CYTOGENETICS OF SELECTED PLANT SPECIES AFFECTED BY INDUSTRIAL EFFLUENTS

Ph.D. THESIS

BY

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Dedicated
To
My Beloved Parents,
Daughter, Son
&
Husband

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Certificate

*This is to certify that this thesis entitled "**Cytogenetics of selected plant species affected by industrial effluents**" submitted by Kazi Kamrun Nahar has been carried out under my supervision in this laboratory. This is further to certify that it is an original work and suitable for submission for the award of doctor of philosophy in Botany.*

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– The Author

Abstract

Two plants *viz. Colocasia esculenta* (L.) Schott and *Ipomoea aquatica* Forssk. commonly growing in the industrial effluent affected areas were selected to observe the effect of industrial effluents on chromosomal and DNA level. Five different industrial areas such as (i) Powertex Fashion Ltd., Gazipur (ii) Shetu Pesticide Ltd., Savar (iii) Salma Leather Industry, Hazaribagh (iv) Monno Ceramic Industries, Nayarhat, Dhamrai and (v) Acme Laboratories Ltd., Tulivita, Dhamrai were selected for this study. A number of different experiments like physico-chemical parameters, essential elements, heavy metals, conventional and fluorescent karyomorphology and RAPD have been done for comparative study with control. The amount of heavy metals in soil, water and plant samples were higher in all affected areas. Dissolved Oxygen was found bellow the permissible limit in all the affected areas (lowest 0.88 mg/l in Hazaribagh) indicating high amount of organic wastes discharge from tannery. Heavy metal uptake in *Colocasia esculenta* is much higher than *Ipomoea aquatica*. The edible portion consumed more heavy metal than root. Diploid chromosome number 2n=28 was found in *Colocasia esculenta* collected from Gazipur garments, Savar pesticide and Hazaribagh leather area as well as control. However, 2n=38 chromosomes of the same species were observed in the sample plants collected from Monno Ceramic and Acme Laboratory area. The probable reason for increasing chromosome number was either the sample a new cytotype of the species or some irregularities occurred during cell division. On the other hand, 2n = 30 chromosomes were observed in all plant samples of *Ipomoea aquatica* including control. In case of both the species, the nature of staining of interphase nuclei and prophase chromosomes of the sample plants were different than those of control. The fluorescent karyotypes of both the species showed significant differences among the samples and with control as well. Fluorescent banding (CMA and DAPI) revealed the probable occurrence of deletion and tandem duplication. The different plant samples of both the species had characteristics RAPD fingerprinting. Lacking of some DNA fragments in some sample plants (which presents in the control plants) may correlate to the deletion of chromosomal parts. Similarly some new fragments in the sample plants (which not found in the control) may corresponds with the tandem duplication of the chromosomal part. Therefore, the substances present in the industrial effluents affected on the chromosome and DNA level of *Colocasia esculenta* and *Ipomoea aquatica*.

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1. Introduction

Industrial effluents are one of the major sources for direct and often continuous input of pollutants/toxicants into aquatic ecosystems with long-term implications (Odeigah and Osanyipeju 1995, Chan *et al.* 2003, Lah *et al.* 2004, Smolders *et al.* 2004). As a result of continuous input of different pollutants, it lowers the quality of life in various aspects and affects health and life span (Grover and Kaur 1999). Rapid industrialization of Bangladesh for the last two decades resulted about 30,000 large and small industries which are destructing our riverine system by discharging their effluents (Rahman *et al.* 2001). Most of the industries and factories are present in and around Dhaka city (Islam *et al.* 2011). The industries are situated on the banks of the river Buriganga, Turag etc. or very close to the river system. Among the industries, textile, leather, paint, pesticides, pharmaceuticals laboratories are northworthy (DOE 1997). Unfortunately, these industries do not follow any biosafety guideline. Even they do not have recommended waste management system and discharging their effluents directly into the different water bodies around the metropolis.

Besides rivers and streams, industrial effluents are also discharging into low-lying lands, causing soil pollution (Rahman *et al.* 2001). Textile, paper pulp, food, fish, tanneries and distillery industries are mainly discharging biodegradable organic wastes (Rahman *et al.* 2001). These wastes undergo aerobic (i.e., using oxygen) decomposition in the receiving water body (Lenore *et al.* 1998). As a result, the oxygen content of water goes down, endangering fish and other aquatic species (Meybeck *et al.* 1996). On the other hand, non biodegradable wastes are mainly discharged by the pesticide, chemical, ceramic and pharmaceutical industries (Rahman *et al.* 2001). Many of the persistent compounds contained in these effluents are toxic and can have a devastating effect on the environment (Chhatwal

et al.1992). Industrial effluents have been found to increase heavy metal load in surrounding agricultural soils (Joardar *et al.* 2005). Among the heavy metals, Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb), Nickel (Ni) etc. are directly health hazardous (Huq *et al.* 2006).

These metals are taken up by plants. *Colocasia esculenta*, *Ipomoea aquatica, Oryza sativa* etc. growing on this contaminated soil can able to uptake and accumulate the toxic substances, even faster than other aquatic organisms (Huq *et al.* 2000). Everyday a huge amount of plants such as *Colocasia esculenta* and *Ipomoea aquatica* are coming from the various affected areas to the local markets and being sold at cheap price. These two plants are popular and a number of people feeding on this plants without knowing the sources. In this way, the toxic substances are entering into the human food chain (Ahmed and Reazuddin 2000, Huq *et al.* 2008).

It has been reported that the heavy metals like As, Pb, Cr, Cd, Ni have carcinogenic effects on both plants and animals (Förstner and Wittmann 1981, Glanze 1996). The heavy metals discharging from different industries might have effect on chromosomal level of these plants growing in and around the effluent affected areas. Moreover, these heavy metals may enter to the human body and increase its level after continuous consumption of these plants. Therefore, it is an urgent need to know whether these effluents cause any change at chromosomal or DNA level of these plants (Xian 1989, Glanze 1996, Zhenbin and Shuman 1997).

Karyotype analysis is one of the important methods for chromosome study. This method is the most reliable and stable. Karyotype of an organism is specific (Nakajima 1963, Hagiwara 1997, Alam *et al.* 2012, Jahan *et al.* 2012). A specimen can be identified authentically through karyotype analysis. Karyotype analysis often plays an important role in determining the taxonomic status of a taxon where

the taxonomic parameters are insufficient. Several taxonomic problems have been solved by comparative karyotype analysis (Alam and Kondo 1995, Alam and Kondo 1996, Nahar and Alam 1998, Nahar and Alam 1999, Alam *et al.* 1999, Alam *et al.* 2000, Akhter and Alam 2005). However, classical karyotype analysis can sometimes be confusing since varieties usually have similar chromosome numbers and other karyotypic parameters. Moreover, problem also arises when different taxa possess same chromosome number and almost similar karyotypic features (Akter and Alam 2005, Zaman and Alam 2009). In this situation, it is hard to distinguish between such taxa by conventional karyotype analysis. Even the consideration of chromosome length, arm ratio, position and number of secondary constrictions are not always sufficient to differentiate individual chromosome. In case of large number and small chromosomes, it becomes very difficult. Minute alteration regarding the distribution pattern of GC- and AT- rich repeats in the karyotypes could not be possible to detect through conventional karyotype analysis. Further, deletion of heterochromatic regions may change the karyotype of a specimen without affecting the morphology (Sumner 1990). To avoid this difficulty, some modern methods may be applied.

One such method is the study of staining properties of interphase nuclei and prophase chromosomes. This is usually done by differential staining with orcein, CMA and DAPI (Hashimoto 1987, Hizume *et al.* 1988, Alam *et al.* 1993, Alam *et al.* 1998, Begum and Alam 2004, Begum and Alam 2005, Begum and Alam 2009, Islam and Alam 2011). Tanaka (1971) classified the different types of interphase nuclei and prophase chromosomes on the basis of orcein staining property. The outcome of his study showed that various taxa including varieties of many plant species could be distinguished by their staining properties.

Another method for karyotype study is concerned to DNA-base specific banding with two common fluorochromes such as chromomycine A3 (CMA) and 4′-6

Diamaidino-2-phenyl Indole (DAPI) (Schweizer 1976). CMA binds with GC-rich repetitive sequences of the genome and gives characteristics yellow color bands. On the other hand, DAPI binds to AT- rich repeats giving characteristics blue color. With this method it is possible to determine the GC- (Guanine-Cytosine) and AT- (Adenine-Thymine) rich DNA segments of the chromosomes (Kondo and Hizume 1982, Jessy *et al.* 2005, Alam *et al.* 2011). Thus it seems that fluorescent banding is quite satisfactory for detailed and critical chromosome analysis such as identification of individual chromosome, determination of amount and site of AT- and GC- rich base sequences on chromosomes (Schweizer 1976).

For molecular characterization, DNA fingerprinting by Randomly Amplified Polymorphic DNA (RAPD) is a method for characterizing germplasms authentically. The term DNA fingerprinting/profiling describes the combined use of several single locus detection systems. This method has been using as versatile tool for investigating various genomic aspects of organism (Kennard *et al.* 1994). It includes characterization of genetic variability, genome fingerprinting, genome mapping, gene localization, analysis of genome evolution, population genetics, taxonomy etc. Genetic analysis using molecular marker technology can provide a powerful approach to understand the organization and distribution of genetic resources in natural and manage populations. At the species level, the identification of taxonomic units and the determination of uniqueness of species are essential information for conservation, systematic, ecological and evolutionary studies (Schierwater 1994).

In this study five different industries have been selected such as (i) Powertex Fashion Ltd., Gazipur (ii) Shetu Pesticide Ltd., Savar (iii) Salma Leather Industry, Hazaribagh (iv) Monno Ceramic Industries Ltd., Nayarhat, Dhamrai and (v) Acme Laboratories Ltd., Tulivita, Dhamrai. These industries are discharging waste effluents directly to either agricultural land or water body. These waste effluents

contain different types of toxic substances like As, Cr, Cd, Pb, Ni, Hg, Al, Co, etc. (Huq *et al.* 2006). Physico-chemical parameters, essential elements and heavy metals of water, soil and the plant samples around the different industrial areas were measured to assess the rate of accumulation and uptake of these substances.

Two very common plants *viz. Colocasia esculenta* (L.) Schott and *Ipomoea aquatica* Forssk. were selected as sample plants because of their wide abundance in the affected areas. Moreover, these two plants from the affected areas are selling to the local markets for human consumption.

In this study, a combined efforts of cytogenetical and molecular analysis of above mentioned two species collected from the five affected areas have been carried out. Each data was compared to that of control sample.

The aims of this present research work were:

(i) to determine the amount of physico-chemical parameters, essential elements and heavy metals of soil, water and plant samples collected from five affected areas as well as control.
(ii) to compare the karyotypes of samples after staining with orcein, CMA- and DAPI.
(iii) to compare the distribution of AT- and GC- rich repeats in the genomes of sample plants.
(iv) to determine the genetic alteration at molecular level using RAPD.

2. Materials and Methods

2.1. Plant materials

Two very common plants were used as a plant material in this study such as –

(i) *Colocasia esculenta* (L.) Schott (Fig. 11)

(ii) *Ipomoea aquatica* Forssk. (Fig. 12)

These plants are widely growing in the various industrial effluent affected areas. Plant samples were collected from 5 different industrial effluent affected areas of Dhaka city and outskirts of Dhaka city. As control, these two species were collected from a village Shibrampur, Post- Burichang, District – Comilla. All of these plant samples have been maintaining in the Botanic Garden, Department of Botany, University of Dhaka.

2.1.1. Brief morphological description of plant samples

2.1.1.1. *Colocasia esculenta* (L.) Schott.

Perennial herb with underground tubers, large main tuber or corm with a few side tubers, tuber usually cylindrical, 35x15 cm. Sometimes stolons are produced from the main tuber or corm. Leaves petiolate, petiole 30–85 cm long, sheathing for about 25–35 cm at the base, leaf blade peltate, 15–45x10–35 cm, ovate acute, cordate, dark green above enlight green beneath, base shallowly cordate, glaucous, venation pinnately reticulate, sinus 1 – 4 cm long. Inflorescences with axillary peduncle, shorter than petiole, solitary, many. Peduncle 15–25 cm long. Speathe constricted, about 35 cm long, glabrous, basal part convolute, persistent, green, 3–5 cm long, upper part lanceolate, yellow, 20–25 cm long. Spadix sessile, shorter then the speathy, male and female zones usually separated by a flat elongate neuters, appendage cylindric, constricted at the base or absent. Female portion 2.5–3.0 cm, neuter portion 1.5–2.0 cm, male portion 5–6 cm and the apical sterile appendix about 3–5 cm long. Female flowers naked, many crowded at the base of the spadix, ovary ovoid with parietal placentation and orthotropus ovules, green, stigma sessile.

Neuter flowers elongate, sub-rhormboidal two hexagonal, occupying the constricted zone of the spadix. Male flowers numerous, each with 6 -8 linear, cream colored and anther lobes, dehiscence by apical pores, appendage shorter than the male portion, cream in color, sub-cylindric, tapering towards the tip. Fruit a berry, ovoid, seeds elongate. Flowering and fruiting: May – October (Siddique *et al.* 2007).

2.1.1.2. *Ipomoea aquatica* Forssk.

A glabrous trailer on mud or floating on water branchletes succulent, usually floating. Stem usually hollow, rooting at the nodes. Leaves 5–9x2–5 cm, ovate, ovate – oblong, deltoid, lanceolate or linear, base cordate, sagittate or hastate. Flowers 1 to few, in axillary symes. Calyx lobes subequal, 7–10 mm long. Corolla funnel shaped, 2.5–5 cm long, pink rarely white. Stamens included, villous at the base, filaments hairy at the base. Carples 2, syncarpus. Fruit a capsule, about 0.8 cm. across, persistent, glabrous, ovoid 2 globes, calyx embracing the fruit. Seeds densely grey – pubescent or glabrous. Flowering and Fruiting: January – December (Ahmed *et al.* 2008).

2.2. Selected industries

Five different industries were selected in this study. The Botanic garden, Department of Botany, University of Dhaka was considered as control area for this study. The selected areas were:

2.2.1. Powertex Fashion Limited, Kolomeshwar, board bazar, Gazipur (23^{o} 56′700″N and 90^{o} 23′007″E). The samples collected about 30 meters (m) the area which from the factory. Dyes are the main waste product discharged directly to the agriculture filed and neighbouring stagnant water body (Figs. 13 and 14).

2.2.2. Shetu Pesticide Limited, Samko Jadur Char, Hemayetpur, Savar, (23^{o} 42′22″N and 90^{o} 20′30″E). The samples collected about 200 meters (m) the area which from the factory. Constituents of DDT are the main waste product discharged directly to the near by Bangshi river (Figs. 15 and 16).

2.2.3. Hazaribagh Tannery Industries, Salma Leather Corporation, 56, Sanaton area, Hazaribagh, Dhaka (23^{o} 47′N and 90^{o} 13′30″E). The samples collected from the area which was distant from the factory about 60m. The main waste product is Chromium, Lead, Nickel etc. which drained into the river Buriganga (Figs. 17 and 18).

2.2.4. Monno Ceramic Industries, Nayarhat, Dhamrai, Dhaka (24^{o} 22′N and 90^{o} 26′10″E). The samples collected from the area which was distant from the factory about 50 m. The main waste product is Porcelain clay, Alluminium, Sillicon etc. which drained near by canal situated about 100 m distant from the factory (Figs. 19 and 20).

2.2.5. Acme Laboratory Limited, Tulivita, Dhamrai, Dhaka (24^{o} 23′N and 90^{o} 28′20″E). The samples collected from the area which was distant from the factory about 20m. The main waste product is Alluminium, Magnesium hydroxide etc. which drained into the nearby canal situated about 300 m distant from the factory (Figs. 21 and 22).

2.3. METHODS

2.3.1. Physico-chemical, essential elements, heavy metal analysis of water sample

The high-density 1-liter PVC bottles were used for sample collection and preservation. One was preserved with toluene for regular water analysis and the other preserved with HNO_3 acid for the heavy metal analysis.

2.3.1.1. Hydrogen ion concentration (pH)

Water pH was determined by glass electrode using pH meter and recorded (Model: Jenway instrument, pH meter 3305 Jackson 1967).

2.3.1.2. Electrical Conductivity (EC)

The EC of six water samples was determined by EC meter and recorded (Moled: HANNA HI-8633) in µs/cm Jackson 1967).

2.3.1.3. Total Dissolved Solids (TDS)

A rapid determination of total dissolved solids (TDS) of water samples was determined by TDS meter and recorded (Model: Mettler-Toledo Ag, DH-8603).

2.3.1.4. Dissolved Oxygen (DO)

Dissolved oxygen of water samples was determined by DO meter and recorded (Model: Mettler-Toledo Ag, CH-8603).

2.3.2. Essential elements of water sample

Water samples were collected from 5 different affected areas as well as control area to study the following five essential elements such as – Sulphate (SO_4), Phosphate (PO_4), Iron (Fe), Copper (Cu) and Zinc (Zn). For this experiment,

200 ml of water sample was taken into a beaker and add 10 ml high purity HNO_3 was added. This was to stand overnight for predigestion. After predigestion this solution was heated 140^o C to 180^o C for complete digestion. For sulphate and phosphate different combination were used such as:

(i) Sulphate: 2 ml sample solution + 1 ml 6 N HCl + 2 ml T-twenty solution + 5 ml distilled water = (Total 10 ml). This was kept for 45 minutes at room temperature and the reading was taken.

(ii) Phosphate: 2 ml sample solution + 2 ml solution A (ammonium molibodate) + solution B (anhydrate ammonium metavanadate) + 6 ml distilled water = (Total 10 ml). This solution was vortexed and kept for 35 minutes at room temperature and reading was taken.

Other essential elements such as - Iron, Zinc and Copper in the water samples collected from different affected areas as well as control area were determined by atomic absorption spectrometry (Model: VARIAN-220) following calibration of the equipment for every 10 ml sample include a certified references materials (CRMs) to ensure QA/QC.

2.3.3. Heavy metal of water sample

Five heavy metal, such as – Arsenic (As), Cadmium (Cd), Chromium (Cr), Nickel (Ni) and Lead (Pb) were analyzed in this experiment. The procedure of water sample preparation was described as section of materials and method 2.3.2. The heavy metal of water samples collected from different affected areas as well as control area were determined by atomic absorption spectrometry (Model: VARIAN-220) following calibration of the equipment for every 10 ml sample include a certified references materials (CRMs) to ensure QA/QC.

2.3.4. Soil sample

2.3.4.1. Preparation of soil sample

The soil samples were air dried and initially sieved through a 2.0 mm sieve for primary removing debries and stored for the experiment. A portion of the samples was broken again and sieved through a 0.5 mm sieve for further analysis.

Approximately 1 g of soil sample of each area was placed in a beaker with 15 ml high-purity HNO_3 – $HClO_4$ (3:1) acid mixture and allowed to stand at room temperature for overnight. These were then heated to 140 – 180^o C for complete digestion. Except Sulpher and Phosporous all other essential and heavy metal such as- Arsenic, Lead, Cadmium and Nickel were analyzed by atomic absorption spectrometer (Model:VARIAN-220) following calibration of the equipment for every 10 ml sample include a certified reference material (CRMs) to ensure QA/QC.

2.3.4.2. Essential elements of soil sample

2.3.4.3. Soil sample were collected from 5 different affected areas as well as control area to study these five essential elements, such as - Sulpher, Phosphorous, Iron, Copper and Zinc.

For Sulpher and phosphorous different combination were used, such as:

(i) Sulpher: 2 ml sample solution + 1 ml 6 N HCl + 2 ml T-twenty solution + 5 ml distilled water = (Total 10 ml). This was kept for 45 minutes at room temperature and the reading was taken.

(ii) Phosphorous: 2 ml sample solution + 2 ml solution A (ammonium molibodate) + solution B (anhydrate ammonium metavanadate) + 6 ml distilled water = (Total 10 ml). This solution was vortexed and kept for 35 minutes at room temperature and reading was taken.

Other essential elements such as- Iron, Zinc and Copper content of the soil samples collected from different affected areas as well as control area were determined by atomic absorption spectrometry (Model: VARIAN-220) following calibration of the equipment for every 10 ml sample include a certified references materials (CRMs) to ensure QA/QC.

2.3.4.3. Heavy metal of soil sample

Soil samples were collected from 5 different affected areas as well as control area to study the following five heavy metal, such as - Arsenic, Cadmium, Chromium, Nickel and Lead.

The heavy metals of soil samples were determined by atomic absorption spectrometry (Model: VARIAN-220) following calibration of the equipment for every 10 ml sample include a certified references materials (CRMs) to ensure QA/QC.

2.3.5. Plant sample

2.3.5.1. Plant sample preparation

The roots of plants were washed with deionized distilled water for several times to remove the soil and ions adhering to root. The aerial parts of plants were also washed. The collected plant samples were first air-dried and then oven dried at $70^{o} \pm 5^{o}$ C for 48 hours. The dry weights of plant samples were measured. The dry plant samples were then grinded and passed through a 0.2 mm sieve for further analysis. Approximately 0.5 gm (where available) of each plant sample was placed in a beaker with 15 ml high-purity HNO_3 – $HClO_4$ (3:1) acid mixture and allowed to stand at room temperature for overnight. These were then heated to 140 – 180^{o} C for complete digestion. Plant samples were analyzed for total arsenic by hydride generated atomic absorption

spectrometry (HG-AAS), while lead and cadmium were analyzed by AAS. Certified reference materials were carried through the digestion and analyzed as part of the quality assurance/quality control protocol (following procedures described by Huq and Alam 2005). Reagent blanks and internal standards were used where appropriate to ensure accuracy and precision in the analysis of arsenic.

2.3.5.2. Essential elements of plants sample

Plant samples (*Colocasia esculenta* and *Ipomoea aquatica*) were collected from 5 different affected areas as well as control area to study these five essential elements such as - Sulpher, Phosphorous, Iron, Copper and Zinc.

For Sulpher and phosphorous, different combination were used such as:

(i) Sulpher: 2 ml sample solution + 1 ml 6 N HCl + 2 ml T-twenty solution + 5 ml distilled water = (Total 10 ml). This was kept for 45 minutes at room temperature and the reading was taken.

(ii) Phosphorous: 2 ml sample solution + 2 ml solution A (ammonium molibodate) + solution B (anhydrate ammonium metavanadate) + 6 ml distilled water = (Total 10 ml). This solution was vortexed and kept for 35 minutes at room temperature and reading was taken.

Other essential elements viz. Iron, Zinc and Copper content of the plant samples collected from different affected areas as well as control area were determined by atomic absorption spectrometry (Model: VARIAN-220) following calibration of the equipment for every 10 ml sample include a certified references materials (CRMs) to ensure QA/QC.

2.3.5.2. Heavy metal of plant sample

Plant samples were collected from 5 different affected areas as well as control area to study these five heavy metals such as - Arsenic, Cadmium, Chromium, Nickel and Lead.

The heavy metals of plant samples were determined by atomic absorption spectrometry (Model: VARIAN-220) following calibration of the equipment for every 10 ml sample include a certified references materials (CRMs) to ensure QA/QC.

The chemical analysis were carried out in soil and environmental laboratory, BCSIR, Dhaka.

2.4. Methods - cytogenetical studies

2.4.1. Preparation of reagents

2.4.1.1. Pre-fixative 8-hydroxyquinoline (0.002 M)

0.29 gm of 8-hydroxyquinoline crystals were thoroughly dissolved in a liter of distilled water by constant stirring and kept at below 20°C for future use.

2.4.1.2. Fixative (45% acetic acid)

45 ml of glacial acetic acid was mixed with 55 ml of distilled water.

2.4.1.3. Preservative (70% alcohol)

70 ml of absolute alcohol was mixed with 30 ml of distilled water.

2.4.1.4. Hydrolyzing agent (1N HCl)

36.5 ml of HCl were thoroughly dissolved in a liter of distilled water by constant stirring and kept for future use.

2.4.1.5. Hydrolyzing solution

Two parts of 1N HCl was mixed with one part of 45% acetic acid.

2.4.2. Stains

2.4.2.1. Aceto-orcein (1%)

1g of orcein dye (Sigma) was added to a flask containing 45 ml (100%) acetic acid. These were heated (not boiling) for about 20 h and messed up to 100 ml by adding distilled water. It was then filtered quickly and stored for future use.

2.4.2.2. Chromomycin A_3 (CMA) (0.1 mg/ml)

1mg of chromomycin A_3 (CMA) (Sigma) was thoroughly dissolved in 10 ml of McIlvaine's buffer supplemented with Mg^{+2} by stirring and kept at -20°C for future use.

2.4.2.3. 4′-6 Diamidino-2-Phenylindole (DAPI) (0.01 mg/ml)

1mg 4'-6-Diamidino-2-Phenylindole (DAPI) (Sigma) was thoroughly dissolved in 100ml McIlvaine's buffer (without Mg^{+2}) by stirring and kept at – 20º C for future use.

2.4.3. Buffer

2.4.3.1. McIlvaine's buffer (pH 7.0)

About 59 (58.99) g of $Na_2HPO_4.12H_2O$ (FW 358.14) and 3.71 g citric acid were measured and mixed in one liter distilled water. It was then autoclaved for 10 m and kept at 4ºC for future use.

2.4.3.2. McIlvaine's buffer with Mg^{+2} (pH 7.0)

About 59 (58.99) g of $Na_2HPO_4.12H_2O$ (FW - 358.14) and 3.71 g of citric acid were mixed in one liter of distilled water. After autoclaving for 10 m, 2.46 g of $MgSO_4$ was added to it and kept at 4ºC for future use.

2.4.4. Antibiotics

2.4.4.1. Distamycin A (0.1 mg/ml)

Two mg of distamycin A (Sigma) thoroughly dissolved in 20 ml of McIlvaine's buffer by stirring and kept at -20^{0}C for future use.

2.4.4.2. Actinomycin D (0.25 mg/ml)

Two mg of Actinomycin D (Sigma) thoroughly dissolved in 8 ml of McIlvaine's buffer by stirring and kept at -20^o C for future use.

2.4.5. Photography

A automatic digital canon camera (8 mega pixels Canon power shoot PC1564 model), Magnification-7x, Mode-Auto and Canon PC1251 Magnification-6x, Mode- Portrate) was used.

2.4.6. Procedure for studying mitotic chromosomes

2.4.6.1. Collection of root-tips (RTs)

Roots were collected from the Botanic garden of the Department of Botany, University of Dhaka. The young healthy roots were cut 0.5 cm away from the tip by a clean blade.

2.4.6.2. Pre-treatment

The collected RTs were soaked on a filter paper to remove surface water and pretreated with 8-hydroxyquinoline (0.002 M) for 2.30 h and 30 m in case of *Colocasia esculenta* and *Ipomoea aquatica* respectively.

2.4.6.3. Fixation

RTs were fixed in 45% acetic acid for 15 m and 5 m in case of *Colocasia esculenta* and *Ipomoea aquatica* respectively at 4ºC.

2.4.6.4. Preparation of slide for orcein-staining

The pretreated RTs were hydrolyzed for 10 s and 4 s for *Colocasia esculenta* and *Ipomoea aquatica* respectively at 60ºC in a mixture of 1N HCl and 45% acetic-acid (2:1). Then the hydrolyzed RTs were soaked on a filter paper and taken on a clean slide. The meristematic region was cut with a fine blade. A drop of 2% aceto-orcein was added to the material and kept in an acetic acid chamber for 30m and 2 h for *Colocasia esculenta* and *Ipomoea aquatica* respectively. After that add 1% aceto-orcein and a clean cover glass was placed on the material. At first the materials were tapped gently by a tooth pick and then squashed by placing thumbs. During tapping and squashing care was taken so that the cover glass should not be moved because a minute displacement of it could damage the entire preparation. The slides were observed under Olympus/Nikon microscope.

2.4.7. Preparation of slide for fluorescent staining

2.4.7.1. Preparation of air-dried slides

After hydrolyzing and dissecting, the materials were tapped and squashed with 45% acetic-acid and kept in -80^{o} C freeze for 3 m. The cover glasses were removed quickly and dried in air for at least 24 h before study.

2.4.7.2. CMA-staining

Method proposed by Alam and Kondo (1995) was followed with slight modifications. After 24 h of air drying, the slides were first pre-incubated in McIlvaine's buffer (pH 7.0) for 30 m. At once one drop of 0.1 mg/ml

distamycin-A was added to the materials of slides and a cover-glass placed on it. The slides were kept in a humid chamber for 10 m. Then the slides were washed with distilled water in such a way that the cover glasses removed. The slides were rinsed mildly in McIlvaine's buffer supplemented with $MgSO_4$ for 15 m. Then one drop of chromomycin A_3 (0.1 mg/ml) was added to the materials of slides and a clean cover glass placed on it. The slides were kept in a humid chamber for 12 m. The slides were washed with distilled water in such a way that the cover glasses removed. The slides were treated again for 10 m in McIlvaine's buffer with Mg^{+2} and 10 m in McIlvaine's buffer without Mg^{+2}. Slides were mounted in 50% glycerol and kept at 4º C for over night before observation. These were observed under Nikon (Eclipse 50i) fluorescent microscope with blue violet (BV) filter cassette.

2.4.7.3 DAPI-staining

For DAPI-staining, method proposed by Alam and Kondo (1995) was followed after slight modifications. For direct DAPI-staining, after 48 h of air drying, the slide was first pre-incubated in McIlvaine's buffer (pH 7.0) for 25 m. The slide was treated in 0.25 mg/ml actinomycin-D for 10 m in a humid chamber. After antibiotic treatment, the slide was washed with distilled water in such a way that the cover glass removed. The slide was immersed again in McIlvaine's buffer (pH 7.0) for 15 m followed by treating in DAPI solution (0.1mg/ml) for 12 m. After rinsing in McIlvaine's buffer (pH 7.0) for 10 m, the slide was mounted with 50% glycerol and kept at 4ºC. These were observed under a Nikon (Eclipse 50i) fluorescent microscope with ultra violet (UV) filter cassette.

2.4.8. Determination of centromeric type, relative length and centromeric index

2.4.8.1. Centromeric type

A procedure proposed by Levan *et al.* (1964) for determining centromeric types of chromosomes was followed with slight modification. In this study, the l/s arm ratio (l = length of the longer arm and s = length of the shorter arm) was considered in the following manner:

	Chromosome type (ct)	l/s ratio
(a)	Metacentric (m)	1.00- 1.50
(b)	Sub-metacentric (sm)	1.51- 3.00
(c)	Acrocentric (ac)	3.01- 7.00
(d)	Telocentric (t)	above 7.00

2.4.8.2. Relative length (RL) of chromosomes

The measurement of relative length was done by dividing the length of a particular chromosome with the total length of the diploid complements. It is represented as follows:

$$\text{Relative length (RL)} = \frac{\text{length of a particular chromosome}}{\text{total length of the diploid complements}}$$

2.4.8.3. Centromeric index (CI)

It was measured by the ratio of short arm to total length of that chromosome, expressed as per cent. It can be shown as below:

$$\text{Centromeric index (CI)} = \frac{\text{length of short arm}}{\text{total length of that chromosome}} \text{ X } 100$$

2.4.9. Classification of fluorescent bands

According to the different fluorescent banding patterns observed in this study, bandings were classified as follows:

β = Satellite.
θ = Band in terminal region.
δ = No band.
γ = Band whole chromosome.
α = Band in centromeric region.
π = Band in two terminal region.
λ = Band in long arm.
φ = Band in short arm.
ω = Band in ¾ th region.

2.4.10. Idiogram

To get an accurate measurement of lengths, chromosomes from at least three metaphase plates were measured for each affected area. Their average arm length was used to prepare the idiogram. The chromosomes were arranged gradually from bigger to smaller in length. The short arm placed on the upper side of the axis and long arm on the lower side.

2.4.11. Magnification

Photographs was taken by using 8 mega pixels canon power shoot A720 model with the magnification of 7x at Auto mode. For measuring the magnification, at first the magnification was calculated by multiplying the magnification of objective (100x), tube length (1.25x) and camera lens (5x, 3.5x). From print the final magnification was calculated.

