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Bioassays based on higher plants as excellent dosimeters for ecotoxicity monitoring: A review

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ABSTRACT

All sorts of pollution on the planet can be traced back to development of industries and the most important among them is water pollution. Clean technologies, management practices and regular monitoring of effluents could be helpful to minimize the contamination of watersheds. *Allium cepa* (*A. cepa*) has been recognized as a promising genetic model to detect the toxicity of industrial wastewater, contaminated soil, river water, nuclear contamination and even for those systems which are considered non toxic. *A. cepa* is distinguished as a low cost test, easy to handle and sensitive to both *in vivo* and *in vitro* models. It offers the detection of damages in genetic material quantitatively and the results can be generalized for other biological and ecological systems. Moreover, the pollutants can be classified on the basis of this test present in industrial effluents and their mechanism of action on genetic material. This review focuses on the studies undertaken to evaluate the toxicity of industrial wastewater, contaminated river and soils.

Capsule Summary: The applications of bioassays for ecotoxicity monitoring are regarded as excellent technique and *Allium cepa* was exemplified for ecotoxicity monitoring of variety of systems.

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INTRODUCTION

From last few decades, there is a growing concern of pollution, which not only affect the animal and plant, but also human beings as well. The water bodies serve as temporary or final receptors of diverse variety of contaminants, which consequently contaminate the entire watershed and adjoining territories (Barbério et al., 2009; Bianchi et al., 2011; Ghodake et al., 2011; Kumari et al., 2011; Salles et al., 2016). The rapid stride in industrialization is major concern and at present is a severe threat to the survival of living organisms and jeopardizing the ecological balance (Abbas et al., 2015; Iqbal et al., 2013a; Iqbal, 2016; Iqbal et al., 2016b; Islam et al., 2015; Manzoor et al., 2013; Mushtaq et al., 2016; Nadeem et al., 2016a; Rashid et al., 2016; Tabrez and Ahmad, 2011; Ullah et al., 2013; Younas et al., 2015).

Among the damages caused by chemical agents to exposed organisms, genotoxic and mutagenic effects have shown to be worrying, which can lead to several health

problems and also affect future generations due to inheritable alterations in genetic material (Leme and Marin-Morales, 2009). Genetic toxicology is involved in detecting compounds capable of causing DNA damage with the aim of understanding potential biological consequences and molecular mechanisms of genetic material. Mutagens screening in environmental samples using *in vivo* method is reliable to measure the extent of pollution load and this biological assessment is compulsory for effluent discharge from industries before being mixed with watersheds and adjoining territories (Akinboro et al., 2011; de Souza Pohren et al., 2013; Hemachandra and Pathiratne, 2015; Kannangara and Pathiratne, 2015b; Rodrigues et al., 2010).

The biological tests, especially short-term bioassays on bacteria or higher plants is the best way to detect and estimate the pollution load in any matrices approach (Gana et al., 2008; Mesi and Kopliku, 2012). Many plant species have a wide range of applications as indicators of cytogenetic and mutagenic effects of environmental physical and chemical polluting agents (Çavuşoğlu et al., 2011; Juchimiuk and Maluszynska, 2005).

The cytogenetic tests in plants are relatively inexpensive and can easily be handled and have shown good correlation with other bio-testing systems (Fiskesjo, 1988) and is also a rapid and sensitive method for environment monitoring. The plants like *Vicia faba, Zea mays, Tradescantia, Nicotiana tabacum, Crepis capillaries, Pisum sativum, Hordeum vulgare* and *Allium cepa* (*A. cepa*) have been used for this purpose (Akintonwa et al., 2009; Andrade et al., 2008; Bagatini et al., 2009; Chandra et al., 2005; do Canto et al., 2013; Fatima and Ahmad, 2006b; Goncharuk et al., 2011; Iqbal et al., 2015a; Iqbal and Bhatti, 2014, 2015a; Iqbal et al., 2014; Iqbal and Nisar, 2015; Srivastava et al., 2005).

Advantages of *A. cepa* test versus other biological assays

A. cepa characteristics make it an excellent genetic model to assess environmental pollutants and can be used for monitoring chemical and physical toxic agents. The application of *A. cepa* is not only due to the sensitivity to detect mutagens, but also to the possibility of assessing several genetic endpoints ranges from point mutation to chromosome aberrations (CA) in meristematc cells as well as F_1 generation (Leme and Marin-Morales, 2009; Paz et al., 2006). First of all Leven (Levan, 1938) introduced the *A. cepa* test for mitotic study since that time it is being used frequently. The *A. cepa* test is considered an excellent presentative of *in vivo* biological test, where the roots grow in direct contact with the substance of interest and enabling to detect the possible damage to the DNA and results can be generalized for divsrse animal and plant biodiversity as a model. Additionally, the structural aberration and numerical chromosomal alterations can be directly visualized. Moreover, *A. cepa* is distinctive regarding its efficiency as compared to other bioassays, good correlation with other test-systems, easy handling, low-cost and have ideal size as well as chromosome number (2n = 16). *A. cepa* can be used for the indication of toxic compounds (mutatoxic, cytotoxic and genotoxic etc) and due to its kinetic characteristics of proliferation as compared to *Vicia faba, Zea mays, Tradescantia, Nicotiana tabacum, Crepis capillaries, Pisum sativum* and *Hordeum vulgare*, is more suitable for chromosomes and nuclear study (Leme et al., 2008; Leme and Marin-Morales, 2009; Magdaleno et al., 2008; Rank, 2003; Smaka-Kincl et al., 1996; Souza et al., 2009).

Furthermore, cytogenetic tests performed are suitable for identification of harmful effects of particular known substances in various concentrations over different exposure of time and is considered one of the best/sensitive method to measure harmful effects of any agent of interest as compared to other physical, chemical, saprobiological, radiological methods on genetic level. The biological tests are indispensable for the evaluation of reactions of living organisms to the complex environmental pollution and for an indication of potential synergistic effects of various pollutants, while physical and chemical analyses provide nothing, but only determination of presence and concentrations of different pollutants. Moreover, they are also laborious and time consuming (Bianchi et al., 2011; Chandra et al., 2005; da Costa et al., 2012b; Guzy et al., 2012; Leme and Marin-Morales, 2008, 2009; Liman et al., 2010a; Smaka-Kincl et al., 1996; Souza et al., 2009).

A. cepa assay is efficient and reliable test systems for the rapid screening of chemicals for mutagenic and clastogenic effect evaluation (Rank and Nielsen, 1997b). This test generally provides estimate of the total toxic effect resulting from the treatment of root tip cells by mixture of wastes (Fiskesjo, 1993). The micronucleus (MN) and chromosome aberration (CA) tests provide a fast opportunity to screen genotoxic effects of chemical substances which are present in the environment. Moreover, *A. cepa* is being often used due to the knowledgement of its cell cycle duration and its reaction in the presence of many known mutagenic agents and on the basis of clastogenic and aneugenic effects, the action mechanism of toxic agent on genetic material can also be evaluated (Evseeva et al., 2003; Njoku et al., 2015). *In situ* application of *A. cepa* is easy as compared to various other biological test (Kovalchuk et al., 1998). The chromosomal features favor carrying out the CA's test, not only for toxic effect assessment, but also for understanding the action mechanisms of the test agent (Fiskejo, 1985; Rank and Nielsen, 1997b). *A. cepa* along with chromosome damages and cell division cycle disturbances also facilitates the evaluation of aneuploidy risks (Leme and Marin-Morales, 2008). The results of the *A. cepa* test are known to be well correlated with the data obtained from prokaryotic and eukaryotic systems (Houk, 1992; Leme and Marin-Morales, 2009). According to Fiskejo (Fiskejo, 1985), *A. cepa test* has similar sensitivity to that of the algal and human lymphocyte and carcinogenity assays in rodents showed the correlation of 82% with *A. cepa* (Rank and Nielsen, 1994). In another study, the effects of infusions of *Maytenus ilicifolia* Mart. and *Bauhinia candicans* Benth with *A. cepa* and bone marrow cells of Wistar rats showed similar results (Camparoto et al.,

2002). Cytotoxicities have been assessed by the *A. cepa* roots tip cell were also found similar to *in vitro* animal toxicity tests (Poonkuzhali et al., 2011b). In a comparative study, Ma et al. (Ma et al., 1995) reported that the *A. cepa* test was more sensitive and efficient for the detection of environmental pollutants. In this regard, the response of *A. cepa* and *Vicia faba* root was studied on the basis of chromosome length, peak sensitivity of the mitotic cells and the regions of the root tip where the MN were formed. Higher efficiency was demonstrated when the MCN frequencies were scored from the F_1 cells in both *A. cepa* and *Vicia* treated with formaldehyde, mitomycin C and maleic hydrazide. The linear regression dose-response curves were established in both *A. cepa* and *Vicia* cell systems and the coefficients of correlations, slope values were used to verify the reliability and efficiency of these two plant cell systems. Based on the dose-response slope value, the *A. cepa* root test was proved to be more efficient test system.

The present review highlights the application of *A. cepa* for the evaluation of industrial wastewater toxicity and its efficiency in evaluating genotoxic and mutagenic agents present in industrial wastewater such as oil refinery, textile and dying industry, pulp and paper mill and metallurgy etc as well as the contaminated soil and watersheds with industrial wastewater.

Endpoints studied in *A. cepa* to evaluate the toxicity

For *A. cepa* root tip assay, the procedure and endpoints studied for toxicity monitoring are shown in Fig. 1. Endpoints are characterized by the genetic categories studied to assess the toxicity of system. For this purpose, following four microscopic genetic endpoints are reported for the assessment of toxicity in *A. cepa,* namely, mitotic index (MI), chromosonal aberrations (CA), nuclear aberrations (NA) and micronuclei (MN) (Leme and Marin-Morales, 2009). Additionally, some researchers have also used macroscopic characteristics such as number of root, length of root and root physical appearance for the indication of toxicity (Bianchi et al., 2011; Fazili and Ahmad, 2014; Lerda et al., 2010; Olorunfemi et al., 2011b; Olorunfemi et al., 2015b; Radić et al., 2010; Roa et al., 2012; Siddiqui et al., 2011; TORRES et al., 2014). These end points are used for the evaluation of cytotoxicity, genotoxicity and mutagenicity of the pollutant.

Cytotoxicity evaluation

The MI is very important endpoint for the evaluation of toxicity and is based on the number of dividing cell in cell cycle and mostly researchers used it as a cytotoxicity indicator (Abdel Migid et al., 2007; Carita and Marin-Morales, 2008; Leme and Marin-Morales, 2008; Porras Torres et al., 2013; Smaka-Kincl et al., 1996). For normal cell division, the MI must be equal to control and MI lowers than the control indicates the abnormality in cell division. The higher value of MI is also an indication of abnormal growth such as cell proliferation and un-control growth versus negative control (Carita and Marin-Morales, 2008; Hoshina and Marin-Morales, 2009). So, the reduction as well as acceleration in MI are important indicators for the assessment of cytoxicity of contaminants (Leme and Marin-Morales, 2009).

The inhibition of MI may be attributed to the effect of environmental chemicals on DNA/protein synthesis of the biological system (Andrade et al., 2008; Chandra et al., 2005). A decrease in MI below 22% versus control causes lethal effects on test organisms, while a decrease below 50% (cytotoxic limit value) usually has sublethal effects (Abdel Migid et al., 2007). Therefore, the inhibition of root growth in fact is a measure of the inhibition of cell division, measured as a decrease in MI (Marcano et al., 2004). The decrease in MI due to the exposure of wastewater/any other physical or chemical agent indicates the presence of cytotoxic agent and can be used for the estimation of pollution level in the sample (Smaka-Kincl et al., 1996). For example, in a study to evaluate the toxicity of *Pterocaulon polystachyum,* Knoll et al. (Knoll et al., 2006a) correlated the inhibition of cell division to the flavonoids in the infusions tested and the authors demonstrated that with an increase in the concentration of the infusions of *P. polystachyum*, lower mitotic index values were recorded (Knoll et al., 2006a). Therefore, due to greater sensitivity of *A. cepa* root to the toxic effects of physical or chemical agents in wastewater, various researchers used the MI as a cytoxicity indicator (Abu and Ezeugwu, 2008; Andrade et al., 2008; Arya and Mukherjee, 2014; Bianchi et al., 2011; Carita and Marin-Morales, 2008; Çelik and Aslantürk, 2009; Chandra et al., 2005; da Costa Machado Matos Carvalho et al., 2011; da Costa et al., 2012b; Espinoza-Quiñones et al., 2009; Geras'kin et al., 2011; Hoshina and Marin-Morales, 2009; Kwasniewska et al., 2012; Olorunfemi et al., 2011b; Rodrigues et al., 2010; Souza et al., 2009). Table 1 shows the MI percentage value versus negative control in *A. cepa* cells exposed to different pollutants.

CA is characterized by change in chromosomal number or structure (Fernandes et al., 2007b). To evaluate the structural abnormalities due to toxic agent, the cell stages like prophase, anaphase, metaphase and telophase were studied well (Fiskejo, 1985; Rank, 2003; Rank and Nielsen, 1993). The CA are caused due to the DNA breakage, inhibition in DNA synthesis and altered DNA replication as a result of contact with physical and chemical polluting agents (Albertini et al., 2000). The CA include chromosome adherence, loss, breakage, bridge, irregular distribution, distortion, lagged, irregular separation, stickiness, vagrant, rings, late separation and un-orientation. Additionally, the C-metaphase, polyploid anaphase, multipolar anaphase, polar slip, drifting away from the metaphase plate, disturbed telophase, bridge at telophase, chromatin degeneration, anaphase with multiple bridges, late anaphase stage with double bridge, disturbed telophase, bridge sticky metaphase, sticky prophase and cytokinetic failure are also the types of CA's abnormalities (Abdel Migid et al., 2007; Abu and Ezeugwu, 2008; Abu and Mba, 2011; Bianchi et al., 2011; El-Shahaby et al., 2003; Gupta and Ahmad, 2012a; Sik et al., 2009).

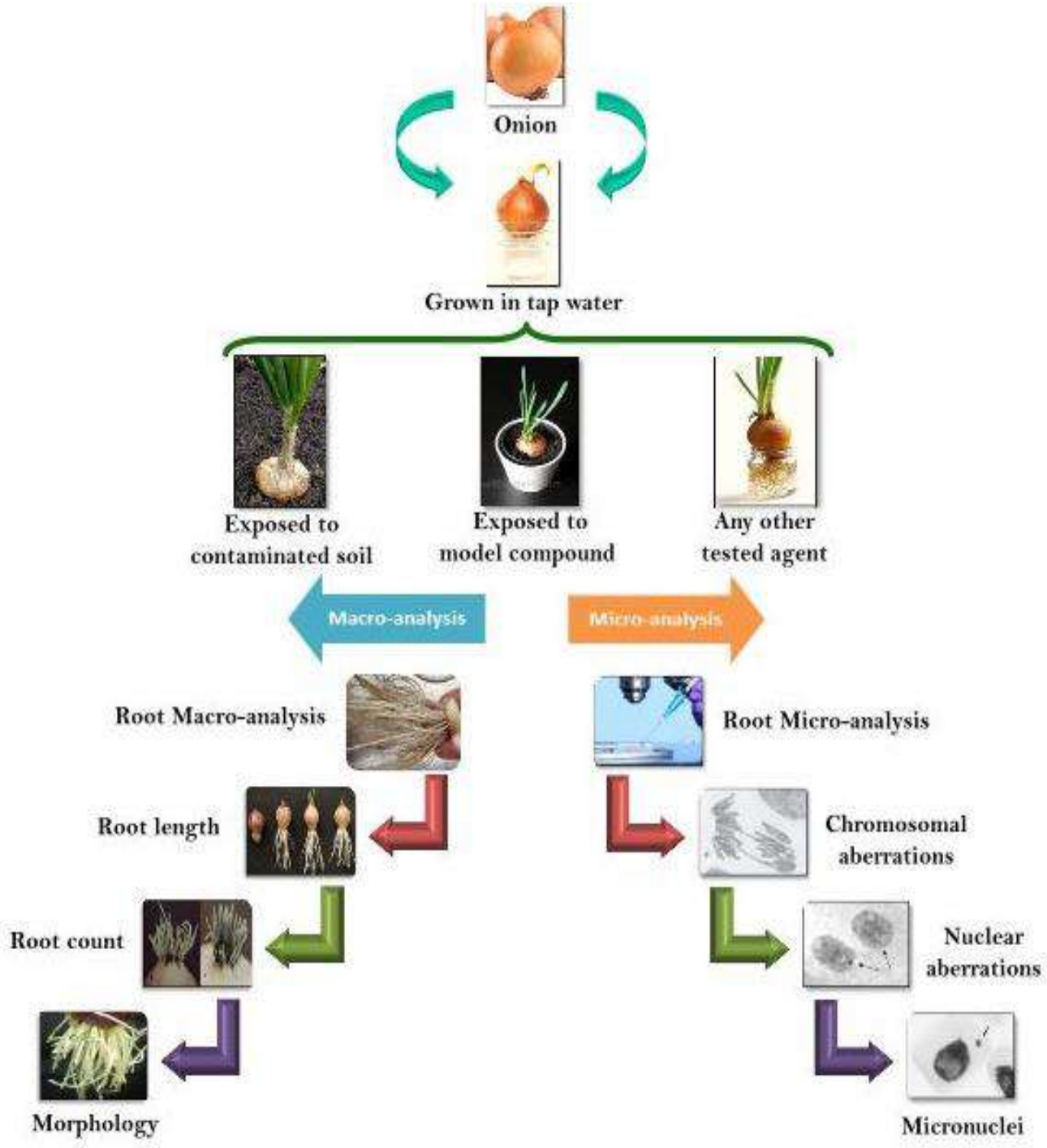


Fig. 1: Procedure and endpoints analysis in *A. cepa* root tip assay for toxicity monitoring

It is reported that metaphase with sticky chromosomes loses its normal appearance and is seen with a sticky "surface" causing chromosome agglomeration. Presence of such type of aberration reflects the toxic effect on chromatin, which generally leads to irreversible cell death.

Chromosomes stickiness is attributed to the formation of complexes of toxic agent with phosphate groups in DNA, on DNA condensation or on formation of inter-and-intra-chromatid crosslinks (El-Ghamery et al., 2003). Additionally, the late segregation of chromosome, C-metaphases and multipolar anaphases suggest the effect on microtubule assembly. The microtubules perform central role during the growth and mitotic cycle such as chromosome migration, cell structure and formation of cell wall (Jordan and Wilson, 1998).

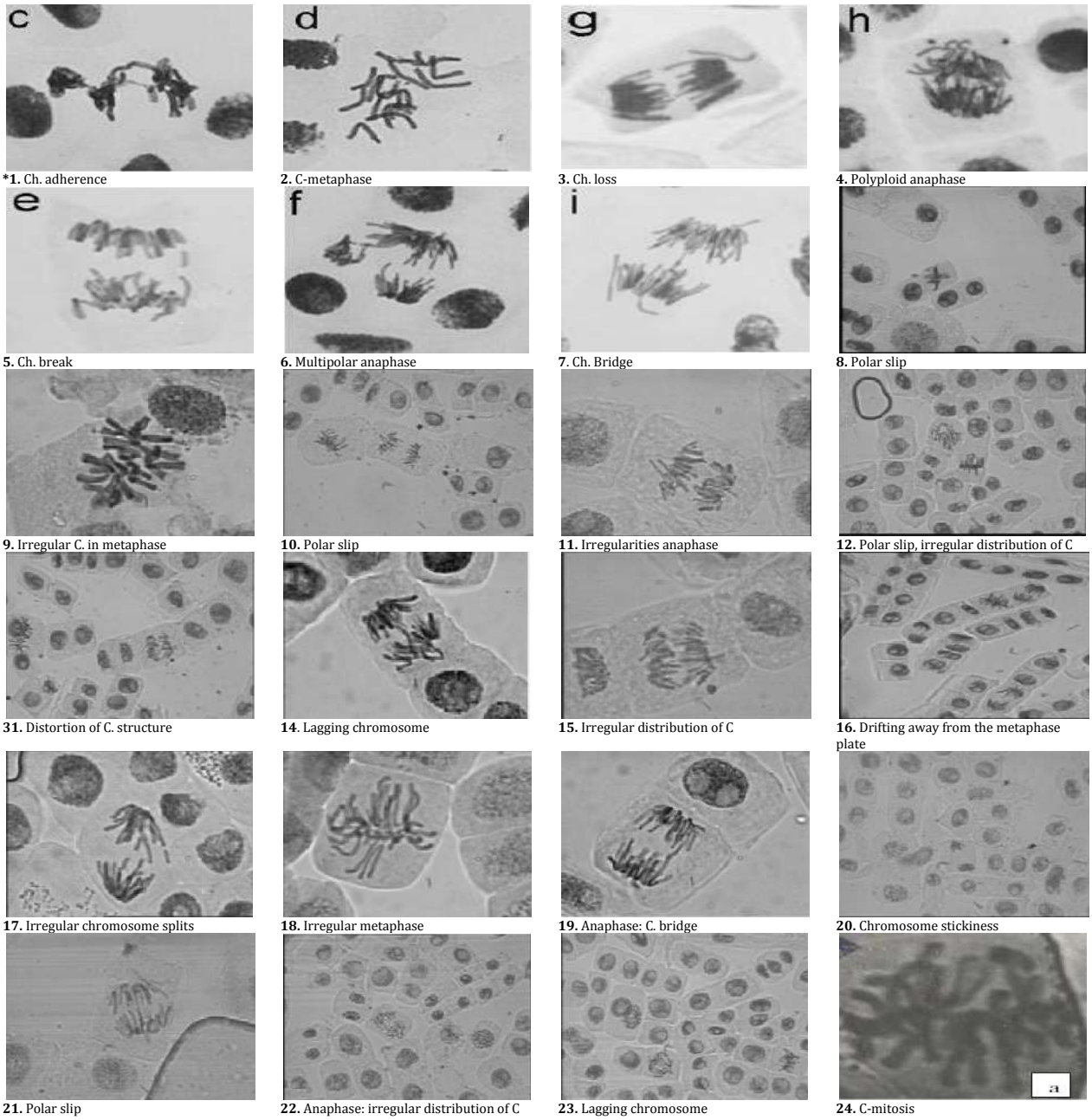
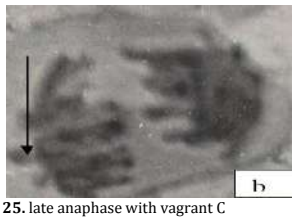
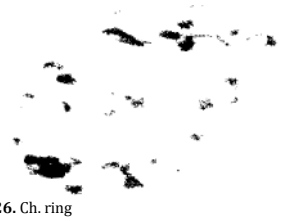
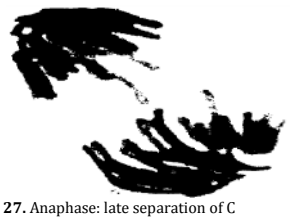


Fig. 2: Chromosomal aberrations and their types observed in A. cepa meristematic cells exposed to different genotoxic agents (1-164). * References and physiochemical properties of wastes: 1-7 (Bianchi et al., 2011): River water mixed with industrial effluents: pH-6.83-7.29, EC-29-114, DO-4.43-7.09 mg/L, T-24.1-25.9 ^{0}C, metal (Cu, Cr, Ni, Cd, Zn, Fe, Mn, Ca). 8-15 (Sik et al., 2009): the water incoming (10%) and outgoing at biological and chemical wastewater treatment from industrial zone (COD-14.18, SS-270.0, Cr-0.62, Pb-1.75, Cu-2.31, Zn-1.81 mg/L, pH-8.6). 16-23 (Sik et al., 2009): the water incoming (25%) and outgoing at biological and chemical wastewater treatment in organized industrial zone (COD-14.18, SS-270.0, Cr-0.62, Pb-1.75, Cu-2.31, Zn-1.81 mg/L, pH-8.6). 24-25 (Abu and Ezeugwu, 2008): chemical industry pH-6.3, TDS-520, SO^{-2}_{4}-71.7, Cl^{-}-90, COD-512, TSS-14700, Na-275, K-42.5, Ca-1062, Mg-48.6, Co-18.85, Cu-1191.11, Cd-43.75, Pd-2.30 mg/L. 26-30 (El-Shahaby et al., 2003): industrial wastewater. 31-33 (Abu and Mba, 2011): pharmaceutical (cotrimoxazole, chlorphiramine, paracetamol, ascorbic acid, ampiclox ampicillin and cloxiacillin). 34-39 (Abdel Migid et al., 2007): industrial effluent before algal treatment. 40-43 (Vujosevic et al., 2001): fertilizer industry wastewater, pH-7.7, EC-461uS/cm, NH_3-<0.05, NO^{-}_{2}-0.03, NO^{-}_{3}-1.58, Cl^{-}-26.8, SO^{2-}_{4}-40.8, PO^{3-}_{4}-0.16, CN^{-}-<0.01, mineral oil (0.045) mg/L and Fe-0.03, Mn, Pb, Cd, Zn, Cr, Hg, As, Cu, <0.05 mg/L and dichlorethane-<0.1), toluene-2.8, xylene-2.8, PCB-<0.001 and PAH's-0.02 ug/L. 44-50 (Odeigah et al., 1997): petroleum wastewater (44-50). 51-58 (Gupta and Ahmad, 2011). 59-63 (Kovalchuk et al., 1998): soil contaminated with radiaoactive material in Chernobyl accident. 64-66 (Kwasniewska et al., 2012): landfill leachates: Pb, Ni, Cd, Hg, benzene, dichloromethane, chloroform, trichlorobenzenes, exachlorobenzene, hexachlorobutadiene, hexachlorocyclohexane, lindane, hexachlorocyclohexane, pentachlorobenzene, pentachlorophenol, PAH's. 67-76 (Leme et al., 2008): refinery wastewater: HC(C_{12}-C_{18}) and PAH's (naphthalene, acenaphthylene, acenaphthlene, fluorine, phenanthrene, pyrene, benzoanthracence and chrysene). 77-86 (Mazzeo et al., 2011): mixture of benzene, toluene, ethylbenzene and xylene. 87-98 (Ventura-Camargo et al., 2011): azo dye. 99-104 (Jadhav et al., 2010): textile effluent. 105-108 (Kumari et al., 2009) 2009: silver nano particle. 109-112 (Herrero et al., 2012): Di(2-ethylhexyl)phthalate, triclosan and propylparaben. 113-119 (Ukaegbu and Odeigah, 2009). 120-123 (Gul et al., 2006): 0.1%, 0.2% and 0.4% concentrations of herbicide avenoxan. 124-129 (Olorunfemi et al., 2011): A. cepa grown in agriculture filed under the effect of pesticides. 130-135 (Ivanova et al., 2005): heavy metal and cyanide contaminated river waters in a mining region. 136-138 (Quilang et al., 2008): polychlorinated biphenyls. 139-144 (Radic et al., 2010). 145-147 (Oriaku et al., 2011): textile effluent (BOD-152, COD-319, TSS-1160, O & G-612, pH-6.9, Cr-1.20, Cu-0.02, NH^{4+}-0.50 mg/L). 148-151 (Pavlica et al., 2000): wastewater from the phosphoric gypsum depot: Ca^{2+}(960), $SO4^{2-}$(3599), P^{5+}(3200-3800), F^{-}(2500-3500), Si^{4+}(800), Na^{+}(1313), Fe^{3+}(55), Mg^{2+}(114), NH^{4+}(23), Cl^{-}(40), K^{+}(53) and SS-9 mg/dm^3. 152-156 (Ivanova et al., 2008). 157-160 (Saxena et al., 2005): 161-164 (Staykova et al., 2005).



25. late anaphase with vagrant C

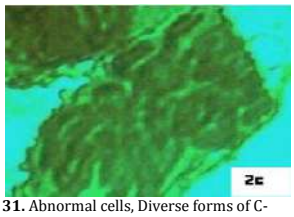

26. Ch. ring


27. Anaphase: late separation of C

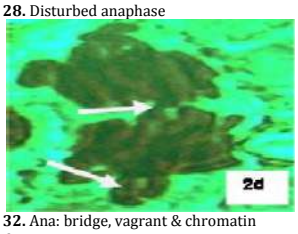

28. Disturbed anaphase


29. Disturbed telophase

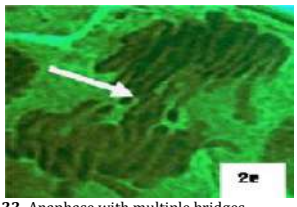

30. Bridge at telophase



31. Abnormal cells, Diverse forms of C-mitotic

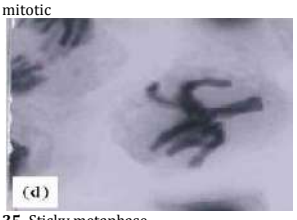


32. Ana: bridge, vagrant & chromatin degeneration



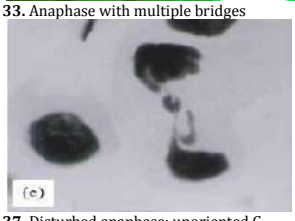
33. Anaphase with multiple bridges

34. Sticky prophase



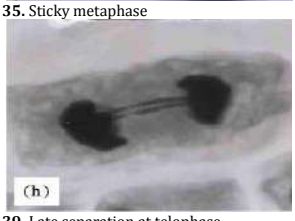
35. Sticky metaphase

36. Disturbed metaphase: unoriented Ch

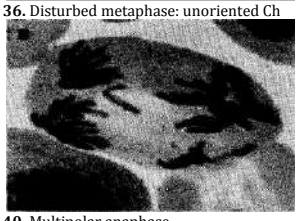


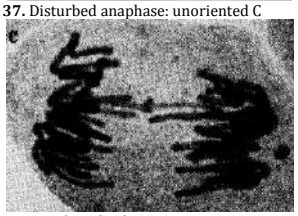
37. Disturbed anaphase: unoriented C

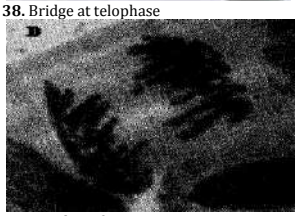
38. Bridge at telophase

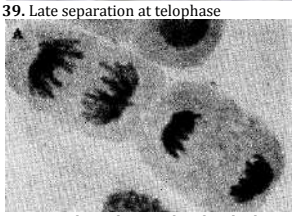


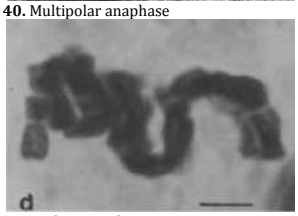
39. Late separation at telophase


40. Multipolar anaphase


41. Anaphase bridge


42. Anaphase fragment

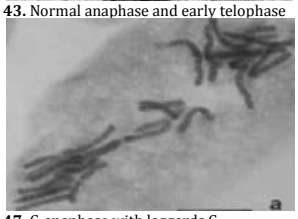

43. Normal anaphase and early telophase

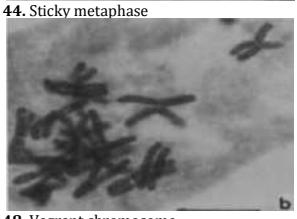


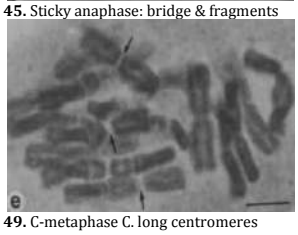
44. Sticky metaphase

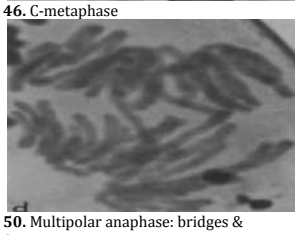

45. Sticky anaphase: bridge & fragments

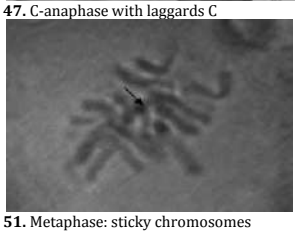

46. C-metaphase

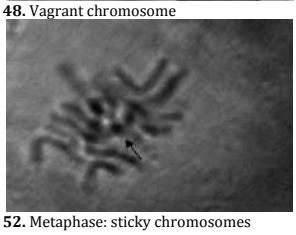

47. C-anaphase with laggards C

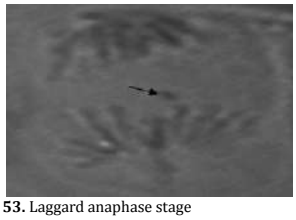

48. Vagrant chromosome

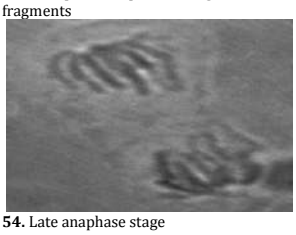

49. C-metaphase C. long centromeres

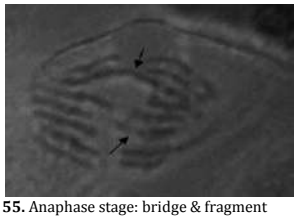

50. Multipolar anaphase: bridges & fragments


51. Metaphase: sticky chromosomes


52. Metaphase: sticky chromosomes


53. Laggard anaphase stage


54. Late anaphase stage


55. Anaphase stage: bridge & fragment

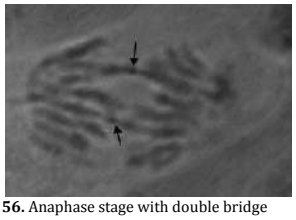

56. Anaphase stage with double bridge

Fig. 2: Continue…

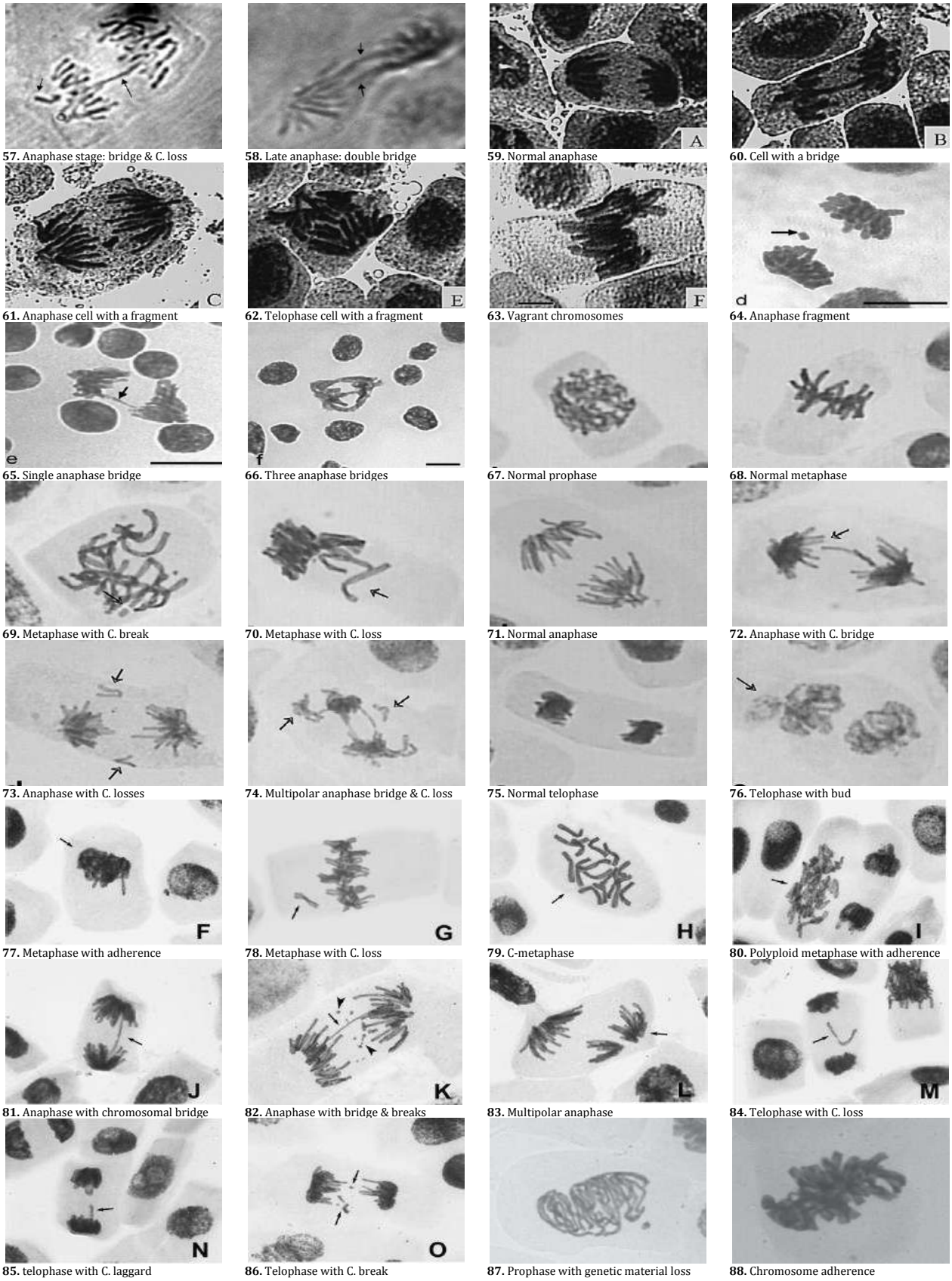


Fig. 2: Continue...

In the C-metaphase number of chromosomes, late segregation and multipolar anaphases are attributed to alterations and disturbances in the dynamics of the microtubules that is also called a spindle poisoning (Andrade et al., 2008; Haroun and Al Shehri, 2001). The C-metaphases are evidence of aneugenic agents, since they provide the complete inactivation of the cell mitotic spindle (Fiskejo, 1985). The spindles are inactivated when no equatorial plate is organized and consequently, the centromere division is blocked (Leme et al., 2008). According to (Fernandes et al., 2007b) and (Kirsch-Volders et al., 2002), the presence of C-metaphases can result in multinuclear cells, although the most frequent result is the induction of MN.

Some authors reported that chromosomal losses, breaks as well as the excess materials, promoted by the DNA replication, can also induce MN, which can be eliminated from the cell in the form of mini cells, i.e., small cytoplasm portions with a reduced fraction of nuclear material (Fernandes et al., 2007b; Leme et al., 2008) and resultantly CA's types are induced from nuclear abnormalities such as nuclear buds, MN, mini cells, lobated nuclei and polinucleated cells (Fernandes et al., 2007b).

It is reported that the CA's aberration such as breaks and fragments are induced due to formation of DNA–DNA and DNA–protein cross-links and heavy metals are considered as major contributor in this regard (Chandra et al., 2005). The mechanisms of CA involve clastogenic and aneugenic actions. Clastogenic action is characterized by the induction of chromosomal breakage during cell division, while aneugenic action comprises the inactivation of a cell structure such as the mitotic spindle, leading to chromosomal losses (Fenech, 2002; Leme and Marin-Morales, 2009).

The chromosomal adherence is another common sign of toxic effects on the genetic material and may cause irreversible effects on the cell, triggering the cell death process (Fiskesjo, 1993; Turkoglu, 2007) and can lead to develop chromosomal bridges and thereby, chromosomal breaks (Marcano et al., 2004). The chromosomal bridges resulted from adherence which can multiply and persist until telophase stage (Leme and Marin-Morales, 2008). Furthermore, chromosomal adherence is also associated with the formation of chromosomal bridges and eventually lead to chromosomal breaks (Marcano et al., 2004). The multipolar anaphases result from a disfunction of the mitotic spindle, which lead to unbalanced chromosome distribution, heading them for more than two poles, opposite to what occurs in the normal division cycle (Rank and Nielsen, 1998).

