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Effect of landfill leachate on cell cycle, micronucleus, and sister chromatid exchange in *Triticum aestivum*

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Abstract

With increasing use of municipal solid waste landfills for waste disposal, the leachate generated has become a serious environmental concern. Therefore, it is important to set up simple and accurate methods for monitoring leachate toxicity. In the present study, the physiological and genetic toxicity of the leachate, generated from Xingou Municipal Landfill in China, were investigated with *Triticum aestivum* (wheat) bioassay. The results indicate that the lower leachate concentrations stimulated the germination, growth and cell division, and did not induce obvious increase in micronucleus (MN) frequency in root tips; while the higher concentrations inhibited the processes, and significantly augmented the MN frequency in a concentration- and time-dependent manner. In addition, pycnotic cells (PNC) and sister chromatid exchange (SCE) occurred in root tips at all leachate concentrations tested, and the frequencies had positive relation with the treatment concentration and time. The results imply that components of leachate from the landfill may be genotoxic in plant cells, and exposure to leachate in the aquatic environment may pose a potential genotoxic risk to organisms. The results also suggest that the wheat bioassay is efficient, simple and reproducible in monitoring genotoxicity of the leachate.

Keywords: Municipal landfill leachate; *Triticum aestivum*; Mitotic index (MI); Micronucleus (MN); Sister chromatid exchange (SCE)

1. Introduction

The use of municipal solid waste (MSW) landfills is the most widely utilized method of solid waste disposal around the world. In China, landfill and/or open dumping of MSW is very common. The main pollution issues associated with landfill sites are the production of potentially explosive gases and liquid leachate. Among these, leachate emissions from landfill sites are of growing concern, primarily due to their toxic impact when released unchecked into the environment, and the potential for landfill sites to generate leachate for many hundreds of years following closure [1]. Landfill leachate, mainly generated due to the penetration of precipitation through the waste, was characterized by its high concentrations of organic matter and included toxic and carcinogenic chemicals [2]. It is reported that small amount of landfill leachate can pollute large volume of groundwater, rendering them unusable for domestic and many other purpose [3]. Therefore, more attention has been paid to research on toxicity of landfill leachate.

The genetic toxicity of landfill leachate has been shown using a battery of microbial bioassays, including the Salmonella/microsome mutagenicity bioassay (Ames test), the *Bacillus subtilis* DNA repair bioassay, and the diploid *Aspergillus nidulans* chromosome damage bioassay [2]. Aquatic animal assay using goldfish (*Carassius auratus*) also indicates that raw landfill leachate induced DNA damage in peripheral erythrocytes, and increased the frequency of micronucleus (MN) in gill cells [4]. Investigation on mammals shows that both municipal landfill leachate and industrial solid waste leachate could cause the increase of MN and chromosomal aberration (CA) frequency, and contribute to the formation of DNA damage in mouse bone marrow and blood cells *in vivo* [5–7], and may be responsible for the DNA damage in human peripheral blood lymphocytes [8]. The cytogenetic abnormalities and DNA damage induced by landfill leachate implicate that humans consuming leachate contaminated water are at increased risk of

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developing adverse health consequences. Consequently, it has become important to monitor the potential toxicity of landfill leachate.

Higher plant toxicity assays, including *Allium cepa*, *Vicia faba*, *Arabidopsis thaliana*, *Hordeum vulgate*, *Triticum aestivum* and *Tradescantia*, etc., have been calibrated with a broad range of chemicals [9–13]. Since these plant systems have unique advantages for *in vivo* monitoring and screening [9–11], it is recommended that high plant systems be accepted by regulatory authorities as an alternative first-tier assay system for the detection of possible genetic damage resulting from pollution or the use of environmental chemicals.

Many plants are known to be affected by landfill leachate under natural and experimental exposure conditions, including their growth, biomass production, physiological changes and genetic stability [3,14–18]. Therefore, it is possible to use the sensitive and tolerant plants for monitoring landfill leachate toxicity. Our previous studies indicate that the leachate from Xingou municipal landfill of Taiyuan, a city in the north of China, increased the frequency of MN in *Hordeum vulgare* and *Vicia faba* root-tip cells [17,18]. Because of the variations in leachate quality and different sensitivity of high plants to environmental chemicals, it is necessary to test different higher plant system, especially common plant in the landfill area, to confirm the implication.

