 4.60E-04   | 0.270 | 0.012      |

**Table 12. Translocation factor (AF), Daily intakes of metals (DIM) ( $\text{mg kg}^{-1} \text{ person}^{-1} \text{ d}^{-1}$ ) and the Health Risk Index (HRI) for heavy metals in food crops grown in Gazipur Sadar upazila**

|    | Metal             | AF    |            | DIM      |            | HRI   |            |
|----|-------------------|-------|------------|----------|------------|-------|------------|
|    |                   | Rice  | Vegetables | Rice     | Vegetables | Rice  | Vegetables |
| Cd | Control Soil      | 0.083 | 0.45       | 2.84E-04 | 4.60E-05   | 0.284 | 0.046      |
|    | Contaminated Soil | 0.075 | 0.304      | 1.02E-03 | 1.30E-04   | 1.024 | 0.130      |
| Pb | Control Soil      | 0.008 | 0.023      | 7.96E-04 | 1.20E-04   | 0.227 | 0.034      |
|    | Contaminated Soil | 0.009 | 0.024      | 1.54E-03 | 2.51E-04   | 0.439 | 0.072      |
| Ni | Control Soil      | 0.011 | 0.017      | 8.53E-04 | 6.39E-05   | 0.043 | 0.003      |
|    | Contaminated Soil | 0.008 | 0.042      | 1.71E-03 | 3.07E-04   | 0.085 | 0.015      |
| Cu | Control Soil      | 0.35  | 0.188      | 7.96E-03 | 1.92E-04   | 0.199 | 0.005      |
|    | Contaminated Soil | 0.356 | 0.205      | 1.82E-02 | 4.09E-04   | 0.455 | 0.010      |

#### References:

- Awashthi, S. K. 2000. Prevention of Food Adulteration Act No. 37 of 1954. Central and State Rules as Amended for 1999; Ashoka Law House: New Delhi, India, p. 2000.
- Balkhair, K. S. and Ashraf, M. A. 2016. Field accumulation risks of heavy metals in soil and vegetable crop irrigated with sewage water in western region of Saudi Arabia. Saudi journal of biological sciences 23: 32-44.
- Bardgett, R. D., Freeman, C. and Ostle, N. J. 2008. Microbial contributions to climate change through carbon cycle feedbacks. ISME J 2: 805–814.
- BBS (Bangladesh Bureau of Statistics). 2018. Statistical year book Bangladesh 2018, 38th edition, Statistics & Informatics Division (Sid), Ministry of Planning, Government of The People's Republic of Bangladesh, Dhaka, Bangladesh.
- Fulthorpe, R. R., Roesch, L. F. W., Riva, A. and Triplett, E. W. 2008. Distantly sampled soils carry few species in common. ISME J 2: 901–910.
- Malik, A. and Ahmed, M. 2002. Seasonal variation in bacterial flora of the wastewater and soil in the vicinity of industrial area. Environ Monit Assess: 73: 263 273.
- Roane, T. M. and Kellogg, S. T. 1996. Characterization of bacterial communities in heavy metal contaminated soils. Can J Microbiol: 42: 593 - 603.

Sagordoy, M. A. and Salerno, C. M. 1983. Number, distribution and characterization of heterotrophic bacteria in some Argentine soils. *Ann EdafolAgrobiol.* 42: 2069- 2081.

Tanu , F. Z. and Hoque, S. 2013. Comparison of bacterial population in industrial and agricultural soils. *Int. J. Curr. Microbiol. App Sci*(4): 155-165.

USEPA, 2005, "Toxicological review of zinc and compounds. Washington, DC. EPA/635/R-05/002, CAS NO.7440-66-6.

Zheng, N., Wang, Q. C., Zhang, X. W., Zheng, D. M., Zhang, Z. S. and Zhang, S. Q. 2007. Population health risk due to dietary intake of heavy metals in the industrial area of Huludao City, China. *Science of the Total Environment.* 387: 96–104.

