# Assessment of cytotoxic and genotoxic effects of Yamuna river water pollutants in an urban metropolis, Delhi (India)

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## ABSTRACT

The present study evaluates the hazardous effects of water pollutants present in the River Yamuna, the lifeline of Delhi. This was done by collecting water samples from seven sites on the River Yamuna, and studying their water quality parameters (WQP). In all cases, tap water was taken as the control, and WQP like pH, salinity, electrical conductivity (EC), etc. were measured. At site 1, water was slightly alkaline, whereas maximum salinity was found at site 4. The TDS, EC, and turbidity at site 5 were found to be the highest among the studied sites. Further, water samples were used to examine the cytotoxic and genotoxic effect of pollutants in the root tip cells of *Allium cepa* after three and seven day's growth. There was a sharp decline in root length and root number down stream. Moreover, the squash preparations showed significant abnormalities; at the cellular level, cell shape and sizes show undesirable changes. At nuclear level binucleate cells, lobulated nuclei, micronuclei at site 3, 4, and 7 were recorded. The chromosomal abnormalities included chromosomal bridges, chromosomal loss, and abnormal orientation at different sites. This report is a cause for significant concern as the River Yamuna is Delhi's primary source of water supply for domestic, agricultural, industrial, energy, and many other purposes.

**Key words** | chromosomal adherence, chromosomal bridge, cytotoxic, genotoxic, laggards, micronucleus

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## HIGHLIGHTS

- The experiment was carried out in Delhi, the capital city of India, which is one of the largest and most polluted cities of the world.
- We categorized the effects of polluted water in three categories of abnormal images namely cellular, nuclear and chromosomal.
- To the best of our knowledge, we reported cell size and abnormal cellular images of *Allium cepa* root tips grown in polluted water for the first time.
- The damaging effects on the *A. cepa* root tip cells could be used as an indicator of the possible damaging effects of polluted Yamuna river water on crops from the health point of view as well.

## INTRODUCTION

Population explosion together with human interference and industrialization has resulted in the emergence of serious new environmental problems at a global scale. Climate change has brought with it extremes of rains, floods, pollution and eventual scarcity of potable water on the planet earth. Population explosion is resulting in increased requirements, enormous waste generation and disposal leading to pollution of water bodies. Water intensive agricultural

doi: 10.2166/ws.2020.165

practices, textile, paper and pulp manufacturing, and land use practices are over utilizing and contaminating groundwater at an alarming rate. Rivers, a living part of the ecosystem, across the nation are getting polluted due to discharge of municipal waste as well as industrial discharges. This results in bioaccumulation of these contaminants and leads to various health hazards (https://www.unwater.org/water-pollution-increasing-global-concern/ accessed on 27 January 2020). Such practices directly affect the food security and increase the vulnerability of poor rural farmers, especially in the arid and semi-arid tropics and Asian and African countries where the river story is not different from the river Yamuna of Delhi, India. Further, three-quarters of Earth's surface is submerged in water, and the major part of the water is seawater, which is not suitable for human consumption. For irrigation, drinking, and other day-to-day activities, a small amount of fresh water comes from the rivers, lakes, and groundwater. Unfortunately, with increasing levels of pollution, the quality of water has become detrimental for human consumption and unable to support the biotic communities in freshwater bodies like river Yamuna (Agrawal *et al.* 2010; Sehgal *et al.* 2012; Abbas & Siddiqui 2019).

The primary source of water for Delhi, as well as many cities, towns, and villages in the neighboring states, is river Yamuna. In the last few decades, there has been a serious concern over the deterioration of the water quality. According to a Central Pollution Control Board (CPCB) report, the Yamuna in Delhi almost carries sewage water (CPCB 2012). As per CPCB records, the distance covered by the Yamuna in Delhi is just 22 km (just 2% of the river's total length) and accounts for the maximum (70%) pollution). Recently Said & Hussain (2019) mapped the Yamuna river segment passing through Delhi using high-resolution GeoEye–2 satellite (Digital Globe, USA) imagery to highlight the issue of water quality status in River Yamuna. This severe condition arises due to diversion of enormous quantities of partially treated or untreated domestic wastewater and industrial effluents directly into the Yamuna, especially between the upstream Wazirabad and downstream Okhla regions of Delhi (https://www.dw.com/en/saving-the-yamuna-river/av-47026687 accessed 27 January 2020). According to estimations, in Delhi, approximately 850 million gallons per day (MGD) of sewage waste is discharged into the River Yamuna daily (Agrawal *et al.* 2010). Once the lifeline of Delhi, Yamuna has now become the most polluted water body in the country, posing risks of heavy metal poisoning (Sehgal *et al.* 2012), cytotoxic, genotoxic and mutagenic effects (Kaushik *et al.* 2008), which eventually cause diseases like premature aging, cancer, atherosclerosis, cardiovascular disorders and many more. In order to assess the cytotoxicity and genotoxicity of these pollutants, bio-assays are done using plant cells, mammalian cells and microorganisms, alone or in combination with chemical analysis (Žegura *et al.* 2009; Leusch 2019). In a previous single-site study, Aleem & Malik (2005) examined the genotoxicity of the water samples collected from Yamuna bank at Okhla by employing Ames Salmonella/mammalian microsome test, DNA repair-defective mutants, and bacteriophage $\lambda$ systems. Their study presented a clear mutagenic response in all three systems. The problems of pesticides in the Indian river system and drinking water was reported by Kaushik *et al.* (2008) and Agrawal *et al.* (2010). Sehgal *et al.* (2012) reported that there is heavy metal (Cd, Ni, Zn, Fe, Cu, Mn, Pb, Cr, Hg, and As) contamination in the Delhi segment of the Yamuna basin. Further, industrial wastes are also responsible for reducing mitosis and an increased percentage of condensed nuclei and genomic instability as demonstrated by the presence of cellular and chromosomal abnormalities in *A. cepa* (Larissa *et al.* 2012).

There are several plants such as *A. cepa*, *Vicia faba*, *Zea mays*, *Tradescantia*, *Nicotiana tabacum*, *Crepis capillaris* and *Hordeum vulgare* that can be used to assess the impact of polluted water (Barbério 2013). Among them, *A. cepa* is the most favorable plant species as it is easy to handle, cost-effective and exhibit a good correlation with other toxicity tests and shows high sensitivity to environmental chemicals (Leme & Marin-Morales 2009; Siddiqui *et al.* 2011; Abbas & Siddiqui 2019). *A. cepa* root tip cells can be used to measure several morphogenetic and cytogenetic parameters to indicate environmental toxicity. It also contains a lower number ($2n = 16$) of large-size chromosomes, which are easy to analyze for chromosomal aberrations including micronuclei and other nuclear changes (Leme & Marin-Morales 2009). The present study attempts an in-depth study to unravel the ill effects of polluted Yamuna river water on the growth of *A. cepa*. The study was undertaken to evaluate water quality parameters

like TDS, EC, pH, salinity etc. The *A. cepa* bioassay was used to unravel the extent of the morphological changes in its roots. Efforts were also made to study the effect of polluted water on the morphological, cellular, nuclear and chromosomal changes in the root tip cells of *A. cepa*. The Yamuna riverbank is also utilized for growing several vegetables for human consumption and the damaging effects on the *A. cepa* root tip cells could be used as an indicator of the possible damaging effects of polluted Yamuna river water on crops from the health point of view as well.

## METHODS

### Water sampling

Water samples were collected seasonally; that is, during winter and summer, from seven different locations along the 22 km stretch of the Yamuna in Delhi. The sampling was done in accordance with the 'American Public Health Association' (APHA 2006) norms. In short, a water container was dipped half in the downstream of water at different locations and waited to fill the container. These locations were designated as follows: Yamuna Ghat (the first accessible location of the Yamuna after entering into Delhi) as site 1 (28.750337 N, 77.225505 E), followed by the downstream sites, Wazirabad Barrage as site 2 (28.711125 N, 77.231041 E), Inter State Bus Terminus (ISBT) as site 3 (28.680062 N, 77.231072 E), Lohapul Bridge as site 4 (28.661556 N, 77.247831 E), I.T.O. Bridge as site 5 (28.627038 N, 77.253496 E), Sarai Kale Khan Bridge as site 6 (28.600614 N, 77.260791 E) and Okhla Barrage, the point where River Yamuna exits Delhi, as site 7 (28.543930 N, 77.313470 E) (Figure 1). Tap water was used as control and sampling was repeated twice each season by collecting five samples from each site. The idea of site selection was based on the entrance and exit point of River Yamuna in Delhi as well as on the points of sewage discharge sites, industrial sites and thermal power stations.

