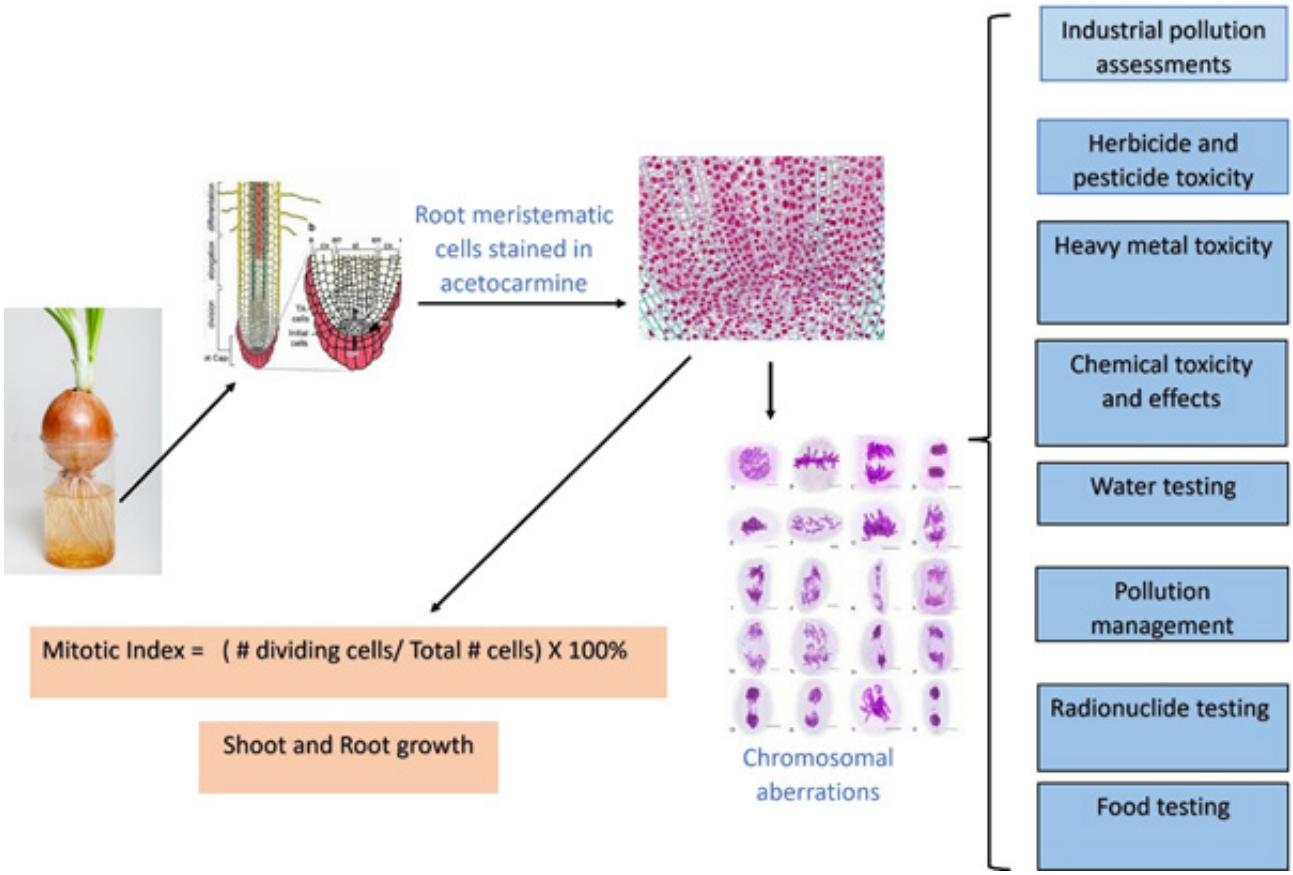
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RESEARCH ARTICLE

Allium cepa Bioassay as an Assessment Tool in Toxicology with Special Emphasis on Environmental Toxicity Assessments

A. M. P. E. K. Atapattu and U. A. Jayawardena

Graphical abstract



Highlights

- *Allium cepa* is an effective, low-cost, and reliable bioindicator widely used for cytotoxicity and genotoxicity assessments in environmental monitoring
- *Allium cepa* bioassay demonstrates strong applicability in evaluating the toxic effects of pesticides, heavy metals, industrial effluents, and contaminated water samples
- Rapid root growth, large chromosomes, ease of handling, and good correlation with mammalian systems make *A. cepa* a valuable alternative to animal-based toxicity testing

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RESEARCH ARTICLE

Allium cepa Bioassay as an Assessment Tool in Toxicology with Special Emphasis on Environmental Toxicity Assessments

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Abstract

Allium cepa has emerged as a valuable model organism for toxicity testing, offering a viable alternative to animal testing worldwide. It is regarded as an efficient bioindicator for genotoxicity testing, primarily due to its rapid root growth rate and the presence of relatively few large chromosomes. The use of *A. cepa* is highly sought after because of its cost-effectiveness, widespread availability, and ease of handling, demonstrating a strong correlation with mammalian test systems. In this review, we emphasize the diverse applications of the *A. cepa* bioassay in environmental assessments. A comprehensive literature search was conducted using Google Scholar and ResearchGate with the keywords *"Allium cepa,* bioassay, environmental assessments, and toxicity testing" over the period of February to April 2024. This search initially yielded 131 articles, which were subsequently narrowed down to 31 relevant studies that specifically assessed the use of *A. cepa* as an evaluation tool. The findings reveal the applicability of the *A. cepa* bioassay in a variety of environmental assessments, including toxicity testing of pesticides, heavy metals, and industrial effluents, as well as monitoring pollution effects and water quality. Additionally, the review discusses chemical testing across a wide spectrum, from pesticides to azo dyes, food preservatives, and hydrocarbons, highlighting various industrial applications. This synthesis of current literature underscores the utility of the *A. cepa* bioassay in contributing to environmental monitoring and pollution control.