Triticum aestivum (wheat) is a common plant in the north area of China, and commonly used for root meristem cytogenetic effect tests and seedling growth assays [19–21]. In the present study, the MN assay and the sister chromatid exchange (SCE) test were performed on root tips of wheat, and mitotic activity and growth alteration induced by Xingou landfill leachate were investigated by monitoring germination, root and bud length, and mitotic index (MI).

2. Materials and methods

2.1. Landfill leachate sample collection

According to the previous reports [17,18,22–24], the leachate sample was collected from Xingou MSW landfill of Taiyuan in October 2006 and stored. Basic physical–chemical properties of the mixed sample were analyzed [22].

2.2. Wheat preparations

Wheat, supplied by Institute of Agriculture Science in Shanxi Province, was selected as the test plant. Dry seeds were soaked for 3 h in distilled water and allowed to germinate on moist filter paper for the use in the test.

2.3. Germination and growth

There were seven groups. Six treatment groups were exposed to the leachate of different concentration by diluting the crude leachate with distilled water (X40, X20, X10, X5, X2 and X1), and the final COD_{Cr} was 80, 160, 320, 640, 800, 1600 and 3200 mg/L, respectively. The negative control group was exposed to distilled water. The germination ratio of 500 soaked seeds in each treatment was measured after exposure for 24, 48 and 72 h. Thirty seedlings, which reached 1.4 cm in root length, were treated for 120 h, and the seminal root and bud length in each treatment was measured after exposure. All the experimental groups were maintained in an incubator at $25 \pm 1\,^{\circ}C$.

2.4. Cell kinetics, micronucleus assay, and pycnosis

To test cell kinetics, micronucleus and pycnosis, roots were treated with the leachate for 24, 48 and 72 h, and then kept in water for 24 h for recovery. After that, the treated roots and negative control were fixed with freshly prepared acetic acid: ethanol (1:3, v/v) solutions. After 24 h, the fixative was replaced with 70% alcohol for storage. For slide preparation and microscopic examination, the root tips were rinsed in distilled water and hydrolyzed in 1 M HC1 at 60 °C for 12–15 min. After staining with Schiff's reagent, 1 mm of the mitotic zone from well-stained root tip was immersed in a drop of distilled water on a clean slide and squashed under a cover glass. The mitotic index was determined by counting the number of mitotic cells among the total cells scored per seedling. The results were expressed per 100 cells scored. The cells with MN and pycnosis were evaluated under 800× magnification with a light microscope (Olympus, Japan). The MN frequency and pycnosis were expressed in terms of the number of cells with MN and the number of cells with pycnosis per 1000 cells scored, resulting from about 5000 examined cells in 10 separate seedlings for each group.

2.5. Sister chromatid exchange test

Seedlings, which reached 1.4 cm in root length, were incubated for 12 h in a solution of 10^{-4} M bromodeoxyuridine (Brdu, Sigma Chemical Co.), 10^{-8} M fluorodeoxyuridine (FdU, Sigma Chemical Co.), and 10^{-6} M uridine (Urd, Sigma Chemical Co.). Thereafter the growing root meristems were treated for 24 h in the tested landfill leachate samples, and in distilled water in the presence of 10^{-4} M thymidine (Thd, Sigma Chemical Co.) and 10^{-6} M Urd. The tissues were kept in 0.05% colchicines for 4–5 h and then fixed in methanol–acetic acid (3:1, v/v) for 24 h at 4 °C. Before fixation the whole procedure was performed in the dark (25 °C). Parallel positive control ($CdCl_2$, 1 mg/L) was incubated and handled alike for each set of treatments. After being rinsed with distilled water, the meristematic regions were excised and digested in a mixture of cellulase and pectinase (2.5% each) for 5 h at 25 °C before the cells underwent hypotonic treatment in distilled water for 1 h. The water was then discarded and the root tips were pounded into a paste. Cell suspensions were made by adding appropriate amounts of fixative to the paste. A few drops of suspension were subsequently dropped onto a clean slide taken from icy water and flame-dried.

