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The sensitivity of an hydroponic lettuce root elongation bioassay to metals, phenol and wastewaters

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ABSTRACT

The root elongation bioassay is one of the most straightforward test methods used for environmental monitoring in terms of simplicity, rapidity and economy since it merely requires filter paper, distilled water and Petri dishes. However, filter paper as a support material is known to be problematic as it can reduce the sensitivity of the test.

The newly developed hydroponic method reported here differs from the conventional root elongation method (US EPA filter paper method) in that no support material is used and the exposure time is shorter (48 h in this test versus 120 h in the US EPA test). For metals, the hydroponic test method was 3.3 (for Hg) to 57 (for Cu) times more sensitive than the US EPA method with the rank orders of sensitivity, estimated from EC_{50} values, being $Cu \geq Cd > Ni \geq Zn \geq Hg$ for the former and $Hg \geq Cu \geq Ni \geq Cd \geq Zn$ for the latter methods. For phenol, the results did not differ significantly; EC_{50} values were 124 mg L^{-1} and 108–180 mg L^{-1} for the hydroponic and filter paper methods, respectively. Lettuce was less sensitive than daphnids to wastewaters, but the root elongation response appears to be wastewater-specific and is especially sensitive for detecting the presence of fluorine.

The new hydroponic test thus provides many practical advantages, especially in terms of cost and time-effectiveness requiring only a well plate, a small volume of distilled water and short exposure period; furthermore, no specialist expertise is required. The method is simpler than the conventional EPA technique in not using filter paper which can influence the sensitivity of the test. Additionally, plant seeds have a long shelf-life and require little or no maintenance.

1. Introduction

As a consequence of human activities and the rapid development of chemical industries, new and potentially harmful substances are being introduced into the environment. The development of modern chemistry has contributed not only to the increase in the number of known chemicals, but also to the synthesis of 1200 new substances every day (Markert et al., 2000). Without proper ecological risk assessment of chemicals and appropriate efforts to formulate effective protective legislation, the natural environment will be endangered by the thousands of potentially damaging chemicals derived annually from industrial and municipal sources (Mallick and Rai, 2002).

Analytical chemistry provides quantitative measurements of the presence of contaminants in the environment but it does not offer ecologically meaningful information on their potential toxic effects. Furthermore, from using chemical methods alone it is not possible to predict whether mixtures of different toxicants will act synergistically, additively or antagonistically. As a consequence, biological toxicity testing is widely employed to assess the toxicological threat posed by different classes of chemicals. Such an

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approach reduces the time and cost associated with blind chemical screening for a wide array of contaminants and provides information for subsequent chemical characterization efforts (Bisson et al., 1989). Moreover, assessment of ecologically meaningful levels of pollution has been made possible which, in turn, can help in evaluating the health of the environment.

Phyto-toxicity bioassays using higher plants have been widely applied for biomonitoring of various environmental toxicants (Wang, 1991), with root elongation being the most commonly used endpoint. The physiological effects on plants may be closely related to the absorption and accumulation of toxicants, and the first organ to be affected is the root. Thus, reduction in root length is considered a valid and sensitive response to exposure to chemicals (Ratsch, 1983). In general the root elongation test merely requires filter paper, distilled water and Petri dishes. However, the use of filter paper to support the plant is known to be problematic. Firstly, the paper is an inconvenience during measurements as roots become strongly attached to the substrate, making the collection of whole roots difficult (Wang, 1993). Secondly, roots tend to grow either horizontally or in a non-linear fashion on filter paper, leading to inaccuracies in measurements and thirdly the paper substrate is known to interfere with toxicant bioavailability, due to adsorption to the paper (Wang, 1993).

Many attempts have been made to replace paper with other supports, in order to overcome these difficulties. One alternative method is the seed tray device which is small, simple, inexpensive, strong, reusable, and does not interfere with test compounds (Wang, 1993). However, roots are easily cut off or remain attached to the upper side of the device, and require individual handling. A variation on this is to sow seeds onto a fine nylon mesh which is then suspended on the surface of a beaker containing the treatment solution (Wong and Bradshaw, 1982). Another method uses seeds immersed in solution within volumetric flasks that are constantly aerated and suspended using compressed air (Berry, 1978). The aerated bottle test offers some advantages over more conventional methods in that planting can be done rapidly, as there is no need to align seeds on a substrate, and root elongation is easily measured since roots grow relatively straight. There is also apparent improvement in sensitivity, perhaps related to uniform exposures to the test chemicals (Ratsch and Johndro, 1986). More recently, an agar test has been developed that demonstrated higher sensitivity to 4 metal species (Cd, Cu, Ni, and Pb) together with its simplicity, rapidity, and low cost as compared with the paper method (Salvatore et al., 2008).