Keywords: *Allium cepa*; Chromosomal aberrations; Genotoxicity; Mitotic indices; Toxicity

INTRODUCTION

The increasing use of chemicals, including pharmaceuticals, pesticides, and petroleum products etc., increases the risk of environmental contamination, causing health implications. Hence, biomonitoring is crucial for minimizing damage to the environment. Special attention should be given to potentially mutagenic chemicals, as they can cause inheritable changes in the genetic materials (Algarni, 2018; Firbas & Amon, 2013; Nabeel et al., 2008). A wide variety of organisms, ranging from viruses, bacteria, plants, and insects to human cell cultures and intact mammals, are used to evaluate the mutagenicity of such chemicals. A variety of tests, including cytogenetic assessments, have been employed to identify the harmful effects of substances at different concentrations and at different times of exposure. The current trend is to use *in vitro* toxicity assessment methods due to ethical concerns related to animal models.

Higher plants such as *A. cepa*, *Vicia faba*, *Zea mays*, *Tradescantia*, *Nicotiana tabacum*, *Crepis capillaris,* and *Hordeum vulgare* are the most frequently used plants for bioassays, which serve as important models for genetic tests to monitor various pollutants present in the environment (Daphedar & Taranath, 2018; Grant, 1999; Kursheed et al., 2018). *Allium cepa* (Onion) has been considered an efficient bioindicator compared to other organisms in genotoxicity testing because of the rapid root growth rate and a small number of large chromosomes (2n = 16) (Bonciu et al., 2018). It is commonly utilized as a short-term and cost-effective indicator of toxicity monitoring (Cabrera & Rodriguez, 1999; Grant, 1999; Jayawardena et al., 2021; Ma, 1999).

Allium cepa assay was first introduced by Levan (1938), when the effects of colchicine were investigated, and since then it has been frequently used (Clarkson, 1965; Kihlman, 1966; Levan & Östergren, 1943; Levan, 1945). Slight modifications have been made to the original form of the test, in order to enable more comprehensive assessment of weak and unknown contaminations to increase the sensitivity (Fiskesjø, 1985). This has been extensively used in environmental toxicity assessments, among other fields (Bagatini et al. 2009; Firbas & Amon, 2013), with a higher emphasis on environmental monitoring, especially in contaminated aquatic environments (Leme & Marin-

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Morales, 2009). This has been validated against evaluating mutagens and detecting toxic substances such as pesticides, azo dyes, food preservatives, and hydrocarbons etc. (El-Shahaby et al., 2003) by measuring the degree of alterations in the test system subjected to any type of chemical causing physical damage and microscopic changes. (Tedesco & Laughinhouse, 2012).

Allium cepa bioassay procedure

A generalized procedure of the *A. cepa* bioassay requires the recruitment of healthy, equal-sized onion bulbs, preferably an organically grown cultivar. The loose outer sheaths and the dry bottom are removed to open the root primordia. The cleaned bulbs are kept in containers (test tubes, etc.) filled with dechlorinated water/distilled water for 2-3 days, touching the water until the bulbs get rooted. The experiments should be performed under a relatively constant room temperature, approximately +25 °C, without exposing them to direct sunlight. The onion bulbs with newly developed roots (about 1 cm long) are then exposed to test samples for 24, 48, 72, or 96 hours, depending on the desired exposure time. A negative control with dechlorinated water (the same water used in the root development phase) and a positive control should be run simultaneously. If the exposure time is longer than 24 hours, the exposure medium should be changed every 24 hours. Onion bulbs are taken out from the test tubes and are placed on soft tissue papers. The top 1 cm of the root tips are harvested with a dissecting blade, and they are heated in a 1:3 1N HCl: Glacial acetic acid solution at 60˚C in a water bath for at least 20 minutes, until the root tips are dipped in the bottom of the boiling tube. Then in the hydrolysis step, the root tips are kept in 1N HCl for 10 minutes and stained with 1% acetocarmine until the very tip of the root tips gets a deep reddish colour. Next, the stained root tip should be placed on a glass slide in a drop of distilled water, and a coverslip is placed on top while applying slight pressure to crush the root tip and release the cells. Meristematic cells are observed with a compound light microscope under high power (400× magnification). Then, the number of dividing cells in each mitotic stage and the number of aberrant cells should be recorded. The Mitotic Index (MI), Mitotic Phase Indices (MPI) for prophase, metaphase, anaphase, and telophase, and the Percentage of Chromosomal Aberrations (PCA) should be calculated by using the following formulae.

MI = (Total number of dividing cells/ Total number of analyzed cells) × 100%

MPI = (Total number of cells in each phase / Total number of analyzed cells) × 100%

PCA = (Total number of aberrant cells /Total number of analyzed cells) × 100%

Common endpoints and toxicity indications

Macroscopic indicators

The exposure of test substances induces both macroscopic and microscopic effects on the *Allium* roots, where root length, shape (root swellings/turgescence/coiled roots/ slightly curved/ curved roots/ Curvy root tips/crochet or hook-like roots), and colour (General white coloration of the root tips/ any changes of colouration/ discoloration) are considered as macroscopic measures (Figure 1a). Each of these measures indicates the different effects of the test substance. In general, root length is measured after the exposure period and expressed as the root growth inhibition as a percentage of the control to compare with each treatment. Growth inhibitions can be further expressed in the retarded shoot and the leaf development along with the root growth. Similarly, percentage shoot growth is also considered as a macroscopic endpoint (Jayawardena et al., 2021).

Microscopic indicators

Cytotoxicity is mostly determined by the decreasing values of MI in the treatments compared to negative controls and the elevations in cell apoptosis/necrosis. Apoptosis is the programmed cell death through a series of molecular steps that lead to the cell's death. Necrosis is the passive, accidental cell death resulting from diseases, infections, injuries, or extreme environmental conditions. Further, the MPI exhibits the frequencies of mitosis and cellular health. Moreover, Fiskesjø (1985) explains that increasing anaphase incidence of incomplete chromosomal separation was considered an indication of a lower rate of cell division.

