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Testing the toxicity of metals, phenol, effluents, and receiving waters by root elongation in *Lactuca sativa* L.

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ABSTRACT

Phytotoxicity tests using higher plants are among the most simple, sensitive, and cost-effective of the methods available for ecotoxicity testing. In the present study, a hydroponic-based phytotoxicity test using seeds of *Lactuca sativa* was used to evaluate the water quality of receiving waters and effluents near two industrial sites (Soyo and Daejon) in Korea with respect to the toxicity of 10 metals (As, Cd, Cr, Cu, Fe, Pb, Mn, Hg, Ni, Zn) and phenol, and of the receiving waters and effluents themselves. First, the *L. sativa* hydroponic bioassay was used to determine whether the receiving water or effluents were toxic; then, the responsible toxicant was identified. The results obtained with the *L. sativa* bioassay ranked the EC_{50} toxicities of the investigated metal ions and phenol as: Cd > Ni > Cu > Zn > Hg > phenol > As > Mn > Cr > Pb > Fe. We found that Zn was the toxicant principally responsible for toxicity in Daejeon effluents. The Daejeon field effluent had a higher Zn concentration than permitted by the effluent discharge criteria of the Ministry of Environment of Korea. Our conclusion on the importance of Zn toxicity was supported by the results of the *L. sativa* hydroponic assay, which showed that the concentration of Zn required to inhibit root elongation in *L. sativa* by 50% (EC_{50}) was higher in the Daejeon field effluent than that of pure Zn. More importantly, we proved that the *L. sativa* hydroponic test method can be applied not only as an alternative tool for determining whether a given waste is acceptable for discharge into public water bodies, but also as an alternative method for measuring the safety of aquatic environments using EC_{20} values, with respect to the water pollutants investigated (i.e., Cd, Cr, Cu, Pb, Mn, Hg, Ni, Zn, and phenol).

1. Introduction

The use of phytotoxicity tests employing higher plants was infrequent in the field of ecotoxicology until the 1970s. During the period 1964–1984, when environmental pollution by herbicides was widespread, most studies used faunal species tests to assess herbicide toxicity rather than tests involving higher plants (Wang, 1991). However, there has been a growing awareness that the impact of herbicides is much greater on flora than on fauna and, since the 1980s, an increasing number of studies have compared the toxicity of metals and herbicides in plants and algae. It has been shown that tests using higher plants are of value for environmental monitoring owing to their simplicity, sensitivity, and cost-effectiveness. Plant tests also offer one of the few options available for monitoring pollution in soil, sediment, rain, water, wastewater, and solid waste (Garten and Frank, 1984; Fletcher et al., 1985; Miller et al., 1985; Thomas et al., 1986). Phytotoxicity tests are therefore now considered as a vital part of comparative toxicological assessment (Wang, 1989).

Lactuca sativa is a model plant species for phytotoxicity tests and has been recommended by many international organizations for the determination of the ecological effects of toxic substances and for standard toxicity tests (ISO, 1995; USEPA, 1996; OECD, 2003). Phytotoxicity tests using *L. sativa* are simple, quick, reliable, and inexpensive and do not require expensive equipment (Wang and Williams, 1990; Charles et al., 2011; Park et al., 2016). In particular, the seed germination and root elongation test is one of the simplest methods of phytotoxicity testing (Wang and Williams, 1988, 1990; Wang and Keturi, 1990; Araújo et al., 2001; Park et al., 2016) because the germinating seed is the first interface of material exchange between the developing plant and the environment (Ernst, 1998). *L. sativa* is more sensitive to

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inhibition of root elongation by chemical contaminants than other plant species, such as cucumber, radish, red clover, wheat (Thomas et al., 1986), Swiss chard, and spinach (Bautista et al., 2013). As a result, *L. sativa* root elongation tests have been widely used for testing the toxicity of pure chemicals (Adema and Henzen, 1989; Castillo et al., 2000; Ronco et al., 2000; Fjällborg et al., 2006; Benzarti et al., 2008; Park et al., 2016), phenolic compounds (Reynolds, 1978; Hulzebos et al., 1993; Park et al., 2016), and effluents (Park et al., 2016). Based on the inhibition of root length in *L. sativa*, Park et al. (2016) identified fluorine as the chemical responsible for the toxicity of wastewaters from the plating industry, indicating that the *L. sativa* root elongation test is a practical bioassay for assessing the toxicity of complex effluent materials.

Many instances of environmental water contamination with potentially adverse effects on human health have been reported; most of these are a result of anthropogenic activities, for example, discharge from industries, and dumping and accidental spills during the transportation of chemicals or hazardous wastes, (Hans et al., 1999; Holt, 2000; WWF-UK, 2004; Giraud et al., 2009; Kjersem et al., 2009; Palma et al., 2010). In particular, discharges from industrial and commercial sources often contain pollutants at high concentrations that might affect the quality of receiving waters or interfere with publicly owned treatment works (POTWs) that receive this discharge. The discharge of effluents into streams (receiving water) can result in the release of heavy metals and phenols into the environment in forms that are easily assimilated by fauna and flora that inhabit or make use of these water sources (Wase and Forster, 1997; Wake and Estuarine, 2005). These toxicants can then accumulate in organisms and be bio-magnified through food webs (Charles et al., 2011). Heavy metals such as As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn can cause adverse effects or potentially affect humans as well as aquatic life in rivers (Reynolds, 1978; Bascombe et al., 1990; Temesagdie and Takano, 1992; Hulzebos et al., 1993; De Boeck et al., 1998; Mujde et al., 1998; Stevens et al., 1998; Blais et al., 1999; Flaten, 2001; Blunden and Wallace, 2003; Giguere and Campbell, 2004; Jha et al., 2009; Park et al., 2016); these metals are primarily pollutants from industrial and municipal effluents (Michalowicz and Duda, 2007; USEPA, Priority pollutant list). In addition to heavy metals, phenols are a ubiquitous environmental pollutant associated with the generation of industrial and municipal effluents (Michalowicz and Duda, 2007).

