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RESEARCH ARTICLE

Bivalent nickel exposure induced mitotic toxicity in onion root tips

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Abstract

Nickel (Ni) is utilized across multiple industries, resulting in significant environmental contamination and exhibiting various genotoxic effects on plants and animals due to its release into the external environment. This study evaluates the cytotoxic effects of different doses of nickel on onion (*Allium cepa* L.) root tips. To evaluate the sensitivity of Nickel, bulbs of onion were treated at 10, 25, 50 and 100 ppm along with a control (0 ppm) using $NiCl_2$ salt as the source of Nickel. At elevated concentrations of nickel (100 ppm) in a hydroponic study, the onion roots exhibited browning and translucency, accompanied by a retardation of growth. Various chromosomal anomalies, including chromosomal breaks, laggards, vagrant chromosomes, bridges, distorted chromosomes, sticky chromosomes, c-mitosis etc. were observed following a 24 hr exposure to toxic doses of nickel. Both types of chromosomal abnormalities, namely spindle fibre abnormality (SFA) and chromosomal abnormality (CA), were observed under Ni treatment. The evaluation of cell death in the treated roots was done using the Evans blue dye test. Uptake of Evans blue by the root cells demonstrated the cell death parameter, which served as an indicator of cytotoxicity.

Keywords: *Allium cepa* L.; cell death; chromosomal abnormality; cytotoxicity; nickel

Introduction

Nickel (Ni) is regarded as a vital trace element for plants (1). It is exceedingly prevalent in the Earth's crust, ranking 22nd in abundance (2). The origins of nickel pollution in the environment mostly stem from its refining, processing, mining, other industrial applications such as electroplating, the manufacture of batteries, the jewellery industry and agricultural applications like fertiliser use, as previously documented (3). Nickel has both natural and anthropogenic sources of emission (3-5). The majority of the nickel that ends up in the environment comes mainly from electric battery components, catalysts used in chemical and food processing and raw materials used in the electroplating and metallurgical sectors (5). Toxic quantities of nickel adversely affect various physiological processes in plants, including photosynthesis, enzyme activity and mineral nutrition (6). The permitted limit of nickel in agricultural soil is estimated to be 100 mg/kg, while in groundwater it is 20 µg/L, levels over this threshold are deemed fatal to living species (7, 8).

Allium cepa L. or onion, is a crucial agricultural crop owing to its extensive culinary applications and economic significance. Nonetheless, its development and production may be negatively impacted by environmental stresses, including nickel poisoning. Worldwide, nickel toxicity from polluted soil and water impairs growth and development, resulting in diminished harvests. Investigating mitotic toxicity in *A. cepa* is justified, since it represents a dependable model for evaluating cytotoxic effects and nuclear anomalies due to its sensitivity to contaminants, offering insights into the overarching consequences for plant vitality and ecosystem equilibrium (9, 10).

Cytological aberrations are an effective monitoring system for detecting environmental pollutants that may pose genetic hazards to plants (11). The International Program on Plant Bioassay (IPPB) has documented the extensive application of *A. cepa* as a test organism for assessing environmental stress effects on chromosomes (12). The *A. cepa* test serves as a straightforward, cost-effective, reproducible and efficient model (Fig. 1) for assessing and monitoring the cytotoxicity and genotoxicity of toxic concentrations of Nickel (9). The features, including a dynamic root system sensitive to toxic metals, distinct mitotic phases with a stable chromosome number, morphological alterations in chromosomes, a stable karyotype and a rare incidence of spontaneous chromosomal damage, have led to its utilization in various experiments focused on chromosomal changes (13). The *A. cepa* assay is well established for determining, identifying and categorising genotoxic substances in various environments. This study focuses on the heavy metal nickel-induced aberrations, utilising *A. cepa* as a screening method for evaluating the genotoxicity associated with various metal toxicities. Considering the information presented, it is essential to assess the degree of chromosomal damage caused by nickel using *A. cepa* root tips after

