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Toxic effect of perfluorooctanoic acid (PFOA) on germination and seedling growth of wheat (*Triticum aestivum* L.)

Lina Zhou [a, b], Mengjie Xia [a], Li Wang [a], Hui Mao [a, b, *]

[a] College of Natural Resources and Environment, Northwest A&F University, Yangling, Shaanxi 712100, PR China
[b] Key Laboratory of Plant Nutrition and the Agri-environment in Northwest China, Ministry of Agriculture, College of Natural Resources and Environment, Northwest A&F University, Yangling, Shaanxi 712100, PR China

HIGHLIGHTS

- PFOA inhibited wheat germination at concentrations >800 mg L^{-1}.
- POD activity in wheat seedlings increased while CAT activity decreased as PFOA increased in the soil.
- Proline content in wheat seedlings accumulated gradually as PFOA increased in the soil.
- Physiological indexes provide a safe way to evaluate PFOA toxicity in wheat.

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ABSTRACT

As a persistent organic pollutant in the environment, perfluorooctanoic acid (PFOA) has been extensively investigated. It can accumulate in food chains and in the human body. This work investigated the effect of PFOA on wheat (*Triticum aestivum* L.) germination and seedling growth by conducting a germination trial and a pot trial. A stimulatory effect of PFOA on seedling growth and root length of wheat was found at <0.2 mg kg^{-1}, while >800 mg kg^{-1} PFOA inhibited germination rate, index, and root and shoot growth. In the pot trial, PFOA concentration in root was double that in the shoot. Soil and plant analyzer development (SPAD) and plant height of wheat seedling were inhibited by adding 200 mg kg^{-1} PFOA. Proline content and POD activity in wheat seedlings increased as PFOA increased, while CAT activity decreased. Using logarithmic equations, proline content was selected as the most sensitive index by concentration for 50% of maximal effect (EC_{50}). Hence, the tolerance of wheat seedlings to PFOA levels could be evaluated on the basis of the physiological index.

1. Introduction

Perfluorooctanoic acid (PFOA) has been commonly applied as a surface protector of metals used in aviation, transportation, electronics and kitchenware (Houde et al., 2006). PFOA can exist in the environment for a long period because of unique properties; furthermore, this substance can be found widely distributed in water, sediments and living organisms, such as wildlife and humans (Olsen et al., 2005). However, PFOA causes developmental toxicity, neurotoxicity, and genotoxicity; PFOA also functions as an endocrine disruptor and accumulates in animals; as such, PFOA has been classified as a common persistent organic pollutant (POP), along with polychlorinated biphenyls, organochlorine pesticides, and dioxins (Beach et al., 2006; Lau et al., 2007; Olsen et al., 2009). Pollutants can be transferred from plants to animals and humans in a food chain or web; for this reason, toxic effects are exacerbated by energy transmission, which can be assessed by the fugacity models used by Passuello et al., to predict POP accumulation and the risks to humans of sewage sludge application on agricultural soil (Passuello et al., 2010).

PFOA levels have been detected in most studied agricultural soils, with an average value of 0.113 ng g^{-1} in South Korea (Kim et al., 2014) and 0.124 ng g^{-1} in another six countries (USA, China, Japan, Norway, Greece and Mexico) (Strynar et al., 2012). These levels have prompted researchers to study PFOA transmission from soil to various plants (Renner, 2008). For instance, spring wheat, oats, potatoes, maize, cabbage and rye grass have

* Corresponding author. College of Natural Resources and Environment, Northwest A&F University, Yangling, Shaanxi 712100, PR China.
E-mail address: maohui@nwsuaf.edu.cn (H. Mao).

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been investigated to evaluate soil-to-plant carryover of PFOA/PFOS compounds; results show that PFOA/PFOS concentrations in plants depend on concentrations applied to soil, and PFOA/PFOS uptake and storage are higher in vegetative portions than relocation in storage organs (Stahl et al., 2009). Similar results have been observed in potato, carrot and cucumber (Lechner and Knapp, 2011).

In this study, seedling growth was observed to evaluate pollutant phytotoxicity because of high efficiency of PFOA uptake in this stage (Munzuroglu and Geckil, 2002). Wheat (*Triticum aestivum* L.), which is a major food crop in northern China, was studied. The hypothesis of this study is that PFOA is toxic to wheat and reduces seedling growth at relatively low concentrations in soil, and the toxic mechanism was through the pro-oxidant pathway. So, this study aimed to determine (1) the effect of wide range of PFOA doses on wheat germination and (2) clarify the inhibition mechanism of PFOA addition on wheat growth, in order to further knowledge of PFOA effect in the environment.