Chromosomal Aberrations (CA) and micronucleus (MN) formation verify the genotoxicity. Common chromosomal aberrations reported in the *Allium* test includes: Sticky chromosomes/ stickiness (ST), C-mitosis/ C-metaphase (CM), vagrant chromosomes (VA)/ laggards (LG), Chromosomal bridges (CBD), Polarity (P), etc. (Figure 1b). These aberrations are indicative of highly toxic, often irreversible effects, leading to cell death.

As explained by Bennett (1977), ST is a result of incomplete replication of chromosomes by defective enzymes. Amin (2002) had explained that it occurs mainly due to immediate reactions of metal ions or toxic agents with DNA during its inhibition periods, causing DNA-DNA or DNA-protein cross-linking. Moreover, these are due to a disturbed balance in the quantity of histones or other proteins responsible for controlling the proper structure of nuclear chromatin. The adherence induced by these mechanisms in turn affects spindle formation, chromosomal arrangement, and separation during the mitotic phase (Fiskesjø, 1985). Stickiness of chromosomes prevents complete separation during anaphase, and it may lead to the formation of CBD.

C-mitosis/C-metaphase (CM) refers to spindle dysfunction and the malfunction of kinetochores. In this context,

chromatin bridges indicate chromatin dysfunction, while chromosome and/or chromatid breaks point to clastogenic effects. Vagrant or laggard chromosomes, which occur due to the unequal distribution of chromosomes or paired chromatids, signify the risk of aneuploidy (Leme et al., 2008). As a result, numerical alterations in chromosome counts can occur. Both chromosomal bridges (CBD) and chromosomal breaks (CBR) are classified as indicators of clastogenic effects, as they lead to alterations in DNA structure. Chromosomal abnormalities linked to aneugenic effects include chromosome losses, delays in movement, adherence, multipolarity, and instances of C-mitosis.

METHODOLOGY

This review emphasizes the diverse environmental assessment applications of the *Allium cepa* bioassay. A comprehensive literature search was conducted using Google Scholar and ResearchGate with the keywords "*Allium cepa*," "bioassay," "environmental assessments," and "toxicity testing" from February to April 2024. The initial search yielded 131 articles, which were narrowed down to 31 relevant studies focusing on the use of *A. cepa* as an assessment tool. The findings from this literature survey illustrate the broad applications of the *A. cepa* bioassay in environmental assessments, including toxicity testing of pesticides, heavy metals, and industrial effluents, as well as monitoring pollution effects. Additionally, the review covers water quality testing and chemical assessments involving a range of substances, from pesticides to azo dyes, food preservatives, and hydrocarbons, highlighting their various industrial applications.

RESULTS AND DISCUSSION

Pesticide and herbicide toxicity assessments

The *A. cepa* bioassay has been utilized to assess cytotoxicity and mutagenicity of pesticides, including Malathion, at concentrations of 0.5 mg/mL and 1.0 mg/mL over a period of 48 hours. This study included a negative control (distilled water) and a positive control (Methyl methane sulfonate solution at 10 mg/L) (Ghisi et al., 2023). Malathion is recognized as an inducer of DNA damage, as it modifies guanine to 7-methylguanine and adenine to 3-methyladenine. These modifications lead to base mispairing and hinder DNA replication, resulting in an increased incidence of anaphase bridges, chromosome delays, and chromosomal losses. Furthermore, in vitro studies support these findings, identifying Malathion as a methylating agent that affects DNA bases.

Rodriguez and colleagues (2015) evaluated the effects of IMI (a neonicotinoid that act on the central nervous system of insects) with *A. cepa* and *Tradescantia pallida* following exposure to different concentrations (40 mg m^{-2}, 20 mg m^{-2}, 80 mg m^{-2}) and found that the selected concentrations had induced chromosomal alterations in *A. cepa* and significantly increased the frequency of micronuclei formation in both *A. cepa* and *T. pallida* compared to the negative control ($p < 0.05$) confirming that IMI in 20 – 80 mg m^{-2} is genotoxic to the test organisms (Table 1). Previous genotoxicity studies have reported the presence of alterations in the chromosomes of human lymphocytes and CHO cells following exposure to IMI (Federal Register, 1995). Datta et al. (2018) had compared the genotoxicity of pesticide-treated soil (PTS) and vermicompost-treated soil (VTS), which showed significant differences in terms of cytotoxicity (root length; mitotic index) and genotoxicity (chromosomal aberrations) in the *A. cepa* test.

Diclofop-methyl (a widespread herbicide belonging to the group of aryloxyphenoxy alkanoic acids and Lindane (An organochlorine insecticide which was banned since 2009 due to neurotoxic effects in humans) tested using onion roots exposed for 24 hours, resulting in a considerable decrease in mitotic index and general sublethal effect in onion root tip cells (Mesi & Kopliku, 2013).

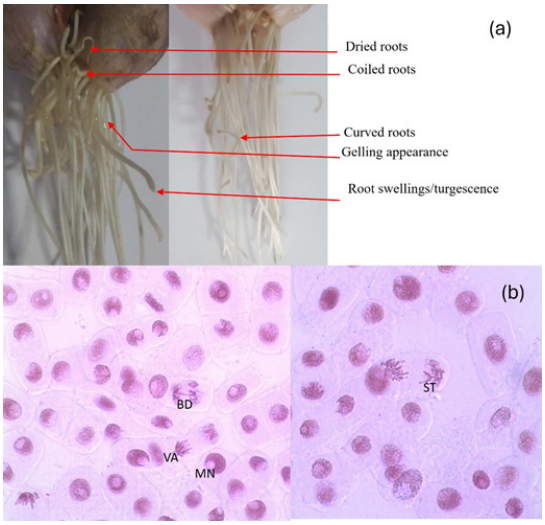


Figure 1: (a) Macroscopic changes observed in *Allium cepa* roots after exposure to a test solution. (b) Examples of chromosomal aberrations in *Allium cepa* root meristematic cells, including: BD - bridging chromosomes; VA - vagrant/laggard chromosomes; MN - micronuclei; ST - sticky chromosomes.