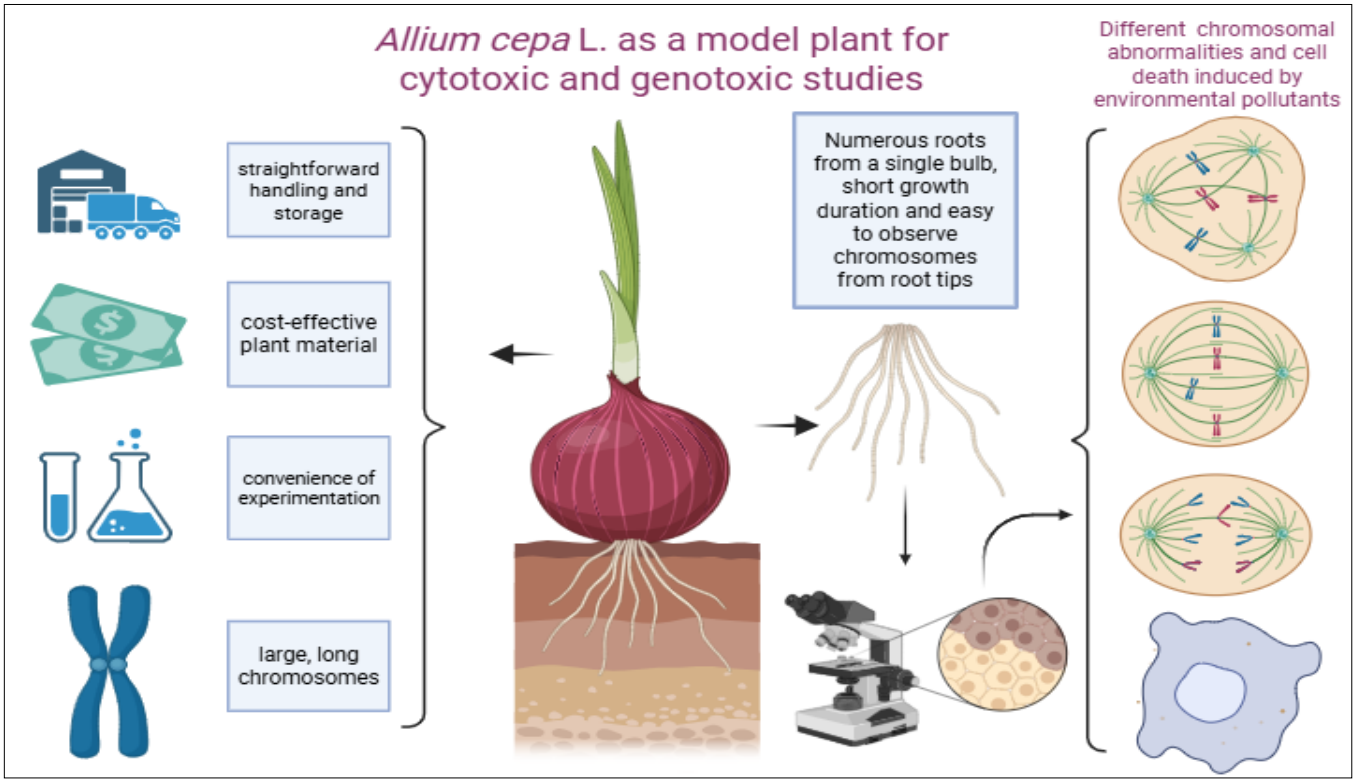

Fig. 1. Schematic representation of onion as a model test plant for cytotoxic and genotoxic studies.

exposure to varying concentrations (10, 25, 50 and 100 ppm) of Ni^{2+}, administered as nickel chloride.

Materials and Methods

Observation of chromosomal anomaly

Onion bulbs (*A. cepa* L.) were cultivated using established protocols (14). Bulbs with roots measuring about 2 cm in length were rinsed under running tap water and subsequently treated with $NiCl_2$ at concentrations of 10, 25, 50 and 100 ppm, in addition to a control group (0 ppm), for a duration of 24 hr at room temperature (Fig. 2). The outer scales of the onion bulbs and brownish bottom plate were removed, leaving the ring of root primordial intact. Root tips were collected, washed 2-3 times and fixed in a solution of acetic acid and ethanol (1:3) overnight at room temperature. Root tips were stained in a solution of 2 % acetocein and 1 M HCl in a ratio of 9:1 for a duration of 3-4 hr at room temperature. A stained root tip was compressed in a drop of 45 % acetic acid on a glass slide and examined under a microscope (Model- Magnus MLX). Digital images were captured for subsequent analysis of various chromosomal anomalies and stages of cell division using a 40x objective (15). Around 200 cells from root tips were scored at random from each slide and the data were pooled from five slides, which account for 1000 cells for each treatment. The mean data were taken from each treatment and each experiment was replicated thrice. There are three replicates for each treatment. The standard error of the mean was calculated for MI values of three replicates. The mitotic index (MI), percentage CA and phase index percentage were calculated using the following formula as follows (15-17).