2.5. Method – molecular study (RAPD analysis)

2.5.1. Collection of leaf sample

To extract genomic DNA, young and actively growing fresh leaves were collected from each affected area including control area of the plant sample of *Colocasia esculenta* and *Ipomoea aquatica*. These leaves were cut apart with sterilized scissors and washed well initially in distilled water and then ethanol. These were dried on fresh tissue paper to remove spore of microorganisms and any other sources of foreign DNA.

2.5.2. Preparation of different stock solutions and working solutions used for DNA isolation

For conducting the isolation procedures, the following stock solutions and working solutions were prepared:

2.5.2.1. 1M stock solution of Tris-HCl pH 8.0 (100 ml)

12.14 g of Trizma base (MW=121.14) was dissolved in 75 ml of distilled water. The pH of this solution was adjusted to 8.0 by adding about 5 ml of concentrated HCl in a fume hood. The volume of the solution was adjusted to a total of 100 ml with de-ionized distilled water. Then it was sterilized by autoclaving and stored at 4º C.

2.5.2.2. 0.5 M stock solution of EDTA pH 8.0 (100 ml)

18.61 g of EDTA (EDTA. $2H_2O$, MW = 372.24) was added to 75 ml of distilled water and stirred thoroughly with a magnetic stirrer. Approximately 2 g of NaOH pellets was added to adjust the final pH to 8.0. It may mention that EDTA alone will not dissolve, unless NaOH is added. The final volume of the solution was adjusted to 100 ml by adding sterile de-ionized distilled water. The solution was sterilized by autoclaving and stored at 4º C.

2.5.2.3. 5 M stock solution of NaCl (100 ml)

29.22 g of sodium chloride (NaCl, MW = 58.44) was dissolved slowly (not at once) in 75 ml of distilled water. The total volume of the solution was adjusted to 100 ml with distilled water. The solution was then heated by oven for 15 s and stirred thoroughly on a magnetic stirrer to dissolve NaCl. It was then sterilized by autoclaving and stored at 4º C.

2.5.2.4. β-Mercaptoethanol

β-Mercaptoethanol (Merck, Germany) was obtained as a 14.4 M solution from company and it was stored in a dark bottle at room temperature.

2.5.2.5. Ribonuclease-A stock solution

10 mg RNase-A (Merck, Germany) was dissolved in 1 ml of deionized distilled water and store in -20^{o} C.

2.5.2.6. Tris-HCl Saturated Phenol

The crystal phenol was melted in a water bath at 65º C for 30 m. Melted phenol (100 ml) was added to same volume of Tris-HCl (pH 8.0). It was mixed initially for at least 10 m with a magnetic stirrer and then kept in rest for 5 m. At this stage, two distinct phases are visible, colorless upper phase and colorful lower phase. With the help of a dropper, the upper phase was removed as much as possible. The same procedure was repeated until the pH of the lower phase rose up to 7.8. Repetition for several times was needed. In this experiment, six times repetitions were done which required about 3.5 h for obtaining the pH 7.75. After saturation, the phenol became the half of the initial volume.

2.5.2.7. Phenol: Chloroform: Isoamyl alcohol (25: 24: 1) (100 ml)

50 ml of Phenol, 48 ml of Chloroform and 2 ml of Isoamyl alcohol were added and mixed properly by vortex mixture under a fume hood. The solution was then stored at 4^{o} C. The solution was shaken well before each use.

2.5.2.8. 70% Ethanol (100 ml)

30 ml double distilled water (ddH_2O) was added in 70 ml absolute ethanol.

2.5.2.9. Stock Solution of TE (Tris-HCl EDTA) Buffer pH 8.0 (100 ml)

1 ml of 1 M Tris-HCl was added to 0.2 ml (200 µl) of 0.5 M EDTA. The final volume was adjusted to 100 ml with sterile de-ionized distilled water. The solution was sterilized by autoclaving and stored at 4^{o} C.

2.5.2.10. 3 M Sodium acetate pH 5.2 (100 ml)

40.824 g of sodium acetate was mixed with 70 ml of ddH_2O and adjust the final volume to 100 ml with ddH_2O and sterilized by autoclaving.

2.5.2.11. Extraction Buffer (Homogenization buffer 100 ml)

To prepare extraction buffer the following components with proper concentrations were used. For the economic use of chemicals, different volume of solutions were prepared as in the tabular form given below:

Table 1. Volume of solutions to prepare extraction buffer

Chemicals	Molecular weight	Stock con.	Working con.	Working volume	
				100 ml	1000 ml
CTAB			2%	2 g	20 g
NaCl	58.44	5 M	1.4 M	28 ml	280 ml
EDTA (pH 8)	372.24	0.5 M	20 mM	4 ml	40 ml
Trisbase (pH 8)	121.1	1.0 M	100 mM	10 ml	100 ml
β- Mercaptoethanol		14.4 M	100 mM	700 µl	7 ml

Steps of Extraction Buffer Preparation (100 ml)

- 10 ml of 1 M Tris HCl (pH 8.0) was taken in a 250 ml conical flask
- 28 ml 5 M NaCl was added to it
- 4 ml of 0.5 M EDTA (pH 8.0) was taken in the conical flask
- The mixture was then autoclaved
- After autoclaving 2 g CTAB was added and stirring very carefully.
- 700 µl β-mercaptoethanol was added to prior use and mixed by glass rod
- Adjust all to pH 5 with HCl and make up to 100 ml by adding sterile de-ionized distilled water

2.5.3. Protocol used for genomic DNA isolation

DNA was isolated using the mini preparation CTAB method (Doyle and Doyle 1987) with minor modification. The protocol was as follows:

2.5.3.1. Required equipments

1. Plant Tissue (leaf tissue in this experiment)
2. Autoclave machine
3. Digital balance
4. Beaker

5. Centrifuge Machine with 10,000-14,000 rpm
6. Conical flasks
7. Eppendorf tubes
8. Gloves
9. Ice machine
10. Water bath capable of maintaining 65^{o} C
11. Micropipettes and nuclease free micropipette tips
12. Mortar and pestle
13. pH meter
14. Refrigerator
15. Water de-ionizer
16. Water distillation plant
17. Fume hood

2.5.3.2. Required Chemicals

1. Liquid nitrogen
2. 100% ethanol
3. Tris base
4. EDTA (Ethylene Diamine Tetraacetic Acid)
5. NaCl (Sodium Chloride)
6. CTAB (Cetyl Trimethyl Ammonium Bromide)
7. Sodium acetate
8. PVP (Polyvinyl Pyrrolidone)
9. β-Mercaptoethanol
10. Phenol `
11. Chloroform
12. Isoamyl alchohol
13. Isopropanol
14. 70% ethanol

2.5.3.3. Procedure

1. Freshly harvested leaf tissue of 1.0 g was grinded to fine powder in liquid nitrogen and taken in 2 ml centrifuge tube. One ml extraction buffer was added into 2 ml centrifuge tubes and vortexed for 5-10 s to mix the contents well. The tubes were put into 65º C pre-heated water bath and invert every 5-10 m to allow mixing. The samples were then cooled down to room temperature.

2. The samples were centrifuged at 13,000 rpm for 10 m at room temperature to remove non soluble debris. The supernatant was transferred to fresh tubes.

3 The supernatant was mixed with equal volume of Phenol: Chloroform: Isoamyl alcohol solution (25: 24: 1) by inverting the tubes and centrifuged at 13,000 rpm for 10 m. This process was repeated 2-3 times.

4. The supernatant was taken into another fresh tubes and add 0.1 vol. of 3 M sodium acetate (pH 5.2) followed by 0.6 vol. of 100% chilled Isopropanol and shaken slowly. In this step, DNA became visible as whitish fibrous in the solution.

5. The solution was centrifuged for 10 m at 13,000 rpm at room temperature. The upper layer of the solution was discarded carefully by using adjustable micropipette.

6. The pellet was washed with 70% ice-cold ethanol. This washing step was repeated at least 2-3 times. Residual CTAB was removed by this step. The liquid was poured out and the tubes put on a paper towel for about 1 h at inverted position. Pellets should neither contain residual ethanol, nor allow for too dry. In both the cases, re-dissolving may be difficult.

7. The dried DNA was dissolved in 500 µl of TE buffer and treated with RNase A for 30 m at 37°C and store at -20°C.

2.5.4. Qualification and quantification of isolated DNA

Before PCR amplification it is important to know the concentration of genomic DNA because different DNA extraction methods produced DNA of widely different purity. It is necessary to optimize the amount of DNA for reproducibility and strong signal in PCR assay. Excessive genomic DNA may result smears lack of clearly defined bands on gel. On the other hand, too little DNA will give non-reproducible patterns (Williams *et al.* 1993). Measurement of isolated DNA concentration can be done by comparing DNA with the standard DNA on agarose gel electrophoresis or by estimating the absorbance of DNA by spectrophotometer at 260 nm wave lengths. Both the methods were carried out in this experiment.

2.5.4.1. Measurement of DNA Concentration and Quality by Agarose Gel Electrophoresis

The following equipments and chemicals were used to conduct agarose gel electrophoresis:

1. A horizontal electrophoresis chamber and power supply
2. Gel casting tray and combs
3. Gel documentation system (BioSciTec, Gelscan 6.0 Professional, German)
4. Gloves
5. Pipette and tips
6. 1 kb DNA ladder
7. Electrophoresis buffer (TAE)
8. 6X sample loading buffer
9. Agarose
10. DNA stain (ethidium bromide)

2.5.4.1.1. Preparation of stock solutions used for gel electrophoresis

For conducting the gel electrophoresis, the following stock solutions and other solutions were prepared:

2.5.4.1.1.1. TAE Buffer (50X, pH 8.3.1 litre)

242 g Trizma base (MW=121.14) was dissolved into 900 ml of sterile de-ionized distilled water. Then 57 ml glacial acetic acid was added to the solution. Finally, 100 ml 0.5 EDTA (pH 8.0) was added in it. These were mixed well. The pH of the solution was adjusted by mixing concentrated HCl to pH 8.3. The final volume of the solution was adjusted to 1000 ml.

2.5.4.1.1.2. Loading dye (10X)

For making 10 ml of 10X loading dye, 40 mg of Bromophenol blue (i.e., the final concentration was 0.4%), 40 mg of Xylene cyanol FF (i.e., the final concentration was 0.4%) and 5 ml of 98% glycerol (i.e., the final concentration was 50%) were added to 4 ml of sterile de-ionized distilled water and mixed properly. The final volume was adjusted to 10 ml with sterile de-ionized distilled water and stored at 4°C.

2.5.4.1.1.3. Ethidium bromide solution

For preparing 1ml solution, ethidium bromide (10 mg) was added to 1 ml of sterile de-ionized distilled water. It was then mixed by hand shaking. The solution was then transferred to a dark bottle and stored at room temperature. Stock solution of 10 mg/ml can be purchased directly from companies.

2.5.4.1.2. Preparation of 1% Agarose Gel

For agarose gel electrophoresis, 100 ml of 1% agarose gel was prepared (100 ml was required for 15 ×15 cm tray with 0.5 cm thickness).

The following steps were followed during casting the gel-

1.0 g agarose powder was measured and put in a 250 ml conical flask. 100 ml of 1X TAE buffer was added to the flask. The agarose was melted in a microwave oven for several short intervals until the solution became clear. The solution did not allow boiling for long period. When the agarose solution was cooled to about 50^{o} C (the flask was cooled enough to hold comfortably with bare hand), 5 µl (from 10mg/ml solution) of ethidium bromide was added so that in the gel the concentration of ethidium bromide maintained 0.8 µg/ml (the final concentration of ethidium bromide in the melted agarose solution ranged from 0.5-1.0 µg/ml) and mixed well by gentle shaking.

2.5.4.1.3. Comb Set-up

The ends of the gel casting tray was sealed or fitted with casting dams and the combs placed in the gel casting tray. The melted agarose solution was poured into the casting tray and let it to cool until solid. The comb was removed carefully from the solidified gel. The casting dams or gates were removed from the edges of the gel tray carefully so that the gel did not slide off the tray.

2.5.4.1.4. Preparation of DNA sample for electrophoresis

At first 2 µl 6X loading dye was placed on a piece of parafilm by a micropipette. 10 µl DNA sample (after thawing from frozen stock) was added to it and mixed 2-4 times with the adjustable micropipette.

2.5.4.1.5. Electrophoresis

The gel was placed in the electrophoresis chamber in such a way that the sample wells remained near to the cathode (negative end generally marked as

black). DNA sample would migrate towards the anode (positive end generally marked as red) during electrophoresis.

The gel was covered by sufficient amount of 1x TAE buffer. The volume of the electrophoresis buffer should not above the maximum buffer mark on electrophoresis system. The prepared samples were loaded slowly and allowed to sink to the bottom of the wells. 10 µl of 1 kb DNA ladder (marker) was also loaded at least in one well (generally the first one). Electrophoresis was carried out at 50 volts and 100 mA for 1.5 hours. The separation process was monitored by the migration of the dye on the gel. When the first dye (bromophenol blue) had reached about three-fourths of the gel length, the electrophoresis was completed and stopped.

2.5.4.1.6. Documentation of the DNA sample

After electrophoresis, the gel was taken out carefully from the electrophoresis chamber and placed in gel documentation system (BioSciTec, Gelscan 6.0 Professional, German) for observing the DNA bands.The DNA was observed as band and photographed using gel documentation system. The electrophoregram of DNA samples of six sample *Colocasia esculenta* (L.) Schott and six sample of *Ipomoea aquatica* Forssk. (Figs. 203, 212).

2.5.5. Quantification and qualification of DNA by spectrophotometer

For more confirmation, DNA was also quantified through spectrophotometer. Spectrophotometer is commonly used in laboratories for the measurement of DNA concentration and purity. The DNA concentration was obtained by multiplying the absorbance at 260 nm wave length by a constant. The DNA purity was measured by dividing the absorbance at 260 nm wave length by the absorbance at 280 nm wave length.

Good quality DNA should give the ratio (A260/A280) ranging from 1.8–2.0. The A260/A280 ratio higher than 2.0 and lower than 1.8 generally indicates RNA and protein contamination, respectively during extraction process. Following Sambrook *et al.* (1989), the DNA concentration was calculated (1 O. D.) at 260 nm corresponds to 50 µl/ml of double standard DNA.

2.5.5.1. Set-up the Spectrophotometer

To estimate genomic DNA concentration, absorbance readings of the DNA samples were recorded at 260 nm using a spectrophotometer (Analylikjena, Specord 50, Germany).

At first, the spectrophotometer UV-lamp was turned on. After warming up the wave length was set at 260 nm. After washing the cuvette (the 'zero' or 'blank' cuvette) was filled with 2 ml sterile distilled water and placed on cuvette chamber. The absorbance reading was adjusted to zero for standardization.

2.5.5.2. Preparation of the DNA samples for Spectrophotometry

The test samples were prepared by taking 2µl of each DNA sample in the cuvette containing 1,998 µl sterile distilled water. The samples were mixed well by using an adjustable micropipette. The reading was taken by viewing the monitor of spectrophotometer at 260 nm and 280 nm wave length. After recording the absorbance readings, the cuvette was rinsed out with sterile distilled water, tamped out on a paper and wiped. The absorbance reading for each sample was recorded in the same way. The ratios of spectrophotometric absorption readings at 260 nm of different samples for quality determination of DNA are shown in the Table 2.

2.5.5.3. Calculation for the Concentration of DNA

Before PCR, DNA concentrations were determined according to the following formula:

$$\text{DNA concentration} = A260 \times \text{Dilution factor} \times \text{Conversion factor}$$

$$= A260 \times \frac{\text{Volume of distilled water (µl)}}{\text{Amount of the DNA sample (µl)}} \times 50$$

$$= (\text{ng}/\mu\text{l})$$

$$= (\mu\text{g/ml})[\text{since } 1\mu\text{g} = 10^{-3}\text{ng i.e., } \mu\text{g/ml} = \text{ng}/\mu\text{l}]$$

A260 = spectrophotometric absorbance reading at 260 nm of DNA sample.

Dilution factor = the ratio of distilled water (µl) to amount of DNA sample (µl).

Conversion factor 50 = the 50µg/ml of DNA contained in a solution which gives the spectrophotometric absorbance reading at 260 nm equal to 1.

Note: The calculated DNA concentration expressed in µg/ml gave a fraction. To avoid fraction it was converted into ng/µl (1µg = 10^{-3} ng) and therefore, multiplied with 1000.

Table 2. Spectrophotometric absorbance readings at 260 nm wave length and concentration of DNA for six plant samples each of *Colocasia esculenta* and *Ipomoea aquatica* collected from 5 different affected area as well as control

Plant Samples	Absorbance Reading at 260 nm	Concentration of DNA (ng/μl)
Colocasia esculenta (Control Plant sample)	0.094	4700
Colocasia esculenta (collected from Gazipur area)	0.098	4900
Colocasia esculenta (collected from ACME Laboratory area)	0.082	4100
Colocasia esculenta (collected from Monno Ceramic area)	0.067	3350
Colocasia esculenta (collected from Pesticide area)	0.058	4100
Colocasia esculenta (collected from Hazaribagh area)	0.083	2900
Ipomoea aquatica (Control Plant)	0.044	2200
Ipomoea aquatica (collected from Gazipur area)	0.078	4150
Ipomoea aquatica (collected from ACME Laboratory area)	0.094	4700
Ipomoea aquatica (collected from Monno Ceramic area)	0.098	3900
Ipomoea aquatica (collected from Pesticide area)	0.094	4900
Ipomoea aquatica (collected from Hazaribagh area)	0.082	4100

2.5.6. Amplification of DNA by Polymerase Chain Reaction (PCR) using RAPD Markers

To perform the amplification of RAPD, a single oligonucleotide of arbitrary DNA sequence was mixed with genomic DNA in the presence of a thermostable DNA polymerase with suitable buffer and subjected to temperature cycling conditions typical for the polymerase chain reaction (PCR).

2.5.6.1. Preparation of working solution (25 ng/µl) of DNA samples for PCR reaction

Original stock solution concentration of each DNA sample was adjusted to a unique concentration (25 ng/µl) using the following formula:

$S_1 \times V_1 = S_2 \times V_2$

$V_1 = S_2 \times V_2 / S_1$

Where,

S_1= stock DNA concentration (ng/µl)

V_1= volume require (µl)

S_2= working DNA concentration (ng/µl)

V_2= working volume of DNA solution (µl)

Original stock DNA (2 µl) was taken in a 2 ml eppendorf tube and required amount of TE buffer calculated from the above formula added to it. Calculated required volume of TE buffer for each sample was shown in Table 3.

Table 3. Preparation of working DNA solution (25 ng/µl) for PCR reaction

Plant samples	Working solution (25 ng/µl) for PCR	
	TE buffer/ de-ionized water (µl) required	DNA (µl) required
Colocasia esculenta (Control Plant sample)	374	2
Colocasia esculenta (collected from Gazipur area)	390	2
Colocasia esculenta (collected from ACME Laboratory area)	326	2
Colocasia esculenta (collected from Monno Ceramic area)	266	2
Colocasia esculenta (collected from Pesticide area)	230	2
Colocasia esculenta (collected from Hazaribagh area)	330	2
Ipomoea aquatica (Control Plant)	174	2
Ipomoea aquatica (collected from Gazipur area)	310	2
Ipomoea aquatica (collected from ACME Laboratory area)	374	2
Ipomoea aquatica (collected from Monno Ceramic area)	390	2
Ipomoea aquatica (collected from Pesticide area)	326	2
Ipomoea aquatica (collected from Hazaribagh area)	340	2

2.5.6.2. Primer test

Primarily 8 decamer primers were tested for RAPD amplification of which eight primers exhibited good quality banding patterns and sufficient variability. These eight primers were selected for further analysis. The details of the eight primers were given in Table 4.

Table 4. Eight random primers used in the present study

Primer code	Sequence (5′—3′)	G+C content (%)
OPA-1	5′-CAG GCC CTT C-3′	70
OPA-2	5′-TGC CGA GCT G-3′	70
OPA-3	5′-AGT CAG CCA C-3′	60
OPA-4	5′-AAT CGG GCT G-3′	60
OPA-6	5′-GGT CCC TGA C-3′	60
OPA-8	5′-GTG ACG TAG G-3′	60
Primer-23	5′-GTC AGG GCA A-3′	70
Primer-24	5′-GGT CGG AGA A-3′	60

2.5.6.3. Preparation of primers

The supplied primers were diluted to 100 µM. To make 100 µM of each primer, the following mathematical deduction was followed:

We know,

n = cv (where n= number of mole)
c= molarity i.e. concentration in molarity
v= volume.

Generally, 100 µM concentration of primer has to prepare as main stock solution. In this case, c=100 µM since 53.4 nM of primer was present in the vial used in the investigation, supplied from the company, n= 53.4 nM.

v= Required volume of TE buffer has to add in the supplied vial to make 100 µM main stocks, using the formula-

$$v = \frac{n}{c}$$

$$v = \frac{53.4\ nmol}{100\ \mu M}$$

$$v = \frac{53.4 \times 10^{-9}\ mol}{100 \times 10^{-6}\ M}$$

$$v = \frac{53.4 \times 10^{-5}\ mol}{M}$$

$$v = \frac{53.4 \times 10^{-5}\ mol}{molL^{-1}}$$

$$v = \frac{53.4 \times 10^{-5}}{L^{-1}}$$

$$v = 53.4 \times 10^{-5}\ L$$

$$v = 53.4 \times 10^{-5} \times 1000\ ml$$

$$v = 53.4 \times 10^{-2}\ ml$$

$$v = 53.4 \times 10^{-2} \times 1000\ \mu l$$

$$v = 534\ \mu l$$

Therefore, 534 µl of TE buffer was added to the vial to make 100 µM main stocks. Using the above deduction method, all primer pairs were diluted to 100µM main stock. All primers were diluted to 50 times i.e. 2 µM to make working solution for use.

2.5.6.4. Preparation of PCR Reaction Mixture/ PCR Cocktail

The following components were used to prepare PCR cocktail (Table 5). The total volume of PCR cocktail was 6.2 µl per sample.

Table 5. Component of PCR cocktail (for 15 reactions)

Sl. No.	Reagents	Amount per sample	Total
1	Sterile de-ionized distilled water	18.8 µl	282.0 µl
2	Taq Buffer A 10X (Tris with 15 mM $MgCl_2$)	2.5 µl	37.5 µl
3	Primer (10 µm)	1.0 µl	15.0 µl
4	dNTPs (2.5 mM each)	0.5 µl	7.5 µl
5	Taq DNA Polymerase (5U/µl)	0.2 µl	3.0 µl
6	Template DNA (25 ng/µl)	2.0 µl	30.0 µl
	Total	25.0 µl	375.0 µl

During the experiment, PCR buffer, dNTPs, primers and DNA sample solution were thawed from frozen stocks, mixed well by vortexing and kept on ice. Template DNA (25 ng/µl) were pipetted (2.0 µl) first into PCR tubes (0.5ml) compatible with the thermocycler. For each DNA sample being tested, a pre-mix was prepared in the following order- buffer, dNTPs, DNA template and sterile distilled water. Taq DNA polymerase enzyme was added to the pre-mix. The pre-mix was then mixed well and aliquoted into the tubes containing primers. The tubes were then sealed and placed in a thermocycler and the cycling started immediately.

2.5.6.5. PCR amplification

PCR amplification was done in an oil-free thermal cycler (Biometra UNOII, Germany). The optimum amplification cycle was as follows:

Denaturation/ Annealing/ Extension		Temperature	Time (min.)
	Initial denaturation	94º C	5 minutes
45 cycles	Denaturation at	94º C	1 minute
	Annealing at	34-36º C	30 second
	Extension at	72º C	3 minutes
	Final extension at	72º C	5 minutes

After completion of cycling programme, the reactions were held at 4º C.

2.5.6.6. Electrophoresis of the amplified products and documentation

The amplified products were separated electrophoretically on 1% agarose gel. The gel was prepared using 1.0 g agarose powder containing ethidium bromide 10 µl and 100 ml 1x TAE buffer. Agarose gel electrophoresis was conducted in 1x TAE buffer at 50 Volts and 100 mA for 1.5 hours. 1kb DNA ladder was electrophoresed alongside the RAPD reactions as marker. DNA bands were observed on UV-transilluminator and photographed by a gel documentation system as before.

3. Results

3.1. Water sample

3.1.1. Physico-chemical parameters

Four different physico-chemical parameters such as, hydrogen ion concentration (pH), Electrical Conductivity (EC), Dissolved Oxygen (DO) and Total Dissolved Solids (TDS) were investigated in water samples collected from 5 different affected areas along with control. Except DO the values of other parameters were within the permissible limit (Table 6, Fig. 1). Only water of control area has DO value within the permissible limit. In contrast, the DO values of 5 different affected areas were below the limit. The most vulnerable area was Hazaribagh in respect of DO value.

3.1.2. Essential elements

Five different essential elements such as, Sulphate, Phosphate, Iron, Copper and Zinc were studied in water samples collected from 5 different affected areas along with control. Sulphate, copper and zinc were found within the permissible limit. However, Phosphate and Iron crossed the permissible limit. Highest amount Phosphate and Iron was found in water sample collected from Pesticide area and Hazaribagh area, respectively (Fig. 2, Table 7).

3.1.3. Heavy metals

Arsenic, Cadmium, Chromium, Nickel and Lead were investigated as heavy metal in water samples collected from 5 different affected areas as well as control area. The amount of Chromium, Nickel and Lead were below detection limit (BDL). On the other hand, Arsenic and Cadmium exceeded the permissible limit (Table 8, Figs. 3, 4) in water samples collected from all affected areas. Hazaribagh was found most vulnerable area in respect of Arsenic and Chromium.

3.2. Soil sample

3.2.1. Essential elements

Five different essential elements such as, Sulpher, Phosphorous, Iron, Copper and Zinc were investigated in soil samples collected from 5 different affected areas along with control. These essential elements were within the permissible limit (Table 9).

3.2.2. Heavy metals

Arsenic, Cadmium, Chromium, Nickel and Lead were observed as heavy metal in soil samples collected from 5 different affected and control areas. It was found that except control Arsenic and Chromium exceeded the permissible limit in other affected areas. Whereas Cadmium, Nickel and Lead present within the permissible limit (Table 10, Figs. 5, 6). Highest amount Arsenic was found in the soil sample collected from Pesticide area (Table 10, Figs. 5, 6).

3.3. Plant samples

3.3.1. *Colocasia esculenta* (L.) Schott

3.3.1.1. Essential elements

Five different essential elements *viz.* Sulpher, Phosphorous, Iron, Copper and Zinc were investigated in *Colocasia esculenta* samples collected from 5 different affected areas along with control. Although these essential elements have crossed the permissible limit, not yet in dangerous level (Table 11).

3.3.1.2. Heavy metals

The amount of 5 heavy metals namely Arsenic, Cadmium, Chromium, Nickel and Lead were tested in *Colocasia esculenta* collected from 5 different affected as well as control areas. Except Lead rest of the heavy metals exceeded the permissible

limit of which amount of Arsenic and Cadmium were the highest (Table 13, Figs. 7, 8). Highest amount of Arsenic and Cadmium were found in the plant sample collected from Monno Ceramic area. It was observed that and leaf portion consumed more heavy metal than root (Table 13, Figs. 7, 8).

3.3.2. *Ipomoea aquatica* Forssk.

3.3.2.1. Essential elements

Five different essential elements such as, Sulpher, Phosphorous, Iron, Copper, Zinc were investigated in *Ipomoea aquatica* collected from 5 different affected areas along with control. These essential elements had just crossed the permissible limit (Table 12).

3.3.1.2. Heavy metals

The amount of Arsenic, Cadmium, Chromium, Nickel and Lead were observed in *Ipomoea aquatica* collected from 5 different affected areas as well as control area. Lead was found below detection level (BDL) in the control and samples collected from Acme Laboratory, Monno Ceramic and Pesticide area. Although a little amount of Lead was found in Gazipur and Hazaribagh area, remained within the permissible limit (Table 14, Figs. 9, 10). Maximum amount of Arsenic and Cadmium were found in the plant samples collected from Acme laboratory and Monno Ceramic areas. The leaf portions of these samples consumed more heavy metals than roots (Table 14, Figs. 9, 10).

3.4. Cytological study

3.4.1. Appropriate season for obtaining maximum number of dividing cells from the root-tip cells (RTCs)

About 85% dividing cells were observed throughout the year in the RTCs of *Colocasia esculenta* collected from both affected and control areas. In contrast, the number of dividing cells was very poor in *Ipomoea aquatica* throughout the year.

3.4.2. Appropriate time for obtaining maximum number of dividing cells from the root tip cells

Roots of *Colocasia esculenta* and *Ipomoea aquatica* were collected from five different affected areas and control area from 8.00 a.m. - 12.30 p.m. in every 30 minutes intervals. It was found that 9.30 a.m. to 10.30 a.m. and 10.10 a.m. were the optimum time for obtaining maximum number of dividing cells (~ 80 %) in *Colocasia esculenta* and *Ipomoea aquatica,* respectively.

3.4.3. Appropriate chemical for pretreatment

Different chemicals like 8-hydroxyquinoline, colchicines, cold water and paradichloro benzene (PDB) were tried as prefixatives to find out proper contraction and intact morphology of chromosomes. It was found that 8-hydroxyquinoline solution (0.002 M) for 2.30 h in case of *Colocasia esculenta* and only 30 minute for *Ipomoea aquatica* gave the best results.

3.4.4. Karyotypes and idiograms

On the basis of overall length and centromeric position, somatic chromosomes of *Colocasia esculenta* collected from Monno Ceramic and Acme Laboratory areas could be assembled in 19 pairs (2n = 38) (Figs. 105-106, 171-172). On the other hand, somatic chromosomes of *Colocasia esculenta* collected from Pesticide

(Savar), Hazaribagh (Tannery), Gazipur (Garments) and control (Botanic Garden, D.U.) area could be assembeled into 14 pairs (2n = 28) (Figs. 103-104, 102, 101, 169, 170, 168, 167). The chromosomes of *Ipomoea aquatica* collected from the affected and control areas could be arranged in 15 pairs (2n = 30). From the data, idiograms were made supplementing the karyotypes (Figs. 113-118, 173-178).

3.5. Interphase nuclei

For every staining, at least 60 interphase nuclei were observed for each sample area.

3.5.1. Orcein staining

Aggregation of heterochromatins were found in the interphase nuclei of *Colocasia esculenta* collected from Hazaribagh and Monno Ceramic areas (Figs. 26, 27). Whereas these were homogeneously distributed in the interphase nuclei of *Colocasia esculenta* collected from Acme Laboratory and Pesciticde areas (Figs 28, 25.). No prominent heterochromatic region was found in the interphase nuclei of this species collected from Gazipur affected area (Fig. 24). In contrast, 6-8 big and darkly stained heterochromatin blocks were found in the interphase nuclei of control plant (Fig. 23).

Ten to twelve darkly stained heterochromatin bodies were found in the interphase nuclei of control *Ipomoea aquatica* (Fig. 29). However, no prominent stained heterochromatin was found in the plant samples collected from different affected areas (Figs. 30-34).

3.5.2. CMA-staining

Six to eight fluoresced bands and 2 non-staining regions were observed in the nuclei of *Colocasia esculenta* control plant (Fig. 35). In the sample collected from Gazipur area, a big fluorescent area was observed (Fig. 36). No prominent band was found in the interphase nuclei of the samples collected from Pesticide,

Hazaribagh, Monno Ceramic and Acme Laboratory areas. Several non staining regions were found in each nuclei of these samples (Figs. 37-40).

Two brightly and 2 lightly stained CMA positive bands were observed in the interphase nuclei of *Ipomoea aquatica* (control) and sample collected from Pesticide area (Figs. 41. 43). At least 4-6 brightly stained round bands were observed in the interphase nuclei of plant samples collected from Gazipur, Hazaribagh, Monno Ceramic and Acme Laboratory areas (Figs. 42, 44, 45, 46).