Further, biomarker function was evaluated with *A. cepa* systems on EROD (7-ethoxy resorufin O-deethylase) activity as a biomarker of pesticide pollution was investigated using the *A. cepa* system (Fatima & Ahmad, 2006) on water samples containing six pesticides (2,4-D, HCB, malathion, carbaryl, DDT and endosulphan) suggesting that *A. cepa* derived EROD as a potential biomarker of certain pesticides since even 1 ppb of total/individual pesticides brought about >10-fold induction of EROD, thus can be used as a presumptive test for the presence of these pesticides in the test water sample before using expensive analytical techniques like HPLC and GC.

Moreover, quizalofop-p-ethyl and cycloxydim were evaluated using the *A. cepa* test at 0.5%, 1%, and 1.5% concentrations for 24 h exposure, indicating a strong cytotoxic and genotoxic effect for both herbicides, a strong mitodepressive effect, chromosomal aberrations, and nuclear alterations: stickiness, fragments, C-mitosis, lobulated nucleus, micronuclei, and nuclear erosion (Rosculete et al., 2019).

Industrial pollution and toxicity assessments

Effluent samples with H_2O_2 (Hydrogen Peroxide) and CH_2O (Formalin) from mining areas in Caraga Region, located in Northern Mindanao, Philippines, were tested, revealing macroscopic and microscopic alterations (Cabuga Jr et al., 2017). Further, Olorunfemi et al. (2011) assayed industrial wastewater from outlets of brewery, rubber, and bottling companies, with the onion bulbs in a concentration series of wastewater (0.01, 0.1, 1, and 10%) for 72 hours. This study had recorded strong growth retardation, which occurred in high concentrations of all three waste waters at 72 hours. The chromosomal aberrations recorded were mostly sticky chromosomes and bridges, and some chromosomes with disturbed spindles and fragments were also present in appreciable amounts. Polluted water samples collected from three different industrial effluents (soap, beverage, and paint industries) in southeast Nigeria resulted in a lowering of mitotic index and the root growth, suggesting a combination of physicochemical analysis and cytogenotoxicity assay as effective in monitoring the pollution (Olusola & Solomon, 2018).

Apart from wastewater, sediments have also been tested with *A. cepa* bioassay, revealing promising results. Wijeyaratne & Wadasinghe (2019) assessed the cytotoxic effects of contaminated sediments in Dandugan Oya, an industrial effluent receiving water body in the western province of Sri Lanka, revealing a significant root length variation due to root growth inhibition increased with increased exposure time. A similar trend has been observed in mitotic division, indicating significantly lower mitotic indices exposed to sediment elutriates than those in water samples.

Moreover, river water samples were assayed with the *A. cepa* system to evaluate the effect of textile effluents (Wijeyaratne & Wickramasinghe, 2020), heavy metal contamination (Annisa et al., 2024), and both industrial effluents and sewage (Paul & Arjun, 2018) affecting the MI, frequency, and types of chromosomal aberrations. Similarly, tannery and textile effluents have been tested in numerous studies, revealing a dose-dependent increase in percentage aberrations and decline in MI (Admas & Kerisew, 2022; Ventura-Camargo et al., 2016).

Cytogenotoxicity of heavy metals

Effects of heavy metals have been extensively investigated with the *A. cepa* test system, recording dose-dependent elevation of percentage aberrations and lowering of cell division activities in all studies. Among these studies, 50 µg/mL ZnO nanoparticles (Sun et al., 2019), individual and mixtures of heavy metals such as Cd, Cr. Pb, Cu, Zn in 0.05- 5 ppm concentration (Jayawardena et al., 2021) revealed both macroscopic and microscopic alterations of the test model. Furthermore, the metal concentrations above 5 ppm recorded direct cytotoxic effects, producing cellular apoptosis or necrosis.

Silveira et al. (2017) conducted a study in Brazil on the determination of toxic effects on *A. cepa* and *Lactuca sativa* after exposure to Methyl Methanesulfonate (MMS), Cadmium, the aluminum industry waste Spent Potliner (SPL), and the herbicide Atrazine. Both plant species had presented sensitive and efficient responses to the applied tests after exposure to MMS, SPL, and the herbicide Atrazine. Overall, *A. cepa* was more efficient in detecting clastogenic changes, arising from DNA breakage. Cadmium (Cd) significantly reduced root growth in lettuce plantlets compared with the control, while no Cd-induced phytotoxic effects were observed with the *A. cepa* model.

Water testing

Drinking water purification process, including chlorination, may result in mutagenic and/or carcinogenic by-products due to the reaction of chlorine with the organic matter in water (Rook, 1974). In fact, an association between the mutagenicity of drinking water treated with chlorine and urinary and gastrointestinal tract cancers has been observed with a low to moderate relative risk (often around 1.1–1.4). (Khallef et al., 2015; Schenck et al., 1998; Tao et al., 1999). These assessments are crucial where the by-products of chlorination may warrant monitoring.

Due to the sensitivity to aquatic environmental pollution, the *A. cepa* test has been used by Ennaceur (2018) to test the cytotoxic and genotoxic effects of drinking water collected from different sources (tap water, bottled water, and distribution network water) in Yanbu city, Saudi Arabia, during the four seasons to estimate possible seasonal influences on DNA damage. A significant statistical decline in the mitotic index (MIs) in root meristematic cells had been observed for tap water samples collected from two regions: Yanbu Al-Balad and Yanbu Al-Sinaiiya, indicating cell division retardation in the root-tip meristematic cells, which might be due to DNA synthesis inhibition or G2

phase blocking during the cell cycle, as explained by Sudhakar et al., 2001.

Similarly, many studies show that treated drinking water can still show mild cytogenetic signals while remaining within regulatory safety limits. Therefore, when the *A. cepa* test showed a decrease in mitotic index in some samples of tap water, it did not point out the chemical agents causing the observed effects. Since the test is a sensitive screening method that reacts to a broad spectrum of natural and man-made chemicals, the observed effects cannot be taken as an indication that the treated tap water is a direct health hazard. Further chemical analysis would be needed to determine the cause of the observed cytogenotoxic effects.

