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Review Article

Genotoxicity monitoring of industrial wastes using plant bioassays and management through vermitechnology: A review

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ABSTRACT

The main objective of this review was to summarize and present a comprehensive account of the cytotoxic, genotoxic and mutagenic potential of various industrial wastes/sludges using some well-known plant bioassays followed by their bioremediation using vermitechnology. Industries are the main origin of discharges of various types of chemical wastes and are the main causes of environmental degradation. The direct application of industrial sludges could also harm the local biota. The genotoxicity of industrial sludges is assessed using various plant bioassays (for example Allium cepa, Vicia faba) and these bioassays are comparatively more sensitive and cost-effective compared to other in-vitro genotoxicity bioassays. In addition, the materials used for toxicity evaluation are easily available and are being routinely used for the monitoring of environmental pollution. In most studies, the increases in root length and mitotic index, as well as the decrease in chromosomal aberrations in post vermicomposted sludges/wastes indicate that earthworms have the ability to reduce the ecotoxicogenetic effects of sludges/wastes. Post vermicompost is considered an excellent material of a homogenous nature as it has reduced levels of contaminants and holds more nutrients over a longer time without affecting the environment. The biotransformation potential of earthworms and their ability to detoxify most of the heavy metals in industrial sludges is because of their strong metabolic system and the involvement of diverse intestinal microflora and chloragocytic cells that reduce toxic forms to nontoxic forms. This unique ability of earthworms confirms the effectiveness of vermitechnology in reducing the toxicity of industrial wastes.

Introduction

Industrialization is believed to cause all types of pollution problems as the balance in the natural ecosystem is affected by the release of hazardous wastes into the environment, threatening the survival of all living beings (Pondhe et al., 1997). Heavy metals and organic wastes produced in industrial sludges can induce genotoxic effects and are thus harmful to human beings and crops (Jain et al., 2004). Presently in most developing countries, raw and unstabilized sludges are either incinerated or dumped unscientifically in open places and landfills, which disturbs the geochemical cycles and natural environment (Sen and Chandra, 2007). The application of industrial wastes/sludges directly as a fertilizer on agricultural fields can also potentially harm humans and useful organisms in the ecosystem (Sangwan et al., 2010). Therefore, there is a dire need to safely dispose or manage such industrial waste through environment friendly and cost effective technology for remediation. Vermicomposting is one such biotechnology that combines microbial degradation with earthworm activity for faster degradation of wastes over a short time with the best final product (Dominguez and Gomez-Brandon, 2013). A large number of industrial wastes/sludges are vermicomposted and converted into organic fertilizer, including paper mill sludge (Elvira et al., 1998; Kaur et al., 2010), sewage sludge (Dominguez et al., 2000), textile industry waste (Garg and Kaushik, 2005; Garg et al., 2009; Bhat et al., 2013), guar gum mill waste (Suthar, 2006), sugar industrial sludges/wastes (Sen and Chandra, 2007; Sangwan et al., 2010; Bhat et al., 2014, 2015a, 2015b, 2016a), winery and distillery wastes (Romero et al., 2007; Singh et al., 2014a), tannery industry waste (Ravindran et al., 2008; Ravindran and Sekaran, 2011; Vig et al., 2011), olive-mill

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waste (Vivas et al., 2009), beverage mill waste (Singh et al., 2010), agro mill waste (Suthar, 2010) and sewage sludge (Hait and Tare, 2011).

The use of various plant bioassays to evaluate the genotoxicity of industrial pollutants is well known in literature/science (Knasmuller et al., 1998). The genotoxicity of industrial wastes/ sludges has been tested on *Salmonella* strains in most studies (Houk, 1992). Other plant bioassays (*Vicia faba*, *Allium cepa*, *Tradescantia paludosa*, *Hordeum vulgare*, *Nicotiana tabacum* and *Crepis capillaries*) have also been used for genotoxicity evaluation of environmental pollutants in sludge, leachates and in soil (Odeigah et al., 1997; Steinkellner et al., 1998; Cotelle et al., 1999; Cabrera and Rodriguez, 1999; Bhat et al., 2014, 2015b, 2016b; Iqbal and Nisar, 2015). According to many researchers (Grant, 1982; Fiskesjo, 1985; Feng et al., 2007) for cyto-genotoxicity analysis, *A. cepa* and *V. faba* root chromosomal aberration assays are more reliable, sensitive and cost-effective standardized tests. Rank and Nielsen (1997) observed that the *A. cepa* test estimates the genotoxic effects of the tested agent as well as evaluating their clastogenic and aneugenic effects. Many researchers in water screening programs have observed that the *A. cepa* bioassay was helpful for the detection of genotoxic pollutants (Rank and Nielsen, 1998; Cotelle et al., 1999; Moraes and Jorado, 2001). Many researchers have used the *A. cepa* bioassay for the evaluation of genotoxicity in industrial wastes/sludges/effluents (Rank and Nielsen, 1998; Grover and Kaur, 1999; Abdel-Migid et al., 2007; Junior et al., 2007; Bhat et al., 2014, 2015b). Other researchers have used *V. faba* bioassay for genotoxicity evaluation including Chandra et al. (2004), Sang and Li (2004), El Hajjouji et al. (2007), Chiochetta et al. (2014), El Hajjouji et al. (2014) and Cotelle et al. (2015).

Treatment methods on the toxicity reduction and decolorization of industrial effluents have been reported by many researchers using an array of assays (Bilal and Asgher, 2015a, 2015b; Iqbal and Bhatti, 2015; Iqbal et al., 2015; Qureshi et al., 2015; Bilal et al., 2016). Bilal et al. (2016) used immobilized manganese peroxidise (MnP) for the decolorization and detoxification of textile industrial effluent. The cytotoxicity and mutagenicity were evaluated using *Allium cepa*, Heamolytic, Brine shrimp and Ames test and were found to be reliable short-term tools to assess the detoxification efficiency of the MnP method. Qureshi et al. (2015) observed cytotoxicity reduction in textile waste effluent treated with an advanced oxidation process (UV/H_2O_2/TiO_2 system). The *A. cepa* test showed an increase in root count and root length after treatment. The study indicated that the initial effluent was cytotoxic in nature whereas after treatment with the advance oxidation method, the cytotoxicity had decreased considerably, evidencing the role of the process in the detoxification of wastewaters. Anacleto et al. (2017) assessed the cytotoxic, genotoxic and mutagenic potential of sugarcane filter cake (SCFC) on the test system *A. cepa*, before and after biodegradation process of 6 months. The initial samples of SCFC showed genotoxicogenetic effects (cytotoxicity, genotoxicity, mutagenicity), whereas after 6 mth of natural attenuation, the toxicogenic effects were reduced through the possible degradation by the microorganisms present in the SCFC samples.

