IJASRM
International Journal of Advanced Scientific Research and Management, Volume 4 Issue 3, Mar 2019
www.ijasrm.com
ISSN 2455-6378

Genotoxic Effect of Copper, Fluoride and Lead Contaminated Ground Water using *Allium cepa* L. (onion) as Test Material

AashaMadiq, R.[*, 1], Abubacker, M. N.[1] and Udaya Kumar, R.[2]

[1] Department of Biotechnology, National College,
Tiruchirappalli-620001, Tamil Nadu, India

[2] Department of Biochemistry, Government Arts College,
Kumbakonam-612001, Tamil Nadu, India

Abstract

Angiosperm plants are recognized as appropriate genetic models to detect pollutant based environmental mutagens and are utilized in monitoring studies. *Allium cepa* (onion), Liliaceae plant has been used to evaluate DNA damage such as chromosome abnormalities and chromosome aberrations in mitotic cycle. The main aim of the present study is to determine the cytotoxic effects of copper, fluoride and lead contaminated ground water source by growing *A. cepa* plant and the root tip squash was analysed for mitotic cell divisions at room temperature 32°C for five days. Mitotic index and chromosomal abnormalities were calculated. It was observed that the contaminated ground water induced different types of chromosomal abnormalities such as chromosome bridge, chromosome break, C-mitosis, vagrant and loculated nuclei. The ranking of genotoxic effect was in the descending orders: Vagrant > Chromosome break > Chromosome bridge > C-Mitosis > Loculated nuclei.

Keywords: *Allium cepa, chromosomal abnormalities, contaminated ground water, mitotic index*

1. Introduction

Ground water contamination is a global problem. It is due to the discharge of hazardous chemicals into the soil, percolates and reaches the ground water source. Ground water is an important natural resource is totally essential to the human life and human survival. The urban areas are mainly associated with large number of population and totally depend on the ground water. The consumption of contaminated ground water directly or indirectly, sometimes acts on the health problem of the population (Mrinalini *et al*., 2015). The consumption of drinking water with copper, lead leads to diarrhea, kidney damage, nervous problems, bone problems, liver damage, cancer and reproductive disorders (Wan Ngah*et al.*, 2002; Gode and Pehlivan, 2006). The consumption of drinking water with fluoride level above 4 mg/l cause dental fluorosis, skeletal fluorosis which associated with bone abnormalities (Saranya and Ann, 2016). Plants do not have any specific mechanisms for copper and fluoride uptake and transport. There are some non-essential metals like lead have unknown biological or physiological function (Pandey Ambuj, 2012; Subhashini and Swamy, 2013). However, hyper accumulation of toxic pollutant by plants dependent on certain physiological mechanisms like higher rates of uptake, efficient translocation and deposition in tissue systems in the growing region such as shoot and root tips. The accumulation of these toxic contaminant cause damages, altering the genetic material in the cell cycle (Girjesh Kumar and Akanksha Srivastava, 2015; Panda *et al*., 2002; Abubacker and Sathya, 2017). To test this *Allium cepa* root tip squash technique was performed and it is a quick and relevant biological test for toxic contaminant interaction in environment and its genetic risk assessment. The test is based on the assessment of genotoxic effect of copper, fluoride and lead found in excess in the ground water source used for the experiment using *A. cepa* by recording mitotic activity (Mitotic Index) and mitotic abnormalities in meristematic root tip cells.

2. Materials and Methods

2.1Collection of Samples

The area under study for this research research work was identified based on the need, diversity and extend of pollutants produced by SIPCOTT industry in and around Cuddalore, Tamil Nadu. The ground

water samples were collected in properly cleaned, sterilized wide mouth glass bottles and stored in the lab and later utilized for the study of physico-chemical properties as per APHA (2005) standard methods.

