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Research article

Uptake and phytotoxic effect of benzalkonium chlorides in *Lepidium sativum* and *Lactuca sativa*

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ABSTRACT

Cationic surfactants such as benzalkonium chlorides (BACs) are used extensively as biocides in hospitals, food processing industries, and personal care products. BACs have the potential to reach the rooting zone of crop plants and BACs might thereby enter the food chain. The two most commonly used BACs, benzyl dimethyl dodecyl ammonium chloride (BDDA) and benzyl dimethyl tetradecyl ammonium chloride (BDTA), were tested in a hydroponic system to assess the uptake by and phytotoxicity to lettuce *(Lactuca sativa* L.*)* and garden cress (*Lepidium sativum* L.*)*. Individually and in mixture, BACs at concentrations up to 100 mg L^{-1} did not affect germination; however, emergent seedlings were sensitive at 1 mg L^{-1} for lettuce and 5 mg L^{-1} for garden cress. After 12 d exposure to 0.25 mg L^{-1} BACs, plant dry weight was reduced by 68% for lettuce and 75% for garden cress, and symptoms of toxicity (necrosis, chlorosis, wilting, etc.) were visible. High performance liquid chromatography-mass spectroscopy analysis showed the presence of BACs in the roots and shoots of both plant species. Although no conclusive relationship was established between the concentrations of six macro- or six micro-nutrients, growth inhibition or BAC uptake, N and Mg concentrations in BAC-treated lettuce were 50% lower than that of control, indicating that BACs might induce nutrient deficiency. Although bioavailability of a compound in hydroponics is significantly higher than that in soil, these results confirm the potential of BACs to harm vascular plants.

1. Introduction

Increasing scarcity of fresh water in many parts of the world has necessitated recycling water from various effluents. Adequately treated municipal wastewater can be used for many purposes, including irrigation of agricultural land, parks and playgrounds (Bixio et al., 2006; Exall, 2004) and industrial applications. Increased demand for water in the United States from 2008 to 2012 led to a 22% increase (from $5 billion to $6.1 billion) in the infrastructural costs associated with distributing recycled water (US EPA, 2016). In southern Europe, 44% of the reclaimed wastewater was used for agricultural irrigation (Bixio et al., 2006). Because of the extensive use of organic compounds, such as pharmaceuticals and personal care products, and their persistence in traditional wastewater treatment facilities (Gros et al., 2010; Clara et al., 2007), these contaminants might be in reclaimed wastewater. Organic micropollutants have been detected in various vegetables (Calderón-Preciado et al., 2012; Dodgen et al., 2013; Wu et al., 2013; Mathews et al., 2014). Up to 13 pharmaceutical contaminants were taken up and translocated by cucumber and pea grown on soil irrigated with reclaimed wastewater irrigation and amended with biosolids (Tanoue et al., 2012).

Benzalkonium chlorides (BACs) are a type of cationic surfactant and represent a subset of quaternary ammonium compounds (QACs) with long alkyl chains of C_8 to C_{18} (Zhang et al., 2011). The two most commonly used BACs are benzyl dimethyl dodecyl ammonium chloride (BDDA; alkyl chain with 12 C atoms) and benzyl dimethyl tetradecyl ammonium chloride (BDTA; alkyl chain with 14 C atoms). Many domestic, agricultural and healthcare applications use BACs as a surfactant and/or a biocide (Gerba, 2015),

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which eventually end up in wastewater treatment plants (Clara et al., 2007). Up to 6.03 mg L^{-1} BACs are found in hospital effluents in several European countries (Kümmerer et al., 1997). During the wastewater treatment processes, most BACs are usually removed by biodegradation in combination with adsorption on sewage sludge and the remainder is discharged in the effluent. For example, a maximum of 0.002 mg L^{-1} of total BACs was measured in the effluent of a wastewater treatment plant in Austria even though a high concentration (0.31 mg L^{-1}) of BACs was found in the influent (Clara et al., 2007). Higher concentrations of BACs could be in the reclaimed wastewater in places where the treatment facilities are inadequate. Depending on the local influent concentrations, high amounts of BACs can adsorb to sewage sludge. For example 9.0 mg kg^{-1} (dry weight; ~0.01% by weight) BACs was measured in sewage sludge in Austria (Martínez-Carballo et al., 2007). BACs are generally biodegradable; however, biodegradation is inhibited when BACs are present in a mixture (Khan et al., 2015). Most of the commercial products use multiple BACs in their formulation, thereby increasing the probability of having a mixture of BACs in an effluent.

There is ample evidence of the presence of BACs in the environment. For instance, 1.1 mg kg^{-1} of BACs was found in river sediments in China (Li et al., 2014); 5 to 30 mg L^{-1} was recorded in roof runoff immediately after repair in France (Gromaire et al., 2015); and 0.005 to 28.5 mg kg^{-1} was measured in soil in Korea (Kang and Shin, 2016). The toxicity of BACs has been reported for various organisms. For examples, 1.0 mg L^{-1} BACs was genotoxic to eukaryotic cells (Ferk et al., 2007), 0.31 mg L^{-1} was cytotoxic to fish gill cells (Chen et al., 2014), and the EC_{50} of BACs for a natural assemblage of algae in sea water was 36.4 μg L^{-1} in 24 h and 63.9 μg L^{-1} in 72 h (Pérez et al., 2009). Recently, Richter et al. (2016) reported that the EC_{50} of BDDA was 0.154 mg L^{-1} to *Lemna minor* (duck weed) and 222 mg kg^{-1} to *Brassica napus* (canola) grown in spiked soil.

