New insights into the toxicity of landfill leachate to zebrafish and mung beans

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Research Article

Keywords: Landfill leachate, Zebrafish, Mung beans, Toxicity

Posted Date: June 2nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1644908/v1

Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on June 19th, 2023. See the published version at https://doi.org/10.1007/s11356-023-28086-8.

Abstract

Landfill leachate have become a major public health concern because of their adverse effects on health. Due to its complex composition, the toxicological effects have yet to be evaluated. In this study, we use two model organisms: zebrafish and mung beans, to assess the toxic effects of landfill leachate. The results showed that low concentrations of waste leachate promoted the growth of mung beans, while high concentrations severely affected the growth and development of seedlings. Furthermore, landfill leachate caused a decrease in chlorophyll levels and malondialdehyde levels increased, significantly increased the rate of root tip micronuclei. In addition, zebrafish embryos exposed with 0.5%, 1%, 1.2%, 1.5% (v/v) landfill leachate, which was shown significantly reduced levels of embryonic incubation rate and heart rate, while the rates of mortality and malformation were increased. 1.0% of the landfill leachate in the experiment can result in a decrease in spontaneous movement frequency of embryos and the light stimulation reaction. The number of black and white area explore and mirror attacks were reduced. In general, these results help to understand the environmental toxicity of the landfill leachate, providing additional reference data for the risk assessment and management of landfill leachate.

Introduction

In recent years, due to the high standard of living, the rapid expansion of industry and commerce, and the mass consumption of packaged products, the generation of solid waste have promoted rapidly. It is estimated that by 2025, the global urban solid waste generation will reach 2.2 billion tons per year (Daniel and Perinaz 2012). Sanitary landfill is the most commonly used treatment method in developed and developing countries due to its simplicity and economic advantages (Bolyard and Reinhart, 2016). However, sanitary landfills are always accompanied by pollution problems, mainly the production of potentially explosive gas and liquid leachate (Sang et al., 2010). Liquid leachate is a very critical issue, not only because it has a toxic effect on the environment, it may contaminate soil, groundwater and surface water, in addition to its potential negative effects on human health (Jones et al., 2006). Previous studies have shown that the standards for the treatment and discharge of landfill leachate are limited to chemical indicators; the assessment of its hazards is also limited to chemical analysis (Ghosh et al.,2017). Many of the lower levels of pollutants that have not been detected toxic effects that cannot be completely ignored. In contrast, biological analysis can make up for the shortcomings of a single chemical analysis to a certain extent. The use of biological assays as an evaluation tool to characterize the pollution characteristics of landfill leachate can achieve the effect of comprehensively evaluating the impact of leachate on environmental organisms (Wilke et al., 2008; Žaltauskaitė et al., 2008).

Therefore, bioassays were used to assess the toxicity effects of waste leachate. As we all know, environmental pollutants can cause various toxic effects. Several studies have investigated the toxicity of landfill leachate and have shown harmful effects on organisms with different nutrient levels (Baun et al., 2004; Bertoldin et al., 2012; Bortolotto T, Bertoldo et al., 2009; Farombi et al., 2012). Mung bean is a common agricultural economic crop in China, and it is also a good biological indicator. As an exposure carrier, mung bean is used to evaluate pollution toxicity. The main indicators include: seed germination

(Sun et al., 2020), plant growth (Nahar et al., 2016), root tip micronucleus, leaf enzyme activity (Shabnam and Kim 2018) and so on. Among them, DNA damage is the joint action of many environmental toxins, and specific DNA damage or damage can trigger cell death (Franco et al., 2009). Higher plants are considered to be excellent genetic models for detecting environmental mutagens.

Zebrafish is a good aquatic model organism that can be used to evaluate the potential ecological hazards of leachate. Previous studies have shown that exposure of leachate to medaka (Osaki et al., 2006) can lead to reducing egg hatchability and larval malformations. After exposure to the leachate, it was found that there were binucleate and micronuclei in the red blood cells (Alimba et al., 2011). Exposure of perch leachate led to oxidative damage to the liver and changes in related enzyme activities (Noaksson et al., 2001). In addition, in toxicity tests, the locomotor activity of fish is the most commonly assessed sublethal endpoint to determine the behavioral changes of pollutants, and therefore is considered to be a key feature for determining fish survival in the natural environment (Gui et al., 2014). Sub-lethal exposure to pollutants results in changes in the motor activity of fish and other aquatic organisms that adversely affect animal behavior, including looking for food, eating and responding to predators, avoiding predators, or reproducing successfully (Steinberg CEW, 2003). In some cases, in a short-term toxicity test using one or several endpoints, the behavioral changes of the fish may not be noticed. Therefore, in order to ensure a more accurate representation of the results and gain mechanistic insights into the toxic pathways induced by single chemicals or multi-component mixtures, it is necessary to integrate different endpoints (Steele et al., 2018).

The leachate produced in the landfill is usually a complex mixture. Therefore, it is necessary to better understand the potential toxicity of these leachates to the environment. This study aims to conduct bioassays on zebrafish and mung beans to solve the following questions: 1) How does the leachate affect the growth and development of mung bean and its genetic toxicity? 2) Does the leachate affect the growth and development of zebrafish? 3) What effect does leachate have on the behavioral toxicity of zebrafish?

Materials And Method

2.1 Landfill leachate eachate sample collection and analysis

Landfill in northern part of a northern city. It had a maximum capacity of 3,500,000 m^3. The waste stream entering the landfill was primarily residential refuse with very little industrial waste. Leachate samples were collected from the landfill in October 2017. The basic chemical properties of the leachate sample were analysed. The detailed procedure for detection chemical properties is provided in the SI (Text S1).

2.2 Model organisms and landfill leachate treatment

2.2.1 Zebrafish and landfill leachate treatment

Wild-type (AB strain) adult zebrafish were purchased from the National Zebrafish Resource Center (Wuhan, China). Randomly selected healthy zebrafish embryos and placed them in a 6-well plate randomly, with 3 parallels for each treatment. Embryos were cultured in an incubator at a constant temperature, the set temperature was 28 ± 1℃, the light was 14 hours, and the darkness was 10 hours. The detailed procedure for embryo exposure is provided in the SI (Text S2).

2.2.2 Plant material and landfill leachate treatment

The mung bean provided by Shanxi Agricultural Science Research Institute was selected as the experimental plant. The pretreated landfill leachate was formulated into leachate with volume concentration of 1%, 5%, 10%, 15%, and 20% (v/v), which was applied to the poisoning of plant seed. There were 30 grains per dish and 4 dishes per treatment group; cultured at room temperature in the dark. Added liquid regularly to keep the filter paper moist. The detailed procedure for plant exposure is provided in the SI (Text S3).