2.2 Collection of Plant materials

A. cepa test consists of obtaining onion bulbs cultivated without the application of herbicides, fungicides or chemical fertilizers from an agricultural field where manure alone used for cultivation. The bulbs obtained were placed initially in 50 ml culture tubes containing distilled water for three days for the emerging of roots. After this period, the bulbs are transferred into the experimental condition in contaminated ground water in triplicate for another three days of exposure for the further growth of roots.

2.3 Genotoxic analysis

The root tips were cut at 1 cm length and immediately fixed in ethanol : acetic acid (3:1) ratio for one hour. Afterwards, the root tips were removed from the fixing solution and transferred to 70% ethanol and stored at 4°C for further experimental work. In the next stage, microscopic slides are used in which the root tips are placed in a drop of 1 N HCl to hydrolysed for one minute for softening of tissues and then stained with 4% acetocarmine with gentle heat and the slides were prepared by gentle tapping the coverslip which was placed over the stained root tip and referred as squash technique. The prepared slide was analysed for genotoxic study, observation was done at 1000X oil immersion in light microscope (Olympus CX211 LED, Camera Magcam DC 10, 10 MP, ½.3" CMOS Sensor, Software Magvision).The cell divisions were analysed 300 cells per one onion bulb and a total of 1200 cells were observed and the mitotic index was calculated (Girjesh Kumar and Akanksha Srivastava, 2015).

$$\text{Active Mitotic Index (AMI) \%} = \frac{\text{Total number of dividing cell}}{\text{Total number of cell observed}} \times 100$$

$$\text{Total Abnormality Percentage (TAP) \%} = \frac{\text{Number of abnormal cell}}{\text{Total number of cell observed}} \times 100$$

3. Results and Discussion

The results of physico-chemical analysis of ground water were present in Tables 1 and 2. The analyses showed many parameters are well within the WHO standards however the availability of copper, fluoride and lead seems to be higher than the WHO permissible standards. The parameters like pH, EC, TH, TDS, DO, COD, carbonate alkalinity, bicarbonate alkalinity, calcium, nickel, mercury, cadmium, arsenic, zinc and chromium values are well within the WHO standard, whereas copper content ranging between 1.69 ± 0.10 to 1.85 ± 0.05 mg/l as against WHO standard 1.0 mg/l. Fluoride content ranging between 1.70 ± 0.10 to 2.09 ± 0.15 mg/l as against the WHO standard 1.5 mg/l whereas lead content ranging between 0.54 ± 0.10 to 0.69 ± 0.05 mg/l, the WHO standard was 1.10 mg/l. The ground water contamination is due to the disposal of effluent without proper treatment into the environment poses major hazards through percolation in soil and also cause biomagnification as reported by Abubacker*et al.* (2018), Rajeswari *et al.* (2013), and Mahawar and Akhtar (2015).

The data of mitotic index and chromosomal abnormalities were presented in Table-3. The statistical analysis was carried out by using Statistical Package SPSS16 version, One-way ANOVA, Post HOC = Tukey Alpha, significant at 0.01 level for physiochemical analyses and also mitotic index and chromosomal abnormalities. The Active Mitotic Index (AMI) characterized by the total number of dividing cells in cell cycle has been used as a parameter to assess the genotoxicity of environmental contaminants. In the present study, these contaminants were absorbed by the root and by meristematic cells of *A. cepa*. The AMI for control was 52.0 ± 1.50 remediated ground water showed 49.0 ± 0.50, whereas for contaminated ground water it was 43.0 ± 0.50. The decrease in AMI is as a result of the contaminant especially copper, fluoride and lead as limiting factor. Such reports were in accordance with the other reports of Panda *et al.* (2002) and Kwankua*et al.* (2012). The primary action of these metals and contaminants on the mitotic spindle, promoted spindle related chromosomal abnormalities during cell division as reported by Preety Singh (2015). The decreased mitotic index also due to disturbances in the cell cycle or chromatin disfunction induced by metal-DNA interaction which led to significant reduction of mitotic index as pointed out by DitikaKopliku and Anila Meri (2013) and Abubacker and Sathya (2017).

