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The effects of pruning and nodal adventitious roots on polychlorinated biphenyl uptake by *Cucurbita pepo* grown in field conditions

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The application of cultivation practices (pruning and nodal adventitious root encouragement) increases phytoextraction of PCBs in C. pepo.

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ABSTRACT

Two cultivation techniques (i-pruning and ii-nodal adventitious root encouragement) were investigated for their ability to increase PCB phytoextraction by *Cucurbita pepo* ssp *pepo* cv. Howden (pumpkin) plants *in situ* at a contaminated industrial site in Ontario (Aroclor 1248, mean soil [PCB] = 5.6 μg g^{-1}). Pruning was implemented to increase plant biomass close to the root where PCB concentration is known to be highest. This treatment was found to have no effect on final shoot biomass or PCB concentration. However, material pruned from the plant is not included in the final shoot biomass. The encouragement of nodal adventitious roots at stem nodes did significantly increase the PCB concentration in the primary stem, while not affecting shoot biomass. Both techniques are easily applied cultivation practices that may be implemented to decrease phytoextraction treatment time.

1. Introduction

Polychlorinated biphenyls (PCBs) were widely used in North America from the 1920s to the 1970s and extensive environmental contamination is still present worldwide. The treatment of PCB-contaminated soil using incineration and landfilling removes or physically destroys the soil matrix. Yet, soil is an important renewable resource that provides nutrients for food crops and serves as the basis of habitation for both wildlife and humans. Phytoremediation may offer an alternative method that preserves and enhances the soil matrix, lowers overall costs and has a high acceptance from the public (Cunningham and Ow, 1996; Pilon-Smits, 2005).

Traditionally, persistent organic pollutants (POPs) such as PCBs were thought of as unlikely candidates for phytoremediation due to their hydrophobicity (log $K_{ow} > 3.5$) (Bacci and Gaggi, 1985; Briggs et al., 1982; Reischl et al., 1989). However, some members of the species *Cucurbita pepo* ssp. *pepo* (zucchini and pumpkin) can accumulate a variety of hydrophobic POPs (Hülster et al., 1994; Lunney et al., 2004; Mattina et al., 2004; Otani et al., 2007; White, 2002) including PCBs (White et al., 2006; Whitfield Åslund et al., 2007; Zeeb et al., 2006). It has been shown that these contaminants are taken up by plant roots and translocated to plant shoots via xylem sap in the transpiration stream (Gent et al., 2007; Whitfield Åslund et al., 2008).

Currently phytoextraction of PCBs is viewed as a potential long-term solution to remediating soil. The amount of contaminant extracted per plant is low and limited to the growing season, which may be quite short in the temperate zone. Decades may be needed to remediate relatively low (<10 μg g^{-1}) concentrations of PCBs in soil. In order to optimize this technology, extraction must be maximized through i) increasing the concentration of the contaminant in the above-ground plant biomass, and/or ii) increasing the amount of plant biomass produced.

PCB concentration in pumpkin plant stems and leaves has been observed to decrease as the distance from the root increases (Whitfield Åslund et al., 2007, 2008). This pattern was reported in a field study which involved a mixture of PCB Aroclors 1254 and 1260. It is hypothesized that the pattern of decreasing concentration with distance is due to progressive adsorption of PCBs to the hydrophobic xylem components including vessel walls and lignins found in the lower part of the stem (Campanella et al., 2002).

Pruning can control the growth pattern of plants. It is generally implemented to promote shorter, bushier plants, which otherwise tend to have a vine-like growth pattern with stem lengths exceeding 6 m in some cases (Stevens, 1994; Whitfield Åslund et al., 2007, 2008). The majority of peer reviewed pruning experiments involve trees and food crops. Early reports (Stein, 1955) indicate that a certain level of pruning will promote stem growth in trees.

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However, Chandrashekara (2007) noted that foliage and branch biomass production was altered due to pruning, but results varied between species. A study conducted by Serna et al. (2004) observed that pruning pipiana pumpkins (*Cucurbita argyrosperma Huber*) had no significant effect on the number of fruits and/or seed weight. In the current study, pruning was implemented to maximize the amount of biomass close to the root (highest PCB concentration), while preventing the development of biomass at increased distance from the root, in an attempt to phytoextract a higher total amount of PCBs from the soil.