To evaluate the CA by using *A. cepa* is not a easy task, since it requires an accurate, adequate and precise knowledge of cell division phases and their possible abnormalities (Leme and Marin-Morales, 2009). Before the work of (Fiskejo, 1985; Rank and Nielsen, 1993), the CA applications for the estimation of polluting agent were uncommon. Afterword, a number of authors used CA's in *A. cepa* to detect the toxicity of wastewater samples of various origin (Andrade et al., 2008; Bernardes et al., 2015; Carita and Marin-Morales, 2008; da Costa Machado Matos Carvalho et al., 2011; Firbas and Amon, 2013; Geras'kin et al., 2011; Holan, 2014; Kwasniewska et al., 2012; Leme et al., 2008; Leme and Marin-Morales, 2008; Mazzeo et al., 2011a; Mishra, 1993; Monarca et al., 2000; Phugare et al., 2011; Tabrez and Ahmad, 2011; Wardini and Notodarmojo, 2015).

Table 1 shows the CA's percentage value versus negative control in *A. cepa* cells exposed to different pollutants, while the pictorial presentation of CA's can be seen in Fig. 2.

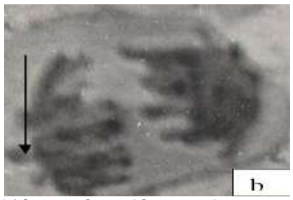
Genotoxicity evaluation

The toxicity evaluation on the basis of NA endpoint was started in the beginning of present decade and is characterized by morphological changes in nuclei during cell division. The NA include bi-nucleated, multi-nucleated cell, lobulated nuclei, nuclei carrying nuclear buds, mini cell, vacuolated nucleus, nucleus with nuclear wall lesions and deformed nuclei. The NA evaluation is a sensitive analysis to detect the toxic effects of any mutagen (Abdel Migid et al., 2007; Abu and Ezeugwu, 2008; Abu and Mba, 2011; Akinsemolu et al., 2015; Bianchi et al., 2011; Chandra et al., 2005; Hoshina and Marin-Morales, 2009; Leme and Marin-Morales, 2009).

The lobated nuclei can result from multipolar anaphases with chromosomal bridges and the presence of multipolarity during the nuclear division seems not to avoid the reorganization of the nuclear envelope and the membrane would follow the unbalanced distribution of the genetic material within the cell, resulting in the lobated nuclei and polynuclear cell (Leme et al., 2008). The presence of NA leads to the induction of cell death process (Leme et al., 2008; Leme and Marin-Morales, 2008). The nuclear bud is another NA type and is considered among the most frequent abnormality. The nuclear bud is well known genotoxic alteration and its formation is related to the initiation of the nuclear envelope formation prior to the total migration of the chromosomes to the opposite poles and consequently, their incorporation into the nuclei. The nuclear bud may originate as a result of chromosome breaks, bridges and rearrangements due to clastogenic action of agents, which hinder the proper reorganization of the chromatin in the nucleus. The nuclear buds may also be the result of cellular activities that promote the elimination of the amplified genetic material (Mazzeo et al., 2011a; Shimizu et al., 2000). Various researchers used this end point for genotoxicity evaluation of pollutants (Asita and Makhalemele, 2009; Chaparro and Pires, 2015; Olorunfemi et al., 2014a; Olorunfemi and Ehwre, 2011; Paiva et al., 2008; Phugare et al., 2010)

Fig. 3 shows the NA's formation reported in literature in the *A. cepa* root cells after exposure to toxic agents, while percentage NA's value versus negative control can be seen in Table 1.

Mutagenicity evaluation

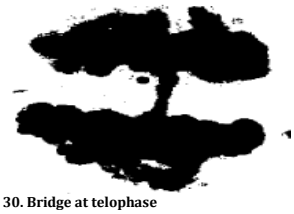

25. late anaphase with vagrant C

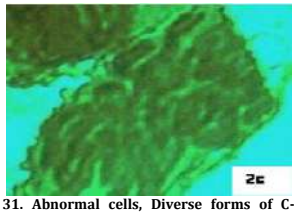

26. Ch. ring

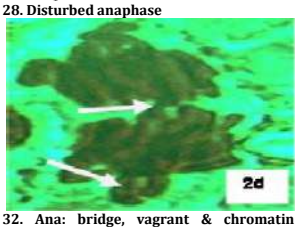

27. Anaphase: late separation of C


28. Disturbed anaphase


29. Disturbed telophase

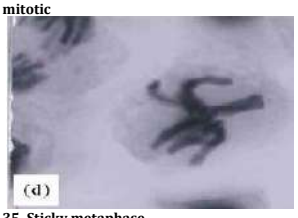

30. Bridge at telophase


31. Abnormal cells, Diverse forms of C-mitotic

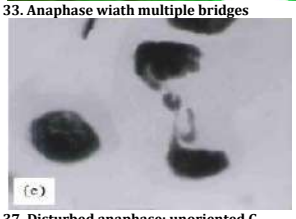

32. Ana: bridge, vagrant & chromatin degeneration


33. Anaphase wiath multiple bridges

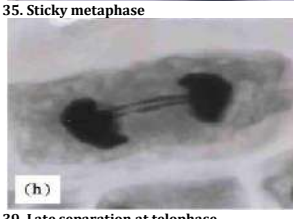

34. Sticky prophase


35. Sticky metaphase

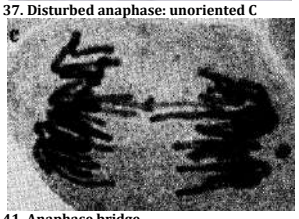

36. Disturbed metaphase: unoriented Ch

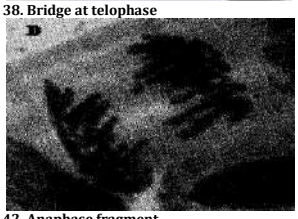

37. Disturbed anaphase: unoriented C

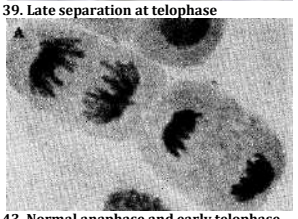

38. Bridge at telophase

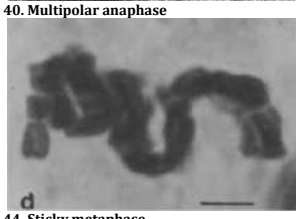

39. Late separation at telophase

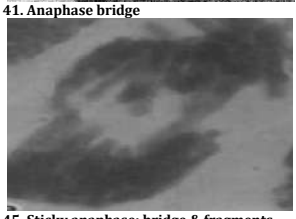

40. Multipolar anaphase

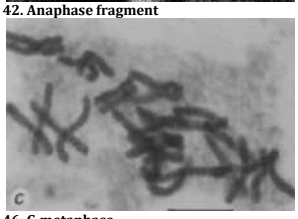

41. Anaphase bridge


42. Anaphase fragment

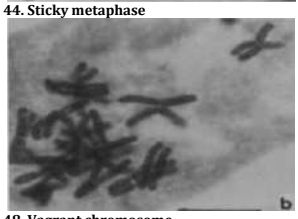

43. Normal anaphase and early telophase

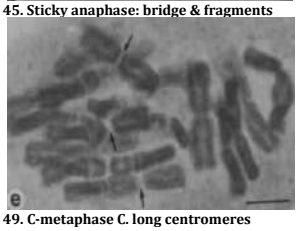

44. Sticky metaphase

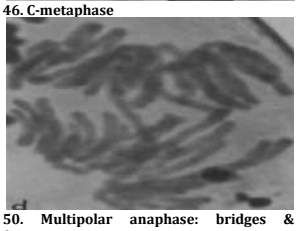

45. Sticky anaphase: bridge & fragments

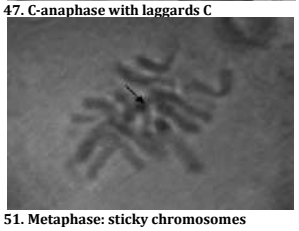

46. C-metaphase

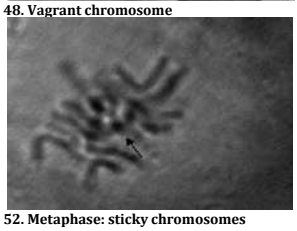

47. C-anaphase with laggards C

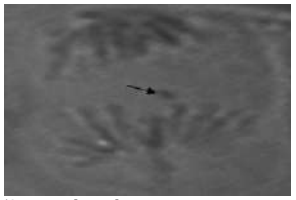

48. Vagrant chromosome

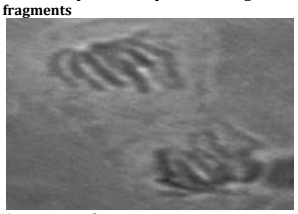

49. C-metaphase C. long centromeres

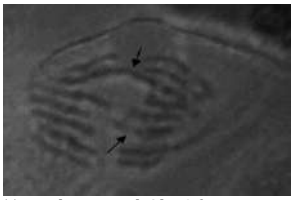

50. Multipolar anaphase: bridges & fragments


51. Metaphase: sticky chromosomes


52. Metaphase: sticky chromosomes


53. Laggard anaphase stage


54. Late anaphase stage


55. Anaphase stage: bridge & fragment

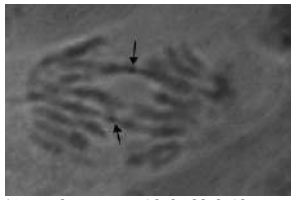

56. Anaphase stage with double bridge

Fig. 2: Continue…

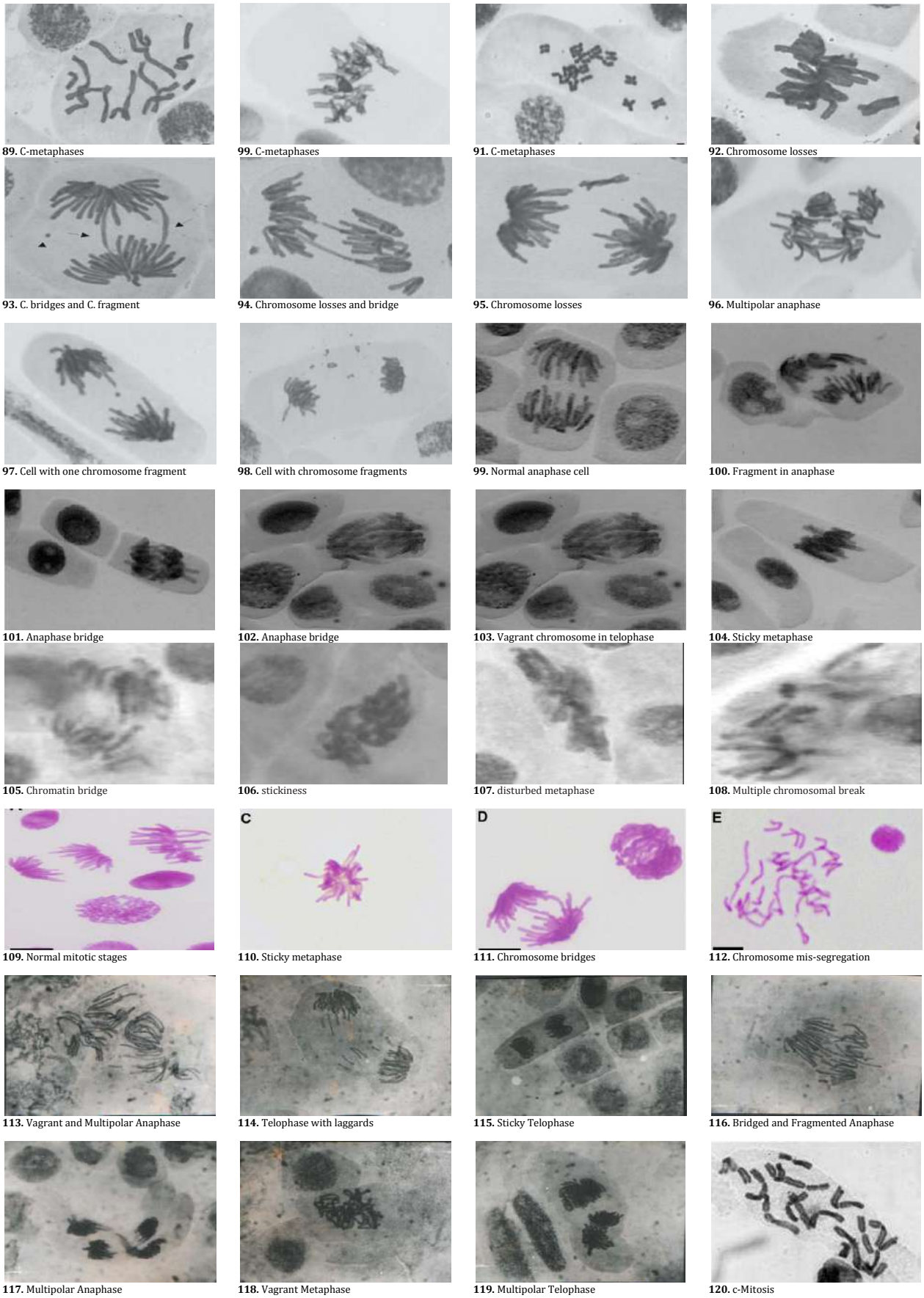
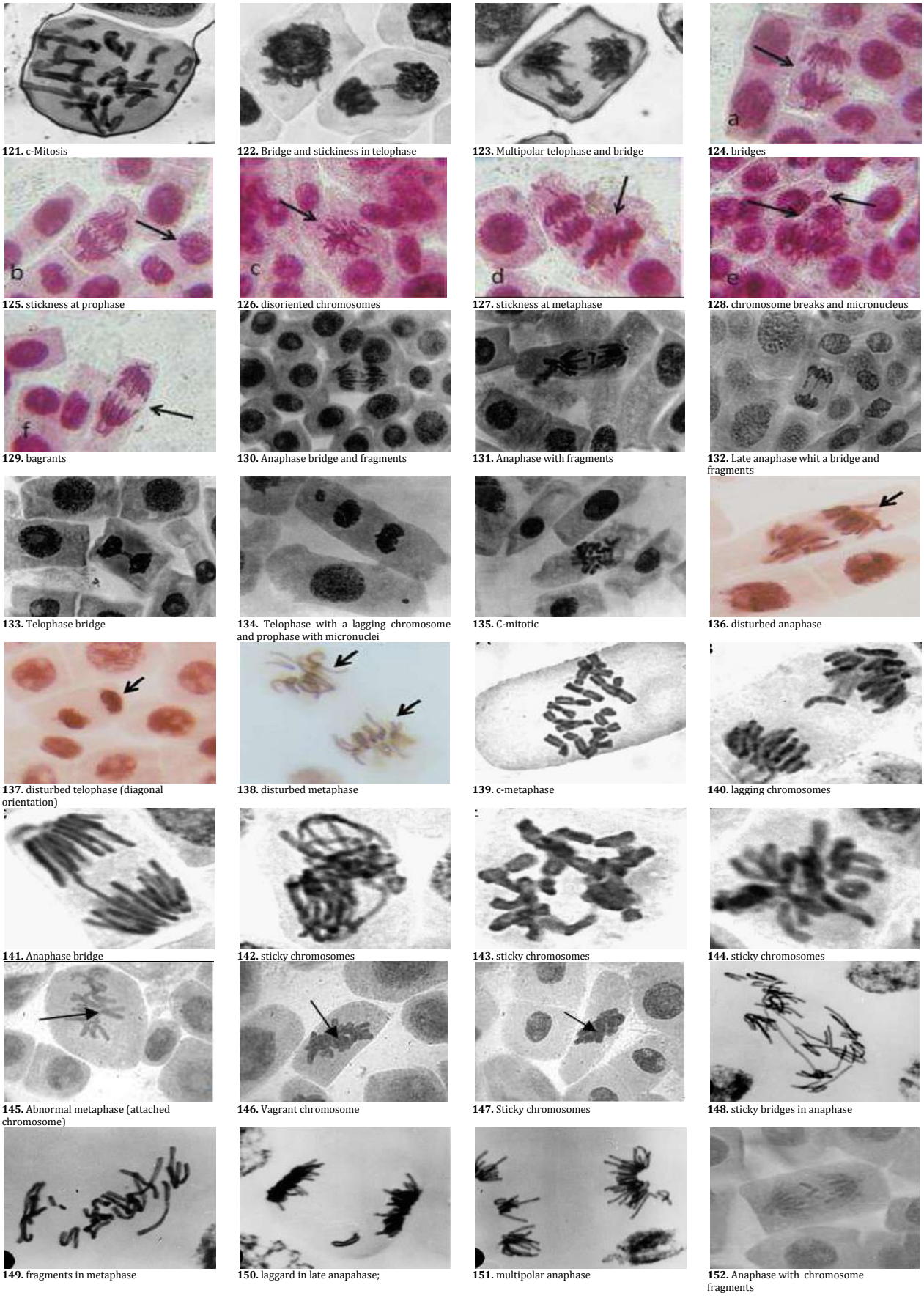
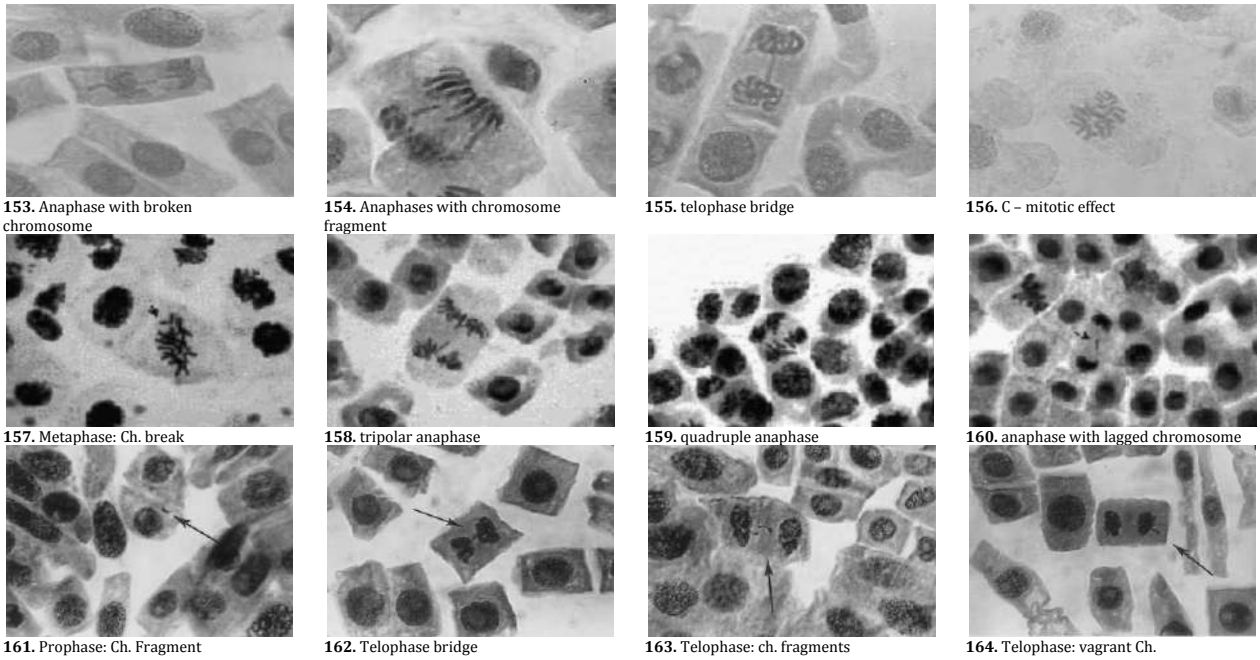


Fig. 2: Continue…



121. c-Mitosis
122. Bridge and stickiness in telophase
123. Multipolar telophase and bridge
124. bridges
125. stickness at prophase
126. disoriented chromosomes
127. stickness at metaphase
128. chromosome breaks and micronucleus
129. bagrants
130. Anaphase bridge and fragments
131. Anaphase with fragments
132. Late anaphase whit a bridge and fragments
133. Telophase bridge
134. Telophase with a lagging chromosome and prophase with micronuclei
135. C-mitotic
136. disturbed anaphase
137. disturbed telophase (diagonal orientation)
138. disturbed metaphase
139. c-metaphase
140. lagging chromosomes
141. Anaphase bridge
142. sticky chromosomes
143. sticky chromosomes
144. sticky chromosomes
145. Abnormal metaphase (attached chromosome)
146. Vagrant chromosome
147. Sticky chromosomes
148. sticky bridges in anaphase
149. fragments in metaphase
150. laggard in late anapahase;
151. multipolar anaphase
152. Anaphase with chromosome fragments

Fig. 2: Continue…



153. Anaphase with broken chromosome
154. Anaphases with chromosome fragment
155. telophase bridge
156. C – mitotic effect
157. Metaphase: Ch. break
158. tripolar anaphase
159. quadruple anaphase
160. anaphase with lagged chromosome
161. Prophase: Ch. Fragment
162. Telophase bridge
163. Telophase: ch. fragments
164. Telophase: vagrant Ch.

Fig. 2: Continue…

The MN endpoint in *A. cepa* test is simple and easy to evaluate as compared to the NA and CA, which results from damaging and wrongly repaired cells. As a result of MN abnormality, the nucleus size in daughter cell is reduced as compared to parent cell. The MN size can be an effective parameter to assess the clastogenic and aneugenic effects in *A. cepa*, since this species present a symmetric karyotype, which is homogeneous in relation to chromosomal size, with large and few chromosomes. Therefore, large MN would indicate an aneugenic effect resulting from a chromosome loss, whereas small MN may indicate a clastogenic action resulting from chromosome break. However, other cytogenetic techniques, such as chromosome banding and *in situ* hybridization, should be applied to make the analysis more reliable and accurate (Leme and Marin-Morales, 2008, 2009).

The MN abnormality is often results from the acentric fragments (clastogenic action) or lagging chromosomes or even from malfunctioning of the spindle/ malformation of the mitotic fuse (aneugenic action) that fail to incorporate into either of the daughter nuclei during telophase of the mitotic cells and can cause cell death due to the deletion of primary genes (Andrade et al., 2008; Sudhakar et al., 2001). The chromosome breakage are also associated with the formation of chromosome fragments and MN cells (Fiskesjo, 1993). Furthermore, the formation of MN may also result from spindle poisoning which is an anomalous disjunction of chromosomes at anaphase stage (Chandra et al., 2005; Grover and Kaur, 1999) or by eliminating the amplified genetic material (Mazzeo et al., 2011a). In general, the induction of MN in root meristems is the manifestation of chromosome breakage and disturbance of the mitotic process due to spindle abnormalities (Grover and Kaur, 1999) and MN are considered an indication of a true mutation effect (Abdel Migid et al., 2007).

The formation of multinucleated cells may be the result of a preceding multipolar mitosis or the failure of cell plate formation, a cytokinesis disturbance (Abdel Migid et al., 2007). Lobate nuclei and polynucleated cells are resultant of CA, as a consequence of multipolar anaphases, which are associated or not with chromosomal adherence, making the cells inviable (Fernandes et al., 2007b). Micronucleated cells can also arise from nuclear buds, which are eliminated from the nucleus through an active process during the S phase (synthesis phase- stage of cell cycle in which DNA is replicated) of the cell cycle (Shimizu et al., 1998).

The clastogenic/aneugenic effects of toxic agents can be evaluated precisely on the basis of MN. The MN derived from clastogenic agents are reported to be smaller than MN derived from aneugenic action of toxic agents (Leme and Marin-Morales, 2008). The nuclear buds are indicative of an initial process of releasing the exceeding nuclear material and, consequently, they can also be related to the MN formation, which might be further eliminated from cytoplasm as mini cell (Fernandes et al., 2007b).

The MN due to induction of chromosomal breaks during cell division, as well as by the expulsion of excess genetic material by nuclear buds, which later eliminated as MN has also been reported (Mazzeo et al., 2011a) and this induction is correlated with inhibitory action of the enzyme topoisomerase II, responsible for the reconnection of fragments of DNA during the replication process, which results in the clastogenic effect of toxic agent (Mondrala and Eastmond, 2010; Whysner et al., 2004). Moreover, Alberts et al. (Alberts et al., 2008) affirm that the inhibition of topoisomerase II leads to a tangle of the daughter chromosomes, which remain intertwined and therefore

unable to separate after replication. Thus, if such damage is not repaired, replication of a damaged DNA may lead to the amplification of the genetic material and extra material is originated by amplification and eliminated from the nucleus as an MN (Fernandes et al., 2007b; Mazzeo et al., 2011a). In view of MN importance, various authors used it as genotoxicity indicator for different systems (da Costa Machado Matos Carvalho et al., 2011; Geras'kin et al., 2011; Hoshina and Marin-Morales, 2009; Junior et al., 2007; Kwasniewska et al., 2012; Leme et al., 2008; Leme and Marin-Morales, 2008; Marcano et al., 2004; Mazzeo et al., 2011a; Misik et al., 2011; Nunes et al., 2011; Smaka-Kincl et al., 1996; Tabrez and Ahmad, 2011).

Fig. 4 shows the MN's that are reported in literature in the *A. cepa* root after exposure to various toxic agents, while percentage effect on MN formation versus negatice control can be seen in Table 1.

Industrial wastewater nature

Industrial effluents are characterized by changes in turbidity, conductivity, total suspended solids (TSS), chemical oxygen demand (COD), biological oxygen demand (BOD) and total hardness (TH). Some industries are contaminating water bodies by toxic materials, while other are responsible for colored effluents (KANU and Achi, 2011). Beside, heavy metals, the industrial wastewater also contains a considerable amount of inorganic materials, organic compounds, salts, detergents, surfactants, dyes, food additives etc, which affect the water physico-chemical properties and toxicity to living organisms (Abbasi et al., 2016; Asadi et al., 2016; Balapure et al., 2016; Barbosa et al., 2014; Barrera-Díaz et al., 2014; Behbahani et al., 2016; Bertani et al., 2016; Bordes et al., 2015; Camacho-Muñoz et al., 2014; Carbajo et al., 2015; Chae et al., 2016; Cheballah et al., 2015; Chong et al., 2015; Chou et al., 2014; da Costa Filho et al., 2016; da Costa et al., 2012b; Expósito et al., 2016; Gatsios et al., 2015; Gatta et al., 2015; Goh et al., 2015; Hoshina and Marin-Morales, 2009; Hu et al., 2015; Huang et al., 2016; Jeon, 2015; Kang et al., 2016; Kuroda et al., 2015; Lee et al., 2016; Lee et al., 2015; Liu et al., 2016; Lovatel et al., 2015; Meneses-Jácome et al., 2016; Olorunfemi et al., 2011b; Pereyra et al., 2015; Polat et al., 2015; Prieto et al., 2015; Sarkar et al., 2015; Sasmaz and Yaman, 2008; Sasmaz et al., 2015a; Sasmaz et al., 2016b; Sasmaz et al., 2016c; Sasmaz et al., 2016d; Sasmaz et al., 2015b; Şaşmaz et al., 2011; Seow and Hauser, 2016; Singh et al., 2016; Sueviriyapan et al., 2016; Tamis et al., 2014; Vasiliadou et al., 2016; Vymazal, 2014; Zakharov and Bondareva, 2015; Zarpelon et al., 2016; Zheng et al., 2015).

The current contamination of water resources, especially as a consequence of anthropogenic discharges, is becoming a major problem in urban regions and changes in water chemistry obviously have deleterious effects on the living organisms inhabiting these areas, especially aquatic biota directly and humans beings indirectly (Adesola et al., 2016; Amadi and Ukpaka, 2015; Babarinde and Onyiaocha, 2016; Iqbal and Khera, 2015; Jafarinejad, 2016a; Jafarinejad, 2016b; Jamal et al., 2015; Majolagbe et al., 2016b; Ohe et al., 2004; Pandey et al., 2016a, b; Peter and Chinedu, 2016a; Qureshi et al., 2015b; Sayed, 2015; Shindy, 2016; Ukpaka, 2016b; Ukpaka et al., 2015; Ukpaka, 2016e, f; Ukpaka, 2016g; Ukpaka and Collins, 2016; Ukpaka, 2016h). Among the lethal and sub-lethal effects of the complex mixtures, fertility disorders as well as cellular, metabolic, and DNA damages are reported (Vianna Villela et al., 2007). Epidemiological studies have failed to clearly characterize these effects, since the experimental procedure to demonstrate this influence is a time-consuming task. This scenario stresses the importance of efficiently and continuously monitoring possible impacted areas using screening assays. The *A. cepa* test has been used to detect a variety of environmental pollutants in industrial wastewater and the results obtained were reported significant versus control (Leme and Marin-Morales, 2009). A number of studies have been performed using A. cepa test system and positive responses of A. cepa have been reported i.e., complex mixture (Aguiar et al., 2016; Andrade-Vieira et al., 2012; Blagojević et al., 2009; Chakraborty and Mukherjee, 2010; Chakraborty et al., 2009; Klauck et al., 2013; Mazzeo et al., 2011b; Pathiratne et al., 2015; Pekol et al., 2012; Singh et al., 2014; Vidaković et al., 1993; Yahaya et al., 2012), anti-toxic agent (Aslantürk and Çelik, 2006; Frescura et al., 2012; Kuhn et al., 2015; Kumari et al., 2013; Majewska et al., 2003; Nantes et al., 2014; Oyeyemi and Bakare, 2013; Santana et al., 2016; Ślusarczyk et al., 2014; Ślusarczyk et al., 2016; Sultan and Çelik, 2009), contaminated soil (Christofoletti et al., 2013; Dash et al., 1988; Kataeva et al., 2012; Kopliku and Mesi, 2012b; Masood and Malik, 2013a; Meier et al., 1997; Oberholster et al., 2008; Paul et al., 2013; Saxena et al., 2004; Sayles et al., 1999), drugs (Firbas and Amon, 2014; Grisolia and Takahashi, 1991; Jos et al., 2003; Lutterbeck et al., 2015; Mišík et al., 2014; Onwuamah et al., 2014), detergents and surfactants (Bellani et al., 1991; Iqbal and Bhatti, 2015a; Pedrazzani et al., 2012; WANG and WANG, 2007), hair and food additive (Adeyemo and Farinmade, 2016; De Lima et al., 2010; Maiti et al., 2016; Onyemaobi et al., 2012; Roychoudhury and Giri, 1989; Tripathy and Rao, 2015), industrial wastewater (Bakare et al., 2012; Bohórquez-Echeverry et al., 2012; Chaparro and Pires, 2011; Gupta and Ahmad, 2012b; Gupta et al., 2012; Matsumoto and Marin-Morales, 2004; Obute et al., 2005; Watharkar and Jadhav, 2014; Xing et al., 1995), leachate/sludge/sewage (Amin, 2002; Bakare, 2001; Bakare et al., 2012; Christofoletti et al., 2012; Corrêa Martins et al., 2016; Garaj-Vrhovac et al., 2013; Klauck et al., 2015; Magdaleno et al., 2008; Mazzeo et al., 2015; Obidoska and Jasińska, 2008; Pelegrini et al., 2007; Roig et al., 2012; Shashank and Suresh, 2013; Tanti et al., 2009), metallic compounds (Gömürgen, 2005; Meng and Zhang, 1992; RENCÜZOĞULLARI et al., 2001; Teerarak et al., 2009; Wierzbicka, 1994; Yi and Meng, 2003; Yıldız et al., 2009), metals (Arambašić et al., 1995; Arya and Mukherjee, 2014; Barbosa et al., 2010; Carruyo et al., 2008; Chang et al., 1997; Geremias et al., 2010; Hemachandra and Pathiratne, 2015; Jiang et al., 2014; Karaismailoglu, 2014; Kopliku and Mesi, 2012a; Kumar et al., 2015; Lerda, 1992; Liman, 2013;

Liu et al., 2003; Liu et al., 1994; Marcano et al., 2002; Marcano et al., 2006; Md Nazmul et al., 2013; Mohan Murali Achary et al., 2008; Pal and Nandi, 1990; Palacio et al., 2005; Panda et al., 1997; Patnaik et al., 2013; Pepper et al., 1988; Qin et al., 2010; Seth et al., 2008a; Sharma et al., 2012; Singh, 2015; Wierzbicka, 1999a; Wierzbicka, 1999b; Wu et al., 2010; Yi et al., 2007b), model compounds (Abdel-Azeem, 2014; Al et al., 2013; Arkhipchuk et al., 2004; Asgher and Iqbal, 2013; Asita and Matobole, 2010; Ateeq et al., 2002; Bhatta and Sakya, 2009; BOKHARI et al., 2015; Cabaravdic, 2010; De Marco et al., 1986; Evandri et al., 2003; Flores Navarro et al., 2015; Freire et al., 2014; Geremias et al., 2011; González et al., 2012; Goujon et al., 2015; Hardy et al., 2011; Hassan and YASSEIN, 2016; Herrero et al., 2012; Jadhav et al., 2012a; Jadhav et al., 2012b; Jha et al., 2009; Jos et al., 2005; LIU et al., 2009; Mellado-García et al., 2016; Mesi and Kopliku, 2014; Mohammed et al., 2015; Pandey et al., 2014; Panneerselvam et al., 2012; Patil and Jadhav, 2013; Patra et al., 2005; Paul et al., 2013; Petriccione et al., 2013; Poonkuzhali et al., 2011a; Quilang et al., 2008; Ragunathan and Panneerselvam, 2007; Rainho et al., 2010; Ramos de Rainho et al., 2013; Rank et al., 2002; Rathore and Choubey, 2005; Ronchi et al., 1986; Tabrez and Ahmad, 2012; Trushin et al., 2013a; Trushin et al., 2013b; Türkoğlu, 2008; Wasi et al., 2011; Yi and Meng, 2003; Younis et al., 1989), disinfected water/tap water (Crebelli et al., 2002; Olorunfemi, 2013), nano materials (Babu et al., 2008; de Andrade et al., 2014; Fernández Freire et al., 2015; Ghodake et al., 2011; Ghosh et al., 2015; Grillo et al., 2015; Klančnik et al., 2011; Kumari et al., 2011; Kumari et al., 2009a; Nagaonkar et al., 2015; Pakrashi et al., 2014; Pesnya, 2013), pharmaceutical, pesticides, herbicides, fungicide & insecticide (Akintonwa et al., 2009; Andrioli et al., 2006; Asita and Makhalemele, 2008; Bakare et al., 2009; Bianchi et al., 2016; Bianchi et al., 2015; Bolle et al., 2004; Çavuşoğlu et al., 2012a; Chauhan et al., 1986; Chauhan and Gupta, 2005b; da Lima et al., 2012; de Campos Ventura-Camargo and Marin-Morales, 2016; Feretti et al., 2007; Fernandes et al., 2007a; Fisun and Rasgele, 2009; Goujon et al., 2014; İLBAŞ et al., 2012; Karaismailoglu, 2015; Kaymak and Muranli, 2005; Lateef et al., 2007; Liman et al., 2010b; Liman et al., 2011; Liman et al., 2015; Marin-Morales, 2015; Maselli et al., 2015; Mesi and Kopliku, 2013; Mishra et al., 2015; Mosuro et al., 1999; Musanovic et al., 2013; Mustafa and Suna Arikan, 2008; Ozakca and Silah, 2013; Özkara et al., 2015a; Özkara et al., 2015b; Palma et al., 2014; Rank and Nielsen, 1997a; Rodríguez et al., 2015; Saxena et al., 2010; Shaikh et al., 2012; Silveira et al., 2015; Sinha and Kumar, 2014; Srivastava and Mishra, 2009; Tripathy et al., 2013; Tripathy and Patel, 2015; Türkoğlu, 2012), plants extracts/vegetable grown in contaminated water and soil (Adegbite and Sanyaolu, 2009; Ali, 2010; Aşkin Çelik and Aslantürk, 2010; Bidau et al., 2004; Bittencourt De Souza et al., 2010; Bratu et al., 2012; Çavuşoğlu et al., 2016; Çavuşoğlu et al., 2012b; Chukwujekwu and Van Staden, 2014; Eren and Özata, 2014; Feretti et al., 2007; Frescura et al., 2012; Glińska et al., 2007; Glińska and Gabara, 2011; Jothy et al., 2013; Knoll et al., 2006b; Kuraś et al., 2006; Kwankua et al., 2010; Leite et al., 2015; Lubini et al., 2008; Mauro et al., 2014; Mekki, 2014; Mendes et al., 2012; Mohammed et al., 2015; Olorunfemi et al., 2011a; Pastori et al., 2013; Prajitha and Thoppil, 2016; Ragunathan and Panneerselvam, 2007; Renjana and Thoppil, 2015; Saleh Alqasoumi, 2012; Sharma et al., 2011; Shibata and Hizume, 2002; Silva et al., 2013; Solanke, 2008; Soliman, 2001; Sondhi et al., 2008; Sultan and Çelik, 2009; Tartar et al., 2006; Timothy et al., 2014; Yuet Ping et al., 2012), air pollution (Blagojević et al., 2009; Glasencnik et al., 2002; Glasenčnik et al., 2004), radioactive metals, radioactive wastes and radiations (Evseeva et al., 2005; Saghirzadeh et al., 2008; Tkalec et al., 2009; Udalova et al., 2014), surface water and contaminated river water (Athanásio et al., 2014; Barbério et al., 2011; da Costa et al., 2012a; de Oliveira Meneguetti et al., 2012; Düsman et al., 2014; Espinoza-Quiñones et al., 2009; Fawole et al., 2008; Fernandes et al., 2010; Geras'kin et al., 2011; Grippa et al., 2012; Júnior et al., 2007; Mazzeo and Marin-Morales, 2015; Mesi and Kopliku, 2011; Oberholster et al., 2008; Olorunfemi et al., 2012; Palma et al., 2010; Roy et al., 2015; Serra de Lima Moraes and Jordao, 2001; Vujošević et al., 2008; Wängberg et al., 1995), treated waste and wastewater (Ameta et al., 2003; Balan et al., 2013; Balan et al., 2012; Bhat et al., 2014; Brkanac et al., 2014; Edemaa and Okungbowa, 2011; Geremias et al., 2012; GRISALES PENAGOS et al., 2012; Gupta and Ahmad, 2012b; Iqbal et al., 2015a; Iqbal et al., 2014; Iqbal and Nisar, 2015; Jadhav et al., 2015; Kern et al., 2013; Kusumaningrum et al., 2012; Machado et al., 2012; Masood and Malik, 2013b; Mazzeo et al., 2011b; Netto et al., 2013; Olorunfemi et al., 2011c; Olorunfemi et al., 2013; Papa et al., 2016; Patel et al., 2015; Patil and Jadhav, 2013; Poonkuzhali et al., 2013; Poonkuzhali et al., 2011a; Prasad et al., 2013; Ravindran et al., 2015; Roa et al., 2012; Satar and Husain, 2011; Sobral et al., 2013; Viana et al., 2014; Wasi et al., 2011; Watharkar and Jadhav, 2014; Watharkar et al., 2013), toxin (Laughinghouse Iv et al., 2012), wastewater (Chaparro and Pires, 2011; Defaveri et al., 2009; Fatima and Ahmad, 2005, 2006a; Frank and Harangozó, 1994; Gupta and Ahmad, 2012b; Gupta et al., 2012; Kern et al., 2015; Kopliku et al., 2012; Magdaleno et al., 2014; Somashekar and Arekal, 1983; Tabet et al., 2015; Viana et al., 2014), complex environmental mixtures, solid waste, surface and ground water of an urban and industrial origin (Smaka-Kincl et al., 1996; Umebese et al., 2013), sludge from municipal treatment stations (Rank and Nielsen, 1998), wastewater contaminated soil (Kwasniewska et al., 2012), water bodies contamination with radioactive material (Evseeva et al., 2003), hospital wastewater (Guzy et al., 2012) and river water receiving industrial wastewater (da Costa Machado Matos Carvalho et al., 2011). In all these studies, author's used *A. cepa* test for toxicity monitoring and positive results have been documented.