Chemical testing

Chemicals of various origins have been extensively evaluated with the *A.cepa* test system, resulting in higher reproducibility. Accordingly, Di (2-ethylhexyl) phthalate, triclosan, and propylparaben revealed that di (2-ethylhexyl) phthalate had no apparent effects; the other two chemicals inhibited *A. cepa* root growth in a dose-dependent manner (Herrero et al., 2012). On the other hand, although all three compounds caused alterations in the mitotic index of root-tip cells, propylparaben was the only one that had no evidence of genotoxicity for chromosomal aberrations and micronuclei formation. Finally, the study clearly indicated that sensitive plant bioassays are useful and complementary tools to determine the environmental impact of contaminants of emerging concern.

Personal care products including hair dyes, cleansings are among the emerging environmental contaminants with the widespread use of such products for cosmetic purposes, which may pose health risks to biota because the chemicals having cytogenotoxic potential, especially aromatic amines are ended up in the aquatic and terrestrial environments with drain waters from salons Maiti et al., (2016) had tested three most popular and commonly used permanent hair dyes (3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyl tetrazolium bromide (3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyl tetrazolium bromide (MTT)) while Nawalage & Pathiratne, (2020) evaluated herbal based hair dyes with the *A. cepa* bulbs, revealing root growth retardations and mitosis suppression. Condensed nuclei, micronuclei, were common among other chromosomal aberrations. Therefore, toxicity assessments, including in vivo test models, are warranted considering human and ecosystem health.

Synthetic food coloring agents, preservatives, have been tested in numerous instances with the *A. cepa* assay. Among these, Brilliant Blue (Hussein et al., 2022), Metanil yellow and Carmoisine (Khan et al., 2020), sunset yellow, bordeaux red, and tartrazine yellow (Gomes et al., 2013), Ponceau 4R food dye (Santana et al., 2015), lemon yellow and orange red (Sun et al., 2019), resulted in a substantial reduction in mitotic index and also produced different kinds of chromosomal aberrations mostly at longer exposure periods, concluding that food coloring agents possess genotoxic effects. Severe DNA damage, membrane damage, and metabolic inactivation observed are indicative of apoptotic cell death. Therefore, the results are useful for the establishment of appropriate tolerable limits for the use of these food additives.

Moura et al. (2016) had studied the toxicity of synthetic Cheese and Cheddar Cheese food flavours at doses of 1.0 and 2.0 mL, at 24 and 48 hours of exposure, revealing significantly reduced cell division. Moreover, genotoxic and cytotoxic effects of monosodium glutamate (MSG) have been studied, recording inhibited root growth of *A. cepa* in all concentrations, 1, 3, 5, and 7 g/L (Adeyemo & Farinmade, 2013). Further, it has reduced the number of roots growing from the primordium in all test concentrations as compared to controls, and the least was observed in the highest concentrations (5 g and 7 g/L). The colour of the root tips had ranged from brownish to dark brown or black in higher MSG concentrations. Sticky chromosomes at telophase were recorded as the most commonly induced aberration type among all the treatments. However, MSG had decreased the mitotic index of *A. cepa* cells at all the test concentrations, but not statistically significant. No significant difference had been observed in total chromosomal aberrations in all experimental setups as compared to the control.

Testing solid waste pollution effects

The disposal of municipal waste in landfills may lead to a huge environmental problem, because the decomposition of such residues generates large volumes of leachate, which may present high toxicity. Klauck et al. (2013) had assessed the toxic effects of untreated leachate collected from a landfill located in the region of Vale do Rio dos Sinos, Southern Brazil, using fish (*Leporinus obtusidens*) and onions (*A. cepa*). Results of the *A. cepa* bioassay for 48 hours had shown high toxicity with significant reduction of root growth and mitotic index in bulbs exposed to the 100% concentration of the leachate. An increase in the frequency of chromosome abnormalities in anaphase–telophase was observed with increasing leachate concentration (5%, 10%, 25% and 50%), with values significantly greater than the control, at the highest concentration. So, the study represented a major source of environmental pollution if not handled with care.

Testing of environmental radioactives

Oudalova et al. (2017) mention that bioassays are rarely applied in cases of chemicals with radionuclide combinations and hence they have used the *A. cepa* test to assess the environmental impact from a nuclear waste storage facility in European and Far-Eastern parts with the concentrations of some radionuclides beyond the permitted limits by testing waters from monitor wells and nearby water bodies.

Test sample	Macroscopic indicators	Microscopic indicators	Interpretation/type of toxicity	Reference/s
		Pesticide and herbicide toxicity		
Malathion	-	CA- Anaphase bridges with a higher incidence, Chromosome delays, Chromosome losses	DNA damage Cytotoxicity	Ghisi et al., 2023
Imidacloprid	-	MI, CA - Anaphases with chromosome breakages, a higher number of binucleated cells, metaphases with chromosome loss, anaphases with chromosome bridges, and anaphases with chromosome loss, significantly high MN	No significant reduction in MI Therefore, no cytotoxicity is detected Genotoxicity is revealed, because a significant dose-dependent increase in the frequency of CAs in *A. cepa* cells	Rodriguez et al., 2015
Pesticide-treated soil (PTS) and Vermicompost-treated soil (VTS)	Root length Presence of root twists (crochet, hooks)	MI, CA - stickiness, laggards, C-mitosis, vagrant chromosomes, fragments, chromosomal bridges	MI is significantly higher in VTS than in the control. A concentration-dependent inhibition of MI in PTS. The highest frequency of CA (54.5%) was found in 100% of PTS in 48 h exposure. Therefore, the PTS extract had significantly more cytotoxic and genotoxic effects on *A. cepa.*	Datta et al., 2018
Diclofop-methyl (herbicide) and Lindane (insecticide)	-	MI, MPI, Interphase nuclear volume, CA types and rates - Stickiness, C-mitosis, C anaphase, Chromosome fragments, Chromosome bridges, Multipolarity, Laggard chromosomes	MI was reduced substantially and dose-dependently with pesticide levels, though no significant correlation was observed. Significant changes in all mitotic phases caused by both pesticides indicate remarkable irregularities in the cell cycle. CA in all corresponding concentrations indicates genotoxicity	Mesi & Kopliku, 2013
Six pesticides: 2,4-D, HCB, Malathion, Carbaryl, DDT, Endosulphan	-	EROD (7-ethoxy resorufin O-deethylase) activity	Pesticide treatments had enhanced the enzyme activity with carbaryl and HCB, causing 63- and 53-fold induction, respectively, with respect to the control at a dose of 1.2 ppb. Industrial wastewater samples from Ghaziabad, Northern India, resulted in a 68-fold rise in the EROD activity, confirming a high pesticide pollution level	Fatima & Ahmad, 2006
Herbicides - Quizalofop-p-ethyl Cycloxydim		MI, CA and nuclear alterations - stickiness, fragments, C-mitosis, lobulated nucleus, MN, nuclear erosion	Mitodepressive effect and percentage CA increased with increasing herbicide concentrations Cytotoxic and genotoxic potential in both herbicides	Rosculete et al., 2018
Industrial pollution				

