

Genotoxic of Diesel on Mitosis and Frequencies of Micronucleus of Root Tips Cells of *Scirpus triqueter*

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Abstract: The biological effects and cytogenetic damage induced by diesel on root tips cells of *Scirpus triqueter* explored in this study. The results showed that the root length and weight decreased obviously with diesel concentrations increasing. The mitotic index in root tip cells of seedlings were increased first and then decreased significantly, while the frequencies of micronucleus increased with the diesel concentrations increasing. However, high concentration of diesel restrained the frequencies of micronucleus. So diesel caused biological changes, root tip cells mitotic divisions and the chromosome mutation in root of *Scirpus triqueter*, which indicated significant genetic toxicity.

Introduction

It is known that wetlands play essential role in sustaining the structure and function of ecosystems while oil spills in wetlands become one of the serious problems[1]. Petroleum may affect the living environment and creatures through selective toxicity and by influencing the exchange of substance in soil [2, 3]. The growth of animals and plants in oil contaminated wetlands were inhibited and hard to recover from these disturbances [4, 5]. The impact of the oil spill on subtidal fish and invertebrate communities was investigated at the oil contaminated site[6]. The dominant plants, a suite of infauna and epifauna, and many species of fish, birds, and marine and terrestrial mammals would affected by the toxic oil spill in the intertidal and shallow subtidal regions [7, 8].

In the oil contaminated soil, the plant showed a retardation of growth, shorten of length, decline of biomass and dead seriously. The root of plant is the most direct and sensitive organ exposure to the oil pollution. Petroleum could lead to decreasing of activity and the main exposure stress on the plant active oxygen metabolism inside the plant cells. Huangpu River plays an important role in water supply, flood discharge and transportation of surrounding areas in Shanghai[9]. *Scirpus triqueter* is the pioneer plant in Wusong estuary and the growth area is the largest. The oil spill pollution is serious in Wusong estuary because of busy traffic, ship spill and oil leakage. The main objective of the current study was to investigate the effect of diesel on the root growth and cell mitosis.

Methods and Materials

Experiment materials

Reagent: deionized water, NaClO solution, Carnot's fixative (Methanol: Glacial acetic acid=3:1), Ethanol, Hydrochloric acid, Carbol fuchsin.

The plump and healthy seeds of *Scirpus triqueter* were selected and disinfection in NaClO solution (5%) for 10min, then soaking in distilled water for 24h.

Experiment methods

After soaking for 24h, the seeds evenly broadcast in petri dishes with two layers of filter paper for 25du incubation, exchange the water every 12h and take experiment for toxicity test when the radical be about 1.5-2.0cm. The roots of *Scirpus triqueter* were put in different concentration of diesel (1000, 5000, 10000, 15000, 20000 mg/kg) and culture in 25 with distilled water as a control for 24h.

Root length and weight were determinate and took mean value. Every 10 root point was chipped and fixed in carnot's fixation at room temperature for 24h, then root tips were cleaned 3times in distilled water and put in 70% ethanol for 4 freeze. After washed with distilled water the root tips were put in the dissociative liquid was made of 95% ethanol and concentrated hydrochloric acid(1:1) for 10min at 25-30 , and then put in carbolic acid fuchsin solution for 30min[10]. The apical portion of root were gently mashed by tweezer, take root tip meristem region for about 1 mm , add 1 drop of distilled water and remove the coverslip tablet, then using 10 times first to find dispersing preferably cells , using 40 times the observed counts at last.

Results

Effects of diesel on root length and weight

The root growth exposure to diesel pollution was showed in Fig.1. The roots turn yellow in color with increasing pollution concentration. In contrast to blank test, the length and weight of root decreased in the diesel-contaminated soil which showed the diesel in soil restraint the elongate and growth of root. Morphological observation showed that the root tip show obvious victims symptoms, such as root over-striking, browning and even rotting with the dose increasing exposure to diesel pollution.

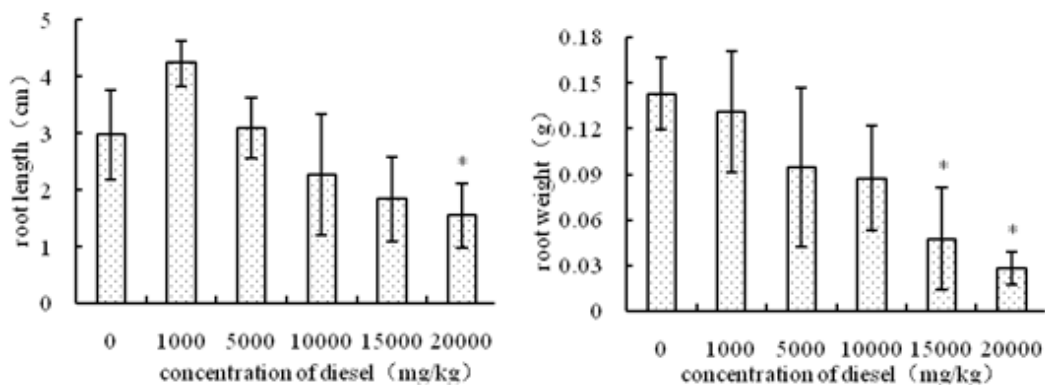


Fig. 1. Effects of diesel on root weight and length

Effects of diesel on the mitosis of root cells

During cell division, chromation become chromosomes to produce all sorts of change and divided into four periods: the early, middle, late and the end. The treatment of different

concentration of diesel on root tip splinter cell in the different periods of mitosis is showed in Fig.2. In the light diesel pollution soil, the total cells in division increased significantly, but the tendency reduced as the treatment concentration rising and it can be seen the number of cells in late and the end period more than in the early period at the same time. The cells in split phase increased in low concentration pollution, then became decreasing when the concentration is higher than 15000 mg/kg and cells in early period changed greatly compared to other periods. Diesel plays a certain role in promoting in cell division of light pollution while been strained after treatment with heavy diesel contamination.