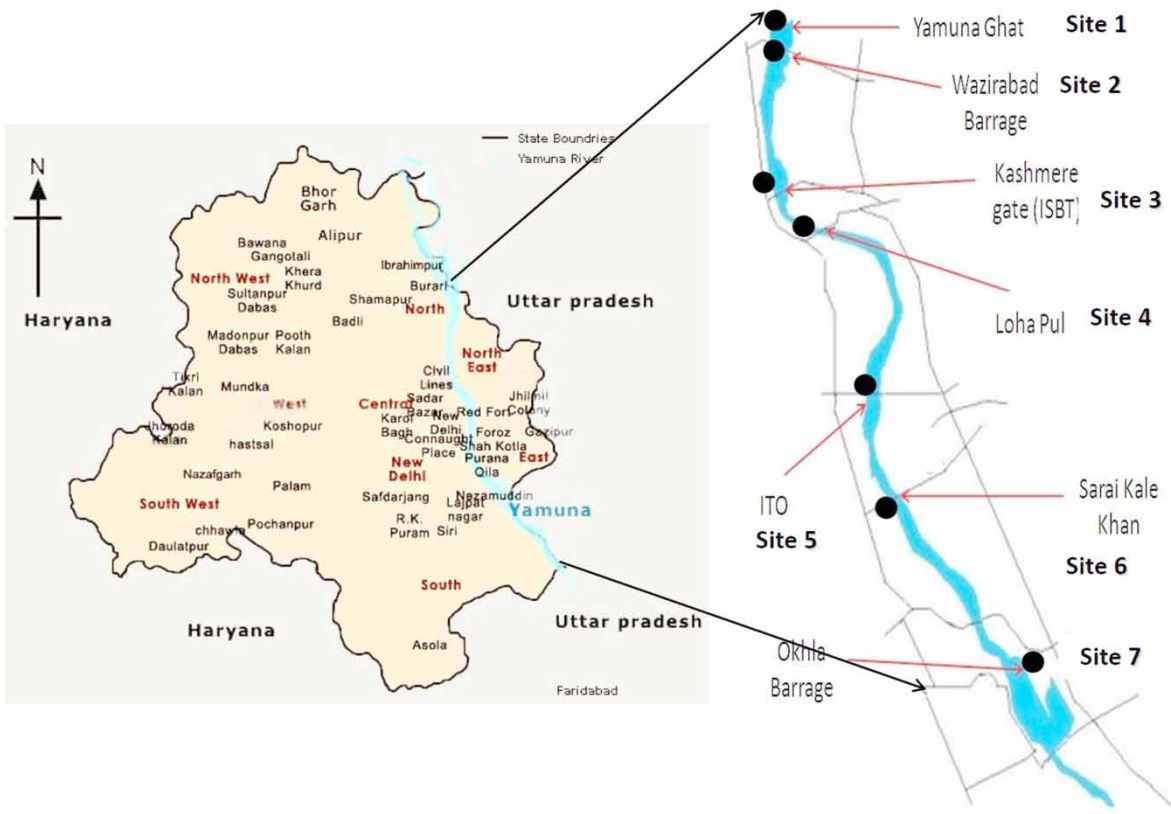


**Figure 1** | Sampling sites in the Yamuna river, Delhi (one of the megacities of the world), India.

### Analysis of water quality parameters

Five water quality parameters, namely electrical conductivity (EC), total dissolved solids (TDS), pH, temperature and salinity were analyzed by an on-site portable instrument named '*Multi-parameter PCS Tester 35*' (Eutech Instruments, OAKTON). To record the data, water samples were taken in a beaker and the Multi-parameter tester's probe was dipped in it for 1 minute. By using '*mode selection*' of the tester, different parameters, EC, TDS, pH, salinity or temperature were selected and five concurrent values per parameter per site were recorded.

### Turbidity

This was measured on-site using a Lutron portable hand-held Turbidity Meter (Model: TU-2016; range- 0.00 to 50.00 NTU and 50 to 1,000 NTU (NTU: Nephelometric Turbidity Unit). The instrument is very simple and easy to use and is provided with a graduated glass vial. In order to measure the turbidity, distilled water was filled in the vial and used as blank. Thereafter, a control as well as river water sample were filled in the glass vial, inserted in the reader slot of the turbidity meter, and five concurrent readings were recorded in terms of NTU.

### Dissolved oxygen (DO)

In the same manner, the DO was measured using a Lutron's portable on-site Dissolved Oxygen Meter (Model: DO-5509 with automatic temperature compensation; range 0–20.0 mg/L; resolution- 0.1 mg/L). To record DO, the sensor of the DO meter was immersed in the control as well as river water samples for 1 minute, and five concurrent readings were noted.

Following recording of the water quality parameters, the water samples from all the collection sites were brought to a laboratory, and were used to test the toxic effects of the water pollutants on live cells by using *A. cepa* root tip meristematic cells as a model.

### *Allium cepa* test

The *A. cepa* toxicity test was carried out by following Fiskesjö (1988) method with slight modifications for the adoption under Indian conditions. In short, equal-sized healthy red onion bulbs were chosen and their outer scales were removed by blade to expose the apices of root primodia. These onion bulbs were germinated for three and seven days in jam bottles containing polluted Yamuna water from seven different locations in Delhi. Tap water was used as control. Then the macroscopic as well as microscopic aspects, as detailed below, were studied extensively. For this purpose, triplicate sets of five onion bulbs each were incubated in these water samples for three and seven days to grow roots.

## MACROSCOPIC (MORPHOMETRIC) PARAMETERS

Following incubations, macroscopic (morphometric) data, such as 'number of roots per bulb', and 'individual root length' were noted down. Thereafter, roots were cut and stored in FAA solution (a mixture of 10 ml formaldehyde +5 ml acetic acid +85 ml absolute alcohol). These roots were used to make root tip squash preparation and microscopic parameters were recorded.

## MICROSCOPIC PARAMETERS

### Onion root tip squash preparation

The root tip squash preparations were made as per Sharma & Sharma (1980). Briefly, all the batches of roots were washed thoroughly with double distilled water, then hydrolyzed with 1 N HCl for 3 min. Following acid hydrolysis, the roots were washed again with double-distilled water, and 1–2 mm of the root tips were cut and stained in a drop of acetocarmine. The stained root tips were then squashed with a metal rod and another drop of acetocarmine was added to the squash. A cover-slip was carefully lowered and sealed with clear nail polish.

### Observation of slides

The slides were observed under a microscope to study various microscopic parameters such as morphological and morphometric details of the cell as well as the nucleus. The parameters studied include cellular (cell size, cell shape), nuclear (size and shape of nucleus, mitotic index

(MI)), as well as chromosomal (abnormalities like chromosomal adherence, stickiness, fragmentation, laggards, bridges, micronuclei etc.) details to ascertain the cytotoxic and genotoxic effects of river water pollutants on the cells of *A. cepa*. The slides were observed under the light microscope at ×400 and ×1,000 magnification. Photography of the cells for cellular, nuclear and chromosomal abnormalities was done by using a Nikon E400 microscope equipped with Vezu Scientific Grade 3 MP CMOS Camera (Vezu Tech Ltd, Model: US300), and VIMAGE proprietary software was used to measure cell size. The data were used to determine the MI, micronuclei formation, and chromosomal aberrations in mitotic phases. For MI calculation, randomly 250 cells per slide were scored (five slides in triplicates were observed for each treatment, each site). All the experiments were repeated twice as explained above, to check the reproducibility and veracity of data (Figure 2).

### Statistical analysis

The data collected were subjected to statistical analysis. Within a given group, variation between treatments (incubation time periods) in terms of mean and standard deviation was tested by Student's T-test. The data of water quality parameters and growth-related parameters such as root length, root numbers, cell size, MI were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$. Values are means of five replicates from two experiments.

## RESULTS AND DISCUSSION

### Water quality parameters

The recorded values of pH, salinity, TDS, EC, and temperature of water samples from different locations tested by Multiparameter PCS Tester 35 showed great variation between samples from various sites (Tables 1 and 2). The ability of water to conduct an electric current is quantified by EC. It is an indirect indicator of pollution due to its direct association of the dissolved salt present in the water bodies, which is generally associated with sewage discharges (Thompson *et al.* 2012). The highest mean value for EC, which is due to the presence of electrolytes in the water, was observed at site 3 during the winter season (1,425.3 ± 115.5 µS), whereas during the summer season it was at site 6 (1,278.4 ± 6.4) (Table 1). At

