Toxicity Assessment of Environmental Complex Mixtures in and around Roadways

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*The aim of this study was to evaluate the toxic effect of heavy metals to bacteria and plant. A spiderwort (*Tradescantis* clone BNL 4430) and coliform bacteria (*Escherichia coli* strain MV1184 and *Comamonas testosteroni* strain TK102) were used in this study. The growth of *C. testosteroni* strain TK102 was inhibited by copper, lead, and cadmium. Cadmium shows the strongest growth inhibition among these three metals. In an assay of *Tradescantis*, zinc reduced the amount of chlorophiles in leaves. Copper inhibited flowering and had a fatal effect on *Tradescantia*.*

Keywords : *Tradescantia, Comamonas testosteroni, heavy metals, growth inhibition*

1. Introduction

In railways, there are a number of metal components, which rub each other and gradually wear off (for example, wheel and rail, slider and contact wire). The resultant metal powders spread along the railroads. Metal species, which are expected to be found, are copper, lead, zinc, chromium and aluminum, for instance. Lubricants, which are used for turnouts and bearings, seem to penetrate into the soil of track.

The environmental quality standard for soil was enacted in 1991 in Japan. After that, the number of the cases of soil pollution reported increased along with the number of investigations of land soil. The major part of the pollutants is lead, arsenic, trichloroethylene and tetrachloroethylene¹⁾. However, there are no reports about the soil pollution in and around railroads. We should maintain railroads clean and safe for passengers, railway workers and residents near the railroad. To monitor the soil of railroad, we apply bioassays. One of the greatest advantages in bioassays is that we can evaluate the toxicity of complex mixtures in the environment. There are different types of assays available in bioassays, for example, the bacterial mutation assay²⁾. In this study, we investigated the growth inhibition of bacteria and the toxic effects on spiderwort (*Tradescantia*) of heavy metals because they are found around railroads³⁾.

2. Materials and methods

2.1 Bacterial strains

This study used *Comamonas testosteroni* strain TK102⁴⁾, which was isolated from soil, and laboratory strain of *E. coli* strain MV1184, which was purchased from Takara Co.. These bacteria were inoculated onto a nutrient medium containing 6.7 g/l of Luria Broth base (Gibco BRL Co.) and 1.5 % of agar. After 24-hour incubation at 30°C, the emerging colonies were transferred to 100 ml of the nutrient medium and incubated at 30°C for 48 hours

on a reciprocal shaker (100 rpm) to obtain an inoculum for bacterial assay.

2.2 Growth inhibition test on bacteria

For the bacterial growth inhibition assay, 10 ml of the inoculum was transferred to 100 ml of the nutrient medium containing heavy metals at various concentrations. The bacterial growth was monitored with respect to the optical density at 660 nm by using a Beckman DU-650 spectrophotometer after 24-hour incubation. The inhibitory effect of heavy metals on the bacterial growth was estimated by a comparison with the growth of bacteria in the nutrient medium without heavy metals.

2.3 A *Tradescantia* strain

Tradescantia clone BNL 4430 used in this study has frequently been used in studies of somatic mutations in the stamen hairs and has been shown to be more sensitive to alkylating agents than other clones.

2.4 Cultivation system for *Tradescantia*

A cultivation system equipped with a nutrient solution circulating (NSC) unit was used to cultivate *Tradescantia* (Fig.1). The NSC cultivation system was designed by Ichikawa, Saitama University⁵⁾. The NSC cultivation system is composed of three-layered growth spaces. Each growth space is equipped with four 40-W white fluorescent tubes and a polyvinyl chloride tray, 560, 400 and 70 mm in length, width and depth, respectively, with a lid having 25 open holes of 30 mm in diameter. The nutrient solution is circulated between the trays and a tank via polyvinyl chloride tubes. To set *Tradescantia* at individual open holes, polyurethane sponges with a slit were used to hold their stems and also to prevent the roots being exposed to light.

Fig. 1 A cultivation system for *Tradescantia*

2.5 Preparation and cultivation of *Tradescantia*

Young inflorescence-bearing *Tradescantia* was distributed from Ichikawa. The nutrient solution used was a 2,500 times diluted Hyponex® solution (HYPONeX Japan Co.). The characteristics of this strain, i.e., many new shoots constantly emerging from the basal nodes one after another and its short height favorable for early flowering, made it possible to prepare a lot of young inflorescence-bearing *Tradescantia* at one time. Usually, approximately 35-50 young inflorescence-bearing *Tradescantia* could be prepared weekly in the NSC cultivation system. New young shoots emerged from the basal nodes were also cut off to grow new young inflorescence-bearing *Tradescantia*. During the cultivation, *Tradescantia* was rearranged depending on their developmental stages once a week, and the nutrient solution was exchanged every two weeks. Whenever the volume of the nutrient solution was reduced, 4,000 times diluted Hyponex® solution was added to the tank. The NSC cultivation system was placed in a room, where the temperature was kept at 19°C and the day length was 16 hours.

2.6 Treatment of *Tradescantia* with heavy metals

To see the effect of copper, *Tradescantia* was cultivated for 20 hours in the nutrient solution containing copper sulfate. Copper sulfate was rinsed out of the root under tapping water and was transferred to the nutrient medium for further cultivation.

To see the effect of lead and zinc, lead sulfate or zinc sulfate was added to the nutrient solution separately and *Tradescantia* was cultivated in it during an experimental period.