MI = (Total number of dividing cells/Total number of cells scored) × 100
(Eqn. 1)

Percentage chromosomal abnormality (% CA) = (Number of cells with aberrations/ Total number of dividing cells) × 100 (Eqn. 2)

Phase index percentage = (Number of cells in particular phase/ Total number of dividing cells) × 100 (Eqn. 3)

Study of cell death in *A. cepa* roots

The Evans blue staining method was used to study the cell death assay (18). Control and treated roots were stained with 0.25 % Evans blue for 10-15 min. The stained tips were cleaned with distilled water for 30 min. Evans blue uptake by the root cells showed the cell death parameter, which served as an indicator of cytotoxicity. Photographs of the stained root tips were taken. For measurement of the cell death and dye uptake, five stained root tips from each treatment were transferred to 1 mL of N,N-dimethylformamide for 60 min at 37 °C. The Optical Density (OD) of the dissolved Evans blue solution was measured at 600 nm in a UV-visible spectrophotometer (19).

Results

The morphological analysis indicates that the root length and structure of *A. cepa* L. were significantly influenced by toxic concentrations of nickel. The toxicity of the tested samples to *A. cepa* roots was evaluated through root length measurements taken 24 hr post-exposure to the test medium. Root growth in the control group was considered to be at its maximum level (100 %). Lengths of five roots from each treatment were considered for calculating the mean root length for each treatment. The experiment was replicated three times for each treatment. Root growth was significantly inhibited at a concentration of 100 ppm nickel during the 24 hr treatment. The root length was reduced by over 50 % at the 50 ppm concentration (Fig. 2 & 3). Roots exhibit browning and translucency as nickel concentration increases.

MI values got reduced with increased Ni stress. Ni stress of

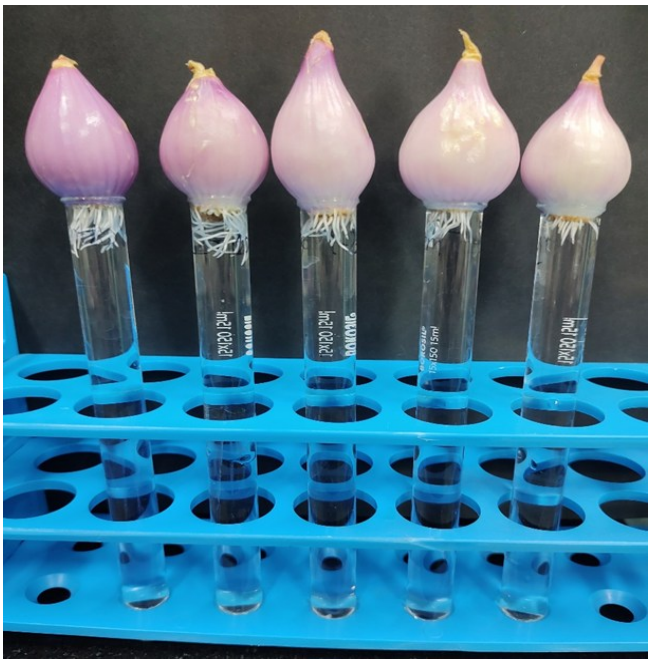

Fig. 2. Experimental set-up of *A. cepa* bulbs in different concentrations of Nickel. From left to right - 0 ppm (Control), 10 ppm, 25 ppm, 50 ppm, 100 ppm.

100 ppm showed a 75 % reduction in mitotic index value after a 24 hr exposure period. Nickel at 100 ppm shows a lethal dose as it reduces the MI value up to 75 % of the control, whereas 25 ppm Ni treatment is considered a sublethal dose and is the cytotoxic limit value as reported from the present study. About 60 % cells lose their ability to divide when exposed to Ni (50 ppm), whereas only one-fourth of cells become actively dividing after exposure to Ni at 100 ppm (Table 1). Variation in mitotic index is highly significant in 25, 50 and 100 ppm Ni treatment (Table 2). The increase in nickel concentration of the phase index for different phases of mitosis also varied significantly. The number of cells in prophase increased with the increase in nickel concentration, whereas the number of cells in metaphase, anaphase and telophase decreased accordingly (Table 3).

Table 1. Mitotic index of root tip cells of *A. cepa* subjected to various concentrations of Nickel

Ni treatment (ppm)	Mitotic index (Mean± S.E.M)	% reduction in mitotic index
Control (0)	26.35 ± 0.81	0.00
10	20.25 ± 0.65	23.15
25	16.89 ± 0.67	35.90
50	10.51 ± 0.56	60.11
100	06.45 ± 0.88	75.52

Several chromosomal aberrations in mitotically proliferating *A. cepa* root cells were observed (Table 4). Exposure to toxic concentrations of nickel resulted in several deformities in the chromosome structure of *A. cepa* root tips. Chromosomal structural and morphological abnormalities were observed across four distinct stages of mitotic division in root tip cells (Fig. 4a-p). The treatment with varying concentrations of Ni revealed a range of chromosomal abnormalities, including c-mitosis, laggard chromosomes, chromosomal breaks, chromosomal bridges and multipolarity of chromosomes, disturbed metaphase, vagrants, nuclear bridge, clumped or sticky chromosomes, nuclear lesions, vagrant in the