The slides were incubated in 2 X Standard saline citrate solutions (0.3 M sodium chloride and 0.03 M sodium citrate) in glass dishes at 80 °C and irradiated simultaneously under UV lamp

(30 W) at a distance of 10 cm for 40 min. After that, the slides were stained in 5% Giemsa solutions for 10 min and rinsed with tap water. The frequency of SCE was expressed as the number of SCEs per chromosome, resulting from at least 30 well-spread metaphases for each treatment.

2.6. Statistical analysis of data

Experiments were repeated three times. Analysis of variance (ANOVA) was used to determine the significant difference (0.05 or 0.01) among the negative control and a series of treated groups.

3. Results

Basic physical–chemical properties of the leachate sample were measured, with the following results. pH, 8.12; NH_3–N, 415.8 mg/L; NO_3–N, 5.6 mg/L; NO_2–N, 85.7 mg/L; phenols, 0.712 mg/L; COD_{Cr}, 3200 mg/L. Heavy metal: Pb, undetected; Cd, 0.015 mg/L; Hg, 0.010 mg/L; As, 0.105 mg/L; Cr, 0.014 mg/L.

3.1. Germination

As shown in Table 1, for 24, 48 and 72 h exposure, germination was not affected by the leachate at lower concentrations (COD_{Cr} 80 and 160 mg/L), and statistically significant ($P < 0.05$ and 0.01) inhibition occurred at higher concentrations. Germination ratio slightly increased with increasing treatment time, except crude leachate group.

3.2. Root growth (RG)

The root growths before and after treating with the municipal landfill leachate are presented in Fig. 1. During the test period, the root growth was not affected by the leachate at low concentrations (COD_{Cr} 80, 160 and 320 mg/L), but was slightly promoted at 80 and 160 mg/L. The leachate at the concentration of 640 mg/L tended to inhibit the root growth, and the difference compared to control was augmented during exposure period. COD_{Cr} 1600 mg/L and crude leachate almost completely inhibited the root growth.

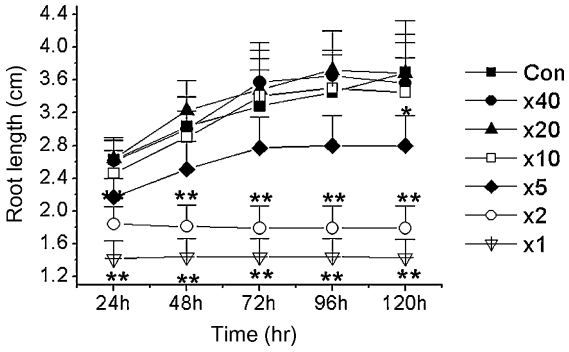

Fig. 1. Effects of Xingou municipal landfill leachate on root length of wheat seedings. Seedlings, which reached 1.4 cm in root length, were treated for 120 h with landfill leachate and with distilled water control, and the seminal root length of 30 seedlings in each treatment was measured after exposure. *$P < 0.05$, **$P < 0.01$ versus control.

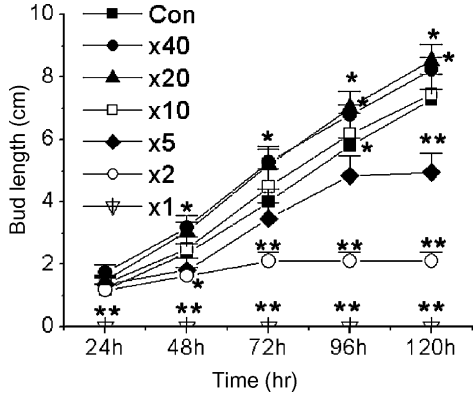

Fig. 2. Effects of Xingou municipal landfill leachate on the bud length of wheat seedings. Seedlings, which reached 1.4 cm in root length, were treated for 120 h with landfill leachate and with distilled water control, and the seminal bud length of 30 seedlings in each treatment was measured after exposure. *$P < 0.05$, **$P < 0.01$ versus control.