3.5.3. DAPI-staining

In *Colocasia esculenta*, no band was found in the interphase nuclei of the control plant and samples collected from Gazipur, Pesticide, Hazaribagh and Acme laboratory areas. Three to four non staining regions were found in the interphase nuclei of these samples (Figs. 47-50, 52). A number of brightly stained round dot like bands was aggregated of sample collected from Monno Ceramic areas (Fig. 51).

Few brightly stained areas were scattered within the nuclei of *Ipomoea aquatica* samples collected from Gazipur, Pesticide and Hazaribagh areas (Figs. 54-56). On the other hand, no such band was observed in the interphase nuclei of plant samples collected from Monno Ceramic, Acme Laboratory and control areas (Figs. 57, 58, 53).

3.6. Prophase chromosomes

3.6.1. Orcein staining

The prophase chromosomes of *Colocasia esculenta* collected from control, Gazipur, Hazaribagh and Monno Ceramic were stained uniformly along the length (Figs. 59, 60, 62, 63). However, prophase chromosomes of sample plants collected from the Pesticide and Acme Laboratory areas stained darkly at the interstitial regions of prophase chromosomes (Figs. 61, 64).

The prophase chromosomes of *Ipomoea aquatica* collected from control area were stained homogeneously along the length (Fig. 65). Darkly stained areas were found within the interstitial regions of prophase chromosomes of the samples collected from Hazaribagh area (Fig. 68). On the other hand, the prophase chromosomes of the samples collected from the rest of affected areas were stained darkly at one end and then gradually faint to the other end (Figs. 66, 67, 69, 70).

3.6.2. CMA-staining

Several CMA bands were found in the prophase chromosomes of *Colocasia esculenta* collected from control, Hazaribagh and Acme laboratory areas (Figs. 71, 74, 76). No band was found in the prophase chromosomes of samples collected from rest of the affected areas (Figs. 72, 73, 75).

Few prominent CMA bands were found in the prophase chromosome of *Ipomoea aquatica* samples collected from Gazipur, Hazaribagh and Monno Ceramic areas (Figs. 78, 80, 81). The prophase chromosomes of samples from other affected and control areas were dully fluoresced (Figs. 82, 79, 77).

3.6.3. DAPI-staining

No prominent DAPI band was found in the prophase chromosomes of all *Colocasia esculenta* samples (Figs. 83-88). In contrast, prophase chromosomes of *Ipomoea aquatica* collected from control and different affected areas were fluoresced brightly (Figs. 89-94).

3.7. Metaphase chromosomes

3.7.1. Orcein staining

Total length, l/s ratio, centromeric index, relative length and centromeric type of each chromosomes of *Colocasia esculenta* and *Ipomoea aquatica* collected from

five different affected area as well as control area were tabulated (Tables 15-20 and 24-29).

Colocasia esculenta

In *Colocasia esculenta* samples (collected from Monno Ceramic and Acme Laboratories area), 2n=38 chromosomes were observed (Figs. 99, 100). The total length of diploid chromosome complements (61.31µm), centromeric formula (30m+8sm) and range of individual chromosomal length (0.92-2.30µm) of the above two samples were similar (Table 21). The smaller ten chromosomes of both the samples were almost in similar size. The length of largest chromosome was almost double to that of the smallest one, although there was a gradual decrease in chromosomal length (Figs. 105, 106, 171, 172, Table 21).

In contrast, 2n= 28 chromosomes were found in the plant samples collected from other four areas (Figs. 95-98). Total length of diploid complements and range of individual chromosomal length were smallest in the specimen collected from Pesticide area, whereas these parameters were more or less similar in other samples (Table 21). The centromeric formulae of all these plant samples were same, i.e. 20m+8sm (Table 21).

Ipomoea aquatica

All the samples of *Ipomoea aquatica* were found to possess 2n = 30 chromosomes. The 30 chromosomes were metacentric (Figs. 107-112). The total length of 2n chromosome complements were more or less similar except samples from Acme laboratory areas (Table 30). The individual chromosome length was almost similar all the samples. No gradual decreased in the chromosomal length was observed (Figs. 113-118, 173-178, Table 30).

3.7.2. CMA-staining

Colocasia esculenta

In control plant sample, 14 CMA-positive bright bands were observed (Fig. 119). The bands were distributed in different locations of chromosomes. Both members of pair III, XIV and a member of pair XIII were entirely fluoresced. Two terminal bands were found at the short arms one in each member of pair II and VIII. A member of chromosome pair IV and both the members of pair XII fluoresced about $3/4^{th}$ of length (Figs. 125, 179). A band in short arms was found in each member of pair X. The total length of CMA banded region was 11.15 µm which covered about 17.25% of the total chromatin length. The CMA banded karyotype formula of this control plant was 5γ+ 4θ+3ω+2φ+14δ (Table 22). No CMA negative band was found.

Neither CMA-positive nor negative band was found in the sample plants collected from Gazipur affected area (Fig. 120). However, a CMA-positive satellite was found at the short arm in both the members of pair I (Figs. 126, 180 arrow). The total length of CMA banded region was 0.69 µm which covered about 1.09% of the total chromatin length. The CMA – positive banded karyotype formula of this Gazipur plant sample was 2β+26δ (Table 22).

Plant sample collected from Monno Ceramic area had two CMA-positive bands, one at the short arm in a member of pair VIII and other in pair XVI (Figs. 123, 129, 183 arrow). The total length of CMA banded region was 0.57 µm which covered about 0.94% of the total chromatin length. The CMA banded karyotype formula of this control plant was 2φ+36δ (Table 22). No CMA negative band was found.

Four CMA-positive bright bands and two CMA-positive satellites were observed in plant samples collected from Acme laboratory area (Fig. 124). A small terminal

band was found in both the members of pair XII and a member of pair XV. On the other hand, a thick band was present on the long arm in a member of pair III that occupied almost the length of long arm. Moreover, a pair of CMA-positive satellites was found in both the members of pair VI (Figs. 130, 184 arrows). The total length of CMA banded region was 1.72 µm which covered about 2.82% of the total chromatin length. The CMA – positive banded karyotype formula of this plant sample was 1λ+ 2β+ 3θ+32δ (Table 22). No CMA negative band was found.

Plant sample of *Colocasia esculenta* collected from Pesticide and Hazaribagh area had no CMA positive and negative band (Figs. 121, 122, 127, 128, 181, 182 and Table 22).

Ipomoea aquatica

Five CMA-positive bright bands were observed in the control plant (Fig. 131). The bands were distributed in different locations of different chromosomes. One terminal dot like band was found in a member of pair I, no band found in its homologue. Both the members of pair VIII had a terminal band. A member of pair III was fluoresced entirely and another one had a thick terminal band (Figs. 137, 185). The total length of CMA banded region was 2.30 µm which covered about 8.93% of the total chromatin length. The CMA banded karyotype formula control plant was 4θ+1γ+25δ (Table 31). No CMA negative band and satellite was found.

Six chromosomes were entirely fluoresced in the plant samples collected from Gazipur affected area (Fig. 132). Both members of pair II, VIII and XII were entirely fluoresced (Figs. 138, 186). The total length of CMA banded region was 2.99 µm which covered about 12.15% of the total chromatin length. The CMA – positive banded karyotype formula of this plant sample was 6γ+ 24δ (Table 31). No CMA negative band was found.

Seven chromosomes of plant sample collected from Pesticide area showed CMA-positive bands (Fig. 133). A terminal bands was found at the short arm in each member of pair II. Only one member of pair X and both member of pair XII and XIV were entirely fluoresced (Figs. 139, 187). The total length of CMA banded region was 4.14 µm which was about 23.52% of the total chromatin length. The CMA banded karyotype formula of this control plant was 2φ+5γ+23δ (Table 31). No CMA negative band was found.

Four CMA-positive bands were found in the sample collected from Hazaribagh area (Fig. 134). Only one member of pair I had a terminal band on the short arm. In pair III, one member was entirely fluoresced. Both members of pair IV had a band at the centromeric region (Figs. 140, 188). The total length of CMA banded region was 2.00 µm which was about 8.05% of the total chromatin length. The CMA banded karyotype formula of this sample plant was 1θ+1γ+2α+26δ (Table 31). No CMA negative band was found.

Plant samples collected from Monno Ceramic area were found to possess 10 CMA-positive bright bands (Fig. 135). The bands were distributed in different locations of different chromosomes. A band was found at the terminal region in the both members of pair IX and pair III. Both members of pair XI, XIV and XV were entirely fluoresced (Figs. 141, 189). The total length of CMA banded region was 3.22 µm which was about 15.81% of the total chromatin length. The CMA – positive banded karyotype formula of this sample was 4φ+6γ +20δ (Table 31). No CMA negative band was found.

Six CMA-positive bright bands were observed in the plant sample collected from Acme laboratory area (Fig. 136). A terminal band was found on the short arm in each member of pair V. Both the members of pair VI and XIV were entirely fluoresced (Figs. 142, 190). The total length of CMA banded region was 2.76 µm which covered about 9.02% of the total chromatin length. The CMA – positive

banded karyotype formula of this sample was 4γ+ 2θ+24δ (Table 31). No CMA negative band was found.

3.7.3. DAPI-staining

Colocasia esculenta

Plant samples collected from control, Gazipur and Pesticide area had no DAPI-positive or negative band (Figs. 143-145, 149-151, 191-193).

Plant samples collected from Hazaribagh area had 5 DAPI-positive bands (Fig. 146). Both the members of pair X and pair XIV were fluoresced entirely. A member of pair III possessed a terminal band on the short arm (Figs. 152, 194). The total length of DAPI banded region was 6.55 µm which was about 9.54% of the total chromatin length. The DAPI-positive karyotype formula of this sample was 4γ+ 1θ +23δ (Table 23).

Nine DAPI positive bands were found in the samples collected from Monno Ceramic area (Fig. 147). Both the members of pair IX and XII were entirely fluoresced. One centromeric band was observed in each member of pair VIII. A terminal band on short arms was found in a member of pair XV and XIX. One member of pair X had a band on both the terminal ends whereas no such band was found in its homologue (Figs. 153, 195 arrow). The total length of DAPI banded region was 5.29 µm which covered about 8.62% of the total chromatin length. The DAPI-positive karyotype formula of this sample was 4γ+2α+ 2θ+1π+29δ (Table 23).

Plant samples collected from Acme laboratory area had in total 4 DAPI positive bands (Fig. 148). One small dot like band was found at the terminal region of the short arm in a member of pair X. No band was found in its homologue. A member of pair XVI and both the member of pair XV were entirely fluoresced (Figs. 154, 196). The total length of DAPI banded region was 4.83 µm which covered about

7.91% of the total chromatin length. The DAPI-positive karyotype formula of this sample was 3γ+ 1θ+ 34δ (Table 23).

Ipomoea aquatica

Plant samples collected from the control area had 6 DAPI-positive bands (Fig. 155). A thick DAPI positive upper terminal band was found in both the members of pair I. One relatively thin upper terminal band was found in both members of pair VII. A centromeric dot like band was observed in a member of pair XI, whereas the other member of this pair was entirely fluoresced (Figs. 161, 197 arrow). The total length of DAPI banded region was 2.41 µm which was about 9.37% of the total chromatin length. The DAPI-positive karyotype formula of this sample was 4θ+1α+1γ+24δ (Table 32).

Plant samples collected from Gazipur area were found to possess 4 DAPI-positive bands (Fig. 156). Both the members of pair III and XI were entirely fluoresced (Figs. 162, 198). The total length of DAPI banded region was 2.07 µm which covered about 8.41% of the total chromatin length. The DAPI-positive karyotype formula of this sample was 4γ+26δ (Table 32).

Plant samples collected from Pesticide area had in total 7 DAPI-positive bands (Fig. 157). A centromeric band was observed in a member of pair IV and in both members of pair VIII. A member of pair V and XIII and both members of pair XIV were fluoresced entirely (Figs. 163, 199). The total length of DAPI banded region was 2.41 µm which was about 13.72% of the total chromatin length. The DAPI-positive karyotype formula of this sample was 3α+4γ+23δ (Table 32).

In total 8 DAPI-positive bands were found in plant samples collected from Hazaribagh area (Fig. 158). A member of pair III was entirely fluoresced. On the other hand, both the members of pair XIII and XIV fluoresced almost along the length. A dot like centromeric band was found in both the members of pair VI. A

thick and prominent centromeric band was found in only a member of pair XII, whereas no such band present in its homologue (Figs. 164, 200 arrow). The total length of DAPI banded region was 2.87 µm which was about 11.57% of the total chromatin length. The DAPI-positive karyotype formula of this sample was 5γ+3α+22δ (Table 32).

Four DAPI positive bands were found in the plant samples collected from Monno Ceramic area (Fig. 159). Both the members of pair IV had one DAPI positive band at the centromeric region. A member of pair III and XIV was entirely fluoresced (Figs. 165, 201). The total length of DAPI banded region was 1.49 µm which was about 7.34% of the total chromatin length. The DAPI-positive karyotype formula of this was 2γ+2α+26δ (Table 32).

Two DAPI positive bands were found in plant samples collected from Acme laboratory area (Fig. 160). Both the member of pair I were almost fluoresced along the length (Figs. 166, 202). The total length of DAPI banded region was 1.38 µm which covered about 4.51% of the total chromatin length. The DAPI-positive karyotype formula of this sample was 2γ+ 28δ (Table 32).

3.8. RAPD analysis

Colocasia esculenta

Eight primer combinations namely OPA-1, OPA-2, OPA-3, OPA-4, OPA-6, OPA-8, Primer-23 and primer-24 were used for RAPD analysis of six plant samples of *Colocasia esculenta* (L.) Schott. Each primer combination showed different banding patterns. The primer wise RAPD analysis of six plant samples of *Colocasia esculenta* (L.) Schott were described below:

3.8.1. Primer OPA-1 (5′-CAG GCC CTT C-3′)

A band of 12000 bp was found in all samples of *Colocasia esculenta* including control. Another band of 1500 bp was observed in all samples except control. Only control and Gazipur samples showed a band of 2000 bp which was absent in other samples (Fig. 204, Table 33).

3.8.2. Primer OPA-2 (5′-TGC CGA GCT G -3′)

A fragment of 12000 and 2000 bp were common in all samples. The fragment of 2000 bp of control plant was brighter than that of other samples (Fig.205, Table 34).

3.8.3. Primer OPA-3 (5′- AGT CAG CCA C -3′)

All the samples showed a common band of 12000 and 1500 bp. Bands of 12000 bp in Gazipur sample was brightest. On the other hand, 1500 bp fragment of sample collected from Acme Laboratory and Monno Ceramic area were brighter (Fig.206, Table 35).

3.8.4. Primer OPA-4 (5′-AAT CGG GCT G-3′)

A band of 12000 bp was common in all samples. Whereas 3000 bp band was only absent the sample collected from Hazaribagh area. This sample showed polymorphism in respect of 3000 bp fragment (Fig. 207, Table 36).

3.8.5. Primer OPA-6 (5′-GGT CCC TGA C-3′)

A band of 12000 bp and 1500 bp was found in all samples of *Colocasia esculenta*. However, 1500 bp band of samples from Acme Laboratory and Monno Ceramic area were brighter (Fig. 208, Table 37).

3.8.6. Primer OPA -8 (5′-GTG ACG TAG G -3′)

All the samples had a band of 12000 bp. A band of 1500 bp was present in all the samples except Hazaribagh. All bands were lightly stained except 1500 bp of control (Fig. 209, Table 38).

3.8.7. Primer-23 (5′-GTC AGG GCA A-3′)

A fragment of 1500 and 1700 bp was unique in the samples collected from Pesticide and Hazaribagh area, respectively. These bands were absent in other samples. A light band of 800 bp and a bright band of 1200 bp were common in control, Gazipur and Acme Laboratory area. Samples of Monno area had only a light band of 800 bp. Moreover, a band size of 800 bp was absent in the sample of Hazaribagh and Pesticide areas. (Fig. 210, Table 39).

3.8.7. Primer-24 (5′-GGT CGG AGA A-3′)

No band was found in samples of Pesticide area. A band of 1100 and 800 bp was unique in the samples collected from Gazipur and Hazaribagh area, respectively. Contorl sample had only a band of 1500 bp. Samples of Acme laboratory and Monno Ceramic area had similar bands of 1000 and 600 bp (Fig. 211, Table 40).

Ipomoea aquatica

Eight primer combinations namely OPA-1, OPA-2, OPA-3, OPA-4, OPA-6, OPA-8, Primer-23 and primer-24 were used for RAPD analysis of six plant samples of *Ipomoea aquatica* Forssk. Each primer combination showed different banding patterns. The primer wise RAPD analysis of six plant samples of *Ipomoea aquatica* Forssk were described below:

3.8.1. Primer OPA-1 (5′-CAG GCC CTT C-3′)

Samples of Hazaribagh showed no bands in this primer. The other samples had only a band of 12000 bp (Fig. 213, Table 41).

3.8.2. Primer OPA-2 (5′-TGC CGA GCT G -3′)

A band of 12000 bp was found in control, Acme Laboratory, Monno Ceramic and Pesticide ares. On the other hand a fragment of 1400 bp was found in Gazipur, Acme Laboratory and Monno Ceramic ares. In contrast no band was found in Hazaribagh area (Fig. 214, Table 42).

3.8.3. Primer OPA-3 (5′- AGT CAG CCA C -3′)

A light band of 11000 bp was found in all sample, except Monno Ceramic area (Fig. 215, Table 43).

3.8.4. Primer OPA-4 (5′-AAT CGG GCT G-3′)

A band of 12000 bp was common in all samples. In the samples collected from Acme Laboratory area had a band of 3000 bp in addition to the former band. The fragment of 3000 bp was unique for this sample since absent in other samples (Fig.216, Table 44).

3.8.5. Primer OPA-6 (5′-GGT CCC TGA C-3′)

No band was found in sample of control, Gazipur and Pesticide area with this primer. However, a band of 11000 bp was common in the sample of Acme laboratory, Monno Ceramic and Hazaribagh area. (Fig. 217, Table 45).

3.8.6. Primer OPA -8 (5′-GTG ACG TAG G -3′)

No band was found in any sample with this primer (Fig. 218, Table 46).

3.8.7. Primer-23 (5′-GTC AGG GCA A-3′)

Fragment size of 1200 bp was common in samples of control, Gazipur, Acme Laboratory and Hazaribagh area. In addition to this, a band of 1700 bp was present in control and Gazipur samples. On the other hand, another band of 800 bp was found in Gazipur and Acme Laboratory area. No band was observed in samples of Monno Ceramic and Pesticide area (Fig. 219, Table 47).

3.8.8. Primer-24 (5′-GGT CGG AGA A-3′)

Only a light band of 1500 bp and a bright band of 1200 bp was found in control and samples of Acme Laboratory area, respectively. No band was found in other samples (Fig. 220, Table 48).

Table 6. Comparative study of physico-chemical parameters of water samples collected from control and different affected areas

Control/affected areas	Sample ID	pH	EC (µS/cm)	DO (mg/l)	TDS (mg/l)
Control	WCTR	7.15	371	6.40	187.80
Gazipur Garments	WGAZ	7.34	1438	4.10	719.00
ACME Lab.	WACME	7.07	765	3.04	381.00
Monno Ceramics	WMONNO	7.09	353	5.63	174.02
Savar Pesticides	WPSTD	6.54	495	4.23	246.00
Hazaribagh Tannery	WHAZ	7.40	1230	0.88	624.00
*Permissible limit	-	6.50 – 8.50	700 – 3000 (µS/cm)	6 and above	500 – 1500 mg/l

*Source: Guide to the environmental conservation at 1995 and rules 1997.

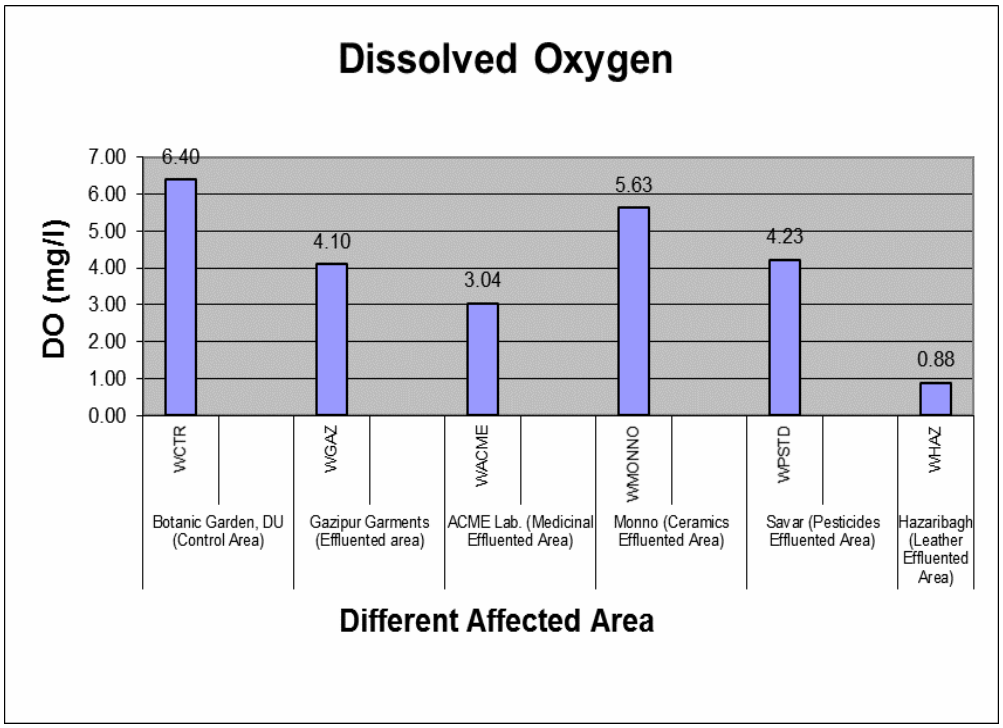


Fig. 1. Dissolved Oxygen concentration (mg/l) in water samples collected from control and different affected areas.

Table 7. Comparative study of essential elements of water samples collected from control and different affected areas

Control/affected areas	Sample ID	Sulfate (SO_4) mg/l	Phosphate (PO_4) mg/l	Iron (Fe) mg/l	Copper (Cu) mg/l	Zinc (Zn) mg/l
Control	WCTR	4.40	BDL	0.25	0.05	BDL
Gazipur Garments	WGAZ	8.85	3.70	1.14	0.24	2.50
ACME Lab.	WACME	115.06	6.60	2.10	BDL	1.50
Monno Ceramics	WMONNO	102.51	14.30	0.10	BDL	2.00
Savar Pesticides	WPSTD	105.88	39.70	1.70	BDL	0.23
Hazaribagh Tannery	WHAZ	20.90	8.50	3.54	1.43	5.01
*Permissible limit	-	200 mg/l	6.0 mg/l	0.30 mg/l	1.0 mg/l	5.0 mg/l

*Source: Guide to the environmental conservation at 1995 and rules 1997.

BDL = Bellow detection limit.

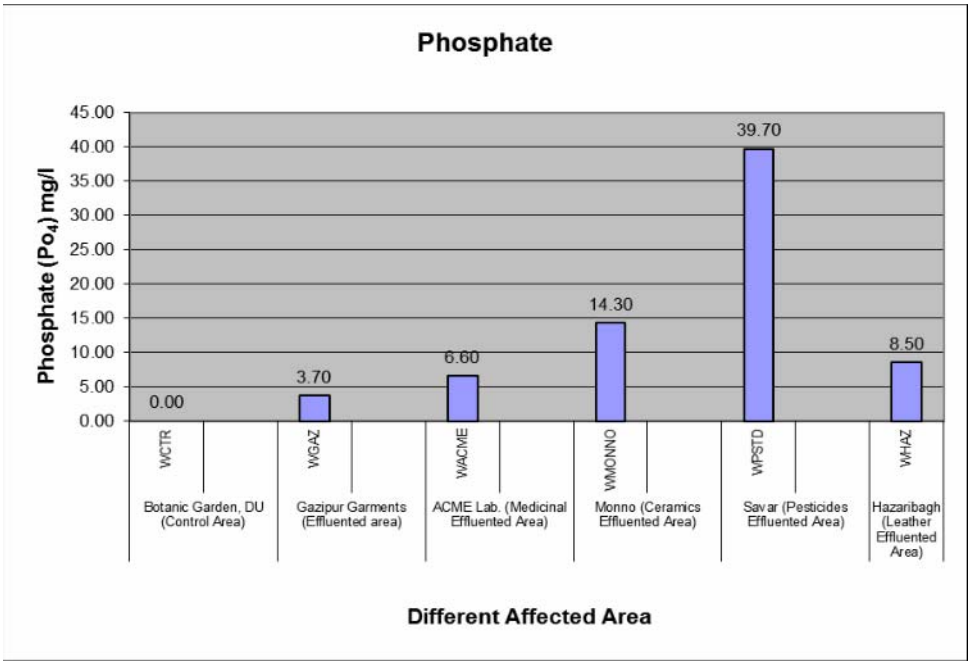


Fig. 2. Phosphate concentration (mg/l) in water samples collected from control and different affected areas.

Table 8. Comparative study of heavy metal analysis of water samples collected from control and different affected areas

Control/affected areas	Sample ID	Arsenic (As) mg/l	Cadmium (Cd) mg/l	Chromium (Cr) mg/l	Nickel (Ni) mg/l	Lead (Pb) mg/l
Control	WCTR	0.06	BDL	BDL	BDL	BDL
Gazipur Garments	WGAZ	2.04	0.09	0.05	BDL	BDL
ACME Lab.	WACME	1.00	0.01	BDL	BDL	BDL
Monno Ceramics	WMONNO	0.92	0.02	BDL	BDL	BDL
Savar Pesticides	WPSTD	0.95	0.01	BDL	BDL	BDL
Hazaribagh Tannery	WHAZ	3.26	0.11	0.08	BDL	0.07
*Permissible limit	-	0.05 mg/l	0.005 mg/l	0.01 mg/l	0.1 mg/l	0.05 mg/l

*Source: Guide to the environmental conservation at 1995 and rules 1997.

BDL = Bellow detection limit.

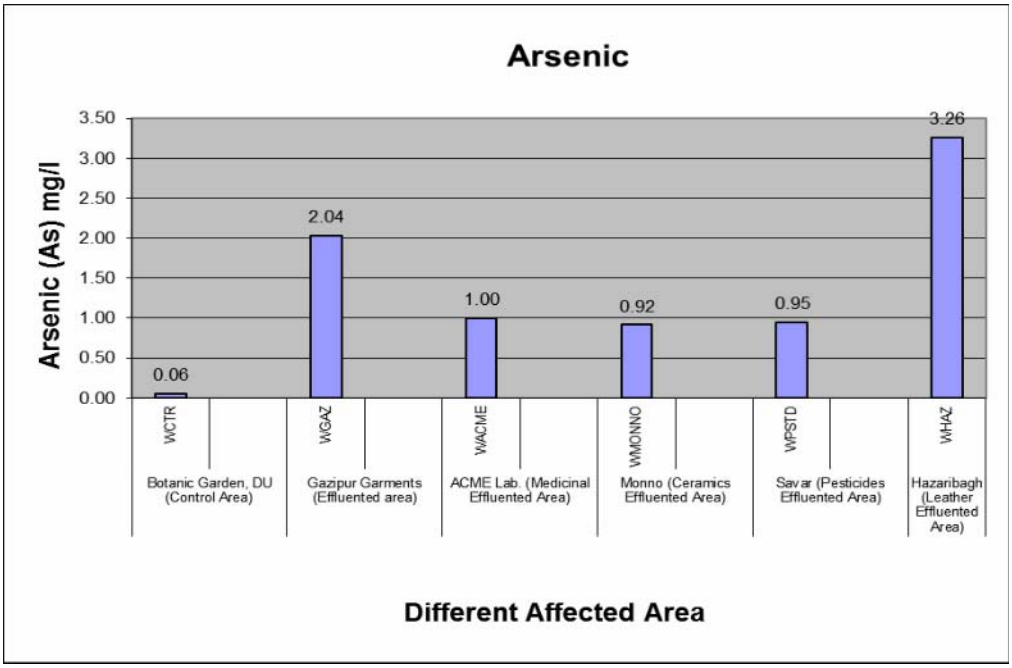


Fig. 3. Arsenic concentration (mg/l) in water samples collected from control and different affected areas.

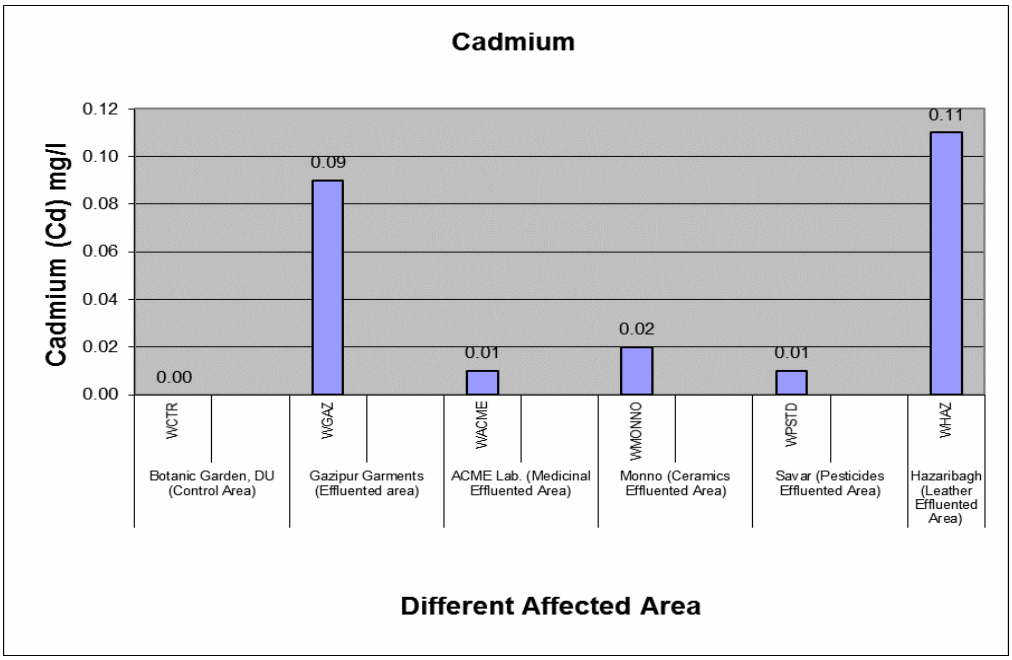


Fig. 4. Cadmium concentration (mg/l) in water samples collected from control and different affected areas.

Table 9. Comparative study of essential elements in soil samples collected from control and different affected areas

Control/affected areas	Sample ID	Sulpher (S) ppm	Phosphorous (P) ppm	Iron (Fe) ppm	Copper (Cu) ppm	Zinc (Zn) ppm
Control	SCTR	4,324.62	778.13	1,076.58	31.03	269.11
Gazipur Garments	SGAZ	4,275.00	1,320.00	11,920.00	18.19	198.00
ACME Lab.	SACME	10,153.85	1,230.77	229.81	23.15	1,185.71
Monno Ceramics	SMONNO	3,071.87	1,125.39	1,057.77	38.60	376.83
Savar Pesticides	SPSTD	112.35	926.23	148.36	22.98	875.39
Hazaribagh Tannery	SHAZ	3,656.66	453.59	739.59	93.66	1,115.24
*Permissible limit	-	3 - 8200 ppm	20 - 6000 ppm	100 - 21000 ppm	2.5 – 60 ppm	1.5 - 2000 ppm

*Source: Guide to the environmental conservation at 1995 and rules 1997.