Table 2. Study of the significance level with respect to control

Ni (ppm)	Mean ± SE	t-value	p-value	Significance
Control	26.35 ± 0.81	—	—	—
10	20.25 ± 0.65	5.85	< 0.01	**
25	16.89 ± 0.67	9.03	< 0.001	***
50	10.51 ± 0.56	16.11	< 0.001	***
100	6.45 ± 0.88	16.65	< 0.001	***

NB: Significance codes: ns = not significant (p > 0.05); ** = p ≤ 0.01; *** = p ≤ 0.001

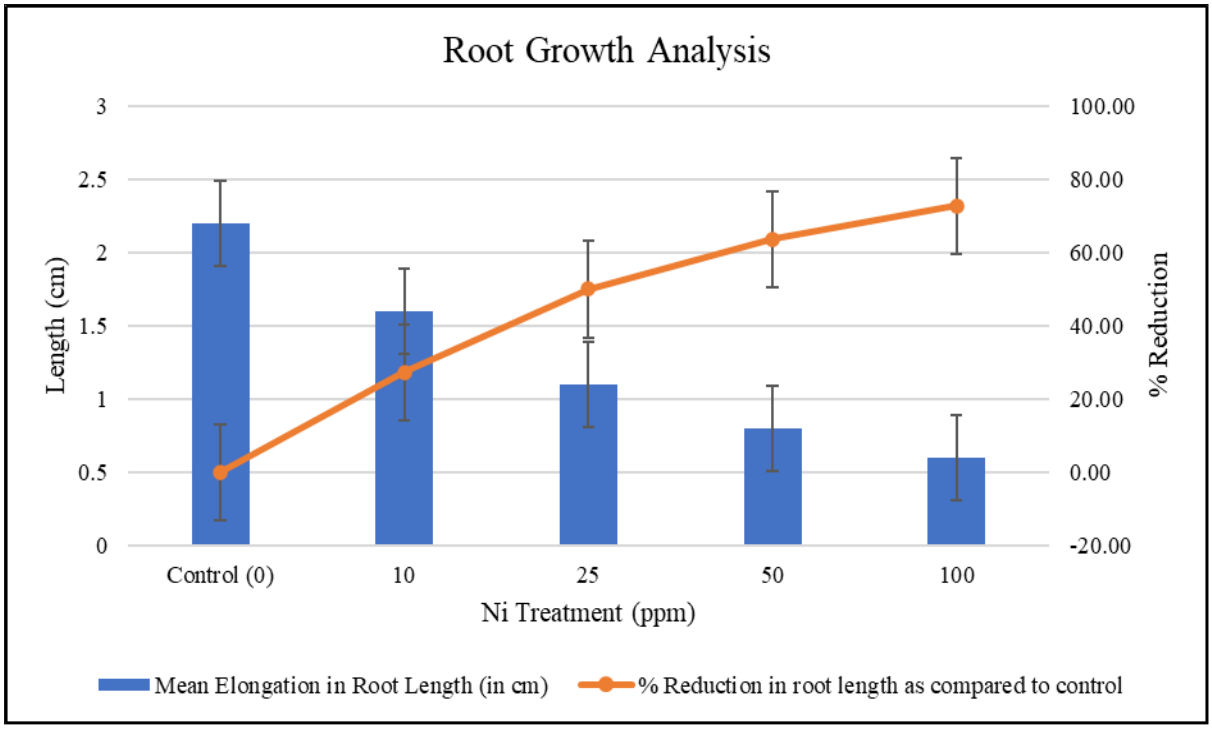

Fig. 3. Root growth analysis of *A. cepa* in response to treatment with various concentrations of Nickel.

Table 3. Phase index of root tip cells of *A. cepa* subjected to various concentrations of Nickel

Ni Treatment (ppm)	Phase Index %			
	Prophase	Metaphase	Anaphase	Telophase
Control (0)	34.47	23.48	20.45	21.59
10	35.47	18.23	21.18	25.12
25	46.15	19.53	15.98	18.34
50	52.38	18.10	11.43	18.10
100	61.54	10.77	10.77	16.92

Table 4. Percentage of different types of abnormal cells and total percentage of abnormal cells in *Allium* root tips under various concentrations of nickel treatment