3.3. Bud growth

Fig. 2 shows the bud growths under the control and the municipal landfill leachate exposure groups. During the test period, the bud growth was significantly stimulated by the leachate at lower concentrations, and the promotion was attenuated, even disappeared, with the increase of leachate concentration. Actually, COD_{Cr} 640 mg/L and higher concentration leachate signifi-

Table 1
Effects of Xingou municipal landfill leachate on germination of wheat seeds

Test substance	Concentration (COD_{Cr}, mg/L)	Germination percentage (% ± S.D.)		
		24 h	48 h	72 h
Negative control	0	97.5 ± 3.67	98.2 ± 3.68	99.8 ± 2.67
Leachate × 40	80	98.8 ± 2.51	100.0 ± 0.00	100.0 ± 0.00
× 20	160	98.8 ± 3.45	99.3 ± 4.12	100.0 ± 0.00
× 10	320	87.8 ± 2.28*	92.7 ± 5.23	94.8 ± 4.89
× 5	640	71.0 ± 4.23**	78.79 ± 4.56**	81.02 ± 3.67**
× 2	1600	13.5 ± 3.14**	14.34 ± 3.21**	14.34 ± 3.21**
× 1	3200	0	0	0

* $P < 0.05$.
** $P < 0.01$ versus control.

Table 2
Effects of Xingou municipal landfill leachate on the mitotic index in wheat root tips

Test substance	Concentration (COD_{Cr}, mg/L)	Mitotic index (% ± S.D.)		
		24 h	48 h	72 h
Negative control	0	19.67 ± 3.68	23.00 ± 2.16	27.33 ± 2.87
Leachate × 40	80	24.00 ± 4.32	32.00 ± 3.27	35.33 ± 2.36*
× 20	160	25.67 ± 3.86	27.33 ± 3.30	31.00 ± 2.94
× 10	320	18.67 ± 3.40	21.33 ± 2.62	22.33 ± 1.89*
× 5	640	13.33 ± 2.49**	11.67 ± 0.94**	9.00 ± 2.16**
× 2	1600	8.00 ± 2.16**	7.33 ± 1.89**	4.33 ± 1.25**
× 1	3200	5.14 ± 1.21**	3.33 ± 1.24**	2.00 ± 0.56**

* $P < 0.05$.
** $P < 0.01$ versus control.

Table 3
Effects of Xingou municipal landfill leachate on MN frequency in wheat root tips

Test substance	Concentration (COD_{Cr}, mg/L)	MN Frequencies (‰ ± S.D.)		
		24 h	48 h	72 h
Negative control	0	2.33 ± 0.47	2.33 ± 0.47	3.00 ± 0.41
Leachate × 40	80	2.45 ± 0.82	2.67 ± 0.94	3.10 ± 0.82
× 20	160	2.89 ± 0.82	3.05 ± 0.94	3.46 ± 0.82
× 10	320	3.33 ± 0.54*	3.67 ± 0.70*	5.00 ± 0.63*
× 5	640	5.00 ± 0.63*	8.00 ± 2.94**	10.33 ± 2.87**
× 2	1600	7.33 ± 1.70**	10.67 ± 2.05**	12.33 ± 4.50**
× 1	3200	5.67 ± 1.52*	9.33 ± 1.70**	11.00 ± 2.16**

* $P < 0.05$.
** $P < 0.01$ versus control.

cantly inhibited the bud growth, and the concentration required to produce the effect was lower in plants that had a longer exposure time.

In addition, after the same concentration exposure, bud growth was significantly increased with increasing treatment time at the concentrations of COD_{Cr} 80–640 mg/L, but the difference compared to the control was enhanced with prolonged exposure time.

3.4. Mitotic index

As indicated in Table 2, MI increased at lower concentrations (COD_{Cr} 80 and 160 mg/L) during the test period, and decreased with further increasing leachate concentration. The statistical inhibition occurred at COD_{Cr} 320 mg/L for 72 h treatment, and at higher concentrations even for 24 and 48 h exposure. After the same concentration exposure, MI augmented at lower concentrations (COD_{Cr} 80, 160 and 320 mg/L), and decreased at higher concentrations with increasing treatment time.