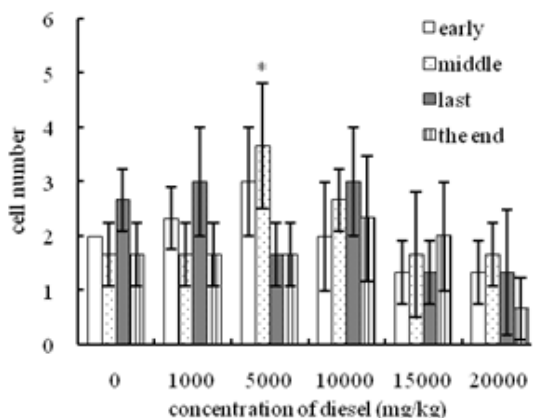


Fig. 2 Effects of diesel on mitosis of cells

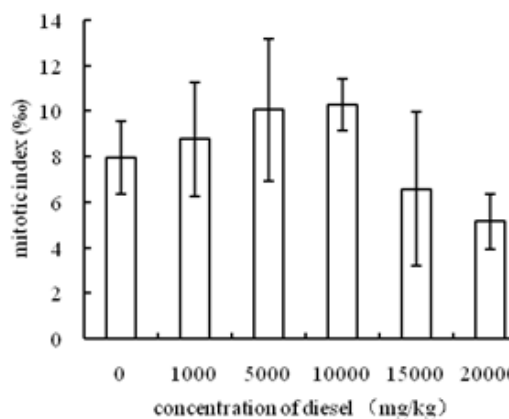


Fig. 3 Effects of diesel on mitotic index

Toxic effects of diesel on mitotic index of root cells

Mitotic index refers to a division organization of cells, the percentage of cells in mitosis issue of total cell numbers which is also an index of cell production activity and can reflect cell physiological activities. The toxic effects of diesel on root cell of *Scirpus triquetar* are different with the pollution concentrations that were showed in Fig.3. The cell division was stimulated when the concentration of diesel was lower than 5000 mg/kg and the cell division frequency increased while the mitotic index declined when concentration was higher than 5000 mg/kg.

The decline of mitosis index in this experiment can be also related to the disorder. The different periods of mitosis change is due to diesel destroyed the mitotic spindle in the process of formation. The slower progression of cells from DNA synthesis phase to mitosis phase which is resulted from the energy shortage for competition of arsenic for ADP in the citric acid cycle and the formation of arsenate-ATP in arsenic-treated samples may cause the decrease of mitotic activity[11] . In an early study, mitosis frequency was associated with increased lipid peroxidation [12].

The produce of micronucleus

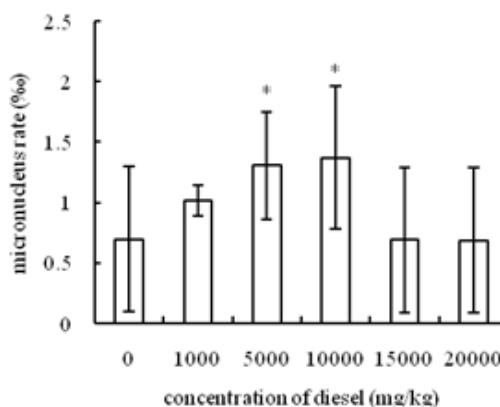


Fig. 4. Effects of diesel on micronucleus rate

Micronucleus which is also called state-II nuclear is biological eukaryotic cells from an unusual structure, a chromosome aberration in inter-phase cell in a form of expression. Micronucleus are always stimulated by all kinds of physical and chemical factors, such as radiation, chemical agents which is circular or elliptic, beside the main nuclear and the size should be less than one-third of main nuclear. The micronucleus frequency (MNF) refers to the proportion of micronucleus cells in 1000 cells. It can be seen in the Fig.4 that between the pollution concentrations from 1000-10000 mg/kg, the micronucleus gained and presented a clear dose-effect relationship.

It is found in study that cell micronucleus declined when diesel concentration is low and in a relatively high concentrations, micronucleus generally increased with the raising of pollution. The micronucleus decreased when the concentration more than a certain range that is because of chromosome structure is destroyed heavier, cell of separatist activities is restrained seriously resulted in micronucleus are difficult to produce. The increasing of micronucleus is caused by many factors, especially oxidative stress [13, 14].

Conclusions

The length and weight of root decreased in the diesel-contaminated soil. The root tip show obvious victims symptoms. The total cells in division increased significantly in the light diesel pollution soil. The number of cells in late and the end period more than in the early period at the same time. Diesel in low concentration can improve the root tip cell mitosis index but reduced gradually at higher concentration. The cell micronucleus decline when diesel concentration is low or relative high, micronucleus generally increase with the diesel concentration increasing.

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