2.3 Embryo/larval behavior detection

2.3.1 Embryo spontaneous activity (ESA) assay

Early developmental neurotoxicity was assessed using the embryo spontaneous activity (ESA) behavioral test, which quantified the spontaneous tail contraction of 24 hpf (hour post-fertilization) embryos, as described in (Raftery et al., 2014). In short, embryos (24 hpf) were exposed to leachate treatment (0.5% and 1% v/v, and control) and kept in the dark at 27 ± 0.5°C. At 24 hpf, a customized video imaging system was used to record behavioral responses under transmitted light. The video file was analyzed with the software of the Danish Instrument Company (Noldus Inc., Wageningen, the Netherlands), which counted the number of embryos' movements per minute.

2.3.2 Larval photomotor response (LPR) assay

As mentioned earlier (Huang et al., 2018), the EthoVision XT 11.5 system and instrument (Noldus Inc., Wageningen, the Netherlands) was used to measure the light motion response behavior of juvenile fish. The larvae exposed to leachate (0.5 and 1% v/v) and the control developed to 120 hpf for the experiment. The total duration of the experiment was 50 minutes, the first 10 minutes of adaptation, no data was collected, the next 10 minutes of dark motion data were collected, and then 10 minutes of light stimulation was given, and then the motion data were recorded for 10 minutes in the dark, with two cycles of light and dark alternating cycles. Recorded the movement speed of the juvenile fish.

2.3.3 Experiment on exploratory behavior of larvae in black and white area

Design the experiment according to the experimental principle of Ju Wang (Wang et al., 2014), and then it was tested and completed by the EthoVision XT 11.5 system. Zebrafish larvae were exposed to 168 hours. Each treatment group selected 32 healthy larvae (swimming larvae) and transferred them to a 24-well plate containing 1.5 mL of exposure solution (half of each well) were shaded to form a black-and-white area exploration device), placed in a light incubator for 15 minutes, and then transferred to a behavior analysis box for data collection.

2.3.4 Larvae mirror attack behavior experiment

According to the literature design experiment (Cachat et al., 2013), it was tested and completed by the EthoVision XT 11.5 system. A mirror was pasted on one side of a custom-made transparent 12 square hole plates to form a mirror exploratory device. In the experiment, a rectangle with a width of about 2 mm was circled in front of the lens as the mirror analysis area. Zebrafish larvae were exposed to 264 h, and 32 healthy larvae were selected for each treatment group, one for each well, and then transferred to the behavior analysis box. The experiment collected the movement of the fish in the observation area, the frequency of reaching the mirror area, and the movement speed under observation.

2.4 Detection of Phytotoxicity of Mung Bean

2.4.1 Determination of seedling root length and bud length

The cleaned seeds were soaked in deionized water at a volume of 2.5:1 for 8 h, and sprouted in the dark at room temperature for 24 h. Selected mung beans with a root length of 1-1.5 cm for cultured, 30 mung beans on each dish, 4 dishes were a treatment group, cultured at room temperature, regularly and quantitatively added the leachate of the corresponding concentration, and kept the filter paper wet, recorded root length and shoot length every day for 7 consecutive days.

2.4.2 Chlorophyll determination and lipid peroxidation determination (MDA)

Chlorophyll determination. For chlorophyll detection, cut 0.1 g of fresh leaves and washed them with deionized water. Soaked in 10 mL of a 1:1 mixed extract of 80% acetone and 95% alcohol, kept 18 h in the dark, took the supernatant, used a spectrophotometer to measure its absorbance at 645 nm and 663 nm. The detailed procedure for chlorophyll calculation formula is provided in the SI (Text S4). The concentration of MDA was determined by the method of Sang (2010).

2.4.3 Determination of the cell mitotic index (MI) and micronucleus (MCN) rate

Determination of the cell MI and MCN rate was determined by the method of Sang (2010). The cell MI and MCN frequency were examined and counted by microscopically observing the number of mitotic and micronucleus cells among the total amount of cells (Corrêa et al., 2016). According to the observation results, calculated the influence of leachate on different treatments.

2.5 Statistical analyses of the data

The results are presented as the mean ± SE, and the statistically significant difference between the control and treated groups was determined using the one-way ANOVA.

Results

3.1 Properties of landfill leachate sample

The basic chemical properties of the landfill leachate samples were showed in Table 3.1. Chemical oxygen demand (COD_{Cr}) content of the leachate was 1759 mg/L, which was 17 times higher than the standard. BOD_5 was 344 mg/L, which was 10 times higher than the emission standard. And, the ratio of BOD_5/COD_{Cr} was relatively low, only 0.19. Studies have shown that BOD_5/COD_{Cr} lower than 0.30 indicated that wastewater has poor biodegradability (GilPavas et al., 2019). The contents of NH_3-N and T-N in the leachate used in this study were relatively high, which were 73 and 52 times the emission standard, respectively. The salt content in the leachate reached more than 10000 mg/L, and the chroma was 6 times the emission standard. The above data shown that the total amount of organic matter in the leachate used this time was relatively high, and the content of biodegradable organic matter was relatively low. The test result of heavy metal content found that the content of heavy metal in the leachate was low, which was lower than the emission standard.

Table 3.1
Characteristics of the crude leachate sample from landfill.

Parameter	Unit	Detection value	Emission concentration limit	Excessive multiple
COD_{cr}	mg/L	1759	100	17
BOD_5	mg/L	344	30	10
NH_3-N	mg/L	1815	25	72
T-N	mg/L	2085	40	53
T-P	mg/L	2.65	3	6
Chroma	Times	256	40	66
Cr	mg/L	0.0048	0.1	Not exceeded
As	mg/L	0.0650	0.100	Not exceeded
Pb	mg/L	0.0061	0.100	Not exceeded
Cd	mg/L	——	0.01	——
Mn	mg/L	0.0187	——	——
Cu	mg/L	0.0185	——	——
Zn	mg/L	0.1464	——	——

3.2 Effects of leachate on germination and growth of mung bean

The germination rate of mung bean for 24 h after leachate treatment (Fig. 3.1 A), the germination rate of the 1% treatment group was higher than the control group (90.8%), the other groups were slightly lower than the control group, however, there was no significant difference. Reflecting the effect of contaminants on plant growth and development is through the root length, lateral root and fresh root weight of seedlings. Figure 3.1 B showed the change of seedling root length. From the point of view of concentration effect, all leachate treatment groups inhibit the growth of seedling roots, and as the treatment concentration increased, the inhibition ratio was higher. The 1% leachate treatment group still increased the number of lateral roots, while the 5%, 10%, 15%, and 20% treatment groups reduced (Fig. 3.1 C).