In following section, the wastewaters and related matrices genetotoxic, cytotoxic and mutatoxic effect in *A. cepa* cells are discussed.

Application of *A. cepa* to detect the toxicity of industrial wastewater and contaminated soil and river water

Table 1: The chromosomal aberrations, nuclear aberrations, aberrant cell, total aberration, micronucli, mitotic index and root length percentage effect observed in A. cepa root cells exposed to different toxic agents

Industry	RL	MI	MN	AC/TA	CA and NA types	Refeences
Metal industry leachates (10 %)	---	---	0.14%*	---	Multipolar ana, Aberrant anaphase, unequal chromosome, bridge (4%), stikiness(83.33%), Frag(10%), Lagg(80%)	(Chandra et al., 2005)
Dye industry leachates	---	---	7.81%	/0.13%	Multipolar anae, aberrant anae, unequal chromosome, stikiness and lagg	(Chandra et al., 2005)
Paint industry WW	---	---	---	3-7%/	Bridges(3-5%), stikiness(6%) and Frag(3-5%)	(Samuel et al., 2010)
Textile WW	---	---	---	4-5%/	Bridges(2-5%), Stikiness(1-2%) and Frag(2-5%)	
Bottling industry (10%)	---	---	1%	5-8%/	Bridges(4-6%), Stikiness(5-7%), Frag(2-3%) and lagg(1-3%)	(Olorunfemi et al., 2011)
Rubber industry (10%)	---	---	1-5%	2-8%/	Bridges(3-5%), Stikiness(1-7%), Frag(1-4%) and lagg(1-5%)	(Olorunfemi et al., 2011)
Brewery (10%)	---	---	1%	5%/	Bridges(2-5%), Stikiness(1-4%), Frag(1-2%) and lagg(1-4%)	(Olorunfemi et al., 2011)
Chemical Industry (100%)	---	---	---	48%/	Abn. Pro(14%), Meta(ND), Ana(100%) and Telo(ND)	(Abu and Ezeugwu, 2008)
Al metal leachates (5%)	---	---	---	---	Cell death (73-90%), C-mitosis (28-33%), multipolar ana (0.08-0.13%), Bridge(25.12%), Stikiness(5.08%) and frag(8.33%)	(Andrade et al., 2008)
Industrial area site	---	-62%	52.00%	/71%	Bridges, ch. rings, frag, late separation, disturbed ch, prophase (-24%), meta(-23%), ana(-36%) and telo(-26%)	(El-Shahaby et al., 2003)
	---	---	---	35.47%/	Bridges and stikiness,	(Abdel Migid et al., 2007)
River water summer	---	---	58.06%	---	CA(30.91%), mitotic cell (-37.90%) and cell death (40.74%)	(Bianchi et al., 2011)
River water autumn	---	---	29%	---	CA(45.18%) and mitotic cell (-36.22%),	(Bianchi et al., 2011)
River water winter	---	---	38%	---	CA(11.09%), mitotic cell (-57%) and cell death(78%)	(Bianchi et al., 2011)
Soil sample irrigated with industrial WW	---	---	53-81%	---		(Cabrera and Rodriguez, 1999)
Textile WW (100%)	---	---	5.00%	---	Ana bridge, ana loss,,microcyte, meta loss, C-meta, delayed telo, telo bridge,binucleated cell, adherence and cell death	(Carita and Marin-Morales, 2008)
Industrial WW contaminated soil	---	-30%	0.2-23%	---		(Cotelle et al., 1999)
Lemon juice factory	-27%	---	---	---	Aberrant ana	(Gana et al., 2008)
Paper factory	-16%	-33%	1%	---	Aberrant ana	(Gana et al., 2008)
Mining industry	-14%	-46%	1.60%	---	Aberrant ana	(Gana et al., 2008)
Sugar industry	-14%	-46%	---	---	Aberrant ana	(Gana et al., 2008)
Acid mine drainage	---		---	---	Chromatid breaks and fragmentation	(Geremias et al., 2012)
	---	-53%	+	/70%	Lagging chromosme, bridges and Stikiness	(Grisolia et al., 2005)
Textile and paper mills	---	---	65%	---	Aberant ana	(Grover and Kaur, 1999)
Refinery WW	-68%	---	90%	/97%	Laggard chromosome(88%), late anap, bridges, stikiness and frag(93%),	(Gupta and Ahmad, 2012)
Petroleum refinery contaminated river water	---	-41%	---	---	Cell death and CA's(88.88%)	(Hoshina and Marin-Morales, 2009)
Textile WW (30%)	-6%	-19%	---	/86%	Chromosome breaks, delayed ana, multipolar ch, binucleated cell, ana with loss, meta with loss, bridges(92%) and lagg(7%)	(Jadhav et al., 2010)
Tannery waste summer	-71%	-9%	---	---	Overall microscopical effects(20%)	(Junior et al., 2007)
Tannery waste winter	-37%	-28%	---	---	Overall microscopical effects(11%)	(Junior et al., 2007)
Pesticide contaminated soil	---	---	---	---	CA, bridges(40%) and Frag(14%)	(Kong and Ma, 1999)
Soil sample (Chernobyl accident)	---	---	---	/81.02%	Multipolar ana(95.65%), C-mitosis, vagrant ch(84.21%), bridges(29.52%), stikiness and frag(12.53%)	(Kovalchuk et al., 1998)
Oil refinery contaminated river water	---	---	---	---	Lobated nuclei, polynucleated cells, nuclear buds, mini cells, C-metaphases(78%), multipolarity(95%), Ch. losses (96%), Lagg(90%), bridges(73%), stikiness(14.89%) and frag(80%)	(Leme et al., 2008)
Chemical industry	---	---	---	---	CA and interphase nucleus aberrations, multinucleated cells, late-separating ch, unoriented ch. bridges, stikiness, lagg	(Abdel Migid et al., 2007)
Sewage (20%)	-2.50%	-41%	---	---	Stickiness, C-mitosis(1%), vagrant(4%) and bridges(3%)	(Ukaegbu and Odeigah, 2009)

Table 1: Continue…

Paper mill	-44.28%	-44%	---	---	Early ana(7.7%), stickiness(3.1%), C-mitosis(12.2%), bridges and fragments(6.8%), banded ch(3.4%) and multipolar ana	(Tipirdamaz et al., 2003)
Disinfected water with ClO_2	---	-10%	---	---	Anaphase aberation	(Monarca et al., 2003)
Disinfected water with NaClO	---	-11%	---	---	Anaphase aberation	(Monarca et al., 2003)
Disinfected water with PAA	---	7.40%	---	---	Anaphase aberation	(Monarca et al., 2003)
Gasoline station WW (50%)	---	-14%	---	60%/	Fragments and laggard chromosomes	(Oliveira-Martins and Grisolia, 2009)
Industrial water contaminated soil extracts	---	---	---	---	Fragments, bridges, lagg, vagrant and Aberrant ana(92.13%)	(Katnoria et al., 2011)
Textile effluents (100%)	-92.82%	-40%	---	/6.8%	Stickiness, vagrant, bridges, fragments and multipolar anaphase	(Oriaku et al., 2011)
Insecticide and shoe polish factory (100%)	---	-50%	---	81%/	Abn. prophase(94%), abn. meta(72%), abn. ana(85%), abn. telo(48% for 75% conc.)	(Abu and Ezeugwu, 2008)
Pharmaceutical WW	-95%	-80%	---	---	C-mitosis, ana bridge, multiple nuclei	(Abu and Mba, 2011)
Al metal industry (25 %)	-98%	---	---	---	Late seg(83%), bridges(70%), stickiness(63.63%), frag, cell death (90%), C-metaphase(33%), multipolar ana	(Andrade et al., 2008)
River wastewater	-48%	-66%	50%	---	Anaphase bridges, ch. fragments and delayed anaphase	(da Costa Machado Matos Carvalho et al., 2011)
Mineral water	---	---	---	---	Bridges, fragments, lagg and C-mitosis	(Evandri et al., 2000)
Radium production industry territory water	---	-50%		/2-4%	Single and double fragments, bridges and vagrant ch.	(Evseeva et al., 2003)
Landfill leachates-drainage pumping stations	---	-80%	---	---	CA	(Kwasniewska et al., 2012)
Refinery contaminated river WW	---	-13%	95% & 98%(F1)	---	CA (89%)	(Leme and Marin-Morales, 2008)
HC mixture	---	---	85%	/79%	Adherence, lagg, C-mitosis, Ch. loss, bridges, Ch. breaks, multipolarity, nuclear buds, polynucleated, polypolid, mini cell, lobulated	(Mazzeo et al., 2011)
Disinfected water	---	---	4%	---	CA (3.02-4.14%)	(Monarca et al., 2002)
Oil field WW	---	-71%		/90%	Stickiness, C-mitosis, vagrant, multipolar ana, bridges and frag	(Odeigah et al., 1997)
Bottling industry (10%)	-33%	---	---	7.72%/	Binucleated cell, lagg, bridges, frag, stickiness, vagrant, C-mitosis and disturbed spindle	(Olorunfemi et al., 2011)
Rubber industry (10%)	-21%	---	---	7.33%/	Binucleated cell, lagg, bridges, frag, stickiness, vagrant and disturbed spindle	(Olorunfemi et al., 2011)
Brewery industry (10%)	-13%	---	---	5.41%/	Binucleated cell, lagg, bridges, frag, stickiness, vagrant, C-mitosis, disturbed spindle	(Olorunfemi et al., 2011)
WW phosphoric gypsum plant	-73%	---	---	/93%	C-mitosis, multipolar ana, lagg ch, sticky and polyploidy	(Pavlica et al., 2000)
Textile WW	---	-10%	---	/87%	Bridges, frag and vagrant	(Phugare et al., 2011)
Treated WW (bioremediation)	---	-1%	---	/55%		(Phugare et al., 2011)
WW treatment plants	---	---	---	/45%	Bridges(31%), frag(66%), vagrant(66%), other berations (66%)	(Rank and Nielsen, 1998)
Oil refinery (100%)	---	-4%	25%			(Rodrigues et al., 2010)
Paint industry (100%)	-71%	---	---	/7% (72% con)	Stickiness, Cc-mitosis, vagrant, bridges, fragment, binuclin multipolar ana, attached chromosome (72% conc),	(Samuel et al., 2010)
Textile WW (100%)	-84%	---	---	/5%	Stickiness, C-mitosis, vagrant, bridges, frag, binuclin multipolar ana, attached chromosome(72% conc),	(Samuel et al., 2010)

Table 1: Continue…

Industrial mixture	---		-50%	---		---	CA (polar slip, irregularities at anaphase, irregular distribution of ch in meta, Ch. distortion, lagg, stickiness and bridges)	(Sik et al., 2009)
Oil refinery landforming	---	---		82%	---		Adherence, breaks, bridges, nuclear buds and other CA(36%)	(Souza et al., 2009)
Industrial waste mixture	---		-47%	---		---	Stickiess, fragments, laggards and bridges	(Tabrez and Ahmad, 2011)
Fertilizer & petrochemical WW	-30%	---		---		/83%	Multipolar ana, anaphase bridge, ana frag, other aberrations (up to 93%)	(Vujosevic et al., 2001)

RL–root length, MI–mitotic index, MN–micronucli, AC–aberrant cell, TA–total aberrations, CA–chromosomal aberration, NA–nuclear aberrations, ana–anaphase, frag–chromosome fragments, lagg–laggards chromosome, Abn–abnormal, pro–prophase, meta–metaphase, telo–telophase, ch–chromosome

Petroleum and petrochemical

Petroleum industry is one of the most important sources of energy, and waste water discharged is a serious problem from environmental point of view. The refinery effluents consist of compounds from original crude oil stock as well as metallic (Zn, Cr, Ni, Pb, Cu) and non-metallic constituents. Phenols are also a major component of refinery wastewater. Moreover, among the hydrocarbons present in crude oil, the polycyclic aromatic hydrocarbons (PAHs) are among the most dangerous environmental contaminants due to their toxicity, carcinogenicity and mutagenicity (Gupta and Ahmad, 2012a). According to Leme and Marin-Morales (Leme and Marin-Morales, 2009), aromatic hydrocarbons are considered hazardous environmental pollutants due to the harmful effects they have on different living organisms.

The genotoxicity of petroleum refinery wastewater was investigated using *A. cepa* assays. Bulbs were germinated in distilled water and exposed to 20%, 40%. 60%, 80% and 100% wastewater for 48 h. Root analysis revealed that the wastewater induced mitodepressive effects in dose dependent manner, culminating in total mitotic inhibition at 100% v/v concentration. Among CA's, stickiness, erosion of chromatin, vagrant chromosomes, fragments and anaphase bridges were observed in root oxposed to 60%-80% of wastewater. Lower concentrations (20%-40%) induced c-mitosis as the major aberration (Obute et al., 2005).

The toxicity of river water impacted by an oil pipeline leak was studied (Leme and Marin-Morales, 2008) and the *A. cepa* meristematic cell exposed to wastewater showed clastogenic and aneugenic effects. CA's inductions were most common, which were attributed to the presence of hydrocarbons. In another study, same authors also reported the CA's and MN induction in *A. cepa* roots when exposed to river water contaminated with petroleum hydrocarbons and polycyclic aromatic hydrocarbons (PAHs). The larger CA's and MN incidence in the meristematic cells of *A. cepa* was documented after exposure to water sample collected during the dry season, at the spring of the river, where the oil leak has arisen. The F_1 cells from roots exposed to such sample were also analyzed for the incidence of MN, also showing a larger frequency of irregularities (Leme et al., 2008).

In order to assess the toxicity before and after landfarming bioremediation of oil refineries wastwater, *A. cepa* MN, NA and CA endpoints were studied (Souza et al., 2009). Authors investigated the landfarming and landfarming with rice hulls amendment before and after hydrocarbons biodegradation. Landfarming presented 13.5 g/kg of total petroleum hydrocarbons which induced strong clastogenic and mutagenic effects in *A. cepa* root cells. After 108 days of biodegradation, the concentration of hydrocarbons decreased by 27% and there was significant reduction in mitotic abnormalities, CA, MN and nuclear bud. Landfarming treated with rice hulls decreased the CA, MN and nuclear bud and correlated this efficiency with the biodegradation efficacy of landfarming. Finally, authors concluded that the *A. cepa* test is suitable to evaluate biodegradation process in soils contaminated by petroleum products. In another study, wastewater collected from petrochemical industry showed negative effect on the growth of *A. cepa* roots (CA's) for 24 h of exposure only (Nielsen and Rank, 1994). The MI, MN and CA's as indicators of toxicity in *A. cepa* exposed to petroleum wastewater were used and results showed that there was a significant alteration in MI and MN and CA's induction (Turkmen et al., 2009).

The toxicity of Mathura refinery wastewater adjoining area of Mathura refinery, Mathura, India was monitored using *A. cepa* roots. In exposed roots a significant formation of MN and CA's (bridges) was observed (Gupta and Ahmad, 2012a). The cytogentic effects of Mathura refinery wastewater and Aligarh wastewater of Northern India were also confirmed using IC_{50} value in *A. cepa* root. *A. cepa* genotoxicity test demonstrated a considerable amount of chromosomal damage. The aberration index for Aligarh wastewater and refinery wastewater was recorded to be 11.2% and 14.7%, respectively, whereas the aquaguard mineral water serving as negative control displayed the aberration index value to be 2.6%. The genotoxicity of both industrial wastewaters was reduced to a remarkable extent in presence of mannitol, the hydroxyl radical scavenger. Chromosomal aberrations were predominantly stickiness and stray chromosomes in case of Aligarh wastewater, while clumping and stickiness in case of refinery wastewater, thereby affirming the genotoxicity of both test waters (Fazili and Ahmad, 2014).

Similarly, the toxicity (root growth inhibition, malformation and CA's formation) of an oil field wastewater in the *A. cepa* root cells was reported (Odeigah et al., 1997). A

series of 10 small bulbs of *A. cepa* were cultivated in various concentrations of the wastewater and after 48 h exposure, root tips were analyzed for cytological studies, while root lengths were measured after 96 h, which resulted in significant dose dependent root growth inhibition. The wastewater treated group of plant showed significantly increased frequency of CA's such as sticky chromosomes, C-mitosis, spindle multipolarity, bridges and fragments. At lower concentrations only C-mitosis was observed.

While studying the nature of Santa Maria da Vitória River, Brazil (receive domestic, municipal and agricultural waste), Grippa *et al.* (2012) employed *A. cepa* test. Water samples were collected from 5 sites in the rainy season (March—high temperatures) and in the dry season (August—low temperatures) in 2006. The CA index was significantly higher at 2 sites in the rainy season compared with the dry season. This genotoxic effect may be related to agricultural influence and to domestic effluents that occur close to these 2 sites and to the presence of fertilizers, pesticides and iron oxide from the basin soils that are leached out by the rain and reach the river. In another study, the genotoxic potential of effluents from oil refineries by *A. cepa* test was studied (Rodrigues et al., 2010). The MN assay revealed the presence of 25% MN and 4% of MI inhibition after 24 h of treatment, however, these values were found non-significant in comparison to control. In order to evaluate the genotoxicity of petrochemical wastewater in *A. cepa,* CA test was employed (Vujosevic et al., 2001). In *A. cepa* root clear growth inhibition was observed. The CA abnormalities were also observed statistically significant in the treated group of plant. In another study, the toxicity and genotoxicity of wastewater from gasoline stations in Brasília, Brazil was studied by assessing CA's and MI in *A. cepa* root cells. The cell proliferation was significantly inhibited, especially at the highest concentrations. These results showed that although the effluent from gasoline stations was processed by an oil/water separation system before being discharged into the main sewage system, the wastewater still contained toxic compounds and further treatment was suggested to reduce the toxicity load (Oliveira-Martins and Grisolia, 2009).

Recently, the toxic effect of Nigerian Agip Oil Company (NAOC), Ogboinbiri, Bayelsa State was studied. Macroscopic analysis showed that *A. cepa* exposed to wastewater revealed concentration-dependent root growth inhibition with an EC_{50} value of 14.8 % at 96 h. Root tip cells of the onion bulbs processed for cytological studies showed chromosomal aberrations at all concentrations. Along with aberrant chromosomes and reduction of MI was also observed in concentration-dependent manner and authors suggested the proper treatment of process water before its discharge into water bodies to avoid cyto-genetic damages to aquatic lives (Olorunfemi et al., 2015).

Similar to petroleum refinery effluents, Tanti *et al.* (2009) studied the toxicity of the oil refinery sludge using *A. cepa* assay. Roots exposed to different concentrations showed high MI values and lower percentage of abnormality in roots treated with diluted sludge. The MI values were decreased with concentrations. Binucleate, sticky anaphase erosion, C-metaphase, polyploidy, unequal distribution of chromosomes, spiral nature of chromosome and distortion of poles were the common CA's. Both duration and concentration of the treatment influenced the cell division. Results revealed that sludge generated by oil refinery may induce negative impact on crop and other biota in the agro-ecosystem. The petrochemicals, air liquid and polyester resin effluents using *A. cepa* assay were also studied (Abu et al., 2015). Chemical analysis of the wastewaters showed high concentration of heavy metals. Mitodepressive were observed and MI vlues were recorded as 14.0, 5.3 and 4.1 in control, air liquid and polyester resin effluents, respectively. Percent abnormal dividing cells (C-mitotic effects to precocious chromosomes and anaphase bridges) increased with increase in wastewater concentration. Results indicates the cytotoxic and genotoxic effects of wastewaters tested, which could serve as indicator of the deleterious effects of wastewaters tested on living organisms at the point of discharge – either on land or water bodies. The need for sound sewerage system that would protect flora and fauna in the ecosystem was suggested.

The genotoxic effects of refined petroleum products (Petrol, Kerosene) has also been assessed using *A. cepa* test (Obute et al., 2016). The roots were treated with 10, 25 and 50% of these chemicals for 1, 3 and 6 h and resultantly, depressive effects on mitosis i.e., reduced MI, mitotic phases abnormalities and CA's (anaphase bridges) were observed in petrol and zobo, laggards and vagrant chromosomes observed in zobo, while sticky chromosomes were observed in all sample tested. MN inductions were observed in zobo and petrol, whereas ghost cells were observed in zobo samples. Results suggested the presence of clastogens agents in tested samples and authors advised minimal consumption, contact and reduction in the duration of exposure.

The effect of oil refineries wastewater on *A. cepa* roots is attributed to changes in the cellular volume and permeability of the cellular membrane, which seems to be associated with changes in the cytoskeleton. One reason for the increase of permeability would be due to lipophilic compounds that enhances the permeability and as a consequence the plasma membrane it loses the protection function (Hoshina and Marin-Morales, 2009; Thakkar et al., 2000). Some PAHs can induce an increase in the production of reactive oxygen species (ROS) and ROS can affect the cytoskeleton by changes in the redox state of regulatory enzymes and ultimately cell death, due to the exposition to petroleum by products such as aromatic hydrocarbons (Gomez-Mendikute and Cajaraville, 2003). In this regard, various authors reported the toxicity by exposing the *A. cepa* roots to aqueous solution of aromatic hydrocarbons (Rank and Nielsen, 1994). The genotoxic and mutagenic potential of benzene, toluene, ethylbenzene and xylene were also reported by applying CA and MN *A. cepa* test system (Mazzeo et al., 2011a).

Metallurgical waste and metals

Table 2: The observed toxicities in *A. cepa* root cells as a result of exposure to industrial waste, contaminated soil and river water

Waste source	Physiochemical properties	Endpoint	Cell Type	Results	References
Engineering plant WW	The wastewater contain heavy metals		MC	TC	(Frank and Harangoz, 1994)
Mixture	Metallic, chemical, textile, petrochemical, pulp and paper industry WW	CA, MI	MC	GT, CT	(Nielsen and Rank, 1994)
Mixture	Composition not reported	MN	MC & F_1	MT	(Ma et al., 1995)
Mixture	Industrial and municipal wastewater, biological treatment plant output water; water from the Drava river	CA, MI, MN	MC	CT, GT, MT	(Smaka-Kincl et al., 1996)
Oil field wastewater	Composition not reported	CA, MI	MC	GT, CT	(Odeigah et al., 1997)
WW treatment plants ludge	pb-51-390, Ni-17-45, Cr-32-137, Zn-691-1420, Cu-82-378 and Cd-0.8-6.2 mg/Kg	MI, CA	MC	CT, GT	(Rank and Nielsen, 1998)
Soil sample (Chernobyl accident)	Cs_{137}- 2287, K_{40}-222), Th_{232}-10 and Sr_{90} Bq/Kg, pH-5.65, N-5.60, P-32.0, K-22.5, humus-3.63%, Cu-2.2, Zn-27.2, Cd-0.5, Cr-0.52, Ni-1.02, Pb-15.32 and Hg-0.008 mg/Kg of soil	MI, CA	MC	CT, GT	(Kovalchuk et al., 1998)
Industrial WW contaminated soil	Metals (Hg, Cd, As, Cu, Ni, Cr, Pb, Zn), PAHs (n = 7), PCBs (n = 9) and other organic compound used as solvents (n = 14)	MI, MN	F_1	GT, MT	(Cotelle et al., 1999)
Textile & paper mills WW	Wastewater and sludge (50%): Composition not reported	CA, MN	MC	GT, MT	(Grover and Kaur, 1999)
Mixture	Municipal sludge sample before and after bioremideation	MI, CA, MN, NA	MC	GT, GT, MT	(Cabrera and Rodriguez, 1999)
Farmland soil	Irrigated with mixture of industrial watewater	MN, CA	MC	GT, CT	(Cabrera and Rodriguez, 1999)
Pesticides contaminated soil	Pesticide (Metolachlor	CA	MC	GT	(Kong and Ma, 1999)
	. Atrazine, Extrazine and 2,4-D) contaminated soil sampling and soil practing organic forming				
Mineral water	commercial non-carbonated mineral waters stored at different condition, PET bottled and glass bottle: pH-7.33, TDS-330, Free CO2-43, HCO3−325, Ca^{2+}-72, Mg^{2+}-24, Ba^{2+}-0.006, Na^{+}-16, Sr^{2+}-0.38, SiO2-7.0, Cl−16.0, SO_4^{2-}25 and NO3−0.9 mg/L	CA, MI, RL	MC	GT, CT	(Evandri et al., 2000)
Phosphoric gypsum depot WW	Composition: Ca^{2+}(960), $SO4^{2-}$(3599), P^{5+}(3200-3800), F^{-}(2500-3500), Si^{4+}(800), Na^{+}(1313), Fe^{3+}(55), Mg^{2+}(114), NH^{4+}(23), Cl^{-}(40), K^{+}(53) and SS-9 mg/dm^3	MI, CA	MC	CT, GT	(Pavlica et al., 2000)
Disinfected drinking water	Chlorine dioxide, ozone, peracetic acid and UV radiation. Before disinfection raw water properties: winter (COD-29.7, SS-4.3, nitrate-10.1, nitrite-<0.2, ammonia-<1, mg/L, pH-7.2, T-12.9^{0}C and redox potential-168 mV and Summar (COD-21.8, SS-4.8, nitrate-10.9, nitrite-<0.2, ammonia-<1, mg/L, pH-7.1, T-21^{0}C and redox potential-79 mV)	MN	MC	MT	(Monarca et al., 2002)
Silk dying industry WW	Composition not reported	MI, NA	MC	GT, CT	(Sudhakar et al., 2001)
Fertilizer and petrochemical WW	pH-7.7, EC-461 uS/cm, NH3-<0.05, NO2 -0.03, NO3-1.58, Cl-26.8, SO4-40.8, PO4-0.16, CN-<0.01, mineral oil-0.045 mg/L and Fe-0.03, Mn, Pb, Cd, Zn, Cr, Hg, As, Cu, <0.05 mg/L and dichlorethane-<0.1, Toulene-2.8, Xylene-2.8, PCB-<0.001 and PAH-0.02 ug/L	CA	MC	GT	(Vujosevic et al., 2001)
Carbon electrode production factory	Soil samples were collected from a contaminated area near a factory, composition: Benzo(a)anthracene, Benzo(b)uoranthene, Benzo(k)uoranthene, Benzo(a)pyrene, Dibenzo(a,h)anthracene are equal to 61.2 mg/Kg and other PAH (Phenantrene, Anthracene, Fluoranthene, Pyrene and Chrysene) and heavy metals (Cd, Pb, Cu, Zn, Hg)	MN	MC	MT	(Monarca et al., 2002)

Table 2: Continue…

Radium production industry	Water collected from natural reservoirs located near the radium production industry storage cell, composition: U^{238}, Th^{232}, RA^{226}, Po^{210}, Pb^{210}, Ca, Mg, K, Na, Cu, Pb, Cd, Zn, Mn, Ni	MI, CA	MC	GT, CT	(Evseeva et al., 2003)
Mixed industrail WW	Composition: pH-8.01, EC-750 umhos/cm, Cl^--0.06%, SO^{2-}_4-0.03%, HCO^-_3-0.12%, CO^-_3-0%, Na-28, Ca-5.7, Mg-43, Co-0.40, Cd-0.10, Zn-0.15, Cu-0.20 and Pb-0.29 mg/L	CA, MN, MI	MC	GT, CT, MT	(El-Shahaby et al., 2003)
Mixed industrail WW	Composition: pH-8.18, EC-650 umhos/cm, Cl^--0.06%, SO^{2-}_4-0.04%, HCO^-_3-0.12%, CO^-_3-0%, Na-33, Ca-1.2, Mg-53, Co-0.20, Cd-0.10, Zn-0.22, Cu-0.40 and Pb-0.43 mg/L	CA, MN, MI	MC	GT, CT, MT	(El-Shahaby et al., 2003)
Mixed industrail WW	Composition: pH-8.20, EC-800 umhos/cm, Cl^--0.08%, SO^{2-}_4-0.04%, HCO^-_3-0.23%, CO^-_3-0%, Na-35, Ca-1.0, Mg-50, Co-0.40, Cd-0.15, Zn-0.1, Cu-0.40 and Pb-0.43 mg/L	CA, MN, MI	MC	GT, CT, MT	(El-Shahaby et al., 2003)
Mixed industrail WW	Composition: pH-8.34, EC-850 umhos/cm, Cl^--0.08%, SO^{2-}_4-0.03%, HCO^-_3-0.20%, CO^-_3-0%, Na-9.1, Ca-26, Mg-7.3, Co-0.40, Cd-0.15, Zn-20.0, Cu-0.40 and Pb-0.43 mg/L	CA, MN, MI	MC	GT, CT, MT	(El-Shahaby et al., 2003)
Sewage water	pH-7.40, T-28.92 ^{0}C, SS-532.40, DO-0.73, BOD-175, COD-1758 mg/L	MI, CA, NA, MN, RL	MC	CT, MT, GT	(Amin, 2002)
Disinfacted drinking water	Disinfacted by NaClO, ClO_2 and peracetic acid	CA	MC	GT	(Monarca et al., 2003)
Pulp and paper WW	Composition: pH-9.35, SS-362.85, COD-432, EC-1525 mmho/cm, Na-227.2, K-24, Ca-72.77, Mg-2.01, Fe-0.12, Mn-<0.2, Cr-<0.4, Ni-<0.2, Cu-<0.1, Zn-0.02, Pb-<0.5 and PO4-0.66 mg/L	RL, MI, CA	MC	CT, GT	(Tipirdamaz et al., 2003)
Mixture	Pulp and paper mill, pharmaceutical, enzyme production plant, oil refinery, polyester production plant and steel factory.	RL	MC	CT	(Pessala et al., 2004)
Metal industry waste	Composition: pH-5.1, COD-21.7, EC-2.3 M mho/cm., Sali-11.9, TN-4.4, TOC-384.1, TDS-309.0, Cl-600.0, N-1120.0, Na-414.0, K-11.7 mg/L, heavy metals (Cd-0.01, Cr-282.2, Cu-1.0, Fe-21.0, Mn-1.0, Ni-9.8, Pb-1.1 and Zn-2.1 ppm)	MI, CA, MN	MC	GT, CT, MT	(Chandra et al., 2005)
Dye industry	Composition: pH-6.7, COD-24.7, EC-1.3 M mho/cm, Sali-6.7, TN-90.7, TOC-138.4, TDS-372.0, Cl^--64.2, Nitrate-870.0, Na-53.3, K-3.6 mg/L, heavy metals (Cd-<0.001, Cr-11.2, Cu-1.6, Fe-4.2, Mn-0.02, Ni-3.6, Pb-0.5 and Zn-1.2 ppm)	MI, CA, MN	MC	GT, CT, MT	(Chandra et al., 2005)
Disinfected surface water	Wastewater samples were collected before and after disinfection, in winter and in summer (peracetic acid and hypochlorite disinfecting agent)	CA, MN, RL	M. C	GT, CT, MT	(Crebelli et al., 2005)
Sewage WW	Sampling form municipal wastewater treatment plant: composition: BOD-199-482, COD-458-815, TSS-70-303, TN-29.8-50.3, TP-5.3-11.7 mg/L	MI, CA	MC	GT, CT	(Grisolia et al., 2005)
Contamianted river water	heavy metal and cyanide contaminated river waters in a mining region of Southwest Bulgaria	MI, CA	MC	GT, CT	(Ivanova et al., 2005)
Contamianted river water	Sampling from river contaminated with industrial wastewater (mixture)	MI, CA, MN	MC	GT, CT, MT	(Srivastava et al., 2005)
Contaminated water	Heavy metal (Cu, As, Cd, Pb) and cyanide contaminated waters	CA, NA, MI	MC	CT, GT	(Staykova et al., 2005)
Complex mixture	Industrial effluents: composition heavy metals (Cd-878, Cu-564, Cr-94, Pb-324, Zn-242, ppm and Hg-242 ppb) and pesticides	CA	MC	GT	(Fatima and Ahmad, 2006b)
Chemical fertilizer WW	Composition: Fe-1.1, Pb-1.2, Cr-0.3 and Cu-0.12 mg/L	NA, CA, MI, MN	MC	GT, MT, CT	(Abdel Migid et al., 2007)
Complex mixture	Industrial effluent	CA, MN	MC	GT, MT	(Carita and Marin-Morales, 2008)
River water	The river receives municipal wastewater as well as industrial and agricultural wastewater	CA, NA, MI, MN, RL	MC	CT, MT, GT	(Egito et al., 2007)
Stream contaminated with tannery WW	Composition: Summar (COD-8, BOD-8, pH-6.8, TSS-6, TN-2, O & G-3, Cr-1.5-13, T-20 ^{0}C, Ph-0.02, DO-6.0 mg/L) and Winter: COD-11, BOD-4, pH-7.7, TSS-17, TN-1.0, O & G-9.0, Cr-ND, T-13 ^{0}C, Ph-ND, DO-6.1 mg/L	MI, MN, RL	MC	GT & TC	(Junior et al., 2007)
Insecticide and shoe polish factory WW	Composition: pH-6.3, TDS-520, SO4-71.7-, Cl-90, COD-512, TSS-14700, Na-275, K-42.5, Ca-1062, Mg-48.6, Co-18.85, Cu-1191.11, Cd-43.75, Pd-2.30 mg/L	CA, MI	MC	CT	(Abu and Ezeugwu, 2008)

Table 2: Continue…

Aluminum industry waste	Spent potliners waste; composition: pH-9.5, EC-9.3 dS/m, Al-0.7, CN^--23.4, F^--47.8, Cd-0.18 mg/L, Cu-0.34 mg/L, Fe-75 mg /L. Pb-0.23 mg/L, Mn-0.18 mg/L, Na-657 mg /L and Zn-0.34 mg/L	MI, CA	MC	CT, GT	(Andrade et al., 2008)
Textile WW	Wastewater contaning dyes (CI Disperse Blue 373, CI Disperse Violet 93, CI Disperse Orange 37) and aromatic amines	MI, CA, MN	MC	CT, GT, MT	(Carita and Marin-Morales, 2008)
Lemon juice factory	Composition: pH(4.46), EC(1260 uS/cm), DO (0 mg/L), T (22.4^{0}C), SS (0.70 g/L), COD (42.23mg/L)	CA, MI, MN	MC	MT, CT, GT	(Gana et al., 2008)
Mining industry	Composition: pH(6.12), EC(2967 uS/cm), DO (5.25 mg/L), T (23^{0}C), SS (1.80 g/L), COD (23.57 mg/L)	CA, MI, MN	MC	MT, CT, GT	(Gana et al., 2008)
Sugar factory	Composition: pH(4.92), EC(627 uS/cm), DO (0 mg/L), T (42^{0}C), SS (0.40 g/L), COD (5566 mg/L)	CA, MI, MN	MC	MT, CT, GT	(Gana et al., 2008)
Paper factory	Composition: pH(6.12), EC(4483 uS/cm), DO (0 mg/L), T (29^{0}C), SS (1.80 g/L), COD (6.27 mg/L)	CA, MI, MN	MC	MT, CT, GT	(Gana et al., 2008)
River water	Contaminated with refinery WW, composition: HC (C_{12}-C_{18}) and PAHs (naphthalene, acenaphthylene, acenaphthlene, fluorine, phenanthrene, pyrene, benzoanthracence and chrysene)	MN, CA	MC & F_1	GT, MT	(Leme and Marin-Morales, 2008)
River water	Oil refinery contamination, composition: HC (C12-C18) and PAHs (naphthalene, acenaphthylene, acenaphthlene, fluorine, phenanthrene, pyrene, benzoanthracence and chrysene)	MI, CA, MN	MC &F_1	GT, CT, MT	(Leme et al., 2008)
Contaminated soil	Composition: Cr, Co, Cu, Mn, Hg, Ni and Zn	CA	MC	GT	(Katnoria et al., 2008)
Mixture	Industrial WW, composition: Cu, As, Cd, Pb and cyanides	MI, CA, MN	MC	CT. MT, GT	(Ivanova et al., 2008)
Hospital waste	Composition is not reported	CA, MN	MC	GT, MT	(Bagatini et al., 2009)
River water	Contaminated with Petroleum refinery WW, composition: Ph-7.4, BOD-252, COD-848,NH3-19.32, DO-0.0, NO3-8.2, NO2-0.32, SS-1.80, metal such as Ba-0.27-Cd-<0.003, Cr-<0.005, Co-<0.005, Cu-0.01, Fe-0.46, Pb-<0.02, Ni-0.02 and Zn-0.18 mg/L and PAH (563-847 ug/kg)	MI, CA, MN	MC	CT, GT, MT	(Hoshina and Marin-Morales, 2009)
silver nanoparticles	The silver nanoparticles were suspended directly in deionized water at different concentrations (25, 50, 75 and 100 ppm).	MI, CA	MC	GT, CT	(Kumari et al., 2009)
Gasoline station WW	Composition: pH-6, COD-404, BOD-180, O & G-30, SS-34, VSS-17, Fixed SS-17, benzene-41.8 ug/L, Toluene-291.5 ug/L, xylene-671.4 ug/L, Ph—0.68, Cu-0.027, NI-0.105 and Zn-0.005 mg/L	NA, CA, MI, MN	MC	GT, CT, MT	(Oliveira-Martins and Grisolia, 2009)
Sewage water	Sample was collected before their discharge into the coastal waters and mile away from discharge point	CA, RL	MC	GT	(Ukaegbu and Odeigah, 2009)
Oil refinery WW	Sampling before and after landforming: pH-5.0, OM-115.0 g/dm^3, K-1.8, Ca-75, Mg-27.0, Al-1.0, Na-195.0, Fe-112.0, Mn-13.8, Cu-3.2, Zn-84.8 mmol/dm^3, Ba-493.4, Cd-<0.01, Cr-400.2, Ni-104.5, Pb-18.8 mg/L	CA, MN	MC	CT, MT	(Souza et al., 2009)
Industrial WW	Incoming and outgoing in biological and chemical wastewater treatment plant in industrial zone	MI, CA	MC	GT, CT	(Sik et al., 2009)
River water	River water contaminated with industrial wastewater	MI, RL, MN	MC	CT, GT, MT	(Barberio et al., 2009)
Textile effluent	Composition: pH-8.2, EC-3.48 uS/m, COD-6760, BOD-670, TDS-1040, TS-9810 and TH-375 mg/L	MI, CA, MN	MC	GT, MT, CT	(Jadhav et al., 2010)
Oil refinery WW	Composition: Toluene-75, Ethylbenzene-126, Xylene-85, pyrene-9, benzoantracence-33 and benzopyrene-61 ppb	MI, MN	F_1	GT, MT	(Rodrigues et al., 2010)
Refinary WW	Composition is not reported	CA, MN		CT, GT	(Gupta and Ahmad, 2011)
River water	Contaminated with municipal wastewater, sugar factory, lead smelter, runoff fromsoil contaminated by agricultural practices, fertilizer plant, pharmaceutical plant and food industries: Composition: pH-7-8, EC-431-1642us/cm, COD-2.15-112.70, BOD-165-16.9, SS-2.0-45.6, nitrate-1.09-4.67, nitrite-0.02-0.35, Al-0.07-1.64 mg/L	RL, MI, CA	MC	GT, CT	(Radic et al., 2010)
Textile and paint WW	Effluents were collected from discharge points of the industries	MI, CA	MC	GT, CT	(Samuel et al., 2010)
Paper mill	bleach plant effluents: sampling after and before biodegradability from a bench-scale horizontal anaerobic immobilized bioreactor	MI, CA, MN	MC	GT. CT, MT	(Chaparro et al., 2010)
Phamarceutical WW	Composition: pH-2.9, TDS-42000, Cu-20.0, Zn-17.3, Pb-2.2, Cl-0.02, SO4-696.2 mg/L	RL, MI	MC	CT	(Abu and Mba, 2011)
River water	Contaminated with untreated industrial wastewater, composition: Summar-pH-7.29, EC-29, DO-7.09, T-24.1 ^{0}C, metal (Cu-ND, Cr-ND, Ni-0.031, Pb-0.136, Cd-0.009, Zn-0.078 and Fe-0.001 mg/L)	CA, MI, MN	MC	GT, CT, MT	(Bianchi et al., 2011)