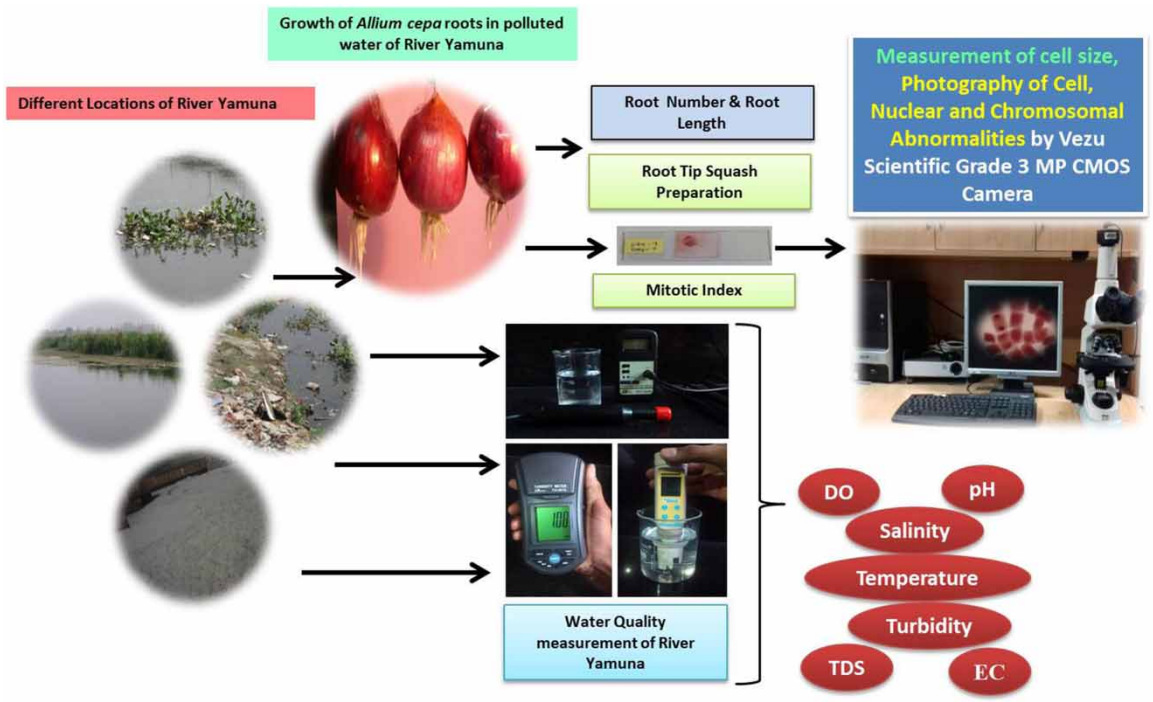


**Figure 2** | Pictorial depiction of material and methods.

**Table 1** | Water quality parameters of samples from different sites of the Yamuna river in Delhi region

| | Electrical conductivity (μS) | | pH | | Salinity (ppm) | |
|---|---|---|---|---|---|---|
| **Sample/Site** | **Winter** | **Summer** | **Winter** | **Summer** | **Winter** | **Summer** |
| Tap water | $461.00 \pm 49.01^{a}$ | $257.00 \pm 0.70^{a}$ | $7.96 \pm 0.043^{a}$ | $7.78 \pm 0.13^{a}$ | $258.67 \pm 27.33^{a}$ | $142.00 \pm 0.44^{a}$ |
| Site 1 | $450.83 \pm 222.43^{a}$ | $671.20 \pm 1.06^{b}$ | $8.05 \pm 0.135^{a}$ | $7.50 \pm 0.03^{b}$ | $370.00 \pm 8.71^{b}$ | $375.20 \pm 1.39^{b}$ |
| Site 2 | $1{,}034.33 \pm 43.65^{b}$ | $262.20 \pm 4.46^{a}$ | $7.50 \pm 0.025^{b}$ | $7.96 \pm 0.09^{a}$ | $594.00 \pm 23.02^{c}$ | $146.60 \pm 2.35^{a}$ |
| Site 3 | $1{,}425.33 \pm 115.51^{c}$ | $1{,}246.75 \pm 61.95^{c}$ | $7.31 \pm 0.101^{b}$ | $7.30 \pm 0.04^{c}$ | $825.67 \pm 66.24^{d}$ | $745.40 \pm 19.75^{c}$ |
| Site 4 | $1{,}135.67 \pm 99.71^{b}$ | $1{,}262.80 \pm 54.24^{c}$ | $7.29 \pm 0.195^{b}$ | $7.10 \pm 0.00^{d}$ | $670.67 \pm 66.69^{c}$ | $685.20 \pm 58.89^{c}$ |
| Site 5 | $1{,}147.67 \pm 105.03^{b}$ | $1{,}064.2 \pm 7.85^{d}$ | $7.21 \pm 0.153^{b}$ | $7.20 \pm 0.00^{e}$ | $660.00 \pm 61.65^{c}$ | $613.00 \pm 4.89^{d}$ |
| Site 6 | $1{,}115.67 \pm 101.53^{b}$ | $1{,}278.4 \pm 6.37^{c}$ | $7.27 \pm 0.161^{b}$ | $7.30 \pm 0.04^{c}$ | $638.00 \pm 64.78^{c}$ | $738.20 \pm 5.42^{c}$ |
| Site 7 | $1{,}302.50 \pm 18.50^{c}$ | $1{,}209.8 \pm 57.66^{c}$ | $7.12 \pm 0.070^{b}$ | $7.40 \pm 0.00^{f}$ | $723.00 \pm 14.00^{d}$ | $702.40 \pm 37.49^{c}$ |

Different sites of sample collection: Site 1: Yamuna Ghat, Site 2: Wazirabad Barrage, Site 3: ISBT, Site 4: Lohapul, Site 5: ITO, Site 6: Sarai Kale Khan, Site 7: Okhla Barrage. Beside these samples, tap water was used as control. Values are means ± standard errors of at least five replicates; within each column, means followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's MRT.

**Table 2** | Water quality parameters of samples from different sites of the Yamuna river in Delhi region

| | Temperature (°C) | | TDS (ppm) | | Turbidity (NTU) | | DO (mg/L) | |
|---|---|---|---|---|---|---|---|---|
| **Sample/Site** | **Winter** | **Summer** | **Winter** | **Summer** | **Winter** | **Summer** | **Winter** | **Summer** |
| Tap water | 16.8 | 30.5 | $328.0 \pm 34.50^{a}$ | $200.0 \pm 0.00^{a}$ | $0.0 \pm 0.0$ | $0.0 \pm 0.00$ | $4.93 \pm 0.14^{a}$ | $3.40 \pm 0.12^{a}$ |
| Site 1 | 10.5 | 32.9 | $459.33 \pm 5.45^{b}$ | $500.0 \pm 0.00^{b}$ | $0.0 \pm 0.0$ | $18.20 \pm 2.31^{a}$ | $9.27 \pm 2.03^{b}$ | $6.56 \pm 0.79^{b}$ |
| Site 2 | 11.0 | 32.2 | $725.67 \pm 28.0^{c}$ | $1{,}800.0 \pm 44.7^{c}$ | $30.15 \pm 2.35^{a}$ | $37.39 \pm 2.31^{b}$ | $6.00 \pm 0.35^{c}$ | $4.98 \pm 0.18^{c}$ |
| Site 3 | 12.2 | 34.3 | $339.00 \pm 31.01^{a}$ | $940.0 \pm 24.49^{d}$ | $37.06 \pm 1.80^{b}$ | $91.00 \pm 8.93^{c}$ | $5.30 \pm 0.60^{c}$ | $3.20 \pm 0.12^{d}$ |
| Site 4 | 12.6 | 34.4 | $819.67 \pm 74.24^{c}$ | $1{,}100.0 \pm 0.00^{e}$ | $49.42 \pm 1.75^{c}$ | $67.80 \pm 3.65^{d}$ | $6.47 \pm 0.21^{c}$ | $3.18 \pm 0.26^{d}$ |
| Site 5 | 15.0 | 39 | $794.00 \pm 69.15^{c}$ | $800.0 \pm 0.00^{f}$ | $83.40 \pm 6.79^{d}$ | $136.20 \pm 31.89^{e}$ | $3.20 \pm 0.50^{d}$ | $2.44 \pm 0.53^{d}$ |
| Site 6 | 17.5 | 34 | $786.00 \pm 64.04^{c}$ | $1{,}000.00.00^{g}$ | $38.62 \pm 4.16^{b}$ | $82.00 \pm 14.02^{c}$ | $4.63 \pm 0.36^{c}$ | $3.74 \pm 0.35^{e}$ |
| Site 7 | 20.5 | 37.3 | $925.50 \pm 22.50^{d}$ | $800.00.00^{f}$ | $112.40 \pm 5.46^{e}$ | $26.66 \pm 1.03^{f}$ | $6.47 \pm 0.17^{c}$ | $3.86 \pm 0.24^{e}$ |

Different sites of sample collection: Site 1: Yamuna Ghat, Site 2: Wazirabad Barrage, Site 3: ISBT, Site 4: Lohapul, Site 5: ITO, Site 6: Sarai Kale Khan, Site 7: Okhla Barrage. Beside these samples, tap water was used as control. Values are means ± standard errors of 5 replicates; within each column, means followed by the same letter are not significantly different at $p \leq 0.05$ according to DMRT.

both sites, direct sewage discharge points were present and high values of EC during winter attributed to the presence of domestic sewage, agricultural runoff, and organic matter with increased ionic concentration $Ca^{2+}$, $Mg^{2+}$, $Cl^{-}$, $SO_4^{2-}$ etc. The pH measures the acidic and alkaline condition of a solution. The slight change in a pH impacts a great change in the chemical and biochemical reactions. Similarly, a high or low value of pH in a water body changes its biota, inhibits recreational uses of water and transform the toxicity of other pollutants (Morrison *et al.* 2001). The pH of the Yamuna water was slightly alkaline and ranged between 7.12 and 8.05 in the winter season, whereas it ranges 7.1–7.9 in the summer season (Table 1). In our results, the seasonal variations in the pH data did not show a huge difference. Perhaps, high values of pH during summer are due to disintegration of organic matter and high respiration rate of aquatic organisms, this results in production of $CO_2$ and decrease in pH. Higher pH at site 1 during winter could be due to bicarbonates and carbonate of calcium and magnesium in water this site is used for religious activities and lot of idols made up of plaster of Paris were observed by us during sample collection. The salinity was found highest at site 3 during winter and summer season ($825.7 \pm 66.2$ and $745.40 \pm 19.75$ respectively) indicating the presence of salts (Table 1).