The average fresh weight of 1% and 5% roots of the treatment group was significantly higher than that of the control group. The fresh roots of the 10%, 15% and 20% treatment groups the weight was lower than that of the control group, which was different from the growth trend of root length (Fig. 3.1 D). The 1% leachate treatment group significantly promoted the growth of the shoots, while the other treatment groups significantly inhibited the growth of the shoots (Fig. 3.1 E). Figure 3.1 F indicated the effect of different concentrations of leachate on the fresh weight of mung bean sprouts. The fresh weight of the 1%, 5%, and 10% leachate treatment groups were higher than that of the control group.

3.3 Effect of leachate on physiological and biochemical indexes of mung bean leaf, mitosis and micronucleus of root tip

The changes in leaf chlorophyll content (Fig. 3.2 A) data indicated that the effect of leachate on mung bean chlorophyll as a whole pointed "low concentration promotion, high concentration inhibition", and the chlorophyll content of mung bean leaves was more obviously affected by the leachate. When the leachate concentration was 5%, the chlorophyll content of mung bean leaves was lower than that of the control group. Figure 3.2 B directed the MDA content of plant leaves after leachate treatment of mung bean. There was no difference between the 1% treatment group and the control, the MDA content of the 5% treatment group was lower than that of the control group, and the MDA content of the 15% and 20% treatment groups were higher than that of the control group. The 20% treatment group was higher than the control group, suggested that the high-concentration group had obvious oxidative damage to the leaves of plants.

There are two main methods for detecting plant root tip cells by leachate, root tip cells MI and MCN. Figure 3.2 C data indicated that the MI of the 1% leachate treatment group was higher than that of the control group; the 5% and 10% treatment groups were lower than the control group, and the 15% and 20% treatment groups significantly inhibited the root tip MI. On the whole, the high concentration of leachate significantly inhibited the mitosis of mung bean root tip cells in a dose-dependent manner. MCN test results (Fig. 3.2 D) indicated that the leachate at a concentration of 1% had a certain inducing effect, but there was no significant difference. The 5%, 10%, 15% and 20% treatment groups were in a dose-dependent manner. Induce root tip cells to produce micronuclei.

3.4 Developmental toxicity of leachates on the embryo development

Developmental indicators show a dual time- and dose-dependent effect (Fig. 3.3 A-D). Figure 3.3 E showed the deformity rate, the 1.2% and 1.5% treatment group's deformity rate was significantly higher than the control group, that was, high concentration the leachate had obvious teratogenic effects. The main manifestations of deformity were: spinal deformity, pericardial edema combined with spinal deformity, yolk cyst combined with spinal deformity, a small amount of head and eye hypoplasia and hypopigmentation (Fig. 3.3 E). Based on the comprehensive data, the leachate exposure inhibited the growth of zebrafish larvae.

3.3 The effect of leachate on larval movement behavior

According to the literature, intermittent spontaneous contraction of the tail can be observed 26 h, and contraction behavior of 21 h embryos appears frequently (Gerlai et al., 2009). At this time, the embryonic nervous system has developed to control the embryo's movement, and the embryo's movement frequency is relatively stable during this period. As shown in Fig. 3.4 A, the frequency of fetal movement in the 0.5%, 1.0%, 1.2% and 1.5% treatment groups was significantly reduced by 14.6%, 24.1%, 27.1% and 57.5% compared to the control group, respectively. The higher the leachate concentration, the more pronounced the inhibition of embryonic spontaneous movement.

Detection of spontaneous movement speed of zebrafish larvae under illumination. Figure 3.4 B indicated that the leachate treatment significantly reduced the spontaneous movement speed of zebrafish. Compared with the control group, the spontaneous movement speed of light by 0.5% and 1.0% decreased significantly by 35.4% and 93.0%. Figure 3.4 C was a heat map of the larval movement position. The brighter the color in the graph, the longer the larva stays in that position. It was clearly observed that 1.0% of the larvae’s movement behavior was reduced and their preference was in the same position. The above data shown that the leachate affects the spontaneous movement ability of zebrafish under light, which was manifested by the reduction of swimming speed and movement behavior.

The effect of leachate exposure on light stimulation behavior of zebrafish larvae. The research data pointed that the larvae swim faster in the dark than in the photoperiod. Compared with the control group (Fig. 3.4 D), the average movement speed of the 0.5% and 1.0% treatment groups was lower than that of the control group, and the difference in speed was more obvious in the dark period. During the dark period before the light stimulation, the average speed of 0.5% and 1.0% was significantly lower than that of the control group by 49.4% and 62.0%; in the dark period after the light stimulation, the average movement speed of the two treatment groups was compared with that of the control group decrease by 11.5% and 38.0%, respectively. Combined with the above analysis, it was shown that exposures to leachate has a significant inhibitory effect on the spontaneous movement speed of larvae; 1.0% leachate treatment significantly inhibited larval response to external stimuli.

The study on exploration behavior of larvae in black and white areas after exposure. The average swimming speed of zebrafish larvae in the white area and the frequency of crossing the two areas (Fig. 3.5 A, B); 0.5% leachate exposured, larval movement speed and crossing frequency in the white area compared with the control group, there was no significant difference; the swimming speed of zebrafish larvae in the white area and the frequency of crossing the black and white area in the 1.0% treatment group were lower than those in the control group, which were 0.55 mm/s and 0.51 times/min, respectively. Figure 3.5 C shown that the larvae of the control and treatment groups stayed in the white area for more than 80%, indicating that zebrafish larvae prefer the white area. Figure 3.5 D was a heat map of larvae behavior trajectory, in which 1.0% treatment group larvae had less exercise behavior and low exercise exploration ability. To sum up, 1.0% leachate exposure significantly reduced the swimming speed and frequency of larval exploration in black and white areas, that was, a certain concentration of leachate will affect the black and white area exploration behavior of zebrafish larvae.

Mirror aggressive behavior is a kind of aggressive behavior, and it is also a process for zebrafish to communicate with their peers and recognize themselves. Analyzing the speed of the larvae in the mirror area (Fig. 3.6 A) found that the swimming speed of the larvae in the leachate treatment group was significantly reduced. In Fig. 3.6 B, the mirror attack frequency of the control group was 4.7 times/min, while the 0.5% and 1.0% mirror attack frequencies were significantly lower than those of the control group, 3.5 times/min and 1.9 times/min, respectively, that was, the higher the leachate concentration, the frequency of larvae attacking the mirror surface was reduced. In Fig. 3.6 C, the stay time in the mirror area of the control group was 25.9%, and the stay time in the 0.5% and 1.0% treatment groups was lower than that in the control group, which was 21.7% and 9.6%, respectively. Figure 3.6 D was a behavior trajectory heat map, in the figure, the 1.0% treatment group was clearly moving away from the mirror area. Based on the above results, it was shown that 1.0% leachate can cause abnormal mirror attacks of larvae, including reduced swimming speed, reduced number of mirror attacks, and reduced residence time.