Ni Treatment (ppm)	Percentage of different types of Chromosomal Abnormality (CA)											Total CA %
	Lg	Bk	Bg	Vg	Sc	Cl	MN	NL	MP	DP	c-mitosis	
Control (0)	0	0	0	0	0	0	0	0	0	0	0	0
10	2.96	4.93	5.42	4.43	4.43	3.94	0.99	6.40	0.49	0.49	0.49	34.98
25	4.73	5.33	4.73	4.14	7.10	5.92	1.18	12.43	2.96	1.18	2.96	52.66
50	6.67	6.67	4.76	5.71	9.52	8.57	1.90	30.48	3.81	2.86	8.57	89.52
100	1.54	1.54	1.54	0	7.69	10.77	1.54	63.08	0	1.54	4.62	93.85

Lg- Laggard; Bk- Break; Bg- Bridge; Vg- Vagrant; Cl- Clumped; MN- Micronucleus; NL- Nuclear lesions; MP- Multipolarity; DP- Diagonal Mitotic phases; C- mitosis

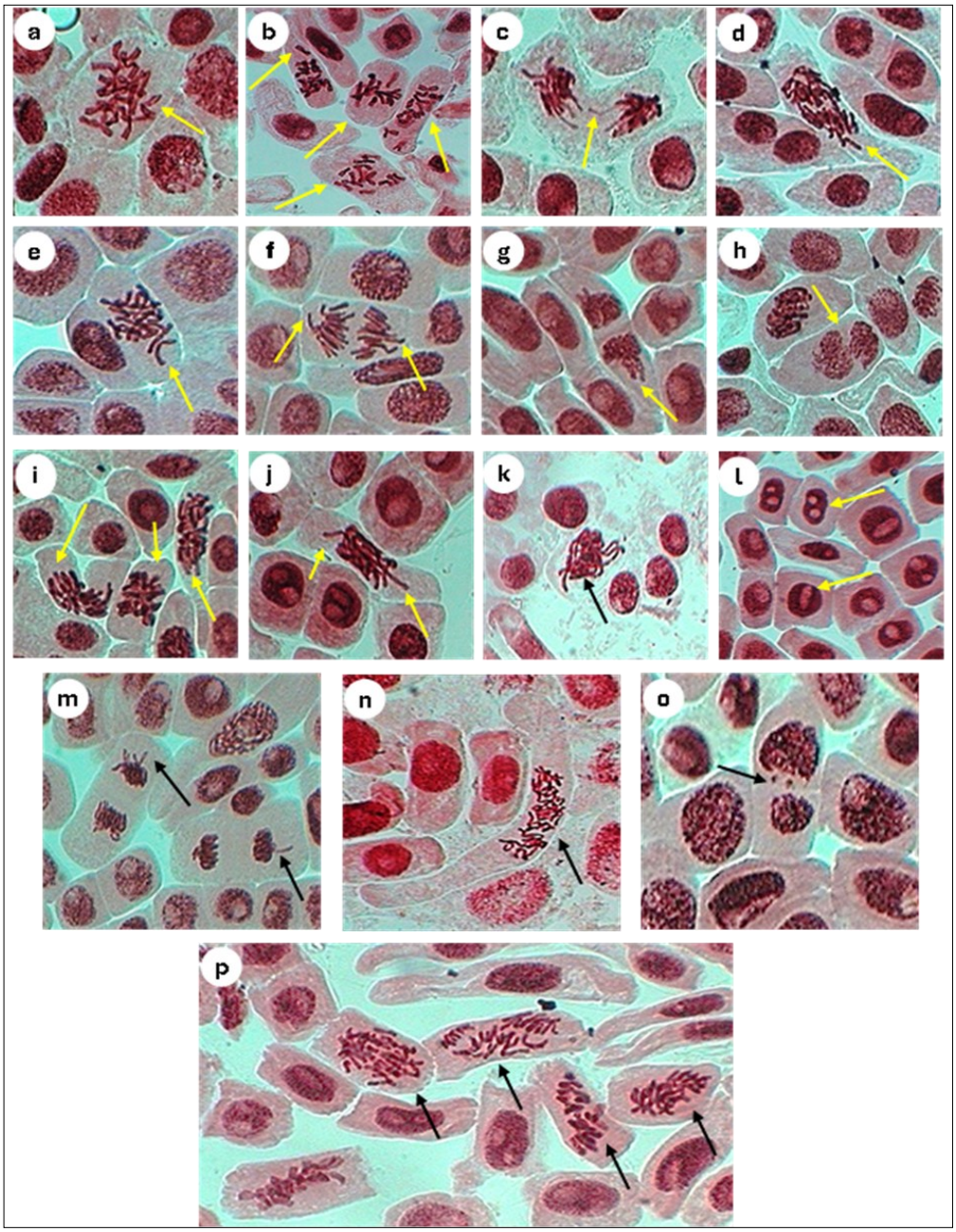

Fig. 4. Cells showing different types of chromosomal abnormalities in response to toxic concentrations of nickel (indicated by arrows); **a-b.** c-mitosis, **c.** laggard chromosomes, **d.** multipolarity with break, **e.** disturbed metaphase, **f.** chromosomal break and vagrant, **g.** telophasic vagrant, **h.** nuclear bridge, **i.** sticky chromosomes, **j.** sticky with vagrant chromosomes, **k.** clumped chromosome, **l.** nuclear lesions, **m.** vagrant, **n.** giant cell showing polyploidy, **o.** micronuclei formation from laggard chromosomes, **p.** plate showing stickiness, multipolarity, clumping, break and c-mitosis.