Table 10. Comparative study of heavy metal analysis of soil samples collected from control and different affected areas

Control/affected areas	Sample ID	Arsenic (As) ppm	Cadbium (Cd) ppm	Chromium (Cr) ppm	Nickel (Ni) ppm	Lead (Pb) ppm
Control	SCTR	15.37	BDL	1,155.36	114.84	23.87
Gazipur Garments	SGAZ	51.9	BDL	2,113.90	316.43	53.9
ACME Lab.	SACME	52.89	0.01	3,222.38	428.51	85.36
Monno Ceramics	SMONNO	48.33	0.49	2,219.75	231.74	31.51
Savar Pesticides	SPSTD	62.34	0.01	2,018.91	347.86	119.85
Hazaribagh Tannery	SHAZ	47.09	0.49	5618.00	511.70	138.69
*Permissible limit	-	0.1 - 40 ppm	0.01 - 0.7 ppm	5 – 3000 ppm	10 – 1000 ppm	2 – 200 ppm

*Source: Guide to the environmental conservation at 1995 and rules 1997.

BDL = Bellow detection limit.

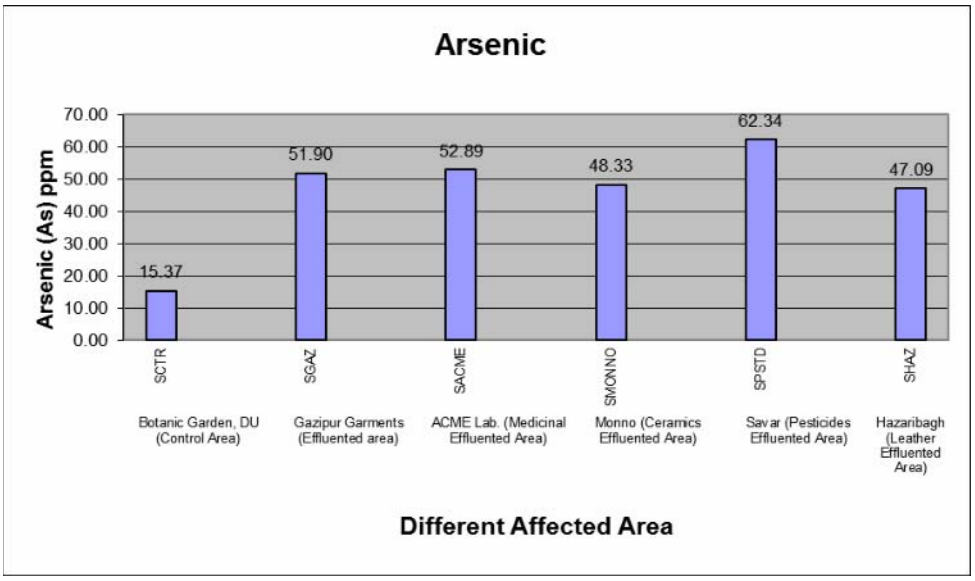


Fig. 5. Arsenic concentration (ppm) in soil samples collected from control and different affected areas.

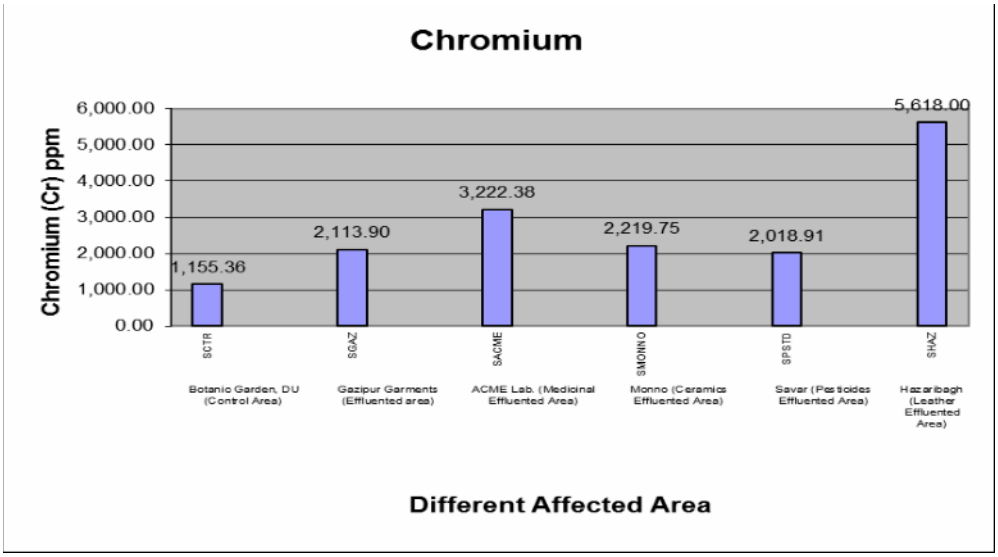


Fig. 6. Chromium concentration (ppm) in soil samples collected from control and different affected areas.

Table 11. Comparative study of essential elements of plant samples [*Colocasia esculenta* (L.) Schott] collected from control and different affected areas

Control/affected areas	Sample ID	Sulpher (S) ppm	Phosphorous (P) ppm	Iron (Fe) ppm	Copper (Cu) ppm	Zinc (Zn) ppm
Control	CCTR (from root)	2,795.20	1,540.59	166.26	3.76	40.31
	CCTR (from leaf)	3,477.01	2,509.62	238.54	4.54	93.21
Gazipur Garments	CGAZ (from root)	1,870.00	1,125.00	100.00	6.40	32.00
	CGAZ (from leaf)	2,079.00	2,140.00	100.00	4.44	42.22
ACME Lab.	CACME (from root)	5,740.00	1,700.00	82.00	8.62	38.84
	CACME (from leaf)	3,382.35	2,394.12	32.35	10.47	40.74
Monno Ceramics	CMONNO (from root)	1,944.44	1,481.48	92.59	10.00	44.76
	CMONNO (from leaf)	2,631.58	2,008.77	102.11	13.33	57.00
Savar Pesticides	CPSTD (from root)	3,550.00	2,080.00	190.00	8.12	28.56
	CPSTD (from leaf)	4,711.54	2,230.77	121.15	11.31	45.10
Hazaribagh Tannery	CHAZ (from root)	4,142.23	1,763.16	114.74	7.26	13.92
	CHAZ (from leaf)	4,937.24	2,568.63	199.24	5.74	31.84
	CHAZ (from shoot)	3,780.36	2,003.72	184.67	8.96	24.67
*Permissible limit	-	1000 ppm	2000 ppm	100 ppm	6 ppm	20 ppm

*Source: Guide to the environmental conservation at 1995 and rules 1997.

Table 12. Comparative study of essential elements of plant samples (*Ipomoea aquatica* Forssk.) collected from control and different affected areas

Control/affected areas	Sample ID	Sulpher (S) ppm	Phosphorous (P) ppm	Iron (Fe) ppm	Copper (Cu) ppm	Zinc (Zn) ppm
Control	ICTR (from root)	947.91	2,259.62	179.25	5.56	80.10
	ICTR (from leaf)	1,465.06	2,430.00	184.34	4.29	80.30
Gazipur Garments	IGAZ (from root)	1,339.00	1,220.00	40.00	11.20	40.10
	IGAZ (from leaf)	1,241.00	2,680.00	42.00	9.76	56.22
ACME Lab.	IACME (from root)	1,166.67	1,806.56	127.78	8.98	17.56
	IACME (from leaf)	2,932.69	2,140.38	130.77	12.78	51.21
Monno Ceramics	IMONNO (from root)	1,152.17	1,060.87	121.74	10.70	16.30
	IMONNO (from leaf)	2,805.56	1,175.93	153.70	12.17	21.48
Savar Pesticides	IPSTD (from root)	2,730.70	2,730.70	104.02	6.82	18.20
	IPSTD (from leaf)	1,264.15	3,264.15	126.45	11.86	13.94
Hazaribagh Tannery	IHAZ (from root)	1,160.00	1,960.00	147.36	8.29	6.88
	IHAZ (from leaf)	1,270.00	2,170.00	142.90	9.61	10.30
*Permissible limit	-	1000 ppm	2000 ppm	100 ppm	6 ppm	20 ppm

*Source: Guide to the environmental conservation at 1995 and rules 1997.

Table 13. Comparative study of heavy metal of plant samples [*Colocasia esculenta* (L.) Schott] collected from control and different affected areas

Control/affected areas	Sample ID	Arsenic (As) ppm	Cadmium (Cd) ppm	Chromium (Cr) ppm	Nickel (Ni) ppm	Lead (Pb) ppm
Control	CCTR (from root)	0.50	0.09	BDL	BDL	BDL
	CCTR (from leaf)	0.90	0.08	BDL	BDL	BDL
Gazipur Garments	CGAZ (from root)	3.02	1.22	4.28	3.00	2.15
	CGAZ (from leaf)	5.55	2.54	3.74	5.22	3.20
ACME Lab.	CACME (from root)	8.11	1.60	2.42	7.74	9.83
	CACME (from leaf)	7.96	1.15	4.74	8.50	12.4
Monno Ceramics	CMONNO (from root)	9.08	1.13	0.30	8.44	6.63
	CMONNO (from leaf)	11.12	2.56	3.16	10.06	8.44
Savar Pesticides	CPSTD (from root)	8.45	0.68	1.26	7.62	BDL
	CPSTD (from leaf)	7.42	1.77	2.33	8.04	BDL
Hazaribagh Tannery	CHAZ (from root)	9.56	1.35	3.63	BDL	5.13
	CHAZ (from leaf)	10.14	1.00	3.92	BDL	6.25
	CHAZ (from shoot)	8.49	2.47	2.24	BDL	3.19
*Permissible limit	-	0.009 – 1.50 ppm	0.013 – 0.22 ppm	0.02 – 0.20 ppm	0.2 – 3.70 ppm	0.1 – 10.0 ppm

BDL = Bellow detection limit.
*Source: Guide to the environmental conservation at 1995 and rules 1997.

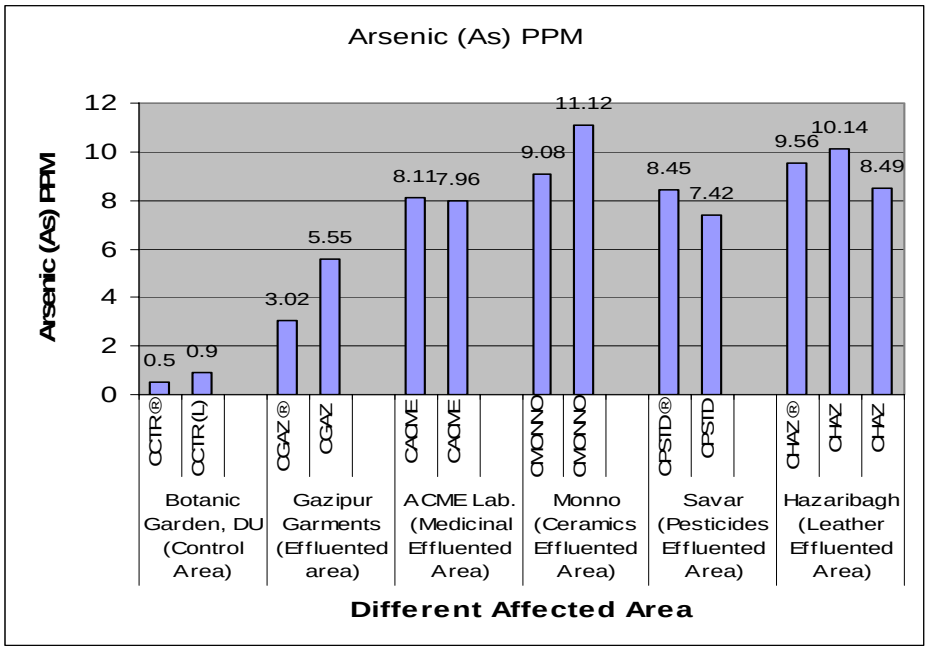


Fig. 7. Arsenic concentration (ppm) in *Colocasia esculenta* (L.) Schott collected from different affected areas.

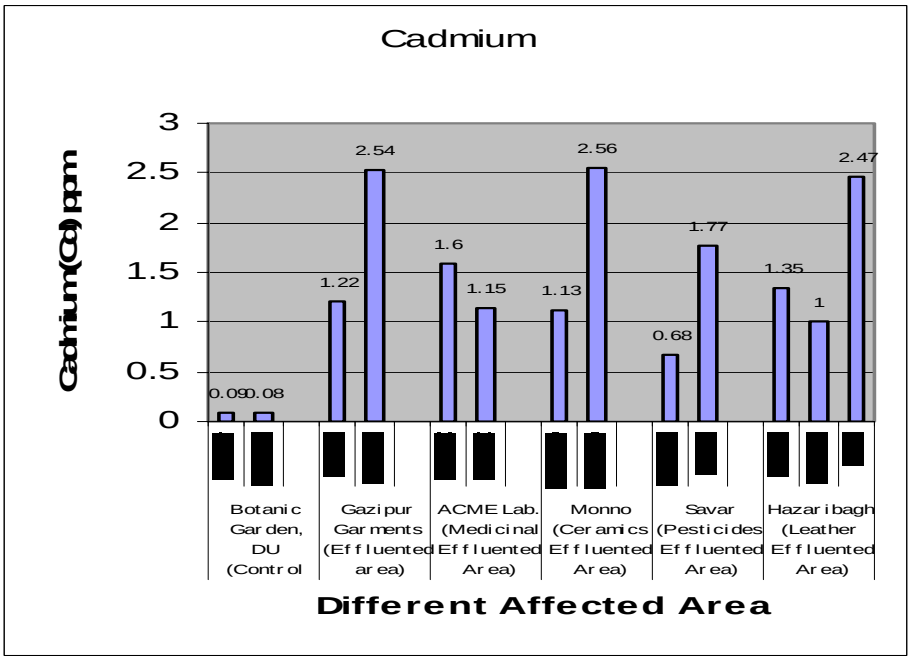


Fig. 8. Cadmium concentration (ppm) in *Colocasia esculenta* in control and different affected areas.

Table 14. Comparative study of heavy metal of plant samples (*Ipomoea aquatica* Forssk.) collected from control and different affected areas

Control/affected areas	Sample ID	Arsenic (As) ppm	Cadmium (Cd) ppm	Chromium (Cr) ppm	Nickel (Ni) ppm	Lead (Pb) ppm
Control	ICTR (from root)	0.04	BDL	BDL	BDL	BDL
	ICTR (from leaf)	0.05	BDL	BDL	BDL	BDL
Gazipur Garments	IGAZ (from root)	4.14	1.11	3.66	6.68	1.50
	IGAZ (from leaf)	5.15	1.13	5.74	5.12	2.22
ACME Lab.	IACME (from root)	11.10	1.13	2.33	3.11	BDL
	IACME (from leaf)	13.15	2.46	7.83	6.55	BDL
Monno Ceramics	IMONNO (from root)	10.23	1.00	3.52	6.94	BDL
	IMONNO (from leaf)	12.15	2.69	7.52	9.08	BDL
Savar Pesticides	IPSTD (from root)	7.67	1.05	0.64	5.42	BDL
	IPSTD (from leaf)	6.96	1.00	1.74	7.99	BDL
Hazaribagh Tannery	IHAZ (from root)	8.25	1.10	2.14	0.22	2.48
	IHAZ (from leaf)	7.80	1.13	4.42	1.42	4.48
*Permissible limit	-	0.009 – 1.5 ppm	0.013 – 0.22 ppm	0.02 – 0.2 ppm	0.2 – 3.7 ppm	0.1 – 10 ppm

BDL = Bellow detection limit.

*Source: Guide to the environmental conservation at 1995 and rules 1997.

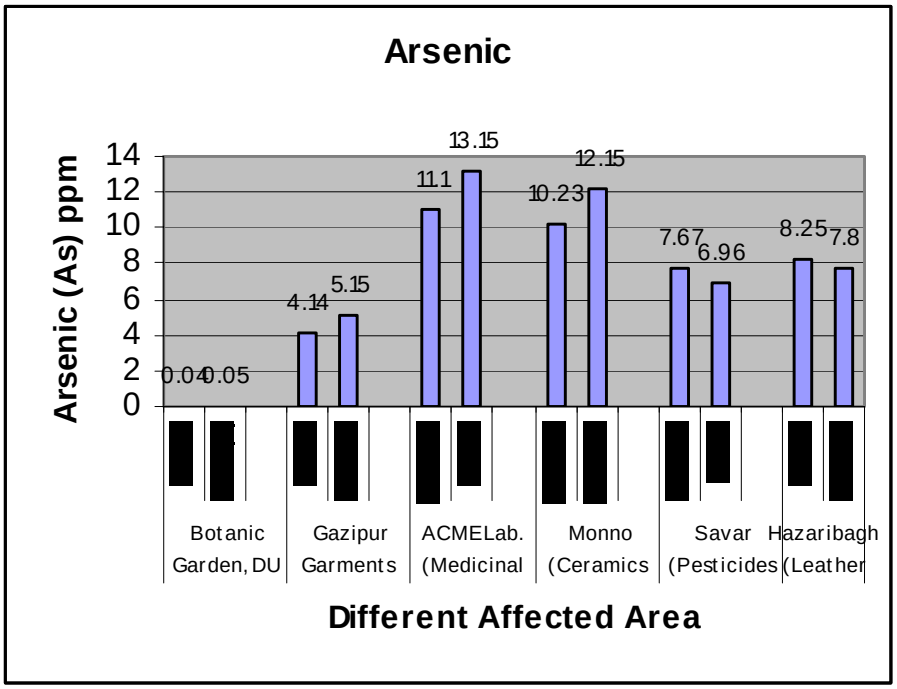


Fig. 9. Arsenic concentration (ppm) in *Ipomoea aquatica* of control and different affected areas.

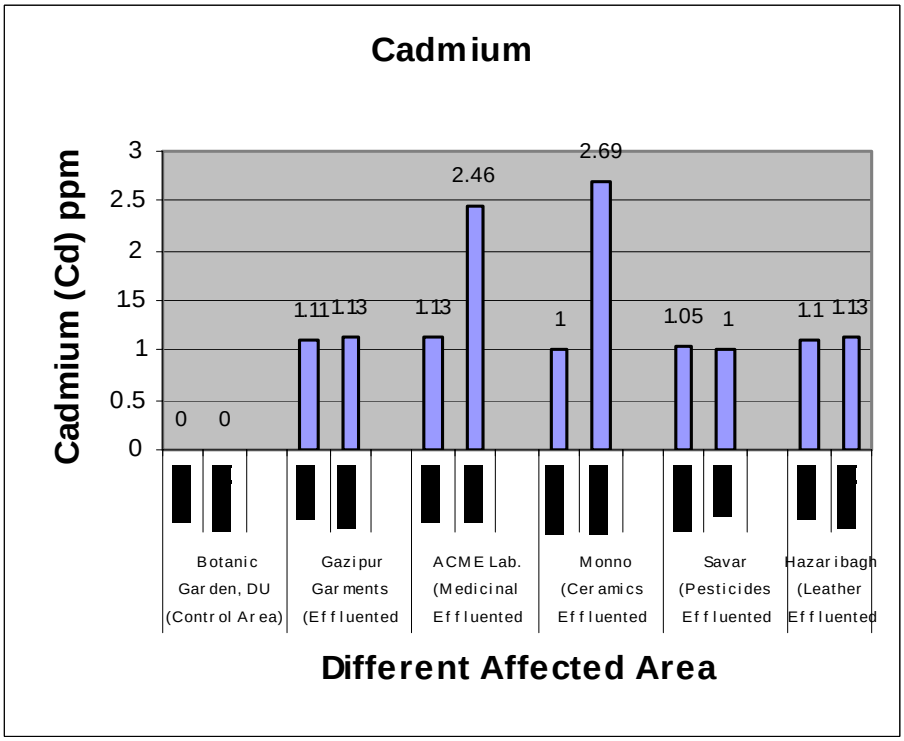


Fig. 10. Cadmium concentration (ppm) in *Ipomoea aquatica* of control and different affected areas.

Table 15. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of *Colocasia esculenta* (L.) Schott (Control plant samples)

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	2.12	0.92	3.04	2.30	0.05	30.26	sm
	2.07	0.92	2.99	2.25	0.05	30.77	sm
II	1.73	1.27	3.00	1.36	0.05	42.33	m
	1.72	1.15	2.87	1.50	0.04	40.07	m
III	1.61	1.27	2.88	1.27	0.04	44.10	m
	1.61	1.15	2.76	1.40	0.04	41.67	m
IV	1.38	1.38	2.76	1.00	0.04	50.00	m
	1.38	1.38	2.76	1.00	0.04	50.00	m
V	1.50	1.15	2.65	1.30	0.04	43.40	m
	1.38	1.15	2.53	1.20	0.04	45.45	m
VI	1.73	0.81	2.54	2.14	0.04	31.89	sm
	1.73	0.69	2.42	2.51	0.04	28.51	sm
VII	1.61	0.69	2.30	2.33	0.04	30.00	sm
	1.50	0.81	2.31	1.85	0.04	35.06	sm
VIII	1.50	0.69	2.19	2.17	0.03	31.51	sm
	1.61	0.58	2.19	2.78	0.03	26.48	sm
IX	1.27	0.92	2.19	1.38	0.03	42.01	m
	1.27	0.92	2.19	1.38	0.03	42.01	m
X	1.15	0.92	2.07	1.25	0.03	44.44	m
	1.04	1.04	2.08	1.00	0.03	50.00	m
XI	1.04	0.92	1.96	1.13	0.03	46.94	m
	0.92	0.92	1.84	1.00	0.03	50.00	m
XII	0.92	0.92	1.84	1.00	0.03	50.00	m
	0.92	0.92	1.84	1.00	0.03	50.00	m
XIII	0.92	0.81	1.73	1.14	0.03	46.82	m
	0.81	0.81	1.62	1.00	0.03	50.00	m
XIV	0.81	0.81	1.62	1.00	0.03	50.00	m
	0.81	0.69	1.50	1.17	0.02	46.00	m

m = metacentric chromosome.

sm = sub-metacentric chromosome.

Table 16. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of *Colocasia esculenta* (L.) Schott (Gazipur plant samples)

Chromosome pair	Long arm (l)	Short arm (s)	Total length (T)	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
	µm	µm	µm				
I	1.73	1.38	3.11	1.25	0.05	44.37	m
	1.61	1.38	2.99	1.17	0.05	46.15	m
II	2.07	0.81	2.88	2.56	0.05	28.13	sm
	1.96	0.81	2.77	2.42	0.04	29.24	sm
III	1.96	0.81	2.77	2.42	0.04	29.24	sm
	1.96	0.69	2.65	2.84	0.04	26.04	sm
IV	1.50	1.15	2.65	1.30	0.04	43.40	m
	1.38	1.15	2.53	1.20	0.04	45.45	m
V	1.38	1.15	2.53	1.20	0.04	45.45	m
	1.27	1.27	2.54	1.00	0.04	50.00	m
VI	1.27	1.15	2.42	1.10	0.04	47.52	m
	1.15	1.15	2.30	1.00	0.04	50.00	m
VII	1.38	0.92	2.30	1.50	0.04	40.00	m
	1.27	1.03	2.30	1.23	0.04	44.78	m
VIII	1.15	1.15	2.30	1.00	0.04	50.00	m
	1.15	1.04	2.19	1.11	0.03	47.49	m
IX	1.38	0.69	2.07	2.00	0.03	33.33	sm
	1.38	0.69	2.07	2.00	0.03	33.33	sm
X	1.15	0.92	2.07	1.25	0.03	44.44	m
	1.15	0.81	1.96	1.42	0.03	41.33	m
XI	1.27	0.69	1.96	1.84	0.03	35.20	sm
	1.27	0.58	1.85	2.19	0.03	31.35	sm
XII	0.92	0.92	1.84	1.00	0.03	50.00	m
	0.92	0.92	1.84	1.00	0.03	50.00	m
XIII	0.92	0.81	1.73	1.14	0.03	46.82	m
	0.81	0.81	1.62	1.00	0.03	50.00	m
XIV	0.69	0.69	1.38	1.00	0.02	50.00	m
	0.69	0.58	1.27	1.19	0.02	45.67	m

m = metacentric chromosome.

sm = sub-metacentric chromosome.

Table 17. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of *Colocasia esculenta* (L.) Schott (Pesticide plant samples)

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.73	0.92	2.65	1.88	0.05	34.72	sm
	1.61	0.92	2.53	1.75	0.05	36.36	sm
II	1.61	0.92	2.53	1.75	0.05	36.36	sm
	1.50	0.92	2.42	1.63	0.04	38.02	sm
III	1.27	1.15	2.42	1.10	0.04	47.52	m
	1.15	1.15	2.30	1.00	0.04	50.00	m
IV	1.15	1.04	2.19	1.11	0.04	47.49	m
	1.15	0.92	2.07	1.25	0.04	44.44	m
V	1.15	0.92	2.07	1.25	0.04	44.44	m
	1.15	0.92	2.07	1.25	0.04	44.44	m
VI	1.38	0.58	1.96	2.38	0.04	29.59	sm
	1.38	0.58	1.96	2.38	0.04	29.59	sm
VII	1.15	0.81	1.96	1.42	0.04	41.33	m
	1.15	0.81	1.96	1.42	0.04	41.33	m
VIII	0.92	0.92	1.84	1.00	0.03	50.00	m
	0.92	0.92	1.84	1.00	0.03	50.00	m
IX	0.92	0.92	1.84	1.00	0.03	50.00	m
	0.92	0.92	1.84	1.00	0.03	50.00	m
X	0.92	0.81	1.73	1.14	0.03	46.82	m
	0.92	0.81	1.73	1.14	0.03	46.82	m
XI	1.15	0.58	1.73	1.98	0.03	33.53	sm
	1.15	0.46	1.61	2.50	0.03	28.57	sm
XII	0.92	0.69	1.61	1.33	0.03	42.86	m
	0.81	0.81	1.62	1.00	0.03	50.00	m
XIII	0.81	0.69	1.50	1.17	0.03	46.00	m
	0.69	0.69	1.38	1.00	0.03	50.00	m
XIV	0.69	0.58	1.27	1.19	0.02	45.67	m
	0.58	0.58	1.16	1.00	0.02	50.00	m

m = metacentric chromosome.

sm = sub-metacentric chromosome.

Table 18. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of *Colocasia esculenta* (L.) Schott (Hazaribagh plant samples)

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.84	1.38	3.22	1.33	0.05	42.86	m
	1.84	1.38	3.22	1.33	0.05	42.86	m
II	1.84	1.38	3.22	1.33	0.05	42.86	m
	1.73	1.38	3.11	1.25	0.05	44.37	m
III	1.96	1.15	3.11	1.70	0.05	36.98	sm
	1.84	1.15	2.99	1.60	0.04	38.46	sm
IV	1.50	1.27	2.77	1.18	0.04	45.85	m
	1.50	1.27	2.77	1.18	0.04	45.85	m
V	1.38	1.38	2.76	1.00	0.04	50.00	m
	1.38	1.38	2.76	1.00	0.04	50.00	m
VI	1.38	1.38	2.76	1.00	0.04	50.00	m
	1.38	1.38	2.76	1.00	0.04	50.00	m
VII	1.50	1.15	2.65	1.30	0.04	43.40	m
	1.50	1.15	2.65	1.30	0.04	43.40	m
VIII	1.73	0.92	2.65	1.88	0.04	34.72	sm
	1.61	0.92	2.53	1.75	0.04	36.36	sm
IX	1.50	0.81	2.31	1.85	0.03	35.06	sm
	1.50	0.81	2.31	1.85	0.03	35.06	sm
X	1.27	1.04	2.31	1.22	0.03	45.02	m
	1.27	0.92	2.19	1.38	0.03	42.01	m
XI	1.04	1.04	2.08	1.00	0.03	50.00	m
	1.04	0.92	1.96	1.13	0.03	46.94	m
XII	0.92	0.92	1.84	1.00	0.03	50.00	m
	0.92	0.81	1.73	1.14	0.03	46.82	m
XIII	1.15	0.58	1.73	1.98	0.03	33.53	sm
	1.15	0.46	1.61	2.50	0.02	28.57	sm
XIV	0.69	0.69	1.38	1.00	0.02	50.00	m
	0.69	0.58	1.27	1.19	0.02	45.67	m

m = metacentric chromosome.

sm = sub-metacentric chromosome.

Table 19. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of *Colocasia esculenta* (L.) Schott (Monno Ceramic plant samples)

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.27	1.04	2.31	1.22	0.04	45.02	m
	1.38	0.92	2.30	1.50	0.04	40.00	m
II	1.15	1.15	2.30	1.00	0.04	50.00	m
	1.15	1.04	2.19	1.11	0.04	47.49	m
III	1.50	0.69	2.19	2.17	0.04	31.51	sm
	1.38	0.69	2.07	2.00	0.03	33.33	sm
IV	1.15	0.81	1.96	1.42	0.03	41.33	m
	1.04	0.92	1.96	1.13	0.03	46.94	m
V	1.04	0.92	1.96	1.13	0.03	46.94	m
	1.04	0.92	1.96	1.13	0.03	46.94	m
VI	0.92	0.92	1.84	1.00	0.03	50.00	m
	0.92	0.92	1.84	1.00	0.03	50.00	m
VII	0.92	0.81	1.73	1.14	0.03	46.82	m
	0.92	0.81	1.73	1.14	0.03	46.82	m
VIII	0.92	0.81	1.73	1.14	0.03	46.82	m
	0.92	0.81	1.73	1.14	0.03	46.82	m
IX	1.15	0.58	1.73	1.98	0.03	33.53	sm
	1.27	0.46	1.73	2.76	0.03	26.59	sm
X	0.92	0.69	1.61	1.33	0.03	42.86	m
	0.92	0.69	1.61	1.33	0.03	42.86	m
XI	1.04	0.46	1.50	2.26	0.02	30.67	sm
	0.92	0.58	1.50	1.59	0.02	38.67	sm
XII	0.69	0.69	1.38	1.00	0.02	50.00	m
	0.69	0.69	1.38	1.00	0.02	50.00	m
XIII	0.92	0.46	1.38	2.00	0.02	33.33	sm
	1.04	0.35	1.39	2.97	0.02	25.18	sm
XIV	0.69	0.69	1.38	1.00	0.02	50.00	m
	0.69	0.69	1.38	1.00	0.02	50.00	m
XV	0.69	0.69	1.38	1.00	0.02	50.00	m
	0.69	0.69	1.38	1.00	0.02	50.00	m
XVI	0.69	0.58	1.27	1.19	0.02	45.67	m
	0.69	0.58	1.27	1.19	0.02	45.67	m
XVII	0.58	0.58	1.16	1.00	0.02	50.00	m
	0.58	0.58	1.16	1.00	0.02	50.00	m
XVIII	0.58	0.46	1.04	1.26	0.02	44.23	m
	0.58	0.46	1.04	1.26	0.02	44.23	m
XIX	0.46	0.46	0.92	1.00	0.02	50.00	m
	0.46	0.46	0.92	1.00	0.02	50.00	m

m = metacentric chromosome.
sm = sub-metacentric chromosome.