Table 2: Continue…

River water	Autumn: pH-5.53, EC-9.0 uS/cm2, DO-7.15, T-19.6 ^{0}C, metal (Cu-ND, Cr-ND, Ni-0.072, Pb-0.192, Cd-0.009, Zn-0.096 and Fe-1.01 mg/L)	CA, MI, MN	MC	GT, CT, MT	(Bianchi et al., 2011)
River water	Winter: pH-6.85, EC-13uS/cm2, DO-8.65, T-20.9 ^{0}C, metal (Cu-ND, Cr-ND, Ni-ND, Pb-0.304, Cd-ND, Fe-0.002 and Ca-1.342 mg/L)	CA, MI, MN	MC	GT, CT, MT	(Bianchi et al., 2011)
River water	Spring: pH-6.55, EC-12 uS/cm2, DO-7.22, T-20.7 ^{0}C, metal (Cu-0.04, Cr-0.03, Ni-ND, Pb-0.14, Cd-ND, Zn-0.15, Fe-1.34, Mn-0.10, Na-1.72 mg/L)	CA, MI, MN	MC	GT, CT, MT	(Bianchi et al., 2011)
Coal mining waste	Composition: Inorganic ions, Metals (Ca, Mg, Na, K, Fe, Mn, Ba, Sr, Zn, Pb, Al, Cr, Cu, Ni, Co, Cd, Hg) and radionuclides (Ra^{226} and Ra^{228})	MI, CA	MC	GT, CT	(Geras'kin et al., 2011)
Contaminated soil	soil Contaminated with zinc coating industry wastewater: pH-3.46, nitrate-1.69, PO4-3.05, K-0.13, Cu-32.86, Co-6.86, Cr-0.0, Hg-0.037, Mn-1.55, Ni-9.66, Zn-6.53 mg/L	CA	MC	GT	(Katnoria et al., 2011)
Mixture	Benzene, toluene, ethylbenzene and xylene (benzene 1.25 ug/L, toluene 42.5 ug/L, ethylbenzene 50 ug/L and xylenes 75 ug/L)	MN, CA	MC & F_1	GT	(Mazzeo et al., 2011)
Sewage and oil refinery	Composition: T-25-26 ^{0}C, EC-119-113, pH-6.8-7.3, DO-3.2-3.3, BOD-2-3, COD-16-22, NH3-1.15-1.54, ON-1.38-1.85, PO4-0.21-0.29, TS-81, srfactatnt-0.023, Cu-0.009, Cl$^-$-7.0, Tot. Cr-0.032-0.041, Cr(III)-0.032, Al-1.86, Fe-2.19, Zn-0.025-0.034 mg/L	MN, MI	MC	GT, MT	(Nunes et al., 2011)
Mixed WW	Sewage, Leather, agriculture, paper recycling industry, composition: T-25-34 ^{0}C, EC-119-113, pH-6.9-7.1, DO-0.3-2, BOD-3 COD-20-22, NH3-1.28-2.33, ON-1.20-2.71, PO4-0.20-0.26, TS-123-164, srfactatnt-0.14, Cu-0.009, Cl-112.2-18, Tol. Cr-0.01-0.03, Cr(III)-0.03, Al-0.52, Fe-1.47, Zn-0.031 mg/L	MN, MI	MC	GT, CT	(Nunes et al., 2011)
Mixture	Sewage and tannery WW, composition: T-17-24 ^{0}C, EC-156-130, pH-7.0-7.1, DO-0.2-0.3, BOD-3 COD-15-85, NH3-2.19-14.3, ON-2.14-5.0, PO4-0.34-0.40, TS-101-637, Phenol-0.035, srfactatnt-0.56, Cu-0.008, Cl-7.4-130.0, Tol. Cr-0.15-0.034, Cr(III)-0.034, Al-4.78, Fe-2.66, Zn-0.102 mg/L	MN, MI	MC	GT, CT	(Nunes et al., 2011)
Mixture	Sewage and matallurgy WW, composition:T-18-23 ^{0}C, EC-92-145, pH-6.7-7.1, DO-1.4-2.0, BOD-2-19, COD-2-18, NH3-0.81-1.91, ON-0.30-0.60, PO4-0.30-1.17, TS-100-110, phenol-0, srfactatnt-0.12, Cu-0.006-0.01, Cl-8.5-11.0, Tol. Cr-0.03, Cr(III)-0.03, Al-1.29, Fe-3.19, Zn-0.023 mg/L	MN, MI	MC	GT, CT	(Nunes et al., 2011)
Mixture	Sewage, footwear companies and leather finish: T-18-23 ^{0}C, EC-471-482, pH-6.7-7.0, DO-0.5, BOD-8-17, COD-85-89, NH3-11-12, ON-5.30-6.9, PO4-1.04-1.46, TS-291-300, phenol-0.008, srfactatnt-1.10-2.52, Cu-0.15, Cl-26-39, Tol. Cr-0.058, Cr(III)-0, Al-0, Fe-0, Zn-0.188-0.31 mg/L	MN, MI	MC	CT, GT	(Nunes et al., 2011)
Contaminated soil	*A. cepa* grown directly in industrial wastewater irrigated agricultural filed	CA, MN, RL	MC	GT, MT, CT	(DI and MW, 2011)
Textile WW	Wastewater before and after treatment: pH-8.1, COD-9860, BOD-880, TDS-8756, TSS-1089, TS-9845, TH-689, F-12, Cl-1254, SO4-1123, Cu-1.3, Fe-1.7, Mg-1.43, Pb-0.63, Ni-0.79, Ca-34.43, K-23.65 and Na-89 mg/L	MI, CA	MC	CT, GT	(Phugare et al., 2011)
Industrial and river water	Composition: heavy metal (Cu > Ni > Cd) and phenolic compounds	CA	MC	GT	(Siddiqui et al., 2011)
Mixture	Industrial wastewater: BOD-82-378, COD-283-1259, TOC-553-2932, Cd-2.1-672, Cr-3.3-74, Cu-8-376, Fe-8.9-104, Pb-1.3-216 and Hg-0.2-0.4 mg/L	MI, CA, MN	MC	GT	(Tabrez and Ahmad, 2011)
River water	Rainy season site A: Sample collection at point of mixing of metabisulfite and EC-374, H-12.24, Cl-93, DO-6.96, BOD-6, N-0.35, NH3-4.00, pH-6.8, Ca, Mg, K, Na (268, 137, 4.0, 1.1 Meq/100 g soil) and site B: EC-392, H-7.90, Cl-16.45, DO-7.12, BOD-2, N-0.20, pH-6.5, Ca, Mg, K, Na (139, 94, 4.6, 0.9 Meq/100 g soil)	MI, CA, MN	MC	GT, CT, MT	(da Costa Machado Matos Carvalho et al., 2011)
River water	Dry season site A: Sample collection at point of mixing of metabisulfite and EC-44.0, H-35.50, Cl-10.73, DO-5, BOD-2.5, N-0.32, NH3-0.03, pH-6.9, Ca, Mg, K, Na (100, 94, 4.6, 1.7 Meq/100 g soil) and site B: EC-50, H-10.50, Cl-12.9, DO-6.5, BOD-2.5, N-0.2, NH3-0.01, pH-6.5, Ca, Mg, K, Na (139, 58, 3.7, 1.0 Meq/100 g soil)	MI, CA, MN	MC	GT, MT, CT	(da Costa Machado Matos Carvalho et al., 2011)

Table 2: Continue…

River water	Upstream to metabisulfite discharge point: contaning sodium metabisulfite (SMB) and the point where sodium metabisulfite is discharged in river	MI, CA, MN, RL	MC	GT, MT, CT	(da Costa Machado Matos Carvalho et al., 2011)
River wastewater	100 m downstream the point of sodium metabisulfite discharge in river	MI, CA, MN, RL	MC	GT, MT, CT	(da Costa Machado Matos Carvalho et al., 2011)
Municipal wastewater	Sampling form treatment plant after ozonation form reactors	MI, MN	MC	GT, MT	(Misik et al., 2011)
Bottling industry (10%)	Composition: T-30^{0}C, pH-10.5, Turb-68FTU, TH-417, TSS-43, TDS-430, BOD-19.90, DO-2.13, Alkalinity-750, TS-715, Nitrate-50, SO_4-78, PO_4-0.91, Cl^--248.50, Ca-306, Cu-0.02, Mn-0.1, Pb-0.1, Fe-0.64, Cd-0.07, Ni-0.12, Zn-0.06 mg/L	MI, CA	MC	CT, GT	(Olorunfemi et al., 2011)
Rubber industry (10%)	Composition: T-31^{0}C, pH-4.75, Turb-98FTU, TH-320, TSS-40, TDS-720, BOD-12, DO-4.24, Alkalinity-340, TS-1012, Nitrate-86.50, SO_4-67.50, PO_4-1.15, Cl^--276.90, Ca-220, Cu-0.1, Mn-0.1, Pb-0.1, Fe-0.24), Cd-0.01, Ni-0.1 and Zn-0.3 mg/L	MI, CA	MC	CT, GT	(Olorunfemi et al., 2011)
Brewery industry (10%)	Composition: T-28^{0}C, pH-5.5, Turb-38FTU, TH-298, TSS-48, TDS-1500, BOD-15.10, DO-2.72, Alkalinity-230, TS-1970, Nitrate-54, SO_4-18, PO_4-0.28, Cl^--335, Ca-250, Cu-0.32, Mn-0.1, Pb-0.1, Fe-0.40, Cd-0.03, Ni-0.15, Zn-2.03 mg/L	MI, CA	M.C	CT, GT	(Olorunfemi et al., 2011)
Textile dyes mixture	Nitroaminoazobenzene based dyes: C.I. Disperse Blue 373, C.I. Disperse Violet 93 and C.I. Disperse Orange 37	NA, CA	MC	CT, GT	(Ventura-Camargo et al., 2011)
Textile WW	Composition: BOD-152, COD-319, TSS-1160, O & G-612, pH-6.9, Cr-1.20, Cu-0.02, NH_4^+-0.50 mg/L	CA, MI, NA	MC	GT, CT	(Oriaku et al., 2011)
Contaminated soil	Agricultural soil irrigated with industrial WW and pesticide sprayed	CA	MC	GT	(Chahal et al., 2012)
River water	Contaminated with industrial wastewater: PHAs, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene,benzo(a)anthracene, chrysene,benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene andbenzo(ghi)perylene.	MI, CA	MC	GT, CT	(da Costa et al., 2012)
Disinfacted drinking water	Disinfacted ground and surface water for drinking purpose	CA, MN, RL	MC	GT, MT	(Feretti et al., 2012)
Acid mine drainage	Before and after calcination of coal mining waste: pH-2.6, Al-28.99, Fe-13.23, Zn-1.19 Cu-0.01, Pb-<0.0001 mg/L SO_4^{-2}-2.17 g/L	CA	MC	GT	(Geremias et al., 2012)
Refinery WW	Composition is not reported	CA, MN, RL	MC	CT, GT	(Gupta and Ahmad, 2012)
Drainage pumping stations	Landfill leachates sampling, composition: Pb, Ni, Cd, Hg, benzene, dichloromethane, chloroform, trichlorobenzenes, exachlorobenzene, hexachlorobutadiene, hexachlorocyclohexane,lindane, hexachlorocyclohexane, pentachlorobenzene, pentachlorophenol, PAHs.	CA, MN, MI	MC	GT, MT, CT	(Kwasniewska et al., 2012)
Olive mill WW	Composition: pH-4.83, EC-11.5 ds/m, COD-65.4, BOD-29.3 g/L, phenolic contents-4.36 ugGA/mL	MI, CA	MC	CT & GT	(Pierantozzi et al., 2012)
Soil sample	Contaminated with Cs-137 and Sr-90	CA	MC	GT	(Kovalchuk et al., 1998)
Surface water	Surface water treated with different disinfecting agent: ClO_2-1.6 mg/L, NaClO-1.2 mg/L, peracetic acid-1 mg/L	MI, CA	MC	GT, CT	(Monarca et al., 2003)
Hospital waste	Hospital incinerator bottom ash leachate samples	CA	MC	GT	(Akinbola et al., 2011)
Mixture	Plasticizer, cosmetics, healthcare and preservative agent containing WW	MI, CA, MN	MC	CT, GT, MT	(Herrero et al., 2012)
Aligarh and Mathura refinery	City wastewater and refinery wastewater	CA	MC	GT	(Fazili and Ahmad, 2014)
Pulp and paper mill	Wastewater bleaching sequence of Kraft cellulose	CA, MN, MI	MC	CT, GT, MT	(Roa et al., 2012)
Nigerian Agip Oil Company	Wastewater at different concentrations	MI, CA	MC	CT, GT	(Olorunfemi et al., 2015)
Paint industry effluent	Concentrations (100%, 50%, 25%, 10%, 5%, 1%, 0.50% and 0.25%)	CA, MI	MC	CT, GT	(Njoku et al., 2015)
Wastewater	Public hospital of Buenos Aires (Argentina)	CA	MC	GT	(Magdaleno et al., 2014)
Wastewater	Buenos Aires (Argentina)	CA	MC	GT	(Paz et al., 2006)
Wastewater	hospital effluent in Santa Maria, Rio Grande do Sul State, Brazil	CA, MN	MC	GT, MT	Bagatini et al., 2009

Table 2: Continue…

Shkodra city wastewater	Sampling from four sites	CA, MI,	MC	CT, GT	(Mesi and Kopliku, 2012)
Wastewater	industrial wastewaters reaching the Dandugan Oya, Srilanka and the downstream water	CA, MI	MC	CT, GT	(Kannangara and Pathiratne, 2015)
Wastewater	Near the Sava River, Croatia	CA, MI	MC	CT, GT	(Radić et al., 2010)
Wastewater	Un-treated and Upflow Immobilized Biomass Anaerobic Reactor hospital treated wastewater	MI	MC	CT	(Torres et al., 2014)
Wastewater	Industrial and contaminated river water	CA, MI	MC	GT, CT	(Siddiqui et al., 2011)
Wastewater	Effluent from tobacco industry	CA, MI	MC	GT, CT	(Akinsemolu et al., 2015)
Wastewater	Distillary waste from a sugarcane factory, Kanpur, India				(Mishra, 1993)
Compost	Compost from TPK Sarimukti, Cipatat, West Bandung (solid & liquid)	CA, MN, MI, NA	MC	CT, GT, MT	(Wardini et al., 2015)
Wastewater	Un-treated and treated textile wastewater by advanced oxidation process	RG	MC	CT	(Iqbal et al., 2015)
Wastewater	Un-treated and photo-catalytic treated textile waster Textile wastewater	RG	MC	CT	(Iqbal and Bhatti, 2014)
River water	Sampling from contaminated river Vallkärrabäcken, Sweden 34 years after the closure of the landfill, Sankt Hans	RG	MC	CT	(Lindqvist, 2015)
Leachate	Landfill leachate collected from city of Araranguá (Santa Catarina state, Brazil)	RG	MC	CT	(do Canto et al., 2013)
Wastewater	Effluents from six pharmaceutical companies in the Lagos province, Nigeria	RG, MI, CA	MC	CT, GT	(Akintonwa et al., 2009)
Wastewater	Industrial effluents contain copper, cadmium and chromium metals	MI, CA, NA	MC	CT, GT, MT	(Hemachandra and Pathiratne , 2015)
Soil sample	Soils contaminated by heavy metals from municipality of Triunfo, state of Rio Grande do Sul, Brazil	MI, CA,	MC	CT, GT	(de Souza Pohren et al., 2013)
River water	Sungai Dua River contaminated with sodium and calcium metals	RG, MI, CA			(Akinboro et al., 2011)
Nanoparticles	ZnO dispersions at 25, 50, 75, and 100 µg/mL concentrations	MI, CA, MN	MC	CT, GT, MT	(Kumari et al., 2011)
Nanoparticles	Cobalt and zinc oxide NPs at 5, 10, and 20 µg/mL concentrations	RG	MC	CT	(Ghodake et al., 2011)
River water	Jaguari River and the Ribeirão Lavapés, Brazil samples collected in dry season (August 2011 and 2012) and rainy season (February 2012 and 2013), Mn concentration 0.13 mg/L, greater than permissible limit	MI, CA, MN	MC	CT, GT, MT	(Salles et al., 2016)
River water	Wastewater collected in the years of 2005 and 2006 from sites in the cities of Tremembé and Aparecida (São Paulo state, Brazil)	RG, MI	MC	CT	(Barbério et al., 2009)
Ecballium elaterium	Roots were exposed to 10L, 20, 50 ml/L and undiluted juice concentrations	MI, CA, MN	MC	CT, GT, MT	(Çelik and Aslantürk, 2009)
River water	Cr(VI) contaminated reiver water	MI, RG	MC	CT	(Espinoza-Quiñones et al., 2009)
Radioactive material	thallium-232 in the presence of different cadmium and potassium concentrations	CA, MN	MC	GT, MT	(Evseeva et al., 2005)
Wastewater	Textile industries, rubber industry, treatment plants of industrial zones	MI, CA	MC	CT, GT	(Pathiratne et al., 2015)
Leachate	Leachate collected from landfill, Vale do Rio dos Sinos, southern Brazil	RG, MI, CA	MC	CT, GT	(Klauck et al., 2013)
Leachate	Leachate-municipal solid waste landfill, Sinos River Valley, southern Brazil	RG	MC	PT	(Klauck et al., 2015)
Textile wastewater	Bio-treated textilel effluent	RG	MC	TC/CT	(Patel et al., 2015)
Wastewater	Industry producing veterinary medicines, before and after treatment	RG	MC	TC	(Maselli et al., 2015)
Stream water	Water samples were collected from three streams located in the urban area of a municipality in the south of Brazil	MI, MN, CA	MC	CT, GT, MT	(Athanásio et al., 2014)
Wastewater	Hospital laundry wastewaters, Rio Pardo Valley, Rio Grande do Sul, Brazil	CA, MI	MC	CT, GT	(Kern et al., 2015)
Sugar industry	Vermicomposted pressmud (waste by product of the sugar cane industry)	CA, MI	MC	CT, GT	(Bhat et al., 2014)
Leachate	Leachate from an old sanitary landfill before and after treatment	CA, MI	MC	CT, GT	(Brkanac et al., 2014)
Dye mixture	Dyes mixture before and after Petunia grandiflora and Gaillardia grandiflora treatment	CA, MI	MC	CT, GT	(Watharkar and Jadhav, 2014)
Contaminated water	Natural waters contaminated with ^{90}Sr and heavy metals near radioactive waste storage, Obninsk, Kaluga region	MN	MC	MT	(Udalova et al., 2014)

Table 2: Continue…

River water	Water collected from Quatorze River, Francisco Beltrão, Paraná, Brazil	MI	MC	CT	(Düsman et al., 2014)
Dye	Direct Blue-1 before and after bio-treatment	CA	MC	GT	(Prasad et al., 2013)
Leachate	Treated landfill leachate from the Piškornica (Croatia) sanitary landfill	MI	MC	CT	(Garaj-Vrhovac et al., 2013)
Wastewater	Liquid waste produced in the process of industrialization of the bitter cassava, olho-junto variety	MI, CA	MC	CT, GT	(Viana et al., 2014)
Wastewater	Metal rich acid mine drainage	CA	MC	MT, GT	(Sobral et al., 2013)
Wastewater	Industrial wastewaters from Jajmau (Kanpur)	MI, CA	MC	GT, CT	(Masood and Malik, 2013b)
Wastewater	Treated and un-treated acid mine drainage	MI, CA,	MC	GT, CT	(Netto et al., 2013)
Contaminated soil	Soil samples from agricultural fields near industrial area, Jajmau, Kanpur (India)	MI, CA	MC	GT, CT	(Masood and Malik, 2013a)
Wastewater	Hospital laundry wastewaters (COD - 3343.8 mg L(-1)), biochemical oxygen demand (BOD(5) - 1906.4 mg L(-1)), total Kjeldahl nitrogen (TKN - 79.8 mg L(-1))	MI, CA	MC	GT, CT	(Kern et al. 2013)
Sludge	Tested 28 sewage sludge samples, Spanish wastewater treatment plants	RG	MC	CT	(Roig et al., 2012)
Wastewater	Treated and un-treated acid mine drainage with calcinated coal mining waste	RG, MI	MC	CT	(Geremias et al., 2012)
Wastewater	Collected from two different stations of northern India contain trichloroethylene	CA	MC	GT	(Tabrez and Ahmad, 2012)
Water	Tested tap water treated in static conditions with gray and black silicon mineral	RG	MC	CT	(Goncharuk et al., 2011)
Dust	Roots were exposed to cement dust over different periods at 100 m from a cement factory	RG, CA, MI	MC	CT, GT	(Yahaya et al., 2012)
Dyes	Un-treated and bio-treated Reactive Blue 198 and triphenylmethane dye	RG	MC	CT/TC	(Balan et al., 2013)
Fly ash	Flay ash from thermal power plant in West Bengal Zn, Pb, Cu, Ni, Cd and As metals	MI, CA	MC	CT, GT	(Chakraborty et al., 2009)
River	Matanza-Riachuelo river sediment contains copper, lead, and chromium	CA	MC	GT	(Magdaleno et al., 2008)
Waste	Collected from sewage effluent, agricultural and informal residential runoff	RG	MC	TC	(Oberholster et al., 2008)
River	Water and sediment samples collected from the Estância Velha stream of southern Brazil	RG	MC	TC	(Júnior et al., 2007)
Wastewater	Wndustrial wastewater samples from Aligarh and Ghaziabad cities	CA	MC	GT	(Fatima and Ahmad, 2006a)
Wasterwater	Wasterwater contains heavy metal (Cd, Cr, Cu, Hg, Pb and Zn)	MI	MC	CT	(Fatima and Ahmad. 2005)
Disinfected water	Urban wastewater disinfected with sodium hypochlorite or peracetic acid	MI, CA	MC	CT, GT	(Crebelli et al., 2002)
Wastewater	From Corumbá and Paraguay River in flood and drought seasons	MI, CA	MC	CT, GT	(de Lima Moraes and Jordao, 2001)
Herbicide	Herbicide ametryn encapsulated in microspheres of poly(hydroxybutyrate) and poly(hydroxybutyrate-co-valerate)				(da Lima et al., 2012)
Waste	Obtained from a sewage treatment plant, vinasse (a by-product of the sugar cane industry), and a combination of both residues	CA, MN	MC, F cell	GT, MT	(Christofoletti et al., 2013)
Sewage sludge	Solubilized and crude sludge from two sewage treatment stations were tested	MI, RG, CA, MN	MC, F cell	PT, CT, GT, MT	(Corrêa Martins et al., 2016)
Sewage sludge	Sewage studge/soil with of 10, 25 and 50% proportions was buried for 0, 2, 6 and 12 months in holes	MI, CA, MN	MC	CT, GT, MT	(Mazzeo et al., 2015)
Hair dye	Recommended dose precisely mentioned in instructions booklet of the hair dye was used	MI, CA	MC	CT, GT	(Maiti et al., 2016)
Food dyes	Food dyes, i.e., fast green FCF, indigo carmine, orange G and tartrazine, and the non-permitted dye metanil yellow	MN, CA	MC	GT, MT	(Roychoudhury and Giri, 1989)
Melted falling snow	Snow samples were collected from two sites, characterized by differences in pollution intensity, Belgrade, Serbia	RG, CA	MC	TC, GT	(Blagojević et al., 2009)
Contaminated soil	Having high level of natural radiation in the soils from Ramsar, Iran	MN, RG	MC	TC, GT	(Saghirzadeh et al., 2008)
Leachates	Spent Pot Liner (aluminum industry waste, composed of organics, fluoride salts, inorganic cyanides, metals, and sodium)	MI, CA	MC	CT, GT	(Andrade-Vieira et al., 2012)
Water and sediment	From the Upper Silesia Coal Basin (USCB), Poland	MI, CA	MC	CT, GT	(Geras'kin et al., 2011)
Industrial waste	Stones i.e., marbles and granites, includes a stage of plate polishing	MI, CA, MN	MC	CT, GT, MT	(Aguiar et al., 2016)

Table 2: Continue…

Contaminated water and soil	Mercuric chloride, methyl mercuric chloride, phenyl mercuric acetate and a methoxy ethyl mercuric chloride based fungicide, Emisan-6	MN, CA	MC	MT, GT	(Dash et al., 1998)
Industrial and domestic	Effluents collected near tannery activities, 2001 and 2002 at different sites along the Sapucaizinho river, Municipality of Patrocínio Paulista, State of São Paulo, Brazil	MI, CA, MN	MC	CT, GT, MT	(Matsumoto and Marin-Morales, 2004)
River and well water	Alamuyo River in Ibadan, Nigeria and wells alongside river	MI, CA	MC	CT, GT	(Fawole et al., 2008)
Radiation	Roots were exposed to radiofrequency electromagnetic fields	MI, CA	MC	CT, GT	(Tkalec et al., 2009)
Wastewater	Un-treated and physical, chemical, and biologically treated acid mine drainage wastewater	MI	MC	CT	(Defaveri et al., 2009)
Textile effluents	Un-treated and treated at the absorbed doses of 5-15 kGy in combination with H_2O_2	RG, MI	MC	CT	(Iqbal and Nisar, 2015)
Textile dye	Treated and un-treated azo dye Remazol orange by *Pseudomonas aeruginosa* BCH in plain distilled water	RG	MC	CT	(Jadhav et al., 2012)
Leachate	Raw and simulated e-waste leachates (Fe, Cd, Mn, Cu, Ni, Cr As, Zn and Pb metals)	MI, CA	MC	CT, GT	(Bakare et al., 2012)
Tannery effluents	Composition: pH (8.5), EC (11.94 dSm-1), BOD (499 mg/L), COD (1382 mg/L) and Cr content (2.32 mg/L)	MI, CA, MN	MC	CT, GT, MT	(Gupta et al., 2012)
River water	Water samples from river Rasina, Serbia from nine different places	MI, CA	MC	CT, GT	(Vujošević et al., 2008)
Air pollution	Roots were exposed to field conditions at six research plots in the most polluted areas, Slovenia	MI, CA	MC	CT, GT	(Glasenčnik et al., 2004)
Air pollution	Roots were exposed at eight sampling plots in the vicinity of major Slovene local emission sources	MI, CA	MC	CT, GT	(Glasencnik et al., 2002)
Contaminated soil	Soil contaminated with Diuron (herbicide)	MI, CA, MN	MC	CT, GT, MT	(Saxena et al., 2004)
Waste	Diluted and un-diluted waste drilling fluid samples	MI, CA	MC	CT, TC, GT	(Vidaković et al., 1993)
Sea water	Roots were exposed to ballast water at 0.5%, 1%, 5%, and 10% concentrations	MI, CA	MC	CT, GT	(Olorunfemi et al., 2012)
River water	Samples collected from Shiroka, Zogaj, Shegan, Kamicë, Stërbeq, Buna bridge, Bahçellek, Zues and Dajç (Shkodra lake, Buna and Drini rivers)	MI, CA	MC	CT, GT	(Kopliku et al., 2012)
Sewage effluent	collected form Mandsaur (Madhya Pradesh), India	RG	MC	CT/TC	(Shashank and Suresh, 2013)
Leachate	Raw and simulated leachate from a rural refuse dump site at Odo Oba, South-West Nigeria	MI, CA	MC	CT, GT	(Bakare, 2001)
Wastewater	Wastewaters from two pharmaceutical production processes, cotrimoxazole B wastewater and Piriton wastewater	MI, CA	MC	CT, GT	(Lateef, 2007)
Wastewater	Water from Dobër wells, Vraka runnel, and Shegan waterside	MI, RG	MC	CT	(Mesi and Kopliku, 2011)
Wastewater	Pharmaceutical effluent at 0.5, 1, 2.5, 5 and 10%; and 1, 5, 10, 25 and 50% concentrations	MI, RG, CA	MC	CT, GT	(Bakare et al., 2009)
Wastewater	electroplating wastewater (metal ions and cyanides)	CA	MC	GT	(Somashekar and Arekal, 1983)
Wastewater	Bio-treated and un-treated bleaching effluent from two kraft pulp mills	MI, CA	MC	CT, GT	(Chaparro and Pires, 2011)
Leachate	Limeira Sanitary LandfillBrazilian state of São Paulo (Al, Cd, Pb, Cu, Cr, Ni, Zn, K, Na, Fe, Mn and pH 8.01, Color – Abs. (400 nm) 2.46, Turbidity (UT) 68.70, Condutivity (mS) 12.21,5 Hardness (mg/L $CaCO_3$) 934.00, TOC (mg/L) 1116.10, AOX (µg/L) 690.70, NH^{4+} (mg/L) 426.00)	MI,RG	MC	CT/TC	(Pelegrini et al., 2007)
Tap water	Borehole water supply in six halls of residence of the University of Benin main campus	MI, CA	MC	CT, GT	(Olorunfemi, 2013)
Wastewater	Advanced oxidation treated and un-treated wastewater produced by the process of rhodamine B dyeing	MI, CA	MC	CT, GT	(Machado et al., 2012)
Water sample	Nën-Shkodra lowland agricultural area after massive 2010-2011 flooding	MI, CA, RG	MC	CT, GT	(Kopliku and Mesi 2012)
Textile dye	Un-treated and bio-treated reactive Navy Blue RX dye	MI, CA	MC	CT, GT	(Watharkar et al., 2013)
Textile dye	Un-treated and bio-treated reactive blue 198	MI	MC	CT	(Poonkuzhali et al., 2011)
River water	River Vale do Jamari, brazil	MI, MN, CA	MC	CT, GT, MN	(de Oliveira Meneguetti et al., 2012)
Mixture	Samples collected from outlets of sewage effluent, agricultural and informal residential runoff	RG, MI	MC	CT	(Oberholster et al., 2008)
Food industry	Monosodium glutamate used as flavor enhancer in foods	RG, MI, CA	MC	CT, MT	(Adeyemo and Farinmade, 2016)

Table 2: Continue…

Waste	Surface water from five sites of Hooghly River in West Bengal, India, along the banks of which many shipbuilding and scrap industries	MN, MI, CA	MC	CT, GT, MT	(Singh et al., 2014)
Water sample	Sodium metabisulfite in sea waters and sediments collected in a shrimp farm in Cajueiro da Praia (Luis Correia), state of Piauí, Brazil,	MN, CA, MI	MC	TC	(Matos et al., 2011)
Wastewater	Upflow Immobilized Biomass Anaerobic Reactor treated and un-treated hospital wastewater	MI,RG	MC	CT/TC	(Porras Torres et al., 2013)
Food industry	Food preservatives (sodium benzoate and sodium metabisulphite) were tested at different concenrations	RG, MI, CA	MC	CT, GT	(Onyemaobi et al., 2011)
Wastewater	Chemical industrial effluent	CA	MC	CT	(Xing et al., 1995)
Waste sludge	Raw and biosolid sludge by diplopods	CA	MC	GT	(Christofoletti et al., 2012)
Leachate	Raw municipal landfill leachates from Radiowo municipal Poland and zeolite treated leachate	RG, CA	MC	PT, GT	(Obidoska and Jasińska, 2008)
Wastewater	Petroleum refinery wastewater tested at 20%, 40%. 60%, 80% and 100% concentrations	CA, MI	MC	CT, GT	(Obute et al., 2005)
Wastewater	Raw and treated effluents of dairy industry	CA, MI	MC	CT, GT	Olorunfemi et al. (2012)
River water	Santa Maria da Vitória River, Brazil, received domestic, municipal and agricultural waste	CA	MC	GT	(Grippa et al., 2012)
Food industry	Dye orange red (amalgamation of two primary food colourants like caramoisine and sunset yellow) was studied	CA	MC	GT	(Tripathy et al., 2015)
Soil sample	Un-treated and treated soil from a site heavily contaminated with polychlorinated biphenyls	CA	MC	GT	(Meier et al., 1997)
Food industry	Chitosan and poly(methacrylic acid) nanoparticles (size 60, 82, and 111 nm) were studied, which are used for food packing	MI	MC	CT	(De Lima et al., 2010)
Wastewater	Un-treated and treated textile wastewater with yeast cell biomass and *S. cerevisiae* MTCC 463	RG, MI, CA	MC	PT, CT, GT	(Phugare et al., 2010)
Pyrolysis oil	Oils produced from different sources (poplar, beech and spruce)	MI, CA	MC	CT, GT	(Holan et al., 2014)
Sludge	Oil refinery sludge tested at different concentrations	MI, CA	MC	CT, GT	(Tanti et al., 2009)
Water sample	São Gonçalo Channel, water supply source of the city of Pelotas, Brazil and the surrounding region	MI, CA	MC	CT, GT	(Paiva et al., 2008)
Water sample	Rural settlements area (Obazuwa community in Ovia North East Local Government Area of Edo State, Nigeria)	MI, CA, MN	MC	CT, GT, MT	(Olorunfemi et al., 2014)
Tannery wastewater	Tannery effluent treated with Aqueous leaf extract	RG, MI	MC	CT	(Poonkuzhali et al., 2013)
Wastewater	Advanced oxidation process (ozone and ozone/UV) treated and un-treated kraft cellulose pulp mill	CA	MC	GT	(Chaparro et al., 2015)
Soil samples	Collected near copper-smelters (the Middle Urals) and Ni-enriched soil from an area of a natural geochemical anomaly (the Polar Urals)	CA, MI, MN	MC	CT, GT, MT	(Kataeva et al., 2012)
Textile dye	Un-treated and treated malachite green with laccase enzyme	RG, MI	MC	TC	Balan et al. (2012)
Wastewater	Un-treated and treated hospital effluent by ozonation	RG	MC	TC	(Grisales Penagos et al., 2012)
Textile dyes	Un-treated and calcium-alginate pectin entrapped bitter gourd peroxidases treated disperse Brown 1 and Disperse Red 17 dyes	RG/MI	MC	TC/CT	(Satar and Husain, 2011)
Waste	Sample contaminated with Cd^{2+}, Cr^{6+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, BHC, 2,4-D, mancozeb and phenols	RG, MI	MC	CT/TC	(Khan and Ahmad (2006)
Textile dye	Treated and un-treated remazol red (a monochloro sulphonated azo dye) by *Pseudomonas aeruginosa* BCH	CA	MC	GT	Jadhav et al. (2011)
Distillery waste	Roots were exposed to distillery effluent (25, 50, 75 and 100%) wastewater				(Hemanth Kumar et al. 2015)
Sugar industry	Vinasse (by-product of the biomass distillation, mainly for the production of ethanol, from different cultures such as sugarcane	MI, CA, MN	MC	CT, GT, MT	(Pedro-Escher et al., 2016)
River water	Vistula river water at selected points in Warsaw (downstream from Gruba KaĞka) (Poland)	MI, GT	MC	CT, GT	(Obidoska et al., 2013)
Pulp and paper	Treated the paper and pulp industry effluent by *Rhodococcus* sp. NCIM 2891 and un-treated wastewater	RG, MI	MC	CT/TC	(Nadaf et al., 2014)
Fingicide	Fungicide tilt in root tip cells of A. cepa. Bulbs were exposed to 0.02%, 0.04%, 0.06%, 0.08 %) concentrations of tilt for 3, 6, 9, 12 h	MI, CA	MC	CT, GT	(Pulate et al., 2014)
Water sample	Roots were exposed to five different water samples collected from boreholes Ring road, Sakponba, Ekosodin, Science and Eddy Grace in Benin City, Edo State, Nigeria for 96-h	MI, CA	MC	CT, GT	(Adelanwa et al., 2014)

Table 2: Continue…

Nanoparticles	Different sizes silica nanoparticles (SiNP) (TM40 (22 nm), HS30 (12 nm), SM30 (7 nm) with concentrations ranging from 0.19 to 163.8 g/L (TM40) and 0.29 to 122.85 g/L (HS30 and SM30) were tested	MI	MC	CT	(Silva, 2014)
Wastewater	Petrochemicals, air liquid and polyester resin effluents	MI, CA	MC	CT, GT	(Abu et al., 2015)
Waste	Complex environmental mixtures (surface water or industrial wastewater)				(Teena et al., 2016)
Water sample	Collected from from a regional radioactive waste repository	MI, CA	MC	CT, GT	(Pyatkova et al., 2009)
Wastewater	Bio-treatment of industrial effluent containing having eleveated values water parameters i.e., COD, color and heavy metals	RG	MC	CT	(Patel et al,, 2015)
Contaminated sea water	Bilge water (water from oceanic vessels is usually discharged through the bilge wells), contains Cu, Mn, Pb, Fe, Cd, Cr, Ag, Ni and Zn heavy metals	MI, CA	MC	CT, GT	(Olorunfemi et al., 2015)
Pesticides	Emulsifiable concentrate of cypermethrin, deltamethrin, lambdacyhalothrin and endosulfan at 1.0, 5.0, 10.0, 20.0 and 40.0 ppm concentrations for 48-72 h exposure	MI, CA	MC	CT, GT	(Yekeen and Adeboye, 2013)
Textile wastewater	textile effluent on A. test at 1, 2, 5, 10, 25, 50 and 100% (v/v; effluent/tap water) concentrations for 96 h, contains Fe, Cd, Mn, Ni, Cr, and other disturbed water quality parameters	MI, CA	MC	CT, GT	(Alimba et al., 2013)
Insecticides and fungicide	Insecticides (Endosri & Nuvan) and fungicide (Kvistin) at different concentrations	RG, MI	MC	CT	(Kuchy et al., 2015)
Wastewater	Un-treated and treated effluents with aquatic macrophytes *Salvinia auriculata* Aublet, Mexico	RG	MC	CT	(Gonçalves et al., 2015)
Textile dyes	Un-treated and bio-treated textile dyes using peroxidases from turnip roots	RG, MI	MC	CT, PT	(Kulshrestha and Husain, 2007)
electronic waste	From Iloabuchi electronic market, Diobu, Rivers State, Nigeria tested at 5%, 10%, 25%, 50%, and 100% concentrations	MI, CA	MC	CT, GT	(Babatunde and Anabuike, 2015)
Hospital waste	Treated hospital effluent applying ozone at different pH conditions (3,0, 6,7, 10)	RG, MI	MC	CT	(Dayana et al., 2012)
Contaminated soil	Four soil types collected from different regions of Amritsar, India	CA	MC	GT	(Soodan et al., 2014)
Effluents	Effluents arising from the ornamental rock polishing	MI, CA	MC	CT, GT	(Teixeira et al., 2015)
Textile effluents	Un-treated and bio-treated effluents from Masood Textile, Kalash Textile, Khyber Textile and Sitara Textile (Faisalabad, Pakistan)	MI, RG	MC	CT	(Bilal et al., 2016)
Contaminated water	Marrakesh's CMR wastewater, Draa Lasfar mine located about 12km south-West of the city of Marrakech, Morocco	MI, CA	MC	CT, GT	(Chaik et al., 2011)
Water sample	Orathupalayam dam Water, India	MI, CA	MC	CT, GT	Gajalakshmi and Ruban (2014)
Waste	Metalworking fluids at 1/250, 1/500 and 1/1000 concentrations	MI, CA, MN	MC	CT, GT, MT	(Pekol, 2014)
Agriculture	Abamectin+Emaamectin benzoate) at 0, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 ml/L concentrations for 24 h exposure	MI, CA, MN	MC	CT, GT, MT	(Ahmed, 2014)
Wastewater	Dandugan Oya, a water canal located in the Western Province of Sri Lanka receives industrial waste from multiple sources	MI, CA, NA, MN	MC	CT, GT, MN	(Kannangara and Pathiratne, 2015)
Electroplating industry	Wastewater from Madurai city, India contains heavy metal (Ni, Cu, Fe, Mn and Pb) higher than permissible limits	MI, CA	MC	CT, GT	(Nagarajan et al., 2014)
Contaminated water	Arsenic contaminated groundwater from Eastern region of Burdwan district, West Bengal	MI	MC	CT	(Bandyopadhyay, 2015)
Nanoparticles	Al_2O_3 nanoparticles at 0.01, 0.1, 1, 10, and 100 µg/mL concentrations	MI, CA	MC	CT, GT	(Rajeshwari, 2015)
Wastewater	Sediment elutriate from Tambis River, Barobo, Surigao Del Sur, Philippines, 1000 g/L and 500 g/L, for 72 h exposure	MI, CA	MC	CT, GT	(Fajardo et al., 2015)
Water sample	Ballast water at 0.5, 1, 5 and 10% concentrations	CA	MC	GT	(Olorunfemi et al., 2014)
Water sample	Different concentrations of bilge water (1, 5, 10, 25 and 50%)				(Olorunfemi et al., 2015)
Food industry	Liquid waste produced in the process of industrialization of the bitter cassava (a plant used as food and an ingredient in industry)	MI, CA	MC	CT, GT	(Viana et al., 2014)
Fly ash	Un-treated and vermicomposted flyash	MI, RG	MC	CT	(Sarojini et al., 2010)
Wastewater	Irrigation water used for vegetables in a Greater São Paulo, Brazil watershed region	CA, MI, MN	MC	GT	(de Maio Lacerda et al., 2014)
Sugar industry	Raw and vermicomposted (*Eisenia fetida* + cattle dung) sugar beet mud and pulp (waste by-products of the sugar industry)	CA	MC	GT	(Bhat et al., 2015)
Contaminated water	Well water from Vazante-MG city, Brazil (zinc producer district)	CA	MC	GT	(da Silva et al., 2013)
Food industry	Synthetic Cheese and Cheddar Cheese (food flavors) at 1.0 and 2.0 mL doses	MI	MC	CT	(Moura et al., 2016)

Table 2: Continue…

Leachate	Gaseous emissions (biogas) from three municipal landfills and Leachate samples	MI, CA	MC	CT, GT	(Feretti et al., 2008)
Textile industry	Un-treated and treated textile effluent using immobilized manganese peroxidase (MnP) and detoxification	MI	MC	CT	(Bilal et al., 2016a)
Textile industry	Un-treated and Ganoderma lucidum IBL-05 MnP immobilized on Sol-Gel matrix treated textile effluent	MI	MC	CT	(Bilal et al., 2016b)
Textile dyes	Un-treated and Agar–agar gel immobilized MnP treated dyes	MI	MC	CT	(Bilal et al., 2016c)
Textile industry	Un-treated and *Ganoderma lucidum* IBL-05 crude ligninolytic enzymes (MnP LiP and Laccase) treated textile effluents	MI	MC	CT	(Bilal et al., 2016d)
Textile dye	Un-treated and N-TiO_2 nanostructure treated textile dye	MI, CA	MC	CT, GT	(Kadam et al., 2014)
Wastewater	Refined petroleum products (Petrol, Kerosene) and locally made soft drink (Zobo) at different concentration were tested	MI, CA, MN	MC	CT, GT, MT	(Obute et al., 2016)
Coal mine waste	used calcinated coal mining waste to treat river water contaminated by residues derived from coal mines from the Urussanga River (Urussanga, Santa Catarina state, Brazil)	MI	MC	CT	(da Silva Francisconi et al., 2013)
Textile dye	Un-treated and UV/TiO2/H_2O_2 treated Disperse Red F3BS	RG, MI	MC	CT	(Bokhari et al., 2015)
Surfactant	Un-treated and gamma radiation/H_2O_2 treated nonylphenol ethoxylates	RG, MI	MC	CT	(Iqbal et al., 2015)
River water	Todos os Santos River (TSR), Brazil receives a high discharge of untreated effluents from various sources	CA, MN	MC	GT, MT	(Blanc et al., 2013)

The metallurgy industries discharge a variety of toxic heavy metals like Zn, Cd, Cr, Cu, Ni, Pb, V, Mo, Co along with other solid waste and is threat to the environment, if improperly managed (Lee and Pandey, 2011). The major waste generation units include smelter flue dust, converter slag, dump slag, tailings, smelting slag, filter press residue, waste-sludge, red mud, metallurgical-waste dump, pyrite sludge spillage, waste and blast furnace slag and sludge in large amount. For example, 300–400 kg of slag is produced in the production of one ton of pig iron and a single aluminum (Al) manufacturing industries generate about 35,000 ton of spent potliners (waste) per annum (Andrade et al., 2008; Kumar et al., 2006).