Vermicomposting is beneficial for industrial sludges as it reduces the sludge toxicity (Bhat et al., 2014). Earthworms have the ability to accumulate heavy metals in polluted soils and specifically, the intestinal microflora and chloragocytic cells of earthworms have the ability to detoxify toxic substances such as heavy metal (Martin and Bullock, 1994; Saxena et al., 1998). Vermicomposting is a complex mechanical, environmentally friendly and biochemical transformation of waste achieved through the action of earthworms and microorganisms to produce fully stabilized organic material with the best physico-chemical parameters (Dominguez and Gomez-Brandon, 2013). The utilization of industrial sludges and organic wastes reduces the production costs and eliminates the need for incineration and landfill disposal (Suthar, 2006). This review article documents the extent and kind of genotoxicity present in various industrial wastes/sludges, its detection methods using various plant bioassays and management through vermitechnology for toxicity reduction.

Allium cepa L. root chromosomal aberration assay in genotoxicity monitoring

Higher plants are considered as an important material for genotoxicity monitoring through evaluation of chromosomal abnormalities and changes in the mitotic cycle, with *A. cepa* (common onion) being considered as an efficient test organism, due to the presence of large-sized chromosomes but in a reduced number with 2n = 16 (Fiskesjo, 1985). Levan (1938, 1945) was the first to introduce this test to examine the effect of colchicines on mitotic spindles and to study the kinds of mitotic aberrations in the root tip cells of *A. cepa*, which were induced by different solutions of organic salts. Its technical modification was developed by Fiskesjo (1985) for the genotoxicity assessment of industrial wastes.

Test organism and procedure

The following procedure is detailed in Bhat et al. (2015b). Healthy and equal-sized onions are chosen from a population of common onion. They are first grown in distilled water and then in various concentrations of test chemicals or industrial sludge extract (Fig. 1). With the help of forceps, the outer scales are carefully removed without disturbing the root primordial. Then, the bulbs are placed in Coplin jars containing the test sample solutions in an incubator at 25 ± 1 °C for different exposure times. After treatment, the root tips (0.5—1 cm) are washed under tap water and are placed in Farmer's fluid (glacial acetic acid and ethanol at a 1:3 ratio) for 24 h. The root tips are hydrolyzed in 1 M HCl for 30 s and then squashed in 1 M HCl and aceto-orcein (1:9) solution after some heating (60 °C) for 2—3 min. Material is kept aside for 20—25 min under cover. The root tips are then immersed in 45% acetic acid and then mounted on a slide with a cover slip, squashed using matchstick to evenly spread the dividing cells and sealed with DPX
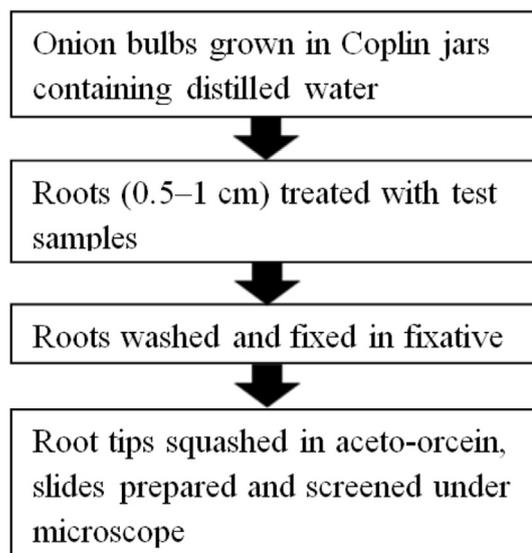

Fig. 1. Schematic representation of *Allium cepa* root chromosomal aberration assay.

Mountant (a mixture of distyrene, a plasticizer (tricresyl phosphate) and xylene). The cells are then scored for their mitotic index and chromosomal aberrations.

The root length test is performed as a 5 d (96 h) treatment test (Rank, 2003). Onion bulbs are put in Coplin jars containing different concentrations of chemicals or industrial sludges. After 96 h exposure, the onions are washed in tap water and then the lengths of roots are measured (Bhat et al., 2015b). Other signs of toxicity such as the presence of twists, swellings, broken root tips, changes in root consistency and color are also examined (Freire et al., 2016).

Genotoxicity and cytotoxicity evaluation

A. cepa root chromosomal aberration assay is an efficient test system for the evaluation of the genotoxic potential of pollutants in the environment (Leme and Marin-Morales, 2009). Nagao (1978) suggested that heavy metals with other chemicals cause carcinogenicity and mutagenicity. Chromosomal aberrations in the *A. cepa* bioassay have been observed by Russel (2002) which characterizes as either a change in total number or chromosomal structure. The various aberrations such as anaphase bridges, breaks and ring chromosomes indicate clastogenic aberrations, whereas chromosome delays, losses, multipolarity, adherence and c-mitosis result from physiological aberrations as shown in Fig. 2 (Bhat et al., 2016b). Some researchers (Dixit and Nerle, 1985) have observed that exposure to various industrial effluents leads to chromosomal abnormalities (bridges) in *A. cepa*. An increase or reduction in the mitotic index (MI) can determine the cytotoxic level of a waste/ sludge (Smaka-Kincl et al., 1996). The MI can be calculated using Equation (1):

$$\text{Mitotic Index} = (\text{Number of dividing cells/Total number of cells}) \times 100 \tag{1}$$