The most frequent loculated nuclei occurred in this study indicate a cell death process, since these abnormalities are related to nuclear abnormalities. The occurrence of C-Mitosis refers to types of chromosome abnormalities, it is due to the loss of microtubules of spindle fibres (Girjesh Kumar and Akanksha Srivastava, 2015). The chromosomal break induced by several factors, such as DNA breaks, inhibition of DNA synthesis and replication of altered DNA results from anthropogenic effects as reported by Walker *et al.* (2012).

4. Conclusion

The present report provides information about the toxic effect of copper, fluoride and lead present in

ground water source by evaluating mitotic index and chromosomal abnormalities in the meristematic root cells of *Allium cepa* L. The result obtained through this research work should be considered a warning of risk the environment, biota and human health may incur by natural and anthropogenic pollutant discharge in soil and subsequent percolation and reaching the ground water source. The *A. cepa* bioassay is an excellent tool in quality monitoring of water bodies.

References

[1] Abubacker, M. N.,Mehala, T., Senthilkumar, S. and Ramu, S. Microbial degradation of textile effluent and its genotoxic effect on *Allium cepa*. *InternationalResearch Journal of Environmental Studies*, 7: 1-7 (2018).

[2] Abubacker, M. N. and Sathya, C. Genotoxic effect of heavy metals Cr, Cu, Pb and Zn using *Allium cepa* L. *Biosciences Biotechnology Research Asia*, 14: 1181-1186 (2017).

[3] APHA. *Standard Methods for the Examination of Water and Wastewater* (21st ed.). Washington: American Public Health Association, American Water Works Association, Water Environment Federation. (2005).

[4] DitikaKopliku and Anila Meri. Assessment of cytotoxic and genotoxic potency of Cr(VI) doped river water of Nen-Sukodra Lowland Albania, on *Allium cepa* L. *Journal of Environmental Research and Development*, 7: 1322-1332 (2013).

[5] Girjesh Kumar and Akanksha Srivastava. Comparative genotoxicity of herbicide ingredients glyphosate and atrazine on root meristem of buckwheat (*Fagopyrum esculentum*Moench). *Jordan Journal of Biological Sciences*, 8: 221-226 (2015).

[6] Gode F. and Pehlivan, E. Removal of Chromium (III) from Aqueous Solutions using Lewatit S 100: The effect of pH, time, metal concentration and temperature. *JHazardousMaterials*,136: 330-337, (2006).

[7] Kwankua, W., Sengsni, S., Muangphra, P. and Euawong, N. Screening for plants sensitive to heavy metals using cytotoxic and genotoxic biomarkers. *Kasetsart J. Natural Science*, 46: 10-23 (2012).

[8] Mahawar, P. and Akhtar, A. Physico-chemical characterization of soil and effluent of dye industries in Kaithun region of Kota Rajasthan.

[9] Int. J. Pure Appl. Biosci., 3: 419-422 (2015).

[10] Mrinalini Kanyal and Anuja Ashok Bhatt. Removal of Heavy Metals from Water (CuandPb) Using Household Waste as an Adsorbent.

[11] J. Bioremed. Biodeg., 6: 269 (2015).

[12] Panda, S. K., Mahapatra, S. and Patra, H. K. Chromium toxicity and water stress simulation effects in intact senescing leaves of green gram (*Vigna radiata* L. var. WilckzeckK851). In: Panda, S. K. (Ed.). *Advance in Stress Physiology in Plants*. Scientific Publisher, India. pp. 129-136 (2002).

[13] Pandey Ambuj. Arsenic contamination of ground water in South and East Asia. *J. Environ. Res. Develop.*, 7: 70-74 (2012).

