Chapter

Pollination Biology and Environmental Water Pollution Indicator of Onion (*Allium cepa* L.)

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Abstract

Numerous genes in flowering plants, including onion (*Allium cepa* L.) govern morphological character differences in structure, shape, orientation, weight and number, etc. arising from their assortment and recombination due to pollination. Pollination in onion flower occurs mainly by insects (91%) and wind (9%), with gravity also contributing to the pollination process. The hybrid vigour seeds through cross pollination as an essential input in enhancing crop productivity. The present study reveals that an onion plant generally takes around 63 days to attain flowering position, and complete flowering condition in 70–72 days, which include 15–18 days for sprouting of the green hollow fleshy shoots along with about 45 days to complete the peduncle formation on the top small part of the bud. *A. cepa* L. takes around 160 days to produce mature seeds with life cycle completion. The onion bulb roots are used for the last 50 years to study chromosomal behaviour as an indicator of environmental water pollution. The presence of different impurities and heavy metals in the polluted water causes reduction in reproductive capacity of cells due to the occurrence of peculiarity from the normal mitotic cell division in onion . Cytotoxicity influences all morphological characters, including root growth retardation, mitotic index, chromosomal aberration, etc. Thus, the present investigation explores the effect of pollutant water on pollination biology, cytotoxicity, root apical meristem cells in onion. We report a significant ($p < 0.05$) in the mitotic index in polluted water as compared to normal water.

Keywords: cross pollination, heavy metals, chromosomal aberration, cytotoxicity and environmental indicator

1. Introduction

The *Allium* (family:Amaryllidaceae) is a large genus of onion or garlic fragrant perennial bulbous herbs and globally represents about 700 species, but only seven species are cultivated. Members of the family are the world's oldest cultivated plants after potatoes, yams, and tomatoes, comprising an important group of vegetables, except the tropics, New Zealand and Australia. *Allium* is a native to southern western Asia with cultivation throughout the world, predominantly in the temperate regions [1].

There are different species of *Allium* which are grown in the field for the next generation in the form of flowers instead of bulbous structures. Generally, the seedlings are used for bulb production as vegetable. Two development phases occur to complete the life cycle of onions. One phase is entirely responsible for the production of seeds only whereas the other herbaceous annual phase is for the production of bulb from the seedling. Pollination in onion takes by an array of insects [2, 3]. The hermaphrodite onion flowers cannot fertilise themselves since the anthers exhibit protandry, releasing sticky and wet pollens before the stigma becomes receptive [4]. Thus, a cross-pollination between two flowers of the same plant (Geitonogamy) or two flowers from different plants (Allogamy) accomplishes seed production in the onion. The cross-pollinated seeds are used for the production of the onion bulb. FAO [5] reported that the production of onion in India is 12.5 ton/ha only whereas it is much lower than the production 41.12 ton/ha in the USA. Different sources of Municipal, Industrial, Agricultural, and advanced technological waste components ingredients can increase significant amounts of impurities in surface water and later on slowly deposited at a lower base as a consequence water pollution contributed a serious problem for the health of the biological organism along with human, those interact with this aquatic ecosystem in developed and developing countries. Those waste materials released from the different sources are mainly toxic metals and metalloids which are not converted into harmless nontoxic forms by the biological process but engaged in the environmental system which react the suppression activity of metabolism and translocation of reserve food materials into low concentration and impose to damage of the growing regions cells of the living organism [6]. Living organisms encounter nasty toxic heavy materials responsible for damage/modification of the genetic materials happening in the cell cycle. Meristematic root tips of *A. cepa* L. are used all over the world for testing the level of environmental water pollution [7–10]. To evaluate water quality being used for cytological studies of *A. cepa* L. root tips squash technique offers one of the best and quick methods, which also provides a reliable estimate for the genotoxic effect of heavy metal and chloride interaction on the environment. The water samples from three locations, Preonagar, Mathpara and Harishnagar 24 Parganas (N), West Bengal, India have been assessed on the basis of mitotic cell activity (Mitotic Index) and different chromosomal abnormalities (CAs) in the meristematic root tips cells of *A. cepa* L. (**Table 1**). The effect was compared with distilled water. The wastewater from the above locations enters, in different ways, either the agriculture fields for irrigation or river flow which makes a hazard to the ecosystem of that environment. With this background, the hazardous elements in the water samples assumingly react with the chromosome of the meristematic part of root tip cells of *A. cepa* L. that acts as an indicator for natural water pollution.