The temperature values of a water body is very important as it influences the physical, chemical and biological

properties of water. Further, increase in water temperature decrease the dissolved oxygen in water bodies. The availability of dissolved oxygen to aquatic organisms influences their metabolism, behaviour, feeding habits, reproduction, geographical distribution and community structure, tolerance to parasites, diseases and pollution (Perlman 2013). The temperature data in winter range from 10.5 °C to 20.5 °C and in summer it was 30.5 °C to 39 °C. The results shows an ascending trend from winter to summer. The changes in water temperature are due to the seasonal changes in air temperatures, sensible heat transfer from the atmosphere, thermal plant effluent discharges into river, convective heat exchange between the free water surface and the atmosphere, the intensity and duration of sunshine (Hassan *et al.* 2017).

Turbidity indicates the degree of clarity/lucidity of water and it is an optical feature. The reason for turbidity is suspended solid materials, *i.e.*, human waste, phyto- and zooplanktons, etc. In general, the water at all sites studied was found highly turbid during summer as compared to winter, with a maximum turbidity at site 5 (136.2 ± 31.9 NTU), whereas the turbidity was maximum during winter at site 7 (112.4 ± 5.5 NTU) (Table 2). The high rate of organic matter decomposition during summer might be attributed to the high value of turbidity. DO is the non-compound, free oxygen available in water bodies. It is an excellent health indicator of a water body. The concentration of DO in natural waters is generally affected by temperature and salinity. The DO solubility decreases with increase in temperature and salinity. Among the various anthropogenic factors that change the DO concentration in the aquatic environment is the addition of organic matter mainly from sewage discharge. During winter season, the DO content was more as compared to summer season. During both the seasons, it was highest at site 1, the cleanest of all sites under this study (Mean DO: 9.27 ± 2.03 mg/L and 6.56 ± 0.79 mg/L during winter and summer respectively) whereas site 5 showed the least amount of mean DO (3.20 ± 0.5 mg/L; 2.44 ± 0.53 mg/L) during the winter and summer respectively (Table 2). The possible reason for low DO content in summer season was possibly due to less oxygen holding capacity of water at high temperature along with increase in DO use for decomposition of organic matter by microorganism. High dissolved oxygen during winter could be attributed to greater dissolution of oxygen in winter at lower water temperature.

Delhi is a city with a population load of 17 million as per 2011 census (Census India 2011). According to an estimate, there are 21 major drains in Delhi, which carry around 850 MGD of sewage daily. Out of this, only 390 MGD is treated in the sewage treatment plants (STPs), and the remaining 460 MGD of untreated sewage, mostly industrial effluents, falls directly into the River Yamuna. Furthermore, in accordance with the CPCB (2012) and Dhillon *et al.* (2013) reports, the present study also confirms the above fact that the water quality parameters of River Yamuna are severely deteriorated. As we move down the stream, masses of gaseous sludge are often noticed floating near the surface of the water. The water is not fit for human consumption, neither for general household purposes nor for irrigation. One of the biggest reasons for this is the discharge of untreated waste form 21 major drains in the studied segment of the River Yamuna. Recently, Said & Hussain (2019) used high-resolution GeoEye-2 imagery for monitoring and mapping pollution levels of Yamuna river in Delhi region, reporting a 'high water quality' before Wazirabad site and 'low water quality' after Wazirabad site. This is in accordance with our findings, where we also found that many of the water quality parameters showed a deterioration beyond sites 1 and 2.

## *A. cepa*: macroscopic parameters

Following incubations, the number of roots per bulb and root length were measured. Root length was expressed as 'mean of root length in centimeters ± SE' and 'mean of no. of roots/bulb ± SE'. In general, the mean number of roots/bulb and mean length of roots remained very low during winter, whereas it was recorded in high numbers during summer following three and seven days of exposure to the water samples. The results were highly varied among various study sites, and among seasons. During winter, for example, the mean root length was longest in control sample (1.87 ± 0.23 cm) and (2.58 ± 0.21 cm) after three and seven days of exposure, respectively, whereas it was shortest in the samples of site 2 (0.6 ± 0.0 cm) and site 7 (0.55 ± 0.05 cm) after three and seven days of exposure, respectively. The reduction in root length was observed as 32.08% and 21.31% after three and seven days respectively as compared to control. During

summers, on the other hand, the longest mean root length recorded was in site 2 (2.54 ± 0.09 cm) and site 1 (6.80 ± 0.79 cm) samples after three and seven days of exposure, respectively. Whereas, at 0.83 ± 0.17 cm for site 3 samples after a 3-day incubation, and 1.7 ± 0.6 cm for site 5 samples after 7 days of incubation, the mean root length is shortest. The reduction in root length was observed as 32.67% and 25% after three and seven days respectively as compared to control (Table 3). Likewise, during winter, the highest number of roots/bulb was noticed in control (2.58 ± 0.21) and samples from site 2 (1.91 ± 0.17) after three and seven days respectively. Whereas, the lowest number of roots/bulb was recorded with the samples from site 3 (0.6 ± 0.05) and site 7 (0.55 ± 0.05) after three and seven days of incubation, respectively. The reduction in roots/bulb was observed as 23.25% and 28.79% after three and seven days respectively as compared to control. On the other hand, during summer, the highest number of roots/bulb was noticed in the samples of site 6 (27 ± 5.29) and control (40.5 ± 23.5) after three and seven days respectively. Whereas, the lowest number of roots/bulb was recorded in those from site 4 (12 ± 2) after three days of incubation, and site 3 (18.5 ± 1.5) and site 4 (18.5 ± 2.5) after seven days of incubation. The reduction in roots/bulb was observed as 44.44% and 45.67% after three and seven days respectively (Table 3). The macroscopic parameters such as root length and root number are very important to ascertain the effects of pollutants on plant growth. In our results, we observed a significant reduction of root length and root numbers at various sites. These results are in accordance with Mesi & Kopliku (2013). Wijeyaratne & Wadasinghe (2019), Iqbal *et al.* (2019), and it is an indicator of rhizotoxicity, a general phenomenon caused by most of the pollutants.

## Microscopic analysis of cytotoxicity and genotoxicity

When the root tip squash preparations were observed under microscope, we could notice cytological, nuclear, and

**Table 3** | Morphological parameters of onion roots grown on water samples from different sites of the Yamuna river in Delhi region

| | Sampling site | | Tap water | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 |
|---|---|---|---|---|---|---|---|---|---|---|
| Root length | Winter Samples | Day 3 | 1.87 ± 0.23$^{a}$ | 1.91 ± 0.17$^{a}$ | 0.6 ± 0.05$^{b}$ | 1.03 ± 0.08$^{c}$ | 0.86 ± 0.08$^{c}$ | 1.25 ± 0.06$^{c}$ | 0.62 ± 0.08$^{c}$ | 1.70 ± 0.13$^{a}$ |
| | | Day 7 | 2.58 ± 0.21$^{a}$ | 2.22 ± 0.18$^{a}$ | 1.71 ± 0.15$^{b}$ | 1.97 ± 0.14$^{b}$ | 1.32 ± 0.12$^{c}$ | 3.67 ± 0.16$^{d}$ | 1.60 ± 0.23$^{c}$ | 0.55 ± 0.05$^{e}$ |
| | Summer Samples | Day 3 | 1.07 ± 0.825$^{a}$ | 1.44 ± 0.91$^{b}$ | 2.54 ± 0.09$^{c}$ | 0.83 ± 0.17$^{a}$ | 0.87 ± 0.13$^{a}$ | 1.26 ± 0.46$^{d}$ | 1.54 ± 0.12$^{b}$ | 1.27 ± 0.11$^{a}$ |
| | | Day 7 | 6.80 ± 0.79$^{a}$ | 4.3 ± 1.6$^{b}$ | 2.95 ± 0.25$^{c}$ | 2.06 ± 1.03$^{d}$ | 2.8 ± 1.1$^{c}$ | 1.7 ± 0.6$^{d}$ | 3.23 ± 0.27$^{c}$ | 4.4 ± 0.2$^{b}$ |
| Root number | Winter Samples | Day 3 | 1.87 ± 0.23 | 1.91 ± 0.17 | 0.6 ± 0.05 | 1.03 ± 0.08 | 0.86 ± 0.08 | 1.25 ± 0.06 | 0.62 ± 0.08 | 1.70 ± 0.13 |
| | | Day 7 | 2.58 ± 0.213 | 2.22 ± 0.18 | 1.71 ± 0.15 | 1.97 ± 0.14 | 1.32 ± 0.12 | 3.67 ± 0.26 | 1.60 ± 0.23 | 0.55 ± 0.05 |
| | Summer Samples | Day 3 | 21.33 ± 9.68$^{a}$ | 14.66 ± 3.38$^{b}$ | 17.33 ± 3.84$^{a}$ | 21 ± 3.51$^{a}$ | 12 ± 2$^{b}$ | 22 ± 10.1$^{a}$ | 27 ± 5.29$^{c}$ | 21.33 ± 5.81$^{a}$ |
| | | Day 7 | 40.5 ± 23.5$^{a}$ | 30.5 ± 0.5$^{b}$ | 23.0 ± 4.0$^{c}$ | 18.5 ± 1.5$^{c}$ | 18.5 ± 2.5$^{c}$ | 20.0 ± 8.0$^{c}$ | 35.0 ± 5.0$^{a}$ | 34.5 ± 5.5$^{a}$ |