Discussion

Landfill leachate contains a variety of inorganic, natural and xenobiotic compounds (Baderna et al., 2019). The mixture of these compounds will affect the growth and development of model organisms, fish and plants (Makaras et al., 2020; Klauck et al., 2013). Our results showed that higher levels of leachate can affect the growth and behavior of zebrafish, higher concentrations of landfill leachate can also affect plant growth, while lower levels of leachate stimulate growth. These results provide additional reference data for risk assessment and leachate management.

The leachate is a kind of complex and highly polluting organic wastewater. Experimental studies on the phytotoxicity of leachate report positive and harmful effects on plants. On the one hand, excessive organic pollutants cause the COD_{Cr} in the collected leachate samples to cause phytotoxicity (Table 1), and other major potentially harmful components, such as heavy metals, nitrogen and salinity, are harmful to the plant system, and are harmful to plants at high concentrations. There is an inhibitory effect. On the other hand, the leachate is rich in nutrients such as nitrogen, phosphorus, and organic matter necessary for plant growth. Therefore, a lower level of leachate promotes the growth and development of mung beans. Our findings are similar to those of previous studies. According to reports, high levels of leachate may lead to decreasing yield and low survival rate (Li et al., 2017); however, low concentration of leachate promotes plant growth and survival (Li et al., 2008). The current research results also show that the vegetation system has a certain tolerance for the stress of the leachate sample at a lower level, but the defense ability to the stress may decrease with the increase of the exposure level.

Plant physiologists and ecological physiologists typically choose chlorophyll analysis to estimate the non-destructive effect of plants, which is one of the most powerful and widely used technology (Ata-Ul-Karim et al., 2011). As lesser increases and contains heavy metal ions, the pigments in the plants are destroyed (Li et al., 2017; Lu et al., 2007). The leachate contains rich organic matter, nitrogen, phosphorus, potassium and manganese, is a nutrient element of plants, and therefore, these nutrients may play a key role in the growth of plants when plants are exposed to lower levels of leachate. However, as the exposure concentration increases, various contaminated components in the leachate may enter and accumulate in plant cells, and therefore, the activity of several enzymes in chlorophyll biosynthesis may be suppressed due to inhibition of chlorophyll synthesis (Kuwano et al., 2017). Our results demonstrated that low levels of leachate increases chlorophyll content, which is due to the effects of nutrients of diaphragm. However, high concentrations of leachate reduced the synthesis of chlorophyll, indicating that leachate affects the pathway of chlorophyll biosynthesis, and changes the relative expression of the enzyme and its activity and synthesis. Studies have shown that chlorophyll content is usually associated with lipid peroxidation, and lipid peroxidation assays with malondialdehyde (Gill and Tuteja 2010). MDA is a symbol of cell membrane oxidation damage, which is the final product of lipid peroxidation, usually caused by oxidative stress, and thus evaluates (Stobrawa and Lorenc-Plucińska, 2007). Our propylene deridedehyde test illustrated the production of high concentrations of leachate induced malondialdehyde, while low concentrations of leachate reduced the content of malondialdehyde.

In order to verify the ecological toxicity of the garbage permeation, the root tip was used to determine MI and MCN. In this study, enhancement of MCN of mung bean root cells and the declining of MI revealed potential genetic injury after the exposure of garbage filtration. The MCN is associated with increased oxidation of lipids, which indicate that MCN is formed in an oxidative stress induced by leachate (Dong and Zhang, 2010). The results of this study illustrated that high concentrations of leachate cause genetic injury of plants, and the ecological risk of leachate still exists, although lower levels of leachate seem to be effective in induced more cell division in mung beans.

Zebrafish is an important model organism due to its short growth, development time, short embryo transparent growth, and development time. The use of zebrafish to assess the toxicity of waste leachate is rare. The toxic effects of landfill leachate on fish have been studied in many fishes, however, due to the different toxic components in the leachate from different landfills, it is difficult to draw conclusions and directly compare the available results (Alkassasbeh et al., 2009). Nevertheless, some studies have shown that the LC_{50} calculated after 96 hours of exposure to the original leachate of a closed sanitary landfill in Malaysia is 3.2% and 5.9% of the fish species Pangu and Snakehead, respectively (Emenike et al., 2012). Alkassasbeh et al. indicated that leachate from three landfills in Malaysia caused 100% of carp deaths at concentrations of 4%, 4.6%, and 7.5% after exposure for 30 hours, 42 hours, and 72 hours, respectively (Alkassasbeh et al., 2009). The crucian carp was exposed to several samples of landfill leachate at a concentration of 10% or higher and died due to high concentrations of sodium chloride or ammonium ions (NH^{4+}) (Deguchi et al., 2007). In this sense, high concentrations of ammonia nitrogen may be responsible for the fish mortality in this study.

Some studies have shown that exposure to leachate can cause varying degrees of behavioral, cellular, physiological, and biochemical changes in fish (Emenike et al., 2012; Klauck et al., 2013; Salem et al., 2014; Budi et al., 2016). In the early life of zebrafish, motor behavior is initiated and controlled by the nervous system, which is usually considered abnormal neurodevelopment sensitive indicators of change (Drapeau et al., 2002; Budi et al., 2016). In the behavioral response, swimming dynamics is the most relevant and studied parameter (d'Amora and Giordani 2018). Previously, it has been demonstrated that the early chemical behavior phenotype of zebrafish can be used as a sensitive endpoint for detecting neurotoxicity induced by environmental toxicants (Rafteryi et al., 2014). Investigations on the toxicity of leachate and its effected on fish (carp) behavior indicated that leachate can cause general activity decline, loss of balance, breathing difficulties, excessive mucosal secretion, and fish gathering on the water surface to breathe (Alkassasbeh et al., 2009). Emenike pointed that in their study, fish (Pangasius Sutchiand Clarias batrachus) exposure, high chemical oxygen demand/biochemical oxygen demand ratio and high ammonia nitrogen value can lead to changes in fish behavior, such as fish gathering on the surface and losing Balance, this is typical neurotoxin toxicity (Emenike et al., 2012). In our case, obtained leachate physical and chemical analysis results were consistent with previous research results, indicated that the leachate had high alkalinity, high chemical oxygen demand/biochemical oxygen demand ratio and high ammonia nitrogen concentration.