Table 20. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of *Colocasia esculenta* (L.) Schott(Acme Lab. plant samples)

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.15	1.15	2.30	1.00	0.04	50.00	m
	1.15	1.15	2.30	1.00	0.04	50.00	m
II	1.15	1.15	2.30	1.00	0.04	50.00	m
	1.15	1.15	2.30	1.00	0.04	50.00	m
III	1.27	0.92	2.19	1.38	0.04	42.01	m
	1.27	0.92	2.19	1.38	0.04	42.01	m
IV	1.45	0.55	2.00	2.64	0.03	27.50	sm
	1.40	0.60	2.00	2.33	0.03	30.00	sm
V	1.04	0.92	1.96	1.13	0.03	46.94	m
	1.04	0.92	1.96	1.13	0.03	46.94	m
VI	1.24	0.60	1.84	2.07	0.03	32.61	sm
	1.24	0.60	1.84	2.07	0.03	32.61	sm
VII	1.04	0.72	1.76	1.44	0.03	40.91	m
	1.02	0.74	1.76	1.38	0.03	42.05	m
VIII	0.98	0.74	1.72	1.32	0.03	43.02	m
	0.98	0.74	1.72	1.32	0.03	43.02	m
IX	1.00	0.68	1.68	1.47	0.03	40.48	m
	1.00	0.68	1.68	1.47	0.03	40.48	m
X	0.90	0.62	1.52	1.45	0.02	40.79	m
	0.90	0.62	1.52	1.45	0.02	40.79	m
XI	0.88	0.64	1.52	1.38	0.02	42.11	m
	0.88	0.64	1.52	1.38	0.02	42.11	m
XII	1.00	0.44	1.44	2.27	0.02	30.56	sm
	1.00	0.44	1.44	2.27	0.02	30.56	sm
XIII	0.98	0.40	1.38	2.45	0.02	28.99	sm
	0.98	0.40	1.38	2.45	0.02	28.99	sm
XIV	0.68	0.58	1.26	1.17	0.02	46.03	m
	0.68	0.58	1.26	1.17	0.02	46.03	m
XV	0.68	0.58	1.26	1.17	0.02	46.03	m
	0.68	0.58	1.26	1.17	0.02	46.03	m
XVI	0.58	0.58	1.16	1.00	0.02	50.00	m
	0.58	0.58	1.16	1.00	0.02	50.00	m
XVII	0.58	0.58	1.16	1.00	0.02	50.00	m
	0.58	0.58	1.16	1.00	0.02	50.00	m
XVIII	0.55	0.55	1.10	1.00	0.02	50.00	m
	0.55	0.55	1.10	1.00	0.02	50.00	m
XIX	0.58	0.46	1.04	1.26	0.02	44.23	m
	0.46	0.46	0.92	1.00	0.02	50.00	m

m = metacentric chromosome.

sm = sub-metacentric chromosome.

Table 21. Comparative karyotype analysis of *Colocasia esculenta* (L.) Schott collected from five different affected areas along with control plant samples

Plant samples collected from different affected areas	2n	Range of chromosomal length (μm)	Total length of 2n chromosome complement (μm)	Centromeric formulae
Control	28	1.50 – 3.04	64.67	20 m+ 8sm
Gazipur Garments	28	1.27 – 3.11	62.89	20 m+ 8sm
Pesticide Savar	28	1.16 -2.65	53.79	20 m + 8sm
Hazaribagh Tannery	28	1.27 -3.22	68.65	20 m + 8sm
Monno Ceramic	38	0.92 – 2.30	61.31	30 m + 8sm
Acme Laboratory	38	0.92 – 2.30	61.31	30 m + 8sm

m = metacentric chromosome
sm = Sub-metacentric chromosome

Table 22. Comparative CMA-banding analysis of *Colocasia esculenta* (L.) Schott collected from five different affected areas along with control plant samples

Plant samples collected from different affected areas	No. of CMA- band		CMA positive banded region		CMA banded karyotype formulae
	Positive	Satellite	Total Length (µm)	%	
Control	14	-	11.15	17.25	5γ+4θ+3ω+2φ+14δ
Gazipur Garments	-	2	0.69	1.09	2β+26δ
Pesticide Savar	-	-	-	-	-
Hazaribagh Tannery	-	-	-	-	-
Monno Ceramic	2	-	0.57	0.94	2φ+36δ
Acme Laboratory	4	2	1.72	2.82	1λ+2β+3θ+32δ

Classification of CMA positive band and satellite:

β = Satellite
θ = Band in terminal region
δ = No band
γ = Band in whole chromosome
λ = Band in long arm
φ = Band in short arm
ω = Band in ¾ th region

Table 23. Comparative DAPI-banding analysis of *Colocasia esculenta* (L.) Schott collected from five different affected areas along with control plant samples

Plant samples collected from different affected areas	No. of DAPI Positive band	DAPI positive banded region		DAPI banded karyotype formulae
		Total Length (µm)	%	
Control	-	-	-	-
Gazipur Garments	-	-	-	-
Pesticide Savar	-	-	-	-
Hazaribagh Tannery	5	6.55	9.54	4γ+1θ+23δ
Monno Ceramic	9	5.29	8.62	4γ+2α+2θ+1π+29δ
Acme Laboratory	4	4.83	7.91	3γ+1θ+34δ

Classification of DAPI positive band and satellite:

θ = Band in terminal region
δ = No band
γ = Band in whole chromosome
α = Band in centromeric region
π = Band in two terminal region

Table 24. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of *Ipomoea aquatica* Forssk. (Control plant samples)

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.00	1.00	2.00	1.00	0.08	50.00	m
	1.00	1.00	2.00	1.00	0.08	50.00	m
II	0.98	0.98	1.96	1.00	0.08	50.00	m
	0.98	0.98	1.96	1.00	0.08	50.00	m
III	0.96	0.94	1.90	1.02	0.07	49.47	m
	0.96	0.94	1.90	1.02	0.07	49.47	m
IV	0.94	0.94	1.88	1.00	0.07	50.00	m
	0.94	0.94	1.88	1.00	0.07	50.00	m
V	0.90	0.90	1.80	1.00	0.07	50.00	m
	0.90	0.90	1.80	1.00	0.07	50.00	m
VI	0.88	0.84	1.72	1.05	0.07	48.84	m
	0.88	0.84	1.72	1.05	0.07	48.84	m
VII	0.78	0.74	1.52	1.05	0.06	48.68	m
	0.78	0.74	1.52	1.05	0.06	48.68	m
VIII	0.76	0.74	1.50	1.03	0.06	49.33	m
	0.76	0.74	1.50	1.03	0.06	49.33	m
IX	0.74	0.70	1.44	1.06	0.06	48.61	m
	0.74	0.70	1.44	1.06	0.06	48.61	m
X	0.74	0.70	1.44	1.06	0.06	48.61	m
	0.74	0.70	1.44	1.06	0.06	48.61	m
XI	0.70	0.68	1.38	1.03	0.05	49.28	m
	0.70	0.68	1.38	1.03	0.05	49.28	m
XII	0.68	0.64	1.32	1.06	0.05	48.48	m
	0.68	0.64	1.32	1.06	0.05	48.48	m
XIII	0.64	0.64	1.28	1.00	0.05	50.00	m
	0.64	0.64	1.28	1.00	0.05	50.00	m
XIV	0.62	0.62	1.24	1.00	0.05	50.00	m
	0.62	0.62	1.24	1.00	0.05	50.00	m
XV	0.62	0.62	1.24	1.00	0.05	50.00	m
	0.62	0.62	1.24	1.00	0.05	50.00	m

m = metacentric chromosome.

Table 25. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of *Ipomoea aquatica* Forssk.(Gazipur plant samples)

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.05	1.05	2.10	1.00	0.09	50.00	m
	1.05	1.05	2.10	1.00	0.09	50.00	m
II	1.00	1.00	2.00	1.00	0.08	50.00	m
	1.00	1.00	2.00	1.00	0.08	50.00	m
III	0.98	0.96	1.94	1.02	0.08	49.48	m
	0.98	0.96	1.94	1.02	0.08	49.48	m
IV	0.98	0.94	1.92	1.04	0.08	48.96	m
	0.96	0.94	1.90	1.02	0.08	49.47	m
V	0.96	0.94	1.90	1.02	0.08	49.47	m
	0.96	0.88	1.84	1.09	0.07	47.83	m
VI	0.96	0.88	1.84	1.09	0.07	47.83	m
	0.94	0.88	1.82	1.07	0.07	48.35	m
VII	0.90	0.86	1.76	1.05	0.07	48.86	m
	0.90	0.86	1.76	1.05	0.07	48.86	m
VIII	0.86	0.84	1.70	1.02	0.07	49.41	m
	0.86	0.84	1.70	1.02	0.07	49.41	m
IX	0.84	0.82	1.66	1.02	0.07	49.40	m
	0.84	0.82	1.66	1.02	0.07	49.40	m
X	0.84	0.82	1.66	1.02	0.07	49.40	m
	0.78	0.82	1.60	0.95	0.07	51.25	m
XI	0.78	0.78	1.56	1.00	0.06	50.00	m
	0.78	0.78	1.56	1.00	0.06	50.00	m
XII	0.78	0.74	1.52	1.05	0.06	48.68	m
	0.72	0.74	1.46	0.97	0.06	50.68	m
XIII	0.72	0.72	1.44	1.00	0.06	50.00	m
	0.70	0.72	1.42	0.97	0.06	50.70	m
XIV	0.70	0.68	1.38	1.03	0.06	49.28	m
	0.70	0.68	1.38	1.03	0.06	49.28	m
XV	0.68	0.68	1.36	1.00	0.06	50.00	m
	0.68	0.68	1.36	1.00	0.06	50.00	m

m = metacentric chromosome.

Table 26. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of *Ipomoea aquatica* Forssk.(Pesticide plant samples)

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.00	0.98	1.98	1.02	0.11	49.49	m
	1.00	0.98	1.98	1.02	0.11	49.49	m
II	0.98	0.96	1.94	1.02	0.11	49.48	m
	0.98	0.96	1.94	1.02	0.11	49.48	m
III	0.96	0.94	1.90	1.02	0.11	49.47	m
	0.96	0.96	1.92	1.00	0.11	50.00	m
IV	0.95	0.95	1.90	1.00	0.11	50.00	m
	0.95	0.95	1.90	1.00	0.11	50.00	m
V	0.90	0.90	1.80	1.00	0.10	50.00	m
	0.90	0.90	1.80	1.00	0.10	50.00	m
VI	0.86	0.84	1.70	1.02	0.10	49.41	m
	0.84	0.84	1.68	1.00	0.10	50.00	m
VII	0.84	0.84	1.68	1.00	0.10	50.00	m
	0.84	0.84	1.68	1.00	0.10	50.00	m
VIII	0.78	0.78	1.56	1.00	0.09	50.00	m
	0.78	0.78	1.56	1.00	0.09	50.00	m
IX	0.72	0.72	1.44	1.00	0.08	50.00	m
	0.72	0.72	1.44	1.00	0.08	50.00	m
X	0.72	0.72	1.44	1.00	0.08	50.00	m
	0.72	0.72	1.44	1.00	0.08	50.00	m
XI	0.68	0.68	1.36	1.00	0.08	50.00	m
	0.68	0.68	1.36	1.00	0.08	50.00	m
XII	0.68	0.66	1.34	1.03	0.08	49.25	m
	0.66	0.66	1.32	1.00	0.08	50.00	m
XIII	0.66	0.66	1.32	1.00	0.08	50.00	m
	0.66	0.66	1.32	1.00	0.08	50.00	m
XIV	0.64	0.64	1.28	1.00	0.07	50.00	m
	0.64	0.64	1.28	1.00	0.07	50.00	m
XV	0.60	0.60	1.20	1.00	0.07	50.00	m
	0.60	0.60	1.20	1.00	0.07	50.00	m

m = metacentric chromosome.

Table 27. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of *Ipomoea aquatica* Forssk. (Hazaribagh plant samples)

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.25	1.25	2.50	1.00	0.10	50.00	m
	1.20	1.20	2.40	1.00	0.10	50.00	m
II	1.10	1.10	2.20	1.00	0.09	50.00	m
	1.10	1.00	2.10	1.10	0.08	47.62	m
III	1.00	0.98	1.98	1.02	0.08	49.49	m
	1.00	0.98	1.98	1.02	0.08	49.49	m
IV	0.98	0.96	1.94	1.02	0.08	49.48	m
	0.98	0.96	1.94	1.02	0.08	49.48	m
V	0.96	0.96	1.92	1.00	0.08	50.00	m
	0.96	0.96	1.92	1.00	0.08	50.00	m
VI	0.95	0.95	1.90	1.00	0.08	50.00	m
	0.94	0.93	1.87	1.01	0.08	49.73	m
VII	0.92	0.90	1.82	1.02	0.07	49.45	m
	0.92	0.90	1.82	1.02	0.07	49.45	m
VIII	0.90	0.86	1.76	1.05	0.07	48.86	m
	0.90	0.84	1.74	1.07	0.07	48.28	m
IX	0.84	0.84	1.68	1.00	0.07	50.00	m
	0.84	0.84	1.68	1.00	0.07	50.00	m
X	0.80	0.80	1.60	1.00	0.06	50.00	m
	0.80	0.78	1.58	1.03	0.06	49.37	m
XI	0.78	0.78	1.56	1.00	0.06	50.00	m
	0.78	0.78	1.56	1.00	0.06	50.00	m
XII	0.76	0.72	1.48	1.06	0.06	48.65	m
	0.76	0.72	1.48	1.06	0.06	48.65	m
XIII	0.72	0.68	1.40	1.06	0.06	48.57	m
	0.72	0.68	1.40	1.06	0.06	48.57	m
XIV	0.68	0.68	1.36	1.00	0.05	50.00	m
	0.68	0.68	1.36	1.00	0.05	50.00	m
XV	0.65	0.65	1.30	1.00	0.05	50.00	m
	0.65	0.65	1.30	1.00	0.05	50.00	m

m = metacentric chromosome.

Table 28. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of *Ipomoea aquatica* Forssk.(Monno Ceramic plant samples)

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.20	1.10	2.30	1.09	0.11	47.83	m
	1.20	1.10	2.30	1.09	0.11	47.83	m
II	1.10	1.00	2.10	1.10	0.10	47.62	m
	1.10	1.00	2.10	1.10	0.10	47.62	m
III	0.98	0.98	1.96	1.00	0.10	50.00	m
	0.98	0.98	1.96	1.00	0.10	50.00	m
IV	0.94	0.94	1.88	1.00	0.09	50.00	m
	0.94	0.94	1.88	1.00	0.09	50.00	m
V	0.93	0.91	1.84	1.02	0.09	49.46	m
	0.93	0.91	1.84	1.02	0.09	49.46	m
VI	0.91	0.91	1.82	1.00	0.09	50.00	m
	0.91	0.91	1.82	1.00	0.09	50.00	m
VII	0.88	0.86	1.74	1.02	0.09	49.43	m
	0.88	0.86	1.74	1.02	0.09	49.43	m
VIII	0.84	0.84	1.68	1.00	0.08	50.00	m
	0.84	0.84	1.68	1.00	0.08	50.00	m
IX	0.78	0.74	1.52	1.05	0.07	48.68	m
	0.78	0.74	1.52	1.05	0.07	48.68	m
X	0.72	0.72	1.44	1.00	0.07	50.00	m
	0.72	0.72	1.44	1.00	0.07	50.00	m
XI	0.70	0.69	1.39	1.01	0.07	49.64	m
	0.70	0.69	1.39	1.01	0.07	49.64	m
XII	0.69	0.69	1.38	1.00	0.07	50.00	m
	0.69	0.69	1.38	1.00	0.07	50.00	m
XIII	0.67	0.65	1.32	1.03	0.06	49.24	m
	0.67	0.65	1.32	1.03	0.06	49.24	m
XIV	0.65	0.64	1.29	1.02	0.06	49.61	m
	0.65	0.64	1.29	1.02	0.06	49.61	m
XV	0.64	0.64	1.28	1.00	0.06	50.00	m
	0.64	0.64	1.28	1.00	0.06	50.00	m

m = metacentric chromosome.

Table 29. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of *Ipomoea aquatica* Forssk.(Acme Lab. plant samples)

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.60	1.60	3.20	1.00	0.10	50.00	m
	1.60	1.50	3.10	1.07	0.10	48.39	m
II	1.40	1.40	2.80	1.00	0.09	50.00	m
	1.40	1.30	2.70	1.08	0.09	48.15	m
III	1.30	1.25	2.55	1.04	0.08	49.02	m
	1.30	1.20	2.50	1.08	0.08	48.00	m
IV	1.20	1.15	2.35	1.04	0.08	48.94	m
	1.20	1.15	2.35	1.04	0.08	48.94	m
V	1.10	1.00	2.10	1.10	0.07	47.62	m
	1.10	1.00	2.10	1.10	0.07	47.62	m
VI	1.00	0.96	1.96	1.04	0.06	48.98	m
	1.00	0.96	1.96	1.04	0.06	48.98	m
VII	0.98	0.94	1.92	1.04	0.06	48.96	m
	0.98	0.94	1.92	1.04	0.06	48.96	m
VIII	0.96	0.96	1.92	1.00	0.06	50.00	m
	0.96	0.96	1.92	1.00	0.06	50.00	m
IX	0.95	0.93	1.88	1.02	0.06	49.47	m
	0.95	0.93	1.88	1.02	0.06	49.47	m
X	0.93	0.93	1.86	1.00	0.06	50.00	m
	0.93	0.93	1.86	1.00	0.06	50.00	m
XI	0.88	0.86	1.74	1.02	0.06	49.43	m
	0.88	0.86	1.74	1.02	0.06	49.43	m
XII	0.86	0.86	1.72	1.00	0.06	50.00	m
	0.86	0.86	1.72	1.00	0.06	50.00	m
XIII	0.86	0.86	1.72	1.00	0.06	50.00	m
	0.86	0.86	1.72	1.00	0.06	50.00	m
XIV	0.84	0.84	1.68	1.00	0.05	50.00	m
	0.84	0.84	1.68	1.00	0.05	50.00	m
XV	0.80	0.80	1.60	1.00	0.05	50.00	m
	0.80	0.80	1.60	1.00	0.05	50.00	m

m = metacentric chromosome.

Table 30. Comparative karyotype analysis of *Ipomoea aquatica* Forssk. collected from five different affected area along with control plant samples

Plant samples collected from different affected areas	2n	Range of chromosomal length (μm)	Total length of 2n chromosome complements (μm)	Centromeric formulae
Control	30	1.24 – 2.00	47.24	30 m
Gazipur Garments	30	1.36 – 2.10	51.24	30 m
Pesticide Savar	30	1.20 -1.98	47.66	30 m
Hazaribagh Tannery	30	1.30 -2.50	52.53	30 m
Monno Ceramic	30	1.28 – 2.30	49.88	30 m
Acme Laboratory	30	1.60 – 3.20	61.75	30 m

m = metacentric chromosome

Table 31. Comparative CMA-banding analysis of *Ipomoea aquatica* Forssk. collected from five different affected area along with control plant samples

Plant samples collected from different affected areas	No. of CMA-positive band	CMA positive banded region		CMA banded karyotype formulae
		Total Length (µm)	%	
Control	5	2.30	8.93	4θ+1γ+25δ
Gazipur Garments	6	2.99	12.15	6γ+24δ
Pesticide Savar	7	4.14	23.52	2φ+5γ+23δ
Hazaribagh Tannery	4	2.00	8.05	1θ+1γ+2α+26δ
Monno Ceramic	10	3.22	15.81	4φ+6γ+20δ
Acme Laboratory	6	2.76	9.02	4γ+2θ+24δ

Classification of CMA positive band:

θ = Band in terminal region
δ = No band
γ = Band in whole chromosome
φ = Band in short arm
α = Band in centromeric region

Table 32. Comparative DAPI-banding analysis of *Ipomoea aquatica* Forssk. collected from five different affected area along with control plant samples

Plant samples collected from different affected areas	No. of DAPI - positive band	DAPI positive banded region		DAPI banded karyotype formulae
		Total Length (µm)	%	
Control	6	2.41	9.37	4θ+1α +1γ+24δ
Gazipur Garments	4	2.07	8.41	4γ+26δ
Pesticide Savar	7	2.41	13.72	3α+4γ+23δ
Hazaribagh Tannery	8	2.87	11.57	5γ+3α+22δ
Monno Ceramic	4	1.49	7.34	2γ+2α+26δ
Acme Laboratory	2	1.38	4.51	2γ+28δ

Classification of DAPI positive band:

θ = Band in terminal region
δ = No band
γ = Band in whole chromosome
α = Band in centromeric region

Table 33. RAPD analysis with primer OPA-1 (5´-CAG GCC CTT C-3´) in six plant samples of *Colocasia esculenta* (L.) Schott collected from five different affected areas along with control

Plant samples collected from different affected areas	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
Control	2 (12,000, 2,000)	2 (12,000, 2,000)	--	--	
Gazipur Garments	3 (12,000, 2,000, 1500)	2 (12,000, 1500)	1 (2,000)	--	
Acme Laboratory	2 (12,000, 1500)	2 (12,000, 1500)	--	--	1 (12,000)
Monno ceramic	2 (12,000, 1500)	2 (12,000, 1500)	--	--	
Pesticide Savar	2 (12,000, 1500)	2 (12,000, 1500)	--	--	
Hazaribagh Tannery	2 (12,000, 1500)	2 (12,000, 1500)	--	--	

Table 34. RAPD analysis with primer OPA-2 (5´-TGC CGA GCT G -3´) in six plant samples of *Colocasia esculenta* (L.) Schott collected from five different affected areas along with control

Plant samples collected from different affected areas	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
Control	2 (12,000, 2,000)	1 (12,000, 2,000)	1 (2000)	--	
Gazipur Garments	2 (12,000, 2,000)	2 (12,000, 2,000)	--	--	
Acme Laboratory	2 (12,000, 2,000)	2 (12,000, 2,000)	--	--	1 (12,000)
Monno ceramic	2 (12,000, 2,000)	2 (12,000, 2,000)	--	--	
Pesticide Savar	2 (12,000, 1500)	2 (12,000, 1500)	--	--	
Hazaribagh Tannery	2 (12,000, 2,000)	2 (12,000, 2,000)	--	--	

Table 35. RAPD analysis with primer OPA-3 (5´-AGT CAG CCA C- 3´) in six plant samples of *Colocasia esculenta* (L.) Schott collected from five different affected areas along with control

Plant samples collected from different affected areas	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
Control	2 (12,000, 1,500)	2 (12,000, 1,500)	--	--	
Gazipur Garments	2 (12,000, 1,500)	1 (1,500)	1 (12,000)	--	
Acme Laboratory	2 (12,000, 1,500)	1 (12,000)	1 (1,500)	--	
Monno ceramic	2 (12,000, 1,500)	1 (12,000)	1 (1,500)	--	2 (12000,1500)
Pesticide Savar	2 (12,000, 1,500)	2 (12,000, 1,500)	--	--	
Hazaribagh Tannery	2 (12,000, 1,500)	2 (12,000, 1,500)	--	--	

Table 36. RAPD analysis with primer OPA-4 (5´-AAT CGG GCT G-3´) in six plant samples of *Colocasia esculenta* (L.) Schott collected from five different affected areas along with control

Plant samples collected from different affected areas	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
Control	2 (12,000, 3,000)	2 (12,000, 3,000)	--	--	
Gazipur Garments	2 (12,000, 3,000)	2 (12,000, 3,000)	--	--	
Acme Laboratory	2 (12,000, 3,000)	2 (12,000, 3,000)	--	--	2 (12,000 ,3000) except Hazaribagh
Monno ceramic	2 (12,000, 3,000)	2 (12,000, 3,000)	--	--	
Pesticide Savar	2 (12,000, 3,000)	2 (12,000, 3,000)	--	--	
Hazaribagh Tannery	1 (12,000)	1 (12,000)	--	--	

Table 37. RAPD analysis with primer OPA-6 (5´-GGT CCC TGA C-3´) in six plant samples of *Colocasia esculenta* (L.) Schott collected from five different affected areas along with control

Plant samples collected from different affected areas	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
Control	2 (12,000, 1,500)	2 (12,000, 1,500)	--	--	
Gazipur Garments	2 (12,000, 1,500)	2 (12,000, 1,500)	--	--	
Acme Laboratory	2 (12,000, 1,500)	1 (12,000)	1 (1,500)	--	2 (12,000 , 1500)
Monno ceramic	2 (12,000, 1,500)	1 (12,000)	1 (1,500)	--	
Pesticide Savar	2 (12,000, 1,500)	2 (12,000, 1,500)	--	--	
Hazaribagh Tannery	2 (12,000, 1,500)	2 (12,000, 1,500)	--	--	

Table 38. RAPD analysis with primer OPA-8 (5´-GTG ACG TAG G-3´) in six plant samples of *Colocasia esculenta* (L.) Schott collected from five different affected areas along with control

Plant samples collected from different affected areas	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
Control	2 (12,000, 1,500)	1 (12,000)	1 (1,500)	--	
Gazipur Garments	2 (12,000, 1,500)	2 (12,000, 1,500)	--	--	
Acme Laboratory	2 (12,000, 1,500)	2 (12,000, 1,500)	--	--	2 (12,000, 1500) except Hazaribagh
Monno ceramic	2 (12,000, 1,500)	2 (12,000, 1,500)	--	--	
Pesticide Savar	2 (12,000, 1,500)	2 (12,000, 1,500)	--	--	
Hazaribagh Tannery	1 (12000)	1 (12,000)	--	--	

Table 39. RAPD analysis with Primer-23 (5´-GTC AGG GCA A-3´) in six plant samples of *Colocasia esculenta* (L.) Schott collected from five different affected areas along with control

Plant samples collected from different affected areas	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
Control	2 (1,200, 800)	1 (800)	1 (1,200)	--	
Gazipur Garments	2 (1,200, 800)	1 (800)	1 (1,200)	--	
Acme Laboratory	2 (1,200, 800)	1 (800)	1 (1,200)	--	
Monno ceramic	1 (800)	1 (800)	--	--	--
Pesticide Savar	1 (1500)	1 (1,500)	--	--	
Hazaribagh Tannery	2 (1,700, 1,200)	2 (1,700, 1,200)	--	--	

Table 40. RAPD analysis with primer-24 (5´- GGT CGG AGA A-3´) in six plant samples of *Colocasia esculenta* (L.) Schott collected from five different affected areas along with control

Plant samples collected from different affected areas	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
Control	1 (1,500)	1 (1,500)	--	--	
Gazipur Garments	1 (1,100)	--	1 (1,100)	--	
Acme Laboratory	2 (1,000, 600)	2 (1,000, 600)	--	--	
Monno ceramic	2 (1,000, 600)	2 (1,000, 600)	--	--	--
Pesticide Savar	--	--	--	--	
Hazaribagh Tannery	3 (1,500, 800, 600)	1 (1,500)	2 (800, 600)	--	

Table 41. RAPD analysis with primer OPA-1 (5´-CAG GCC CTT C-3´) in six plant samples of *Ipomoea aquatica* Forssk. collected from five different affected areas along with control

Plant samples collected from different affected areas	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
Control	1 (12,000)	1 (12,000)	--	--	1 (12,000)
Gazipur Garments	1 (12,000)	1 (12,000)	--	--	
Acme Laboratory	1 (12,000)	1 (12,000)	--	--	
Monno ceramic	1 (12,000)	1 (12,000)	--	--	
Pesticide Savar	1 (12,000)	1 (12,000)	--	--	
Hazaribagh Tannery	--	--	--	--	

Table 42. RAPD analysis with primer OPA-2 (5´-TGC CGA GCT G -3´) in six plant samples of *Ipomoea aquatica* Forssk. collected from five different affected areas along with control

Plant samples collected from different affected areas	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
Control	1 (12,000)	1 (12,000)	--	--	
Gazipur Garments	1 (1,400)	1 (1,400)	--	--	
Acme Laboratory	2 (12,000, 1,400)	2 (12,000, 1,400)	--	--	
Monno ceramic	2 (12,000, 1,400)	1 (12,000)	1 (1,400)	--	--
Pesticide Savar	1 (12,000)	1 (12,000)	--	--	
Hazaribagh Tannery	--	--	--	--	

Table 43. RAPD analysis with primer OPA-3 (5´-AGT CAG CCA C- 3´) in six plant samples of *Ipomoea aquatica* Forssk. collected from five different affected areas along with control

Plant samples collected from different affected areas	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
Control	1 (11,000)	1 (11,000)	--	--	
Gazipur Garments	1 (11,000)	1 (11,000)	--	--	
Acme Laboratory	1 (11,000)	1 (11,000)	--	--	
Monno ceramic	--	--	--	--	--
Pesticide Savar	1 (11,000)	1 (11,000)	--	--	
Hazaribagh Tannery	1 (11,000)	1 (11,000)	--	--	

Table 44. RAPD analysis with primer OPA-4 (5´-AAT CGG GCT G-3´) in six plant samples of *Ipomoea aquatica* Forssk. collected from five different affected areas along with control

Plant samples collected from different affected areas	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
Control	1 (12,000)	1 (12,000)	--	--	
Gazipur Garments	1 (12,000)	1 (12,000)	--	--	
Acme Laboratory	2 (12,000, 3,000)	2 (12,000, 3,000)	--	--	
Monno ceramic	1 (12,000)	1 (12,000)	--	--	1 (12,000)
Pesticide Savar	1 (12,000)	1 (12,000)	--	--	
Hazaribagh Tannery	1 (12,000)	1 (12,000)	--	--	

Table 45. RAPD analysis with primer OPA-6 (5´-GGT CCC TGA C-3´) in six plant samples of *Ipomoea aquatica* Forssk. collected from five different affected areas along with control

Plant samples collected from different affected areas	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
Control	--	--	--	--	
Gazipur Garments	--	--	--	--	
Acme Laboratory	1 (11,000)	1 (11,000)	--	--	
Monno ceramic	1 (11,000)	1 (11,000)	--	--	--
Pesticide Savar	--	--	--	--	
Hazaribagh Tannery	1 (11,000)	1 (11,000)	--	--	

Table 46. RAPD analysis with primer OPA-8 (5´-GTG ACG TAG G-3´) in six plant samples of *Ipomoea aquatica* Forssk. collected from five different affected areas along with control

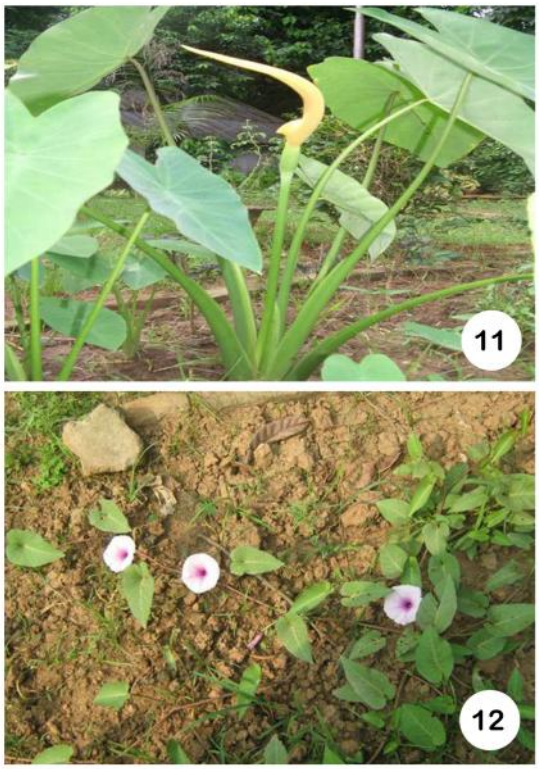
Plant samples collected from different affected areas	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
Control	--	--	--	--	
Gazipur Garments	--	--	--	--	
Acme Laboratory	--	--	--	--	
Monno ceramic	--	--	--	--	---
Pesticide Savar	--	--	--	--	
Hazaribagh Tannery	--	--	--	--	

Table 47. RAPD analysis with Primer-23 (5´-GTC AGG GCA A-3´) in six plant samples of *Ipomoea aquatica* Forssk. collected from five different affected areas along with control

Plant samples collected from different affected areas	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
Control	2 (1,700, 1,200)	2 (1,700, 1,200)	--	--	
Gazipur Garments	3 (1,700, 1,200, 800)	2 (1,700, 800)	1 (1200)	--	
Acme Laboratory	2 (1,200, 800)	1 (800)	1 (1200)	--	
Monno ceramic	--	--	--	--	--
Pesticide Savar	--	--	--	--	
Hazaribagh Tannery	1 (1,200)	1 (1,200)	--	--	

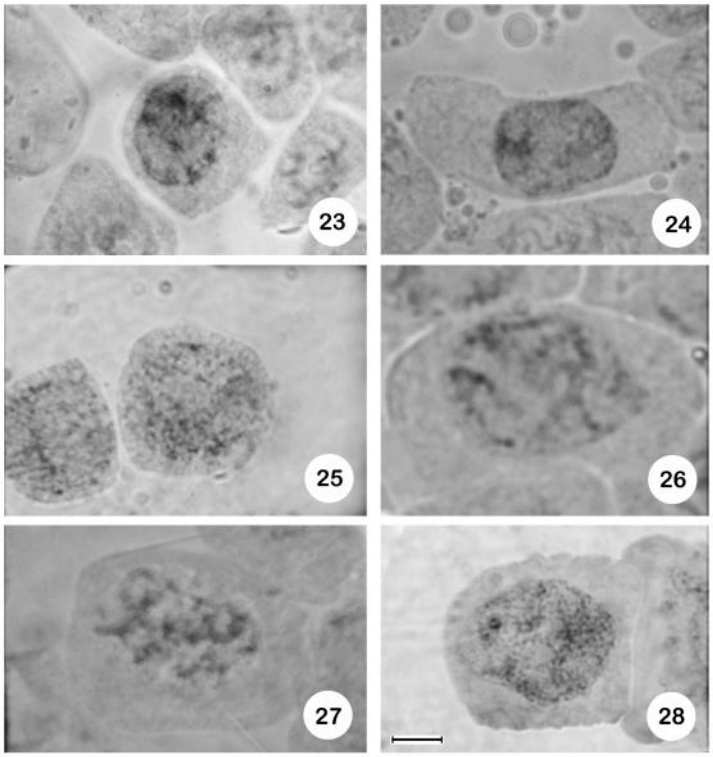
Table 48. RAPD analysis with primer-24 (5´- GGT CGG AGA A-3´) in six plant samples of *Ipomoea aquatica* Forssk. collected from five different affected areas along with control

Plant samples collected from different affected areas	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
Control	1 (1,500)	1 (1,500)	--	--	
Gazipur Garments	--	--	--	--	
Acme Laboratory	1 (1,200)	--	1 (1,200)	--	
Monno ceramic	--	--	--	--	--
Pesticide Savar	--	--	--	--	
Hazaribagh Tannery	--	--	--	--	

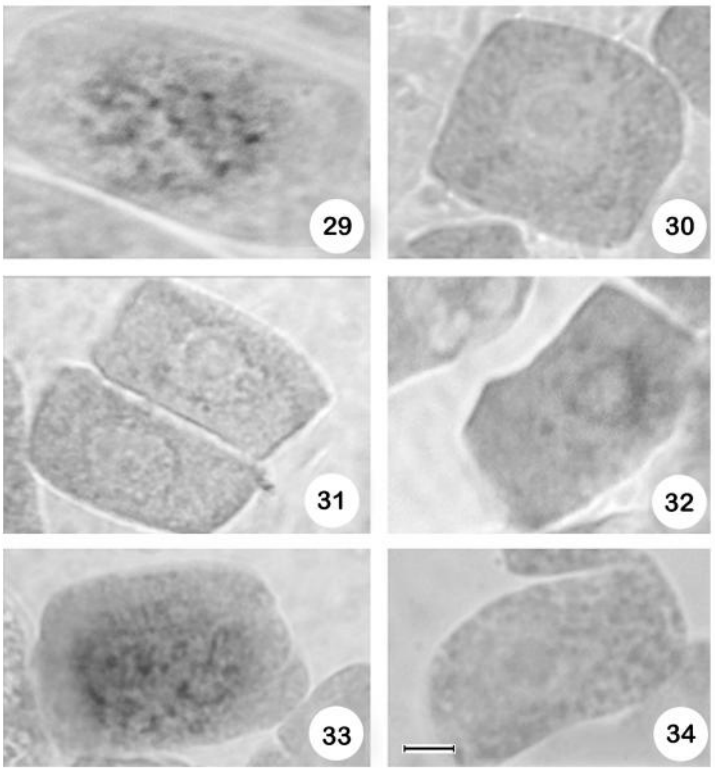


Figs. 11-12. Photographs of control plant samples maintained in Botanic Garden, Department of Botany, University of Dhaka. 11. *Colocasia esculenta* (L.) Schott and 12. *Ipomoea aquatica* Forssk.