Wu et al. (2013) reported the uptake of 16 organic pollutants by lettuce, spinach, cucumber and pepper. Many of these were found in the leaves, including atorvastatin, a lipid-lowering drug with a molecular mass of 558.64 g mol^{-1}. Benzalkonium chlorides, with molecular masses around 300 Dalton (BDDA and BDTA are 339.99 and 368.04 g mol^{-1}, respectively) and with solubility greater than 500 g L^{-1} for both compounds, have the potential to be taken up from soil by plant roots and transported to the shoots.

While Richter et al. (2016) reported phytotoxicity and uptake of BDDA to duck weed and canola, to the best of our knowledge, neither BDTA nor other crop plants have been similarly studied, nor has a mixture of BDDA and BDTA been studied in this context. Therefore, the objective of this study was to evaluate the uptake potential and possible phytotoxicity of two BACs in two edible plants: lettuce (*Lactuca sativa* L.) and garden cress (*Lepidium sativum* L.). BACs have both hydrophobic (alkyl chain) and hydrophilic (positive charge) characteristics; whether the accumulation in plants is related to their lipid content is unknown. Lettuce has lower lipid content (0.15% by weight) than garden cress (0.7% by weight; United States Department of Agriculture (USDA); https://ndb.nal.usda.gov/ndb/search/list). We hypothesized that the relatively higher lipid content of garden cress will result in higher uptake of BACs as compared to lettuce. To represent the impact of using reclaimed wastewater in the greenhouse industry and to determine the worst case scenario arising from irrigation of fields with contaminated water, the experiment was conducted in hydroponics. Phytotoxicity of the test BACs was assessed at three stages of plant development: germination, seedling emergence and during vegetative growth. The effect of BACs on macro- and micronutrient content of roots and shoots was also investigated.

2. Materials and methods

2.1. Germination and emerging seedling experiment

Lettuce (*Lactuca sativa*) and garden cress (*Lepidium sativum*) seeds were purchased from the Ontario Seed Company Ltd. (Kitchener, ON Canada) and were stored in a sealed container at 4 °C. BDDA and BDTA with purity ≥99.0% were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Twenty seeds of garden cress or lettuce were spread over filter paper (VWR 314) placed in a Petri dish, and moistened with 7 mL of 0, 0.5, 1, 3, 5, 10, 25, 50 or 100 mg L^{-1} of BDDA or BDTA individually or a 2:1 solution of BDDA:BDTA, each prepared in reverse osmosis (RO) water, with 3 replicate dishes per treatment. The 2:1 ratio was chosen because the environmental ratio of BDDA:BDTA is between 1.5:1 and 2.1:1 (Clara et al., 2007). The concentrations of total BACs in the mixtures were the same as for individual BACs; for example, 10 mg L^{-1} of 2:1 BDDA:BDTA solution contained 6.67 and 3.33 mg L^{-1} of BDDA and BDTA, respectively. The Petri dishes were placed in a controlled environment chamber maintained at a 16 h day (photon flux = 205 ± 10 μmol m^{-2} s^{-1}) at 20 °C and an 8 h night at 16 °C, and constant 60% relative humidity. Percentage germination was measured after 24 h, when all seeds in the control treatment had germinated, and the emergent seedling length from radicle tip to end of the emerging cotyledon, was measured after 65 h, when the control seedlings reached the maximum size for a Petri dish.

2.2. Phytotoxicity of BACs to seedlings

To evaluate the toxic effect of BACs, plants were grown in nutrient solutions that were dosed with BACs. For these experiments, seeds of both plants were germinated as described in section 2.1 but without any BACs. When the radicle length was 2–3 cm, the seedlings were transferred to pots filled with sand that had been saturated with nutrient solution with the following composition: 1.0 mM $Ca(NO_3)_2 \cdot 4H_2O$, 1.0 mM K_2HPO_4, 0.4 mM KNO_3, 0.3 mM $Mg(NO_3)_2 \cdot 6H_2O$, 0.3 mM NH_4NO_3, 0.1 mM K_2SO_4, 10.0 μM $FeCl_3 \cdot 6H_2O$, 10.0 μM Na_2EDTA, 6.0 μM H_3BO_3, 2.0 μM $MnCl_2 \cdot 4H_2O$, 0.5 μM $ZnSO_4 \cdot 7H_2O$, 0.15 μM $CuSO_4 \cdot 5H_2O$ and 0.1 μM Na_2MoO_4, prepared using reverse osmosis (RO) water and adjusted to pH 6. The sand was kept moist with RO water until the shoot length was approximately 4 cm, at which point pairs of seedlings of the same species were transferred to glass jars containing 100 mL nutrient solution. Each jar was covered with aluminum foil to prevent algal growth in the solution. The seedlings were suspended over the solution by a piece of foam.

When the shoots were approximately 7 cm, each seedling pair was transferred to a glass jar containing 1.4 L of nutrient solution to which environmentally relevant concentrations of BACs were added: 0 (control), 0.025 or 0.25 mg L^{-1} BDDA and BDTA, either as single compounds or in a 2:1 mixture of BDDA:BDTA, with 6 replicate jars per treatment. A preliminary study determined that the BACs did not adsorb on the container wall. Solutions were aerated using air pumps (TopFinAir 8000) connected to plastic tubing. Each jar was covered with aluminum foil and custom made lids were used to support the seedlings. The volume of solution in each jar was maintained daily using RO water and after 6 d of growth, nutrient solutions were replenished with another dose of $Ca(NO_3)_2 \cdot 4H_2O$, K_2HPO_4, KNO_3, $Mg(NO_3)_2 \cdot 6H_2O$, NH_4NO_3, and K_2SO_4 to avoid macronutrient deficiency.

Garden cress and lettuce were harvested after 12 d of BAC treatment, near the end of the vegetative growth phase. Roots were rinsed with RO water then soaked for 30 min in 10 mM $CaCl_2$