telophase phase, giant cells and micronucleus formation (Fig. 4a-o). Common clastogenic aberrations observed in root tips subjected to nickel treatments exceeding 50 ppm include nuclear bridges and chromosomal breaks. Nuclear lesions were observed to be maximal in nearly all cells treated with 100 ppm Ni. Analysis of Ni treatment revealed changes in nuclear morphology, characterised by the presence of irregular nuclei, chromosomal breaks, bridges and laggards. The cell death assay with Evans Blue dye demonstrated an elevation in cell death activity following treatment with elevated amounts of nickel (Fig. 5). The exposure of roots to a higher concentration of 100 ppm of Nickel resulted in an increased number of blue patches as well as increased colour

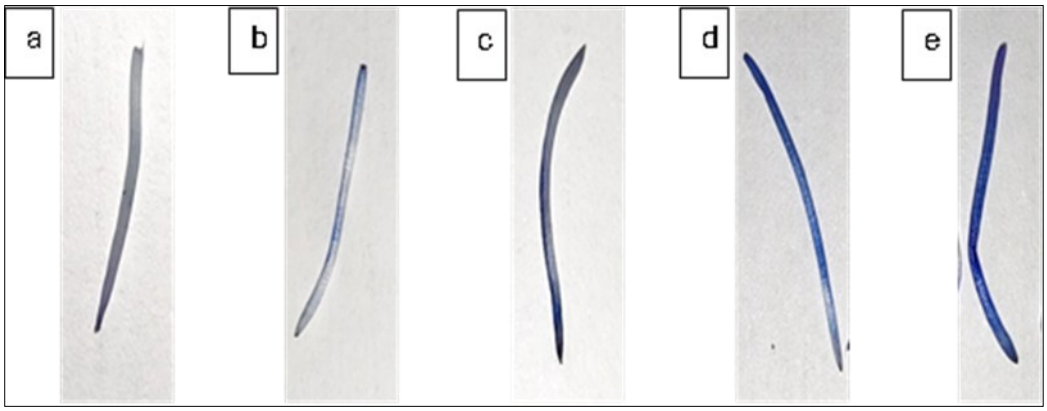

Fig. 5. Uptake of Evans blue dye by dead cells of *A. cepa* root tip cells in different concentrations a-e. Control (0 ppm), 10 ppm, 25 ppm, 50 ppm, 100 ppm respectively.

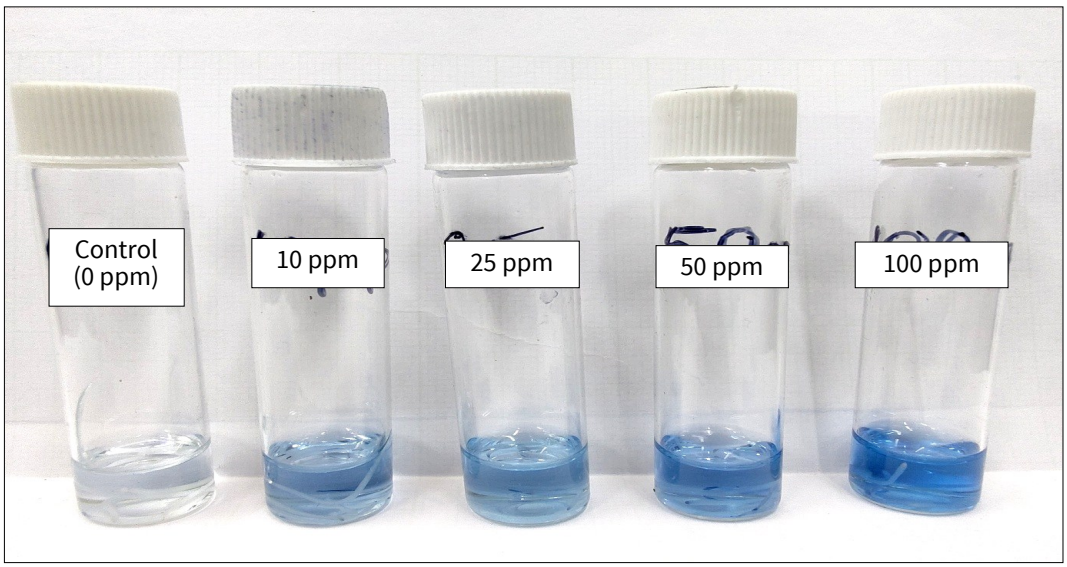

Fig. 6. Release of Evans blue dye by the *A. cepa* root tips in N, N -dimethyl formamide for further spectrophotometric analysis.