H_2O_2, CH_2O, water from the mining areas	Root length Root colour Morphological deformities	MI, CA - stickiness, bridges, laggards	H_2O_2 and CH_2O were potential transporters of CA- suggesting genotoxicity Water samples collected from the mining areas show statistically non-significant toxicity	Cabuga Jr et al., 2017
Industrial wastewater from Ikpoba and Iguosa rivers in Nigeria	Root length Morphology of roots - Twists and crotchet roots	MI, CA- Stickiness, Chromosomal bridges, Chromosomes with disturbed spindles, Chromosomal fragments	Strong growth retardation in high concentrations of all waste waters at 72 hours Concentration-dependent decrease in root growth Statistically significant level of CA at all tested concentrations ($p<0.05$), suggesting genotoxicity.	Olorunfemi et al., 2011
Soap, beverage, and paint industry effluents	-	MI, CA - laggards and sticky chromosomes	Statistically significant ($p<0.05$) decrease in MI with increasing effluent concentrations indicates cytotoxicity. All three effluents induce CA, confirming genotoxicity	Olusola & Solomon, 2018
Sediments and water samples in Dandugan Oya	Root length	MI, Nuclear abnormalities	Non-significant root growth variation in 48 hours of exposure to water samples. Due to the presence of significant root length variations for sediment elutriates for 48-hour exposure, general toxicity is present. Cytotoxicity and genotoxicity of sediment elutriates are present due to low MI and high CA.	Wijeyaratne & Wadasinghe, 2019
Water samples from a water body receiving treated textile effluents	-	MI, MPI, CA - C metaphases, Chromosomal adherence, Chromosomal bridges, Disturbed anaphase, Vagrant chromosomes, Chromosomal breaks	MI and MPI are affected by the treated textile effluents released to the water body Presence of potentially genotoxic and mutagenic substances in the water due to the presence of CA.	Wijeyaratne & Wickramasinghe, 2020
Water from the Citarum River with risk of heavy metal contamination	Root length	MI, Frequency and types of CA -Stickiness (most common type), Chromosome loss, Chromosome bridges, C-mitosis, Binucleated cells, Multipolar Micronuclei, Chromosome break	Root length was not affected; hence, general toxicity is not present Cytotoxicity and genotoxicity in the upstream sites of the Citarum River is confirmed.	Annisa et al., 2024

Tannery wastewater samples	Root length Root morphology -Root/Root tip color/ Root tip fatter/ blunt	MI, CA- Stickiness, Laggard chromosomes, Fragmentation, Chromosome bridge, Binucleated nuclei, Morphologically changed nuclei, MN	A decrease in mitotic index with increasing effluent concentrations. Effluents had induced CA. Tannery Effluents cause cytotoxicity and genotoxicity	Admas & Kerisew, 2022
Industrial effluents and domestic sewage	Root length Root morphology	CA- C-metaphase, Irregular anaphase, Binucleated cells, Telophase bridges, Adherent/ damaged nuclei, Metaphase with numerical alteration	Both effluent and sewage cause a decrease in MI with increasing concentrations. Significantly increased CA ($p<0.05$) in both effluent and sewage with the increasing concentrations. Cytotoxicity and genotoxicity is induced by both industrial effluents and sewage.	Paul & Arjun, 2018
River water in Tremembe and Aparecida, Brazil	Root length	MI Frequency of MN	Significant decrease in the MI values. Micronuclei frequency is not affected by the river water treatments. Therefore, genotoxicity is not assumed for the sampled river water, though cytotoxicity is present.	Barberio et al., 2009
Black dye is used in the textile industries of a biological wastewater treatment plant	-	CA - Stickiness, nuclear buds, and binucleated cells	Significant frequencies of chromosome and nuclear aberrations occur with bacterially biodegraded dye. Therefore, metabolites generated by degradation are more genotoxic than the chemical. Cytotoxicity is confirmed by significant frequencies of cell death in biodegraded samples	Ventura-Camargo et al., 2016
		Heavy metal toxicity		
ZnO NP solutions (5 µg/mL and 50 µg/mL)	Root length	MI, CA - Chromosome disorder, Chromosome break, Chromosome bridges, MN, Stickiness, Chromosome ring	Root length was significantly inhibited at both concentrations. Confirmed that ZnO-NPs are cytotoxic and genotoxic in plant cells	Sun et al., 2019
Heavy metal mixture from Bellanwila-Attidiya sanctuary	Root length, Shape of the roots, Root colour, Turgescence	Mitotic divisions, MI, Types and numbers of CA- adherences, vagrant and laggard chromosomes, C-metaphase, Chromosomal bridges, Cytotoxic effects - apoptosis or necrosis	General toxicity confirmed by a significant reduction of root growth in a dose-dependent manner. Metal concentrations above 5 ppm had direct cytotoxic effects, producing cellular apoptosis/necrosis. Metal mixture causes significant effects on general toxicity, cytotoxicity, and genotoxicity at >5 ppm concentrations	Jayawardena et al., 2021