2. Materials and methods

2.1 Selection of land

To avoid harbouring of root rot or wilt pathogen, the selection of healthy edaphic conditions is the first priority for a normal luxuriantly growing plant. Neutral pH with loam clay loam soil was selected for the experiment at the Village-Belu, P.O. Madhappur, Amdanga, North 24 Parganas (Latitude 22°11′6″ N to 23°15′2″ N and Longitude 88°20′ to 89°5′ E) West Bengal, India.

Sl. No.	Heavy metals, Chloride and pH	Limit as per IS 10500: 2012		Different elements presence in the three locations waste water samples		
		Minimum	Maximum	Preonagar	Mathpara	Harishnagar
1	Mn (mg/l)	0.01	0.30	0.321	0.275	0.101
2	Cr (mg/l)	0.001	0.05	< 0.001	< 0.001	< 0.001
3	Cu (mg/l)	0.001	1.5	< 0.001	< 0.001	0.006
4	Cd (mg/l)	0.001	0.003	< 0.001	< 0.001	<0.001
5	Fe (mg/l)	0.001	0.30	0.340	0.501	0.520
6	Pb (mg/l)	0.001	0.01	< 0.001	< 0.001	< 0.001
7	Zn (mg/l)	0.001	15.0	0.450	< 0.001	0.002
8	Ni (mg/l)	0.001	0.02	0.006	< 0.001	0.002
9	Cl (mg/l)	N/A	1000	89.19	79.55	269.98
10	pH	-	-	5.30	5.15	5.10
11	Colour	-	-	Bluish	Blackish	Blackish

Table 1.
Heavy metal, Chloride and pH analysis of three Experimental locations water samples (Preonagar, Mathpara and Harishnagar).

2.2 Bulb selection and environmental condition

Onion bulbs around 5–6 cm in diameter were selected for sowing of the above field. The good flowerings were obtained by cool weather after bulb planting. It was also noticed that the good sunshine at the time of full blooming stages helped in attracting the beneficial pollinators for the higher rate of hybrid vigour seeds through cross-pollination. One more important parameter was kept in mind that relative humidity (RH) would be in the lower range during the time of seed development. As for the farmer's concern, the experiment was started in the month of the middle of October for sowing the bulb in the field of the above area.

2.3 Experiential laying out

The buds were planted in beds of size 1×3 m with 20–25 cm spacing during October. The experiments were conducted in Randomised Complete Block Design (RCBD) with 3 replications.

2.4 Floral biology

Morphology, physiology and phenology of plant flowering play a vital role in the reproductive capability of individual plant species. A flower's attractiveness to the visiting fauna and efficiency of pollen transfer to those visitors depend on the morphological characters, i. e. shape, colour and flower architecture [11]. The cross-pollinated onion plants are completely dependent on the different types of insects for their pollination [12]. Complete field observation was conducted to invent the onion floral characteristics, flower (bud) initiation and duration, number of days for maximum flowering, number of peduncles per bulb. The number of florets per

inflorescence, life of single floret, colour of the florets and odour were observed in the field materials.

Phenology

Plant phenology is concerned with the timing of recurring events such as

- leaf flushing
- flowering and
- fruiting
- The timing, intensity and duration of the flowering among plants dictate effective successful insect visits and cross-pollination, resulting in the success of the plant reproductive cycle (**Table 2**).