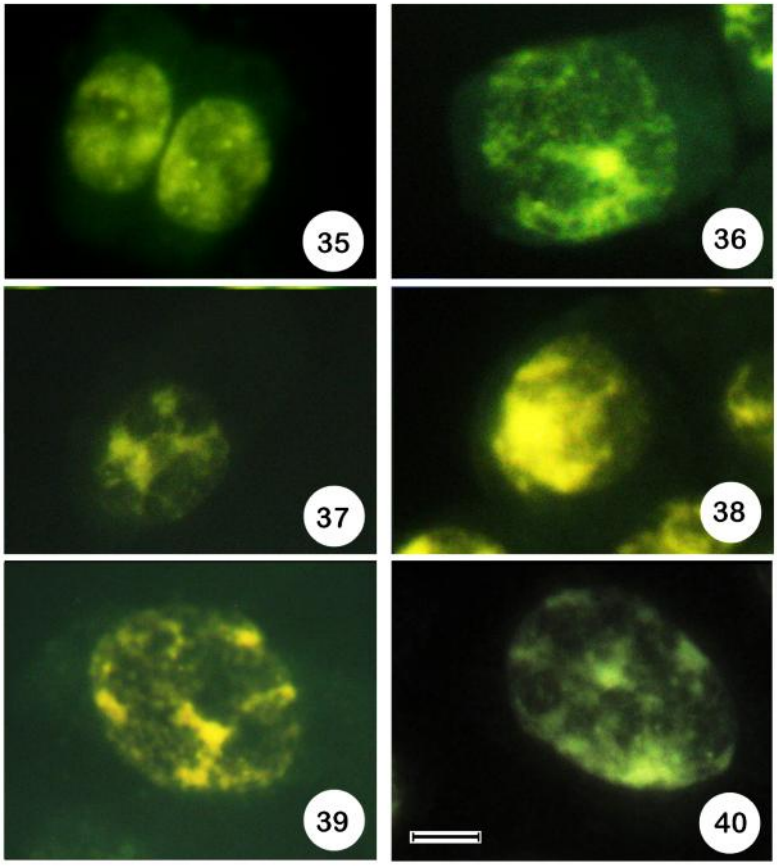
Figs. 13-22. Photographs of different Industrial areas with vegetation. 13. Power Tex Fassion Ltd., Gazipur. 14. Sample collection from Power Tex Fassion Ltd., Gazipur area. 15. Shetu Pesticide Ltd., Savar. 16. Affected vegetation around Shetu Pesticide Ltd., Savar. 17. Salma Leather Industries, Hazaribagh. 18. Affected vegetation around Salma Leather Industries, Hazaribagh. 19. Monno Ceramic Industries, Nayarhat, Dhamrai. 20. Affected vegetation around Monno Ceramic Industries, Nayarhat, Dhamrai. 21. Acme Laboratories Ltd., Tulivita, Dhamrai. 22. Affected vegetation around Acme Laboratories Ltd., Tulivita, Dhamrai.



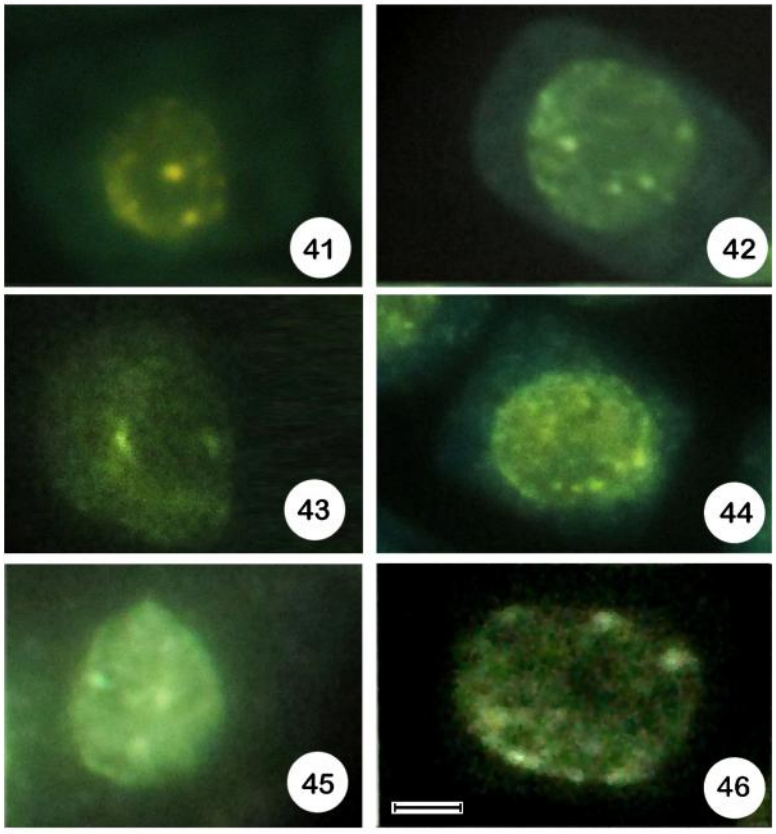
Figs. 23-28. Orcein-stained mitotic interphase nuclei of *Colocasia esculenta* (L.) Schott collected from different affected areas. 23. Control, Botanic Garden, Department of Botany, University of Dhaka. 24. Power Tex Fassion Ltd., Gazipur. 25. Shetu Pesticide Ltd., Savar. 26. Salma Leather Industries, Hazaribagh. 27. Monno Ceramic Industries, Nayarhat, Dhamrai. 28. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



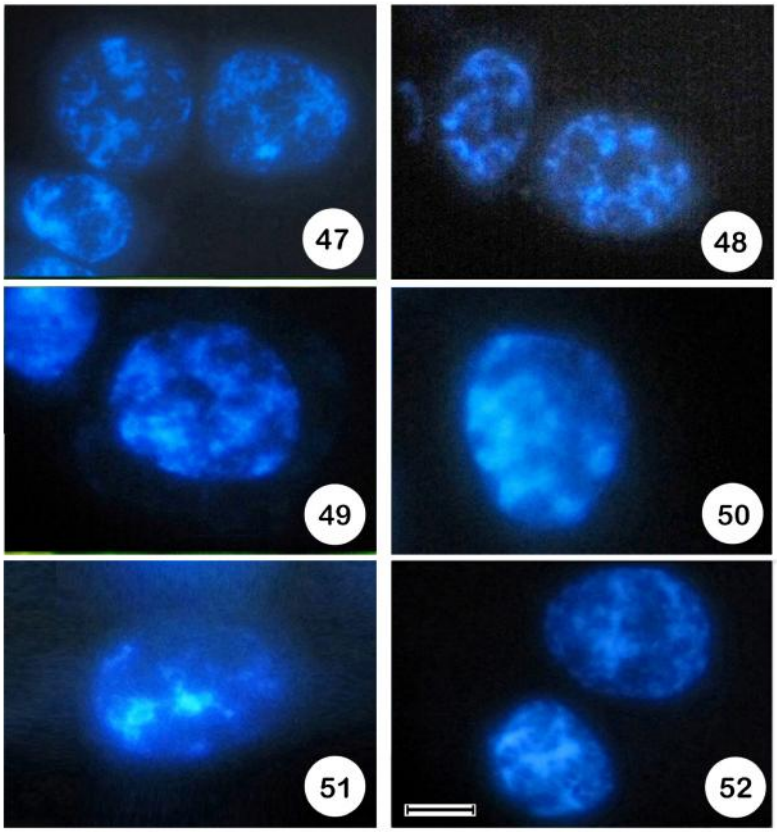
Figs. 29-34. Orcein-stained mitotic interphase nuclei of *Ipomoea aquatica* Forssk. collected from different affected areas. 29. Control, Botanic Garden, Department of Botany, University of Dhaka. 30. Power Tex Fassion Ltd., Gazipur. 31. Shetu Pesticide Ltd., Savar. 32. Salma Leather Industries, Hazaribagh. 33. Monno Ceramic Industries, Nayarhat, Dhamrai. 34. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



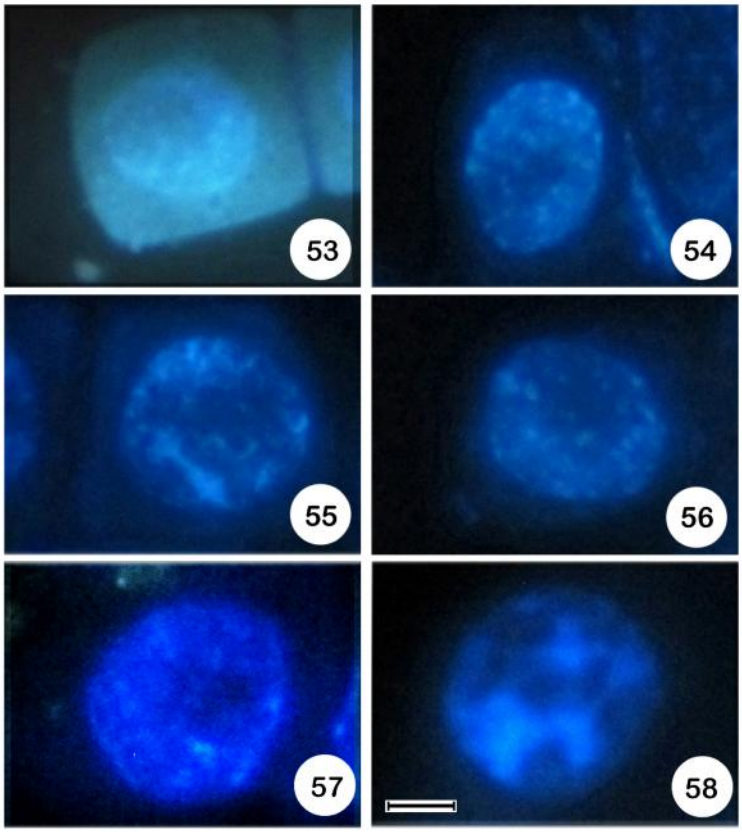
Figs. 35-40. CMA-stained mitotic interphase nuclei of *Colocasia esculenta* (L.) Schott collected from different affected areas. 35. Control, Botanic Garden, Department of Botany, University of Dhaka. 36. Power Tex Fassion Ltd., Gazipur. 37. Shetu Pesticide Ltd., Savar. 38. Salma Leather Industries, Hazaribagh. 39. Monno Ceramic Industries, Nayarhat, Dhamrai. 40. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



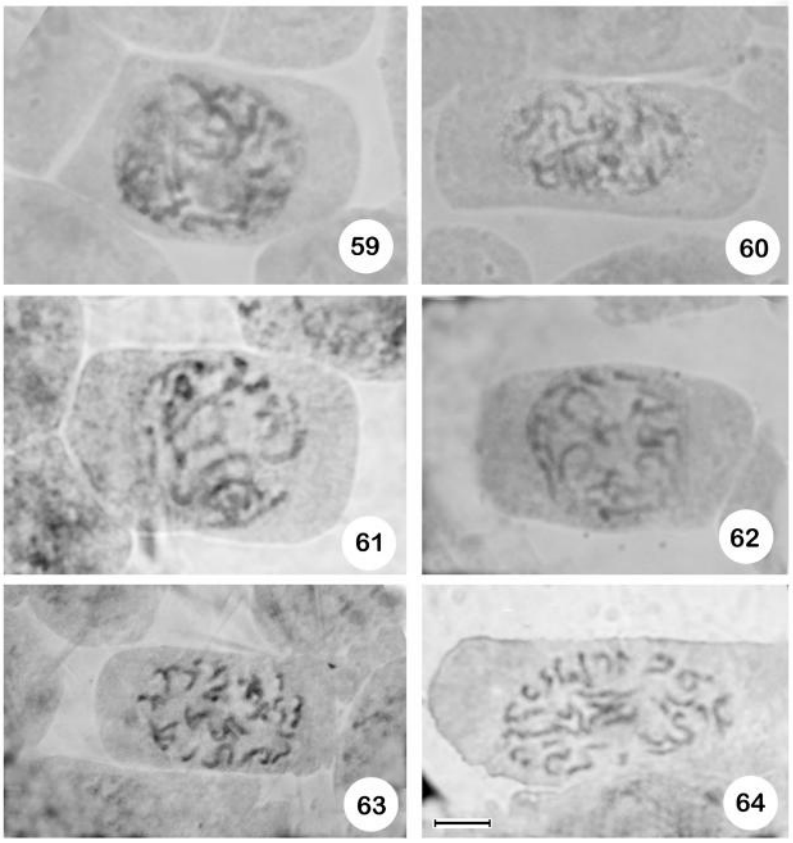
Figs. 41-46. CMA-stained mitotic interphase nuclei of *Ipomoea aquatica* Forssk. collected from different affected areas. 41. Control, Botanic Garden, Department of Botany, University of Dhaka. 42. Power Tex Fassion Ltd., Gazipur. 43. Shetu Pesticide Ltd., Savar. 44. Salma Leather Industries, Hazaribagh. 45. Monno Ceramic Industries, Nayarhat, Dhamrai. 46. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



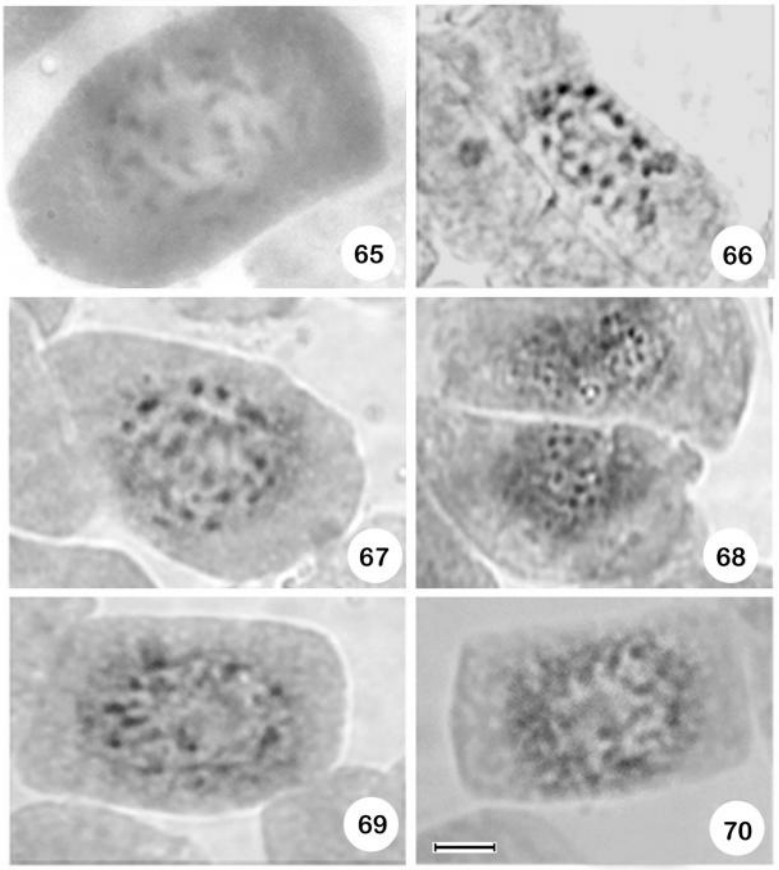
Figs. 47-52. DAPI-stained mitotic interphase nuclei of *Colocasia esculenta* (L.) Schott collected from different affected areas. 47. Control, Botanic Garden, Department of Botany, University of Dhaka. 48. Power Tex Fassion Ltd., Gazipur. 49. Shetu Pesticide Ltd., Savar. 50. Salma Leather Industries, Hazaribagh. 51. Monno Ceramic Industries, Nayarhat, Dhamrai. 52. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



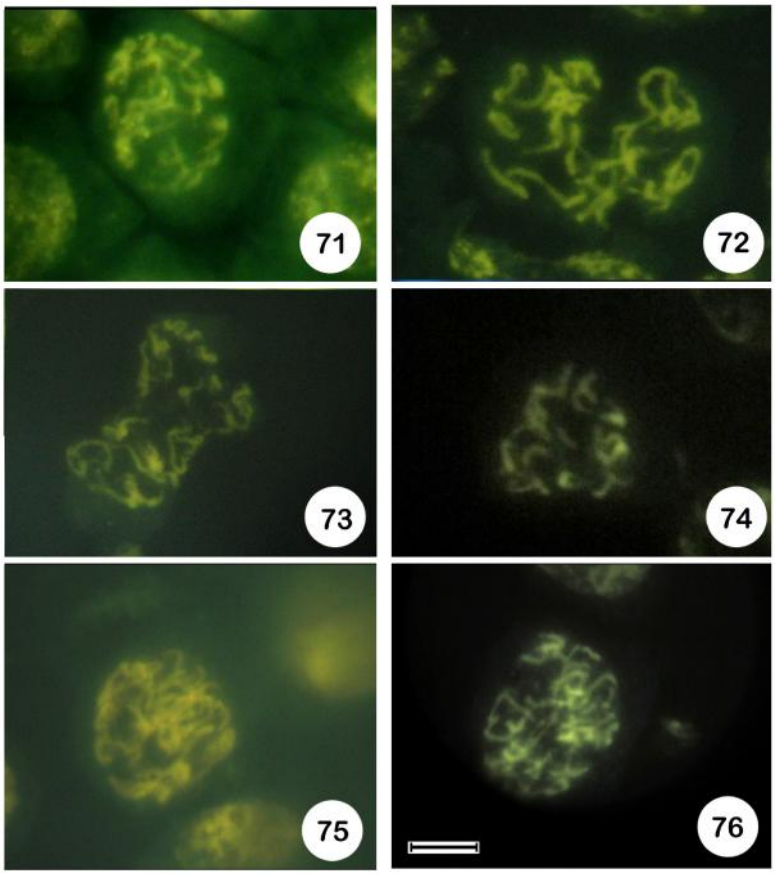
Figs. 53-58. DAPI-stained mitotic interphase nuclei of *Ipomoea aquatica* Forssk. collected from different affected areas. 53. Control, Botanic Garden, Department of Botany, University of Dhaka. 54. Power Tex Fassion Ltd., Gazipur. 55. Shetu Pesticide Ltd., Savar. 56. Salma Leather Industries, Hazaribagh. 57. Monno Ceramic Industries, Nayarhat, Dhamrai. 58. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



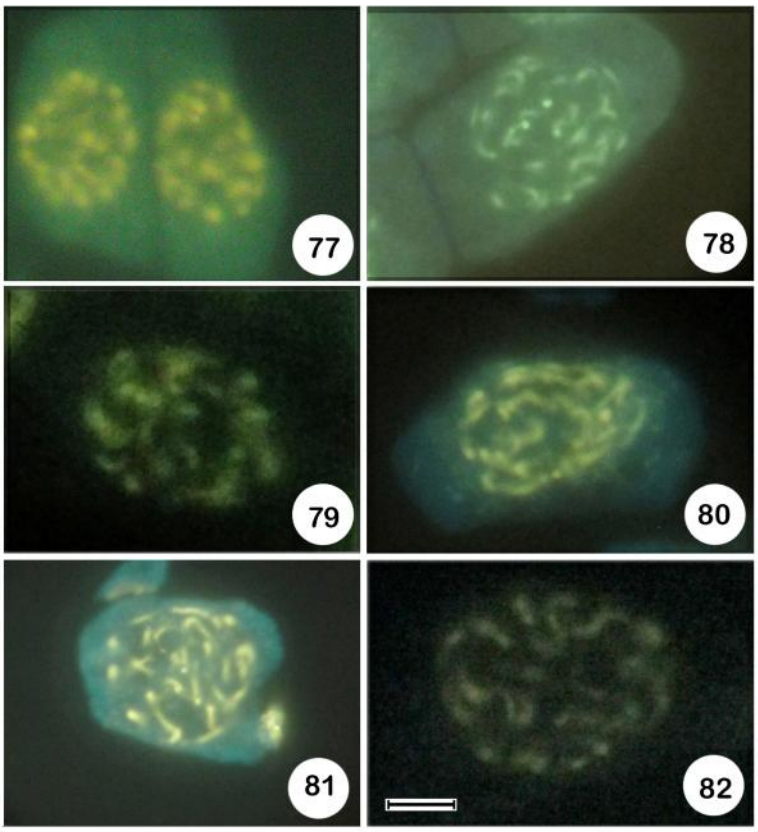
Figs. 59-64. Orcein-stained mitotic prophase chromosomes of *Colocasia esculenta* (L.) Schott collected from different affected areas. 59. Control, Botanic Garden, Department of Botany, University of Dhaka. 60. Power Tex Fassion Ltd., Gazipur. 61. Shetu Pesticide Ltd., Savar. 62. Salma Leather Industries, Hazaribagh. 63. Monno Ceramic Industries, Nayarhat, Dhamrai. 64. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



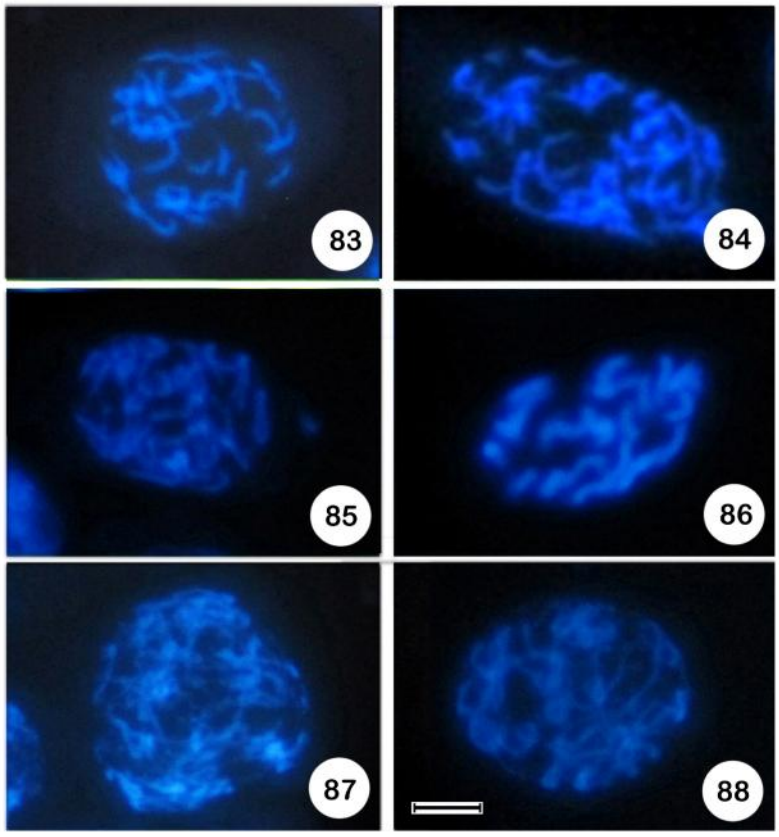
Figs. 65-70. Orcein-stained mitotic prophase chromosomes of *Ipomoea aquatica* Forssk. collected from different affected areas. 65. Control, Botanic Garden, Department of Botany, University of Dhaka. 66. Power Tex Fassion Ltd., Gazipur. 67. Shetu Pesticide Ltd., Savar. 68. Salma Leather Industries, Hazaribagh. 69. Monno Ceramic Industries, Nayarhat, Dhamrai. 70. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



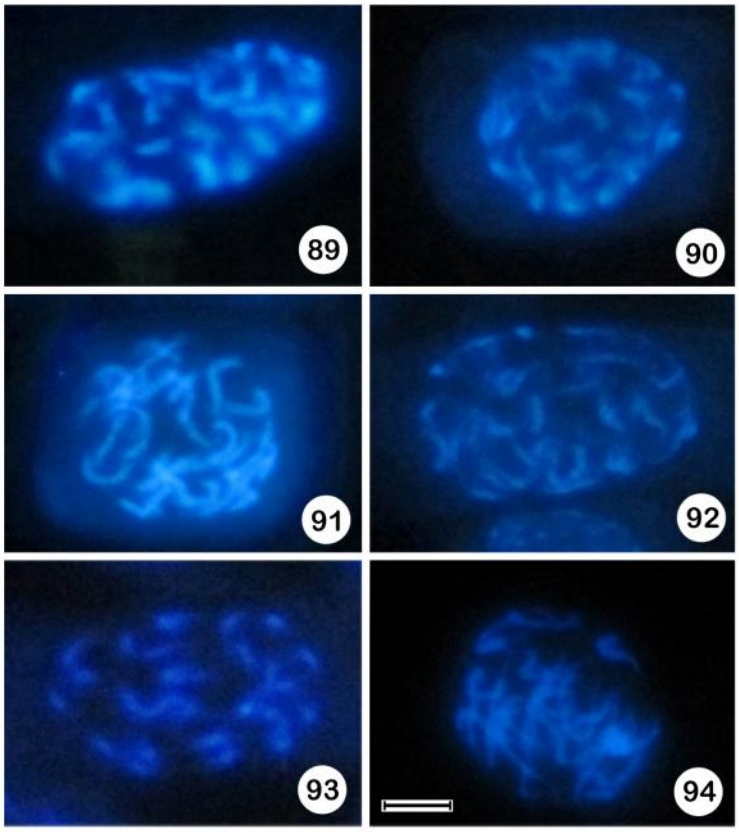
Figs. 71-76. CMA-stained mitotic prophase chromosomes of *Colocasia esculenta* (L.) Schott collected from different affected areas. 71. Control, Botanic Garden, Department of Botany, University of Dhaka. 72. Power Tex Fassion Ltd., Gazipur. 73. Shetu Pesticide Ltd., Savar. 74. Salma Leather Industries, Hazaribagh. 75. Monno Ceramic Industries, Nayarhat, Dhamrai. 76. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



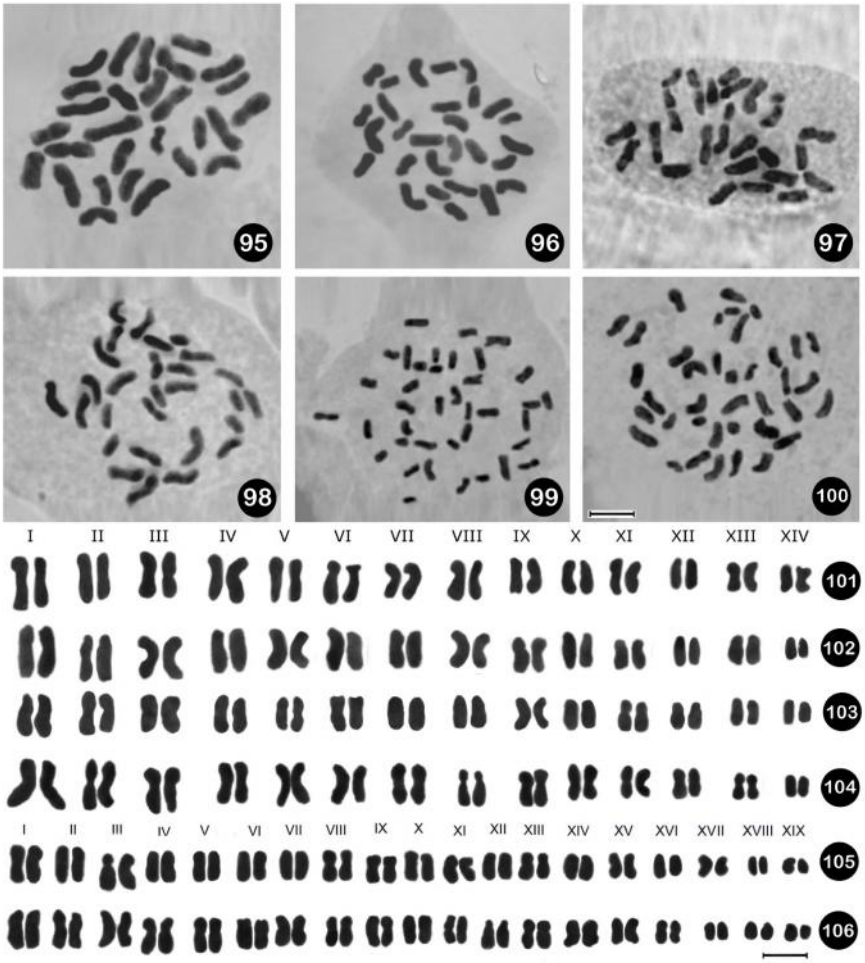
Figs. 77-82. CMA-stained mitotic prophase chromosomes of *Ipomoea aquatica* Forssk. collected from different affected areas. 77. Control, Botanic Garden, Department of Botany, University of Dhaka. 78. Power Tex Fassion Ltd., Gazipur. 79. Shetu Pesticide Ltd., Savar. 80. Salma Leather Industries, Hazaribagh. 81. Monno Ceramic Industries, Nayarhat, Dhamrai. 82. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