Conclusions

In summary, the present study was designed to determine the effect of landfill leachate. The results show that landfill leachate present development toxicity and genetic toxicity to mung bean. For the zebrafish, exposure to waste leachate may affect it growth and development. In addition, the exposure of the landfill leachate can lead to behavioral exception. That was, the embryonic spontaneous movement frequency was significantly reduced, including the light stimulation reaction and the black and white area exploresr, significantly declined mirror attack. The behavioral results showed that the swimming speed of zebrafish was diminished, the response to external stimuli was weakened, and the residence time was shortened after exposure. These results are designed to provide more reference data for the risk assessment and management of the landfill leachate.

Declarations

Acknowledgements

We thank the reviewers for their valuable comments and suggestions.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Yue Wang performed the experiments and wrote the paper. Lin Li and Xia Ning conducted the part of the experiment. Guangke Li and Nan Sang reviewed and edited the manuscript. All authors read and approved the manuscript.

Funding

This study was supported by the National Science Foundation of China (22076108).

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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Figures

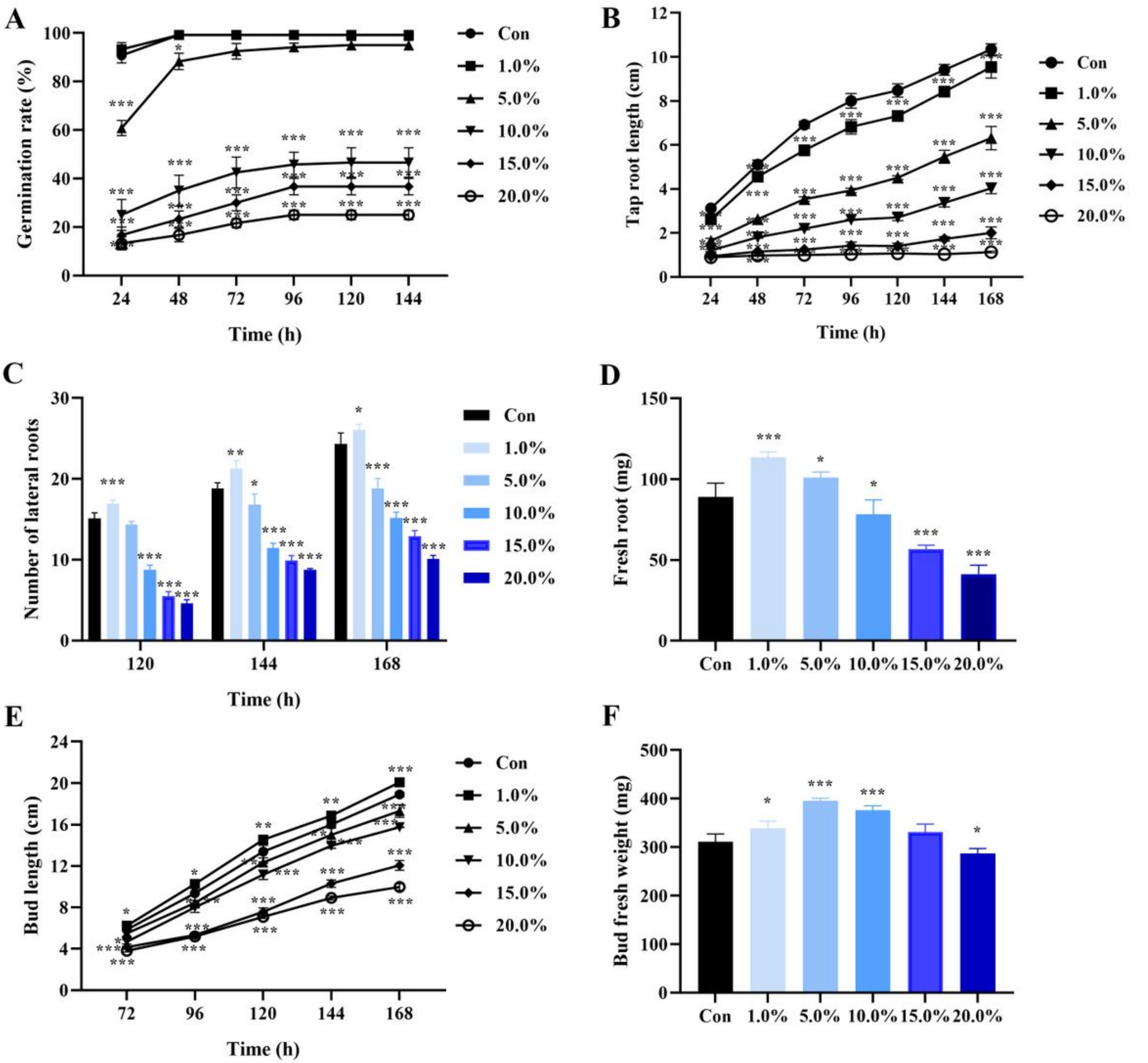

Figure 1

Effects of landfill leachate on the developmental parameters of zebrafish embryos. (A) Mortality rate, (B) hatching rate, (C) deformity rate, (D)morphological malformations, (E) heart rate and (D) body length. Values are expressed as the mean ± SE of six replicate samples and analyzed by one-way ANOVA followed by an LSD test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus the control group.