Sl. No.	Floral characters	Remarks
1.	Sprouting of the green fleshy shoots	15.67± 2.45 days
2.	Days from showing to peduncle formation	45.03± 0.98 days
3.	No. of days for maximum plants in flowering	62.21± 0.65 days
4.	Bud breaking and Flower initiation	70.05± 1.09 days
5.	Number of Peduncle per bulb	4.25 ± 1.00 cm
6.	Length of Peduncle	68.45± 0.58 cm
7.	Type of inflorescence	Umbel
8.	Number of Florets per inflorescence	306.78 ± 29.0
9.	Life of single floret	7.01 ± 0.52
10.	Colour of Inflorescence	Dull white in colour
11.	Type of overy	Superior
12.	Length of pistil	4.50 ± 0.58 mm
13.	Length of style	3.01± 0.67 mm
14.	Number of anthers per floret	6
15.	Type of anther	Bilocular
16.	Length of Stamen	0.71 ± 0.19 cm
17.	Length of Filament	0.52 ± 0.13 cm
18.	Length of Anther	0.22 ± 0.10 cm
19.	Mode of anther dehiscence	Longitudinal
20.	Time of Anthesis	Early in the morning
21.	Anther dehiscence	Whole day
22.	Mode of pollination	Mostly done by insects
23.	Type of Pollination	Cross pollination

Table 2.
Different morphological characters of the Allium cepa L.

Volume of nectar

- Insulin-pushing syringes were used for the collection of nectar from 15 number of florets from each inflorescence of the 20 number peduncle of each replication of the experiment.

- The portable instrument Refractometer was used to quantify the total soluble solids (TSS) of nectar.

Pollinators

- In 15 number of randomly selected inflorescence were used for counting the pollinators in 10 minutes of an hour of the day.

- Visitors were observed under the dissection microscope for identification of the pollinators through trapping and killing by the rectified spirit.

2.5 Collection of effluents sample

The coloured polluted sewage water was collected from the municipality channel of the Preonagar, Mathpara and Harishnagar, North 24 Parganas (22.61680 N, 88.40290 E), West Bengal, India in the winter season from three locations at the depth of 5–7 inches from five random points within the municipality drains of each location. The dirty water was filtered by muslin cloth so many times to remove all visible muddy materials present in the water sample and later on storage having been done in a clean container for conducting physiochemical analysis followed by use in cytological studies of the experimental onion crop.

2.6 Physiochemical parameters

The heavy metals (Manganese, Nickel, Copper, Zinc, Cadmium, Chromium, Iron and Lead) and chloride responsible for different chromosomal mutations were estimated for their concentration (mg/l) in the water sample of the different parts of North 24 Parganas (N), West Bengal (**Table 1**).

Sample assay.

Onion (*A. cepa* L.)

Onion (*A. cepa* L.) bulbs of about the same size (42 mm) weighing about 33 gm and 10 months old were selected by removing the loose outer scale, older roots without damaging meristematic tissues and scrapped the bottom part with the help of a blade so that root primordia were formed in wastewater samples of different locations (treatments) and distilled water (control condition). Keep in mind that the bottom part of each bulb slightly emerged out of the water. The incubated time of the sample in each treatment was 48 hours at the temperature of 22°C in dark conditions.

2.7 Chromosome preparation

The squash preparation demonstrated by Sharma and Sharma [13] was used for the chromosome preparation of the treated onion roots. The following methods were used to investigate the root meristematic tissue exhibiting mitotic cell division:

Pre-treatment

- The developing root tips 1 cm in length were cut and pre-treated with super-saturated aqueous solution of pDB-Aesculine initially kept in an ice chamber (0°C) of a refrigerator for 10 minutes in the form of adequate fixation with good preserved chromosome structure.
- After that, the refrigerator temperature was changed to 10°C for 3 hours.
- After washing in distilled water, the onion roots were fixed in Carnoy's fluid-I (Glacial acetic acid: Dehydrated alcohol = 1:3) for overnight at 20°C (about 24 hours).