3. Results and discussion

3.1 Bacterial assay

3.1.1 Inhibitory effect of copper

To investigate the inhibitory effect of copper on bacterial growth, a copper sulfate pentahydrate was added to the nutrient medium at the concentration of 1, 10, 100, and 1,000 mg/l. At the concentration of 1 and 10 mg/l, growth inhibition was not observed in either bacterium. Although the growth of *C. testosteroni* strain TK102 was greatly inhibited (above 80%) by addition of 100 mg/l of copper sulfate, the growth inhibition in *E. coli* strain MV1184 was not so strong (less than 20%) as in the strain TK102 (Fig. 2A).

3.1.2 Inhibitory effect of lead

Lead acetate trihydrate or lead monoxide was added to the nutrient medium at the concentration of 1, 10, 100, and 1,000 mg/l. There was no inhibitory effect observed in either bacterium in the case of the lead acetate trihydrate. In contrast, when lead monoxide was added, the growth of the strain TK102 was inhibited by 20% and 60% at the concentration of 100 and 1,000 mg/l, respectively (Fig. 2B). The inhibitory effect of lead was weaker than that of copper. The growth of the strain MV1184 was not inhibited

(A) Copper sulfate

(B) Lead oxide

(C) Cadmium sulfate-water

Copper sulfate (A), lead oxide (B) and cadmium sulfate-water (C) were added to the nutrient medium

Fig. 2 Growth inhibition of heavy metals in bacteria

at all even at the concentration of 1,000mg/l. It seemed that strain TK102 is more susceptible to heavy metals than strain MV1184. So, the strain TK102 was chosen for the test strain in the following experiment.

3.1.3 Inhibitory effect of cadmium

Cadmium sulfate-water (3/8) was added to the nutrient medium at the concentration of 1, 2, 4, 6, 8, and 10 mg/l. The growth was inhibited by around 20% and 60% at the concentration of 4 and 8 mg/l, respectively (Fig. 2C). At 10 mg/l, the growth was inhibited completely. Cadmium showed the strongest growth inhibition among three heavy metals tested in this experiment.

3.2 Plant assay

3.2.1 Growth inhibition of lead

To investigate the growth inhibition of lead on *Tradescantia*, lead sulfate was added to the nutrient solution at the concentration of 1,000 mg/l. There was no apparent deterioration observed on *Tradescantia* after the cultivation for one month. However, lead sulfate hardly dissolves into water around neutral pH. According to literature, the maximum concentration in water was 0.45 mg/l at pH 7. Although lead sulfate dissolves into water at acidic pH better than at neutral pH, *Tradescantia* does not grow in acidic environment well. So, we can not conclude whether lead sulfate is harmful to *Tradescantia*.

3.2.2 Growth inhibition of zinc

To investigate the growth inhibition of zinc on *Tradescantia*, zinc sulfate heptahydrate was added to the nutrient solution at the concentration of 20 mg/l. As shown in Fig. 3, a chlorolysis was observed on the leaves (whitish leaves), which were emerged after *Tradescantia* was transferred into the nutrient solution containing zinc sulfate. Chloroplasts were extracted from leaves and the concentration of chlorophyll was determined by Maclachlan's protocol⁶⁾. A reduction of 60% of chlorophyll was observed in whitish leaves when compared to that in the healthy leaves. Besides the chlorolysis, development

Fig. 3 Effect of zinc in *Tradescantia*

of inflorescence was completely inhibited and flowering was not observed during the cultivation period for one month.

3.2.3 Growth inhibition of copper

To investigate the growth inhibition of copper on *Tradescantia*, copper sulfate pentahydrate was added to the nutrient solution at the concentration of 10, 25.4, 63.5, and 125 mg/l. *Tradescantia* died out within seven days after the treatment of copper sulfate at 63.5 and 125 mg/l (Fig. 4). In the treatment of copper sulfate at 25.4 mg/l, a few buds were dried up (Fig. 5) and flowering was delayed by 10 days when compared to the control specimen. Once it started flowering, it had a flower each day during the following two weeks. With the treatment of copper sulfate at 10 mg/l, the delay of flowering or the dying out was not observed. However, from the observation of a stamen hair with a dissecting microscope, it seemed that the number of branching on a stamen hair increased by the treatment. The stamen hair consists of 20 to 30 cells in a line. Branches seemed to result from the fact that copper sulfate might affect the number and/or the direction of the cell division. The influence of copper sulfate upon *Tradescantia* varies with its concentration. To find out whether this concentration dependent effect is general among other heavy metals, we should repeat the same experiment with different kinds of heavy metals.

Fig. 4 Effect of copper in *Tradescantia*

Fig. 5 Dried up buds by copper sulfate treatment

4. Conclusion

According to the report published by the Ministry of the Environment in 1984, the average concentration of copper and zinc in soil in Japan was 25 and 55 mg/kg, respectively. In this study, the growth inhibition around those concentrations was observed. We believe that bioassays using *Comamonas testosteroni* strain TK102 and *Tradescantia* clone BNL 4430 will be useful as a tool to assess soil toxicity. We plan to investigate the genotoxicity (bad effect on DNAs) of heavy metals below the concentration, at which we observed growth inhibition in this experiment. To develop a bioassay to monitor the soil in railroads, we must take into consideration not only heavy metals but also chemicals found along the track, such as polyaromatic hydrocarbons (PAHs) used as a wood preservative³⁾. It is hard to deduce the health risk of soil from only the results of chemical analysis. There is no data available referring to the biological toxicity of environmental complex mixtures, especially in the case when the concentration of each contaminant was below the quality standards. So, bioassays will play an important role to monitor the environmental contamination in the future.

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