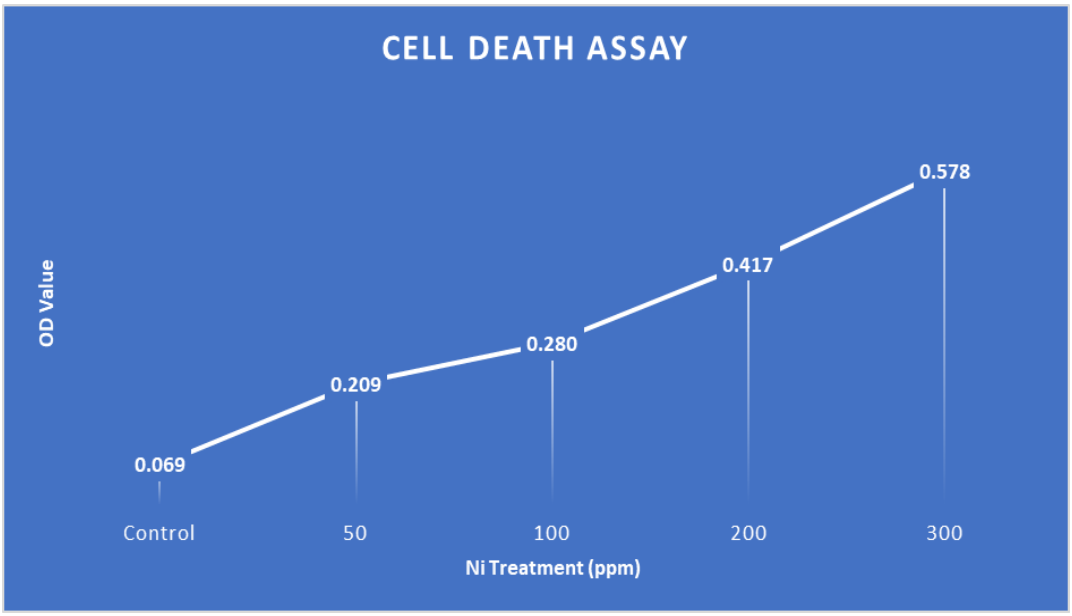

Fig. 7. Graph showing release of Evans blue dye into N,N-dimethylformamide solution indicating cell death induced by nickel in root tissues of *A. cepa*.

intensity after being treated with N-N dimethyl formamide, indicating metal toxicity (Fig. 5 & 6) (20). The OD values taken at 600 nm for the estimation of uptake of the Evans blue dye by the dead cells of the onion root tips clearly indicated the metal toxicity (Fig. 7). With an increase in concentration of nickel, the OD value increased, indicating a rise in cell death.

Discussion

Nickel is known to penetrate cells rapidly, causing osmotic lysis of cells and resulting in cell death (21). The decrease in mean root length elongation with an increase in nickel concentration indicates an index of general toxicity. Reduced mitotic index reveals the inhibition in mitotic activities. Mitotic index was found to be dose-dependent. The mitotic index serves as a marker for assessing nickel-induced cytotoxicity in *A. cepa* and is thus utilized as a parameter for biomonitoring heavy metal stress in the environment. The notable decrease in the quantity of dividing cells with elevated Ni concentration can be ascribed to the stress effects of Ni, which inhibit further DNA synthesis and the growth phase (G_2) during the cell cycle (22).

The percentage of chromosomal abnormalities increased drastically with the increase in nickel concentration. Chromosomal bridges arise from enhanced adhesion and delayed chromosomal separation, leading to nondisjunction of these sticky chromosomes during anaphase (23). The elevated occurrence of chromosome bridges and fragments arising from breaks in cells subjected to high metal stress may result from root toxicity induced by elevated nickel concentrations (23). Chromosome fragments are another characteristic of anomalies, observed randomly in various cells. Chromosome bridges were observed during metaphase and anaphase, indicating the connection between one or more chromosomes under varying doses of Ni treatment; certain chromosomal bridges exhibited chromosomal stickiness. Laggard chromosomes suggest the potential of Ni as a spindle inhibitor (24).