Staining

- Before staining, the treated root tips were kept in 45% acetic acid for 10 minutes at room temperature.
- The staining of all the roots were done in a mixture of 2% Aceto-Orcein (Sigma-aldrich) acid and 1 N HCl (9:1) for 45 minutes after slightly warming at 60°C by a sprit lamp.
- The deeply stained tip portion of onion roots (1 mm size) were cut and placed in a drop of 45% acetic acid which has a very remarkable penetrating property even higher than alcohol due to its smaller ions.
- Squash preparation was made using thumb pressure on a clean grease-free slide.

Observation

- The slides were prepared from random samples each of five root tips for each treatment along with control.
- The five random microscopic fields were scored for each slide.
- The mean mitotic index for each sample and each treatment were compared with those corresponding to control and "t" test was applied to find out the significant difference, if any.
- The slides were temporarily sealed with paraffin wax for observation.
- The optical microscope used in the investigation was Olympus with the Prog-Res Capture Pro 2.1 photosystem.
- *Mitotic index (MI)* was computed using following formulae:

$$\text{Active Mitotic Index}\left(AMI\right)\% = \frac{\text{Total number of dividing cells}}{\text{Total number of cells observe}} \times 100$$

- *Total Abnormality Percentage (TAP) %* was computed using following formulae:

$$\text{Total Abnormality Percentage}\left(TAP\right)\% = \frac{\text{Number of abnormal cells}}{\text{Total number of cells scored}} \times 100$$

2.8 Statistical analysis

The experiments were conducted according to randomised complete block design (RCBD) with three replications. Data were expressed as mean ± standard error (SEM) [14].

3. Result

3.1 Phenological and growth variable

The onion (*A. cepa* L.) is a biennial crop that completes the life cycle in 2 years i.e., 1 year after plantation, using the nutrients from the soil, we get the onion bulb which is the vegetable. Second year that particular size of vegetable bulbs is used for the production of fleshy peduncles and seeds. In the study after sowing the onion bulbs the peduncle started flowering within 45.03 ± 0.98 days (**Figure 1b** and **Table 2**). The flowers are raised on the top of single very compressed hollow internode as a peduncle. Each plant contained around 4.25 ± 1.00 number of peduncles with many other chlorophyll less fleshy leaves and each hollow peduncle length was about 68.45 ± 0.58 cm. Each peduncle held on the top of an umbel inflorescence consisted of 306.78 ± 29.0 florets (**Figure 1f**). Initially, small size inflorescence of *A. cepa* L. covered by 2 layers of white colour membrane called spathe. These spathes protect the juvenile buds of the umbel inflorescence. Due to the increase in the internal pressure of the florets, the spathe splits open which took 8–10 days. It was noticed that around 6 O'clock early in the morning anthesis takes place but the temperature is the crucial factor for the initiation of the anthesis. Fertility of the pollen along with stigma receptivity was observed the highest on the day of anthesis. In two whorls arrangement hold both the perianth and six number stamens. The stamen was found to be 0.71 ± 0.19 cm in length and consists of a bilocular anther 0.22 ± 0.10 cm long (**Figure 1j**). The anther splits longitudinally to release the pollen grains and takes the entire day for its dehiscence. Pistil length (with superior ovary) was 4.50 ± 0.58 mm (**Figure 1i**, **j**). In the normal condition, only one pedicel was carried by a single floret but in this experiment, it was observed that there were two florets attached to the single pedicel (**Figure 1g**, **h**). Different species of the genus *Allium* show the variable colour combination among the inflorescences (**Figure 1c**). The onion flower releases a very strong odour which attracts various insects for cross-pollination. The presence of nectar in flowering plants attracts insects which makes interspecific relationship for cross-pollination in the plants for hybrid vigour seeds production. Morphologically bowl-shaped florets of the onion inflorescence produce nectar with hidden nectarines (**Figure 1o**, **p**) thus act as a good food reward and attract a wide range of vectors among which few of them are pollinators. These vectors belong to different families. One of the family Apidae including honey bees such as *Apis cerana* and *Apis florea* were found in the field most of the time (**Figure 1l**). Another insect was noticed with elongated siphon which might help to suck the nectar from inside of the floret

(**Figure 1 k**, **m**, **n**). Very good relationships between pollinators and temperature were observed as the pollinator movement was maximum in first half of the day and slowly decreased when temperature increased.