To study the toxicity of metallurgical wastes and metals, various authors reported the sensitivity of *A. cepa*. While studying the aluminum manufacturing waste,spent potliners waste was found to be toxic to *A. cepa* root cells (Andrade et al., 2008). A strong inhibition of root growth at higher spent potliners concentrations has been observed. The CA's such as bridges, fragments, stickiness, multipolar anaphase, later segregation and cell death was reported as a result of spent potliners exposure. Other studies also revealed the CA induction and decrease in MI when root were exposed to aluminum metal solution (Fiskesjo, 1988; Roy et al., 1989).

The cytogenetic effect of Cu mine waste at different concentrations (10%, 75% and 100%) was also studied using CA endpoint in *A. cepa* root assays (Inceer et al., 2000). Authors recorded a dose-dependent relationship with the inhibition of MI and at 100% concentration the highest cytotoxicity was recorded. The authors also reported the chromosomal breaks, bridges and adherences. They concluded that Cu wastes can cause deleterious effects on *A. cepa* root cells. While studying the genotoxic effects of leachates from solid waste of a metal industry (Chandra et al., 2005), reported the CA, MI and MN alterations in the *A. cepa* root cells. The *A. cepa* bulbs were exposed for 48 h to 2.5-10% leachates wastes and resultantly, significant inhibition of MI, induction of CA, mitotic aberrations and MN formation were found in all experimental groups exposed to metal waste leachate. The toxic effects were found concentration dependent and MI was severely inhibited at 10% leachate aqueous solution exposure. These toxic responses may have relied on raised heavy metals (Cr, Ni and Fe) concentrations in leachates wastes. The CA inductions and root growth inhibition in *A. cepa* root exposed to the metallic industry wastewater for 24 h was also observed (Nielsen and Rank, 1994).

Few studies also highlighted the individual effects of metal on CA's, MI, NA's and MN's abnormalities. For example, stickiness, C-mitosis, deformed nuclei and decrease in the MI is correlated with Cu metal and Mn with sticky chromosomes (Abdel Migid et al., 2007; Fiskesjo, 1988). In the presence of Zn, MI inhibition and sticky chromosomes, C-metaphases, bridges and metaphase disturbed by lagging chromosomes were observed (El-Ghamery et al., 2003) and Cd induced chromatin fragmentation, changes in the nucleolus and ultimately cell death (Andrade et al., 2008), while Pb reduced root growth and caused mitotic irregularities, including C-mitosis, anaphase bridges and chromosome stickiness. Furthermore, MN formation during interphase, irregularly shaped nuclei and nuclei with decomposed nuclear material were observed in the presence of Pb (Behboodi and Samadi, 2004; Liu et al., 1994). Analyses of *A. cepa* meristematic cells exposed to water samples with high Ni and Pb levels, indicated the CA's abnormalities (Bianchi et al., 2011).

Recently, the use of nanoparticles in various field has attracted the attention (Kumari et al., 2009b) and in this regard, the cytotoxic and genotoxic impacts of silver nanoparticles using root tip cells of *A. cepa* have been investigated. The *A. cepa* root tips were treated with four

different concentrations (25, 20, 75, and 100 ppm) of size <100 nm and studied the MI, distribution of cells in mitotic phases, various types of CA's such as disturbed metaphase, sticky chromosome, cell wall disintegration and breaks. The MI value was 27.62% in treated group of plants as compared to control (60.3%) and with increasing concentration of the nanoparticles decrease in the mitotic index was noticed. The authors revealed that silver nanoparticles could penetrate plant system and may impair stages of cell division causing chromatin bridge, stickiness, disturbed metaphase, multiple chromosomal breaks and cell disintegration and recommendedtthe importance of *A. cepa* in the ecosystems for evaluation of toxicological impact of the nanoparticles in the environment. The ZnO nanoparticles were also revealed to have cytogentic effects (Kumari et al., 2011). *A. cepa* roots were treated with the dispersions of ZnO NPs at four different concentrations (25, 50, 75, and 100 µg/mL). The MI value decreased along with significant inductions of MN and CA's. Results demonstrated the clastogenic/genotoxic and cytotoxic nature of ZnO NPs . Therefore, *A. cepa* cytogenetic test is suggested to evaluate the genotoxicity nanomaterials. Similarly, Al_2O_3 nanoparticles (Al_2O_3 NPs) were also investigated for their cytogenetic potential in *A. cepa* root tip cells at 0.01, 0.1, 1, 10, and 100 µg/mL concentrations (Rajeshwari et al., 2015). MI value reduced in dose-dependent manner (42 to 28 %) along with CA's (sticky, multipolar and laggard chromosomes, chromosomal breaks, and the formation of binucleate cells). The interaction between roots and NP's were studied by optical, fluorescence, confocal laser scanning microscopy and FT-IR techniques. In another study, toxicity of cobalt and zinc oxide NPs using growth, root and cell morphology enpoints was studied (Ghodake *et al.,* 2011). *A. cepa* roots were treated with dispersions of the cobalt and zinc oxide NPs at 5, 10, and 20 µg/mL concentrations. The roots growth inhibited by both the cobalt and the zinc oxide NPs in concentration dependent manner.

The sensitivity of *A. cepa* to cadmium (Cd) stress was investigated. The endpoints screened for genotoxicity included chromosomal aberrations, micronuclei frequency and mitotic frequency. The results indicated that exposure to Cd induced dose-dependent increase in chromosomal aberrations, DNA fragmentation and micronucleus frequency in *A.cepa* roots (Arya and Mukherjee, 2014).

The CA, MN, MI and NA are attributed to the presence of metals in metallurgical wastes and a number of authors showed these aberrations in other systems also in the presence of metals. For example, Cu contaminated drinking water was reported to affect the *A. cepa* root merimistematic cell (Fiskesjo, 1981) and Zn^{2+} and Cd^{2+} ions were also reported as CA causing agents in the *A. cepa* meristematic root cells (Leme and Marin-Morales, 2009). The metals ions such as Hg, Cu, Ni, Cd, Be, Al, Mn, Li induced the CA's like C-metaphases and chromosome adherence in *A. cepa* (Fiskesjo, 1988). Arsenic showed genotoxicity and induced MN in meristematic as well as in F_1 cells of *A. cepa* (Yi et al., 2007a). Water sample containing chromium ions also showed CA and MN formation in *A. cepa* meristematic cells (Matsumoto et al., 2006) and wastewater contaminated with Fe, Pb, Cr and Cu showed CA's in *A. cepa* roots (Abdel Migid et al., 2007). Significant effects on MI and CA and MN induction were also documented when *A. cepa* roots were exposed to Cd metal (Seth et al., 2008b). A considerable CA were also reported in *A. cepa* roots in the presence of Pb, Ni, Cr, Zn, Cu, and Cd (Rank and Nielsen, 1998). For the detection of genotoxicity of boron, Konuk et al. (Konuk et al., 2007) reported significant induction of CA's in *A. cepa* meristematic root cells.

Pulp and paper mill

The pollutants discharged from the pulp and paper industry affect the water bodies adversely (Pokhrel and Viraraghavan, 2004). Change in plankton population has been reported due to untreated paper mill effluent discharge into the system (Baruah and Das, 1997) and (Yen et al., 1996) reported the sub-lethal effects on the aquatic organisms. Another study narrated the effects of treated pulp mill effluents on irrigated soil and revealed the serious adverse soil chemistry changes (Howe and Wagner, 1999). Paddy field was also affected as a result of pulp and paper mill effluents (Dutta, 1999) and high loads of organic pollutants and trace metals in paper mill wastewater (Gupta, 1997; Skipperud et al., 1998). In view of toxic nature of pulp and paper mill wastewater, various authors evaluated the toxicity by *A. cepa* test system.

The MN and anaphase aberrations were performed by exposing *A. cepa* roots to paper mill wastewater. The acetone extracts of the sludge samples from paper mill were also used for the treatment of *A. cepa* roots and resultant abnormalities were recorded (Grover and Kaur, 1999). In a study, *A. cepa* root tip cells were analyzed for CA after 24 h of exposure to paper mill wastewater and results showed considerable effect on CAs and genotoxicity (Nielsen and Rank, 1994). The toxicity of Kraft mill effluent was examined by Tipirdamaz et al. (Tipirdamaz et al., 2003) by *A. cepa* test system. They treated the *A. cepa* bulbs with wastewater samples and observed the inhibition of root growth (55.72%). The *A. cepa* also showed a significant induction in cytological abnormalities such as C-mitosis, anaphase with laggard chromosome, fragment and bridges and chromosome stickness in root-tip cells. C-mitosis of 47.7% was observed in root tip cells treated with paper mill (Kraft) effluent. Taking the total aberrations into account, percentages of laggard chromosomal fragment and bridge formations were 26.6% and 33.3%, respectively and chromosome stickiness was found to be 12.2% versus negative control. The banded chromosome was observed at the frequency of 13.3% of aberrant cells. Chaparro et al. (Chaparro et al., 2010) evaluated the genotoxicity of bleach plant effluents (paper mill) by applying the CA, MN and MI in *A. cepa* root cells. The largest CA and MN incidence in the meristematic cells of *A. cepa* were recorded after exposure to the untreated bleach plant effluent, however, anaerobic biodegradability reduced the CA's and MN formation. In a study to evaluate the genotoxic effects of paper mill, Gana et al. (Gana et al., 2008)

revealaed the induction of toxicity in *A. cepa* roots exposed to the tested samples. In another study, the toxicity and genotoxicity tests were performed on root cells of *A. cepa* in order to evaluate wastewater from the bleaching sequence of Kraft cellulose from radiata pine (*Pinus radiata*). Kraft cellulose from an industrywas specifically obtained from the first extraction stage of the ECF bleaching sequence (Roa et al., 2012). The results revealed a toxic effect of the effluent, with inhibition of meristem growth and generally lower values of metaphase, anaphase and telophase indices at pH 10.5 than pH 7 for all effluent concentrations. The genotoxicity effect was different from the toxic effect which indicated that the micronucleus and the chromosomal aberration tests in anaphase-telophase cells were low over all ranges of the studied effluent concentrations.

Chaparro and Pires (2011) treated the bleaching effluent from two kraft pulp mills in anaerobic reactor and analyzed for physico-chemical parameters and toxicity *A. cepa* along with other bioassays. Bio-treatment improved the water quality along with cytotoxic, genotoxic and mutagenic effects reductions. Based on results, authors suggested the biotreatment of wastewater before discharge the wastewater and monitoring the treated water using bioassays.

Chaparro et al. (2015) applied an advanced oxidation process (ozone and ozone/UV) to treat kraft cellulose pulp mill and ecotoxicity and genotoxicity were measured before and after treatment with ozone and ozone/UV. The AOX, color and phenol removed up to 40%, 79% and 32%, respectively and A. cepa test showed good sensitivity to measure the genotoxicity of treated and un-treated wastewater. In another study, paper and pulp industry effluent treated by *Rhodococcus* sp. NCIM 2891 were evaluated for cytogentic effects. Along with improvement in water quality parameters (COD, BOD, TDS, heavy metals) toxicity was also reduced considerably (Nadaf and Ghosh, 2014). Results revealed effectiveness of bio-treatment of pulp and paper wastewater and efficiency of *A. cepa* test to evaluate the toxicity difference between treated and un-treated effluents.

Textile industry

The textile industry generates a huge volume of wastewater which contains a varieties of toxic agents such as heavy metals, carboxymethyl cellulose, polyvinyl alcohol, sodium hypochlorite, chlorine, acids, alkali, surfactants, dyestuffs, reducing agents, oxidizing, agents, wetting agents, binders, thickeners and cross-linkers (Adeel et al., 2017; Adeel et al., 2015; Bouatay et al., 2016; Gulzar et al., 2015; Husaain et al., 2015; KANU and Achi, 2011; Mahmood and Malik, 2014; Muneer et al., 2016; Saeed et al., 2016). Researches reported the toxicity of textile wastewater in *Daphnia magna* (Eremektar et al., 2007), microbil toxicity (Koparal et al., 2007), male reproductive systems of albino rats and mice (Suryavathi et al., 2005). Regarding toxicity evaluation by *A. cepa,* they indentified and studied numerous potentially mutagenic endpoints.

In a study, the CA's, NA and MN were reported due to continuous exposure of *A. cepa* bulb to textile effluent in different concentrations (0.3%, 3%, 10%, and 100%). The mutagenic effect of the effluent at concentrations of 10% and 100% were observed. However, at lower concentrations (0.3% and 3%) no abnormities were recorded (Carita and Marin-Morales, 2008). In another study, genotoxic effects of leachates from solid waste of a dye industry have been recorded. The *A. cepa* bulbs were exposed for 48 h to 2.5–10% leachates aqeous solution and results revealed the cytogenetic effects in *A. cepa* root. Significant inhibition of MI, CA's and MN inductions were found in all experimental groups exposed to dye waste leachate aqueous solution (Chandra et al., 2005). Chandra et al. (Chandra et al., 2005) applied the CA test in *A. cepa* to assess the genotoxic effects of dye waste leachate from chromium pigments manufacturering plant. The authors also reported MI inhibition and CA's and MN induction in the roots exposed to leachate extracts.

The genotoxicity of wastewater samples from textile industrial effluent from the Amritsar, India were investigated using the *A. cepa* MN and anaphase aberration assays and effluent exposure to *A. cepa* showed negative responses both in the MN and NA (Grover and Kaur, 1999). In another study, the toxicological analysis of effluents before and after treatment (decolorization and degradation) was performed using *A. cepa* test and results revealed the cytotoxicity and genotoxicity significantly reduced after treatment (Phugare et al., 2011). While studying the toxicity of textile industry wastewater by *A. cepa*, Nielsen and Rank (Nielsen and Rank, 1994) revealed that the wastewater showed a considerable effect on the growth of *A. cepa* roots in five days exposure and CA analyzed after 24 h of exposure also revealed the CA's effect as compared to negative control. In another study, the cytogenotoxic effects of the textile industrial effluents at different concentration (1.6, 4, 8 and 16%) were reported by *A. cepa* assay. The root length, MI and CA's were recorded after 96 h exposure and results showed that the root growth inhibition was concentration dependent. The MI decreased with increasing concentrations of textile industrial effluents and the CA's such as vagrant chromosome, bridges and fragments and sticky chromosomes were observed in treated group of *A. cepa*. At lower concentrations bridges and fragments were the most common aberration (Samuel et al., 2010). Cytotoxicity and genotoxicity studies were performed before and after decolorization of the textile effluent by *A. cepa* root test (Jadhav et al., 2010). Results indicated strong genotoxic effects of the effluents in the mitotic cells before treatment. The control exhibited the MI value of 11.68%, while cells exposed to effluents at a concentration of 30% showed MI of 9.42% and further by applying the concentrated solution, the MI decreased significantly. An increase in the incidence of polyploidy cells was also detected and correlated it with effects of the dyes on the -SS and -SH containing proteins of the spindle apparatus. The biotreated samples showed the MI in the range of the control.

The CA test in *A. cepa* roots was applied to evaluate the genotoxic potential of a silk dyeing industry effluent and response revealed MI inhibition and induction of several mitotic abnormalities in roots cells exposed to the tested effluent (Sudhakar et al., 2001). In order to assess the genotoxic effects of textile effluent Oriaku et al., (Oriaku et al., 2011) investigated the MN, CA's and NA abnormalities in *A. cepa* along with physico-chemical characterization of wastewater. The textile effluent reduced the root length and higher CA's and NA frequencies such as vagrant, sticky chromosomes, binucleus and C-tumors in exposed *A. cepa* root tips as compared to control. The results obtained from this study indicate that textile effluent discharged into the watershed is capable of causing significant ecological disruption in the receiving environment and *A. cepa* is a sensitive method for indication disruptions. Few others studies were also performed in order to evaluate the cytotoxic, genotoxic and mutagenic actions of dyes aqueous solution i.e., the toxicity of azo dye at different concentrations (1, 10, 100 and 1000 µg/L) was evaluated by *A. cepa* test and it was found that dye induced cell death as well as CA's, NA and MN even at low concentration (Ventura-Camargo et al., 2011). The CA's in *A. cepa* roots was correlated with to the presence of azo dyes (CI disperse blue 373, CI disperse violet 93 and CI disperse orange 37) in textile wastewater (Carita and Marin-Morales, 2008).

Balan et al., (2012) treated textile dye malachite green with laccase (white-rot fungus *Pleurotus florida* NCIM 1243 enzyme) and treatment efficiency was evaluated on the basis of decolorization and detoxification. Under optimized treatment conditions, up to 96% decolorization of dye was achieved and A. cepa showed toxicity (root growth inhibition) in dye concentration dependent manner. Prasad et al. (2013) used A. cepa test to evaluate the ability of halophilic bacterial strain to degrade the complex azo dye Direct Blue-1 (DB-1). Complete decolorization of DB-1 (100 mg/L) was achieved in 6 h at 37 °C, pH 8 and with 70 g/L NaCl. Phytotoxicity study revealed the less toxic nature of the metabolites compared to the parent dye. Genotoxicity test confirmed the toxic nature of DB-1 since several CA's were observed which reduced significantly after treatment. Mansour et al. (2011) examined the performance of *Pseudomonas putida* mt-2 in treating TE. *P. putida* was able to completely decolorize the studied effluent after 8 h of incubation under agitation in an aerobic bioreactor. Genotoxicity of effluent, before and after biodegradation was evaluatedand chromosome aberrations were compared with untreated mice. Results indicated that TE showed a significant ability to induce DNA damage, which was evaluated in both SOS (IF = 3.52) and chromosome aberration assay systems (23.5%). In addition, TE reduced strongly the acetylcholinesterse (60%) and butyrylcholinesterase (51%) activities and induced a remarkable lipid peroxidation effect (increased markedly by approximately fivefold). This toxicity was imputed to the presence of dye compounds of TE. However, toxicity in TE was significantly reduced after 8 h of aerobic incubation with *P. putida*mt-2 strain. The study demonstrated that *P. putida* mt-2, incubated under aerobic conditions, had a metabolism that enabled it to degrade TE and, especially, to detoxify the effluent mixtures.

The enzymatic (peroxidases from turnip) treated textile dyes toxicity was evaluated using *A. cepa* assay. Bio-treated effluents showed less toxicity versus un-treated wastewater (Kulshrestha and Husain, 2007). Similarly, remazol red (RR) was treated with *Pseudomonas aeruginosa*. Apart from 97% dye degradation, the dye toxic effect was also reduced (Jadhav et al., 2011). Phugare et al. (2010) treated textile wastewater with yeast cell biomass (waste product from the distillery industry) and *S. cerevisiae* MTCC 463 (SC) and toxicity was evlauted before and after treatment using *A. cepa* test. Textile effluent was decolorized up to 78% using SC along with heavy metals removal with both the treatment process. Cytotoxicity, genotoxicity and phytotoxicity studies were performed to evaluate the toxicity of metabolites produced after decolorization of textile effluent, which revaled the efficiency of *A. cepa* for detoxification evaluation. Satar and Husain (2011) employed calcium-alginate pectin entrapped bitter gourd peroxidases for the treatment of disperse Brown 1 (DB 1) and Disperse Red 17 (DR 17). Toxicity was evaluated before and after bio-treatment. Maximum decolorization of 98% DR 17 and 71% DB 1 dyes were achieved at optimized conditions and toxicity tested by *A.m cepa* test showed good efficiency for the evaluation of bio-treatment effect. Jadhav et al. (2012) treated textile azo dye Remazol orange by bacterium *Pseudomonas aeruginosa* BCH in plain distilled water and detoxification was checked by *A. cepa*. Results revealed less toxic nature of the dye after bacterial treatment. Similarly, in another study calcinated coal mining waste contaminated river water and residues derived from coal mines from the Urussanga River (Urussanga, Santa Catarina state, Brazil) were assessed using root growth inhibition test. Untreated water caused significant toxicity, which reduced after treatment (da Silva Francisconi et al., 2013). Iqbal and Bhatti (2014) evaluated the toxicity of un-treated and photo-catalytic treated texile wastewater. Cytotoxicity was evaluated through *A. cepa* and heamolytic bioassays. *A. cepa* test showed that photo-catalytic effluents showed less cytotoxicity and authors revealed that processed wastewater could possibly be used for irrigation purposes in view of significant toxicity reduction. In another study N-TiO_2 nanostructure was used for textile dye treatment and and apart from 97% degradation, the cytotoxic effect of teated wastewater reduced to a greater extent versus un-treated sample (Kadam et al., 2014). Iqbal et al. (2015) also evaluated detoxification of textile effluents treated by advanced oxidation process. The cytotoxicity of un-treated and treated effluents was measured by root growth. The wastewater irradiated to the absorbed doses of 5kGy, 10kGy, and 15kGy, and subjected to toxicity tests. The gamma radiation showed promising efficiency for detoxification of pollutants in wastewater. The root length and root count increased up to 31.10% and 38.34%, respectively when roots exposed to wastewater treated to the absorbed dose of 5 kGy.

A. cepa test showed good correlation with heamolytic and brine shrimp test used for cytotoxicity evaluation. Results showed that gamma radiation has promising efficiency for the degradation of toxic agents present in effluents and *A. cepa* proves to be efficient for monitoring the toxicity of treated effluents. Iqbal and Nisar (2015) also treated textile effluents by gamma radiation in combination with hydrogen peroxide and cytotoxicity and mutagenicity was investigated using A. cepa along with shrimp amd Ames tests. Textile effluents were irradiated to the gamma radiation absorbed doses of 5 kGy, 10 kGy and 15 kGy in combination with 20 mM hydrogen peroxide. Before treatment, textile effluents showed a significant cytotoxic and mutagenic signs, which reduced significantly after treatment. *A. cepa* showed reduction in cytotoxicity upto 50%. Results revealed that gamma radiation in combination with hydrogen peroxide can be implemented for the detoxification of textile effluents and *A. cepa* assay is excellent in evaluating the toxicity. Machado et al. (2012) also investigated the detoxification of the wastewater produced by the process of rhodamine B dyeing using oxidation processes (Ozonization, ultraviolet irradiation and O_3/UV). Cytotoxicity and genotoxicity were assessed of un-treated and treated wastewater using *A. cepa* test along with other assays. Advanced oxidation treatment significantly degraded the rhodamine B. Toxicity results showed that inspite of complete decolorization, dye treated solution was found to be toxic, which might be due to the generation of toxic by-product as a results of destruction of complex dye. While studying the effect of immobilized manganese peroxidase (MnP) on detoxification (Bilal et al., 2016d), *A. cepa* along with other bioassays was employed. At optimized conditions, up to 87.4% degradation was achieved along with significant reduction in cytotoxicity i.e., root length, root count and mitotic index increased up to 38.46%, 43.47% and 41.83%, respectively versus un-treated effluents, which was also confirmed by heamolytic, brine shrimp and Ames tests. Same authors, investigated the efficiency of *Ganoderma lucidum* IBL-05 MnP immobilized on Sol-Gel matrix and reported that the cytotoxicity was reduced after bio-treatment (Bilal et al., 2016b). Agar–agar gel immobilized MnP treated textile dyes was also monitored for toxicity and *A. cepa* test revealed that the toxicity was reduced significantly after bio-treatment (Bilal et al., 2016c). Another study also confirmed the *A. cepa* test efficiency for toxicity monitroring of *Ganoderma lucidum* IBL-05 crude ligninolytic enzymes (MnP LiP and Laccase) treated textile effluents (Bilal et al., 2016f). Patel et al. (2015) also evaluated the efficiency of *A. cepa* to monitor the effectiveness of bio-treatment of industrial effluent. Toxicity study of the effluent using *A. cepa* revealed that detoxification of the effluent as a result of bio-treatment was significant. The detoxification of textile wastewater using *Ganoderma lucidum* IBL-05 crude ligninolytic enzymes was also monitored by *A. cepa* test (Bilal et al., 2016f). *A. cepa* test was applied for cytotoxicity evaluation along with bioassays. The degradation of Masood Textile, Kalash Textile, Khyber Textile and Sitara Textile (Faisalabad, Pakistan) effluents were achieved up to 87.29%, 80.17%, 77.31% and 69.04%, respectively at optimized conditions. The cytotoxicity tests revealed that biodegradation significantly detoxify the toxic agent in wastewater and author's suggested the use of biodegradation for the remediation of textile effluents along with/ and detoxification monitoring using bioassay to evaluate the bio-efficiency of a treatment method.

Balan et al. (2013) investigated the ability of *Pleurotus florida* (laccase) on coconut flesh as a solid substrate fermentation. The decolorization of two structurally different dyes such as azo (Reactive Blue 198) and triphenylmethane dye (Malachite Green) were analysed and toxicity before and after treatment was evaluated by *A. cepa* test. The decolorization of Reactive blue 198 and Malachite Green at 8 h was 93% and 63%, respectiely. *A. cepa* root inhibition was measured to demonstrate the potential of laccase in the detoxification and bioremediation process and positive response was observed. Jadhav et al. (2015) treated textile effluents by *Pseudomonas sp.* SUK1 and toxicity was evaluated before and after treatment for comparison. *A. cepa* test confirmed the severe toxicity of effluent before treatment, which reduced significantly after bacterial treatment. Watharkar and Jadhav (2014) evaluated dyes mixture treated by *in vitro* grown *Petunia grandiflora* and *Gaillardia grandiflora.* Toxicity was evaluated before and after treatment. Metabolites formed after the degradation of dyes revealed the reduced cytogenotoxicity on *A. cepa* roots cells when compared with untreated dye mixture solution. Phytotoxicity study exhibited the less toxic nature of dye mixture after treatment. Watharkar et al. (2013) also treated reactive Navy Blue RX (NBRX) dye by synergistic phytoremediation potential of *Petunia grandiflora* with its rhizospheric bacterial isolate *Bacillus pumilus* strain. The biodegradation degraded dyes up to 80.01% and 76.80% by *P. grandiflora* and *B. pumilus* cultures, respectively, whereas consortium decolorized NBRX up to 96.86%. The treated dye exhibited less cytogenotoxicity effect on *A. cepa* roots when compared to NBRX un-treated solution. Similarly, Poonkuzhali et al. (2011) degraded treated azo dye (reactive blue 198) biologically and analyzed for toxicity. *A. cepa* toxicity test indicates that root growth inhibition was dye concentration dependent, which reduced when roots were exposed to treated dyes solution. Similarly, the cytotoxicity and DNA damaging effects of textile effluent at 1, 2, 5, 10, 25, 50 and 100 (%) were studied. Roots were exposed for 96 h and subjected to macro- and micro-analysis (Alimba et al., 2013). Root length inhibition was 50% and EC50 values were of 16, 35, 6.5 and 8% for 24, 48, 72 and 96 h, respectively. Decrease in cell proliferation and higher CA's (binucleated cells, sticky chromosomes, chromosome fragments and anaphase bridges) were also observed. These cytogenetic effects were correlated with Fe, Cd, Mn, Ni, Cr, and other disturbed physic-chemical parameters.

The toxic potential of flyash by *A. cepa* assay and vermicomposting effect on toxicity reduction was studied (Sarojini et al., 2010). Exposed *A. cepa* roots were found coiled and wavy along with MI reduction, however,

vermicompost did not induce any negative effect on root morphology. The root grown in cow dung, fly ash, cow dung + fly ash and vermicomposted fly ash mixture showed MI values of 9.52, 7.30, 11.43 and 15.76, respectively. Results revealed that fly ash was toxic, which reduced in vermicomposted fly ash and may be beneficial to apply vermicomposted in agriculture.

Recently, the potential toxicity of selected industrial wastewaters reaching the Dandugan Oya in Srilanka, and the downstream water was assessed using *A. cepa*. The exposure of *A. cepa* bulbs to wastewater and downstream water from the Dandugan Oya resulted in the reduction of root growth (24 – 62 %) and mitosis (31 – 55 %), induction of MN (up to 0.6 %), nuclear abnormalities (3 - 14 folds) and CA's (3 - 21 folds) in comparison to control. Results revealed that wastewaters under investigation contained cyto-genotoxic agents and authors suggested the use of suitable bioassays as additional assessments in water quality monitoring as indicator of cyto-genotoxic agents in wastewater receiving inland surface waters (Kannangara and Pathiratne, 2015).

Fertilizer and chemical industry waste

Among chemical fertilizers, the urea and NPK (nitrogen-phosphorous-potasium) fertilizers are used most commonly and contributed immensely towards high pollution load in agricultural runoff effluent and ultimately water bodies and soil is contaminated. Typically, a urea plant produced the wastewater has ammonia level of 1381.3 and 1479.7 mg/L and NPK 2684.5 mg/L. In general the effluent discharged from fertilizer and chemical units may be highly alkaline or acidic having high amount of florides and are responsible for eutrophication, skeletal fluorosis and abnormal calcification of bones (Sridhar et al., 2009).

The genotoxicity tests of fertilizer industry effluent were conducted with *A. cepa* before and after treatment with different kinds of algal biofilters. Root cells were exposed for 24 h to different dilutions of both raw and treated wastewater and cytogenetic endpoints (CA, MI and NA) were used to assess the potencies of the effluents. Before algal treatment, effluents showed a strong genotoxic effects represented by severe inhibition in mitotic activity of meristematic cells with high frequency of both CA and NA. After algal treatment, the cytotoxic effects of 30% and 60% concentrations of the treated effluent were comparable to those of 5% and 10% concentrations before treatment, respectively and the frequency of CA's and NA declined by 50%. Statistical analysis of the data indicates a significant reduction in genotoxicity associated with a remarkable reduction in heavy metals concentrations after bioremediation by algal biofilters (Abdel Migid et al., 2007). In another study, *A. cepa* response was studied of the chemical factory effluent in Enugu, Nigeria (Abu and Ezeugwu, 2008). The MI decreased as effluent concentration and treatment durations increased. Significant differences in MI at different concentrations and treatment durations were recorded. Conversely, percentage of abnormal dividing cells significantly increased as the concentration and treatment durations increased. Diverse CA's and cytotoxic problems were observed, ranged from a mild C-mitotic effect at lower concentrations to vacuolated nucleus and even cytokinetic failure at higher concentrations. In another study, the effect of wastewater from a phosphoric gypsum depot on *A. cepa* roots, showed the CA and mitotic irregularities. In comparison to oak, *A. cepa* was found to be less tolerant to wastewater from the phosphoric gypsum depot (Pavlica et al., 2000). The chemical industry wastewater induced anaphase-telophase disturbance. A significant difference was observed regarding CA's and root growth inhibition in exposed roots versus negative control (Vujosevic et al., 2001).

Tannery and leather industry

Wastewater expelled from leather and tannery industries are of great concern and considered to be one of the ten most harmful to the environment, responsible for extreme pollution of water resources and generating substances leading to deterioration and death of a wide range of living organisms (Junior et al., 2007). Various authors reported the toxicity of tannery wastewater against *A. cepa* exposed to tannery waste, which led to significant decrease in mitosis and root growth and attributed it to the presence of Cr and Ni compounds in it (Chandra et al., 2004). The genotoxicity of leachates of tannery solid waste through aqueous and soil medium has been reported in root meristematic cells of *A. cepa*. The experiment was conducted in two sets in which one set of bulbs was placed over aqueous concentration of leachates (2.5, 5 & 10%), while in other experiment bulb were exposed to contaminated soil. Root tips, sampled after 48 h revealed higher frequency of aberrations in aqueous medium as compared to leachate-contaminated soil. However, mitotic abnormalities, CA's and MI inhibition were observed in both experiments (Chandra and Gupta, 2002). In another study, while evaluating the genotoxicity of water contaminated with leather industry wastewater, Junior et al. (Junior et al., 2007) reported evidences of CA's in *A. cepa* root tip cells. The water impacted by tanneries wastewater, also showed significant frequencies of CA's and MN in exposed *A. cepa* meristematic cells and the abnormalities were correlated with the presence of Cr ions (Matsumoto et al., 2006).

Matsumoto and Marin-Morales (2004) conducted a study to assess the toxicity and cytotoxicity of water bodies receiving industrial and domestic effluents using *A. cepa* test. To assess the toxicity and mutagenicity of water possibly contaminated with chromium, derived from tannery activities, seasonal water samplings were performed in 2001 and 2002 at five different sites along the Sapucaizinho River, Municipality of Patrocínio Paulista, State of São Paulo, Brazil. *A. cepa* seeds were germinated in waters from the different collection sites. Results showed that the collection sites most heavily compromised by chromium since low mitotic indices and a higher frequency of mitotic changes such as irregular anaphases (disorganized, multipolar, laggard), cells with

chromosomal adherences, cells with MN, and binucleate and/or multinucleate cells were observed in exposed roots.

Gupta et al. (2012) studied the cyto genotoxic effects of tannery effluent and chromium (Cr) in *A. cepa*, from "Up flow Anaerobic Sludge Blanket" (U.A.S.B) Jajmau, Kanpur. *A. cepa* roots were exposed to various concentrations of tannery effluent (0.0, 3.125, 6.25, 12.50 and 25.0%) and Cr (0.0, 1.0, 2.0, 4.0, 8.0 mg/L) for 48 and 168 h. Effluent analysis showed pH (8.5), EC (11.94 dSm-1), BOD (499 mg/L), COD (1382 mg/L) and Cr content (2.32 mg/L), which were much higher than the prescribed permissible limits. A significant reduction in root length, MI and CA's, mitotic aberration and MN were observed. Reduction of 81.15 and 79.71% in MI, and induction of 6.8 and 4.8% in CA, 29.24 and 26.66% in mitotic aberration and 0.52 and 0.43% in MN were found at 12.50% of effluent and 4 mg/L Cr treated roots versus control.

Poonkuzhali et al. (2013) evaluated the chelating ability of aqueous leaf extract of *Aerva lanata*. The aqueous leaf extract showed a dose dependent decrease in chelating ability using $FeCl_2$. Aqueous leaf extract removed the chromium from tannery effluent by 43 mg/g. *A. cepa* toxicity test was performed on tannery effluent treated with aqueous leaf extract that increased the root length significantly, which revealed that *Aerva lanata* efficienctly removed the chromium from wastewater.

Pharmaceutical, pesticides and herbicides

Pharmaceutical agent and pesticides are the most common products found in waters. The wastewater discharged from pharmaceutical industries is responsible for microorganisms growth inhibition, reduction in the cell-aqueous phase transfer rates, a sedimentation hindrance due to the development of filamentous microorganisms, development and flotation of sludge with poor activity and clogging (Lateef, 2004).