**12. Research highlight/findings:**

- Untreated industrial effluents have severely contaminated the agricultural field
- Increasing trend of Heavy metal (Zn, Cu, Pb, Cd, Ni, Fe) accumulation found in the agricultural soil due to the untreated industrial waste water discharges
- Bacterial population including beneficial bacteria decreased dramatically in the contaminated soils as compared to the uncontaminated soils
- Community structure of soil bacteria changed due to the contamination of agricultural soil by untreated toxic industrial effluents
- The heavy metal tolerant bacterial isolates could effectively be utilized for the bioremediation of metals from the contaminated soil
- The HRI values through the consumption of rice grown in the contaminated areas were very close to the maximum allowable level which indicates that consumption of food crops grown in the contaminated area might create serious human health hazards.

**B. Implementation Position**

**1. Procurement:**

| Description of equipment and capital items | PP Target                                 |           | Achievement                               |          | Remarks |
|--|---|-----------|---|----------|---------|
|  | Phy (#)                                   | Fin (Tk)  | Phy (#)                                   | Fin (Tk) |         |
| (a) Office equipment                       | Laptop, Scanner                           | 70000.00  | Laptop, Scanner                           | 100%     |         |
| (b) Lab & field equipment                  | Autoclave, micro pipette, COD & BOD meter | 930000.00 | Autoclave, micro pipette, COD & BOD meter | 100%     |         |
| (c) Other capital items                    | By cycle                                  | 15000.00  | By cycle                                  | 100%     |         |

**2. Establishment/renovation facilities: Not Applicable**

| Description of facilities | Newly established |             | Upgraded/refurbished |             | Remarks |
|---------------------------|-------------------|-------------|----------------------|-------------|---------|
|                           | PP Target         | Achievement | PP Target            | Achievement |         |
|                           |                   |             |                      |             |         |
|                           |                   |             |                      |             |         |

### 3. Training/study tour/ seminar/workshop/conference organized: Not Applicable

| Description  | Number of participant |        |       | Duration (Days/weeks/<br>months) | Remarks |
|--------------|-----------------------|--------|-------|----------------------------------|---------|
|              | Male                  | Female | Total |                                  |         |
| (a) Training |                       |        |       |                                  |         |
| (b) Workshop |                       |        |       |                                  |         |

### C. Financial and physical progress

Fig in Tk

| Items of expenditure/activities             | Total approved budget | Fund received  | Actual expenditure | Balance/ unspent | Physical progress (%) | Reasons for deviation |
|---|-----------------------|----------------|--------------------|------------------|-----------------------|-----------------------|
| A. Contractual staff salary                 | 814655                | 807666         | 744770.00          | 1223.00          |                       | fund not released yet |
| B. Field research/lab expenses and supplies | 1122000               | 1079500        | 1122090.00         | -90.00           |                       |                       |
| C. Operating expenses                       | 150000                | 150255         | 145097.00          | 4903.00          |                       |                       |
| D. Vehicle hire and fuel, oil & maintenance | 200000                | 188000         | 200000.00          | 0.00             |                       |                       |
| E. Training/workshop/seminar etc.           | 0                     | 0              | 0.00               | 0.00             |                       |                       |
| F. Publications and printing                | 75000                 | 28622          | 0.00               | 0.00             |                       | fund not released yet |
| G. Miscellaneous                            | 60000                 | 60000          | 60140.00           | -140.00          |                       |                       |
| H. Capital expenses                         | 1015000               | 978950         | 1015000.00         | 0.00             |                       |                       |
| <b>Total</b>                                | <b>3436655</b>        | <b>3292993</b> | <b>3287097.00</b>  | <b>5896.00</b>   |                       |                       |

### D. Achievement of Sub-project by objectives: (Tangible form)

| Specific objectives of the sub-project   | Major technical activities performed in respect of the set objectives  | Output(i.e. product obtained, visible, measurable)   | Outcome(short term effect of the research)  |
|--|--|--|---|
| Investigate the accumulation and spatial distribution of heavy metals in industrial contaminated soils | Site selection, identification of probable sampling points through Projection of digital map and Arc GIS software, collection, processing and analysis of soil, plant and waste water samples, preparation of heavy metal distribution maps of contaminated and uncontaminated soils | Obtained clear dataset regarding basic soil parameters and accumulation of heavy metals in industrial contaminated and uncontaminated soils, generated spatial distribution maps of heavy metals | provided clear message to the environmental scientists and policy makers regarding the accumulation of toxic heavy metals in agricultural soil due to the discharge of industrial waste water |
| Illustrate the risk of heavy metal pollution merging spatial and analytical data                       | Compilation of spatial and analytical data of heavy metals of the study sites, collecting reference data regarding maximum allowable limits of heavy metals in soil, interpreting the analytical data with the standard reference data   | Generated data regarding the risk of heavy metal pollution due to the discharge of untreated industrial waste water to the agricultural soils  | Provided deep insight about the risk of heavy metal pollution to the scientific community for the possible remediation of contaminated agricultural soil for safe food production             |