Many plant species have been recommended for ecotoxicological testing using root elongation of germinated seeds (Wang and Keturi, 1990) among which lettuce seed (*Lactuca sativa*) has been recommended by the U.S. Environmental Protection Agency (EPA) (1982), the U.S. Food and Drug Administration (1987), and the Organization for Economic Cooperation and Development (OECD) (1984).

The aims of this study were to evaluate if (1) hydroponically grown lettuce seeds with no conventional substrate could be used for the root elongation toxicity test, (2) measurements of root elongation 48 h after seed germination was as sensitive as the conventional 96 h test and (3) this lettuce root elongation test was as sensitive as the 48 h *Daphnia magna* mortality test for assessing the toxicity of wastewaters.

2. Materials and methods

2.1. Plant materials

Packets of lettuce seeds with a storage time of 1–2 year(s) were obtained from commercial outlets. After use, the remaining seeds were stored at 5 °C in a 50 ml conical tube (115 × 30 mm^2, SPL, Korea) with a lid that had 10 holes of 1 mm diameter to provide ventilation. The maximum storage periods were 3 months.

2.2. Preliminary experiments

Before carrying out the main study, preliminary experiments were conducted to determine how many seeds were appropriate for toxicity testing and how long seeds should be exposed to toxicants, using $CuSO_4$ (CAS No. 7440-50-8) as a reference toxicant. The number of seeds tested ranged from 1 to 11, and the exposure periods ranged between 48 and 168 h. There were five diluted solutions plus one control with 10 mL distilled water.

2.3. Standardization of testing conditions

For determination of optimal testing conditions, three cell plates each consisting of 6 wells (15 ml volume per well; SPL, Korea) filled with 10 mL of distilled water were prepared. Five lettuce seeds were then placed and floated on the surface of distilled water in each well and the cell plates then sealed with parafilm before exposure to various environmental conditions for 48 h: pH (3–10), photon irradiance (0–150 μmol photons m^{-2} s^{-1}) and temperature (5–25 °C). While testing each single factor, the other environmental conditions were kept optimal: pH 5–6, photon irradiance at 0 μmol photons m^{-2} s^{-1}, and temperature at 20 °C. Distilled water was not replaced during the test period. After 48 h exposure, seeds were harvested for determination of their root elongation using a computer-assisted imaging analysis system (Moticam 2000, Ted Pella Inc., USA).

2.4. Toxicity testing

Under optimal environmental conditions, toxicity testing was conducted by dispensing 10 mL distilled water into one cell as control and five different concentrations of 10 mL test solution into the other cells in a six-well plate. Hydroponic culture was established by floating five lettuce seeds on the surface of control or test solution in each well. A lid was placed over the cell plate (s) and then sealed with a sealing tape to prevent evaporation of the solutions. Three replicate cell plates were prepared and were incubated in the dark at 20 ± 1 °C. After a 48 h incubation period, germinated seeds were harvested and their root lengths measured. Seed germination occurred simultaneously in all treatments and the germination percentage within 24 h was > 90% so smaller roots were not due to delayed germination but a direct effect of the treatment on root growth. The test toxicant concentration ranges used were 0.0625–1.0 mg L^{-1} for Cd (CAS No. 7440-43-9) and Cu 0.125–5.0 mg L^{-1} for Hg (CAS No. 7439-97-6), 0.156–5.0 mg L^{-1} for Ni (CAS No. 7440-02-0), 0.312–5.0 mg L^{-1} for Zn (CAS No. 7440-66-6) and 0.03125–0.5 mg L^{-1} for phenol (CAS No. 108-95-2). Stock solutions of concentrated standards were diluted with distilled water and adjusted to optimal pH with 1 N sodium hydroxide (NaOH) (Junsei, Tokyok, Japan).

Direct comparison of the hydroponic root elongation method with the US EPA filter paper assay was made simultaneously. The US EPA test involved dispensing 4 ml control (distilled water only) and test solutions into Petri dishes (100 × 15 mm^2, SPL, Korea), each containing filter paper (Ø 90 mm, Whatman No. 3 filter). Five lettuce seeds were placed on the filter paper in a Petri dish that was then covered and sealed with a sealing tape to prevent evaporation of the solution before incubation at 24 ± 2 °C. After 120 h, germinated seeds were carefully harvested using plastic tweezers and the respective root lengths were measured with an image analyzer.