In order to assess the genotoxicity of pesticides on *A. cepa*, farms contaminated with pesticide and forms where organic forming is used were monitored for toxicity by anaphase aberration and MN induction (Kong and Ma, 1999). Results showed that the soil contaminated with pesticide showed 2.78-3.01 fold higher anaphase aberrations as compared to organic farming soil, while the MN induction was increased 1.66-4.75 fold in pesticide contaminated soil. Lateef (2007) examined wastewaters from two pharmaceutical production processes, cotrimoxazole B wastewater (BWW) and Piriton wastewater (PWW) for genotoxicity using *A. cepa*. The effluents induced various types of CA's such as disturbed spindle, vagrant and chromosome bridge, and also showed a dose-dependent reduction in the number of dividing cells. The mitotic inhibition ranged from 38.6 to 67.2%. The mean root length at 20% of BWW and all concentrations except 1% of PWW were significantly different from the control. The EC_{50} of the root growth inhibition was 4.17 and 12.45% for PWW and BWW, respectively. However, the wastewater physico-chemical analysis revealed that most parameters were within the permissible limits.

Bakare et al. (2009) investigated the potential genotoxicity of a pharmaceutical industry effluent by *A. cepa* test along with other assays. The *A. cepa* roots were exposed to different effluent concentrations. The root growth was inhibited. The MI value was also decreased along with CA inductions in a concentration-dependent manner. Results revealed the presence of cytogenotoxic constituents in test samples that may induce adverse health effects in exposed individuals.

Agricultural land receiving pharmaceutical effluents were studied for toxic effects using *A. cepa* on the basis of root growth, MI reduction and aberrant (Abu and Mba, 2011). The results revealed a significant reduction in the number and length of roots. The MI reduced up to 52, which was concentration and treatment time dependent. Aberrant cells observed ranged from a C-mitotic effect to anaphase bridges and multiple nuclei induction. In another study, the genotoxicity potential of a pharmaceutical effluent in *A. cepa* root cells by analyzing the CA's and MN abnormalities were investigated (Bakare et al., 2011). The *A. cepa* assay was carried out in concentrations of 0.5%, 1%, 2.5%, 5% of the effluent. There was a statistically different, concentration-dependent inhibition of root growth and MI as well as frequest CA inductions. Akintonwa et al (2009) evaluated the mutagenic potentials of effluents from six pharmaceutical companies in the Lagos province, Nigeria using *A. cepa* assay by measuring root length, MI and CA end points. Onion bulbs were exposed to 5, 10 and 15% concentration of the pharmaceutical effluents. All the six effluents showed mitodepressive and toxic effects to the *A. cepa* root. In view of toxicity observed, authors recommended that pharmaceutical companies should treat effluents before being discharged into water bodies. Maselli et al. (2015) compared the sensitivities of five ecotoxicity tests using aquatic and terrestrial organisms including *A. cepa* test to evaluate the toxicity of effluents from the production of veterinary medicines before and after treatment. Raw and chemically treated effluent samples were highly toxic. However, toxicity was reduced in biologically treated effluents and bioassays were found to be highly sensitive for toxicity evaluation of raw and treated samples. In another study, cytotoxic effect of the fungicide tilt in root tip cells of *A. cepa* were investigated (Pulate and Tarar, 2014). Roots were exposed to 0.02%, 0.04%, 0.06%, 0.08 %) concentrations of tilt for 3, 6, 9, 12 h and as a result of exposure C-metaphase, sticky metaphase, diagonal metaphase, disturbed metaphase, multipolar anaphase, precocious anaphasic chromosome, chromosome bridge at anaphase and laggard were observed.

In order to verify the genotoxic effects of pharmaceutical and pesticides effect, various researchers also evaluated the effect of pesticides and pharmaceutical agents as a model compound (Rank and Nielsen, 1993) i.e., diuron, cypermethrin and fenvalerate (Chauhan et al., 1999; Chauhan et al., 1998), 2,4-D, atrazine & butaclor (Bolle et al., 2004), isoproturon/diuron and deltamethrin (Chauhan and

Gupta, 2005a), atrazine commercial formulation (Srivastava and Mishra, 2009), metolcarb (Liman et al., 2010a), commercial formulation of the pyrethroid (Saxena et al., 2005), afugan (Yüzbaşioglu et al., 2003), abamectin & emaamectin benzoate (Ahmed, 2014), emulsifiable concentrate of cypermethrin, deltamethrin, lambdacyhalothrin & endosulfan (Yekeen and Adeboye, 2013). The pesticides, herbicides and pharmaceutical were found to induce CA's incuding stickiness, C-mitosis, bridges, lagging chromosomes, fragment, multipolarity, disturbed anaphase–telophase, pro-metaphase disturbance, polyploidy, binuclear cells and disturbed nucleus. The MN formation was also observed along with root growth inhibition, and decreased MI in *A. cepa* roots exposed to pesticides, herbicides and pharmaceutical agents.

Other industries

Xing et al. (1995) studied the effect of chemical industrial effluent (from Lanzhou Chemical Industry Corporation) on *A. cepa* root tip cells and it was observed that the mitosis process was disturbed by the industrial effluent. The mitosis of *A. cepa* root tip cell was proved to be very sensitive to environment mutagens, especially to industrial effluent. Therefore, the *A. cepa* test was suggested as a warning of environment monitoring. In another study, electroplating industry wastewater from Madurai city, India was studied for cytogentic effect (Nagarajan et al., 2014). Proportional increase in phtotoxicity percentage with increase in effluents concentration revealed toxicity. Histological abnormalities like bridge formation, fragmentation, multipolarity and stickiness during mitotic cell division in root tip meristamatic cells of *A. cepa* revealed the genotoxic impacts of the effluents. The geno-toxicological effects of olive mill wastewater, olive wet husk and olive brine were compared (Pierantozzi et al., 2012). The roots showed the inhibitory effects on germination and growth. At high concentrations, olive wastes inhibited/suppressed the mitosis process. Authors suggested that direct use of olive wastes for agricultural purposes should be limited owing to their possible genotoxic, chemotoxic and phytotoxic effects. Olorunfemi et al. (2012) studied the toxicity of raw and treated effluents of dairy industry along with physico-chemical analysis. The zinc, iron, manganese, nitrates and sulphates were higher than Standards Organisation of Nigeria permissible limits. *A. cepa* roots exposed to wastewater for 96 h inhibited root growth in concentration-dependent manner. Root exposed for 48 h to 0.5%, 1.0%, 2.5%, 5.0%, and 10% (v/v) concentrations showed CA's (sticky and laggards chromosomes) at all concentrations, however, toxic effects were reduced in treated wastewater. Authors revealed that combination of physico-chemical analysis and genotoxicity assay is effective in assessing the mutagenic components of industrial effluents for environmental monitoring of pollutants. The treated wastewater from the dairy industry, if discharged into water bodies without further treatment, could pollute the receiving water bodies and impair biolife.

In order to assess the cytogenotoxic effects of paint industry effluents, the *A. cepa* roots were exposed to 7.2%, 18%, 36% and 72% concentrations for a period of 96 h (Samuel et al., 2010). The exposed roots growth was inhibited, MI and CA's were observed at all concentration tested and at higher concentration, the aberrations were more severe. The CA's observed in root tip cells of *A. cepa* were of vagrant, bridges, fragments and sticky chromosomes types. At lower concentrations only bridges and fragments were observed. While studying the toxic effects of lemon juice factory, sugar factory and mining works wastewater, Gana et al. (Gana et al., 2008) reported positive results of genotoxicity in *A. cepa* roots exposed to the tested samples.

Wastewater discharged from brewery industries has high nitrogen, cleaning and washing reagents (Kanu et al., 2006). The cytotoxicity and genotoxicity of brewery, rubber and bottling industries wastewater was evaluated by CA test in *A. cepa* meristematic cells (Olorunfemi et al., 2011b). After 72 h of exposure, the cytotoxic (growth retardation and CA's) effects were observed. The root growth inhibition values were recorded to be 35%, 50% and 62% for bottling, rubber and brewery effluents, respectively. The CA's induced were mostly of sticky chromosomes, bridges, disturbed spindles and fragments. Based on the EC_{50} values, the bottling wastewater was found most toxic followed by rubber and brewery. Recently, the genotoxic effects of the paint industry effluent (100%, 50%, 25%, 10%, 5%, 1%, 0.50% and 0.25%) (Ikotun, Lagos) was evaluated based on MI and CA endpoints (Njoku et al., 2015). The root of *A. cepa* exposed to the various concentrations of the effluents showed significant difference in root lengths. There was a significant difference in phase index and mitotic inhibition and decrease MI values were recorded in concentration dependent manner. The effluent induced CA's in the meristematic cells of *A. cepa* root tips and laggards were the most frequently observed aberrations. Akinsemolu et al. (2015) evaluated the effect of industrial effluent from tobacco industry. Healthy sprouted onion roots were treated with concentrations of 20%, 50% and 100% for 6 to 18 h. The MI value changed significantly at 6 and 12 h exposure time. However, exposure of root cells beyond 12 led to a complete inhibition of mitosis division even at lowest concentration,. Moreover, the treatment concentrations were also reported to induce CA aberrations like stickiness, C-metaphase, bridges, unequal distribution of chromosomes, breaks, laggards, vagrant and ring chromosomes at concentrations of 20%, 50% and 100% in 6 and 12 h exposed roots. Observations revealed that tobacco wastewater had genotoxic potential and capable of damaging DNA, wheras A. cepa proved to be very sensitive to the toxicity for each concentration and exposure time. Mishra (1993) monitored the cytotoxic effects of distillary waste from a sugarcane factory (Kanpur, India) by measuring CA's. Exposure roots revealed that the distillary waste induced damage i.e., the mitosis was disturbed even at 1.5% and 2.0% concentration of effluents.

Raw and vermicomposted (*Eisenia fetida* + cattle dung) sugar beet mud and pulp (waste by-products of the

sugar industry) waste toxicity was monitored (Bhat et al., 2015). *A. cepa* root exposed to raw and vermicomposted were analysed for CA's abberations. Vermicomposted sugar beet mud waste reduced the toxicity in the range of 18–75 %. Firbas and Amon (2013) tested the communal waste water cleaning plant of the type LIMNOWET® Constructed Wetlands for toxicity by measuring aberrations of the metaphasic chromosomes of the *A. cepa* exposed roots. However, constructed wetlands plant reduced the degree of genotoxicity in the range of 3.5% to 29.0%. Bhat et al. (2014) investigated the genotoxicity of pressmud (sugar cane industry waste), which was mixed with cow dung at different ratios and vermicomposted using *Eisenia fetida*. Different concentrations of 100% pressmud sludge extract (10%, 20%, 40%, 60%, 80% and 100%) were analyzed for CA's before and after vermicomposting. Percent aberration was highest (30.8%) after exposure to 100% pressmud extract after 6 h expure, which reduced to 20.3% after vermicomposting. Among CA's, c-mitosis, delayed anaphase, laggards, stickiness and vagrant aberrations were observed along with significant inhibition of MI. However, after vermicomposting the MI value was increased. Author's revealed that vermicomposting could be an important tool to reduce the toxicity of pressmud as evidenced by the results of genotoxicity evaluated by *A. cepa* test. Viana et al. (2014) evaluated the cytotoxicity and the mutagenicity of liquid waste produced in the process of industrialization of bitter cassava (olho-junto variety). The liquid wastes werwe characterized as press water, which is obtained when the cassava roots were pressed; pond water, which was press water stored in impounded ponds; and a solution of sodium thiosulfate, pure and with other waste. Treatment with saline solution was cytotoxic for *A. cepa*, which reduced the cell division. The thiosulfate solution was clastogenic and induced CA's. Based on results of toxicity assesd by *A. cepa*, authors suggested prohibition of sodium thiosulfateuse. Masood and Malik (2013) evaluated the genotoxicity of industrial wastewaters from Jajmau (Kanpur) using *A. cepa* anaphase-telophase test. Amberlite resins concentrated wastewater was found to be more mutagenic as compared to those of liquid-liquid extracts (hexane and dichloromethane extracts). The MI values were decreased significantly when roots were exposed to 5, 10, 25, 50, and 100 % (v/v) wastewater concentration. Complementary to the lower levels of MI, the wastewaters showed higher CA's in all cases investigated. In another study, the cytotoxic and genotoxic effects and the anatomical changes caused by the effluents arising from the ornamental rock polishing industry (Nova Venécia, State of Espírito Santo, Brazil) were assessed using *A. cepa* assay. The roots were exposed to the effluent at 12.5, 25, 37.5, 50, 75, and 100 % concentrations for 20 days. MI value decreased in concentration dependent manner along with CA inductions and NA's. A high frequency of cells death was observed in the roots exposed to the higher concentrations. Similarly, the toxicity of wastewater and physico-chemical analysis was peformed using *A. capa* (Olorunfemi et al., 2015a). A bilge water (1, 5, 10, 25 and 50%) showed DNA polymorphism which was reflected by changes in the RAPD profiles as variation in band intensity, disappearance of bands and appearance of new bands, was induced by the different concentrations of bilge water on the genome of *Allium cepa* root cells. The genetic distances shown on the dendrogram revealed that the genotoxicity of the wastewater was concentration-dependent. This study has shown that polymorphism detected by RAPD can be considered as a powerful molecular marker assay for the detection of the genotoxic effect of bilge water. These studies revealed that the wastewater samples from most of the industires induced cytogentic effects, however, *A. cepa* assay found to be highly sensitive to monitor the toxicity of raw and treated efflunets at various concentration and all researchers suggested the toxicity monitoring of efflunets to avoid contamination of water bodies with toxic agents.

Contaminated river water/surface water/sea water

The contamination on account of industrial growth directly affects not only the soil and water systems in industrial areas, but also agricultural fields as well as the beds of rivers and canals. Among different types of pollutants in the soil system, heavy metals are of great concern since heavy metals (Cr, Cu, Ni, As, Hg, Pb and Zn) are well known as carcinogens and deposited in soils and are mobilized either by leaching or by uptake through plants (Katnoria et al., 2011).

The contamination of soil and water sheds and their effects on living systems have been documented in various studies and a number of studied highlighted the genotoxic effects of soil, contaminated with industrial wastewater i.e., the genotoxicity of contaminated soil with industrial wastewater near Metz, France using *A. cepa* MN assay was evaluated. Results showed formation of MN in treated *A. cepa* roots (Cotelle et al., 1999). Similarly, CA, MN, MI and cell death in *A. cepa* root tip cells have been recorded, exposed to river water contaminated with industrial effluents (Bianchi et al., 2011). In another study, the Todos os Santos River (TSR) in Brazil which receives a high discharge of untreated effluents was tested for toxicity (Blanc et al., 2013). The samples were collected at different points (two upstream of an urban area, two in the urban area, and two downstream of the urban area). The concentrations of Al (21.63–1688.84 µg/L), P (38.59–1760.87 µg/L), and Fe (478.9–8296.3 µg/L) were beyond the permissible limits. Based on the *A. cepa* test, all river water samples induced genotoxic and mutagenic effects. Simialrly, sediment elutriates from Tambis River, Barobo, Surigao Del Sur, Philippines were invetigated for possible cytotoxicity in *A. cepa* roots (Fajardo et al., 2015). Result showed that at higher concentrations (1000 g/L and 500 g/L, for 72 h expoure) exerted mitodepressive effect, C-mitoses, spindle damage and CA's (sticky metaphases and anaphase bridges). Results confirmed the cytotoxic and mutagenic effects of sediment elutriate from Tambis River and A. cepa test was found to be very sensitive in detecting toxicity.

The toxicity and mutagenicity resulted from sodium metabisulfite in sea waters and sediments collected in a shrimp farm in Brazil was investigated(da Costa Machado

Matos Carvalho et al., 2011). *A. cepa* roots were exposed towater and sediment at concentration of 25, 50, and 10% for 72 h (collected in the dry and in the rainy seasons). At the end of 72 h exposure, *A. cepa* roots revealed considerable negative effects on MI, CA's and MN. The Taquari river water (Brazil) also showed cytotoxicity against *A. cepa* root meristematic cells such as MI inhibition and chromosomal abnormalities (da Costa et al., 2011). In another study, the *A. cepa* root test was used to evaluate the genotoxicity of the Pitimbu river water, Natal (Brazil). The water was collected from five sites along the river and one sample was obtained after the treatment (flocculation, chlorination and pH correction). All raw river water samples increased the frequency of CA's and MN and two of the water samples affected the MI value. Two of the water samples also altered root growth and two produced morphological modifications in the *A. cepa* roots. Water collected from a site near an industrial area was found to be most toxic (Egito et al., 2007).

Water reservoir from Vazante-MG city (Brazil) (zinc producer district) was tested for toxicity and all collected samples revealed higher concentration of heavy metals and genotoxic signs in *A. cepa* exposed roots, which revealed contamination of well-water in the city since genotoxity was correlated well with heavy metal load detected in water samples (da Silva et al., 2013). Paiva et al. (2008) examined the São Gonçalo Channel (water supply source of the city of Pelotas, Brazil and the surrounding region) for mutagenic activity. Water samples were collected from four different stations (Lock Dam, Santa Bárbara Channel, Pelotas Creek, and Barra do Laranjal). The channel water showed a significantly greater number of abnormalities (MI, mitotic anomalies, interphase anomalies and total anomalies) and in comaprison to previous years, toxic substances in the channel were considerable higher. Orathupalayam Dam water (India) was tested for physico-chemical parameters and toxicity. *A. cepa* roots grown in the Orathupalayam dam water sample revealed a cytological damage (Gajalakshmi and Ruban, 2014). de Oliveira Meneguetti et al. (2012) studied mutagenicity in river Vale do Jamari. The MI value reduced as well as MN and CA induction (anaphase and telophase bridges) were frequent in roots exposed to contaminated river water. Author recommended application of *A. cepa* test for monitoring of river water. Júnior et al. (2007) also studied the genotoxicity and toxicity of water and sediment samples collected from the Estância Velha stream of southern Brazil (a stream transporting both domestic sewage and effluents from regional factories working in the leather industry). Samples were collected from stream headwaters (Site 1), downstream of an urban area (Site 2), and near the basin outfall (Site 3). *A. cepa* results showed no evidence of chromosomal mutation, either in water or in sediment, during winter or summer seasons, but samples collected below Site 1 showed high toxicity. Results suggested that the synergy among different compounds in domestic and industrial sewage discharges could make it difficult to maintain system stability. Similarly, Vujošević et al. (2008) evaluated water samples from river Rasina (Serbia) for potential toxic and genotoxic effects using the *A. cepa* anaphase–telophase test. Inhibition of root growth was observed. Anaphase and telophase analysis revealed that seven among nine samples were highly toxic. Changes in the relation between spindle and chromosome types of aberrations were found in few samples, indicating differences in the potential genotoxic substances present at the analyzed sites. The samples collected at the highest level of river water indicated that the river water might be more toxic during periods of low flow. So, *A. cepa* anaphase–telophase test was recommended as a monitoring system, which can serve as an alert of contamination of system with toxic agents.

In order to evaluate the toxicity of river water contaminated with industrial effluents in areas of Tucuman (Argentina) CA and MN tests were applied (Gana et al., 2008). A significant aberrant anaphase and MN inductions were observed. In another study, while studying the toxicity of river water from the Upper Silesia Coal Basin (Poland) Geras'kin et al., reported clear genotoxic effect such as chromatid (single) fragments and bridges, chromosome (double) fragments and bridges, lagging chromosomes and multipolarity in *A. cepa* root meristem cells (Geras'kin et al., 2011). Genotoxicity and toxicity by *A. cepa* test of Sinos river, Rio Grande do Sul State (Brazil), receiving wastewater from industries (shoes, leather, petrochemical and metallurgy) and domestic wastewater was evaluated (Nunes et al., 2011) and all water samples showed cytotoxic, genotoxic and mutagenic effects. In another study, the microscopic and macroscopic effects in *A. cepa* meristematic cells, exposed to water from the Drava river, Maribor have been observed. The *A. ceap* roots were exposed to raw water and biologically treated plant output water. The raw water caused sub-lethal and lethal effects and the root length and MI were decreased up to 74% and 66%, respectively and 10 time more aberrant cells were observed. The *A. cepa* roots exposed to biologically treated water showed only 3% reduction in MI (Smaka-Kincl et al., 1996).

Fawole et al. (2008) analysed pollution load in water collected from Alamuyo River in Ibadan (Nigeria) and surrounding wells. Water sample analysis and *A. cepa* results revealed toxic substances in all the water samples. High microbial load was observed in river water samples compared to the well water samples throughout the sampling periods with overall highest value observed at late rainy season upstream water sample. Microscopic evaluation of *A. cepa* cells showed decreasing number of dividing cells and MI values, however, this behavior was not dose dependent. Chromosome fragments, bridge, lag and disturbed spindle were the aberrations observed in the study. The results showed presence of toxic substances and high microbial load in river water samples which have also been infiltrated into the surrounding wells. Using MN and anaphase analyses and mitotic indices in *A. ceap* Ivanova et al. (2005) studied the river water from the region of Panagjurishte, Southwest Bulgaria contaminated with heavy metal and cyanide

(Ivanova et al., 2005). The data obtained showed decreased cell reproduction and the presence of deviations from the normal mitosis in the form of MN, anaphase and telophase bridges and fragments, lagging chromosomes and C-mitosis. The cytogenetic analysis was found very effective and suitable for biomonitoring of heavy metal and cyanide contaminated water.

The genotoxicity potential of contaminated Taquari river (Brazil) was studied on the basis of CA's induction in *A. cepa*. The *A. cepa* test showed significant cytotoxicity and mutagenic index (da Costa et al., 2012b). From same country (Junior et al., 2007), reported the genotoxicity of water from Estancia Velha stream under the effect of domestic sewage and leather industry effluents. Kopliku et al. (2012) used *A. cepa* grown in water samples from Shiroka, Zogaj, Shegan, Kamicë, Stërbeq, Buna bridge, Bahçellek, Zues and Dajç to evaluate the cytotoxic and genotoxic effects, by measuring root length, MI, Phase Index and CA. The root length decreased in the order tap water, Stërbeq, Kamicë, Shiroka, Buna bridge, Zogaj, Bahçellek, Dajç, Zues, Shegan. The root length and MI values of Dajç, Zues and Shegan were compared to tap water. Most frequent CA types were: stickiness, bridges and fragments. CA formation was in following increasing order tap water, Kamicë, Stërbeq, Buna Bridge, Shiroka, Dajç, Bahçellek, Zogaj, Shegan and Zues. The number of abnormal dividing cells was significant at Dajç, Bahçellek, Zogaj, Shegan and Zues. The CA higher rate was observed at Zogaj. The results indicated slight water pollution in Dajç, Shegan, Bahçellek, Zogaj and Zues samples, serving as a first alert of chemical pollution environmental impact, even at low concentrations.

While studying the Atibaia river toxicity under the influence of petroleum refinery (Hoshina and Marin-Morales, 2009), MN and CA tests were applied. *A. cepa* were germinated in waters collected from five different sites and also in treated water. Results revealed presence of CA's and MN induction and treated samples (physicochemical, biological and stabilization pond) also induced the CA's and MN abnormalities in meristematic cells of *A. cepa*, however, the effect was lower in comparison to untreated sample and authors suggested an alternative treatment method for wastewater treatment before being discharged in to water bodies. In another study (Smaka-Kincl et al., 1996) *A. cepa* bulbs were grown in undiluted industrial and municipal waste water; biological treated and water from the Drava river upstream and downstream of the city of Maribor, Slocenia and effects were evaluated on the basis of aberrant cells in metaphase and anaphase, index of MN appearance and inhibition of cell division. The untreated industrial and municipal waste water caused lethal and sub lethal effects and the inhibition of root growth, decrease of mitotic index, interphase cells with micronuclei and aberrant cells were found 74%, 66%, 3% and 10% in comparison to control test.

While studying the cytotoxic and genotoxic effect of heavy metal and cyanide contamination (Ivanova et al., 2008) reported the MI inhibition, CA and MN effects in *A. cepa*. Among CA's, chromosome fragments, anaphase and telophase bridges, lagging chromosomes, C-mitosis were the major abnormalities and these abnormalities were correlated with the presence of Cu, As, Cd, Pb and cyanides. Similar to this study (Staykova et al., 2005) also reported the cytogenetic effect of heavy metals and cyanide polluted waters from the region of Panagjurishte, Southwest Bulgaria by *A. cepa* test. A decreased cell division rate and deviation from the normal mitosis was observed along with chromosomal mutations. In order to assess Danube river water toxicity contaminated with petrochemical complex, oil refinery and chemical industry effluents (Vujosevic et al., 2001) reported the anaphase-telophase aberrations in *A. cepa* cells even after five months of contamination. Clear inhibition of growth and CA were seen in sample, taken just after entry of the canal in to river, while upstream sampling showed no effect. In another study, the CA's and MN in *A. cepa* roots in Belgrade, Yugoslavia were linked to hydrocarbons and polycyclic aromatic hydrocarbons found in river water. The larger CA's and MN incidence in the meristematic cells of *A. cepa* was observed after exposure to water sample collected during the dry season, where the oil leak has arisen (Leme and Marin-Morales, 2008). (Barberio et al., 2009) studied the cytotoxic and genotoxic potential of water from the River Paraíba do Sul (Brazil) and found the inhibition of root length, MI and MN induction. Similarly, (Geras'kin et al., 2011) evaluated the genotoxic potential of water samples from the coal mining area, Poland and clear genotoxic effects of contaminated water were observed in *A. cepa* root cells with severe cytogenetic abnormalities. de Lima Moraes and Jordao (2001) evaluated the cytogenotoxic effects of wastewater from Corumbá and Paraguay River water in flood and drought seasons. *A. cepa* root meristems were exposed to 20 and 72 h water and MI, frequency, and aberrant cells types were measured. The wastewater was genotoxic and flood and drought influenced the levels of genotoxicity in river water. Siddiqui et al (2011) germinated *A. cepa* seeds in industrial waste water and river water (India) to measure toxicity/genotoxicity. *A. cepa* responded significantly with the test range of heavy metals and phenolics. The toxicity of heavy metals was in the order of Cu > Ni > Cd, whereas 2,4-dinitrophenol was the most toxic among the phenolic compounds. Among CA's, bridges, stickiness, and fragmentations were recorded with both the industrial wastewater and river water samples in *A. cepa* roots. Akinboro et al. (2011) collected four water samples immediately and five days after rainfall from two locations form Sungai Dua River and tested for toxicity using the *A. cepa* assay. The water samples were found to contain sodium and calcium and exposed roots showed inhibitions in root growth and MI. However, the inhibitory effects were not dose-dependent. The results showed the presence of agents in water samples of Sungai Dua River that have caused mitodepressive and genotoxic effects.

The toxicity and genotoxicity of Vistula river water (Poland) from selected points in Warsaw (downstream from Gruba KaĞka) using *A. cepa* test was also studied (OBIDOSKA and LICHMIRA, 2013). The city water-intake

facility 1-GK (Gruba KaĞka) was nontoxic, however, downstream toxicity and genotoxicity increased until 3-MP (Most Poniatowskiego). The highest toxicity and genotoxicity was observed at point 5-KB (Kolektor Burakowski), receiving untreated municipal sewage directly into the Vistula and below point 6-OC (Czajka Treatment Plant) no toxicity was observed. Düsman et al. (2014) monitored the cytotoxic potential of water of Quatorze River, Francisco Beltrão, Paraná, Brazil, along its route in the rural area, using the root meristematic cells of *A. cepa*. The results showed that the water at points 2, 3, and 4 were not cytotoxic because the rates of *A. cepa* cell division were unaltered and at point 1, MI value was decreased, indicating that this water contained substances with mitogenic capacity. However, the MI values decreased along the route of the river (point 1 to point 4), possibly indicating a mechanism of self-purification, despite having received other sources of pollution. Thus, from results of this study it was concluded that the water of the Quatorze River should be monitored to evaluate contamination and to maintain the quality of the river water. Lindqvist (2015) investigated contamination of river Vallkärrabäcken, Sweden 34 years after the closure of the landfill, Sankt Hans. The *A. cepa* bioassay was used to analyze the water quality in this study, including root growth inhibition and other macroscopic effects. Water samples were taken 0.05 km away from Vallkärrabäcken to the Sankt Hans landfill as well as 0.5 km and 2 km downstream. *A. cepa* bulbs were grown in Styrofoam frames which floated giving *A. cepa* constant contact to the samples of the river water for 4 days. The *A. cepa* roots growth inhibited along with fewer roots mass and number in water sample taken 0.05 km downstream. At 0.5 km downstream the landfill, the bulbs were significantly inhibited in mass of roots and number of roots. At 2 km downstream the landfill, the bulbs were only significantly inhibited in number of roots. Mazzeo and Marin-Morales (2015) exposed *A. cepa* seeds to water samples from a river that receives untreated urban effluent and to the trifluralin herbicide (0.84 mg/L concentration), both analyzed in two different seasons (summer and winter seasons). Samples induced significant frequencies of CA, NA and MN. The herbicide caused a significant increase in the number of nucleoli and MN. Tabet et al. (2015) examined the mutagenicity and genotoxicity of urban wastewater, Guelma city, Algeria between April 2012 and April 2013 using *A. cepa* CA and MI end points. The samples were collected from five different sampling stages (S1-S5). A significant decrease in MI was observed with a decrease in the percentage of cells in the prophase and an increase in the telophase. Among CA's, anaphase bridges, disturbed anaphase-telophase cells, vagrants and stickiness in anaphase-telophase cells were observed. All treatments of wastewater in April 2012, at S5 in July 2012, at S1 and S5 in November 2012, at S5 in February 2013, and at S1 in April 2013 induced CA's. Athanásio et al. (2014) investigated the genotoxic, mutagenic, and cytotoxic potentials of surface waters in urban streams using *A. cepa* test. Water samples were collected from three streams located in the urban area of a municipality in the south of Brazil. The frequent MN and CA induction and decrease in the MI value indicate the cytotoxicity and mutagenicity naure of collected samples. The Pedras stream receives mixed industrial and urban wastewater, while the Lajeado and Preto streams receive wastewater predominantly domestic in nature, and A. cepa shown to be extremely sensitive in detecting the genotoxicity of environmental water samples because significant difference was observed among samples tested and this test can be applied as the first tool for environmental health hazard identification and prediction. Roy et al. (2015) studied the toxic effects of tannery effluent (TE) from a town (Ambur), located on the banks of Palar River, India. The phytotoxic effect of TE was tested on *A. cepa* roots. Exposed roots revealed inhibition of growth. Results revealed that the Palar River of Ambur has been contaminated with agents, which can exert adverse effects on living organisms even at very low concentrations.

Barbério et al. (2009) investigated the cytotoxic and genotoxic potentials of water, River Paraíba do Sul (Brazil) by *A. cepa* assay using root growth, mitotic indices and MN end points. *A. cepa* roots were exposed for 24 to 120 h to wastewater collected in the years of 2005 and 2006 from sites in the cities of Tremembé and Aparecida (São Paulo state, Brazil). The roots treated with samples of the river water collected in 2005 decreased the root length. However, MI values were reduced for samples collected from both sites. Considering the data involving root length growth and MI values, a cytotoxic potential was suggested for the water of River Paraíba do Sul at Tremembé and Aparecida. Espinoza-Quiñones et al. (2009) studied Cr(VI) contaminated river water using *A. cepa* root assay and clean river water caused a 50% reduction in root length with 4.2 mg/L EC_{50} value, while in organically polluted samples similar root growth inhibition occurred at 12.0 mg/L. The results suggested that there was a dislocation to higher values in toxic chromium concentration in polluted river water due to the eutrophization level of river water. Matos et al. (2011) evaluated the toxicity and mutagenicity caused by sodium metabisulfite in sea waters and sediments collected in a shrimp farm in Cajueiro da Praia (Luis Correia), state of Piauí, Brazil, using the *A. cepa* assay. Water and sediment samples were collected in the dry and in the rainy seasons from three sites: upstream the shrimp farm (Site 1), at the point where sodium metabisulfite is discharged (Site 2), and 100 m downstream the farm (Site 3). Roots were exposed to 50%, 25% and 10% dilutions for 72-h and then subjected to MI, CA and MN analysis. The *A. cepa* assay revealed that the water and sediments samples collected in the Piauí coast contaminated with sodium metabisulfite induced toxicity and authors revealed that assay may be used as a regular tool in the analysis of water in shrimp farms in the coast of Piauí state for preserving the region's marine ecosystem. More recently, Salles et al. (2016) collected water samples from Jaguari River and Ribeirão Lavapés, Brazil in dry season (August 2011 and 2012) and rainy season (February 2012 and 2013) and evaluated for toxic effects. MN, MI and mitotic anomalies were measured in exposed roots. The stickiness, c-

mitosis, multipolarity, chromosome bridges were detected in roots exposed to sample collected in rainy season (8.61 ± 3.65) and dry season (7.07 ± 2.96). The MN formation and less MI value were higher in the February 2012 samples than in the August 2012 samples. The aberrations were positively correlated with manganese concentration (0.13 mg/L), which was higher than permitted concentration of <0.1 mg/L. Overall, samples collected in rainy season were more toxic than the dry season samples.

Wastes contaminated soil

Meier et al. (1997) employed *A. cepa* test to evaluate the treated soil from a site heavily contaminated with polychlorinated biphenyls (PCBs). Although the PCB content of the soil was reduced by 99% (after remediation), however, the phytotoxicity and genotoxicity were higher for the remediated soil compared to the untreated soil. The toxicity remaining after treatment appeared to be due to residual solvent introduced during the remediation process, and/or to heavy metals or other inorganic contaminants not removed by the treatment. Results demonstrated the need for including toxicity measurements in the evaluation of technologies used in hazardous waste site remediations for determining the efficiency of remediation processes. While monitoring the toxicity of mercury in aquatic and terrestrial environments, Dash et al. (1998) applied *A. cepa* MN assay. Four mercurial derivatives, namely mercuric chloride (MC), methyl mercuric chloride (MMC), phenyl mercuric acetate (PMA) and a methoxy ethyl mercuric chloride based fungicide, Emisan-6 were tested to assess the sensitivity and versatility of the *A. cepa* MN assay. *A. cepa* bulbs were set directly on water and soil contaminated with known levels of mercurial derivatives (0.0001–10.00 ppm) for five days and then, measured the root length, mitoses with spindle abnormality and MN inductions in root meristems. The effective concentrations of the test chemicals that caused 50% of root length as compared to control (EC_{50}) were determined from dose-response curves so obtained. The lowest effective concentration tested (LECT) and highest ineffective concentration tested (HICT) for each of the mercurial derivatives for the induction of spindle malfunction and MN were determined. It was found that EC_{50}, LECT and HICT values for mercurial derivatives in soil were higher than those in water. The frequencies of cells with MN and mitosis with spindle abnormality were highly correlated indicating that MN is a good parameter of spindle malfunction. The approach applied confirmed the sensitivity of the *A. cepa* assay by 10-fold, the detection limit being 0.001–0.1 ppm and 0.1–1.0 ppm in aquatic and terrestrial environments respectively, depending on the species of mercury.

The general toxicity (root growth inhibition and malformation) and genotoxicity (induction of chromosome aberrations in root cells) of an oil field wastewater have been investigated by the *Allium* test. A series of 10 small bulbs of *Allium cepa* L. were cultivated in various concentrations of the wastewater, and after 48 h one root tip from each bulb was harvested and processed for cytological studies by the aceto-orcein squash technique. After 96 h, mean lengths of root bundles were obtained and the Effective Concentration (EC) values were calculated. Treatment with wastewater resulted in significant dose-dependent root growth inhibition. EC_{50} (96 h) was 28.5% while a total phytotoxic effect was induced by the undiluted sample. The wastewater is mitodepressive and increased significantly the frequency of chromosome aberrations in root cells (sticky chromosomes, c-mitosis, spindle multipolarity, bridges and fragments). At lower concentrations c-mitosis was the most common aberration. The suitability of the *Allium* test in genotoxicity screening was highlighted and the impact and significance of positive results on the environment and human health were discussed. Saxena et al. (2004) evaluated toxicity of soil contaminated with Diuron (herbicide) using root meristem cells of *A. cepa.* Diuron 22.5, 45.0, and 90.0 ppm were mixed in soil and roots were exposed to soil for 48 h and analysed for mitotic/CA's. Dose-dependent inductions of mitotic as well as chromosomal breaks were observed. More frequency of mitotic aberrations was observed than CA's. Mild percentage of MN and binucleated cells were observed. Results revealed the possible interaction of diuron with DNA. From Saharanpur and Aligarh, India, Tabrez and Ahmad (Tabrez and Ahmad, 2011) reported the toxicity of soil contaminated with industrial effluents tested using *A. cepa* test. The water sample from two territories increased the frequency of CA, MN along with MI reduction. Among CA's, bridges and fragmentation of chromosome were the predominant effects of the Saharanpur water sample, while the Aligarh sample induced mainly fragments.

In order to assess the genotoxicity potential of soil from agricultural fields (irrigated with industrial wastewater) from Amritsar, India, Katnoria et al. (Katnoria et al., 2008) employed *A. cepa* root anaphase aberration assay. The genotoxic potential of soil samples was correlated with content of heavy metals like Cr, Co, Cu, Mn, Hg, Ni and Zn. It was observed that soil samples, which showed the highest percentage of aberrations, also contained higher amount of one or more metals studied. Same authors later, (Katnoria et al., 2011) employed the *A. cepa* anaphase aberration assay in order to assess the genotoxicity and mutagenicity of soil samples contaminated with Zn coating industry and copper sulphate manufacturing industry wastewater. The anaphase aberrations ranged from 3.63-10.67% and 0.38-4.83% for soil samples contaminated with zinc coating industry and copper sulphate manufacturing industry wastewater, respectively. In another study (Crebelli et al., 2005) showed MN induction and MI inhibition in *A. cepa* roots exposed to industrial waste contaminated soil near Metz, France. Monarca et al. (Monarca et al., 2000) reported the genotoxicity of contaminanted soil with industrial wastewater using *A. cepa* from industrialized area in the Lombardy region, in Northern Italy and treated group of plant showed strong effect on MN formation and soils sample showed high concentrations of PAHs and heavy metals. Chahal et al. (Chahal et al., 2012) reported the genotoxicity of

pesticides, inorganic fertilizers and industrial effluents contaminated soil from Nangli village of Amritsar, Punjab, India by employing *A. cepa* root CA's. The treatments resulted in different types of CA's like lagging, vagrant, C-mitosis, delayed anaphase, stickiness, chromosomal breaks and chromatin bridges (clastogenic aberrations) and 12.81% aberrant cells were found in treated sample as compared to negative control. de Souza Pohren et al. (2013) evaluated solubilized soils (three points) contaminated by heavy metals from municipality of Triunfo, state of Rio Grande do Sul, Brazil considering germination index, MI, CA and mutagenicity index by *A. cepa*. Significant responses of CA were observed in the two samples of contaminated soil, but mutagenicity was significant only for soil 3. *A. cepa* test system was sensitive to investigate the genotoxicity of the soil samples and can be used as an alert in studies on soil contamination.