|  |   |  |  |
|--|---|--|--|
| Studying the bacterial biodiversity of contaminated soils as compared to the uncontaminated soil | Collection of soil samples from contaminated and uncontaminated areas, enumeration of bacterial population, isolation and characterization of bacteria, Identification of bacteria through DNA sequencing | Prepared spatial distribution maps of bacterial population, screened heavy metal tolerant bacterial isolates | Created the possibility of using heavy metal tolerant bacterial isolates as possible candidates for the bioremediation of contaminated soils   |
| Calculate the health risk index (HRI) of different heavy metals                                  | collection, processing and analysis of soil and plant samples collected from contaminated and uncontaminated areas, estimate the Daily Intake of Metals (DIM) and translocation factor                    | Estimated the HRI values of different heavy metals through the consumption of rice and vegetables            | Created awareness of the crop growers and consumers about the heavy metal toxicity through food chain, provided clear information to the policy makers and industry owners regarding the human health hazards due to unplanned discharge of industrial waste water |

**E. Materials Development/Publication made under the Sub-project:**

| Publication                                     | Number of publication |                         | Remarks (e.g. paper title, name of journal, conference name, etc.) |
|---|-----------------------|-------------------------|--|
|   | Under preparation     | Completed and published |  |
| Technology bulletin/ booklet/leaflet/flyer etc. |                       |                         |  |
| Journal publication                             | 02                    |                         |  |
| Information development                         |                       |                         |  |
| Other publications, if any Master's thesis      | 02                    |                         |  |

**F. Technology/Knowledge generation/Policy Support (as applied):**

**i. Generation of technology (Commodity & Non-commodity)**

The screened heavy metal tolerance bacterial isolates could be utilized for the bioremediation of industrial waste water contaminated agricultural soils

**ii. Generation of new knowledge that help in developing more technology in future**

Estimated data regarding heavy metal accumulation in soil and HRI will help the scientific community to develop technology for the possible remediation of contaminated agricultural soil for safe food production

**iii. Technology transferred that help increased agricultural productivity and farmers' income**

N/A

**iv. Policy Support**

Generated data regarding heavy metal pollution in soil and health risk index values will aware the policy makers to take necessary action against the discharge of untreated industrial waste water to the agricultural soil

**G. Information regarding Desk and Field Monitoring:**

**i) Desk Monitoring:**

Two monitoring workshop was organized by Soil unit, Natural Resources Management Division, BARC and one monitoring workshop was organized by BSMRAU. The monitoring workshops guided the project personnel for the better implementation of the project activities.

**ii) Field Monitoring (time & No. of visit, Team visit and output): Not Applicable**

**H. Lesson Learned/Challenges (if any):**

- i) Timely release of fund is important for procurement as well as smooth running of the project activities
- ii) Regular field monitoring and desk monitoring enhances the research activities

**I. Challenges (if any):**

The most important part of the present study is to collect the samples (soil, plant and waste water) properly. Due to late flood and late heavy rainfall throughout the country including the sampling areas, it was not possible to collect the samples as per schedule. Therefore, sample processing and analysis was delayed. Procurement was delayed due to late approval of procurement plan (took about 2 months) and late release of the funds severely hampered the research activities. Moreover, part of the allocated fund was not released at all. Therefore, it was a great challenge to run the project activities smoothly.

Signature of the Principal Investigator  
Date .....  
Seal

Counter signature of the Head of the  
organization/authorized representative  
Date .....  
Seal

**Project related Photos:**



**Contaminated Agricultural Field**



**Collection of soil, plant and waste water samples**



**Collection of soil samples**



**Analyzing the collected samples and microbial study by the Laboratory Technician and Research Student instructed by the Principal Investigator**