[14] Preety Singh. Toxic effect of chromium genotoxicity and cytotoxicity by use of *Allium cepa* L. *International Journal of Research in Engineering and Applied Sciences*, 5: 1-10 (2015).

[15] Rajeswari, K., Subashkumar, R. and Vijayaraman, K. Physico-chemical parameter of effluents collected from Tirupur textile dyeing and CETP and analysis of Heterotropic bacterial population. *Journal of Microbiology and Biotechnology Research*, 3: 37-41 (2013).

[16] Saranya, S. R. and Ann, N. Comparative study of fluoride removal from synthetic waste water by using bio-adsorbents. *International Journal of Scientific and Engineering Research*, 7: 300-303 (2016).

[17] Subhashini, V. and Swamy, A. V. V. S. Phytoremediation of Pb and Ni contaminated soil using *Catharanthus roseus* L. *Universal Journal of Environmental Research and Technology*, 3: 465-472 (2013).

[18] WanNgah, W. S.,Endud, C. S. andMayanar, E. R. Removal of Copper(II) Ions from Aqueous Solution on to Chitosan and Cross-linked Chitosan Beads.

[19] Reactive and Functional Polymers,50:181-190, (2002).

Table-1: Physico-chemical analysis of ground water collected at different sites near SIPCOTT area in Cuddalore District, Tamil Nadu, India

Parameters	Unit	Site-I	Site-II	Site-III	Site-IV	Site-V	WHO Standards
Odour	-	No odour little muddy	No odour little muddy	No odour little muddy	No odour muddy	No odour muddy	-
Colour	-	Light brown	Light brown	Light brown	Light brown	Light brown	-
pH	-	6.8 ± 0.10	7.0 ± 0.15	6.8 ± 0.10	6.8 ± 0.10	7.0 ± 0.15	7.0-8.5
Electrical Conductivity (EC)	µmhos/cm	611.0 ± 1.5	608.0 ± 0.2	617.0 ± 11.0	628.0 ± 15.2	730.0 ± 1.5	500-1500
Total Hardness as $CaCO_3$(TH)	mg/l	57.9 ± 0.2	59.6 ± 0.3	60.8 ± 0.1	83.4 ± 0.15	63.7 ± 0.12	500
Total Dissolved Solids (TDS)	mg/l	393.1 ± 0.4	400.8 ± 0.45	409.8 ± 0.3	444.0 ± 0.35	416.0 ± 0.20	850-1500
Dissolved Oxygen (DO)	mg/l	6.4 ± 0.20	6.3 ± 0.1	6.4 ± 0.15	4.4 ± 0.10	4.3 ± 0.20	≤ 6.0
Carbonate Alkalinity	mg/l	5.64 ± 0.30	5.56 ± 0.15	5.79 ± 0.2	8.64 ±0.3	8.58 ± 0.2	200
Bicarbonate Alkalinity	mg/l	52.8 ± 3.5	54.9 ± 0.6	52.1 ± 2.7	54.4 ± 2.0	56.2 ± 2.5	50
Chemical Oxygen Demand (COD)	mg/l	8.9 ± 0.12	8.6 ± 0.15	9.3 ± 0.05	15.1 ± 0.2	11.6 ± 0.12	250
Calcium	mg/l	20.03 ± 0.61	21.9 ± 0.50	24.7 ± 0.20	68.0 ± 0.35	24.6 ± 0.15	75-200
Fluoride	mg/l	1.70 ± 0.10	1.8 ± 0.15	1.80 ± 0.15	2.09 ± 0.1	2.09 ± 0.15	1.50

* The mean difference is significant at 0.01 level

IJASRM
International Journal of Advanced Scientific Research and Management, Volume 4 Issue 3, Mar 2019
www.ijasrm.com
ISSN 2455-6378

Table-2: Heavy metal analysis of ground water collected at different sites near SIPCOTT area in Cuddalore District, Tamil Nadu, India (AAA analysis)