The increase or decrease in MI is an important indicator in environmental monitoring and for the evaluation of toxic substances that have cytotoxic potential (Hoshina, 2002). The genotoxic effects of metal and dye industry leachates using *A. cepa* bioassay have been determined by Chandra et al. (2005); the results confirmed that metal and dye waste leachate contained a maximum content of Ni, Fe and Cr, that induced cytogenetic aberrations. Fernandes et al. (2007) measured the increase or decrease of MI by determining the cytotoxicity level of trifluralin, where MIs
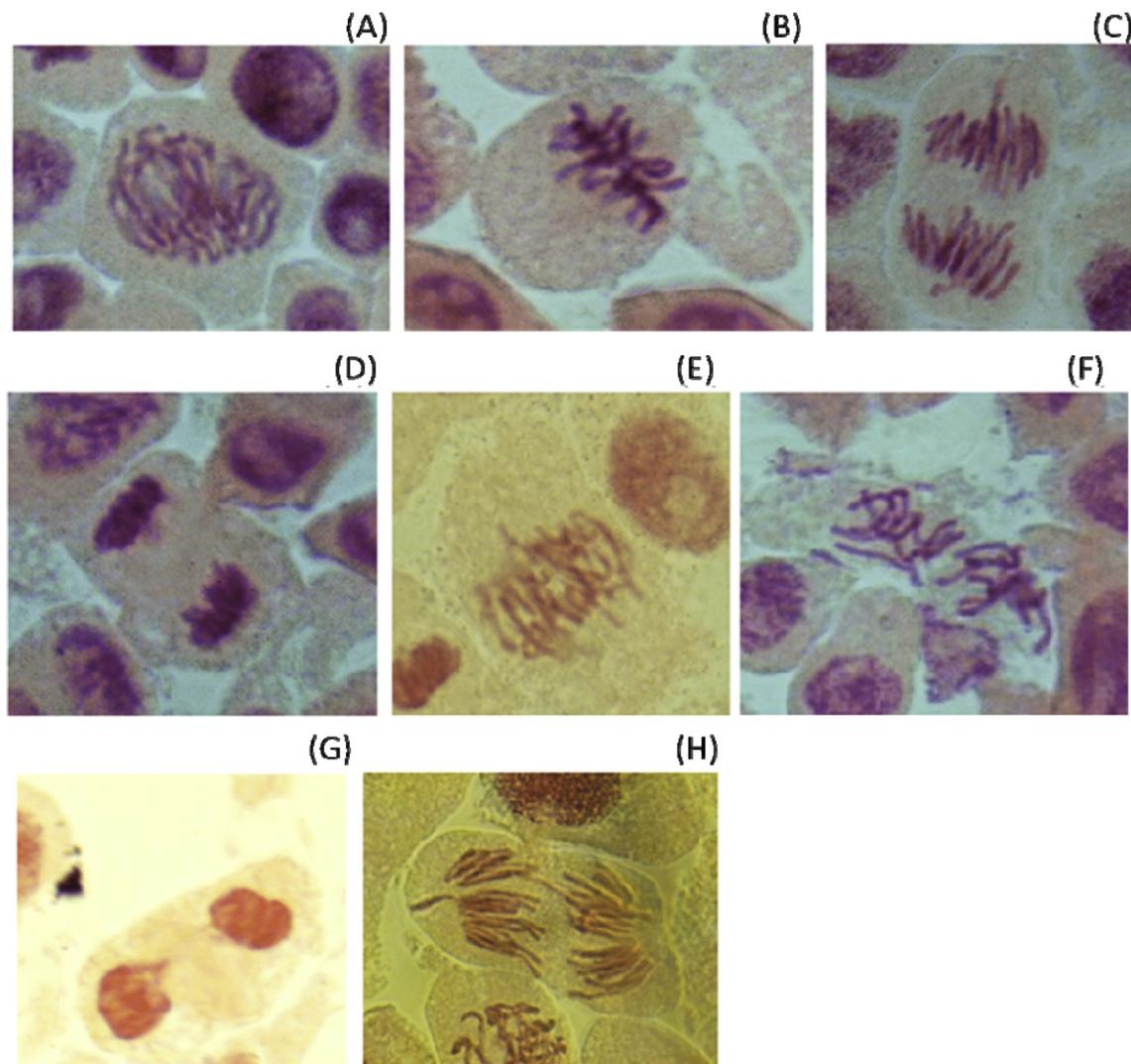

Fig. 2. Root tip cells of *Allium cepa* showing normal stages: (A) prophase; (B) metaphase; (C) anaphase; (D) telophase; and chromosomal aberrations (E) delayed anaphase; (F) c-mitosis; (G) chromosome bridge (H) stickiness.

less than the negative control indicate an alteration of chromosomes due to the chemical action on the exposed organisms, whereas, MIs greater than the negative control indicate an increase in cell division, which can cause disordered cell proliferation and tumor tissue.

Chakraborty et al. (2009) evaluated the genotoxicity of coal fly ash combined with a comet assay in *A. cepa* root tip cells. A 100% concentration of fly-ash on *A. cepa* test inhibited mitotic indices and root growth. The toxicity could have been due to the heavy metal content of fly-ash (Zn, Pb, Cu, Ni, Cd and As). They concluded that the *A. cepa* test combined with a comet assay test can give complete data. Chromosomal aberrations and a micronucleus induced in *A. cepa* by effluent from a petroleum refinery have been studied by Hoshina and Marin-Morales (2009). Their results indicated that final refinery effluent could induce a micronucleus and chromosomal aberrations in the root tip cells of *A. cepa* even after the treatment and could interfere in the quality of river water. The cytogenotoxicity evaluation of paint and textile effluents using the *A. cepa* assay was reported by Samuel et al. (2010) who suggested that the paint and textile effluents in root tip cells of *A. cepa* induced chromosomal aberrations with a maximum 6.93% in paint industrial effluent. The most frequently observed aberrations in this case were bridges, fragments, vagrant and sticky chromosomes.

Induction of aberrations in *A. cepa* root cells exposed to ballast water (collected from a multipurpose vessel carrying a dry cargo) was investigated by Olorunfemi et al. (2012) who concluded that the genotoxic effects of ballast water on the root cells of *A. cepa* may have been due to the observed heavy metals and other physico-chemical parameters in the wastewater. The mitotic index decreased significantly with increasing concentration of ballast water. Mutagents in tannery effluent have been confirmed by Masood and Malik (2013) who suggested this indicated mutagenic hazards in the wastewater. de Souza Pohren et al. (2013) investigated the sensitivity of the *A. cepa* test to evaluate the genotoxic effect of soil exposed to heavy metals using evaluation of genotoxicity by the *Allium* test with the parameters of mutagenicity index, mitotic index, germination index and chromosomal aberrations. The results indicated that the *A. cepa* bioassay was sensitive to the genotoxicity of the soil samples and could be very useful as an alert for an initial screening in biomonitoring.

Fazili and Ahmad (2014) evaluated the genotoxic and phytotoxic potential of mathura refinery wastewater and aligarh wastewater using an *A. cepa* chromosomal aberration assay. The results showed that the *A. cepa* genotoxicity assay of test samples demonstrated a considerable amount of chromosomal damage. Stickiness and stray and clumped chromosomes were the main chromosomal aberrations induced. Monitoring the toxic effects of domestic sewage sludge using the *A. cepa* test has been evaluated by Mazzeo et al. (2015). Their results indicated that the raw sludge induced toxicity, even after 1 yr of natural attenuation, whereas the association of sludge/soil showed no toxicity after 1 yr of natural attenuation. *A. cepa* bioassay has been proved to be an efficient tool to monitor the toxic effects of domestic sewage sludge. For example, Njoku et al. (2015) evaluated the genotoxic effects of industrial effluent from a paint manufacturing company using the root tips cells of *A. cepa*. Their results showed that paint effluent has a genotoxic effect on *A. cepa* root tips and laggards were the main aberrations induced. Genotoxicity monitoring of environmental substances using nucleolar alteration analysis in cells of *A. cepa* has been studied by Mazzeo and Marin-Morales (2015). Their results showed that the samples induced significant frequencies of chromosomal, micronuclei and nuclear aberrations, as observed in cells submitted to conventional chromosomal staining. The use of *A. cepa* root chromosomal aberration assay in various samples is summarized in Table 1.