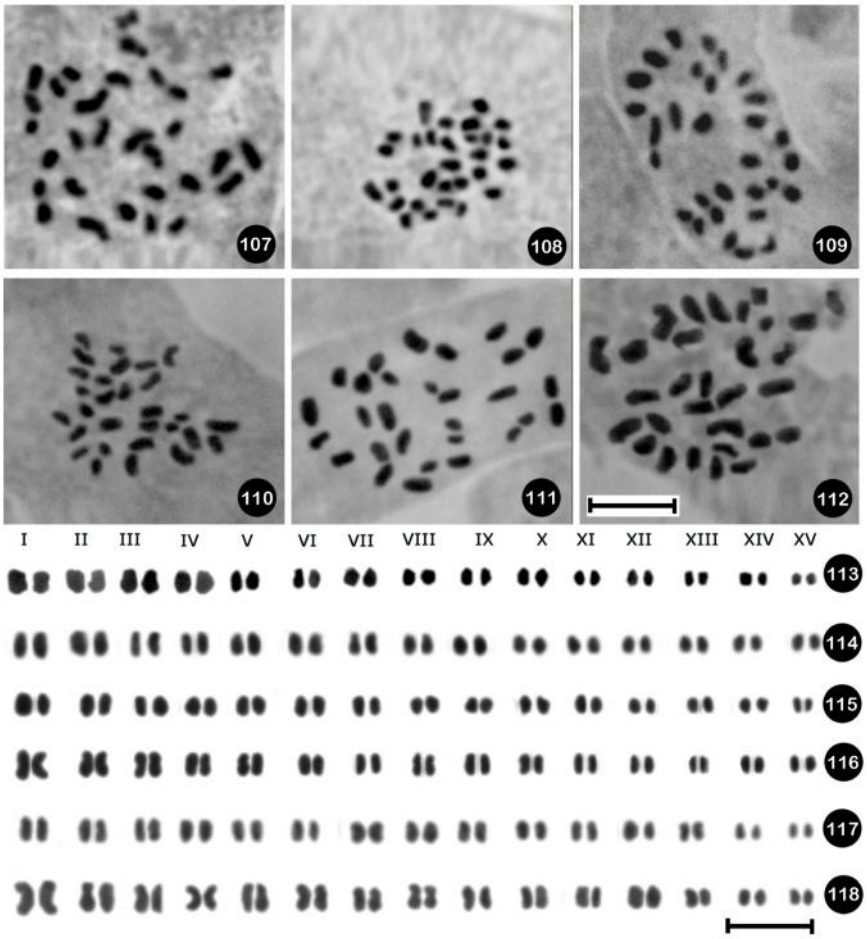
Figs. 83-88. DAPI-stained mitotic prophase chromosomes of *Colocasia esculenta* (L.) Schott collected from different affected areas. 83. Control, Botanic Garden, Department of Botany, University of Dhaka. 84. Power Tex Fassion Ltd., Gazipur. 85. Shetu Pesticide Ltd., Savar. 86. Salma Leather Industries, Hazaribagh. 87. Monno Ceramic Industries, Nayarhat, Dhamrai. 88. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



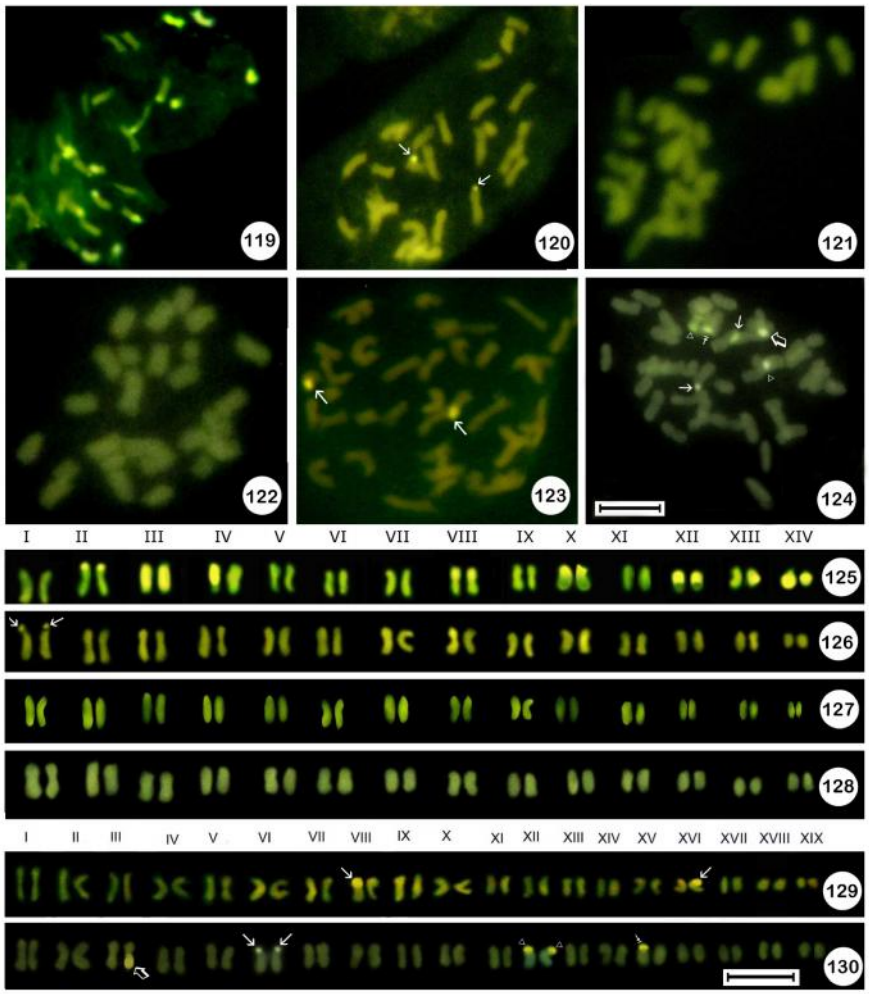
Figs. 89-94. DAPI-stained mitotic prophase chromosomes of *Ipomoea aquatica* Forssk. collected from different affected areas. 89. Control, Botanic Garden, Department of Botany, University of Dhaka. 90. Power Tex Fassion Ltd., Gazipur. 91. Shetu Pesticide Ltd., Savar. 92. Salma Leather Industries, Hazaribagh. 93. Monno Ceramic Industries, Nayarhat, Dhamrai. 94. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



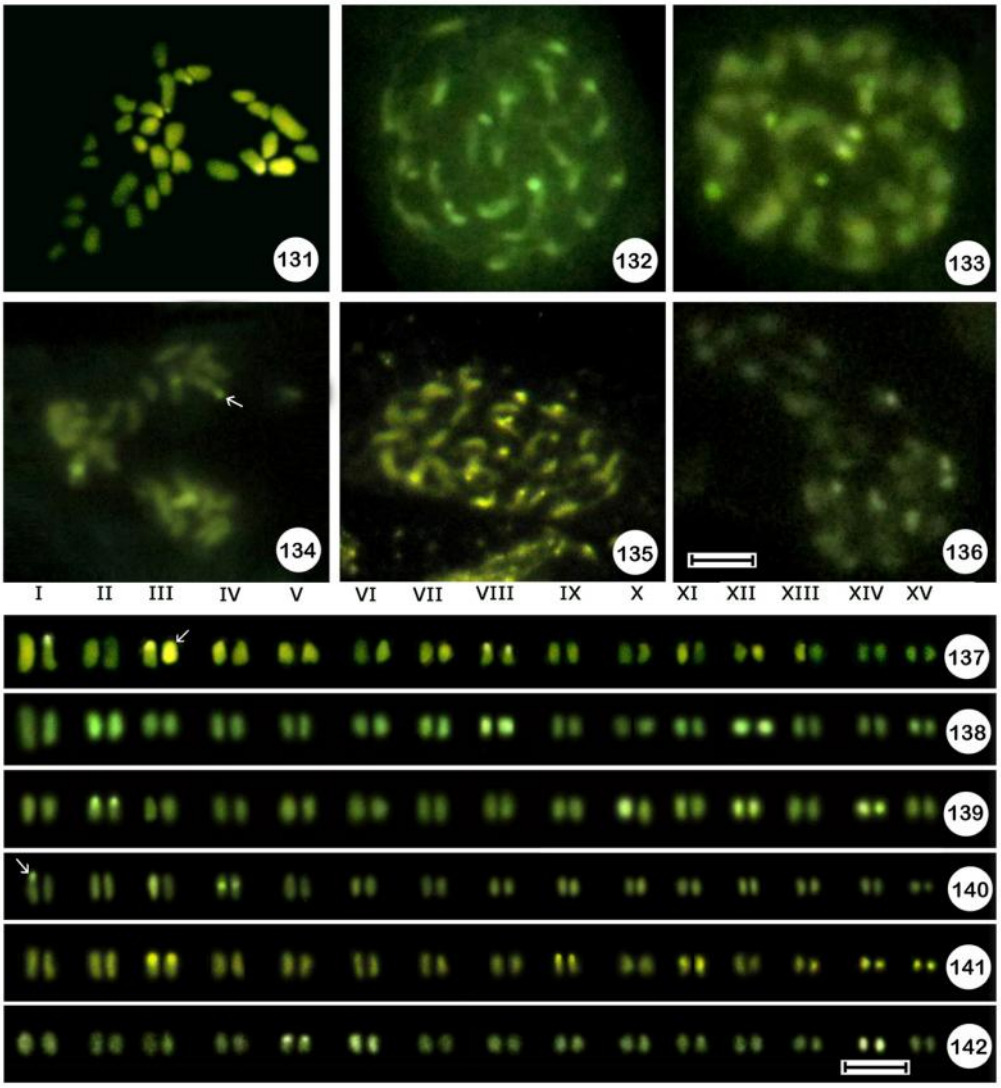
Figs. 95-106. Orcein-stained mitotic metaphase chromosomes and karyotypes of *Colocasia esculenta* (L.) Schott collected from different affected areas. 95. and 101. metaphase and karyotypes of control sample. 96. and 102. metaphase and karyotypes of affected plant from Power Tex Fassion Ltd., Gazipur. 97. and 103. metaphase and karyotypes of affected plant from Shetu Pesticide Ltd., Savar. 98. and 104. metaphase and karyotypes of affected plant from Salma Leather Industries, Hazaribagh. 99. and 105. metaphase and karyotypes of affected plant from Monno Ceramic Industries, Nayarhat, Dhamrai. 100. and 106. metaphase and karyotypes of affected plant from Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



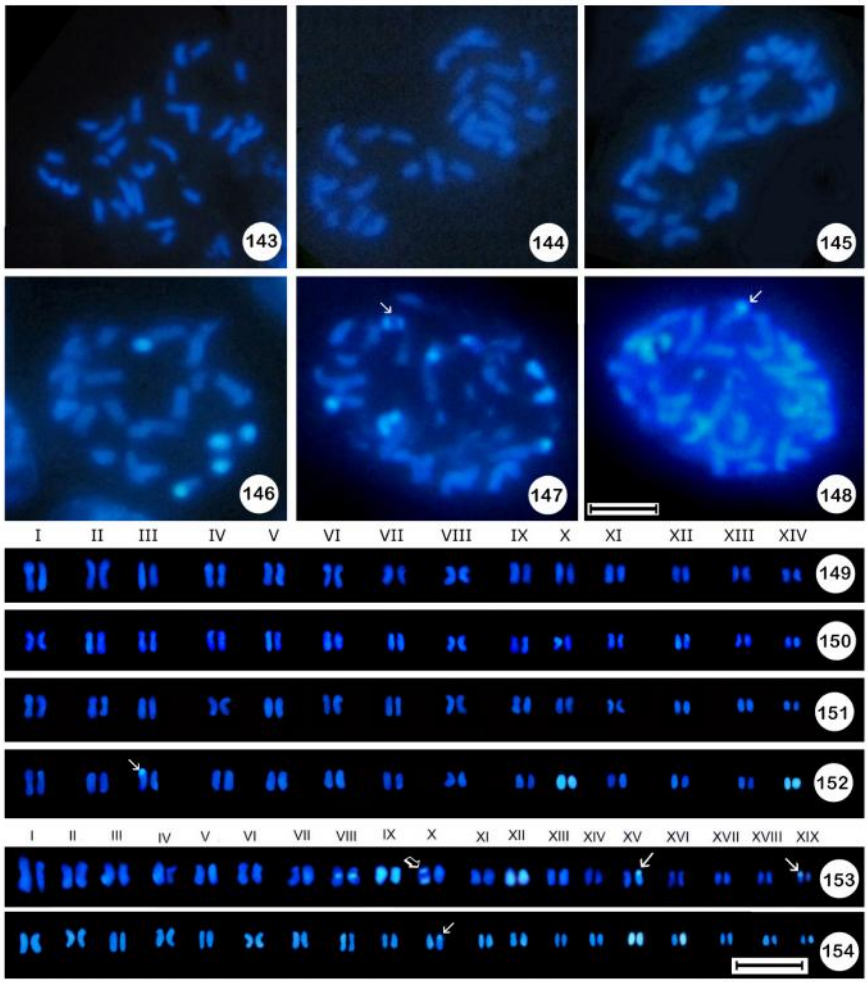
Figs. 107-118. Orcein-stained mitotic metaphase chromosomes and karyotypes of *Ipomoea aquatica* Forssk. collected from different affected areas. 107. and 113. metaphase and karyotypes of control sample. 108. and 114. metaphase and karyotypes of affected plant from Power Tex Fassion Ltd., Gazipur. 109. and 115. metaphase and karyotypes of affected plant from Shetu Pesticide Ltd., Savar. 110. and 116. metaphase and karyotypes of affected plant from Salma Leather Industries, Hazaribagh. 111. and 117. metaphase and karyotypes of affected plant from Monno Ceramic Industries, Nayarhat, Dhamrai. 112 and 118. metaphase and karyotypes of affected plant from Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



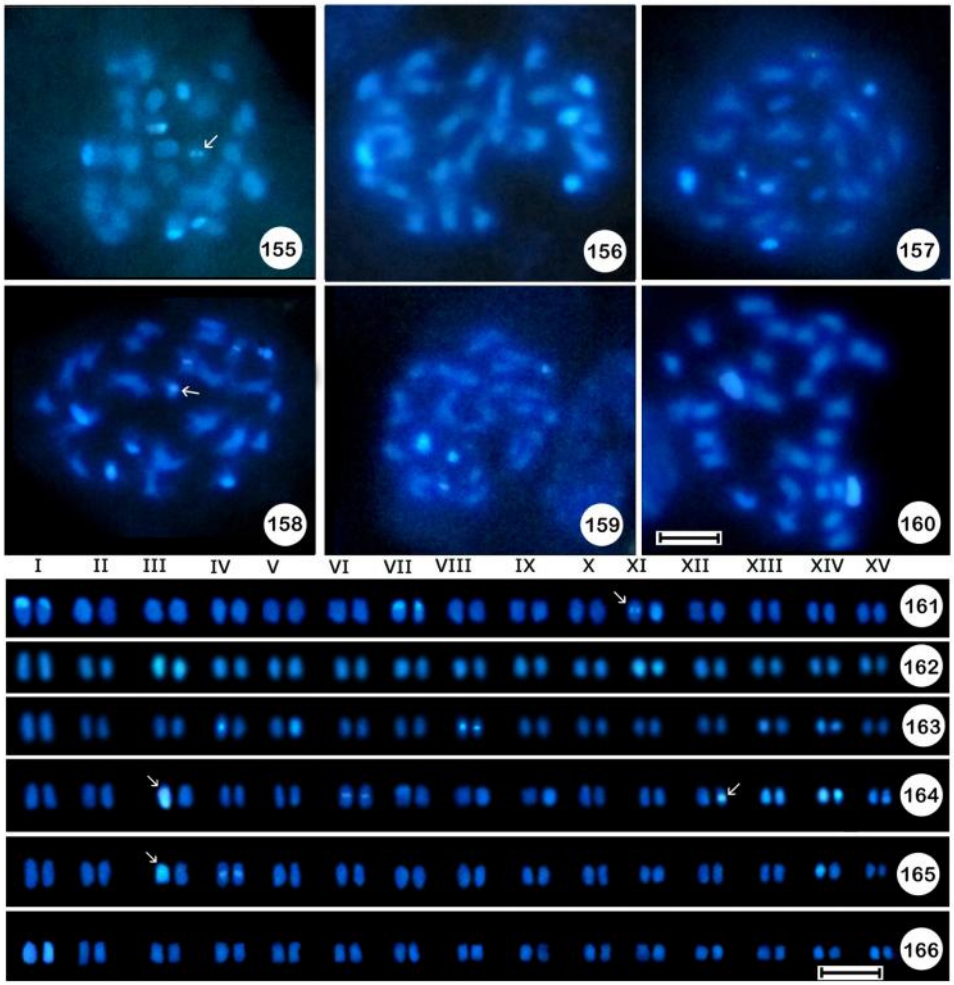
Figs. 119-130. CMA-stained mitotic metaphase chromosomes and karyotypes of *Colocasia esculenta* (L.) Schott collected from different affected areas. 119. and 125. metaphase and karyotypes of control sample. 120. and 126. metaphase and karyotypes of affected plant from Power Tex Fassion Ltd., Gazipur. 121. and 127. metaphase and karyotypes of affected plant from Shetu Pesticide Ltd., Savar. 122. and 128. metaphase and karyotypes of affected plant from Salma Leather Industries, Hazaribagh. 123. and 129. metaphase and karyotypes of affected plant from Monno Ceramic Industries, Nayarhat, Dhamrai. 124. and 130. metaphase and karyotypes of affected plant from Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



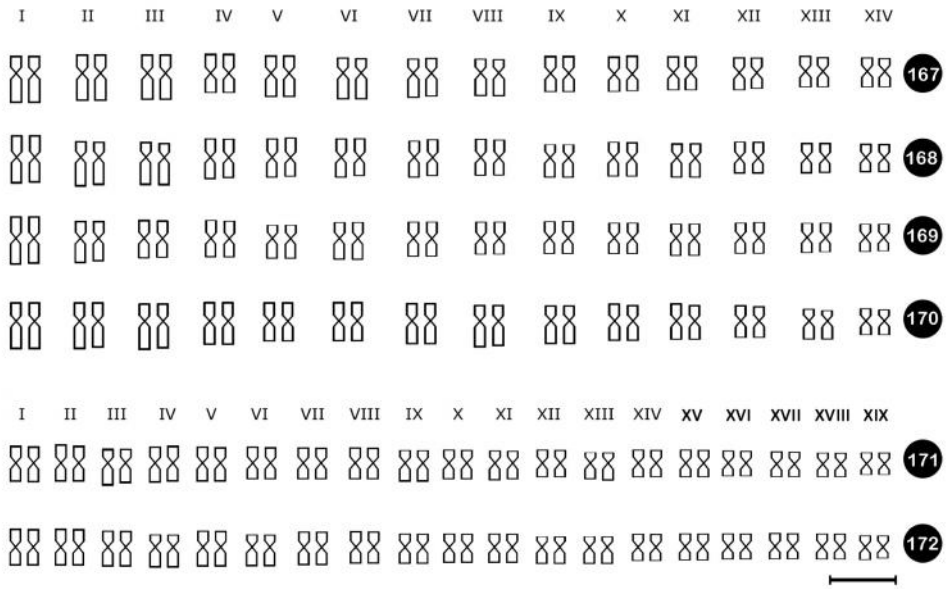
Figs. 131-142. CMA-stained mitotic metaphase chromosomes and karyotypes of *Ipomoea aquatica* Forssk. collected from different affected areas. 131. and 137. metaphase and karyotypes of control sample. 132. and 138. metaphase and karyotypes of affected plant from Power Tex Fassion Ltd., Gazipur. 133. and 139. metaphase and karyotypes of affected plant from Shetu Pesticide Ltd., Savar. 134. and 140. metaphase and karyotypes of affected plant from Salma Leather Industries, Hazaribagh. 135. and 141. metaphase and karyotypes of affected plant from Monno Ceramic Industries, Nayarhat, Dhamrai. 136 and 142. metaphase and karyotypes of affected plant from Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



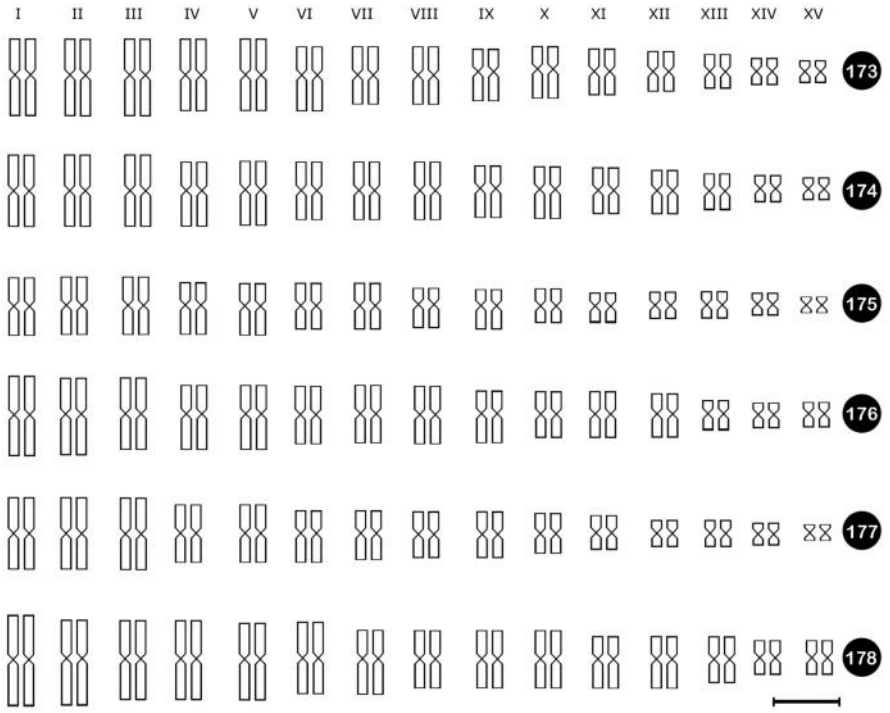
Figs. 143-154. DAPI-stained mitotic metaphase chromosomes and karyotypes of *Colocasia esculenta* (L.) Schott collected from different affected areas. 143. and 149. metaphase and karyotypes of control sample. 144. and 150. metaphase and karyotypes of affected plant from Power Tex Fassion Ltd., Gazipur. 145. and 151. metaphase and karyotypes of affected plant from Shetu Pesticide Ltd., Savar. 146. and 152. metaphase and karyotypes of affected plant from Salma Leather Industries, Hazaribagh. 147. and 153. metaphase and karyotypes of affected plant from Monno Ceramic Industries, Nayarhat, Dhamrai. 148. and 154. metaphase and karyotypes of affected plant from Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



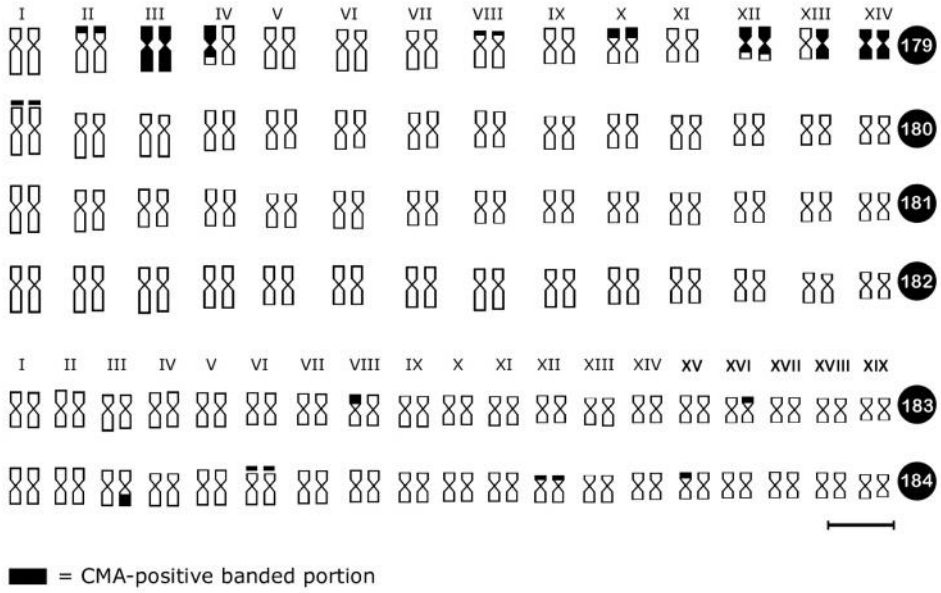
Figs. 155-166. DAPI-stained mitotic metaphase chromosomes and karyotypes of *Ipomoea aquatica* Forssk. collected from different affected areas. 155. and 161. metaphase and karyotypes of control sample. 156. and 162. metaphase and karyotypes of affected plant from Power Tex Fassion Ltd., Gazipur. 157. and 163. metaphase and karyotypes of affected plant from Shetu Pesticide Ltd., Savar. 158. and 164. metaphase and karyotypes of affected plant from Salma Leather Industries, Hazaribagh. 159. and 165. metaphase and karyotypes of affected plant from Monno Ceramic Industries, Nayarhat, Dhamrai. 160. and 166. metaphase and karyotypes of affected plant from Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



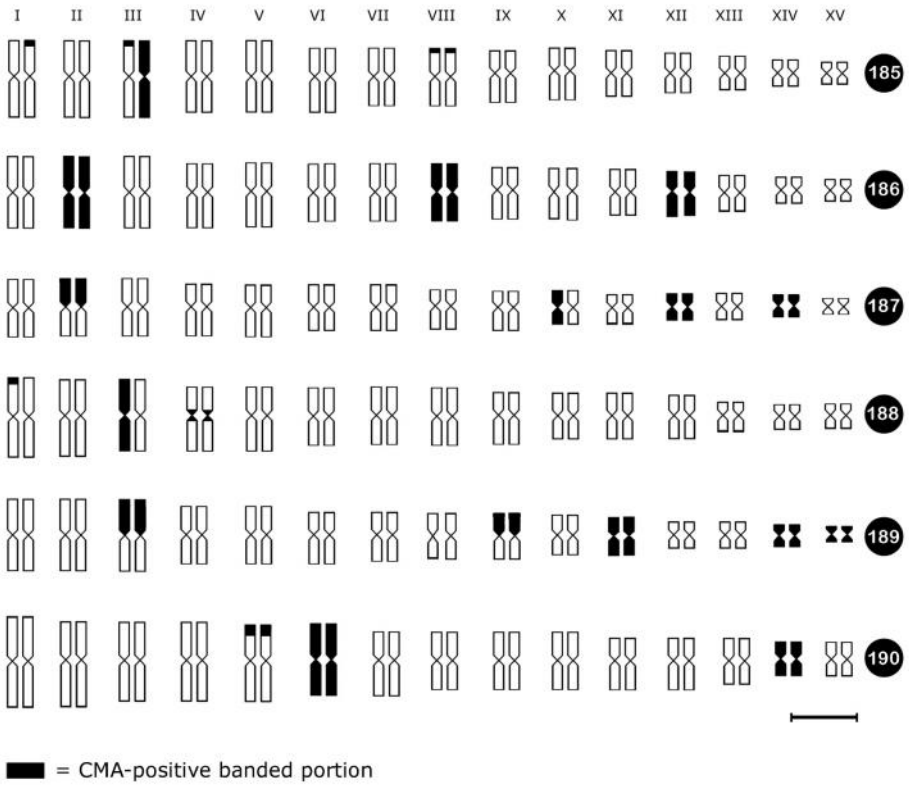
Figs. 167-172. Idiogram prepared from orcein-stained mitotic metaphase chromosomes of *Colocasia esculenta* (L.) Schott collected from different affected areas. 167. Control, Botanic Garden, Department of Botany, University of Dhaka. 168. Power Tex Fassion Ltd., Gazipur. 169. Shetu Pesticide Ltd., Savar. 170. Salma Leather Industries, Hazaribagh. 171. Monno Ceramic Industries, Nayarhat, Dhamrai. 172. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



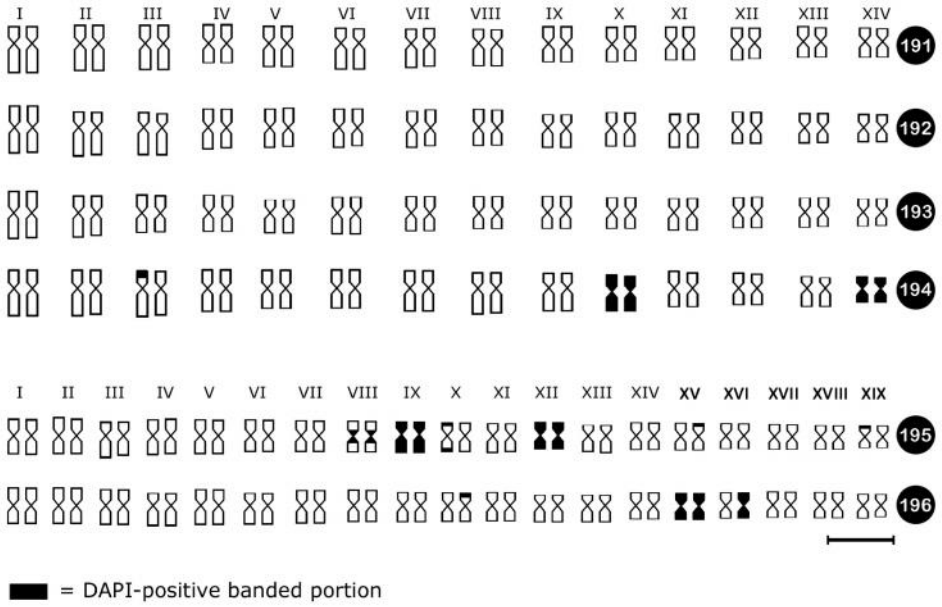
Figs. 173-178. Idiogram prepared from orcein-stained mitotic metaphase chromosomes of *Ipomoea aquatica* Forssk. collected from different affected areas. 173. Control, Botanic Garden, Department of Botany, University of Dhaka. 174. Power Tex Fassion Ltd., Gazipur. 175. Shetu Pesticide Ltd., Savar. 176. Salma Leather Industries, Hazaribagh. 177. Monno Ceramic Industries, Nayarhat, Dhamrai. 178. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



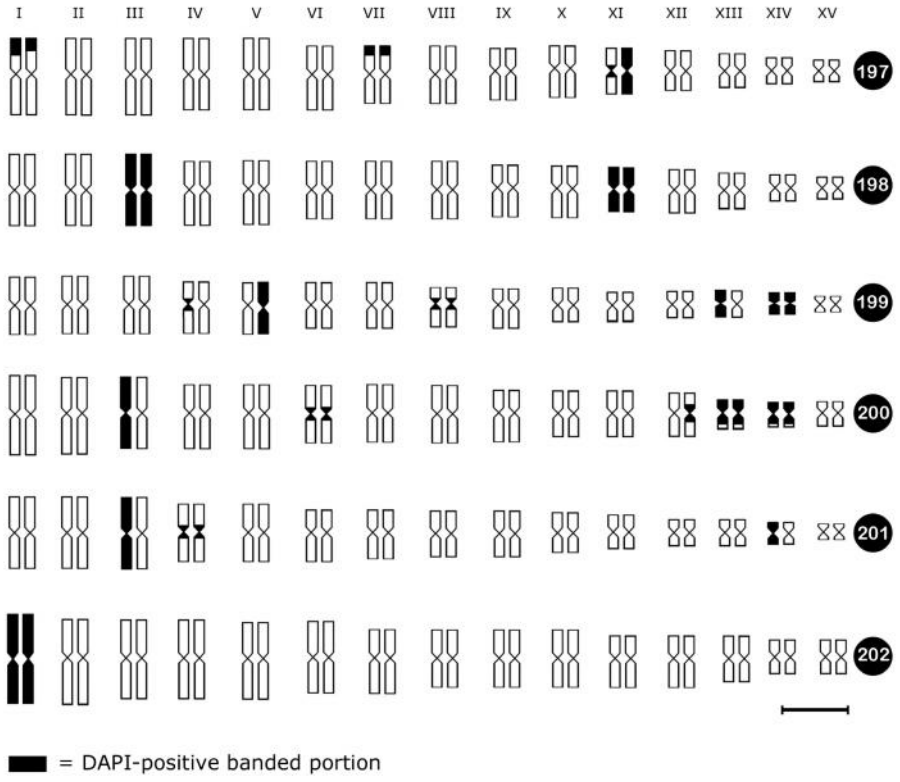
Figs. 179-184. Idiogram prepared from CMA-stained mitotic metaphase chromosomes of *Colocasia esculenta* (L.) Schott collected from different affected areas. 179. Control, Botanic Garden, Department of Botany, University of Dhaka. 180. Power Tex Fassion Ltd., Gazipur. 181. Shetu Pesticide Ltd., Savar. 182. Salma Leather Industries, Hazaribagh. 183. Monno Ceramic Industries, Nayarhat, Dhamrai. 184. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