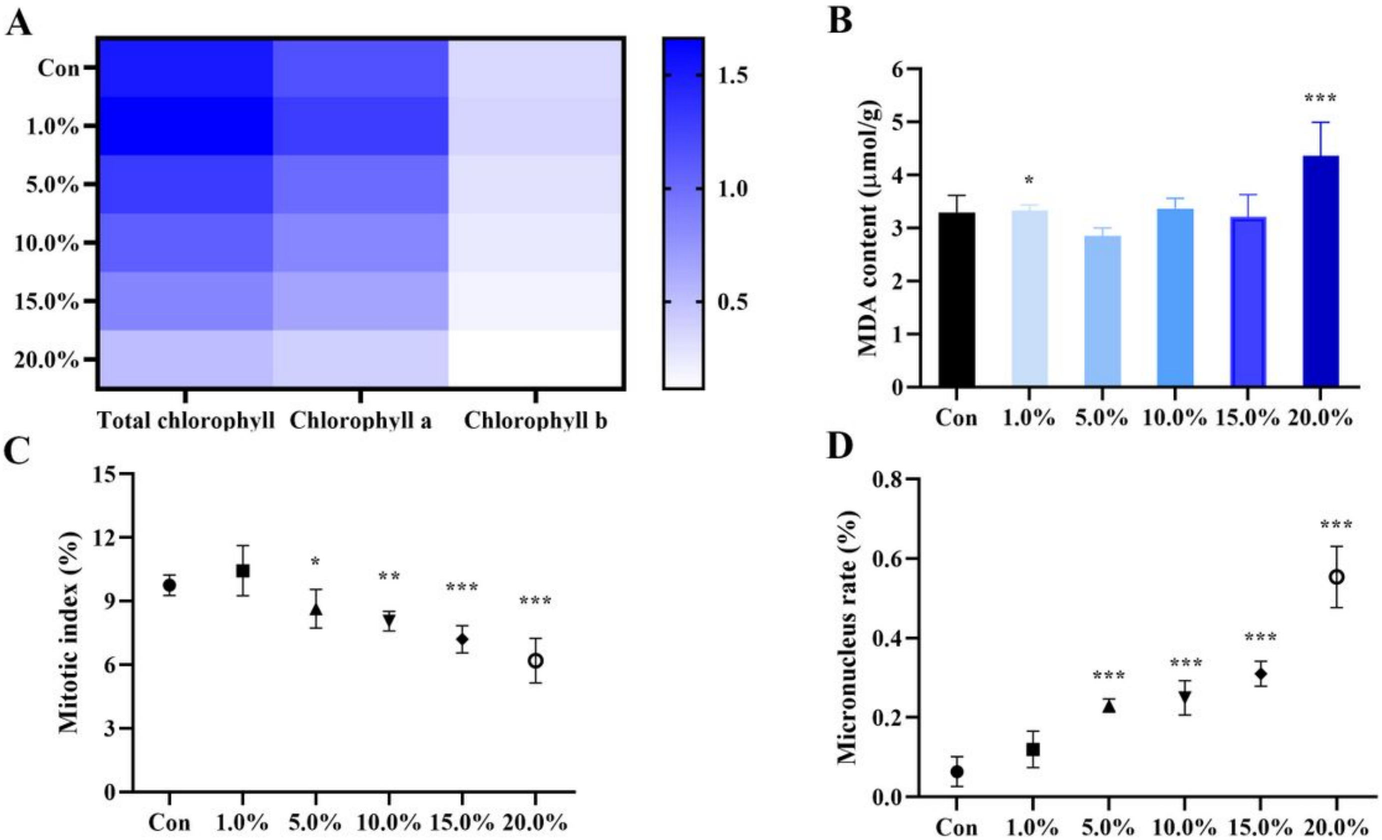

Figure 2

Locomotor behavior of the zebrafish larvae after exposure to the landfill leachate. (A) Spontaneous movement, (B) spontaneous movement speed, (C) locomotor traces and (D) average swimming speed. Values are represented as the mean ± standard error of the mean (SE) and expressed as the fold change relative to the control. *p < 0.05, **p < 0.0 1, ***p < 0.001 versus the control group.

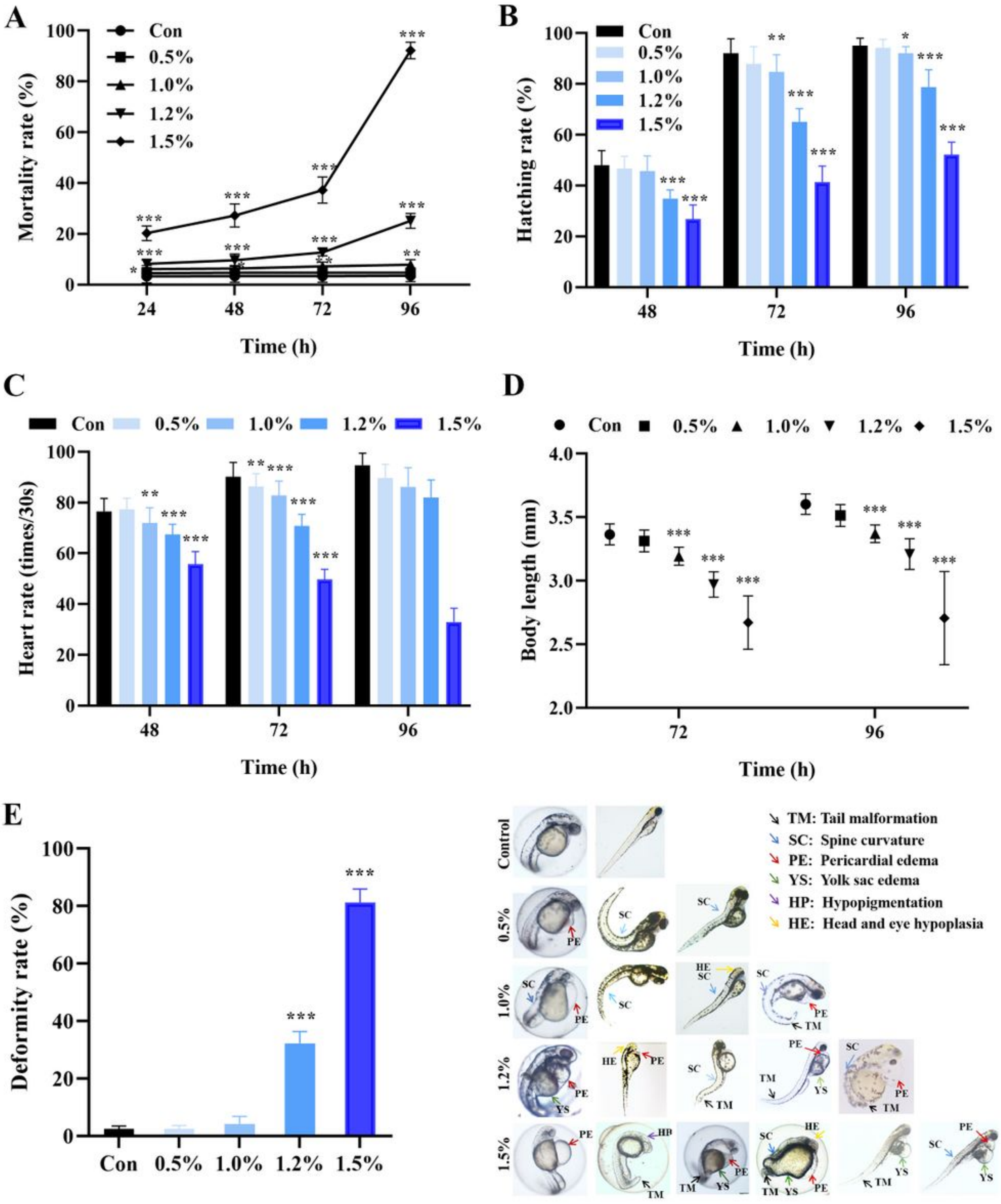

Figure 3

Exploration behavior in black and white areas of the zebrafish larvae after exposure to the landfill leachate. (A) Average speed (B) number of times across black and white areas (C) the proportion of time in the white area and (D) heat map of larvae behavior trajectory. Values are represented as the mean ± standard error of the mean (SE) and expressed as the fold change relative to the control. ***p < 0.001 versus the control group.