Kataeva et al. (2012) used *A. cepa* root MN bioassay to determine the toxicity of 5 soil samples i.e., copper-smelters (the Middle Urals) and Ni-enriched soil from an area of a natural geochemical anomaly (the Polar Urals). *A. cepa* roots were exposed to aqueous soil extracts for 30 h with recovery duration for 20 and 44 h. Nuclear anomalies were observed in exposed root meristematic cells. High Ni concentrations decreased the roots growth along with MN inductions. The total number of anomalies and contribution of extrusions among them were higher for contaminated soils with prevalence of Cu. Masood and Malik (2013) collected and extracted soil samples from agricultural fields in the vicinity of industrial area of Jajmau, Kanpur (India), using dichloromethane (DCM) and hexane solvents, and subjected to genotoxicity testing using *A. cepa* CA assay. All the tested concentrations of soil extracts (5-100%) affected mitotic index in a dose-dependent manner and among CA's, C-mitosis, anaphase bridges, laggards, binucleated cells, stickiness, broken and unequal distributions of chromosomes at anaphase stage of cell division were observed. Based on results, it seems that large number of pollutants has been accumulated in soil and might have adverse impact on soil health. Oberholster et al. (2008) studied the Macroinvertebrate communities in Rietvlei nature reserve wetland area and their relationship with water quality. Samples were collected from sewage effluent, agricultural and informal residential runoff. A large increase in nutrient concentrations was observed downstream from discharged treated sewage with an associated decrease in species richness. *A. cepa* along with other bioassays (*D. magna, H. attenuate, L. sativa, P. adspersus*) showed toxicity and authors revealed the deterioration of the wetland possibly due to factors such as increasing urbanization, industrialization, agriculture runoff and rapid human settlement in the Hennops River catchment area and its principal tributaries. Christofoletti et al. (2013) assessed the toxic potential of biosolid, obtained from a sewage treatment plant, vinasse (a by-product of the sugar cane industry), and a combination of both residues using *A. cepa* assay. *A. cepa* test revealed genotoxic since frequesnt CA's were observed. Micronuclei and chromosome breaks on meristematic cells and F cells with micronuclei were examined to assess mutagenicity of samples. After 30 days, the genotoxic effects were significantly reduced in the soil + biosolid and soil + biosolid + vinasse groups as well as the mutagenic effects in the soil + biosolid + vinasse group. Based on results, authors suggested bioprocessing of residues by diplopods as feasible alternative prior to application to crops to improve degraded soils and/or city dumps.

While studying the presence of potential toxic chemicals in water sources of Nën-Shkodra lowland agricultural area after massive 2010-2011 flooding, Kopliku and Mesi (2012) employed *A. cepa* root tips assay considering root length, MI, phase index and CA endpoints. Roots were grown in four water samples, analysed and compared with controls. Inhibition of root growth, cell division and induction of mitotic and CA's were observed. The most polluted water sample caused inhibited root growth up to 36%, MI decreased up to 38%, and CA's up to 7.8%. Among CA's, bridges and fragments, stickiness and C-metaphase were observed. The results indicated a slight toxic tendency of analyzed natural waters and A. cepa served as an alert of chemical pollution after flooding.

Sugar Industry

The onion bulbs were treated with different concentrations of the distillery effluent (25, 50, 75 and 100%) at room temperature (Hemanth Kumar et al., 2015) and resultantly, MI and relative division rate were decreased in concentration dependent manner. The chromosomal abnormalities (abnormalities include sticky metaphase, disturbed chromosome, sticky telophase, multipolar chromosome, laggards, fragmented metaphase, fragmented anaphase, scattered anaphase and chromosomal bridge) also increased as the concentration of the effluent increased. A. cepa test showed that the higher concentrations of distillery effluents inhibited cell division significantly. In another study, Pedro-Escher et al., studied vinasse (by-product of the biomass distillation, mainly for the production of ethanol, from different cultures such as sugarcane and used as fertilizer) for toxicity using *A. cepa* test (Pedro-Escher et al., 2016). using *A. cepa,* bulbs were exposed to raw vinasse (RV) (from two different harvests (I and II)) and control soil + raw vinasse (SV); vinasse diluted in water at 50% + control soil (V 50%); vinasse diluted in water at 25% + control soil (V 25%); vinasse diluted in water at 12.5% + control soil (V 12.5%). The chemical characterization of vinasse samples showed a low pH and high concentration of potassium. The results demonstrated that the two RV samples tested are toxic, since no germination was observed. The cytotoxic potential was observed in the sample II of SV and V (50%). All groups evaluated in samples I and II, induced chromosomal alterations. SV (Sample I) and all groups evaluated in samples II induced MN. Based on toxicity results, author warned not to use vinasse as fertilizer since it can cause genetic damage. Similarly, the genotoxicity of four soil types collected from different regions of Amritsar, India employing chromosomal

aberration assay in root tip cells of *A. cepa* has also been studied (2014) (Soodan et al., 2014). Roots exposed to different types of soil revealed the CA's like physiological aberrations (c-mitosis, delayed anaphases, stickiness, laggards, vagrants) and clastogenic aberrations (chromosomal breaks, chromatin bridge and ring chromosomes). These studies revaled that sugar industry waste is also potential source of toxicity and *A. cepa* test efficiently applied for toxicity monitoring.

Disinfected water and mineral water/tap water

Disinfection of drinking water is one of the most important steps in the potabilization process, since it ensures microbiological risk control associated with water consumption. Nevertheless, the use of disinfectants in this process can increase chemical reactions with substances naturally present in the water or with pollutants, generating disinfection by-products, for which the genotoxic and carcinogenic activities have been reported (Boorman, 1999; Feretti et al., 2008; Leme and Marin-Morales, 2009; Melnick et al., 1994). For example, super-chlorination the production of chloroform and trihalomethanes along with haloacetic acids and haloacetonitriles were identified (Moudgal et al., 2000) and the use of ClO_2 in the potabilization process has increased now a days and by mixing with water is reduced to chlorite (ClO_2^-) and to chlorate (ClO_3^-) ions, for which the adverse effects to the exposed organisms are not totally understood (Leme and Marin-Morales, 2009). The wastewater disinfection helps prevent the spread of pathogens and chlorination is the most widely used method for the disinfection of wastewater, but it can cause the formation of mutagenic, carcinogenic and toxic by-products which are potentially harmful to human and aquatic organisms (Monarca et al., 2000). In this regard, the disinfectant agent alternative to chlorine, such as chlorine dioxide, ozone, peracetic acid and UV radiation, on the formation of mutagenic and toxic compounds in wastewater has been studied. Wastewater samples were collected before and after disinfection in summer and in winter to detect genotoxicity using *A. cepa* test and found higher MN frequency in peracetic acid treated wastewater sampled in winter. The same author in subsequent year reported the genotoxicity of surface water treated with different disinfecting agents. The toxicity assays (MN and CA tests) were performed to detect the clastogenic and aneugenic effects. The study was carried out at a pilot plant using lake water after sedimentation and filtration. This water supplied four stainless steel basins: three basins were disinfected with sodium hypochlorite, chlorine dioxide, and peracetic acid and the fourth basin containing untreated lake water was used as a control. *A. cepa* was exposed *in situ* in the basins and resultantly, the *A. cepa* showed MN and CA in roots treated with disinfected water (Monarca et al., 2003).

The CA test was applied in order to evaluate the toxicity of the ClO_2^- and ClO_3^- ions. The authors reported positive results for the *A. cepa* test, concerning a significant CA induction by ClO_2^- and ClO_3^- ions, even in concentrations below the limits established by the Italian normative law (0.2 mg/L) and by the World Health Organization (WHO) guidelines (0.7 mg/L) (Feretti et al., 2008). While studying the properties of mineral water packed in polyethylene terephthalate (PET) bottles, Evandri et al. (Evandri et al., 2000) revealed the toxicological effects of mineral water packaged on the basis of *A. cepa* test. Two commercial mineral water brands, bottled both in PET and glass, stored under different conditions (no direct light exposure, storage water (40°C for 10 days in the dark) and exposure to sunlight at varying temperatures. After assayed by *A. cepa,* the root length and color were affected. The water samples bottled in PET induced cytogenetic aberrations and the signs of toxicity were evident even after 8 weeks of bottling, which is well within the recommended expiry date and suggested that storage conditions were very important, as that CA's were particularly apparent after exposure to direct sunlight. Crebelli et al. (2002) analyzed the genotoxic and ecotoxicologic effects of urban wastewater disinfected with sodium hypochlorite or peracetic acid. The mutagenic activity of disinfected effluents was similar to the corresponding untreated wastewater both sampled in four different periods. Therefore, the disinfection process did not seem to contribute to aquatic mutagenicity in the examined range of biocide concentration. Dissociation compounds (hydrogen peroxide and acetic acid) and possible by-products of peracetic acid did not seem to contribute significantly to the toxicity of sewage treated with peracetic acid.

In contrary to above reports Crebelli et al. (Crebelli et al., 2005) investigated the influence of two widely used disinfectants, peracetic acid and sodium hypochlorite (NaClO), on the formation of mutagenic by-products. After disinfection, the water samples were tested for toxicity *A. cepa* root (anaphase aberration) and no negative impact was observed in the *A. cepa* anaphase aberration test. These results indicate that, in the conditions applied, wastewater disinfection with PAA and NaClO does not lead to the formation of significant amounts of toxic by-products. Furthermore, Egito et al. (Egito et al., 2007) also reported non toxicity in chlorinated water used for human consumption.

Goncharuk et al. (2011) tested tap water treated in static conditions with gray and black silicon mineral using *A. cepa* test. Silicon-activated water stimulates metabolic processes during the germination and root growth was stimulated, which confirmed the sensitivity of *A. cepa* for toxicity evaluation and Silicon-activated water was found to be safe resulting in the stimulation of the growth and development. In another study, Marrakesh's CMR wastewater, Draa Lasfar mine located about 12km south-West of the city of Marrakech, Morocco for toxicity using *A. cepa* assay was screened (Chaik et al., 2011) and results revealed that wastewater at 100 % concentration inhibited root growth and MI; induced binucleated cells, but was nontoxic at very low concentrations. Un-controlled cell division led to polyploidy. The wastewater showed the

presence of Zn, Pb, Cu and Cd, whose presence could partly be responsible for the toxicity of wastewater. Author's concluded that water should be used at very low concentrations in order to protect the ecosystem from any potential adverse effects. Olorunfemi (2013) investigated the cytotoxic and genotoxic potentials of borehole water supply in six halls of residence of the University of Benin main campus using *A. cepa* test. The tested samples inhibited the root growth and induction CA's, which were correlated with water physico-chemical nature and heavy metals present in high concentrations in the borehole water samples than tap water. Findings suggested that the investigated water samples may contaminate by wastewater from sewage and solid waste leachates and may cause health risk in case of exposure during consumption. Bohórquez-Echeverry et al. (2012) compared various bioassays (five acute toxicity bioassays) to monitor the quality/toxicity of the affluent and effluent of three drinking-water treatment plants**.** Results revealed that all organisms were potentially useful in the assessment of water quality. Olorunfemi et al. (2014) monitored the rural settlements area (Obazuwa community in Ovia North East Local Government Area of Edo State, Nigeria) water source (lake water during the dry season, lake water during the wet season and borehole water) for physico-chemical and cyto-genotoxic properties. The physico-chemical analysis revealed the pH, chromium, copper, chlorides, nickel, iron, zinc, cadmium, lead and manganese in lake water in both seasons higher than WHO permissible limits. The MI values in exposed roots decreased along with CA's (bridges, fragments, sticky chromosomes, disoriented chromosomes) and MN inductions.

The cytotoxicity and genotoxicity in selected borehole water using *A. cepa* assay was also studied. Roots were exposed to five different water samples collected from boreholes Ring road, Sakponba, Ekosodin, Science and Eddy Grace in Benin City, Edo State, Nigeria for 96-h and results clearly shown that water and environmental pollutants in Benin metropolis is reached to high level (Adelanwa et al., 2011). Bandyopadhyay (Bandyopadhyay, 2015) evaluated arsenic contaminated groundwater from Eastern region of Burdwan district, West Bengal. Treatment for 4 days of newly developed roots of *A. cepa* with water samples with arsenic content 50 µg/L exhibited stimulation in mitotic activity whereas samples with arsenic 1000 µg/L showed inhibition of mitotic activity apparently indicating hormesis. Results indicated that contaminated groundwater depending on the magnitude of the arsenic concentration, might either be mitogenic or mitostatic/toxic, which in turn has obvious implications in agriculture and human health.

Radioactive wastes contaminated soil and water bodies

The soil and water bodies contaminated with radioactive material also revealed the toxic effect (Kovalchuk et al., 1998). There are few studies in the literature on the evaluation of the hazards of radioactive soil and watershed contamination. However, available studies report the sensitivity of the *A. cepa* to assess this pollutant class. The CA test was applied to evaluate the genotoxic potential of watershed near radium production industry storage cell. The concentration of radioactive material (^{262}Ra, ^{228}U, ^{232}Th, ^{210}Pb and ^{210}Po) in all samples were found below the permissible limit. However, the heavy metal ions (Zn and Mn) concentration exceeded the maximum permissible concentration for the natural reservoirs. All water samples showed a significant increase in CA's frequency as compared to control. The regression analysis revealed an increase in MI parallel to Zn ion levels, while MI decrease was correlated with ^{238}U higher concentrations. Authors suggested the combination of two methods, toxicity evaluation and estimation of chemical composition of the system under test, which allows the identification of the elements that require constant biological monitoring such as Zn and ^{238}U which were found most effective regarding genotoxicity in *A. cepa* root (Evseeva et al., 2003). Later, Evseeva et al. (2005) also studied the mutagenic and cytotoxic effects of concentrations of cadmium, potassium and thallium-232 in *A. cepa* root meristem. The combined action of ^{232}Th (0.8 mM) with cadmium in non-toxic (0.009 mM) and toxic (5 mM) concentrations resulted in sinergetic increase of the frequency of aberrant cells in *A. cepa* root meristem. Decrease of the mutagenic effect to the additive level and antagonism with respect to the cytotoxic one was observed only at the certain concentrations of ^{232}Th (0.8 mM) and Cd (0.09 mM) and the time of impact 30 h. In contrast to the heavy metal cadmium the essential for plants potassium at all studied concentrations (0.008, 6, 13 mM) decreased the number of cytogenetic aberrations in control experiments and under the effect of ^{232}Th. The maximum protective effect of potassium was detected at the concentration 13 mM. In another study, while studying the genotoxic effect of radioactive explosion, Ukraine (Chernobyl accident), applied the CA test in *A. cepa* root meristematic cells to estimate the soil contamination, contaminated by cesium (^{137}Cs) and strontium (^{90}Sr) (Kovalchuk et al., 1998). Authors showed a dose-dependent relationship between the increase in the percentage of aberrant mitosis and the increase in the radioactive contamination in the soil samples and the same relationship was verified for MI inhibition. The authors also depicted a strong correlation between the ^{137}Cs activity and the cytotoxic and genotoxic action observed. Finally, they concluded that the *A. cepa* test is efficient and sensitive to evaluate the genotoxicity of soils impacted by radioactive pollutants. Udalova et al. (2014) evaluated the cytotoxicity and genotoxicity of natural waters contaminated with ^{90}Sr and heavy metals in the vicinity of the radioactive waste storage, Obninsk, Kaluga region. Exposed root analysis showed mutagenic potential of waters collected in the vicinity of the radioactive waste storage. Saghirzadeh et al. (2008) estimated the impact of high level of natural radiation in the soils of inhabited zones of Ramsar, Iran using DNA damage in *A. cepa*. The average specific activity of natural radionuclides measured in the soil samples for ^{226}Ra was 12,766 Bq kg^{-1}, whereas in the control soils it was in the range of 34–60 Bq kg^{-1}. A positive strong significant

correlation of the DNA damage in nuclei of the root cells of *A. cepa* seeds germinated in the soil of high background radiation areas with ^{226}Ra activity of the soil samples was observed. The results showed high genotoxicity of radioactively contaminated soils. While assessing the cytogentic activity of water from a regional radioactive waste repository (Pyatkova et al., 2009), *A. cepa* test was applied. Biotesting of natural waters from the site has demonstrated that negative biological effects were generated under the influence of water from well, located near the source of contamination. The percentage of aberrant cells in water samples from well 4 was 2 times higher than in the control or other sources. Effects of water composition at the genome level were found season independent. The *Allium*-test was shown to be highly sensitive and effective in testing a combined effect from radiation and chemical factors in field conditions.

Complex mixtures

Complex mixtures characterized most of the environmental samples, since they can suffer the influence of several contamination sources. Thus, several studies have been conducted by the *A. cepa* test to evaluate toxicity. In order to evaluate the potential mutagenic effect of raw and simulated leachate from a rural refuse dump site at Odo Oba, South-West Nigeria, Bakare (2001) applied A. cepa MI and CA end points. Roots of about 2—3 cm long were treated with 1%, 2.5%, 5%, 10% and 25% concentrations of the leachate for 24 h. analysis showed different types of CA's at all doses tested except at 1% concentration. The number of dividing cells also reduced at the tested concentrations. Results revealed the genotoxic chemicals found in the leachate samples and findings were foundvery useful in the practical aspects of waste management and for the assessment of the hazardous effects of the chemicals in the leachate from solid waste dumpsites. Similarly, metalworking fluids (MWFs) EC_{50} values using the *A. cepa* test at 1/250, 1/500 and 1/1000 concentrations were also studied using *A. cepa* assay (Pekol, 2014). Decrease in MI as well as CA's (stickiness were observed to occur; whereas abnormalities such as c-mitosis, fragments, bridges, vagrants) alongwith MN inductions were observed in concentration dependent manner. Feretti et al. (2008) evaluated the clastogenic effects of gaseous emissions (biogas) from three municipal landfills using *in situ A. cepa* test. Leachate samples inhibited root growth and tip development in *A. cepa*. Genotoxicity of the leachates was evaluated in diluted samples and a significant increase in chromosomal metaphase aberrations in one of the samples was observed. Biogas was nontoxic, whereas leachates were found to display elevated toxicity and author suggested to treat leachates before releasing into the environment to avoid ecological damages. Pelegrini et al. (2007) evaluated toxicity in natural leachate samples collected from Limeira Sanitary Landfill, Limeira, Brazilian state of São Paulo using *A. cepa* along with other test organisms. *A. cepa* (onion) indicated that the diluted leachate up to 50% showed similar toxicity to the phenol solution 2000 mg/L and Cr(VI) concentration of 2000 mg/L. Obidoska and Jasińska (2008) monitored the toxic and genotoxic potential of municipal landfill leachates from Radiowo municipal, Poland and evaluated the efficiency of zeolite as a leachate purifier using *A. cepa* assay. A significant phytotoxicity and genotoxicity of Radiowo landfill leachate was observed and zeolite filtration did not reduce genotoxicity, however, phytotoxicity was decreased. Oberholster et al. (2008) studied macroinvertebrate communities in Rietvlei nature reserve wetland area and their relationship with the aim to evaluate their use as potential indicators of pollution. Samples were collected from outlets of sewage effluent, agricultural and informal residential runoff. A. cepa bioassay was performed for toxicity evaluation along with other assays (*D. magna*, *H. attenuate*, *L. sativa* and *P. adspersus*). Multitrophic level bioassays revealed the deterioration of the wetland possibly due to factors such as increasing urbanization, industrialization, agriculture runoff and rapid human settlement in the Hennops River catchment area and its principal tributaries.

While evaluating the efficiency of biological and chemical wastewater treatment plant Sik et al., showed that after wastewater treatment a significant reduction in MI and CA was observed (Sik et al., 2009). For comparison, the *A. cepa* were kept in 100% concentrations of the treated water and 10%, 25%, 50% and 100% concentrations of untreated water and a significant difference was noted. Wastewater reduced the rate of the mitotic division at different concentrations and increased the mitotic anomalies. The MI was found to be 33.8%, 31.2%, 23.6% and 16.7% in the control, treated water, 10% and 25% concentration of the untreated water, respectively and similar rates in mitosis for four groups of plants were observed. The CA's as high frequency of lagging chromosome, irregular distribution, polar slips, horizontal division and sticky chromosome were also observed in group of plant exposed to untreated wastewater and authors suggested the use of biological and chemical wastewater treatment before being discharged into watershed. El-Shahaby et al. (El-Shahaby et al., 2003) applied the CA and MN test in *A. cepa* to evaluate the genotoxicity of industrial effluents collected from Sandub area, Dakahlia, Egypt. High genotoxicity was reported for all the water samples and correlated with the presence of some heavy metals (Pb, Zn, Co, Cd and Cu). In this study, the authors concluded that the CA and MN tests in *A. cepa* are recommended for aquatic pollution biomonitoring due to its sensitivity to detect mutagens in wastewater. While studying the toxicity of industrial effluents of two cities in India contaminated mainly by heavy metals and pesticides, Fatima and Ahmad (2005) showed a significant increase in the CA frequency in *A. cepa* meristematic cells exposed to these samples. Waste sludge was analysed using *A. cepa* genotoxicity test. Samples collected during three winter periods from three Danish municipal wastewater treatment plants differing in size and industrial load were tested. The toxicity of the sludge was tested using *A. cepa* root inhibition assay, and the results were expressed as EC30 and EC50.

Results showed that the toxicity correlated positively with industrial load. However, when genotoxicity was tested at concentrations corresponding to the EC30 and EC50 values in the *A. cepa* anaphase-telophase assay, only two sludge samples from the smallest plant with the lowest industrial load induced significant chromosome aberrations. Concentrations of the heavy metals Pb, Ni, Cr, Zn, Cu, and Cd were also determined and could partly be correlated with the toxicity of the sludge and the industrial load of the treatment plants (Rank and Nielsen, 1998).In another study Carita and Marin-Morales studied (Carita and Marin-Morales, 2008) sludge from different urban sewage treatment stations in southeastern Brazil, and reported sensitivity of the *A. cepa* test to assess the genotoxic and mutagenic potentials of the sludge. The different responses may be related to the preparation of the test samples, e.g. solubilization procedures to obtain aqueous extracts, during which some chemicals may be lost or even retained in the raw material, depending on the procedure used. Furthermore, the difference in observations may also be related to the type of sewage in each studied region.

An assessment of the vermicompost efficiency, a bioremediation process, used to reduce toxic potential of a municipal sludge sample, in which heavy metals were detected, was made by some authors (Srivastava et al., 2005). MI inhibition for raw sludge and an increase of this parameter in the bioremediated sludge were reported by the *A. cepa* test system. The exposition of *A. cepa* roots to raw sludge also induced significant CA frequencies, which was not observed for bioremediated sludge. In this way, the authors showed that vermitechnology can be a good technique to remediate municipal sludge. *Pseudomonas fluorescens* strain isolated from the soil of industrial estate of Aligarh, India, which was resistant to Cd^{2+}, Cr^{6+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, BHC, 2,4-D, mancozeb and phenols up to high level and for the detoxification pollutants. The decrease in toxicity determined by the *A. cepa* test was 62.5% for the model water containing the mixture of test heavy metals, 71.9% for the pesticides, 73.2% for phenols, and 58.5% for combination of all these pollutants. The efficiency of bioremediation for certain heavy metals at the same concentrations by means of immobilized cells of the test *Pseudomonas fluorescens* isolate was estimated to be 75.9% for cadmium, 74.2% for hexavalent chromium and 61.0% for lead during the 24 hours' treatment. Authors concluded that isolated strain was highly effective to degrade the pollutant and A. cepa was efficient to estimate toxicity before and after treatment (Khan and Ahmad, 2006). The municipal wastewater is composed of highly varied substances with considerable contents of toxic agents (White and Rasmussen, 1998) and several authors reported the cytotoxicity, genotoxicity and mutagenicity by *A. cepa* test system. In order to assess the toxicity of domestic wastewater Bianchi et al., reported a significant induction of CA's, MN, cell death and inhibition of the M I (Bianchi et al., 2011). The chemical analysis revealed the presence of Pb, which may be responsible for the effects observed in *A. cepa* cells. In a study Kwasniewska et al., assessed (Kwasniewska et al., 2012), the genotoxicity of aqueous leachates from municipal solid waste landfill sites in Southern Poland. Six leachate samples out of 22 showed cell divisions abnormalities and the authors revealed more sensitivity of *A. cepa* as compared to Ames test for leachates genotoxicity evaluation. In another study, the municipal wastewater treatment plants sludge was found to be genotoxic to *A. cepa* (CA's) and chemical analysis revealed the presence of heavy metals (Pb, Ni, Cr, Zn, Cu and Cd), which may be responsible for CA's in *A. cepa* (Rank and Nielsen, 1998), whereas Smaka-Kincl et al., reported (Smaka-Kincl et al., 1996), that the municipal waste water effects on root growth, MI and MN up to 74%, 66% and 3% and aberrant cells were also observed in *A. cepa* root cells and Nielsen and Rank (Nielsen and Rank, 1994) observed the CA's in *A. cepa* root cells exposed to treated municipal wastewater for five days. Mesi and Kopliku (2011) monitored the toxicity of some Malësia e Madhe aqueous sources by root length, form, turgescence, color and MI end points. Roots were exposed to water from Dobër wells, Vraka runnel, and Shegan waterside. Along with change in root macroscopic properties, the MI values decreased in following order, distilled water, CuSO4 solution, acetone solution, Shegan, Vraka, Dobër.

While studying the possible toxicity of sludge in view of its use in agriculture, Christofoletti et al. (2012) studied biosolid sample toxicity under laboratory conditions up to 30 days using diplopods Rhinocricus padbergi and plants *Allium cepa* (onion) as test organisms. The data obtained demonstrated that the biosolid raw sample had genotoxic potential. *A. cepa* analysis showed genotoxicity, but this effect was reduced after 30 days of bioprocessing by diplopods. Study highlighted the importance of *A. cepa* as well as waste management for application to agriculture soil to avoid toxic effects on soil as well as plants. In order to asses the genotoxicity of sewage effluent, Ukaegbu and Odeigah (Ukaegbu and Odeigah, 2009) applied the morphological and CA assay. There was a significant decrease in root length of the experiment. Also the MI decreased as concentration increased and the total aberrations were also increased parallel to MI at higher concentration. The results demonstrated that the *A. cepa* test is a useful screening test for the evaluation of toxicity in sewage effluent. A study to evaluate the genotoxic effects of municipal wastewater by *A. cepa* test showed significant CA values and MI inhibition for all the test samples. In the *A. cepa* test, samples from four stages (crude sewage, primary, secondary and tertiary effluent) of the wastewater treatment plant was analyzed. The numbers of aberrant cells found in the *A. cepa* roots did not differ among the four stages tested. At all stages, the most concentrated sample was more toxic than the respective diluted samples, as demonstrated by the decreased MI (Grisolia et al., 2005). While studying the toxicity of leachates from municipal solid waste landfill sites in Southern Poland through various biological assays Kwasniewska et al., (Kwasniewska et al., 2012) revealed that aqueous leachates from municipal solid waste landfill affected *A. cepa* normal growth. Among the bioassays used in the study, the *A. cepa*

test proved to be more sensitive than bacterial tests for the investigation of leachate toxicity. The results suggested that the cytogenetic bioassay was efficient and simple for genotoxicity studies of leachates and biological effects were correlated with chemical parameters that have a genotoxic potential against *A. cepa*. A study to evaluate the toxic effects of hospital incinerator bottom ash leachate by *A. cepa* root cells (Akinbola et al., 2011), applied the CA test at concentrations ranging from 1% to 50% of the leachates and results showed a significant inhibition in root growth and induction of CA's and effects were correlated with contents of heavy metals.

The mutagens in complex environmental mixtures i.e., surface water or industrial wastewater was detected near the Sava River, Croatia using *A. cepa* root assay (Radić et al., 2010). The morphology of the A. cepa roots, inhibition of root growth, cell division and induction of mitotic and CA end points were observed. The most highly polluted water samples (industrial effluents) caused an inhibition of root growth up to 50% and MI was decreased up to 40% along with considerable CA's as compared to control. In view of positive results, authors suggested the application of mutagenicity/genotoxicity assays along with conventional chemical analysis for quality monitoring to quantify the mutagens in surface and wastewaters. Also, the toxic impact of raw waste leachates from Shkodra city wastewater was investigated using *A.cepa* test. Leachates were collected from Rusi vegetable market, University campus, centre city domestic and the main collector runoff to the lake open dump sites. The onion bulbs were grown in 1%, 2.5%, 5%, 20% concentrations and root length, EC50, MI and CA end points were studied. Growth was restricted in all leachate in increasing concentrations manner. The EC50 values were recorded as 2.9%,, 3.5%, 5.5% and 6.2% for Rusi vegetable market, University campus, a centre city domestic and the main collector runoff to the lake open dump sites leachate samples. The genotoxicity and root growth inhibition showed linear relationship. Among CA aberrations, stickiness, chromosome bridges and fragments, laggard and vagrant chromosomes were observed (Mesi and Kopliku, 2012). Bakare et al. (2012) investigated the toxicity using raw and simulated e-waste leachates by *A. cepa*. Roots were exposed to 1, 5, 10, 25 and 50% (v/v; leachate/tap water) concentration of each of the leachate sample. Cytogenetic and root length inhibition analyses were evaluated at 48 and 72 h, respectively. The root growth and cell proliferation inhibited dose-dependently. The leachates also induced morphological modifications in the roots and CA's (anaphase bridge, sticky chromosomes and binucleate cells) and toxicity was correlated with Fe, Cd, Mn, Cu, Ni, Cr As, Zn and Pb metals. These observations indicate that e-waste leachate contained cytotoxicity and mutagenic agents, which might be the environmental and public health issue in view of e-waste exposure. Shashank and Suresh (2013) collected sewage effluent form Mandsaur (Madhya Pradesh), characterized (physico-chemical) and subjected to toxicity test. *A. cepa* roots were treated with different concentrations for 10 days and the roots length was measured every day, decreased when the effluent concentration increased. *A. cepa* showed better growth at 20% dilution of water and authors suggested the use of diluted water for irrigation since toxicity was observed at concentrated wastewater. do Canto et al (2013) evaluated the toxicity of landfill leachate in the city of Araranguá (Santa Catarina state, Brazil) using *A. cepa* bioassay. Roots were exposed to leachate for 7 days and the phytotoxic effect was evaluated by determining the root growth and mass of the roots, and the mass gain of the bulbs. The results indicated that the leachate significantly affected the root growth and mass of the roots and the mass gain of the bulbs. Leachate has toxic potentials to *A. cepa* that were associated with the presence of contaminants in solution. Klauck et al. (2013) assessed the toxic and genotoxic effects of an untreated leachate sample. The leachate was collected in a landfill located in the region of Vale do Rio dos Sinos, southern Brazil. The bulbs of *A. cepa* were exposed to concentrations of 5%, 10%, 25%, 50% and 100% for 48 h. Results showed high toxicity, with significant root growth retardation as well as reduction in MI in bulbs exposed to 100% concentration of the leachate. The CA like abnormalities in anaphase-telophase was observed in accordance with increase in the concentration of leachate (5%, 10%, 25% and 50%). Andrade-Vieira et al. (2012) evaluated the cytogenetics effect of Spent Pot Liner (SPL) (a complex solid waste from the aluminum industry, composed of organics, fluoride salts, inorganic cyanides, metals, and sodium) using *A. cepa* bioassay. Roots were exposed to 0, 10, and 25% concentrations of leachates from SPL-soil mixtures for 4, 8, 12, 24, and 36 h. The results showed an overall mitodepressive effect accompanied by an increased percentage of condensed nuclei and genomic instability as evidenced by cellular and CA inductions.

Garaj-Vrhovac et al. (2013) investigated landfill leachate from the Piškornica (Croatia) sanitary landfill treated by different methods. Chemical treatment procedure combined with chemical precipitation with CaO followed by coagulation with ferric chloride and final adsorption by clinoptilolite. Electrochemical treatment approach included pretreatment with ozone followed by electrooxidation/electrocoagulation and final polishing by microwave irradiation. Cytotoxic effect of the original leachate was obtained for both exposure periods (4 and 24 h) while treated samples showed no cytotoxic effect even after prolonged exposure time. Both methods were found suitable for the treatment of complex waste effluent due to high removal efficiency of all measured parameters for toxicological safety of the treated effluent. Grisales Penagos et al. (2012) evaluated the removal of the organic matter present in hospital effluent by ozonation and in response to 70% biodegradability increment, the acute toxicity decreased by 62%, which revealed sensitivity of A. cepa and efficiency of ozonation for hospital wastewater treatment.

Treated hospital effluent applying ozone at different pH conditions (3,0, 6,7, 10), and other parameters such as UV254, biodegradability ratio (COD/BOD) and color (VIS436)

were measured along with toxicity monitoring (Dayana et al., 2012). In response to biodegradability of 70% the toxicity decreased by 62%. The ozone application seems to be a viable alternative to treat hospital effluents as a pretreatment of a biological process and *A. cepa* was to be sensitive to evauate biodegradability. Similarly (Kern et al., 2013), treated hospital laundry wastewaters by advanced oxidative processes (photocatalytic ozonation O_3, UV, UV/O_3, UV/O_3/Fe^{2+} 50 mg/L and 150 mg/L). The analysis of the investigated wastewater revealed high chemical oxygen demand (COD-3343.8 mg/L, biochemical oxygen demand (BOD_5-1906.4 mg/L, total Kjeldahl nitrogen (TKN - 79.8 mg/L. The treated and un-treated wastewater was tested for genotoxic using *A. cepa*. After treatment COD (59.1%), BOD_5 (50.3%) and TKN (86.8%) were reduced. Normalization of the MI and reduction of micronucleated cells were observed in *A. cepa* after the treatments. Results demonstrated that these methods were efficient in the degradation of hospital laundry wastewaters, representing a thriving alternative for the removal of pollutants that caused toxicity and genotoxicity and A. cepa was found an efficient test to detect the toxicity load before and after treatment. Roig et al. (2012) studied the chemical and ecotoxicological properties of 28 sewage sludge samples from Spanish wastewater treatment plants in order to assess their suitability for agricultural purposes. Sludge samples were classified into five categories according to specific treatment processes in terms of digestion (aerobic/anaerobic) and drying (mechanical/thermal). Composted samples, as indicative of the most refined process, were also considered. The concentrations of seven metals (Cd, Cr, Cu, Pb, Zn, Ni, Hg) and organic substances (phenolic compounds, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, polychlorinated naphthalenes, polybrominated diphenyl ethers, and perfluorinated compounds) were present in samples. Root elongation test with *A. cepa* along with other test organism was conducted. Significant differences were found in the following parameters: dry matter, electrical conductivity, nitrogen, organic matter and its stability, phytotoxicity and ecotoxicity, depending on the sludge treatment. In turn, no significant differences were found between categories in the concentrations of most metals and organic pollutants, with the exception of free phenolic compounds. Furthermore, no correlation between total heavy metal burden and ecotoxicity was observed. However, a good correlation was found between phenolic compounds and most ecotoxicological tests. These results suggested that sludge stability (conditioned by sludge treatment) might have a greater influence on sludge ecotoxicity than the pollutant load. Composting was identified as the treatment resulting in the lowest toxicity. Magdaleno et al. (2008) tested Matanza-Riachuelo river sediment composition and genotoxicity in order to develop a cost-effective, environmentally sound option for disposal and management of contaminated dredged materials. Sampling was performed in a rural area, in a solid waste dumpsite and an urban and industrial area. The concentrations of total heavy metals increased from the upper basin to the lower basin. The concentrations of copper, lead, and chromium in the leachates exceeded the permissible levels for the protection of aquatic life. CA's were observed in *A. cepa* roots exposed to sediment samples and authors classified sediments as genotoxic hazardous waste.

The genotoxicity of industrial wastewater samples from Aligarh and Ghaziabad cities using *A. cepa* test has also been studied. Samples from both sites induced anaphase aberrations. Fragmentation of the chromosome was the predominant effect of the Aligarh water sample, wherease sample from Ghaziabad induced chromosome stickiness and correlated the toxicity with heavy metals and pesticides in water samples (Fatima and Ahmad, 2006a). Fatima and Ahmad (2005) also investigated heavy metals (Cd, Cr, Cu, Hg, Pb and Zn) pollution in water using *A. cepa* test system and positive results were obtained in parallel to other biassays used. Geras'kin et al. (2011) evaluated genotoxic potential of two environmental compartments (water and sediment) from the Upper Silesia Coal Basin (USCB), Poland by employing root meristem cells of *A. cepa*. The clear genotoxic effect of water and sediment sampled was shown, with an important contribution of severe types of cytogenetic abnormalities. Results of simultaneous use of conventional monitoring methods and biological tests suggested that contemporary levels of persistent pollutants in post-mining areas of the USCB may enhance the risk both for human health and biological components of natural ecosystems.

Bio-remediation of pollutant have been found to be efficienct (Bilal et al., 2016a; Bilal et al., 2016e; Obek and Sasmaz, 2011; Sasmaz, 2008, 2009; Sasmaz et al., 2016a; Sasmaz and Obek, 2009, 2012; Sasmaz et al., 2008; Sasmaz and Sasmaz, 2009) and the effect on bio-treatment on toxicity have also been studied i.e., the efficiency of the aquatic macrophytes Salvinia auriculata Aublet in purification of polluted effluents, and toxicity was monitored by *A. cepa* test (2015) (Gonçalves et al., 2015). Three collections were performed in September 2011, the first analysis was performed with water collected directly from River Santa Catarina, Mexico and sampling was performed seven days after the contact of the effluent with the macrophytes, and then after fifteen days. According to the results of the A. test, the aquatic macrophyte *S. auriculata* was efficient in the removing of pollutants after fifteen days in contact with the effluent. While evaluating the performance of an Upflow Immobilized Biomass Anaerobic Reactor (UIBAR) for treating hospital wastewater, Porras Torres et al. (2013) applied *A. cepa* root growth inhibition test and toxicity was reduced up to 50% after treatment. The capacity of the anaerobic immobilized process to remove the compounds that produce toxicity was verified by *A. cepa* test.

Brkanac et al. (2014) assessed the toxic and genotoxic potential of leachate from an old sanitary landfill prior to and following chemical and electrochemical treatments by *A. cepa* test. Untreated leachate inhibited *A. cepa* cell division and induction of mitotic and CA's before treatment and after treatment leachate did not show

genotoxicity and electrochemical method was found to be more efficient in removing toxic substances present in landfill leachate and thus more suitable for treating such leachates prior to their discharge into the environment. A. cepa resonse of treated and un-treated leachate demonstrated that the ecotoxicity/genotoxicity assays should be used in leachate risk assessment together with physico-chemical analysis. Pathiratne et al. (2015) assessed the efficacy of *A. cepa* test for screening cytotoxicity and genotoxicity of treated effluents originated from textile industries, rubber industry, and treatment plants of industrial zones. The heavy metal/metalloid levels of the effluents varied depending on the industry profile. In the A. cepa test system, the undiluted effluents induced significant root growth retardation, mitosis depression, and CA's. The results support the use of a practically feasible *A. cepa* test system for rapid screening of cytotoxicity and genotoxicity of diverse industrial effluents discharging into inland surface waters.

Klauck et al. (2015) evaluated leachate toxicity of a municipal solid waste landfill located in the Sinos River Valley region (southern Brazil). The roots of *A. cepa* were exposed to raw leachate and treated leachate (biological treatment). The levels of metals detected in both samples of leachate were low, and raw leachate showed high values for ammoniacal nitrogen and total Kjeldahl nitrogen. *A. cepa* showed a phytotoxic response to landfill leachate, showing reduced root elongation. The results indicated phytotoxicity in municipal solid waste landfill leachate, even after biological treatment. The cytotoxicity insecticides (Endosri & Nuvan) and fungicide (Kvistin) using *A. cepa* root inhibition test at concentrations; Endosri (2000, 1000, 500, 250, 125, 60, 30, & 15ppm), Nuvan (400, 350, 300, 250, 200, 150, & 100ppm) and Kvistin (3000, 2000, 1000, 750, 500, 250, & 125 ppm) for 5 days was studied amd EC_{50} values obtained for Endosri, Nuvan and Kvistin were 60, 200 and 500 ppm, respectively. Both Endosri and Nuvan showed highly toxic effects and decreased in both root length and root count. Kvistin was less toxic and results indicated that toxicity could also contribute to several afflictions in other organisms after exposure to higher concentrations and a negative impact on the crop as well (Kuchy et al., 2015). While studying the nature of sewage sludge generated by wastewater treatment plants (WWTP), Mazzeo et al. (2015) applied A. cepa to evaluate the detoxification efficiency of treatment. Sewage studge/soil with 10, 25 and 50% proportions was buried for 0, 2, 6 and 12 months in holes. Chemical analysis revealed the presence of high concentration of *m*- and *p*-cresol, especially for samples analyzed after 0 or 2 months. Both, raw sewage studge and aqueous sewage studge extracts induced DNA damage in *A. cepa*, even when associated with soil. However, this effect was observed to decline during the attenuation period, although significant effects were detected for the highest tested concentration (100%) even at the end of attenuation period. Based on A. cepa results, authors suggested the necessity of stabilization of sewage sludge and soil for a period of at least 12 months since *A. cepa* test proved to be sensitive tool to assess the efficiency of sewage sludge detoxification process. The physico-chemical characteristics of water from Dandugan Oya, a water canal located in the Western Province of Sri Lanka receives industrial waste from multiple sources along with toxicity using *A. cepa* was assessed (Kannangara and Pathiratne, 2015a). Root exposed to wastewater and downstream water resulted in the reduction of root growth (24–62%) and mitosis (31–55%), MN (0.6 %), NA (3-14 folds) and CA's (3 - 21 folds) in downstream water. No significant difference between the control and the upstream water was found in relation to the measured biological effects. Results revealed that the tested wastewaters contained cyto-genotoxic agents and authors suggested using bioassays for water quality evaluation along physico-chemical analyses.