In this study, we tested 10 metals (As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn), phenol, and effluent and receiving water samples collected from two industrial sites in Korea. These were tested using the *L. sativa* hydroponic method that has been standardized by Park et al. (2016). We performed toxicity testing using *L. sativa* root elongation to determine whether given effluents are suitable for general discharge. These tests and comparisons will enable us to assess whether toxicity testing using *L. sativa* root elongation can accurately determine whether aquatic environments are safe for the living organisms that inhabit them.

2. Materials and methods

2.1. Plant materials

L. sativa seeds were purchased from a local seed market (Incheon, Korea). After extracting seeds for use in the experiment, the remaining seeds were stored at 5 °C in a 50-mL conical tube (115 mm length, 30 mm diameter; SPL, Korea) with a lid containing 10 1-mm holes to provide ventilation, as described by Park et al. (2016).

2.2. Toxicants toxicity testing

Based on the optimal testing conditions for *L. sativa* (Park et al., 2016), toxicity testing for this study was conducted by dispensing 10 mL distilled water into one well of a six-well plate to serve as a control, and dispensing 10 mL of five concentrations of a test solution into the other wells of the same plate (6-well plate, SPL, Korea). Then, five *L. sativa* seeds were placed on the surface of the control or test solutions in each well (Park et al., 2016). A lid was placed over the plate (s) and sealed with parafilm to prevent evaporation of the solutions. Three replicate plates were prepared and incubated in the dark at 25 ± 1 °C. After 2 d, 5 d, and 10 d, germinated seeds were harvested and their root lengths were measured using a computer-assisted imaging analysis system (Moticam 2000, Ted Pella Inc., USA) as described by Park et al. (2016). Stock solutions of concentrated standards (1000 mg L^{-1}) were diluted with distilled water, decreasing in binary steps. The test toxicant concentration ranges used were 0.156–5.0 mg L^{-1} for As (CAS no. 7440-38-2), Cr (CAS no. 7440-47-3), Fe (CAS no. 7439-89-6), Pb (CAS no. 7439-92-1), and Mn (CAS no. 7439-96-5), and 0.0625–1.0 mg L^{-1} for Cd (CAS no. 7440-43-9), Cu (CAS no. 7440-50-8), Hg (CAS no. 7439-97-6), Ni (CAS no. 7440-02-0), Zn (CAS no. 7440-66-6), and phenol (CAS no. 108-95-2). For each test solution, the pH was adjusted to 7.0 using 1 N sodium hydroxide (NaOH) (Junsei, Tokyo, Japan).

2.3. Receiving waters and effluents toxicity testing

Effluents discharged from a wastewater treatment plant and receiving waters in an adjacent stream were collected from two industrial sites in Soyo (N 37°56′47.1″; E 127°03′32.9″) and Daejeon (N 38°00′22.2″; E 127°04′56.4″) in Korea, during March and April 2016. The points of sampling receiving waters were chosen at distances of 50 m upstream and downstream from the discharge point, respectively (Fig. 1 in Supplementary file). Water samples were transported in sterilized polyethylene containers on ice to the laboratory and stored at 4 °C before experiments were conducted. Initial toxicity tests and water quality analysis were conducted upon the arrival of the samples as described previously (Pandey et al., 2018). Briefly, metals (Cu, Fe, and Zn) in the water samples were analyzed by an inductively coupled plasma-optical emission spectrophotometer (ICP-OES; Varian Vista PRO, CA, USA) using 15-mL water samples. All the samples collected for analyses of metals were previously filtered through 0.45-μm filters (Whatman® ReZist 13 syringe filter, product code Z648264, ALDRICH) without acidification.

The toxicity screening tests of receiving waters and effluents were conducted using the original water samples. After 2 d, the measurements of seed germination and root elongation of *L. sativa* were performed with a computer-assisted imaging analysis system (Moticam 2000, Ted Pella Inc., USA) to preliminarily characterize the toxic water samples. Based on this, a definitive toxicity test was conducted for the toxic water by using a concentration series of 100%, 50%, 25%, 12.5%, and 6.5% of the original water sample. After 48 h, root elongation was measured to determine the concentration that caused 50% inhibition in the root elongation of *L. sativa*.

2.4. Statistical analysis

Results are reported as EC_{50} (effective concentration at which 50% root elongation inhibition occurred) at 95% CI (confidence intervals) estimated by linear interpolation (ToxCalc 5.0, Tidepool Scientific Software, California, USA). One-way analysis of variance (ANOVA), followed by the least significant difference (LSD) post-hoc test at $P < 0.05$ were used to determine the significance of differences between treatments.

3. Results and discussion

3.1. Effects of toxicants on root elongation in L. sativa

The relative toxicities of essential and non-essential elements were tested in *L. sativa* using our hydroponic method (Park et al., 2016).