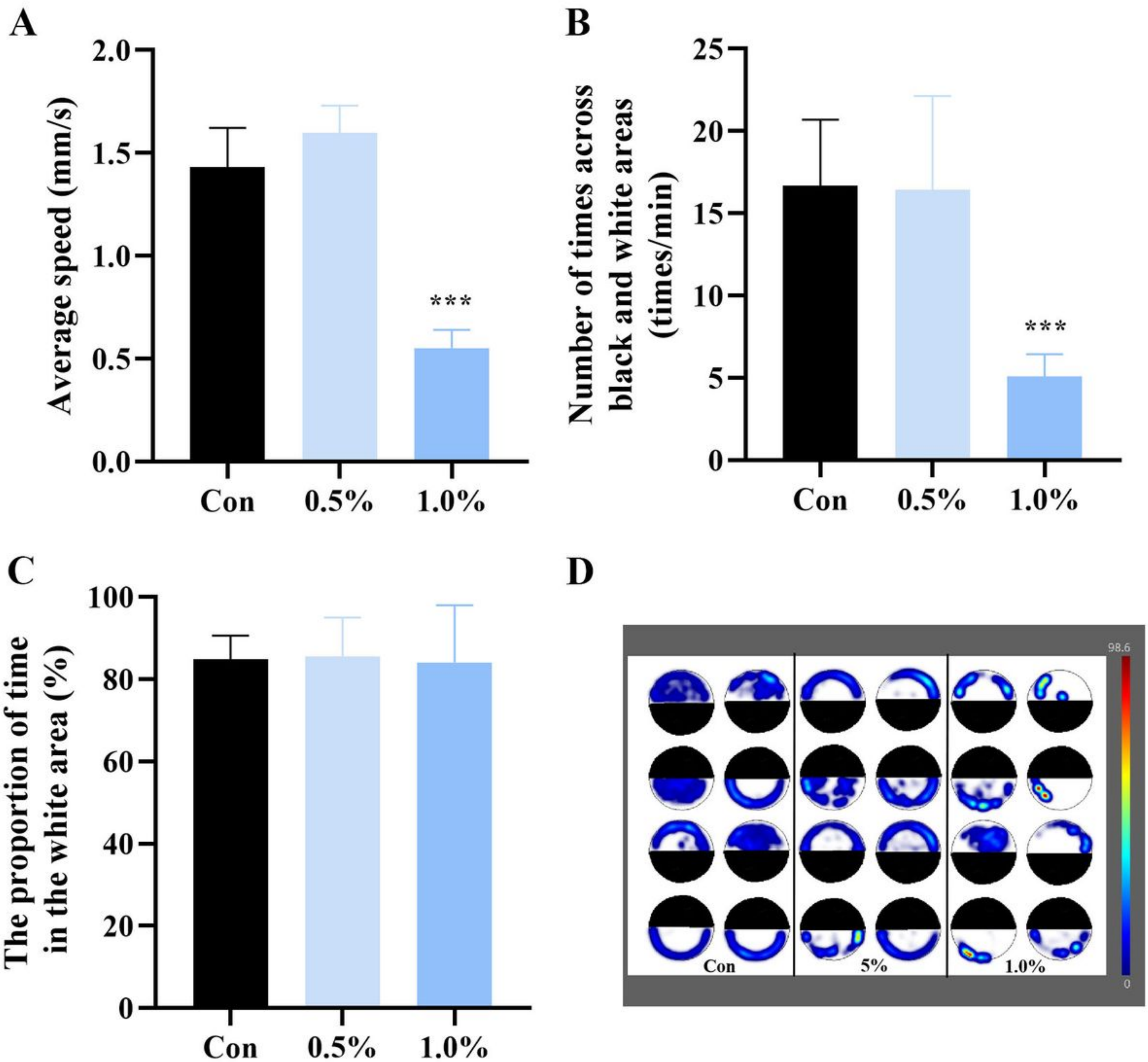

Figure 4

Mirror aggressive behavior of the zebrafish larvae after exposure to the landfill leachate. (A) Average speed (B) number of times attacks of mirror (C) mirror area residence time and (D) heat map of larvae behavior trajectory. Values are represented as the mean ± standard error of the mean (SE) and expressed as the fold change relative to the control. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus the control group.