Root growth inhibition test

Root growth reflects the toxicity in the elongation zone of *Allium cepa*, as root growth inhibition is caused by the occurrence of chromosomal aberrations and a reduction in mitotic activity (Grant, 1982). Samuel et al. (2010) observed a reduction in the root length of *A. cepa* treated with paint and textile industrial effluents. The results confirmed that the textile industrial effluent (EC_{50} 16% 96 h) was more toxic (4.5 times) than the paint industrial effluent (EC_{50} 72% 96 h). There was no root growth in *A. cepa* exposed to concentrations greater than 30% of textile effluent whereas high growth was observed in lower concentrations of paint/textile effluent. Sharma et al. (2012) observed an increase in the length and number of roots exposed to a chloroform extract of *Brassica juncea* (Indian mustard) seed extract compared to the untreated control samples. In *A. cepa*, root growth is due to cell expansion in the root tip of the elongation zone (Rank, 2003). Bhat et al. (2014, 2015b) observed that initial sugar mill sludge decreased the root growth compared to the final feed mixtures. Thus vermin-remediation decreased the toxicity of sugar industrial sludge. Sumitha and Thoppil (2016) observed a root length reduction in *Hyptis* and *Leucas* aqueous plant extracts with increasing treatment concentrations. The authors suggested that the weed extracts contain substances that impair cell expansion and differentiation in the root tip of *A. cepa*.

Vicia faba L. bioassay in genotoxicity monitoring

V. faba (broad bean) bioassay is used extensively to evaluate toxic substances in the environment and is an excellent bioassay for toxicological observations (Iqbal, 2016). *V. faba* is more sensitive and economical to use compared to other bioassays (Feng et al., 2007). The chromosomes are large enough for scoring chromosome aberrations and their number in *V. faba* is low (2n = 12) (Kihlman, 1975).

Procedure for Vicia faba bioassay

The protocol for the standardization of *V. faba* bioassay has been reported by many researchers around the world (Kanaya et al., 1994; Souguir et al., 2008; Adam and El-Ashry, 2010). In brief, seeds of *V. faba* are purchased and soaked for 10 h in tap water at room temperature (Fig. 3). The *V. faba* is germinated on a moist cotton in a BOD incubator for 4—5 d at 22 ± 1 °C. When the primary roots are 2—4 cm long, the tips are cut off to increase the growth of lateral roots. Seedlings are then moved to different industrial wastes/effluents/chemicals with different exposure times. The root tips are then fixed in Farmer's fluid (glacial acetic acid and ethanol at a 1:3 ratio) for 24 h. As described earlier in Test organism and procedure, the same procedure with *A cepa* is followed after fixation of the root tips.

Genotoxicity and cytotoxicity evaluation

Chromosomal aberrations induced by industrial wastes/sludges have been studied using *V. faba* bioassay. In evaluation using *V. faba* bioassay, chromosome aberration (CA) and mitotic indices (MI) are used to determine genotoxicity, and nuclear aberration (NA) and micronuclei (MN) are used for cytotoxicity and mutagenicity, respectively (Kristen, 1997; Jing-Jing, 2011). Chandra et al. (2004) employed *V. faba* roots to study the genotoxicity of tannery industry waste leachates. The roots were treated for 5 d with 2.5%, 5% or 10% concentrations of tannery waste leachate. The results indicated the minimum MI was in exposed roots, and chromosome aberrations were also higher in roots exposed to solid tannery

Table 1
Summary of use of *Allium cepa* L. root chromosomal aberration assay for genotoxicity assessment in different samples.

| Sample number	Tested agents/samples used	Methods used	Genotoxicity/cytotoxicity studies	References
Solid samples				
1	Sugarcane filter cake (SCFC)	Genotoxicogenetic assays (cytotoxicity, genotoxicity, mutagenicity)	- Higher concentrations of SCFC without biodegradation induced cytotoxic, genotoxic and mutagenic potentials. Whereas after 6 mth of natural attenuation, the genotoxicogenetic effects were decreased.	Anacleto et al. (2017)
2	Coal fly ash	Chromosome aberration and micronucleus assay	- Mitotic index was decreased with increasing concentrations of fly ash - A dose-dependent increase in the percentage of chromosome aberrations (breaks, sticky bridges) was observed at the highest concentration of fly ash	Jana et al. (2017)
3	Bagasse waste	Root growth inhibition and genotoxicity bioassay	- Nuclear abnormalities of binucleate cells were high at concentrations of 1:8 and 1:4 (fly ash:water (weight per volume). - Root length and mitotic index were increased in the final vermicompost extract. - Chromosomal aberrations (delayed anaphase, c-mitosis, laggards, vagrants, stickiness, chromosomal bridges and breaks) also decreased in the final vermicompost compared to initial waste.	Bhat et al. (2016b)
4	Domestic sewage sludge	Chromosomal aberrations and micronuclei assays	- Natural attenuation proved to be sufficient to decrease the toxicity of the sludge. - Chromosomal aberrations (losses, bridges, delays, adherence), nuclear abnormalities (budding, binucleated cells, lobulated nuclei) - Cytotoxicity through mitotic index, mutagenic potential through presence of micronuclei in meristematic and F1 cells decreased after natural attenuation (12 mth) of sewage sludge.	Mazzeo et al. (2015)
5	Sugar beet mud	Root growth inhibition and genotoxicity bioassay	- Root growth and mitotic index increased in final vermicompost - Chromosomal aberrations (delayed anaphase, c-mitosis, laggards, vagrants, stickiness, chromosomal bridges and breaks) decreased in final vermicompost.	Bhat et al. (2015b)
6	Pressmud sludge	Root growth inhibition and genotoxicity bioassay	- Root growth test, mitotic index increased whereas chromosomal aberrations (delayed anaphase, c-mitosis, laggards, vagrants, stickiness, chromosomal bridges and breaks) decreased after vermicomposting.	Bhat et al. (2014)
7	Sewage sludge and vinasse	Cytotoxicity, genotoxicity and mutagenicity	- Bioprocessing of residues by diplopods reduced the toxicity of sewage sludge and vinasse - Cytotoxic, genotoxic and mutagenic effects in meristematic cells of *Allium cepa* were observed in initial waste. - The genotoxic as well as the mutagenic effects of sewage sludge were significantly reduced after 30 d treatment with diplopods.	Christofoletti et al. (2013)
8	Soil contaminated with heavy metals	Genotoxicity and mutagenicity index	- Chromosomal aberrations (c-metaphase, loss chromosome, multipolar anaphase, chromosomal bridge, micronuclei, and chromosomal breakage) observed after exposure. - Cytotoxic activity from the mitotic index.	de Souza Pohren et al. (2013)
9	Electronic waste leachate	Cytogenetic and root length inhibition analysis	- Morphological modifications and cytological aberrations (bridge, stickiness, binucleate cells) were induced by electronic waste leachate. - Inhibition of root growth and cell proliferation.	Bakare et al. (2012)
10	Cement dust	Cytotoxicity and mutagenicity	- Chromosomal aberrations observed in the root tip cells were stickiness, c-mitosis, bridge, fragmentation, vagrant, bi-nucleus chromosomes and multi-polar anaphase. - Chromosomal aberrations increased significantly with the length of exposure of cement dust.	Yahaya et al. (2012)
11	Soil samples contaminated with effluents of zinc coating and copper sulphate industry	Genotoxicity/mutagenicity	- The samples induced chromosomal aberrations (delayed anaphase, c-mitosis, laggards, vagrants, stickiness, chromosomal bridges and breaks) with 0.38–4.83% for soil samples.	Katnoria et al. (2011)