Adventitious roots occur on many species of dicotyledonous plants in several forms including aerial, strangling, stilt, nodal and nest roots (Costello, 2005; Paolillo and Zobel, 2002) *C. pepo* plants are able to form nodal adventitious roots which are similar in function and structure to a primary root, yet form at atypical locations such as stem nodes (Neuman and Hansberry, 2006). As in primary roots, the principle function of adventitious roots is the absorption of water, oxygen, mineral elements and nutrients as well as hormone synthesis (Barlow, 1986; Costello, 2005). It has been demonstrated that *C. pepo* ssp *pepo* plants have the ability to mobilize POPs from soil into their roots and then transfer them throughout the plant via the transpiration stream (Gent et al., 2007; Whitfield Åslund et al., 2008). Therefore it is possible that the encouragement of nodal adventitious roots could also lead to increased POPs uptake. This hypothesis was supported by observations from Whitfield Åslund et al. (2008), who suggested the presence of nodal adventitious roots in *C. pepo* may have altered the gradient of PCBs normally observed in shoot tissue. In that study, one treatment included growing pumpkins in potting soil but allowing the shoot to access surrounding contaminated soil. The plants produced nodal adventitious roots into the surrounding contaminated soil possibly to aid in nutrient and water uptake. The presence of these roots altered the typical PCB gradient from primary root > lower shoot > upper shoot to primary root < lower shoot > upper shoot.

The present study was designed to increase the phytoextraction of PCBs through both pruning and the encouragement of nodal adventitious roots.

2. Materials and methods

2.1. Site description and soil preparation

This study was carried out at a former industrial site located in southern Ontario, Canada. From the 1950s–1970s, PCBs were inadvertently released onto the soil at various locations across the site, and at times migrated into the adjoining creek. Following a site investigation (by the Ontario Ministry of the Environment in 2000), an environmental consulting firm (CH2M HILL) provided and implemented a remedial action plan to clean up the site in July 2005. Soil and sediments from an impacted creek were disposed of in a hazardous waste disposal facility (>45 μg g^{-1} PCBs), a licensed industrial waste facility (25–45 μg g^{-1} PCBs), or placed in a containment cell constructed on site (0.3–25 μg g^{-1} PCBs), consistent with the Ontario and Canadian laws and guidelines for contaminated soil and sediment on industrial property.

A research area measuring 12 m × 12 m was made available to the Royal Military College of Canada in the spring of 2006 for phytoremediation research. Prior to homogenization, the soils ranged from <0.5–33.6 μg g^{-1} PCB with an Aroclor 1248 signature. The soil is predominantly clay with: 4.3% total organic carbon, 441 μg g^{-1} Kjeldahl nitrogen, 2.5 μg g^{-1} nitrogen as ammonia, <2.0 μg g^{-1} nitrogen as nitrite, 25.7 μg g^{-1} nitrogen as nitrate, 749 μg g^{-1} total phosphorus, 29.2 μg g^{-1} extractable phosphorus, and 813 μg g^{-1} acid extractable phosphorus. Total organic carbon was analyzed by loss on ignition. Total Kjeldahl nitrogen (TKN), total phosphorus and extractable phosphorus were analyzed by RPC Laboratories, Fredericton, New Brunswick. The methods used were colorimetric (APHA 4500-NH3 G; American Public Health Association et al., 2005), ICP (EPA 3050B; United States Environmental Protection Agency, 1986) and sodium citrate extraction followed by ICP analysis respectively. For extractable ammonia, nitrate and nitrite, a 2 M KCl solution was used. Moisture content was first determined and then a 5 g of dry equivalent sample of the wet soil weighed out and 25 mL of KCl added. Vials containing the resultant mixture were shaken for 30 min at 200 rpm, then filtered through P5 filter paper and analyzed on Technicon Autoanalyzers for each analyte. In order to prepare the site for phytoextraction, it was homogenized using a gas powered rototiller, and amended with top soil (3 cm across the field) and fertilizer (140 g m^{-2}, 6:12:12 C-I-L Tomato Food) to encourage plant growth.