3.2 Different elements present in the samples

Table 1 enlists the different types of toxic metals along with chloride in different water samples. Water samples from three locations attained a higher range of iron quantities as per the standard limits of iron concentration (0.001–0.30 mg/l). Heavy metals like Cd, Cr, Cu and Pb were found in all water samples, lesser than the permissible limits (0.001 mg/l). Nevertheless, manganese (0.32 mh/l) was found in higher concentration in Preonagar water sample than Mathpara and Harishnagar samples as compared to standard limits (**Figure 2**).

3.3 Root growth inhibition

The onion root growth inhibition effect was clearly visible in plants receiving wastewater from the three locations compared with the healthy, elongated roots in distilled water (**Figure 3**).

3.4 Effect on mitotic phase frequency

A study of the mitotic phase frequency revealed that the total number of cells in wastewater of different locations was less than the cells present in distilled water treatment. It exhibited that dividing cells present in distilled water was higher than the remaining three sources of wastewater. In the anaphase stages, it was noticed in the sample of Mathpara to have a higher number of metaphase stages as compared to Preonagar, Harishnagar and distilled water samples (**Figure 4**).

Figure 1.
Sprouting shoots along with floral parts of the A. cepa L. *(a) Sprouting shoots of the onion (15 days old) (b) Cylindrical fleshy leaf with Peduncle (45 days old) (c) Very good No. of Peduncle with Inflorescence (70 days Old) (d) Immature florets (e) Pedicel of a about equal length all arising from the apex of the Peduncle with single open floret (f) Onion inflorescence at mature bud and bloom stage 98% (100 days old) (g) Two florets are attach with single pedicel (h) Distinctly showing the two parts attached at lower part of pedicel (i) Pistil of the floret (j) Petals, Pistil and Stamens of the floret (k, l, m, n) Vector/pollinator (arrow) observed on the individual flower (o, p) Floral nectar (arrows).*