Table 1 (continued)

Sample number	Tested agents/samples used	Methods used	Genotoxicity/cytotoxicity studies	References
12	Coal fly ash	Phytotoxicity, genotoxicity, cytotoxicity, comet assay	- Fly ash inhibited root growth, decreased divisional frequency and increased binucleated cell formation. - Exposure of fly ash increased the percentage of tail DNA, tail length and olive tail moment.	Chakraborty et al. (2009)
13	Municipal sludge	Morphological, phytotoxic and genotoxic studies	- Morphological studies showed coiled and wavy roots. - Mitotic index increased in the vermicompost leachate. - Chromosomal aberrations, micronucleus formation and binucleate cells were observed in initial leacheate, whereas they were reduced after vermicomposting.	Srivastava et al. (2005)
14	Leachates from metal and dye industry	Heavy metal content, genotoxicity	- Leachates contained high amounts of chromium, nickel and iron that induced cytogenetic alterations. - Inhibition of mitotic index, induction of chromosomal aberrations and micronuclei formation were also present.	Chandra et al. (2005)
Liquid samples				
1	Sugarcane vinasse	Chromosomal aberrations and micronucleus induction	- Sugar cane vinasse showed genotoxic effect. - Chromosomal aberrations induced by sugarcane vinasse were nuclear buds, anaphasic bridges, micronucleus, chromosomal loss and chromosomal break. Presence of micronucleus in sugarcane vinasse indicates the mutagenic potential of the waste.	Garcia et al. (2017)
2	Silk dyeing industry effluent	Root growth inhibition, cytotoxicity, genotoxicity studies	- Results of root growth test showed strong root growth retardation capability and root growth decreased significantly with increasing concentration of effluent. - Mitotic index rapidly decreased with increasing concentrations of dye effluent showing the cytotoxic nature of the silk dye. - Different chromosomal abnormalities and chromosome disintegration were observed in all concentrations of silk dye effluent.	Rahman et al. (2017)
3	Hyptis suaveolens and Leucas indica aqueous leaf extracts	Root growth inhibition, cytotoxicity	- Reduction in root length mitotic index observed in both Hyptis and Leucas extracts. - Chromosomal aberrations (ball metaphase, binucleate cell, bridge at anaphase, chromosome laggards and vagrant, nuclear lesion) observed in both the extracts.	Sumitha and Thoppil (2016)
4	Paint effluent	Genotoxicity studies	- Mitotic index decreased with increasing concentrations of the effluent. - Chromosomal aberrations (laggard, bridged, multipolar, vagrant, stickiness, c-metaphase, binucleate and spindle) were observed in the effluent, thus indicating the cytotoxic potential of paint effluent.	Njoku et al. (2015)
5	Shipyard contaminants (surface water)	Genotoxicity studies (micronuclei assay, DNA laddering assay)	- Shipyard contaminants induced morphological distortions, formation of micronuclei and chromosomal aberrations (disturbed anaphase, stickiness, bridges, fragmented metaphase, unipolar anaphase, binucleate) in A. cepa. - Damage to genomic DNA of calf thymus.	Singh et al. (2014b)
6	Tannery effluent	Genotoxicity/mutagenicity	- Cytological parameters such as mitotic index were lower in effluent. - Numbers of chromosome abnormalities (c-mitosis, anaphase bridges, laggards, stickiness, binucleated cells) were higher. - Study confirmed the origin of mutagens in the effluent.	Masood and Malik (2013)
7	Tannery effluent and chromium	Phytotoxicity/genotoxicity	- Total chlorophyll and protein content decreased significantly. - Root length and mitotic index also reduced. - Chromosomal aberrations (break, fragments, c-metaphase, stickiness, multipolar, bridge, laggard) and micronuclei were also observed.	Gupta et al. (2012)
8	Paint and textile industrial effluent	Root growth inhibition and genotoxicity bioassay	- Root growth and mitotic index decreased with increasing concentrations of industrial effluents. - Chromosomal aberrations such as vagrant, bridges, fragments and sticky chromosomes were most frequently observed.	Samuel et al. (2010)

Table 1 (*continued*)