Methyl Methanesulfonate (MMS), Cadmium, aluminium industry waste Spent Potliner (SPL), Atrazine	-	DNA breakage	No Cytotoxicity of Cd had been verified (MI values had not differed from the control)	Silveira et al., 2017
		Water testing		
Wastewater from the Sava River, Croatia	Root lengths	MI, CA - C-mitosis, laggards, chromosome breaks, anaphase bridges, Stickiness	General toxicity confirmed by inhibition of root growth of over 50%. A decrease in MI over 40% confirms the cytotoxicity. A considerable increase in CA leads to genotoxicity. Strongest cytotoxic and genotoxic effects occurred in wastewaters collected near the azithromycin production industry, baker's yeast fermentation industries, and an artificial fertilizer plant	Radic et al., 2010
Tap water, bottled water, and distribution network water in Yanbu city, Saudi Arabia	-	MI, CA – Stickiness, Vagrant chromosomes, Anaphase bridges	Bottled water - exhibited acceptable values of MI. Decreases in MI in local water distribution network samples did not have any statistical significance ($p>0.05$). The highest inductions of CA were in tap water and only one of the distribution network water samples. Relatively low values of CA in bottled drinking water. Genotoxicity caused by tap water is confirmed	Ennaceur, 2018
		Chemical testing		
Di (2-ethylhexyl) phthalate, triclosan, and propylparaben	Root length	MI, CA - MN	Di (2- ethylhexyl) phthalate had no apparent effects; the other two chemicals inhibited *A. cepa* root growth in a dose-dependent manner, indicating general toxicity All three compounds caused alterations in the mitotic index, revealing cytotoxicity Propylparaben was the only one that had no evidence of genotoxicity for chromosomal aberrations and micronuclei formation	Herrero et al., 2012

Permanent hair dyes (3-(4,5-dimethyl thiazolyl-2)-2, 5-diphenyl tetrazolium bromide (3-(4,5-dimethyl thiazolyl-2)-2, 5-diphenyl tetrazolium bromide (MTT))	-	Mitotic index Chromosomal abnormalities - Nuclear bud, Disrupted Anaphase, Anaphase Bridge, Sticky Metaphase, Chromosomal fragmentation	Low mitotic index values confirmed cell division retardation, and hence, hair dyes are cytotoxic	Maiti et al., 2016
Three Herbal-based hair dyes	Root lengths	MI, Chromosomal aberrations - Condensed nuclei (Most frequent nuclear abnormality), binuclei, Micronuclei, Chromosomal anaphase with a laggard chromosome, Late anaphase with polar slipped chromosomes, Anaphase with chromosomal bridges, Anaphase with a chromosomal fragment	Statistically significant root growth retardation effects ($p<0.05$) in all dyes leading to genotoxicity Induction of micronuclei and chromosomal aberrations even at the post exposure stages Persistent genotoxicity of the hair dyes due to the effects of chemical mixtures present in the commercial hair dye formulations	Nawalage & Pathiratne, 2020
Brilliant Blue (A synthetic food colorant)	Root Length	Mitotic Index, Chromosomal aberrations - Laggard chromosomes, C-mitosis, Multipolar anaphase, Sticky metaphase, Star-shaped anaphase, Sticky anaphase, Chromosome loss	Brilliant blue has a significant ability to inhibit root growth, hence showing general toxicity. As it decreases the mitotic indices of root meristematic cells in *Allium cepa* and causes various mitotic abnormalities, it is cytotoxic and genotoxic	Hussein et al., 2022
Metanil yellow and Carmoisin (Azo dyes)	-	Mitotic indices, Chromosomal Aberrations - Disorientation at metaphase, Metaphase and anaphase stickiness, Anaphase bridge, C-mitosis, Chromosome breaks	Both dyes cause a significant reduction in mitotic index and produce different kinds of chromosomal aberrations, mostly at higher concentrations and longer exposure periods Therefore, both dyes have cytotoxicity and genotoxicity	Khan et al., 2020
Sunset yellow, Bordeaux red, Tartrazine yellow (Food dyes)	-	Mitotic index	All three dyes had recorded a statistically significant number of cellular aberrations as compared to their controls, indicating they are cytotoxic.	Gomes et al., 2013

Ponceau 4R (food dye)	-	Mitotic Index Cellular aberrations - Micronuclei, Colchicine metaphases, Anaphase bridges, Gene amplifications, Cell adhesions, Nuclear buds, Multipolar anaphases	Concentrations of 0.25 and 0.50 g L^{-1} at the 48-hour exposure, and the concentration of 0.75 g L^{-1} at 24 h and 48 h exposure times had significantly reduced ($p < 0.05$) the cell division rates, and since all the selected concentrations at both exposure times had caused cellular aberrations in significant numbers, the selected dye is cytogenotoxic.	Santana et al., 2015
Lemon yellow, Orange red (Synthetic food colorants)	Root lengths	Mitotic index, Clastogenic aberrations - chromatin extrusion, nuclear budding, nuclear lesion, nuclear erosion, nuclear peaks, chromatin fragmentation, giant nucleus, chromosome bridges, chromosome coagulation, ring chromosome, stickiness, and C-anaphase. Non clastogenic aberrations - binucleate cells, tetranucleate cells, micronucleus, ball metaphase, ball anaphase, hypoploid cells, polyploid cells, cytostasis, pole-to-pole arrangement of chromosomes, vagrant chromosomes, laggard formation, displaced chromosomes, early cell plate formation, equatorial separation of chromosomes	Root growth inhibition indicates the general toxicity of both dyes. They had induced significant DNA damage, micronucleus formation, and other severe chromosomal aberrations at genetic level and inhibited cell division. Severe DNA damage, membrane damage, and metabolic inactivation observed are indicative of apoptotic cell death. Therefore, both dyes are cytotoxic and genotoxic.	Vazhangat & Thoppil, 2016
Synthetic cheese and cheddar cheese flavorings	-	Number of cells in interphase and under division, Mitotic index, Number of micronucleate cells in colchicine metaphases, Anaphase telophase bridges, Gene amplifications, Cells with adhesions, Nuclear buds, Multipolar anaphases	Chi-square test ($p<0.05$) employed for calculating mitotic indices and cellular aberrations revealed that flavourings under study significantly reduced the cell division rate both individually and in combination, indicating the possible cytotoxicity	Moura et al., 2016