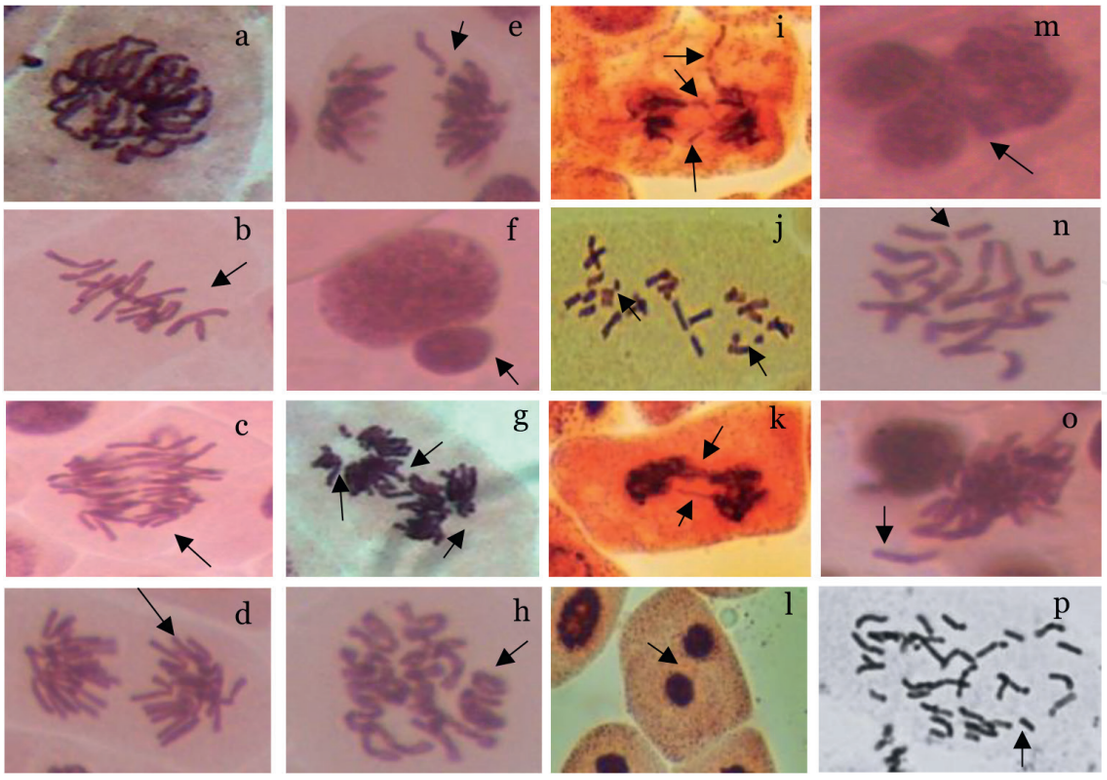

Figure 2.
Normal division and Chromosomal abnormalities observed in A. cepa L. (2n=16) meristematic cells exposed to three different polluted waters with control (distilled water) (a) Normal Late Prophase (b) Normal Metaphase (c) Normal Early Anaphase (d) Normal Telophase respectively treated with distilled water (e) Telophase with chromosome break (f) Micronucleus of large size in Interphase (g) Multipolar telophase with chromosome bridge (h) Tripolar anaphase respectively treated with Preonagar sample (i) Anaphase bridge with laggard chromosome (j) Fragmented chromosome (k) Anaphase with multi chromosome bridges (l) Bi-nucleus cell, respectively treated with Mathpara sample and (m) Multi nucleus cell (n) Fragmented chromosome (o) Metaphase with chromosome adherence and chromosome break (p) C-metaphase chromosome respectively treated with Harishnagar sample.

3.5 Chromosomal abnormalities (CA)

Meristematic cells present in root tips of *A. cepa* L. were exposed to 72 hours of the three different locations water sample and exhibited various chromosomal aberrations induced by chemical agents in the form of changes either in chromosomal structure or in the total numbers of chromosomes compared with distilled water as control. The different types of observed abnormalities included telophase with chromosome break, micronucleus, multipolar telophase with chromosome bridge, tripolar anaphase, anaphase bridge with laggard chromosome, fragmented chromosome, anaphase with multi chromosome bridges, bi-nucleus cell, multi nucleus cell, metaphase with chromosome adherence and chromosome break and c-metaphase chromosome (**Figure 2**). Studies indicated that all the toxic elements present in the different locations sample water caused a significant increase in the total aberrant cells except distilled water (**Figure 3**). The toxic elements in wastewater samples induced the highest aberrant cells frequency of about 18% which was observed to be in Mathpara, whereas medium in Preonagar (11%) and the lowest frequency in Harishnagar (8%) water samples (**Figure 3**). The analysis of chromosomal abnormalities was made mainly on the anaphase and telophase stages of the cell cycle division.