Sample number	Tested agents/samples used	Methods used	Genotoxicity/cytotoxicity studies	References
9	Hospital effluents	Genotoxicity	- Chromosomal disruptions, anaphasic bridges, and micronuclei were observed in *A. cepa* exposed to hospital effluent.	Bagatini et al. (2009)
10	Industrial effluents contaminated with azo dyes	Cytotoxicity, genotoxicity/ mutagenicity	- Effluents induced chromosomal and nuclear aberrations (bridges, laggards, c-metaphases, loss of chromosomes, and binucleated cells). - Study revealed a mutagenic effect of the industrial effluent at concentrations of 10%, whereas lower concentrations did not induce mutagenic alterations in the *A. cepa*.	Carita and Marin-Morales (2008)
11	Industrial wastewaters	Genotoxicity/mutagenicity	- Reduced mitotic index. - Chromosomal aberrations (fragments, stickiness, laggards, bridges, micronuclei) were observed in the *A. cepa* root tip cells. - Genotoxicity of the industrial wastewaters may have been largely due to the presence of pesticides or heavy metals.	Fatima and Ahmad (2006)
12	Silk dyeing industry effluent	Cytotoxicity, genotoxicity.	- Mitotic index decreased with increase in effluent duration and concentration. - Silk and dyeing industry effluent also induced chromosomal abnormalities (stickiness, fragments, bridges, laggards, binucleate cells, vacuolated nuclei). - The study revealed that the industrial effluents acted as potential mutagens.	Sudhakar et al. (2001)
13	Copper mine waste	Cytotoxicity, genotoxicity.	- Inhibition of mitotic index was observed after exposure to copper mine waste - Waste induced chromosomal aberrations (bridges, breaks, scattered chromosomes, laggards, stickiness).	Inceer et al. (2000)
14	Sewage and industrial effluents of textile dyeing mill and paper mill	Micronucleus and aberration bioassay	- Effluents induced both anaphase aberrations (acentric fragments, dicentric chromosomes, chromatin bridges) and micronuclei, suggesting that these pollutants are genotoxic.	Grover and Kaur (1999)
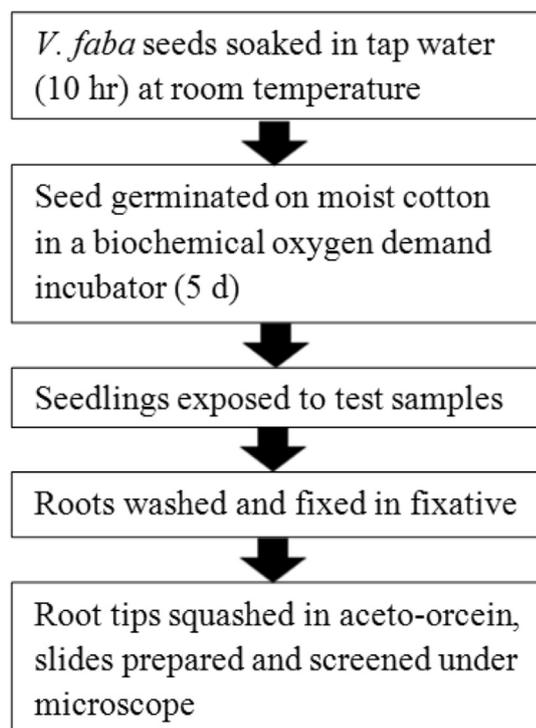
Fig. 3. Schematic representation of *Vicia faba* root chromosomal aberration assay.

waste. Sang and Li (2004) investigated the genotoxicity potential of (municipal) landfill leachate by employing *V. faba* bioassay. The landfill leachates treated in different seasons produced different MN induction and MI values. The values of MI, MN, CA increased clearly with increasing concentrations of leachates. Feng et al. (2007) studied metals (Al, Ba, Co, Cd, Cr, Cs, Cu, Fe, Hg, Mo, Mn, Pb, Sr, Se and Zn) in municipal waste leachate by employing *V. faba* bioassay. The results revealed that the roots exposed with leachate increased MN induction at higher concentrations, which revealed the genotoxic potential of the municipal leachate. The authors suggested *V. faba* bioassay for monitoring wastes prior to their disposal.

The genotoxic nature of olive mill effluent using the micronuclei test in *V. faba* root tip cells has been investigated by El Hajjouji et al. (2007). The results revealed that 10% of olive mill effluent induced micronuclei induction and was correlated with phenolic compounds present in the olive mill effluent. In another experiment, by ZhiGang and QiaoGu (2009), the genetic toxicity of heavy metal ions (Cd^{2+}, Cr^{3+} and Pb^{2+}) was used to study the MN and MI assays. The results indicated that MN aberrations were induced by heavy metal ions with the increase in concentration; the maximum MI value of the heavy metal ions occurred at lower concentrations and the minimum was at higher concentrations.

The genotoxic potential of coke plant effluent employing *V. faba* was also studied by Dong and Zhang (2010). The authors recorded MN induction, sister chromatid exchange (SCE) formation and MI inhibition and suggested *V. faba* bioassay for genotoxicity monitoring of coke plant effluent. Chandra and Singh (2012) observed the toxicity of raw and treated pulp and paper industry effluent

using *V. faba* bioassay. The results showed that the untreated pulp and paper industry effluent was toxic, whereas in biologically treated water, there was a 40% reduction in toxicity. The study revealed that *V. faba* bioassay could be used to evaluate the contamination load and detoxification of pulp and paper mill effluents. Chiochetta et al. (2014) studied the toxicological features of fresh and stabilized agro-industrial sludge leachates employing *V. faba* bioassay. The results revealed that the phytogenotoxic potential of the leachates generated maximum genotoxic effects in less diluted leachate concentrations compared to the negative control. The mitotic index also decreased in higher concentrations of leachates which indicated the cytotoxic nature of stabilized and fresh leachates. The *V. faba* bioassay was proven to be a cost effective, reliable, reproducible and short term bioassay. Thus *V. faba* bioassay was shown to be useful for genotoxicity evaluation of all types of wastes/sludges/effluents and the use of this bioassay in various solid and liquid samples is summarized in Table 2.

Vermitechnology and its importance

Earthworms growth in organic material is called vermiculture and the bioconversion of organic materials into organic manure by earthworms is known as vermicomposting (Dominguez et al., 2000). Vermicomposting is an environmentally-friendly process used to treat organic waste in the presence of earthworms and microorganisms (Suthar, 2006). Vermicompost is one of the highest-grade and most nutrient-rich natural organic fertilizers in the world and has shown to have several positive impacts on plant growth and health (Gajalakshmi and Abbasi, 2002). Vermicomposting is a suitable means of transforming biological wastes (animal, agro-wastes) into nutrient rich organic manure (Nath and Singh, 2016). Vermicompost contains highly enriched nutrients (nitrogen, potassium, phosphorus) and this process gradually makes them easily available to plants (Atiyeh et al., 2001). It contains an above-average number of micro-organisms which revive the soil and provides essential elements in available form by plants (Ndegwa and Thompson, 2001). Thus, vermicompost is increasingly used in agriculture and horticulture as a promising alternative to inorganic fertilizers (Wang et al., 2010). Earthworms act as mechanical blenders, as they modify the physico-chemical characteristics of organic waste, gradually reducing its total organic carbon and C:N ratio, and consequently, the soil retains more available nutrients and further mineralization of organic waste (Bhat et al., 2013, 2016c). Several earthworm species-*Eisenia fetida*, *E. andrei*, *Eudrilus eugeniae*, *Perionyx excavatus* and *P. sansibaricus*—have been identified as potential candidates to degrade industrial, urban and agricultural wastes (Wong and Griffiths, 1991; Suthar, 2007; Sonowal et al., 2014). Vermicomposts contain higher populations of bacteria, fungi and actinomycetes compared to traditional composts (Nair et al., 1997). Humic acids derived from final vermicompost increase the number of plant roots, giving the plant improved capability to scavenge nutrients from the soil for development and growth (Alvarez and Grigera, 2005). Tomati et al. (1995) observed that vermicompost contains growth hormones (auxins, cytokinins and gibberellins) secreted by worms. Vermicompost has a maximum water holding capacity and the humic and fulvic acids are important to plants for proper growth (Muscolo et al., 1999). The evaluation of vermicompost maturity is necessary before it can be used for agricultural practices. For example, changes in the physico-chemical (organic carbon, C:N ratio) parameters of vermicompost are also indicative of vermicompost stability and maturity (Bhat et al., 2017a). Recently Bhat et al. (2017b), described earthworms and their associated microorganisms as waste managers and biofertilizer producers in utilizing and changing the physico-chemical properties of various organic wastes. The authors suggested that vermicompost

application can significantly improve plant growth and the fertility of agricultural soil; the final vermicompost produced from various organic wastes was higher in available nutrients like N, P, K. So vermicomposting and vermiculture technology are an economically sound, environmentally safe technology for organic waste degradation and can create employment opportunities for weaker sections of society. In India, large amounts of organic solid waste are available that could produce millions of tons of vermicompost that could reduce the use of toxic chemical fertilizers (Bhat et al., 2017c).