Different sites of sample collection: Site 1: Yamuna Ghat, Site 2: Wazirabad Barrage, Site 3: ISBT, Site 4: Lohapul, Site 5: ITO, Site 6: Sarai Kale Khan, Site 7: Okhla Barrage. Beside these samples, tap water was used as control. Values are means ± standard errors of 5 replicates; within each column, means followed by the same letter are not significantly different at $p \leq 0.05$ according to DMRT.

chromosomal changes related to cytotoxic and genotoxic effects of pollutants (Figure 3). In our study, it was observed that after three days of incubation during the winter, the root tip cells of the control sample showed the highest mitotic index (MI) (0.11 ± 0.01) whereas at site 1, it was minimum (0.03 ± 0.003) (Figure 4(a)). On the contrary, after three days of incubation during summer, the MI was highest at site 2 (0.071 ± 0.025) and minimum at site 3 (0.023 ± 0.003) (Figure 4(b)). MI is an excellent indicator of plant growth as it gives a clear picture of the changed mitotic cell division kinetics and suggests if cell proliferation is affected. MI is also an indicator of cytotoxicity and a low MI denotes a few dividing cells (Barbério *et al.* 2011). Our results demonstrated that MI significantly decreased as we move down stream from site 1 to site 7. These results are in consensus with previous findings, which reasoned a decrease in MI was due to exposure to a number of pollutants including complex mixtures such as industrial and municipal waste, heavy metals and pesticides (Siddiqui *et al.* 2011; Mesi & Kopliku 2013; Hemachandra & Pathiratne 2015; Iqbal *et al.* 2019) which are possibly present in the polluted Yamuna water.

The morphometric data of onion roots grown on water samples from different sites of the Yamuna river in Delhi region are recorded in Table 4. We want to specify herewith that, to the best of our knowledge, this is the first study to measure cell size in this type of assay. During winter, the cells become massive; that is, 5,316.61 $\mu^2$ and 2,266.85 $\mu^2$ at site 6 and site 7, respectively, after three and seven days of incubation (Table 4). In summer, the same trend followed, but this time, cell size was comparatively smaller than their winter counterparts. The cell size and growth kinetics are fundamental cellular properties with important physiological implications. In multicellular organisms, the rules for cell size regulations are not fully recognized but a comparative account of cell size regulations is present in yeast and bacteria (Willis *et al.* 2016). Recently, Neurohr *et al.* (2019) suggested excessive cell growth causes cytoplasm dilution and contributes to senescence. In our results, massive cell sizes were at site 6 and 7, which suggested that cells are undergoing senescence and therefore root length was small as compared to control. Besides that, in our study, different types of cell shapes were observed that were different from the healthy cells. Serrano-Mislata *et al.* (2015) showed that plant meristem cells can actively maintain a target size, which is required to generate the fine details during development, similar to the way appropriate pixel sizes are required to make details in digital images. The abnormal pictures of cells in our results suggested the harmful effects of pollutants that vary the cell size and shapes. The abnormal shapes of the cells include spiral, lobed, bottle shape, horse-shoe shape, elongated, notched etc. (Figure 5). At sites 3, 4 and 7, cells were observed with elongated ends/ tips and with notches. The devastating and distorting

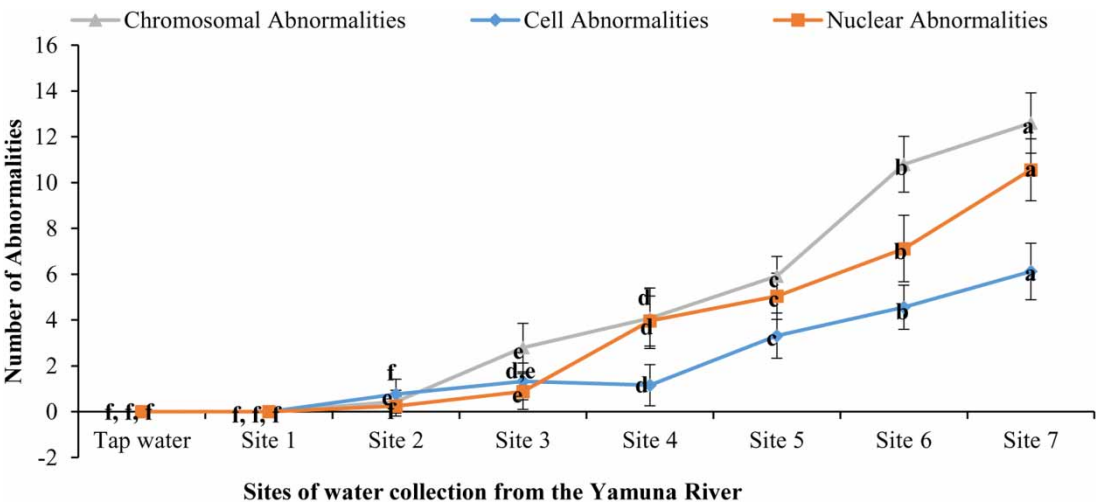


**Figure 3** | Different types of abnormalities in onion root tip cells subjected to treatment with water samples from different sites of the Yamuna river in Delhi region. Different sites of sample collection: Site 1: Yamuna Ghat, Site 2: Wazirabad Barrage, Site 3: ISBT, Site 4: Lohapul, Site 5: ITO, Site 6: Sarai Kale Khan, Site 7: Okhla Barrage. Beside these samples, tap water was used as control. Values are means ± standard errors of 5 replicates; within each abnormality, means followed by the same letter are not significantly different at $p \leq 0.05$ according to DMRT.