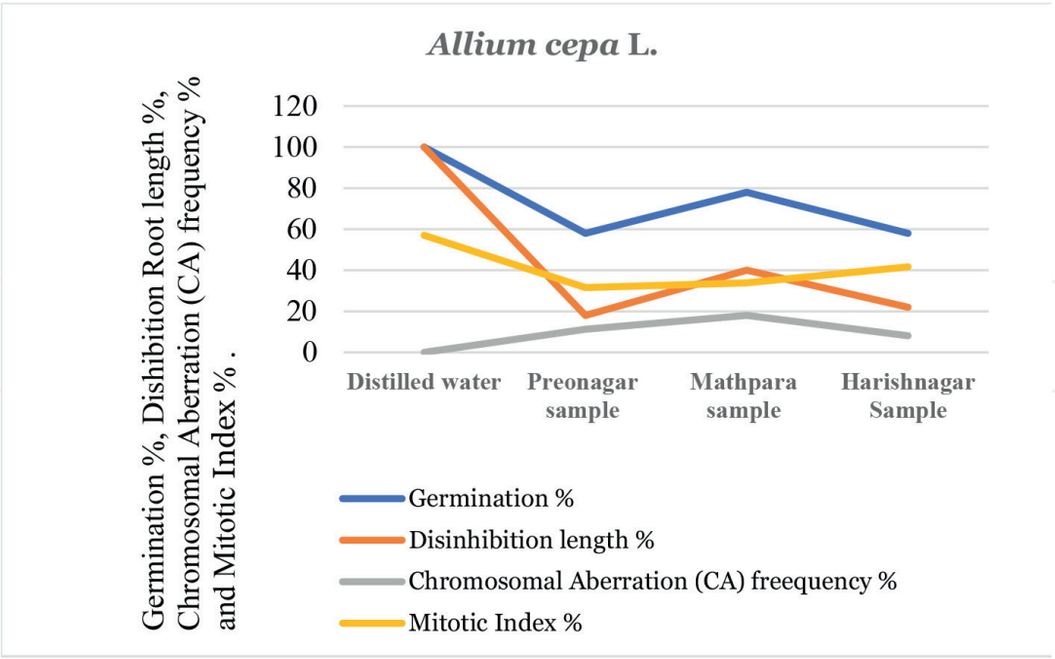

Figure 3.
Effect of Distilled water, Preonagar, Mathpara, and Harishnagar water samples on Germination %, Disinhibition Root length %, Chromosomal Aberration (CA) frequency % and Mitotic Index (MI) % in A. cepa L.

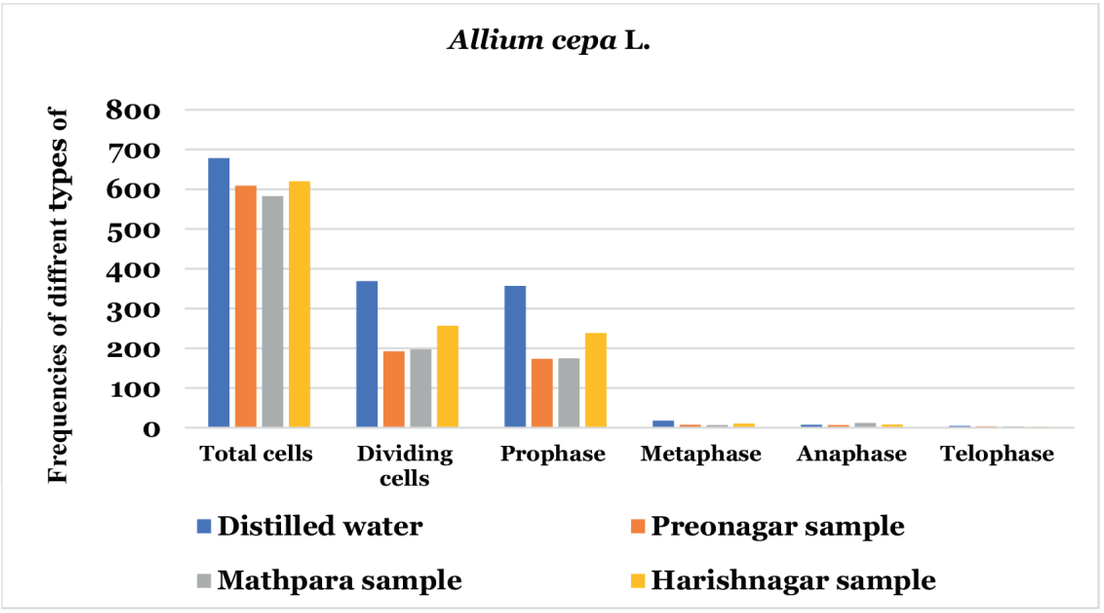

Figure 4.
Frequencies of different types of cells after treatment with different water samples (Distilled water, Preonagar, Mathpara and Harishnagar water samples).