Reduction in genotoxicity of industrial wastes/sludges through vermicomposting

The most beneficial effect of vermicomposting is the reduction in genotoxicity. For example, Fischer and Koszorus (1992) observed that in *E. fetida*, mitochondrial and cytoplasmic fractions can convert highly toxic forms of heavy metals to nontoxic forms. Jain et al. (2004) successfully converted fly ash into vermicompost. The results suggested that *E. fetida* accumulates heavy metals and also reduces Cr (VI) to Cr (III). Thus vermicomposting is an important bioconversion process for heavy metal reduction in fly ash. Active and closed municipal waste landfill leachate contaminates surface water and groundwater (Hancock et al., 1995; Flyhammer, 1997; Ding et al., 2001). Vermicomposting and genotoxicity evaluation of municipal sludge has been studied by Srivastava et al. (2005). The authors suggested that vermiconversion reduces the phytotoxicity and genotoxicity of municipal sludge. The results revealed that the MI reduction is concentration dependent in genotoxic studies and the control value of 11.76 decreased to 5.40 at 10% municipal sludge leachate whereas the MI was maximized (9.48) using 10% vermicomposted sludge leachate. Oleszczuk (2008) studied the influence of composting on municipal sewage sludge phytotoxicity from heavy metals using the physico-chemical properties and the polycyclic aromatic content. The wastes were composted for 76 d. The study showed that sludge composting limited the negative influence of most of the phytotoxicity parameters. Sarojini et al. (2010) investigated the potential of vermicomposting to reduce the genotoxic effects of coal power plant flyash in the common onion (*A. cepa*). The authors reported that the morphological studies of *A. cepa* roots showed coiled and wavy roots after initial exposure to fly ash, whereas after vermicomposting no root disorders were reported. MI also increased in vermicomposted fly ash. The study concluded that the vermicomposting of fly ash reduces its toxic nature and can be used for agricultural purposes without any ill effects. According to Jordao et al. (2011) vermicompost is able to bioremediate soils containing metallic species and this ability also extended to other organic substrates (Kavamura and Esposito, 2010). A phytotoxicity change of sewage sludge-amended soils has been reported by Oleszczuk et al. (2012). The phytotoxicity of soils mixed with the sewage sludges was lowest in the final sludge compared to the beginning of the experiment. Bhat et al. (2014) reported that vermicomposting reduced the genotoxicity of pressmud sludge as shown by the genotoxicity results. The initial greatest (30.8%) after treatment with 100% pressmud sludge, whereas it was least (20.3%) after vermicomposting with *E. fetida*. The authors found that the chloragocytic cells of earthworms and microbiota have the ability to detoxify the metals. The study concluded that vermicomposting is an efficient method of toxicity reduction in pressmud sludge and can play a major role in industrial solid waste remediation. Bhat et al. (2015b) also observed that vermicompost reduces the genotoxicity of sugar beet mud. The genotoxicity analysis of final vermicompost mixtures of sugar beet mud indicated an 18–75% decline in chromosome aberrations. The results revealed that vermicomposting decreases the genotoxic potential of sugar beet mud

Table 2
Summary of use of *Vicia faba* L. bioassay for genotoxicity assessment in different samples.

Sample number	Tested agents/samples used	Methods used	Genotoxicity/cytotoxicity studies	Reference
Solid samples				
1	Diesel exhaust particulate matter	Micronucleus test	- Genotoxicity of diesel particulate matter may have been due to the content of metals and organic compounds in it. - Exposure of this particulate matter significantly increased the frequency of micronuclei and decreased the mitotic index.	Correa et al. (2016)
2	Cr (VI) from activated sewage sludge	Micronucleus test	- Micronucleus frequency was higher in compost aqueous extracts, than in the direct contact. - After 6 month of co-composting, the micronucleus rate decreased significantly with decreasing concentration of Cr (VI). - Mitotic index increased significantly after co-composting.	Loubna et al. (2015)
3	Fresh and stabilized agro-industrial organic sludge leachates	Micronucleus number and mitotic index	- The phytogenotoxic potential of the leachates generated maximum genotoxic effects in less diluted leachate solutions (75% and 100% stabilized and fresh leachate) compared to the negative control. The mitotic index also decreased at higher concentrations of leachates.	Chiochetta et al. (2014)
4	Municipal waste leachate incineration bottom ash	Micronucleus assay	- Roots exposed with municipal waste leachate increased micronuclei inductions at higher concentration, indicating the genotoxic nature of the municipal leachate. - Increase in heavy metal concentration in the leachates, increased the toxic effects on *Vicia faba* root cells.	Feng et al. (2007)
5	Tannery waste leachates	Cytotoxicity, genotoxicity	- Chemical analysis revealed that chromium and nickel in the tannery waste leachate may have caused genetic abnormalities. - The mitotic index value decreased in exposed roots. - Chromosome aberrations (fragments, breaks, stickiness, laggards, bridges, multipolar) were also higher in roots exposed with soild tannery waste. - The frequency of aberrations was higher through the aqueous medium than those exposed through the soil medium.	Chandra et al. (2004)
6	Municipal landfill leachate	Cytogenetic bioassay (mitotic index, micronucleus, anaphase aberration)	- The values of mitotic index, micronuclei and chromosome aberrations increased greatly with increasing concentrations of leachates. - Mitotic index reduction of 82% and 61% observed for the highest concentration of landfill leachate. - Chromosomal aberrations (fragments, gaps, laggards, bridges, stickiness) increased significantly after exposure to landfill leachate.	Sang and Li (2004)
7	Fly ash	Cytogenetic assay	- Cytogenetic examinations of root meristems of *Vicia faba* exposed to fly ash mixtures showed significant inhibition of mitotic index, induction of chromosome aberrations and increased frequency of mitotic aberrations. - Chemical analysis of initial fly ash revealed high concentrations of heavy metals (Cr, Cu, Pb, Zn, Ni). - Cytogenetic analysis of post-vermicomposted mixtures of fly ash revealed a 15–45% decline in the chromosome aberrations as well as 10–50% decline in the heavy metal concentrations.	Jain et al. (2004)
Liquid samples				
1	Semi-coking wastewater	Genotoxicity evaluation (micronuclei)	- Micronuclei frequencies in *Vicia faba* root tip cells were induced by wastewater samples. - Results of genotoxicity assessment showed that the effluent from coagulating sedimentation units of semi-coking wastewater had significant mutagenic properties.	Liu et al. (2017)
2	Pulp and paper mill effluent	Seed germination test	- The untreated effluent was found to be toxic, whereas, in biologically treated water, there was 40% reduction in toxicity. - The seed germination test on *V. faba* showed the reduction of inhibitory compounds present in the effluent.	Chandra and Singh (2012)