Nickel-induced genotoxicity is evidenced by spindle fibre abnormalities (SFA) and chromosomal abnormalities (CA) (25). SFA arises from chromosome stickiness, chromosomal clumping and multipolarity of chromosomes, while CA encompasses chromosomal breaks, chromosomal bridges, laggard chromosomes and distorted chromosomes. Several researchers indicate that stickiness results from the physical adhesion of the proteinaceous matrix of chromatin material, which leads to clustering (24). Stickiness occurs during chromosome condensation due to intrachromatidic links, causing double-strand breaks (26). Chromosomes were observed in a highly condensed state, in fragmented forms and were randomly scattered within the cell (22). Some cells exhibit chromosomal bridges, while others demonstrate chromosomal stickiness. The stickiness of chromosomes suggests the toxic nature of nickel, which likely results in cell death, a process that is typically irreversible, as documented in numerous studies on heavy metal-induced cytotoxicity (22). Chromosomal stickiness represents an irreversible alteration that results in apoptosis and cell death, potentially attributed to a reduction in the mitotic index, as documented by various researchers (27).

Various treatment concentrations of Ni resulted in chromosomal abberrations consistent with those induced by other toxic substances, as documented in previous studies (28-30). Instances of cells exhibiting misaligned metaphase and laggard chromosomes have been documented (31). Lagging chromosomes frequently result in the formation of micronuclei, which may induce cellular death through the deletion of essential genes (32-33). The dose and exposure period are critical factors in inducing cytotoxicity in the roots of *A. cepa*. Chromosomal bridges and breaks represent the most prevalent clastogenic aberrations identified under nickel stress, consistent with findings from other studies conducted by researchers (34). Chromosomal bridges that occur during the anaphase and telophase stages and can result in chromosomal breaks (Fig. 4d).

An increase in chromosome condensation, depolymerisation of DNA, or leaching of nucleoproteins might account for the wide variety of abnormalities seen in this study as a result of varying Ni concentrations (35). Various morphological changes in the nucleus induced by toxic concentrations of nickel, particularly at 100 ppm, have been documented in studies involving other metals such as cadmium, lead and mercury as well (14, 29, 36). Research indicates that acentric chromosome fragments contribute to the formation of micronuclei during the interphase and prophase stages of the cell cycle (37, 38, 39). The current study regards Ni stress-induced micronuclei formation as either clastogenic or aneugenic (40).

Evans blue staining is a valuable tool in cytogenetic studies for assessing the effects of toxic agents on plant cells. This method leverages the properties of Evans blue dye to visualise cell death, providing insights into the cytotoxic effects of various toxicants. Evans blue is a non-vital stain that selectively enters cells with compromised membranes, such as dead or dying cells (41). Live cells exclude the dye, while dead cells take up the stain, allowing researchers to distinguish between viable and non-viable cells. This property makes Evans blue an effective marker for cell death in toxicological studies. Healthy cells maintain membrane integrity and do not absorb Evans blue, while damaged cells do, indicating cell death (41). Research indicates that increased Evans blue uptake correlates with higher concentrations of heavy metals, leading to significant cell death in plant roots (20, 42). Heavy metals induce oxidative stress, which is linked to increased Evans blue absorption, further confirming cell damage (20, 43).

Conclusion

The research investigated the effects of varying concentrations on the induction of chromosomal abnormalities. The percentage of chromosomal abnormalities rises with higher concentrations of nickel treatment. Treatment with nickel at a concentration of 100 ppm resulted in the presence of the maximum occurrence of nuclear lesions. This indicates the toxic potential of nickel to induce chromosomal damage and diminish the capacity of cells to proliferate actively. The induction of chromosomal abnormalities in the root apical meristem of onions treated with various doses of nickel reveals the mutagenic and genotoxic properties of nickel. Chromosomal aberrations may serve as biomarkers for assessing the degree of nickel toxicity, thereby facilitating the screening of environmental toxicity attributed to nickel. Among various chromosomal abnormalities, sticky chromosomes, bridges, clumping and breaks are the most prevalent. These abnormalities indicate the disruption caused by Ni in the normal growth of plants, resulting in changes to the genetic makeup of the offspring from exposed plants. This, in turn, indirectly impacts humans who consume these plants as food,

leading to further complications. Evans blue staining is a useful technique for visualising the effects of toxic agents on *A. cepa* root tips by identifying dead or dying cells. The application of Evans blue aligns with the broader goal of assessing cytotoxicity and genotoxicity in plant cells. This approach enhances the ability to quantify and visualise the impact of toxicants, contributing valuable insights to cytogenetic studies. This method of identifying nickel as a genotoxic and mutagenic pollutant through the *A. cepa* assay can be effectively utilised for monitoring environmental toxicity.

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Authors' contributions

MM conceptualised, designed the study, supervised and corrected the draft article. SM carried out the work, performed the statistical analysis and prepared the draft article. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflicts of interest to declare.

Ethical issues: None

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