3.5. Micronucleus

The results on leachate-induced MN in wheat are summarized in Table 3. Among the exposure groups, the leachate at COD_{Cr} 80 and 160 mg/L did not affect the frequency of MN formation; but higher leachate concentration (COD_{Cr} 320–1600 mg/L) significantly increased the frequency of MN in a concentration-dependent manner for all tested time. The concentration–response curves could be fitted well with the equations: $y = 1.187x - 0.639$ ($\gamma = 0.94$), $y = 2.095x - 0.673$

Table 4
Effects of Xingou municipal landfill leachate on the pycnosis in wheat root tips

Test substance	Concentration (COD_{Cr}, mg/L)	Frequencies of pycnosis (‰ ± S.D.)		
		24 h	48 h	72 h
Negative control	0	0	0	0
Leachate × 40	80	3.00 ± 1.16**	4.33 ± 2.05**	5.67 ± 1.25**
× 20	160	5.00 ± 2.55**	6.33 ± 2.19**	8.33 ± 3.92**
× 10	320	10.33 ± 2.87**	14.00 ± 2.83**	20.67 ± 2.62**
× 5	640	13.33 ± 2.05**	16.67 ± 3.09**	25.33 ± 2.62**
× 2	1600	19.33 ± 4.78**	23.33 ± 3.09**	28.33 ± 3.30**

** $P < 0.01$ versus control.

Table 5
Effects of Xingou municipal landfill leachate on SCE in wheat root tips

Test substance	Concentration (COD_{Cr}, mg/L)	SCE frequency (number of SCEs/chromosome)	SCE ratio (to negative control)
Negative control	0	1.03 ± 0.29	
Leachate × 40	80	1.63 ± 0.30*	1.58
× 20	160	1.89 ± 0.25**	1.83
× 10	320	2.18 ± 0.41**	2.12
× 5	640	2.34 ± 0.35**	2.27
× 2	1600	2.21 ± 0.26**	2.15
Positive control	1 mg/L	2.67 ± 0.24**	2.59

* $P < 0.05$.
** $P < 0.01$ versus control.

($\gamma = 0.93$) and $y = 2.533x - 0.755$ ($\gamma = 0.95$). However, MN frequencies decreased after crude leachate treatment. The results also show that after the same concentration exposure, MN frequencies increased with increasing treatment time.

3.6. Nuclear pycnosis

The results on leachate-induced pycnotic cells (PNC) in wheat root tips are given in Table 4. Among the exposure groups, 80 mg/L leachate induced PNC formation after 24, 48 and 72 h exposure, and the frequencies of PNC significantly increased with increasing concentration of leachate from 80 to 1600 mg/L. After the same concentration exposure, the frequencies of PNC increased with increasing treatment time.

3.7. Sister chromatid exchange (SCE)

As indicated in Table 5, the leachate influenced the formation of SCE in wheat root tips. Exposure to 80–1600 mg/L leachate for 24 h caused concentration-dependent significant increases in SCE frequency as compared with the negative control. However, metaphase chromosomes were hardly found and no SCE could be scored after wheat was exposed to the crude leachate.

4. Discussion

The present study indicates that the tested municipal landfill leachate caused double-effects (promotion and inhibition) on the physiological and genetic index of wheat. The leachate at lower concentrations tended to stimulate the germination, growth and cell division of wheat, and did not induce obvious increase of MN frequency in root tips. While at higher concentrations the leachate inhibited the germination, growth and cell division, and also significantly augmented the MN frequency in concentration- and time-dependent manners. However, probably because of mitotic delay and acute cell toxicity, MN frequencies decreased after crude leachate treatment. In addition, PNC and SCE occurred in root tips at all leachate concentrations tested, and PNC and SCE frequencies had a positive relation with the treatment concentration and the exposure time. The results also indicate that the concentration required to produce physiological toxicity and cytogenetic damage was lower in cells that had a longer exposure time.

4.1. Municipal landfill leachate composition and its double-side effect

As reported, many of the contaminants in the leachate are toxic and capable of inducing mutation [3], and their potential to cause cancer in man and hazards to public health and water quality are also known [2,5,23,24]. At the same time, some nutrient elements for plant, such as N and P, etc., are also included in the leachate [1]. When diluting leachate to lower concentrations, the presence of nutrient elements and decreased toxic organic complex- and heavy metal-level contributed to the positive effects on wheat germination, growth, cell division and fewer genetic injuries of root tips. While the high level of toxic organic complexes and heavy metals contained in the higher leachate concentration concealed the action of nutrient elements, and showed the inhibitory effects on the physiological and genetic process of wheat.