Table 2 (*continued*)

Sample number	Tested agents/samples used	Methods used	Genotoxicity/cytotoxicity studies	Reference
3	Urban surface waters	Cytotoxicity, genotoxicity	- Polluted rivers showed high cytotoxicity and genotoxicity (expressed as RMCN, the relative frequency of micronucleus). - The ecotoxicity of the organic toxicants indicated that the cytotoxic and genotoxic effects were related to the pollutant source of rivers. - RMCN was also correlated to the mitotic index reduction rate.	Ma et al. (2012)
4	Textile effluents	Mutagenicity and phytotoxicity tests	- The textile raw effluent showed maximum cytogenetic and mutagenic effects whereas biologically treated effluents showed minimum toxicity, suggesting the efficacy of biological treatment to reduce the toxicity of textile effluent. - Mitotic index was reduced from 19.58% to 3.82% of root tips grown in textile effluent. - Micronuclei were also increased from 0.46% of the control to 6.56% of the effluent (3 d treatment) and from 0.88% to 5.92% (7 d treatment).	Giorgetti et al. (2011)
5	Coking wastewater	Cytotoxicity, micronucleus	- Authors reported micronuclei induction, sister chromatid exchange formation and mitotic index inhibition. - There was an 85.31% and 82.75% reduction of mitotic index was observed in *V. faba* and *Hordeum vulgare* root tips, respectively, after exposure with highest concentration of coking wastewater. - A 2% concentration of coking waste water induced significant increases in micronucleus frequency (24 h treatment) and the frequency increased significantly with incremental coking wastewater concentrations.	Dong and Zhang (2010)
6	Petroleum refinery effluent	Cytological analysis (germination percentage, root length, weight gain, micronucleus)	- The MI value was low in roots exposed with petroleum refinery effluent. - Root length and weight were also affected in seedlings of *V. faba*. - Micronucleus significantly increased in all the seeds exposed to petroleum refinery effluent. - Study concluded that *Ginkgo biloba* supplementation may decrease toxic damages induced by petroleum effluent.	Cavusoglu et al. (2010)
7	Coke plant wastewater	Cytogenetic bioassay (mitotic index, micronucleus, anaphase aberration)	- Exposure of coke plant wastewater decreased the mitotic index and increased the micronuclei and chromosome aberrations. - Mitotic index reduction of 36%, 42%, and 54% observed for the highest concentration treated for 24, 48, and 72 h, respectively, in root tip cells of *V. faba*. - Chromosomal aberrations (fragments, breaks, lagging chromosomes) increased with increasing concentration and treatment time.	Liu and Lu (2009)
8	Olive mill effluent	Micronuclei test	- Raw olive mill effluent induced significant micronuclei formation. - Genotoxicity of olive mill effluent decreased after aerobic treatment (45 d).	El Hajjouji et al. (2007)

on final products. Genotoxicity and cytotoxicity evaluation of olive mill waste compost was studied by Chowdhury et al. (2015). Their results revealed that all initial mixtures of composts presented cytotoxic effects whereas the majority of mature composts did not have any genotoxic and cytotoxic effects. The genotoxic effects of pre and post vermicompost bagasse waste were analyzed on the root tips cells of *Allium cepa* by Bhat et al. (2016b). Genotoxicity evaluation of post vermicompost mixtures of bagasse waste revealed a 21—44% reduction in the chromosome aberrations compared to initial bagasse waste, with the maximum reduction (44.50%) in the 75% waste mixture. In the final vermicompost mixtures of bagasse waste, root length and mitotic index also increased. A decrease in chromosomal aberrations as well as an increase in the root length and mitotic index in the final vermi-compost mixtures of bagasse waste indicated that earthworms

have the capability to reduce the genotoxicity of bagasse waste and the final product could be used safely in agricultural fields.

Most of the results demonstrated that the bioconversion of the industrial waste by earthworms is a simple and low cost technique for the biosafe disposal and breakdown of complex chemicals in sludges/wastes to non toxic forms. However, these wastes need to be premixed with cattle dung in the ratios of 50:50 or less (Bhat et al., 2016b). Co-composting with cattle dung helps to improve physico-chemical characteristics of organic wastes (Bhat et al., 2015a).

From the analysis of the results reported in this review, it can be concluded that plant test models (*A. cepa* and *V. faba*) are effective and appropriate assays for measuring the cytotoxicity/genotoxicity/ mutagenicity of wastes/sludges before and after the application of remediation technologies. The use or dumping of industrial wastes/

sludges in agricultural soils without proper treatment may contaminate the soil and environment. Vermitechnology is a useful technique to minimize the genotoxicity of industrial wastes/sludges as evaluated by the results of many authors in this review. The bioconversion potential of earthworms and their ability to detoxify most of the heavy metals in industrial sludges is due to their strong metabolic system and the involvement of diverse intestinal microflora, enzymes and chloragocytic cells that reduce toxic forms to nontoxic forms. The remediation of even larger quantities of industrial wastes would offer even greater reductions in cytotoxicity and genotoxicity. From the results of the studies reviewed here organic wastes/sludges mixed with other organic substrates (cattle dung) can be recommended for fertilizing after vermicomposting for 3–4 mth. The final vermicompost produced from organic wastes/sludges was non toxic and reduced the cytotoxic and genotoxic effects in the meristematic cells of *A. cepa*. Various studies suggested valorization and detoxification of industrial wastes/sludges using earthworms and the use of plant bioassays as sensitive and cost effective tests for genotoxicity monitoring. The earthworms have the ability to detoxify heavy metals in industrial wastes/sludges, because of their strong metabolic system that reduce toxic forms to nontoxic forms. This ability of earthworms confirms the effectiveness of vermitechnology in reducing the genotoxicity of industrial sludges.

Competing interests

The authors declare that they have no competing interests.

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