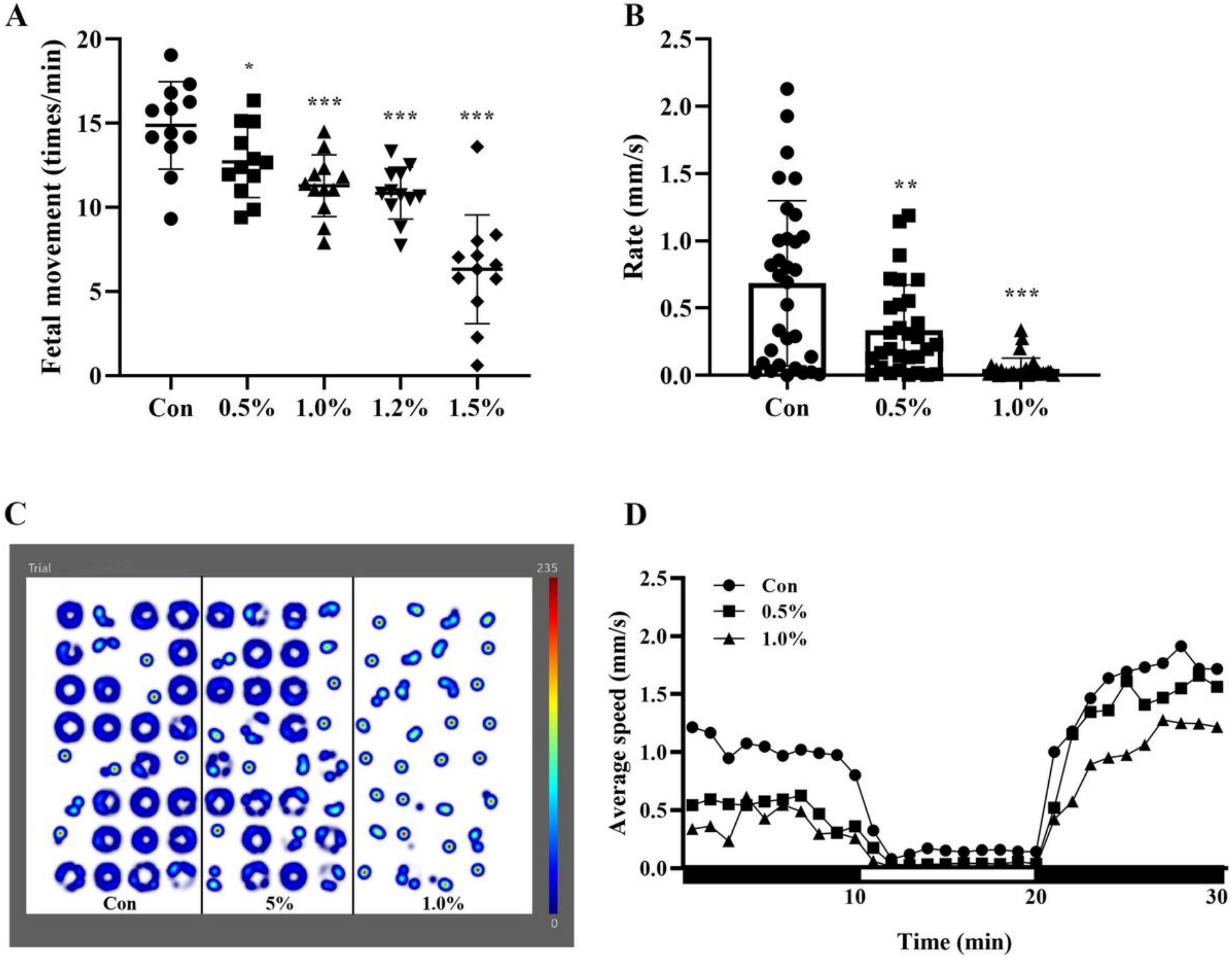

Figure 5

Effects of landfill leachate on the morphological traits of seedlings of mung bean seed (A) Germination rate, (B) root length, (C) number of lateral roots, (D) fresh root biomass, (E) bud length and (F) bud fresh weight. The results are the mean ± SE of triplicates with 6 seedlings each. *p < 0.05, **p < 0.01, ***p < 0.001 versus the control group.

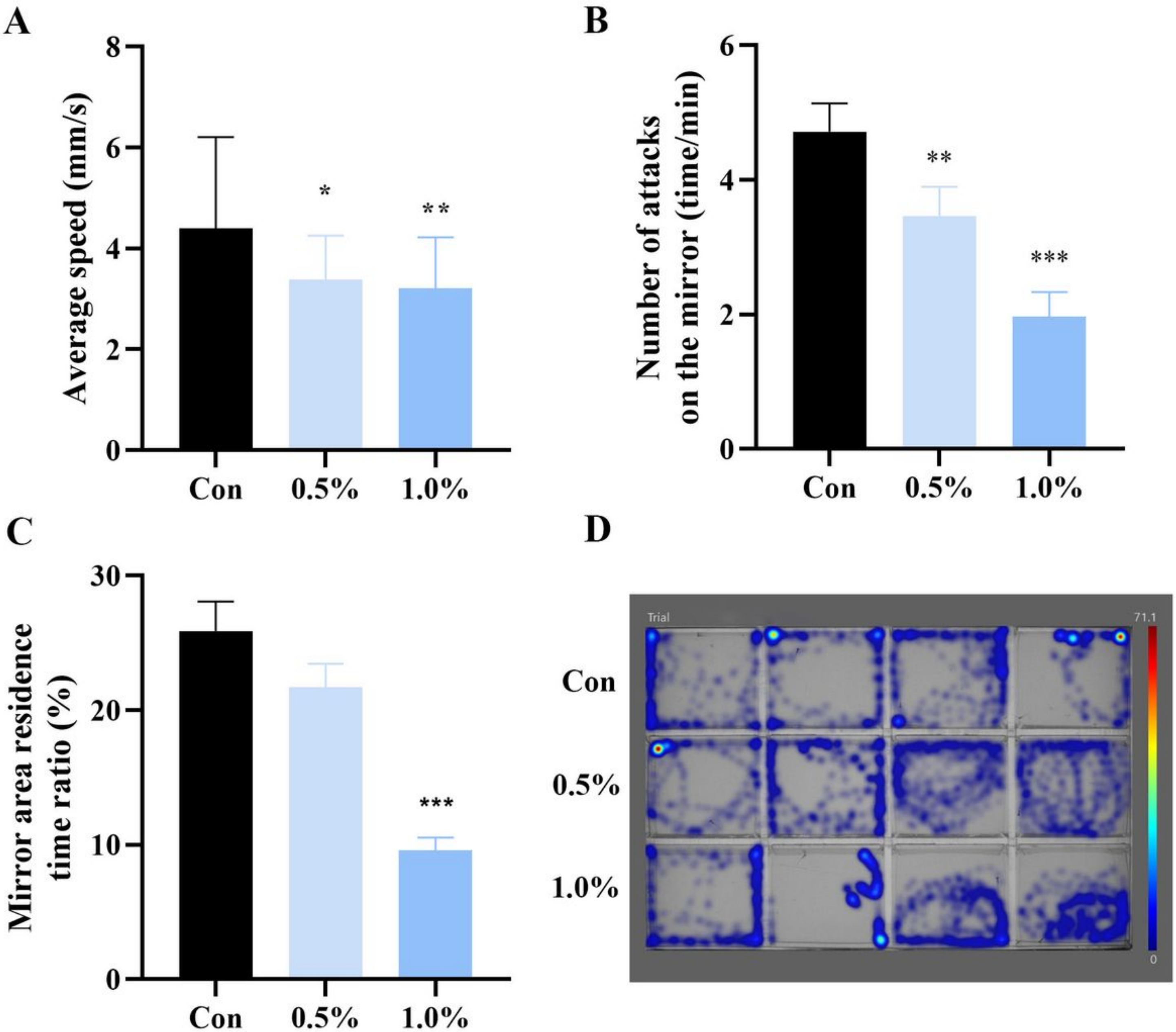

Figure 6

Chlorophyll determination and oxidative damage in mung bean of caused by landfill leachate. (A) chlorophyll content, (B) MDA content. Effects of leachate exposure at the seed soaking and germination stages on MI (C) and MCN (D) of root tip cells in mung bean, respectively. The results are the mean ± SE of six replicate samples. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus the control group.

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