More recently, Papa et al. (2016) evaluated the performance of Wastewater Treatment Plants (WWTPs) using various toxicity tests i.e., algal growth inhibition, bioluminescence inhibition and acute toxicity test (baseline toxicity); an E-Screen-like assay (estrogenic activity); Ames, *Allium cepa* and Comet tests (mutagenic/genotoxic activity). All tests revealed good performance of WWTP which displayed the ability of WWTP to enhance effluent quality and efficiency of *A. cepa* to detect the toxicity. Corrêa Martins et al. (2016) also reported the cytotoxic, genotoxic and mutagenic potential of sewage sludge using *A. cepa* bioassay. Solubilized and crude sludge from two sewage treatment stations were tested. Treated wastes showed less phytotoxicity, cytotoxicity, genotoxicity and/or mutagenicity. Despite negative results for MN F1 (micronuclei counted in F1 root cells, derived from meristematic cells) the authors recommended the use of genotoxic and mutagenic activities of sewage sludge to avoid negative effect on soil in view of their application to the agricultural soils. Recently, (Teena et al., 2016) also screened mutagenicity of complex environmental mixtures (surface water or industrial wastewater). Sewage effluents were collected from Kunnamkulam market, India and roots were exposed for 3, 6, 12, 24, and 48 h. A significant CA (bridges, fragments, vagrant chromosomes, sticky chromosomes, C-mitosis, spindle abnormalities and disoriented chromosomes) along with decreased MI values were observed in exposure dependent manner. Authors suggested the treatment of sewage wastewater before use and toxicity evaluation using A. cepa test.

Treated effluents/toxic compounds

Industrial effluent contains toxic pollutants with elevated values of water quality parameters i.e., COD, color and heavy metals have been successfully treated using different methods. However, their might be generation of toxic by-product, which needs monitoring using reliable assay and various researcher applied the *A. cepa* test to monitor the efficiency of treatment methods (Ashar et al., 2016; Babarinde et al., 2016; Babarinde and Onyiaocha, 2016; Bhatti et al., 2016; Bilal et al., 2016e; Gangadhara and Prasad,

2016; Iqbal et al., 2013b; Iqbal, 2016; Iqbal et al., 2015b; Iqbal and Bhatti, 2015b; Iqbal et al., 2016a; Iqbal et al., 2016b; Iqbal and Khera, 2015; Iqbal and Nisar, 2015; Jafarinejad, 2016a; Jamal et al., 2015; Majolagbe et al., 2016a; Mumtaz et al., 2016; Mushtaq et al., 2016; Nadeem et al., 2016b; Nisar et al., 2017; Nisar et al., 2016; Pervaiz et al., 2015; Peter and Chinedu, 2016b; Qureshi et al., 2015a; Rashid et al., 2016; Sayed, 2015; Shindy, 2016; Shoukat et al., 2017; Tahir et al., 2016a; Tahir et al., 2016b; Ukpaka, 2016a; Ukpaka, 2016b; Ukpaka, 2016c, d). For example, mineral salt medium supplemented with as low as 0.02% (w/v) yeast extract and glucose was found to remove 70% ADMI, 69% COD and >99% sorption of heavy metals in 24 h from the effluent by consortium TSR. Toxicity study of the effluent showed 90% germination, which was 20%-30% of the untreated effluent for *A. cepa* along with other plant species (Patel et al., 2015)**.** Olorunfemi et al. (Olorunfemi et al., 2015c) evaluated bilge water (water from oceanic vessels is usually discharged through the bilge wells) for toxicity using *A. cepa* test from Nigerian marine environment. Exposure of the onion roots to the wastewater at 1, 5, 10, 25 and 50% (v/v; wastewater/tap water) showed strong concentration-dependent root growth inhibition with an EC_{50} value of 52.6% at 72 h and induced a variety of chromatid and chromosome structural aberrations at 6, 12, 24 and 48 h. The bilge also disturbed the spindle fibre apparatus at all the concentrations and toxicity was correlated with heavy metals in tested water (Cu, Mn, Pb, Fe, Cd, Cr, Ag, Ni and Zn). Mitodepressive and low MI values were also observed. Results revealed toxic chemicals presence in the bilge water, which may contribute to the toxicological assessment of the risk associated with its indiscriminate discharge into the environment of bilge water.

Toxic agents of environmental concern

While studying the toxicity of waste water samples (mixture of substances) from reserve pit, Vidaković (1993) applied *A. cepa* test. All undiluted waste drilling fluid samples showed cytotoxicity as well as toxicity, which was proved by cytogenetic parameters such as high inhibition of mitotic activity or low MI values and high percentage of chromosomal and genomic aberrations. The toxicity was significantly reduced by increasing the degree of sample dilutions.

Ochratoxin toxicity was evaluated using *A. cepa* root test. Analyses of root growth and the root meristematic zone in response to Ochratoxin A treatment showed Ochratoxin A toxicity to root growth at a concentration of 10 ug/mL associated with inhibition of proliferation activity. Cytological changes observed in the *Allium* chromosome aberrations assay, at a concentration of 5.0 ug/mL showed that Ochratoxin A was able to induce genotoxicity at the chromosome level. These results indicate that A. cepa is very sensitive to the mycotoxin Ochratoxin A since toxicity and cytogenetic injuries were observed in A. cepa exposed roots (Lerda et al., 2010). The toxicity of glyphosate on *A. cepa* was also studied (Çavuşoğlu et al., 2011). Germination percentage, root length, seedling weight, MN, CA's and MI end points were used along with root anatomy of *A. cepa*. Glyphosate different doses,100, 250 and 500 mg/L were applied and results showed significant alterations in the germination percentage, root length, seedling weight, MN, CAs and MI frequency depending on doses of glyphosate. Mitodepressive action on mitosis was also observed and the MI was decreased depending on the dose of applied-glyphosate. Unclear vascular tissue, unclear epidermis layer, cell deformation, unusual form of cell nucleus (usually flat) and binuclear cells were also observed. Each dose of glyphosate caused severe toxic effects on *A. cepa* root cells and the strongest toxic effect was observed at the dose level of 500/L.

The toxic and genotoxic chemical compounds and using A. cepa test, the toxicity and genotoxicity of wastewaters from the public hospital of Buenos Aires (Argentina) was investigated (Magdaleno *et al.,* 2014). The effluent from the sewage treatment plant serving around 10 million inhabitants was also evaluated. The *A. cepa* test showed that 40% of the samples were genotoxic. In view of genotoxicity, authors suggested the establishment of advanced and effective effluent treatment plants in the hospitals, which are merely dumping the wastewaters in the municipal sewerage system. Similar, the untreated wastewaters from health centers was also evaluated for toxic and genotoxic chemicals. The genotoxicity and toxicity from Hospital San Martín wastewaters was evaluated. This General Hospital releases approximately 560 m^3 of effluents daily to the municipal sewer system. The samples collected in summer 2003 revealed presence of toxic and genotoxic agents. However, samples collected in autumn 2004 were non genotoxic to *A. cepa* (Paz et al., 2006). In another study, the *A. cepa* test was used to evaluate the genotoxicity of a hospital effluent in Santa Maria, Rio Grande do Sul State, Brazil (Bagatini et al., 2009). The CA's, anaphasic bridges, and MN during telophase were observed, which indicated the environmental toxicity risk of hospitals wastes. In another study, performance of an Upflow Immobilized Biomass Anaerobic Reactor in treating real hospital wastewater was evaluated using *A. cepa* test. The toxicity was treated and un-treated wastewater was assessed by measuring the growth inhibition of *A. cepa L* roots. A. cepa showed reduction in toxicity up to 50%. Authors revealed that the A. cepa was able to verify the toxicity removal capacity of the anaerobic immobilized treatment system (Torres et al., 2014). Kern et al. (2015) also assessed the genotoxicity of hospital laundry wastewaters generated from a regional hospital, Rio Pardo Valley, Rio Grande do Sul, Brazil. A cepa revealed IC_{25} value of 51.25% and cytotoxicity was observed five times the wastewater concentrations. The results warned about the necessity to develop treatment methods to mitigate the environmental impacts caused by the ecotoxicity and genotoxicity of hospital laundry wastewaters. Bernardes et al. (2015) determined toxicity of environmental pollutants i.e., difenoconazole (DZ) and tebuconazole (TZ) on A. cepa by macroscopic (germination and root growth) and microscopic

(MI, CA, and NA) analyses. A reduction was observed in the germination, root growth, and MI at higher concentrations of DZ and TZ. CA and NA were also detected in treated roots and DZ and TZ proved to be genotoxic, cytotoxic, and phytotoxic. Wardini et al. (2015) exposed *A. cepa* roots to compost produced in TPK Sarimukti, Cipatat, West Bandung for toxicity evaluation. Tests carried out by treated *A. cepa* roots with liquid compost (2,5%, 5%, 10% and 12,5% (w/v)) or solid compost (25%, 50%, 75% and 100% (w/v)) for 48 h. Results showed reduced root growth and MI in concentrations dependent manner. MN increased with increase in liquid compost concentration. MN found at very high frequencies in highest solid compost concentration (100%), but very low at lower concentrations. Cells with binuclei and cell necrosis increased with increasing concentrations of compost. NA found in high frequency in 75% and 100% solid compost. Based on results it was concluded that liquid compost is more toxic because it can reduce MI and root growth rate at lower concentrations than solid compost. Both types of compost have genotoxic properties because it can induce CA, MN, binuclei and NA formation. Hemachandra and Pathiratne (2015) studied the tolerance limits for copper, cadmium and chromium containg industrial effluents into inland surface waters by *A. cepa* bioassay. The *A. cepa* roots were exposed to Cu (3 mg L) individually or in mixtures with Cd (0.1 mg L) or/and Cr (0.1 mg L) exhibited the highest growth inhibition, MI depression and NA. Root tip cells exposed to Cr or Cd alone or in mixture displayed significant CA's. The EC_{50s} for root growth inhibition showed that Cu was more toxic followed by Cd and Cr. Authors suggested the industrial effluent discharge regulatory limits for metals to avoid cyto-genotoxicity to biological systems. da Lima et al. (2012) analysed the genotoxic effect of controlled release formulations of the herbicide ametryn encapsulated in microspheres of poly(hydroxybutyrate) and poly(hydroxybutyrate-co-valerate) through cytogenetic end points in *A. cepa*. The results showed that the rate of chromosome breakdown caused by unencapsulated ametryn was much higher than that caused by ametryn encapsulated in the polymer microspheres, which indicates that controlled release delivery systems employing the polymer formulations should be significantly safer.

Çelik and Aslantürk (2009) investigated the potential genotoxic effects of Ecballium elaterium fruit juice using the *A. cepa* test system. *A. cepa* were treated with 10 mL/L, 20 mL/L, 50 mL/L concentrations and undiluted *Ecballium elaterium* fruit juice for 72 h and dose-dependent inhibition of root growth and mitodepressive effects on cell division in *A. cepa* root tip cells were observed. Among CA's, breaks, stickiness and pole deviations as well as MN inductions were also observed. Bratu et al. (2012) measured the phytotoxicity/toxicity of aqueous solutions of powder extract using *A. cepa* as a test organism. Aqueous solutions of the fruit powder have reasonably expressed mutagenic activity in vivo on the radicles of *A. cepa*, especially when they are used at a concentration of 1 g/dL for a prolonged time (48 h). At lower concentrations (0.1 g/dL), however, the mutagenic effect was not observed. On the basis of results, authors suggested the use of *Sambucus nigra* fruit extract powder at low concentrations for possible applications in the food industry. Hile studying the toxicity of irrigation water used for vegetables in a Greater São Paulo watershed region, *A. cepa* test was applied (de Maio Lacerda et al., 2014). The samples were collected from small watercourses located in a sub-basin of High Tietê, Cotia-Guarapiranga, Embu-Mirim River, a major tributary of the Guarapiranga Reservoir. From April to September 2011, water and sediment samples were collected at three points (small watercourses) used for irrigation of leafy vegetables inside private agricultural properties (April, June and September). Surface water genotoxic effect was detected in 2 out of the 3 points and mutagenic effect in all three sampled points, as well as in the sediment.

The cytotoxicity and the mutagenicity of liquid waste produced in the process of industrialization of the bitter cassava (a plant used as food and an ingredient in industry), *olho-junto* variety was also studied using *A. cepa* test (Viana et al., 2014). The cassava root contains wastewater, popularly known as *manipueira*, which is a toxic substance. Its ingestion by animals causes poisoning although they react positively to treatment with sodium thiosulfate. The liquid waste was obtained when the cassava roots are pressed; pond water, which is press water stored in impounded ponds; and a solution of sodium thiosulfate, pure and with other waste. The toxicity was evaluated by root *A. cepa* meristematic cells. Treatment with saline solution showed cytotoxicity in *A. cepa* and reduced cell division significantly. Clastogenic effect was also observed through CA test. Based on results, authors suggested the use of sodium thiosulfate only in emergency conditions in which the benefits exceed the risks.

While studying the food industry ingredient used as flavoring agent, Moura et al. (2016) employed *A. cepa* assay for toxicity monitoring of synthetic Cheese and Cheddar Cheese (food flavors) at 1.0 and 2.0 mL doses. Genotoxiicty was not observed, however, flavoring agents significantly reduced the cell division rate. Adeyemo and Farinmade (2016) studied genotoxic and cytotoxic effects of monosodium glutamate (MSG) used as flavor enhancer in foods using the *A. cepa* test. A. cepa roots were exposed to 1, 3, 5 and 7 g/L of MSG concentrations and subjected to macroscopic (morphology and color of roots) and microscopic (MI and CA's) analysis. MSG inhibited growth of *A. cepa* roots at all tested concentrations. MSG also reduced the number of roots growing from primodium in all tested concentrations and the least was observed in 5 g and 7 g/L concentration. Color of root tips range from brownish to dark brown or black in higher MSG concentrations was observed. Sticky chromosomal aberration at telophase was most observed at all the MSG concentrations. MSG also decreased MI values slightly.

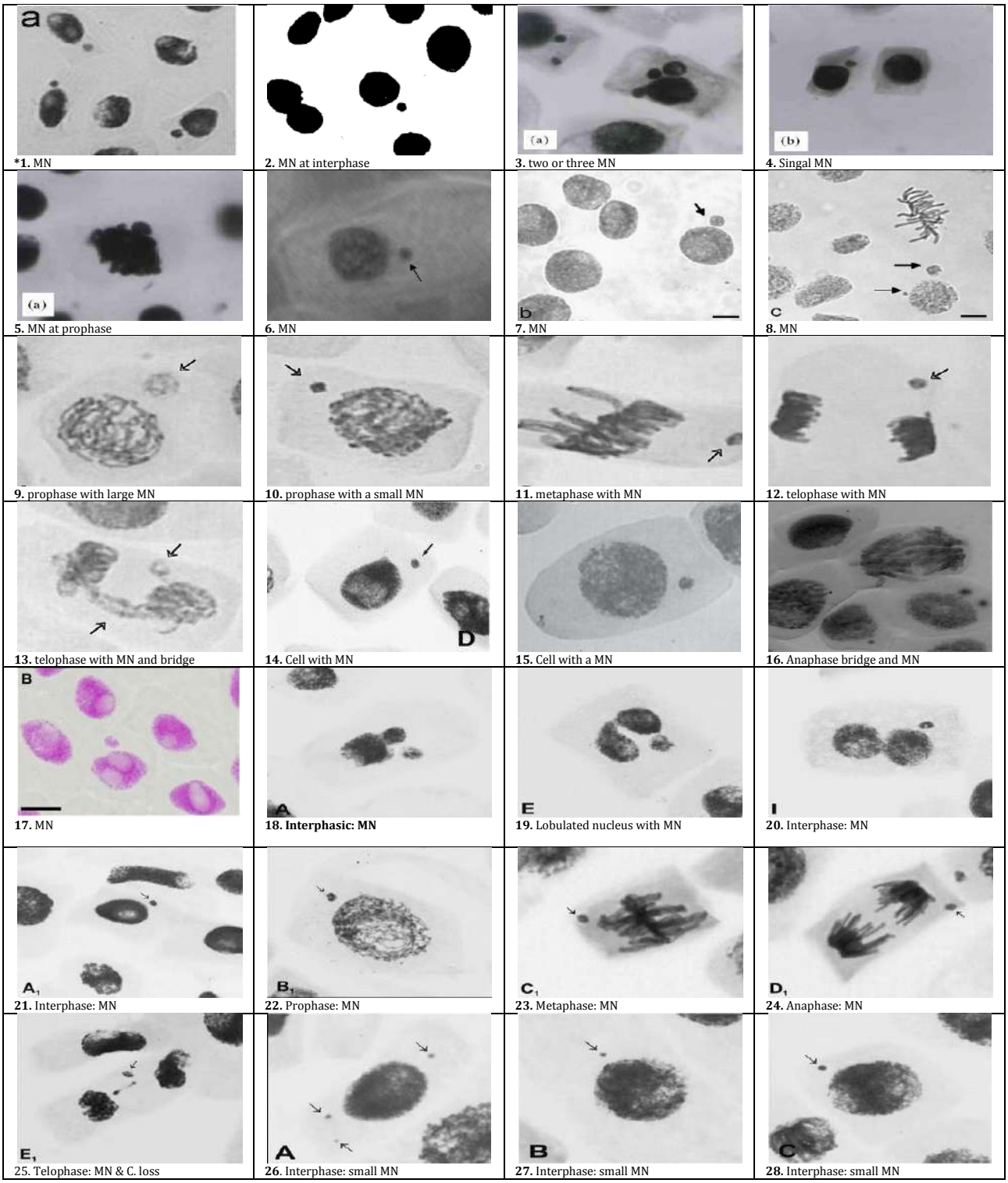
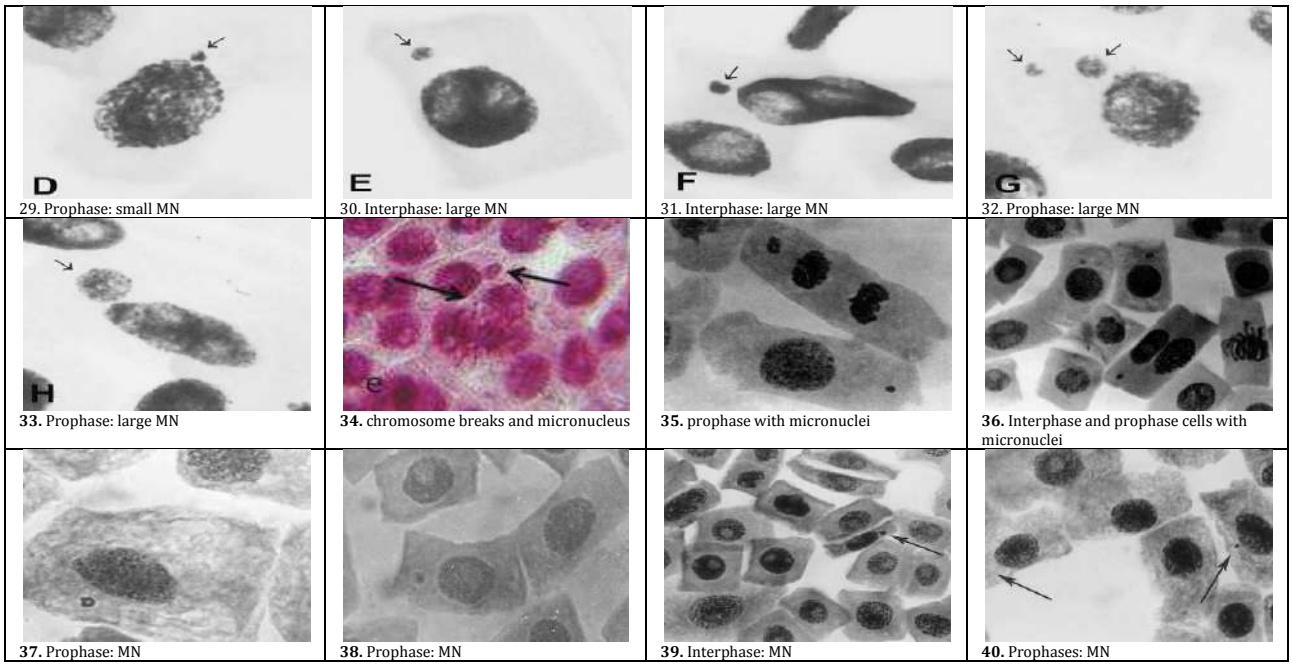


Fig. 4: *A. cepa* meristematic cells carrying micronuclei and their types as a result of different mutagenic agent exposure (1-40). *References and physiochemical properties of wastes: 1 (Bianchi et al., 2011): river water mixed with industrial effluents: pH-6.83-7.29, EC-29-114, DO-4.43-7.09 mg/L, T-24.1-25.9 ^{0}C and metal (Cu, Cr, Ni, Cd, Zn, Fe, Mn, Ca). 2 (El-Shahaby et al., 2003): industrial wastewater. 3-4 (Abdel Migid et al., 2007): fertilizer industrial effluent before algal treatment. 5 (Abdel Migid et al., 2007): industrial effluent before algal treatment. 6 (Gupta and Ahmad, 2011): 7-8 (Kwasniewska et al., 2012): landfill leachates: Pb, Ni, Cd, Hg, benzene, dichloromethane, chloroform, trichlorobenzenes, exachlorobenzene, hexachlorobutadiene, hexachlorocyclohexane, lindane, hexachlorocyclohexane, pentachlorobenzene, pentachlorophenol, PAH's. 9-13 (Leme and Marin-Morales, 2008): refinery wastewater: HC(C_{12}-C_{18}) and PAH's (naphthalene, acenaphthylene, acenaphthlene, fluorine, phenanthrene, pyrene, benzoanthracence and chrysene). 14 (Mazzeo et al., 2011): mixture of benzene, toluene, ethylbenzene and xylene. 15 (Ventura-Camargo et al., 2011): azo dye. 16 (Jadhav et al., 2010): textile effluent. 17 (Herrero et al., 2012): Di(2-ethylhexyl)phthalate, triclosan and propylparaben. 18-33 (Leme and Marin-Morales, 2009). 34 (Olorunfemi et al., 2011): A. cepa grown in agriculture filed under the effect of pesticides. 35-36 (Ivanova et al., 2005): heavy metal and cyanide contaminated river waters. 37-38 (Ivanova et al., 2008). 39-40 (Staykova et al., 2005).



29. Prophase: small MN
30. Interphase: large MN
31. Interphase: large MN
32. Prophase: large MN
33. Prophase: large MN
34. chromosome breaks and micronucleus
35. prophase with micronuclei
36. Interphase and prophase cells with micronuclei
37. Prophase: MN
38. Prophase: MN
39. Interphase: MN
40. Prophases: MN

Fig. 4: Continue…

In order to evaluate the cytogenetic effects of food preservatives (sodium benzoate and sodium metabisulphite) Onyemaobi et al. (2011) employed *A. cepa* assay considering root length, CA and MI end points. The MI decreased with increasing concentration of both sodium benzoate and sodium metabisulphite. Cytological aberrations observed were clumping, fragmentation, pulverization, lagging, binucleate cells and reduction in chromatin materials. The percentage of CA's increased with increase in concentration of the food preservatives. The effects of sodium metabisulphite at different concentrations were very detrimental as more aberrations were recorded even after the recovery experiment. Authors suggested the banning of these substances as food preservatives since irreversible cytotoxic effects at certain doses were observed. Tripathy et al. (2015) studied the toxicity of dye orange red (amalgamation of two primary food colourants like caramoisine and sunset yellow). Assuming the potential genotoxicity of the dye, A. cepa mitotic aberrations end point and gradual decondensation or little condensation of chromosomal arms in abnormal metaphases and anaphases were observed. Unequal cytokinesis and karyokinesis and formation of bi-nucleated cells were also appeared. The frequencies of MA showed good correlation with the concentration of dye.

In view of use of nano materials for food packing, De Lima et al. (2010) proposed that such nanoparticles might harm food quality and tested the chitosan (CS) and poly(methacrylic acid) (PMAA) nanoparticles (size 60, 82, and 111 nm) for genotoxicity using the *A. cepa* cytogenetic tests. Test substrates were exposed to solutions containing nanoparticles at polymer mass concentrations of 1.8, 18, and 180 mg/L and particles of 82 and 111 nm nanoparticles reduced MI values at the highest concentration tested (180 mg/L), indicating that the nanoparticles were toxic. In the case of the 60 nm CS/PMAA nanoparticles, no significant changes in the MI was observed at the concentration levels tested, indicating that these particles were not toxic. The techniques used show promising potential for application in tests of nanoparticle safety envisaging the future use of these materials in food packaging. Lutterbeck et al. (2015) evaluated the toxic effects of four frequently used anti-cancer drugs (Cyclophosphamide (CP), Methotrexate (MTX), 5-Fluorouracil (5-FU) and Imatinib (IM). Cytotoxicity, genotoxicity and mutagenicity investigations were performed with *A. cepa* assays. Significant differences in the MI were observed in three of the studied compounds (MTX, 5-FU and CP), indicating potential cytotoxic activity of these substances. CA's were registered in cells that were exposed to 5-FU, CP and IM. All the four compounds caused the formation of micronucleated cells indicating mutagenic potential. Besides, the assays performed with MTX samples presented a high number of cell apoptosis (cell death). So, authors suggested the use of *A. cepa* for the screening of environmental contamination.

Sobral et al. (2013) studied the toxicity of metal rich acid mine drainage (AMD) using CA end point. Root was exposed to 100, 10, 1, and 0.1 (%) of AMD. Chromosomal aberrations, cell division phases and cell death were quantified after 24 and 48 h recovery periods. The AMD revealed to be mutagenic and genotoxic, even after diluting it to 1 and 0.1 %. Recovery tests showed that AMD genotoxic effects persisted after the exposure. Netto et al. (2013) also evaluated that the carboniferous activity generates acid mine drainage (AMD) which is capable of unleashing toxic effects on the exposed biota. The untreated-AMD and AMD treated with calcinated sediment was tested for toxicity and it was revealed that untreated-AMD presented low pH values and

elevated concentrations of the metals Fe, Al, Mn, Zn and Cu. A. cepa roots showed sub-chronic toxicity and genotoxicity and DNA exposed to untreated-AMD. Treatment of AMD with calcinated sediment reduced the acidity and metals, which in trun showed less toxic and genotoxic effects. Similarly, Defaveri et al. (2009) evaluated the efficiency of physical, chemical, and biological treatment of acid mine drainage using alternative biological indicator *A. cepa*. Samples were collected at four stations that are located at specific treatment system: 1) pH control and precipitation, 2) biological damping pond outlet, 3) wetland inlet, and 4) wetland outlet. Toxicity in A. cepa was observed as root growth inhibition after a seven-day exposure period at 100% effluent concentration and in comparison to shrimp and comet assay, results revealed that *A. cepa* is only suitable for evaluating genotoxicity and in evaluating treatment process efficiency in eliminating toxicity and genotoxicity. Geremias et al. (2012) evaluated the efficacy of the treatment of acid mine drainage (AMD) with calcinated coal mining waste using *A. cepa*. The pH values and the concentrations of aluminum, iron, manganese, zinc, copper, lead and sulfate were determined before and after the treatment of the AMD with calcinated coal mining waste. *A. cepa* roots were exposed to untreated and treated AMD. The results indicated that the AMD treatment with calcinated coal mining waste resulted in an increase in the pH and an expressive removal of aluminum, iron, manganese and zinc. A high sub-chronic toxicity was observed when *A. cepa* was exposed to the untreated AMD. However, after the treatment no toxicity was detected. In conclusion, the use of calcinated coal mining waste associated with toxicological tests on *A. cepa* represents an alternative system for the treatment and biomonitoring of these types of environmental contaminants.

Tabrez and Ahmad (2012) analysed Trichloroethylene (TCE) genotoxic, which is usually present in the air, soil and water as pollutant. For the genotoxicity analysis of TCE in natural milieu, wastewater collected from two different stations of northern India namely Saharanpur and Aligarh, U.P were supplemented with 50 and 100 mg/L of trichloroethylene. TCE alone was found to be non-genotoxic up to the range of 1000 mg/L concentration. However, the test water samples supplemented with 100 mg/L of TCE, exhibited a significant increase in the genotoxicity. *A. cepa* genotoxicity test also showed around 25% increase in CA's frequencies following 100 mg/L TCE supplementation. However, supplementation of 50 mg/L TCE to the test water samples could not enhance the genotoxicity to a significant extent. From results, it can be concluded that TCE itself was non-genotoxic, but it may be toxic at a concentration of 100 mg/L under certain environmental conditions. Yahaya et al. (2012) monitored the cytotoxicity and mutagenicity of cement dust. *A. cepa* roots were exposed to cement dust over three different periods of time at about 100 m from a cement factory. The test groups (groups 2-4) were exposed to the dust for 2 weeks, 4 weeks and 6 weeks, respectively. The elemental analysis of the A. cepa in the test groups revealed significant levels of calcium, silicon, aluminum, chromium and lead compared to the control group. Also, significant differences were found to exist among the levels of the elements detected in the A. cepa in various test groups. There was a direct linear relationship between the concentrations of calcium and root length growths of the *A. cepa* across the groups. The CA's such as stickiness, c-mitosis, chromosomal bridge, chromosome fragmentation, vagrant chromosomes, bi-nucleus chromosomes and multi-polar anaphase were observed, which increased with the length of exposure. The findings of the research highlight the toxicity of cement dust and the need for pollution control measures to safeguard plants and animals in the environment. Similarly, Aguiar et al. (2016) studied the toxicity of industry treating stones i.e., marbles and granites, includes a stage of plate polishing, in which resins and abrasives are used, producing a fine grained waste with high moisture content. The A. cepa bulbs were germinated in Petri dishes with filter paper moistened in the liquid phase of the effluent and germinated roots were prepared for analysis of CA's. The MI values were same in all treatments, however, seeds exposed to the polishing waste showed high MN induction, nuclear buds and other CA's. Authors concluded that the analysed wastes have mutagenic potential that is correlated with high content of phenolic compounds identified in the samples.

Chakraborty et al. (2009) tested fly ash (by-product of coal-fired electricity generation plants) for toxicity using A. cepa assay from thermal power plant in West Bengal, India. Fly ash sample was mixed with sand in different proportions and A. cepa bulbs were germinated in fly ash for five days. The results indicated that fly ash at 100% concentration inhibitted root growth and mitotic indices; induced binucleated cells as a function of the proportion, but was not toxic at very low concentration. The metals like Zn, Pb, Cu, Ni, Cd and As were present in fly ash sample and partially correlated with fly ash toxicity. Authors suggested the use of fly ash at very low concentrations in order to protect the ecosystem from any potential adverse effects. Maiti et al. (2016) studied the toxicity of hair dye and estimated it's discharge to the environment. Recommended dose precisely mentioned in instructions booklet of the hair dye was used to expose *A. cepa* roots along with other bioassays. *A. cepa* MN assay showed various CA's along with cell division retardation. Including A. cepa results, all bioassays confirmed cytotoxicity and mutagenicity of hair dyes on living cells.

Roychoudhury and Giri (1989) reported the effects of 4 permitted food dyes, i.e., fast green FCF, indigo carmine, orange G and tartrazine, and the non-permitted dye metanil yellow on chromosomes of *A. cepa* . A significant increase in polyploid cells was observed in all cases. High doses of dyes induced chromosome breaks and MN formation. Although all dyes produced mitotic aberrations, metanil yellow and fast green FCF showed comparatively stronger clastogenic activity.

Blagojević et al. (2009) evaluated melted snow using *A cepa* anaphase–telophase test from Belgrade, the capital city of Serbia. Falling snow collects pollutants from the air

and could cause genotoxicity. Snow samples were collected from two sites, characterized by differences in pollution intensity. At the more polluted site the analyses showed a very high degree of both toxicity and genotoxicity in the first year of the study. At the other site the situation was much better but not without warning signals. The results showed that standard analyses for the presence of certain contaminants in the air do not give an accurate picture of the possible consequences of urban air pollution because the genotoxic potential remains hidden. The *A. cepa* test has been demonstrated to be very convenient for evaluation of air pollution through analyses of melted snow samples.

Glasencnik et al. (2002) assessed the air pollution on the genetic material of *A. cepa* cytogenetic analysis. Roots were exposed at eight sampling plots in the vicinity of major Slovene local emission sources in 1999 and CA's were measured at each sampling plot. No significant differences in the frequency of CA were found among localities in the clay pot experiment. However, under field experiments the significantly differed CA frequencies were observed among sampling plots. Results confirmed that genotoxic agent in air were present. Similarly, Glasenčnik et al. (2004) later exposed *A. cepa* to field conditions at six research plots in the most polluted areas, Slovenia in the vegetation seasons in 1999 and 2000 and MI and CA end points were analysed. Significant differences in the MU and CA's at different sampling plots were observed and the correlation between the ozone concentration and the MI was determined. Based on results, the selected sites were decleared polluted.

Tkalec et al. (2009) measured the effects of exposure to radiofrequency electromagnetic fields (RF-EMFs) on seed germination, primary root growth as well as mitotic activity and mitotic aberrations in root meristematic cells of *A. cepa*. Seeds were exposed for 2 h to EMFs of 400 and 900 MHz at field strengths of 10, 23, 41 and 120 Vm^{-1}. The effect was also measured for longer exposure time (4 h) and field modulation 23 Vm^{-1} . Germination rate and root length did not change significantly after exposure to radiofrequency fields under any of the treatment conditions. At 900 MHz, exposures to EMFs of higher field strengths (41 and 120 V m^{-1}) or to modulated fields showed a significant increase of the MI value, while the percentage of mitotic abnormalities increased after all exposure treatments. On the other hand, at 400 MHz the MI increased only after exposure to modulated EMF. At this frequency, higher numbers of mitotic abnormalities were found after exposure to modulated EMF as well as after exposure to EMFs of higher strengths (41 and 120 V m^{-1}). The types of aberration induced by the EMFs of both frequencies were quite similar, mainly consisting of lagging chromosomes, vagrants, disturbed anaphases and chromosome stickiness. Results showed that non-thermal exposure to the radiofrequency fields can induce mitotic aberrations field frequencies dependent manner as well as field strength and modulation.

Olorunfemi et al. (2012) studied the cytotoxic and genotoxic effects of ballast water using *A. cepa*. *A. cepa* roots were treated with different concentrations (0.5%, 1%, 5%, and 10%) of ballast water for 48 h and analysed for cytological effects. All concentrations induced CA's in the root tip cells along with MI reduction in increasing concentration manner and root growth inhibition with an EC_{50} value of 15% after 72 h was recorded. Authors highlighted that lack of enforced regulations on the exchange of ballast water from oceangoing ships can led to indiscriminate discharge of toxic agent in the environment. Alos (Olorunfemi et al., 2014b) reported DNA damage in *A. cepa* by exposing roots to ballast water at 0.5, 1, 5 and 10% concentrations.

Singh et al. (2014) studied the genotoxic effects of surface water from five sites of Hooghly River in West Bengal, India, along the banks of which many shipbuilding and scrap industries are located using MN and CA's end points in *A. cepa*. *A. cepa* exposed roots showed morphological distortions, MN inductions and CA's. The MI was lower than 50 % in the treated samples and authors concluded that the workers of local shipbuilding and scrap industries, the residents of nearby areas and the aquatic biodiversity are vulnerable to health hazardous if exposed to contaminated water.

In view of application of pyrolysis for biomass conversion to oil, Holan et al. (2014) studied the pyrolysis of oils produced from different sources (poplar, beech and spruce) for any toxic and/or genotoxic effects using *A. cepa* assay. The oils were diluted with water to gain different concentrations and water-soluble fractions of the oils were tested for toxicity. The macroscopic and microscopic analysis of roots, exposed to different concentrations affectd the roots (shape and root length) depending on oil concentrations as well as the MI values were also found variable among treatment. Other abnormalities i.e., damaged dividing cells were in following order poplar-oil > beech-oil and so on. The results indicate that all the three pyrolysis oils exert a toxic, cytotoxic and genotoxic effect. The differences in toxicity and genotoxicity between the oils are probably due to variations in chemical composition, as a result of the different biomass feedstocks used in the pyrolysis oils. *A. cepa* test proved to be a sensitive indicator of toxicity and genotoxicity.

Silva (Silva, 2014) evaluated the toxicity and genotoxicity of different sizes of silica nanoparticles (SiNP) using *A. cepa* along with other bioassays. Specimen were exposed to TM40 (22 nm), HS30 (12 nm), SM30 (7 nm) with concentrations ranging from 0.19 to 163.8 g/L (TM40) and 0.29 to 122.85 g/L (HS30 and SM30), and tested for germination rate, growth and DNA damage. Within each test SiNP present a size dependent chronic toxicity. All particles revealed phytotoxicity in *A. cepa* along with genotoxicity. Author revealed that SiNP size can strongly influence toxicity.

Electronic waste is very common in the environment, and was also investigated for cytogenic effect on *A. cepa* from Iloabuchi electronic market, Diobu, Rivers State, Nigeria (Babatunde and Anabuike, 2015). Roots exposed to concentrations of 5%, 10%, 25%, 50%, and 100% showed root growth inhibition at all concentrations in concentration dependent manner. Various morphological defects of the onion roots were observed including short,

crochet roots, C-tumor roots and severe toxic effects. In the *in vivo* genotoxity assay, all samples lowered the frequency of mitotic cells in concentration dependent along with significant induction of aberrations cells, which were correlated with higher metal concentration in e-waste leachate.

CONCLUSIONS

From literature survey, it is concluded that the watersheds and soil contaminated with industrial wastewater have cytotoxic, genotoxic and mutagenic properties. All kinds of wastes, wastewater, and soils irrigated with wastewater and contaminated river water showed severe effects on genetic material of *A. cepa* from root inhibition to DNA damage. However, chromosomal aberrations were found most common.. Furthermore, *A. cepa* test was found to be sensitive and efficient to detect the toxic agents present in industrial effluents and other contaminated bodies. Thus, *A. cepa* can be used for evaluation of toxicity of industrial wastes as well as other adjoining territories and their results can be used as a warning for biological and ecological systems. Furthermore, there is need to standardize the *A. cepa* test, because factors such as solvent extraction, sampling season, pH, temperature and exposure of *A. cepa* seed to background pollutants may affect the sensitivity of this test. Because every sphere of life is contaminated and the establishment of international centers of stock seed for the production and supply of unexposed seed for experimentation may be useful and results, thus obtained can be used as biomarker to identify toxicological status of the industrial wastewater with assurance of authenticity.

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