Parameters	Unit	Site-I	Site-II	Site-III	Site-IV	Site-V	WHO Standards
Arsenic	mg/l	0.04 ± 0.11	0.052 ± 0.10	0.058 ± 0.15	0.072 ± 0.10	0.065 ± 0.20	0.010
Cadmium	mg/l	0.003 ± 0.12	0.003 ± 0.10	0.001 ± 0.05	0.003 ± 0.10	0.001 ± 0.05	0.003
Chromium	mg/l	0.04 ± 0.15	0.06 ± 0.10	0.06 ± 0.10	0.06 ± 0.15	0.05 ± 0.20	0.050
Copper	mg/l	1.85 ± 0.05	1.80 ± 0.10	1.82 ± 0.01	1.75 ± 0.02	1.69 ± 0.10	1.000
Lead	mg/l	0.66 ± 0.01	0.69 ± 0.05	0.80 ± 0.05	0.54 ± 0.10	0.58 ± 0.15	0.100
Mercury	mg/l	0.0008 ± 0.01	0.0001 ± 0.05	0.0001 ± 0.02	0.0002 ± 0.01	0.0002 ± 0.05	0.006
Nickel	mg/l	0.22 ± 0.10	0.22 ± 0.15	0.32 ± 0.10	0.38 ± 0.05	0.32 ± 0.15	0.006
Zinc	mg/l	5.40 ± 0.10	5.70 ± 0.10	5.84 ± 0.15	5.05 ± 0.15	5.88 ± 0.10	0.040

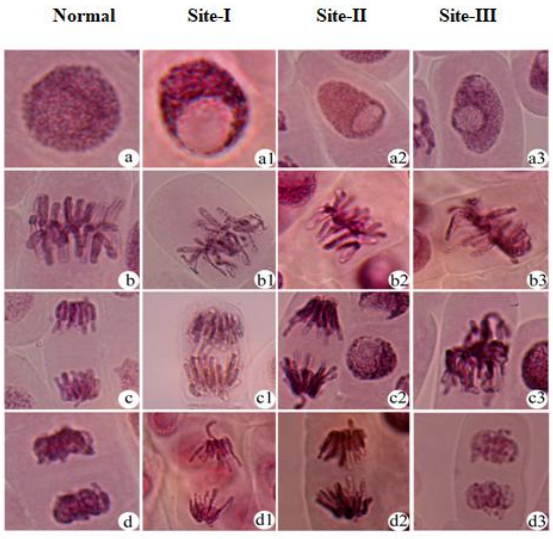
* The mean difference is significant at 0.01 level

Table-3: Genotoxic Studies - Mitotic Index and Chromosomal Abnormalities in *Allium cepa* L.

Treatment	Total number of Cell Examined	Number of Dividing Cells	Active Mitotic Index (AMI)	Abnormalities in Mitotic Cycle					Total number of cells showing abnormalities	Total abnormalities % (TAP)
				CB	CBr	CM	V	CN		
Control (Deionized Water)	300	180	52.0 ± 1.5*	0	0	0	0	3	3	0.010 ± 0.14
Contaminated Ground Water	300	120	43.0 ± 0.5*	20	18	28	14	56	32	0.106 ± 0.50
Remediated Ground Water	300	160	49.0 ± 0.5*	0	0	0	2	6	8	0.026 ± 0.10

CB = Chromosomal Bridge
CBr = Chromosome break
CM = C-Mitosis
V = Vagrant
LN = Loculated Nuclei

Genotoxic effect of copper, fluoride and lead contaminated ground water using *Allium cepa* L. (onion) test (× 1000)



a : Prophase
b : Metaphase
c : Anaphase
d : Telophase
a1, a2, a3 : Loculated nuclei
b1, b2, b3 : C-Mitosis
c1, d2 : Chromosome bridge
c2, d1 : Chromosome break
c3 : Vagrant
d3 : Normal Telophase