The upward direction of Chromosomal aberrations (CA) of the *A. cepa* root tips meristematic cells reflected a significant decrease in the Mitotic Index (MI) due to taxological elements existing in the wastewater samples (**Figure 3**).

4. Discussion

Environment and its interaction with the genome help in the expression of physio-morphological characters of all organisms. The inflorescences *A. cepa* L. is of dull white colour whereas *Allium aflatunense* bears violet colour then slowly turned into purple colour [15. *Allium giganteum* inflorescences produce red-coloured florets from the purple colour [16]. Nevertheless, the onion inflorescence displays a very strong flavour and odour due to the presence of chemical alteration of a volatile secondary metabolite like S-methyl cysteine sulphoxide compound [17]. Temperature strongly influences the reproductive structure of the onion [18]. Interspecies relation may be a cause of the very good method for cross-pollination in the different types of organisms [19]. It is no wonder that nectar offers as a food reward to the insect vectors pollinating the plant to their pollen vectors [20]. In the present investigation, different species of the Genus *Apis* viz., Apis dorsata, *A. cerana,* Apis mellifera and *A. florea* visit the onion inflorescence florets as a gatherer of pollens and nectar (**Figure 4**) [21].

The present study establishes a steady relationship between the wastewater heavy metal toxicity and abnormal cellular behaviour of onions having been indicated by frequencies of the different mitotic phases, MI and the types of structural chromosomal abnormalities. The cytological studies of the meristematic cells of the *A. cepa* L. roots assay provide one of the most reliable and useful protocols for the investigation of environmental pollution, biological monitoring and determination of the toxicity of the different elements present in the different sources.

The lower level of the MI in the *A. cepa* L. meristematic cells can be indicated as a reliable process to determine the presence of cytotoxic agents such as heavy metals in the environment and considered as a real test to evaluate the pollution level in the natural water bodies [22]. Our observation corroborates earlier investigations using the Mitotic Index evaluation as a tool for the detection of cytotoxicity mediators and pollution agents present in the environment [10, 23, 24].

Fiskesjo [25] has established drinking water contamination by copper using the *A. cepa* L. test. Subsequently, the author has successfully extended the same test for others to detect different toxic metals such as Hg, Ni, Cu, Cd, Ne, Al, Mn and Li, establishing chromosomal abnormalities in the form of C-metaphase to be associated with heavy metal Ni [26].

A. cepa L. meristematic root cells assay efficiently evaluates different aqueous concentrations of copper mine waste causing cytogenetic effects such as chromosomal abnormalities. The 100 percent concentration of copper mine waste i.e., raw sample presented the highest toxicity and exhibit a relationship with the inhibition of MI along with chromosome breaks, delay, bridges and adherence were the most frequent Chromosomal aberration (CA) observed [27].

Borboa and Torre [28] have assessed heavy metals Zinc and Cadmium genotoxicity linked with chromosomal abnormalities in the *A. cepa* L. test system with cadmium inflicting a greater genotoxic effect. Seth et al. [29], in evaluation studies of the genotoxicity effects of cadmium by the *A. cepa* L test, have revealed inhibition of CA with MN induction.

Contamination by tannery effluents found in river water causes significant frequencies of chromosomal abnormalities and micronucleus in *A. cepa* L. meristematic cells noticed [30].

5. Conclusion

A. cepa L. test in the present investigation emerges as the best model and gentle assay to detect the presence of environmentally hazardous elements, which causes genotoxicity and mutagenesis. Thus, this test provides an important tool for screening environmental contaminants and their result can be used as a warning signal for human health.

Conflicts of interest

The author declares no conflict of interest.

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