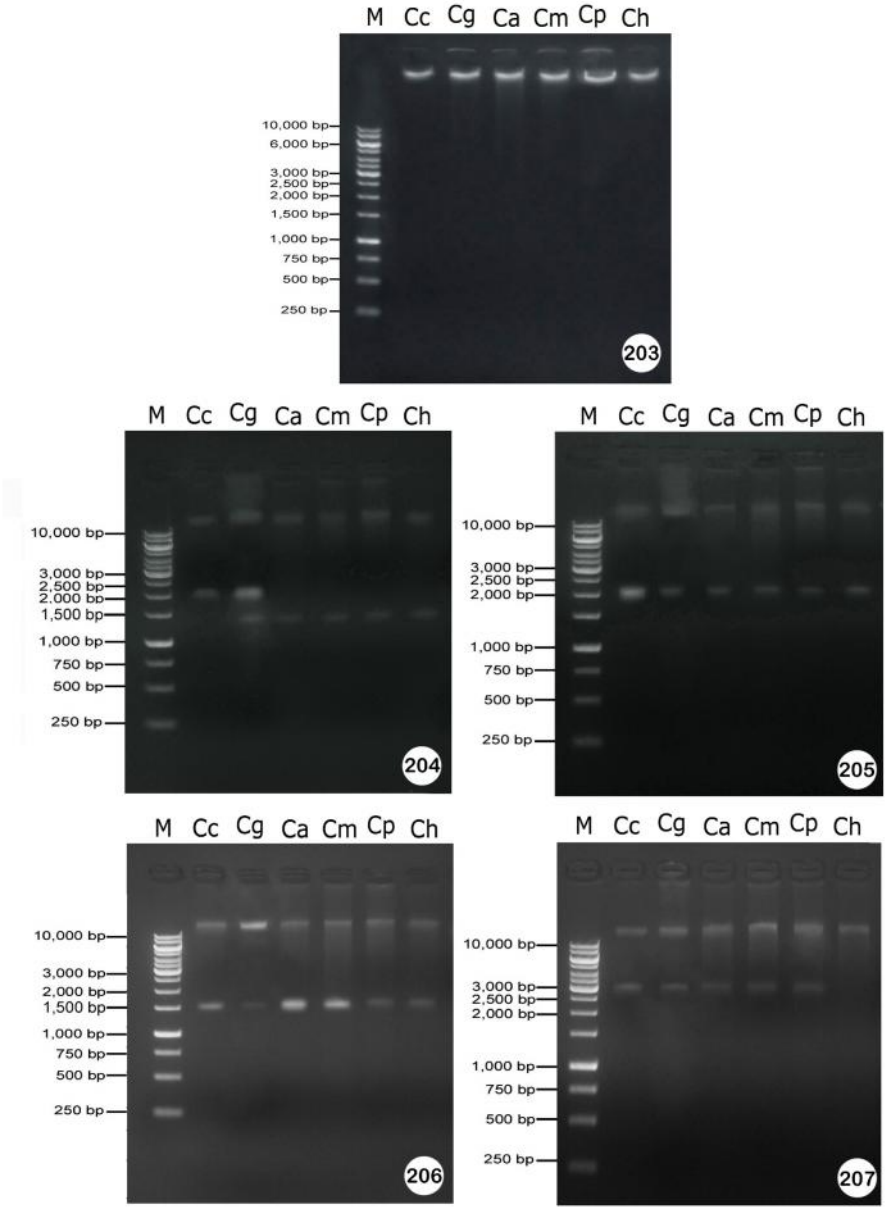
Figs. 185-190. Idiogram prepared from CMA-stained mitotic metaphase chromosomes of *Ipomoea aquatica* Forssk. collected from different affected areas. 185. Control, Botanic Garden, Department of Botany, University of Dhaka. 186. Power Tex Fassion Ltd., Gazipur. 187. Shetu Pesticide Ltd., Savar. 188. Salma Leather Industries, Hazaribagh. 189. Monno Ceramic Industries, Nayarhat, Dhamrai. 190. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



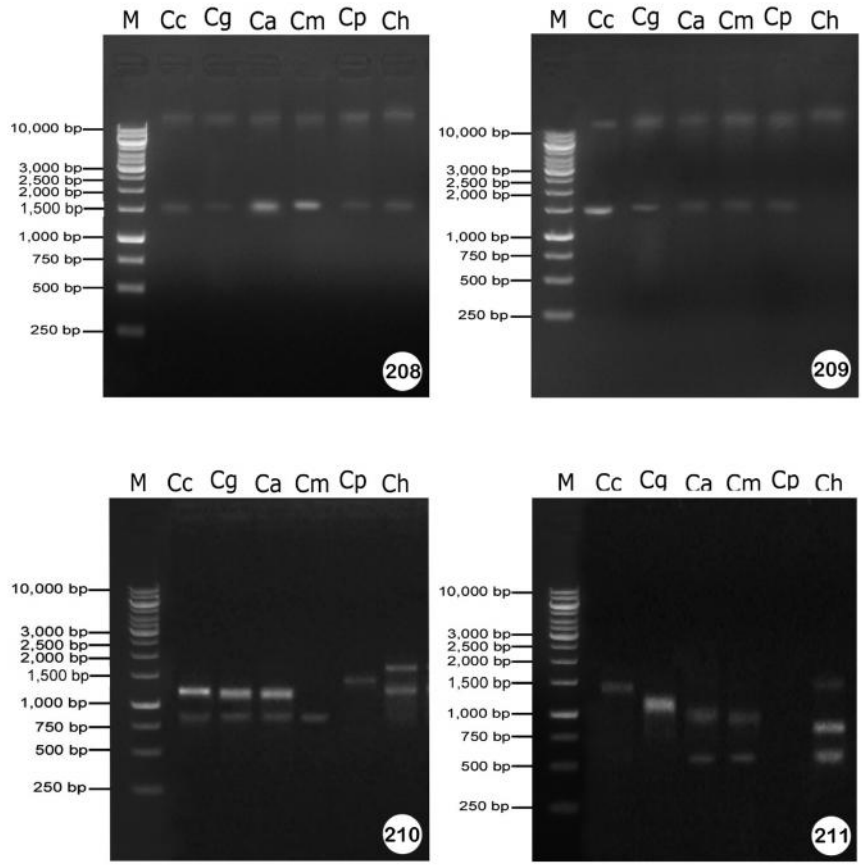
Figs. 191-196. Idiogram prepared from DAPI-stained mitotic metaphase chromosomes of *Colocasia esculenta* (L.) Schott collected from different affected areas. 191. Control, Botanic Garden, Department of Botany, University of Dhaka. 192. Power Tex Fassion Ltd., Gazipur. 193. Shetu Pesticide Ltd., Savar. 194. Salma Leather Industries, Hazaribagh. 195. Monno Ceramic Industries, Nayarhat, Dhamrai. 196. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



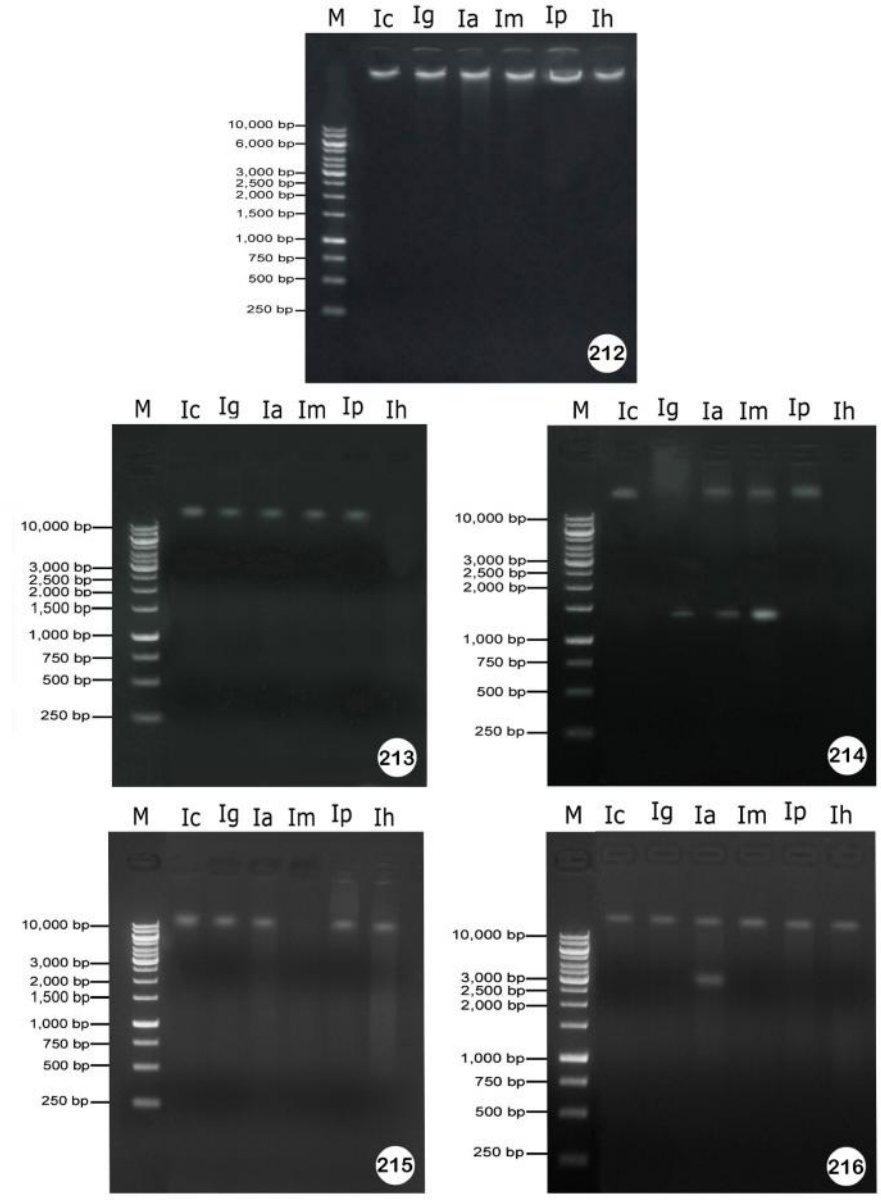
Figs. 197-202. Idiogram prepared from DAPI-stained mitotic metaphase chromosomes of *Ipomoea aquatica* Forssk. collected from different affected areas. 197. Control, Botanic Garden, Department of Botany, University of Dhaka. 198. Power Tex Fassion Ltd., Gazipur. 199. Shetu Pesticide Ltd., Savar. 200. Salma Leather Industries, Hazaribagh. 201. Monno Ceramic Industries, Nayarhat, Dhamrai. 202. Acme Laboratories Ltd., Tulivita, Dhamrai. Bar = 5 µm.



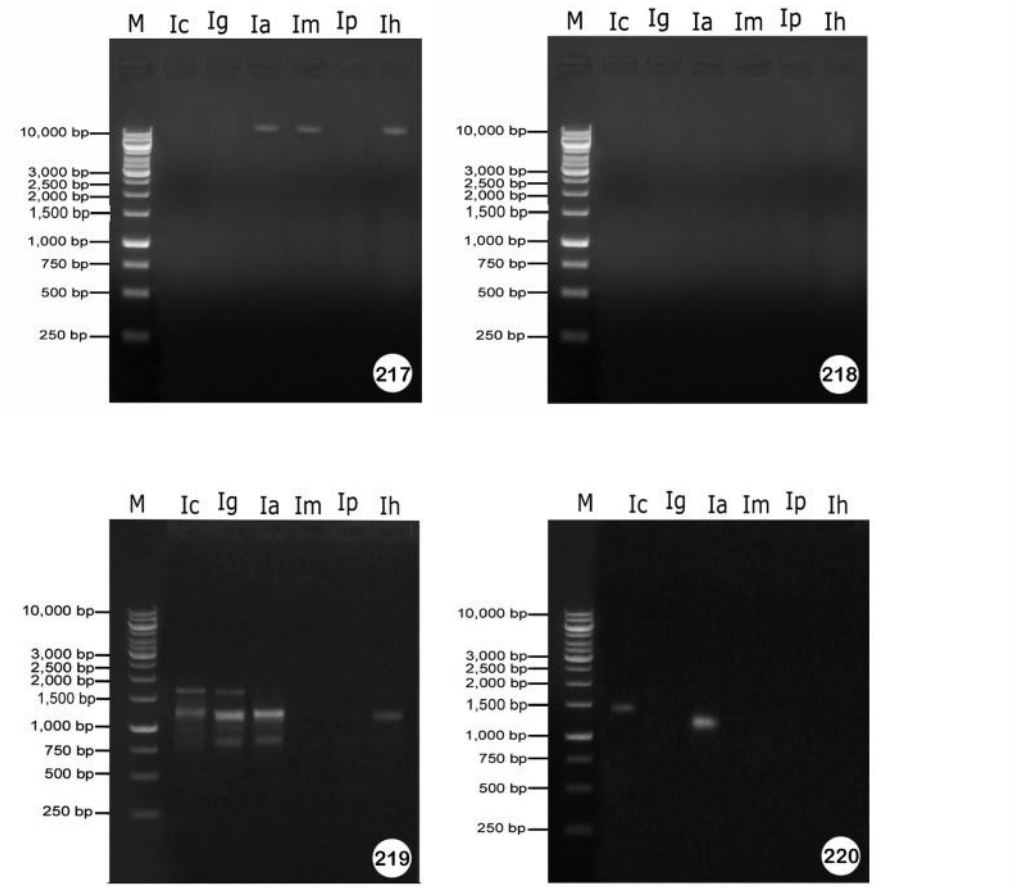
Figs. 203-207. RAPD analysis. 203. Electrophoregram of Ethidium Bromide stained genomic DNA of six plant samples of *Colocasia esculenta* (L.) Schott 204. with primer sequence OPA-1 (5'-CAG GCC CTT C-3'). 205. OPA-2 (5'-TGC CGA GCT G-3'). 206. OPA-3 (5'-AGT CAG CCA C 3') and 207. OPA-4 (5'-AAT CGG GCT G-3'). M=1 Kb DNA ladder, Cc= *Colocasia* control, Cg= Gazipur, Ca= Acme laboratory, Cm= Monno ceramic, Cp= Pesticide Savar and Ch= Hazaribagh leather.



Figs. 208-211. RAPD analysis. 208. with primer sequence OPA-6 (5'-GGT CCC TGA C-3'). 209. OPA-8 (5'-GTG ACG TAG G-3'). 210. primer-23 (5'-GTC AGG GCA A-3') and 211. primer-24 (5'-GGT CGG AGA A-3') in six plant samples of *Colocasia esculenta* (L.) Schott M=1 Kb DNA ladder, Cc= *Colocasia* control, Cg= Gazipur, Ca= Acme laboratory, Cm= Monno ceramic, Cp= Pesticide Savar and Ch= Hazaribagh leather.



Figs. 212-216. RAPD analysis. 212. Electrophoregram of Ethidium Bromide stained genomic DNA of six plant samples of Ipomoea *aquatica* Forssk. 213. With primer sequence OPA-1 (5'-CAG GCC CTT C-3'). 214. OPA-2 (5'-TGC CGA GCT G-3'). 215. OPA-3 (5'-AGT CAG CCA C 3') and 216. OPA-4 (5'-AAT CGG GCT G-3'). M=1 Kb DNA ladder, Ic= *Ipomoea* control, Ig= Gazipur, Ia= Acme laboratory, Im= Monno ceramic, Ip= Pesticide Savar and Ih= Hazaribagh leather.



Figs. 217-220. RAPD analysis. 217. With primer sequence OPA-6 (5'-GGT CCC TGA C-3'). 218. OPA-8 (5'-GTG ACG TAG G-3'). 219. Primer-23 (5'-GTC AGG GCA A-3') and 220. Primer-24 (5'-GGT CGG AGA A-3') in six plant samples of Ipomoea *aquatica* Forssk. M=1 Kb DNA ladder, Ic= *Ipomoea* control, Ig= Gazipur, Ia= Acme laboratory, Im= Monno ceramic, Ip= Pesticide Savar and Ih= Hazaribagh leather.

4. Discussion

A number of industries have been established in Dhaka city and outskirts of Dhaka city. The industries are of different kinds *viz.* tannery, textile, paint, pesticide, pharmaceutical etc. These industries are using different kinds of toxic substances containing various heavy metals and other hazardous chemicals. Unfortunately most of the industry has no waste management system. As a result the industrial wastes are directly drained to nearby water bodies and agricultural land. As a consequence, the heavy metal concentrations are gradually increasing in the soil and water of those areas (Moniruzzaman *et al.* 2009).

Colocasia esculanta (L.) Schott and *Ipomoea aquatica* Forssk. are widely growing plants in these affected areas. Moreover, these plants are being sold to the local market for human consumption. Therefore, it is an urgent need to observe whether the heavy metals or other hazardous chemicals enter the human food chain through these plants. In this study, chemical analysis of water, soil and plants of different affected areas have been done. The cytological behavior of the interphase nuclei, prophase- and metaphase chromosomes of both the plant species collected from the different affected areas were compared with the control (non affected plants). Besides, the molecular analysis by RAPD has been undertaken for comparison of these affected plants with control.

4.1. Chemical analysis

The amount of Dissolved Oxygen (DO) was below the permissible limit in all water samples except control (Table 6, Fig. 1). The lowest amount of DO was found in the water sample of Hazaribagh area. The amount of DO indicates the quality of the water helpful for survival of the aquatic organisms (Ahmed *et al.* 2000). The present results revealed that the water quality in the affected areas below the ambient/survival level and therefore, becomes a threat to the aquatic

organisms. Moniruzzaman *et al.* (2009) reported that the DO of Hazaribagh affected area collected at different times of year ranged from 2.20 – 6.62 mg/l. The present results showed that the DO value of Hazaribagh was 0.88 mg/l (Table 6) which was much less than the earlier report. This result indicated that the DO is gradually decreasing in Hazaribagh area and thus, becoming a threat to aquatic organisms.

The amount of 5 essential elements investigated in this study in water and soil samples from different affected areas were within the permissible limit (Table-7 and 9). Although these essential elements in the plant samples were a bit above the acceptable limit, not much hazardous (Tables 11 and 12).

Among the 5 heavy metals considered in this work, Arsenic and Cadmium surpassed the tolerable limit in the water sample of all affected areas. It was maximum in the water sample collected from Hazaribagh area (Table 8, Fig. 3, 4).

Arsenic and Chromium have crossed the permissible limit in the soil samples collected from 5 different areas. The amount of these elements were highest in Hazaribagh area (Figs. 5, 6 and Table 10). In both the plant species, except lead the other 4 heavy metals crossed the permissible limit (Figs. 7-10, Tables-13 and 14). Arsenic and Cadmium were maximum in both the plant samples. The plant collected from Monno Ceramic and Acme Laboratory areas had the highest amount of Arsenic and Cadmium. It was found that the edible part has more Cadmium and Arsenic than roots (Figs. 7-10, Tables 13 and 14), revealing quick uptaking of those heavy metals. From this study, it is clear that this heavy metals are entering into the human body through the edible parts of these plants.

4.2. Conventional karyotype analysis

In *Colocasia esculenta*, 2n = 28 chromosomes were found in the control and the plants collected from other affected areas except Monno Ceramic and Acme Laboratory area. A detail karyotype analysis with different parameters like total length of chromosome complements, centromeric formula, relative length etc. of this species has been undertaken (Tables 15-18). Similar chromosome number with almost same karyotypic features for this species have been reported earlier (Alam and Deen 2002).

In contrast, the samples of *Colocasia esculenta* collected from Monno Ceramic and Acme Laboratory areas were found to possess 2n = 38 chromosomes. No report of 2n = 38 chromosomes (Tables 19, 20) for this species was found in the available literature (Alam and Deen 2002). This specimen is morphologically quite similar to that of control and other specimens collected from different affected areas, therefore could not be differentiated phenotypcially (Hosne Ara, Director current-charge, Bangladesh National Herbarium – Personal communication). If this plant is considered as triploid, then it should have 2n = 42 chromosomes (Figs. 99, 100). Diploid chromosome number 2n = 38 did not support the specimen as triploid. The probable reason for increasing chromosome would i) either the sample plant were a new cytotype of this species or ii) some irregularities occurred during cell division. It was observed that Arsenic and Cadmium were rich in the soil, water and plant samples of Monno Ceramic and Acme Laboratory area (Table 13). The carcinogenic properties of As and Cd were established (Glanze 1996). Therefore, these heavy metals might have negative impact on cell division.

Although there was no major difference regarding karyotypic features, a sharp variation found in the heterochromatic regions of interphase nuclei and prophase chromosomes of both the species. Tanaka (1971) described the nature of

heterochromatin in the interphase nuclei and prophase chromosomes based on orcein staining properties. In this study, several darkly stained bodies were found in the control plant of both the species (Figs. 23, 29). In the interphase nuclei of plants collected from different affected areas, no such distinct darkly stained bodies was found rather the heterochromatins were fused somehow in the nuclei (Figs. 24-28 and 30-34).

On the other hand, the prophase chromosomes of both the control samples were stained uniformly along the length (Figs. 59, 65). Whereas the prophase chromosomes of the affected plants were stained differently. In some cases, one end of chromosome stained darker and than gradually lighter to other end. In some sample, prophase chromosomes were stained at the interstitial regions (Figs. 60-64 and 66-70).

The above results indicated that the distribution of heterochromatins were different in the interphase nuclei and prophase chromosomes of the affected plants from that of control.

4.3. Fluorescent banding analysis

Schweizer (1976) introduced fluorescent banding using different DNA base specific fluorochromes. CMA and DAPI are two fluorochromes specific to GC- and AT- rich base sequences, respectively. Fluorescent banding gives critical analysis of karyotype, even chromosome having similar morphology and other conventional karyotypic features. In this study, these two fluorochromes were used for critical karyotypes analysis of plant samples collected from different affected area including control.

4.3.1. CMA fluorochromes

In control plant of *Colocasia esculenta*, 10 – 14 CMA positive bands were found in the interphase nuclei and prophase chromosomes (Figs. 35, 71). In metaphase, 14 chromosomes showed CMA bands in the different location (Figs. 119, 125, 179 and Table 22). Similar number of CMA positive bands in interphase nuclei, prophase- and metaphase chromosomes indicated that these bands were very stable and kept their domain intake throughout the cell cycle. In contrast, samples collected from Pesticide and Hazaribagh areas had no band (Figs. 121, 122, 127, 128, 181, 182). In the samples collected from Acme Laboratory area, four chromosomes had CMA positive band with 2 CMA positive satellites (Figs. 124, 130, 184 arrow). Only 2 chromosomes showed CMA positive band in the samples collected from Monno Ceramic area (Figs. 123, 129, 183 arrow). The sample collected from Gazipur affected area was found to possess only 2 CMA positive satellites (Figs. 120, 126, 180 arrow). Therefore, the number of CMA bands were remarkably decreased in the samples collected from different affected areas in comparison to control indicating the probable effect of industrial effluents on DNA level.

Ipomoea aquatica (control and sample collected from Pesticide affected areas) had 2 big spherical and prominent bands in the interphase nuclei. The prophase chromosomes were less fluoresced (Figs. 41, 43, 77, 79). On the other hand, a number of CMA positive bands was found in the interphase nuclei and prophase chromosomes in rest of the samples (Figs. 42, 44-46, 78, 80-82). Only 5 metaphase chromosomes showed CMA positive bands in the control sample. Whereas, 6–10 chromosomes showed CMA positive bands in rest of the samples. The percentage of CMA positive banded region in control and sample plants was ranging from 8.05–23.52 (Table 31). The banding patterns of the control and

affected samples were remarkably different. This result indicate the probable effect of heavy metals on the distribution of GC- repetitive sequences.

4.3.2. DAPI banding pattern

No DAPI band was found in the interphase nuclei, prophase- and metaphase chromosomes of *Colocasia esculenta* collected from control, Gazipur and Pesticide areas (Figs. 47-49, 83-85, 143-145, 149-151, 191-193). DAPI usually binds with AT- rich segments having at least 5000 base pairs. Lack of DAPI positive bands in the 3 samples revealed the absence of AT- rich repetitive segments.

In contrast, several darkly DAPI fluorescent areas were found at the interphase nuclei and prophase chromosomes of samples collected from other areas (Figs. 56-58, 86-88). Five chromosomes of Hazaribagh samples, 9 chromosomes of Monno Ceramics samples and 4 chromosomes in Acme Laboratory samples showed DAPI positive bands. However, the DAPI banding patterns of these samples were different (Figs. 146-148, 152-154, 194-196 and Table 23). The probable reason for appearing DAPI positive bands in these samples was that the heavy metals might influence the tandem duplication of AT- rich repetitive sequence in the respective chromosomes.

On the other hand, DAPI positive bands were found in every samples of *Ipomoea aquatica* collected from different affected areas and as well as control (Figs. 155-166). The highest number of AT- rich regions was found in sample collected from Pesticide area followed by samples collected from Hazaribagh effluent affected area (Table 32). In the control plant, 6 DAPI positive bands were found and the percentage was 9.37 (Table 32). Only 4 DAPI positive bands were found in plant samples collected from Gazipur and Monno Ceramics areas with AT- rich percentage of 8.41 and 7.34, respectively (Table 32). In the later two samples, the

band size was bigger than the control. It indicated that the tandem fusion of AT-rich regions, thus the number of bands lowered than the control. Only two DAPI bands were found in the Acme Laboratory sample with 4.51 percent of AT- rich regions (Table 32). This result revealed the deletion of AT rich regions from the respective chromosomes.

4.3.3. Heteromorphic bands

Heteromorphicity in respect of CMA- and DAPI- banding pattern was found in plant samples of both *Colocasia esculenta* and *Ipomoea aquatica*. In *Colocasia esculenta* (plant sample collected from Monno Ceramic area), an upper terminal CMA band was found in a member of pair VIII and XVI. No such band was found in their homologue (Figs. 123, 129, 183 arrow). Only one upper terminal band was found in a member of pair XV in *Colocasia esculenta* (sample collected from Acme Laboratory area), whereas it was absent in its homologue (Figs. 124, 130, 184 arrow).

In the sample collected from Monno Ceramic area, one upper terminal DAPI band was found in a member of pair XV and XIX (Figs. 147, 153, 195 arrow). Only one terminal band was found in a member of pair X, in the sample collected from Acme Laboratory area while no band found in its homologue (Figs. 148, 154, 196 arrow).

In *Ipomoea aquatica* (sample collected from Hazaribagh area), one terminal CMA positive band was found in pair I and a DAPI interstitial band in pair XII, however, no such band was found in their homologue members (Figs. 134, 140, 188, 158, 164, 200).

The absence of band (CMA and DAPI) in the members of homologue pairs revealed the probable deletion of the banded portion from the respective

chromosomes (Hashimoto 1987, Alam *et al*.1993, Begum and Alam 2004, Begum and Alam 2005, Begum *et al*. 2009, Fawzia and Alam 2011). The smaller size of the deleted chromosomes supported deletion of the segment from the terminal regions.

It may be noted that the heteromorphicity of the banding patterns were found in the samples collected from Hazaribagh, Monno Ceramics and Acme Laboratory areas, where uptake of heavy metals were more (Figs. 3-10, Tables 8, 10, 13, 14). Therefore, the excessive heavy metals might cause chromosomal deletion.

4.3.4. Satellite

In *Colocasia esculenta*, two CMA positive satellites were found in each member of pair I (collected from Gazipur area) and pair VI (collected from Acme Laboratory area) (Figs. 120, 126, 180, 124, 130, 184 arrows). However, no satellite could be found after orcein and DAPI staining of these samples. A number of orcein and DAPI staining slides of these samples were observed. No satellite was observed in more than 50 slides. Why the satellites were not found in DAPI and orcein staining is unknown. Alam and Kondo (1995) reported stain specific satellites in *Drosera helodes* and *Drosera sewelliae* after a sequential staining with orcein, Giemsa, CMA and DAPI. The CMA positive satellites found in this study perhaps stain specific in nature.

4.3.5. Marker chromosomes

Few chromosomes of *Colocasia esculenta* and *Ipomoea aquatica* showed unique fluorescent banding pattern. In *Colocasia esculenta*, (collected from Acme Laboratory area) the entire long arm of a chromosome in pair III was brightly fluoresced with CMA (Figs. 124, 130, 184 arrow). A chromosome of pair X in the

same species collected from Monno Ceramic area had a DAPI band on both the terminal ends (Figs. 147, 153, 195 arrow).

One thick and prominent centromeric DAPI band was found in a member of pair XII in *Ipomoea aquatica* collected from Hazaribagh effluent affected area (Figs. 158, 164, 200 arrow). In the control plant of same species, a chromosome of pair XI had 2 dot like centromeric DAPI bands (Figs. 155, 161, 197 arrow).

The above chromosomes were unique in respect of banding patterns since this type of banding absent in other chromosomes of these samples. Therefore, these chromosomes could be used as a marker chromosomes of these specimens.

4.3.6. RAPD analysis

Eight different primers were used in the RAPD analysis of 6 plant samples of both *Colocasia esculenta* (L.) Schott and *Ipomoea aquatica* Forssk. to find out the polymorphism of DNA fragment among the affected samples including control. Samples of both the species showed few common bands in different primer combination indicating the sharing of common fragments. This was usual since the samples are belonging to the same species. Which is not usual that these samples showed some unique bands characteristics for specific samples. For example -

In primer OPA-1, a band of 2000 bp was found in only control and Gazipur samples. One band of 1500 bp was present in all samples except control (Fig. 204, Table 33). In OPA-4, a fragment of 3000 bp was absent in the sample collected from Hazaribagh area (Fig. 207, Table 36). In OPA-8, all samples had a band of 1500 bp except Hazaribagh (Fig. 209, Table 38). In the primer-24, a band of 1100 and 800 bp was unique in the samples collected from Gazipur and Hazaribagh area, respectively (Fig. 211, Table 40). In *Ipomoea aquatica*, a fragment of 3000

bp was unique in the sample collected from Acme Laboratory area with OPA-4 (Fig. 216, Table 44).

The above results indicated that some bands were absent in the samples collected from different affected areas which present in the control. On the other hand, few unique fragments were found in the sample plants collected from different affected areas which absent in the control. The RAPD analysis showed a polymorphism regarding the absence and presence of DNA fragments in comparison to the control.

In fluorescent banding, deletion and tandem duplication of chromosomal segments were found in the samples collected from different affected areas. The absence and appearance of certain unique RAPD bands may be correlated with chromosomal deletion and tandem duplication.

Conclusions

From the forgoing discussion it becomes clear that the industrial effluents affected *Colocasia esculenta* and *Ipomoea aquatica* on chromosome and DNA level. It is very likely that these toxic heavy metals from the industrial effluents enter to human body through the edible parts of the plants.

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