Monosodium glutamate (MSG - A food flavor enhancer)	Root length Number of roots growing from primodium Colour of root tips	Chromosomal aberrations- Sticky chromosomes at telophase (Most commonly induced aberration type)	Reduced the number of roots in all concentrations compared to the control. Inhibition of root growth at all test concentrations and changes in color (ranging from brownish to dark brown/ black in higher concentrations) of root tips indicate general toxicity. No significant difference had occurred in total chromosomal aberrations in all concentrations compared to the control MSG had decreased the mitotic index at all the tested concentrations, but not statistically significant Though not statistically significant, it concludes the potential cytogenotoxic activity of MSG	Adeyemo & Farinmade, 2013
		Pollution testing		
Untreated leachate collected from a landfill in Southern Brazil	Root length	Frequency of CA – CBD, chromosome breakage, LG Frequency of MN	High toxicity with significant reduction of root growth and MI in bulbs exposed to 100% concentration of leachate. An increase in the frequency of CA in anaphase–telophase with increasing concentration of leachate (5%, 10%, 25% and 50%), with values significantly greater than the control, at the highest concentration Untreated leachate generated by landfills causes general toxicity, cytotoxicity, and genotoxicity.	Klauck et al., 2013
Water samples in monitor wells and water bodies closer to nuclear waste storage	-	MI, Frequency of CA - LG, CBD and tripolar mitoses	Significantly decreased MI in the samples collected from the radionuclide contaminated areas below the controls - cytotoxic effect in water samples. Tested waters have an enhanced mutagenic potential	Oudalova et al., 2017

CA - Chromosomal Aberrations, CBD- Chromosomal Bridges, LG- Laggard Chromosomes, MI - Mitotic Index, MPI- Mitotic Phase Index, PTS - Pesticide-treated soil, VTS - Vermicompost-treated soil, MN -Micronuclei

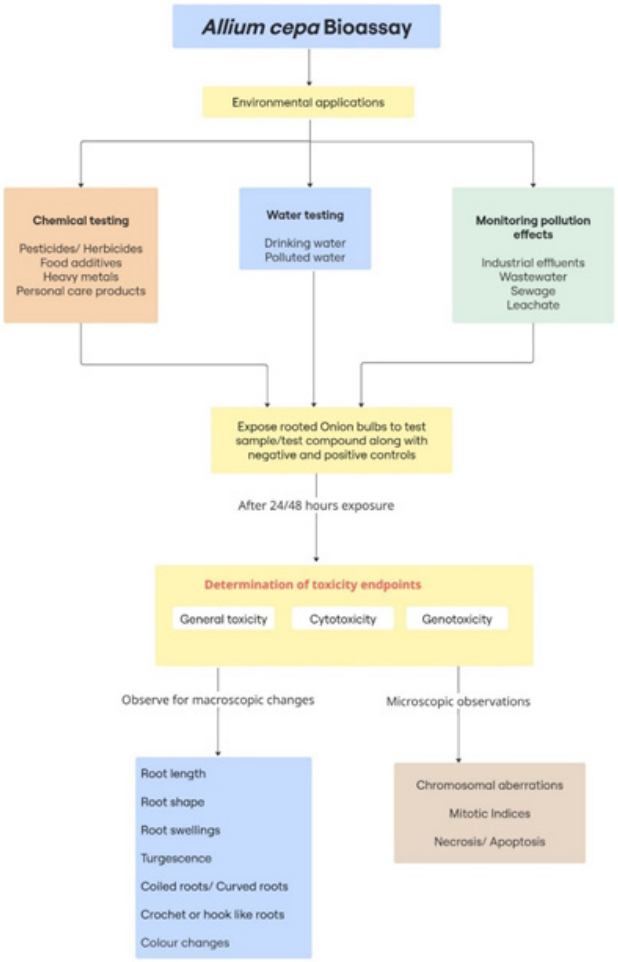


Figure 2. Common endpoints of the *Allium cepa* assay used in environmental toxicity assessments include macroscopic indicators and microscopic indicators for evaluating cytotoxicity, genotoxicity, and general toxicity.

MI values had decreased below the controls in the samples collected from the radionuclide contaminated areas, which have statistically significant values (absolute value only to 10-13% of the control level), indicating a cytotoxic effect. Mutagenicity estimated via the frequency of aberrant cells in the root meristematic cells in the same samples had also shown the inhibitory effect on the proliferative activity. Moreover, there was a close correlation between MI and aberrant cell occurrences, and finally, they drew a conclusion that the waters have an enhanced mutagenic potential. Collectively, A. cepa bioassay provides a comprehensive tool for environmental monitoring, detecting a wide range of constituents through multiple, validated endpoints (Figure 2).

CONCLUSION

This review highlights the broad applicability of the *Allium cepa* bioassay in evaluating cytotoxicity and genotoxicity induced by a wide range of environmental contaminants and chemical compounds. The assay has been extensively utilized in water quality assessment, industrial pollution monitoring, pesticide and herbicide evaluation, heavy metal toxicity studies, and the testing of various chemical agents. Chromosomal and mitotic abnormalities observed in the root meristematic cells of *A. cepa* serve as reliable indicators of genotoxic effects, while alterations in the mitotic index reflect cytotoxicity.

Due to its sensitivity, simplicity, cost-effectiveness, and reliability, the *A. cepa* assay is recognized as an effective bioindicator for environmental toxicity assessment. Although it primarily serves as a preliminary screening method, the assay provides valuable insights into the potential hazardous effects of pollutants and chemicals. Furthermore, its application offers an important alternative approach for scientific investigations by reducing the dependence on animal-based toxicity testing.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflict of interest regarding the publication of this paper.

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DATA AVAILABILITY STATEMENT

Data sharing does not apply to this article, as no datasets were generated or analyzed during the current study.

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