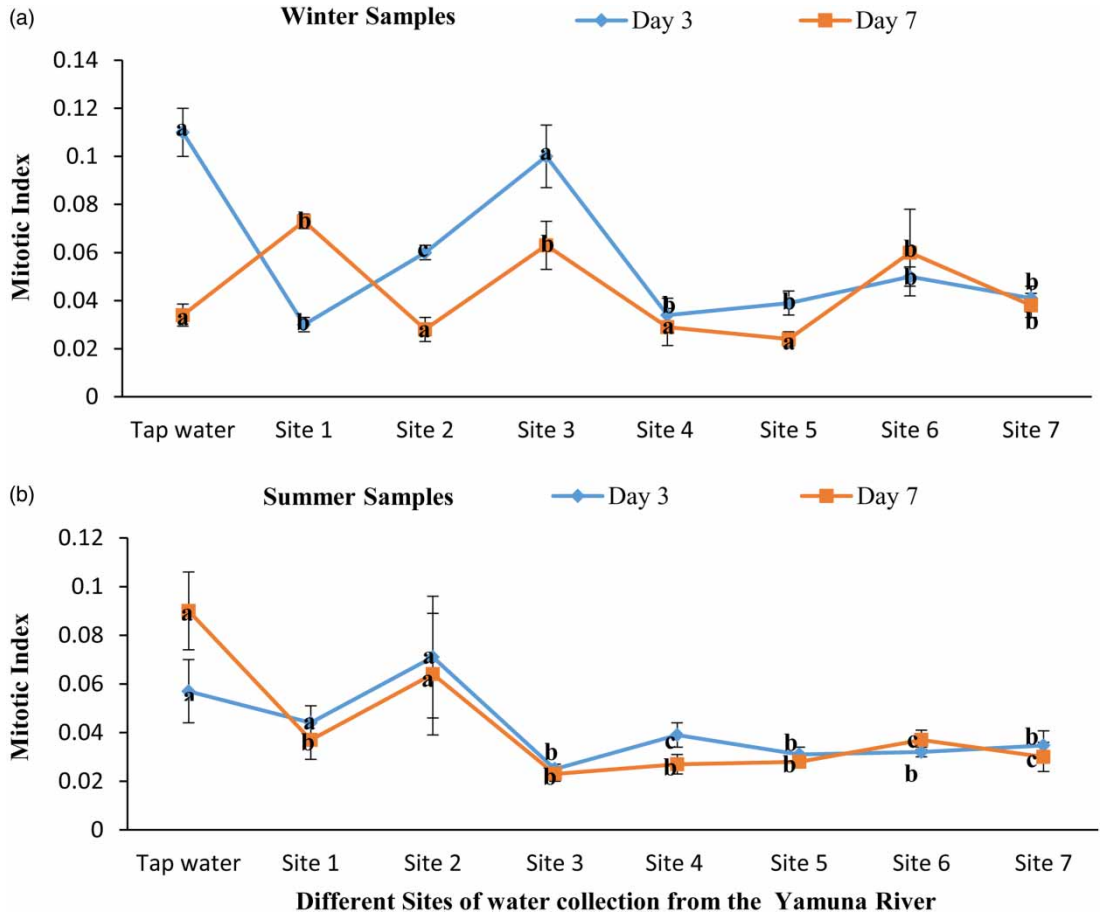


**Figure 4** | Mitotic index of onion roots grown on winter and summer water samples from different sites of the Yamuna river in Delhi region. Different sites of sample collection: Site 1: Yamuna Ghat, Site 2: Wazirabad Barrage, Site 3: ISBT, Site 4: Lohapul, Site 5: ITO, Site 6: Sarai Kale Khan, Site 7: Okhla Barrage. Beside these samples, tap water was used as control. Values are means ± standard errors of 5 replicates; within each day, means followed by the same letter are not significantly different at $p \leq 0.05$ according to DMRT.

**Table 4** | Morphometric data of onion roots grown on water samples from different sites of the Yamuna river in Delhi region

| | Sampling site | | Tap water | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 |
|---|---|---|---|---|---|---|---|---|---|---|
| Cell size ($\mu^2$) | Winter samples | Day 3 | 1,109.53$^a$ | 941.78$^a$ | 810.78$^a$ | 835.35$^a$ | 2,496.44$^b$ | 535.35$^a$ | 5,316.61$^c$ | 2,266.85$^b$ |
| | | Day 7 | 1,015.40$^a$ | 2,769.12$^b$ | 863.92$^a$ | 1,986.91$^d$ | 1,511.75$^d$ | 505.065$^a$ | 7,017.22$^e$ | 6,586.008$^e$ |
| | Summer samples | Day 3 | 899.52$^a$ | 665.77$^b$ | 469.36$^c$ | 3,274.24$^d$ | 974.46$^a$ | 689.06$^b$ | 643.91$^b$ | 1,387.76$^e$ |
| | | Day 7 | 446.002$^a$ | 742.40$^b$ | 539.43$^c$ | 1,464.42$^d$ | 501.49$^c$ | 570.76$^c$ | 816.32$^b$ | 483.016$^a$ |

Different sites of sample collection: Site 1: Yamuna Ghat, Site 2: Wazirabad Barrage, Site 3: ISBT, Site 4: Lohapul, Site 5: ITO, Site 6: Sarai Kale Khan, Site 7: Okhla Barrage. Beside these samples, tap water was used as control. Values are means ± standard errors of 5 replicates; within each column, means followed by the same letter are not significantly different at $p \leq 0.05$ according to DMRT.

effects of pollutants were more prominent in the cells at sites 2, 3, 4, and 7 than those of control ones and in the cells at the remaining sites (Figures 3 and 5). These types of images are possibly reported for the first time in *A. cepa* bioassay.

Nuclear changes, which are also known as '*nuclear abnormalities*' (NA) and generally observed in interphase nuclei, were very prominent in our root tip squash preparations at different sampling sites (site 1, 3, 5 and 7) as compared to the control sample during summer and winter. The abnormally distorted nuclear shapes observed include horseshoe-shaped and lobulated at site 3 and site 7, respectively, whereas spiral-shaped nuclei, as well as binucleated cells were observed at site 5. Further, at site 7, the nuclei were distorted into U- and hook-shapes, showing

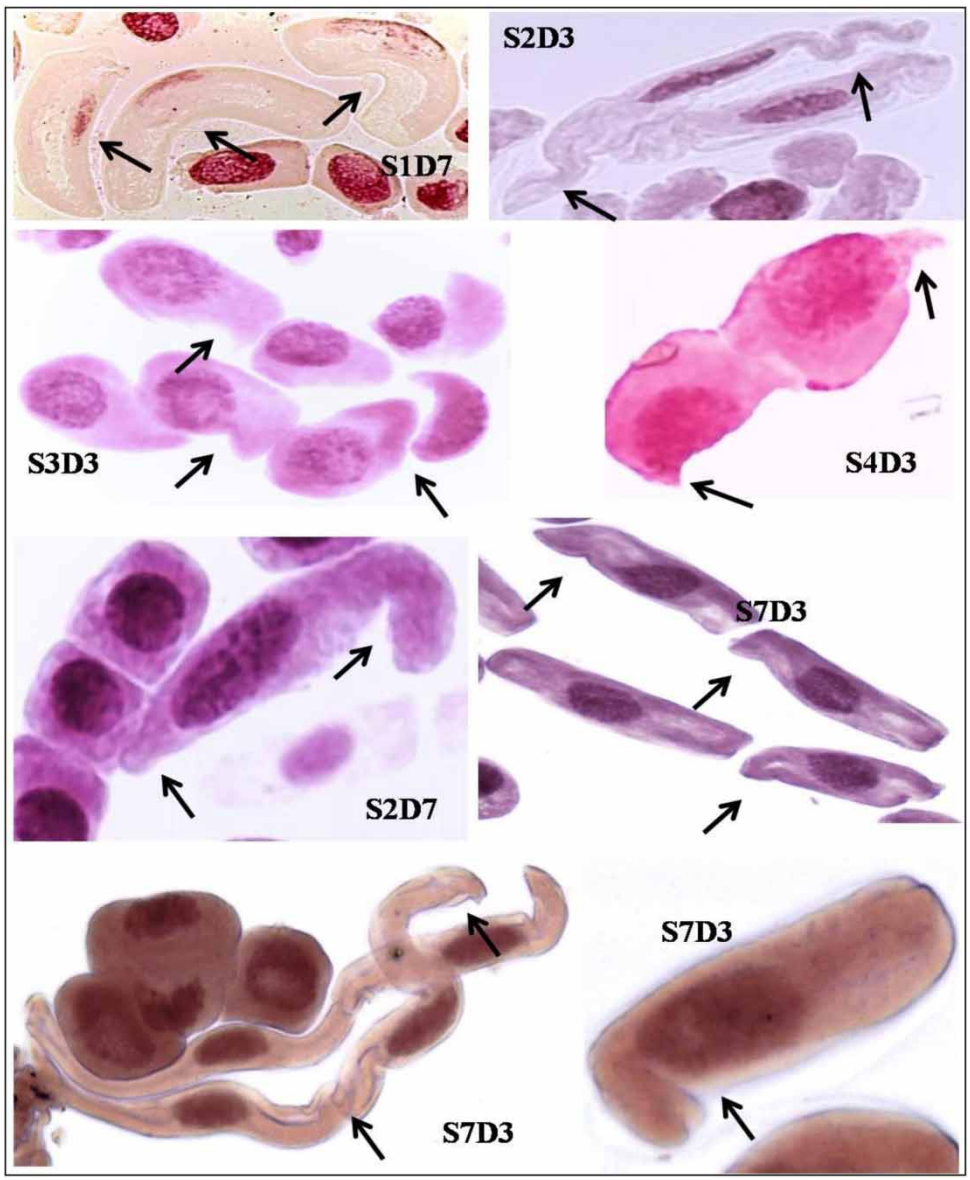


**Figure 5** | **C-shaped cell with fractured nucleus:** S1D7; **spiral shape cell:** S2D3, S7D3; **lobed cell:** S7D3, S2D7; **cell with elongated ends and notches:** S3D3, S4D3.

the life-devastating effects of pollutants in the river water, which is being used not only for human consumption but livestock and agricultural purpose as well (Figures 3 and 5). NA has been included as an endpoint on testing cyto-genotoxicity of environmental chemicals because its analysis makes the investigation more accurate regarding their effects on the DNA of exposed organisms (Leme & Marin-Morales 2009). Due to the effects of pollutants, the meristematic cells in their interphase showed micronuclei, lobulated nuclei, nuclear bud, and chromosomal breaks. (Mazzeo & Marin-Morales 2015; Iqbal *et al.* 2019; Wijeyaratne & Wadasinghe 2019). The presence of lobulated nuclei and polynuclear cells is an indicator of cell death process. The presence of nuclear buds is associated with pollutant exposure, which may arise as a result of the elimination of exceeding genetic material derived from the polyploidization process (Rosculete *et al.* 2018). In addition to the above nuclear deformities, micronuclei were observed at sites 1, 3, 4, 5 and 7 (Figure 6). The formation of micronuclei and chromosomal abnormalities are considered as a sign

of genotoxicity caused by chemicals and heavy metals (Barbério *et al.* 2011; Mazzeo & Marin-Morales 2015; Iqbal *et al.* 2019). At the banks of river Yamuna, farmers use different types of herbicides and pesticides that fall directly into the river. Recently, Mazzeo & Marin-Morales (2015) reported that herbicide significantly increases the number of nucleoli and micronuclei formation, in order to remove the excessive nucleolar material resulting from the induction of polyploidization. So, the presence of micronuclei in our results infers the damaging genotoxic effect of heavy metals and pesticides present in the river water.