During the first year of access in 2006, a preliminary study was undertaken to measure the feasibility of pumpkin growth in this soil. Six pumpkins were successfully grown and phytoextracted PCBs from the soil (mean root PCB concentration: 39.5 ± 12.5 μg g^{-1}; mean 0–20 cm shoot PCB concentration: 19.1 ± 4.9 μg g^{-1}). The current study was undertaken in June 2007. Prior to planting, the soil was again fertilized, at the recommended rate and homogenized using a rototiller. The plot was divided into twelve 9 m^2 subplots and soil samples were collected at a depth of 0–20 cm from the centre of each subplot. Soil samples were placed in labeled Whirlpak bags and refrigerated at 4 °C until time of analysis.

After soil samples were collected, a black woven polypropylene ground cover (constructed by Canadian Hydrogardens Ltd., Ancaster, Ontario) was laid over the planting area to minimize weeds and soil contact with growing plant shoots.

2.2. Planting and maintenance

In the centre of each of the 12 subplots, the groundcover was cut and *C. pepo* ssp *pepo* cv. Howden (pumpkin) seeds (obtained from the Ontario Seed Company, Waterloo, Ontario) were planted in mounds with five seeds per mound. After germination, the plants were thinned to allow the largest plant to continue growing without competition. Plants were monitored daily and watered as necessary. Once a week, female flowers were removed to prevent fruit development, and records of plant description and shoot lengths were updated.

The field experiment consisted of two treatments, pruning (P) and encouragement of adventitious roots (R), with the treatment present (+) or absent (−). These two treatments were crossed to form four treatment combinations (P−R−, P+R−, P−R+, P+R+) which were replicated in triplicate in randomly assigned subplots.

In the pruned treatment (P+), secondary shoots were trimmed (a maximum of 112 cm of stem removed per treatment application and composted) four times over the growing season and tertiary stems were removed to encourage bushy growth near the base of the plant (Fig. 1c,d). Plants that were not pruned (P−) were allowed to grow naturally (Fig. 1a,b). In the adventitious root treatment (R+), stem nodes were buried on a weekly basis and kept moist to encourage nodal adventitious root development (Fig. 1b,d), and the polypropylene ground cover was cut to facilitate the necessary soil contact. In the R− treatment, natural nodal adventitious roots were prevented from forming by the presence of the polypropylene ground cover; in the rare cases where formation occurred, the nodal adventitious roots were removed (Fig. 1a,c).

2.3. Site harvesting

Plants were harvested after 76 days of growth and separated into roots (below ground biomass) and shoots (above-ground biomass). The roots and shoots were washed with water to remove soil and dust particles and the lengths and fresh masses of tissues were recorded. Only final plant weight was recorded. Pruned material was immediately composed on site and the weight was not recorded. The presence and location of any nodal adventitious roots and/or secondary stems was also noted and recorded for each plant. Soil was collected from the root zone of each plant and stored in labeled Whirlpak bags at 4 °C until time of analysis.

For each harvested plant, the main root was collected whole. For plants with nodal adventitious root encouragement, the nodal adventitious roots were collected from the entire plant and combined into one composite sample. Due to the length of plant shoots (some greater than 5 m), representative subsamples (Whitfield Åslund et al., 2008) were collected as follows. For each plant, 20 cm shoot subsamples were collected from the bottom of the shoot, the end of the shoot, and two equidistant intervals along the plant shoot length (for a total of four primary shoot subsamples). Four 20 cm long shoot samples were also collected from secondary shoots. When possible, the secondary shoot samples were collected at the same distance from the root as the primary stem samples (Fig. 2). All shoot subsamples were separated into leaves (including petioles) and stems. A total of 146 plant samples and 24 soil samples were collected.

All cutting of plant tissues was carried out with metal blades which were rinsed with methanol or acetone between cuts. Plant samples were placed in labeled Whirlpak bags and frozen until time of analysis. All plant matter not collected for analysis was cut into small segments and placed in an on-site compost bin.

2.4. Analytical procedures

All samples were analyzed at the Analytical Services Unit (ASU) at Queen's University in Kingston, Ontario. Soil and plant tissues were analyzed for total Aroclor concentrations as described in detail by Whitfield Åslund et al. (2007, 2008).

Prior to extraction, soil samples were well mixed in the Whirlpak bags and subsamples of approximately 10 g wet soil were collected for PCB analysis. An additional 10 g subsample of wet soil was collected and dried at 100 °C for 24 h prior to measuring weight to determine the wet/dry ratio of each soil. Approximately 10 g of each plant sample was cut into small pieces with scissors and dried in a vented