4.2. Leachate exposure and cytogenetic damage

The municipal landfill leachate-enhanced MN and SCE in wheat root cells in the present study reveal the potential genetic injuries of leachate in plant systems. Increases in both MN and SCE indicate that the chemicals damage DNA and are, therefore, mutagen and potential carcinogen. A possible mechanism for leachate-induced cytogenetic damages involves the formation of free radicals, either via autoxidation or by enzyme-catalyzed oxidation of organic compounds and heavy metals in the leachate, such as chlorinated and nonchlorinated hydrocarbons, including carbon tetrachloride, chloromethane, chloroethane, chloroethylene, decanoic acid, nonanoic acid, Cd, Hg, As, Cr, etc. These free radicals could react with lipids and lead to lipid peroxidation of cell membrane in tissues, causing the breakage of the DNA chain by oxidating the base in DNA and covalent binding between the product of lipid peroxidation and DNA [25,26]. They could also react with proteins, affect the structures and functions of enzymes, and alter membrane properties [27]. In addition, these free radicals could attack nucleic acids, especially some spots in purine and pyrimidine, resulting in base substitution and breakage of DNA, and eventually induce mutation [27].

Among the experimental groups, 80 mg/L is the lowest concentration, which induced a significant increase in SCE in wheat root tips. However, leachate treatment at 80 and 160 mg/L,

did not cause marked MN formation, while it could stimulate seedling growth as opposed to the high-concentration treatments. These results indicate that MN and SCEs are two different genetic events under same environmental stresses. MN, which often results from the acentric fragments or lagging chromosomes that fail to incorporate into either of the daughter nuclei during telophase of the mitotic cells, can cause cell death due to the deletion of primary genes. But at low concentrations SCE occurrence could cause cellular adaptability to the environments instead of cell death. SCE, resulting from chromatid break and then recombination of DNA molecules between two sister chromatids, may influence gene expression due to positional effects. It is possible that leachate-related genes expression may occur following SCEs, resulting in a rapid adaptive detoxification reaction, which needs further study. Although leachate induced both genotoxicity and growth inhibition in wheat roots, the genotoxicity occurred earlier and could influence the mitotic activity and growth under the stress. Results of the SCE test indicate that genotoxicity of leachate occurred at lower concentration for a short exposure, due to penetrating into cells and inducing the formation of oxidation products. It also implies that the wheat root SCE test is very sensitive and efficient for genotoxicity monitoring of environmental leachate exposure.

4.3. Species difference of cytotoxicity induced by leachate

After exposure to the same concentration of Xingou municipal landfill leachate, pycnotic cells were observed in *Triticum aestivum* and *Hordeum vulgare* root tips, but did not occur in *Vicia faba* root tips [17,18]. The difference in pycnosis formation could result from the difference of sensitivity among these species and different chemical levels in root cells after treatment. Several studies have clearly demonstrated that nucleolar morphological characteristics are indicators of the most important molecular-genetic processes and are useful markers of cell metabolism [28].

Our present study also shows that double-side effects occurred in cell division of *Triticum aestivum* root tips, but not in *Vicia faba* and *Hordeum vulgare* root tips [17,18]. In addition, the lowest concentration, which induced a significant increase in MN frequency in *Triticum aestivum* root tips, is higher than that in *Vicia faba* and *Hordeum vulgare* root tips, implicating that *Triticum aestivum* posed more tolerant than *Hordeum vulgare* and *Vicia faba* to the leachate from Xingou municipal landfill in China.

5. Conclusion

The present results confirm that the landfill leachate may act as a physiological and genotoxic agent in plant cells. It implies that the mixture could result in water contamination even at dilute concentrations either due to faulty design and/or construction of the landfill. Exposure to leachate contaminated aquatic environment may thus pose a potential risk for induction of cytogenetic damage in organisms. The results also suggest that the *Triticum aestivum* bioassay can be used as efficient toxicity, especially genotoxicity, test of landfill leachate, and as monitor of leachate pollution in the aquatic environment.

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