Chromosomal abnormalities can be interpreted by changes in the number and structure of chromosomes. The changes at chromosomal level were recorded in the form of abnormal mitosis, improper alignment of metaphase plate as well as the disorientation of chromosomes during anaphase. Further, chromosomal bridges (at sites 1, 2, 3, 4, 5 and 7), chromosomal adherence at metaphase

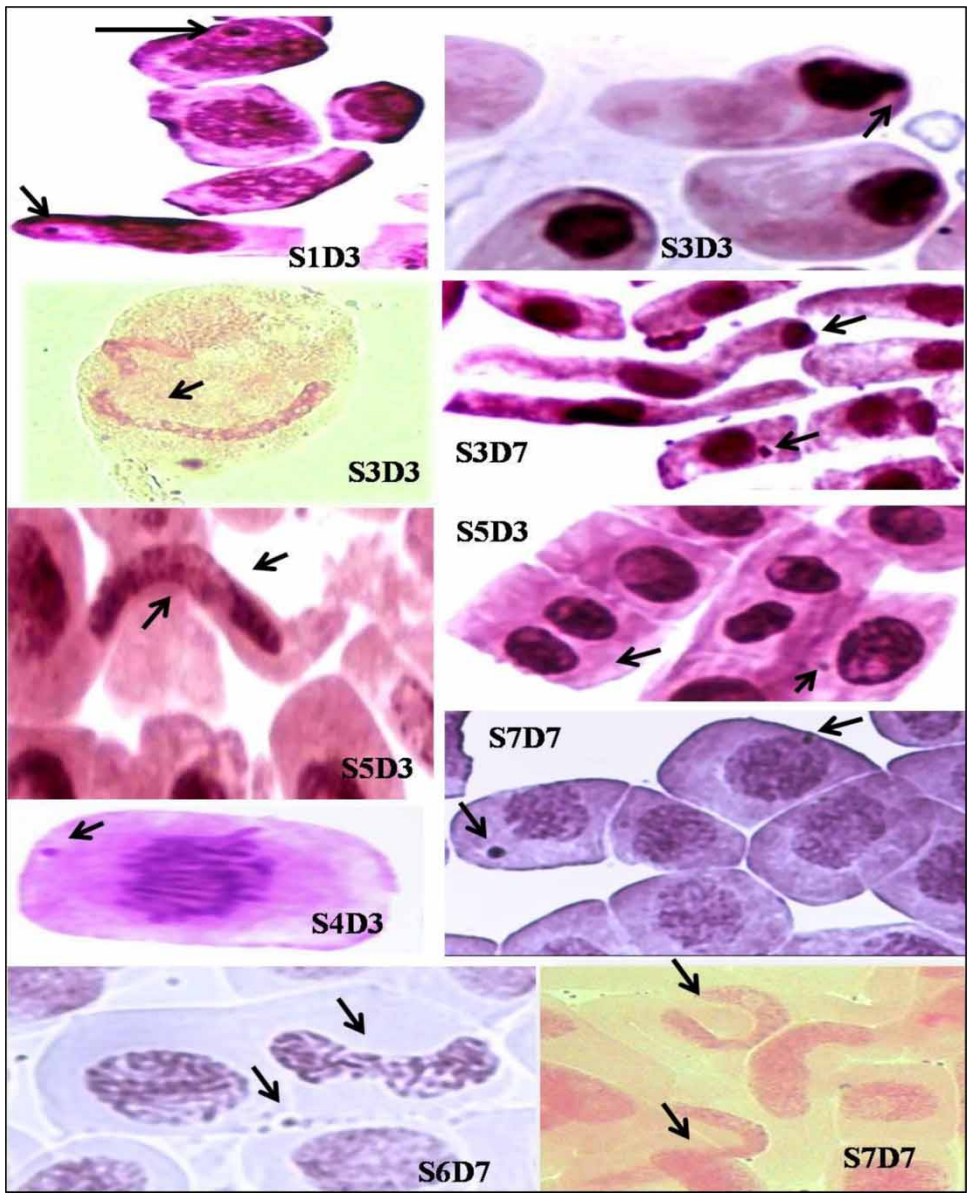


**Figure 6** | **Micronucleus:** S1 D3, S4D3, S5D3, S3D7, S7D7; **lobed nucleus:** S3D3, S6D7; **spiral shape nucleus:** S5D3; **binucleate cell:** S5D3; **horse shoe shape nucleus:** S3D3, S7D7, **hook shape nucleus:** S7D7.

and telophase (at sites 4, 5 and 6), chromosomal loss (at sites 2, 5 and 7) were also observed (Figures 3, 7 and 8). Moreover, the presence of chromosomal fragments is an indication of chromosomal breaks and can be a consequence of anaphase or telophase bridges. These changes are due to the effects of certain chemicals/pollutants (Leme & Marin-Morales 2009; Iqbal *et al.* 2019). The present study substantiates the above facts as we have also recorded scores of chromosomal abnormalities (Figure 7 and 8) at site 4, 5 and 7. The reasons for the chromosomal loss and breaks is the abnormal segregation of chromosomes. This phenomenon can occur either spontaneously or by the action of aneugenic agents (Leme & Marin-Morales 2009; Iqbal *et al.* 2019). In our study, we observed abnormal metaphase, anaphase and telophase quite frequently. The possible reason for this might be the presence of drainage systems that fall into the River Yamuna and the pesticides, herbicides, domestic as well as industrial detergents, industrial dyes, garbage leachates,

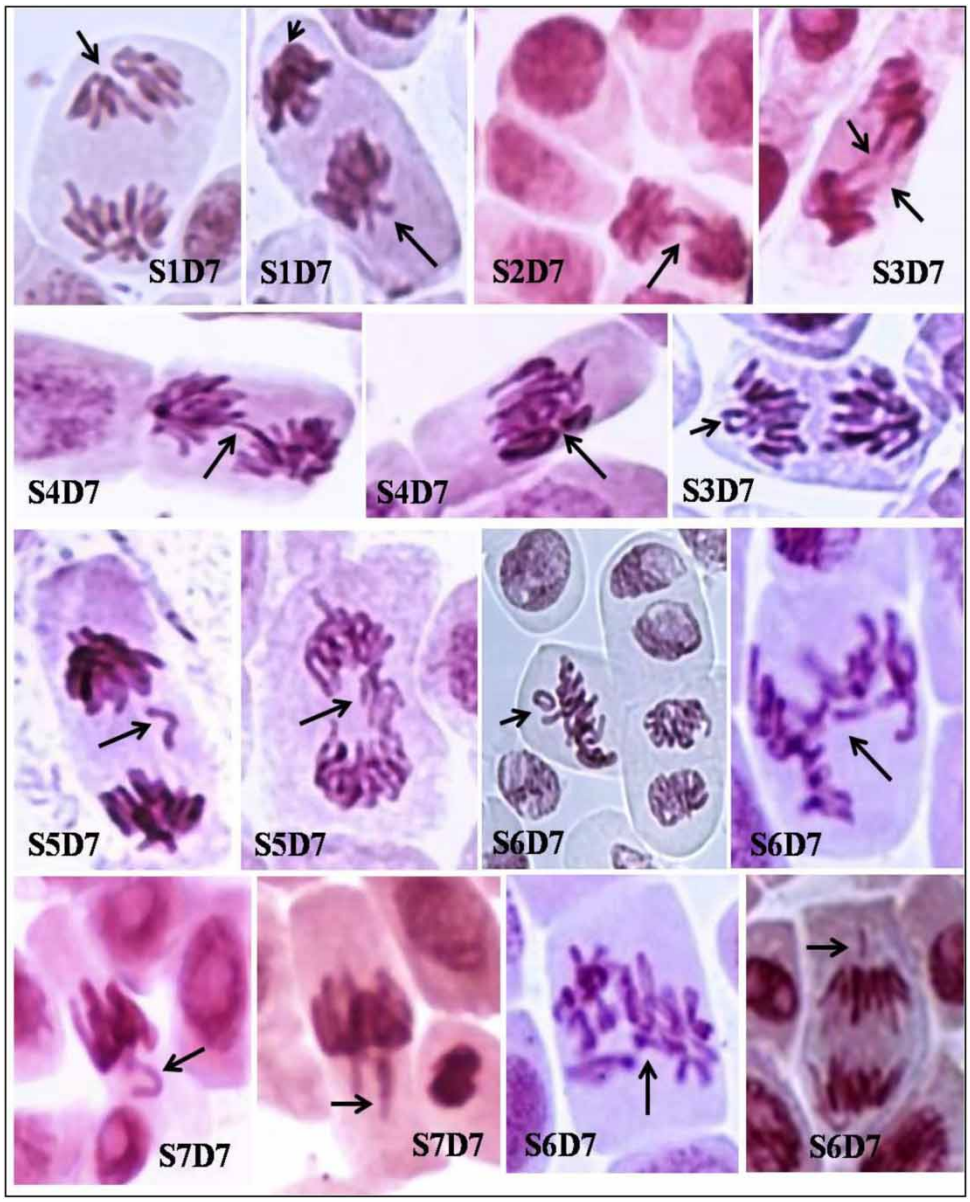


**Figure 7** | Abnormal anaphase: S1D7, S6D7; abnormal telophase: S1D7; chromosomal bridge: S2D7, S3D7, S4D7, S5D7; metaphase adherence: S4D7, S7D7; chromosomal loop: S3D7, S6D7; chromosomal loss: S5D7, chromosomal laggard: S6D7, S7D7.

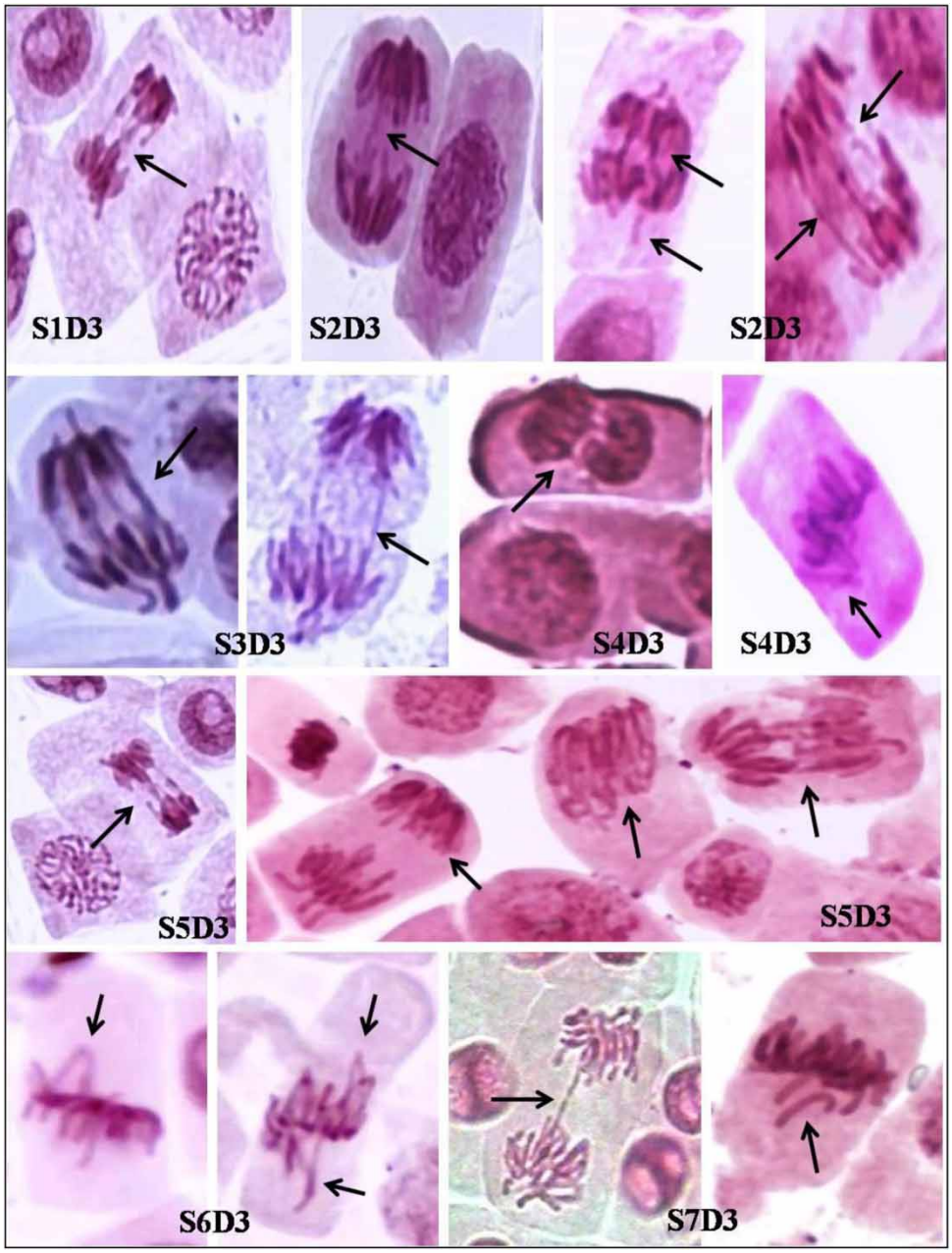


**Figure 8** | **Chromosomal bridge:** S1D3, S2D3, S3D3, S4D3, S5D3, S7D3; **metaphase adherence:** S4D3, S5D3, S6D3; **telophase adherence:** S5D3; **compact telophase:** S4D3; **chromosomal loss:** S2D3, S7D3.

tannery effluents and heavy metals released from the two thermal power stations situated on the riverbank.

Further, drainage systems release organic substances, detergents, shampoo etc. and chemicals from industry. These substances forms surfactants and their interaction with water breaks the surface tension and results in mixing of air, creating foam. The creation of foam is in the same manner as it forms in our sinks and washing machines. This foam is not a normal part of the river system; it is a sign of pollution and it also emits a foul smell (Joshi 2018; https://www.crwa.org/hubfs/foamfactsheet.pdf accessed 24 September 2019). During our sample collection at site 7, a heavy dense white froth/ foam was observed floating on the water surface hindering the access to the water, along

with complaints of itching, eyes and airway irritation, nauseating smell and breathlessness. The presence of froth was very persistent and gives the look of snow-filled surface and technically decreases dissolved oxygen by inhibiting the contact of surface water with air. In our preliminary study, we have detected heavy metal ions like copper, chromium, arsenic, and zinc in the water samples from various sites, and the source of these ions in river water can be traced to the nearby Rajghat and Indraprastha Thermal Power Stations located at site 5 and site 6, respectively (data not shown). However, to reach a rational conclusion, more work is required to be done to elucidate the effects of complex mixtures of various types of pollutants causing cytological, nuclear and chromosomal abnormalities. Perhaps, arsenic was present in the highest concentration at all the sites; at site 7 an industrial area is located that directly discharge untreated effluents directly into the Yamuna river.

## CONCLUSION

The data obtained in our results are a significant cause of concern and indicates the synergistic cellular, nuclear, and chromosomal abnormalities of polluted Yamuna water on the meristematic cells of *A. cepa*. Our results further corroborate the available literature with regards to the toxicity of pesticides and heavy metals. Further, our research also showcases the harmful effects of pollutants on the cellular and nuclear morphology and chromosomal abnormalities, which ultimately leads to the activation of cell death program (apoptosis) by cytotoxic and genotoxic compounds present in polluted water. In this report, perhaps to the best of our knowledge we reported cell size measurement and cytological changes in *A. cepa* cell under the influence of polluted water of River Yamuna. The abnormal images of cells and nuclei can be used for a mass awareness campaign to educate people and farmers about the harmful effects of water pollution. This study further highlighted the fact that cytotoxicity and genotoxicity bioassays should be used as an essential method for evaluation of wastewater toxicity before its discharge into the environment. Moreover, this work can be further extended towards detailed estimation of heavy metals and pesticides present in River Yamuna to ascertain the main reasons behind the cytotoxic and genotoxic effects.

## ACKNOWLEDGEMENTS

Authors gratefully acknowledge the Innovation Desk, University of Delhi, India, for the financial support (DAAP-2582/26.11.13); Principal, Zakir Husain Delhi College, JLN Marg, University of Delhi, New Delhi-110002, India, for providing the infrastructural support, and Dr Ankur Maheswari for critical reading and reviewing the article. Authors also acknowledge the hard work put in by the undergraduate 'part-time student fellows' of Zakir Husain Delhi College, including Rohan Kapoor, Pragati Shakya B.Sc. (H) Botany; Neha, Shubham, Prashant, Anoop, Debarati Chanda B.Sc. (H) Zoology; and Nisha Bhardwaj B.Sc. Life Sciences for their active participation.

## CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest.

## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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First received 14 February 2020; accepted in revised form 10 July 2020. Available online 27 July 2020