2. Materials and methods

2.1. Materials

PFOA (>99% purity) was purchased from Aladdin Reagent Company (Shanghai, China). The soil used in the pot trial was collected from the first agricultural cultivation station of Northwest A&F University, which was used as a control treatment without any fertilizer or pesticide input in former research. The variety of tested wheat (*Triticum aestivum* L.) was Xiaoyan 22, which is the main local variety. All of the reagents used in this research were of analytical grade, purchased from the same company as the PFOA.

2.2. Experimental methods

2.2.1. Germination experiment

Wheat seeds were sterilized with 1% hydrogen peroxide solution for 10 min and then thoroughly washed with distilled water. Fifty wheat seeds were selected at random and placed in Petri dishes (diameter = 10 cm) with filter papers. 25 mL solutions of 11 different PFOA levels: (0 (control), 0.02, 0.2, 2, 20 and 200 μg kg^{-1}, and 2, 20, 200, 800 and 1600 mg kg^{-1}) were added to the dishes with four replications, similar to a previous study (Qu et al., 2010). All of the dishes were placed with covers in an incubator without light at a temperature of 30 ± 1 °C. Seeds were germinated for 8 d. Germination rate, root and shoot lengths, and seedling biomass were then determined.

2.2.2. Pot experiment

Soil was air-dried at room temperature and passed through a 2 mm sieve. A plastic basin (diameter = 15 cm and height = 20 cm) with a pot pad was prepared to fill with 1.5 kg soil. Nitrogen and phosphorus obtained respectively from 0.322 g urea kg^{-1} soil and 0.145 g KH_2PO_4 kg^{-1} soil were added. Five concentrations (0 (control), 2, 20, 200, and 800 mg kg^{-1} air-dried soil) of PFOA (diluted in water) were prepared in three replicates and mixed with the soil. Each soil mixture was air-dried and sieved again, then added to the basin. The moisture was maintained at 70% of the maximum capacity of soil. Seeds were selected the same as in the germination experiment. Fifteen seeds were planted in each pot and the wheat seedlings were harvested after 28 days' growth. Height and chlorophyll content were determined thrice during growth while POD, CAT and proline content were determined at harvest of wheat seedlings.

2.3. Sample analysis

In the germination experiment, the number of germinated seeds was recorded each day. Root and shoot lengths were measured using a ruler. At the end of germination, the fresh plant samples were oven-dried at 70 °C to ensure constant weight and to determine the dry weight of seedlings. The following equations were used:

Germination potential (%) = number of germinated seeds in 3rd day/total seed number for testing × 100%

$$\text{Germination index}(GI) = \sum(G_t/D_t)$$

$$\text{Vitality index}(VI) = \sum(G_t/D_t) \times S$$

where G_t is the germination rate at day t, D_t is day t, and S is the length of the seedlings (Wang et al., 2010).

In the pot trial, chlorophyll contents in the leaves were estimated by using a SPAD meter (Minolta, Japan-SPAD-502) (Mao et al., 2015) and plant height was measured with a ruler. The enzyme activities were determined using fresh plant samples, POD activity assay was performed by using the guaiacol colorimetric method (Smith et al., 1949); CAT activity was determined using the ultraviolet absorption method (Mukherjee and Choudhuri, 1983). The proline content was measured using the indene three ketone colorimetric method (Troll and Lindsley, 1955).

For PFOA determination, the plant and soil, after being sampled and dried with vacuum freeze-drying machine (FD-1D-50, Biocool Experimental Instrument Co., Ltd. Beijing), was mixed with a 5 mL mixture of dichloromethane and methanol (v:v, 2:1), ultrasonicated for 30 min, and then centrifuged for 30 min at 25 °C. Supernatant was collected and dried to 1 mL under a gentle nitrogen flow. The solution was then loaded onto a silica solid-phase extraction (SPE) cartridge (Restek, 6 mL, 1000 mg) that was preconditioned with 5 mL of hexane. After sampling, the column was eluted with 20 mL hexane and air dried for 5 min. The cartridge was then eluted with 5 mL acetonitrile. The eluent was collected, blown dry by a nitrogen flow, and finally reconstituted to 1.0 mL with acetonitrile for PFOA quantification on LC-MS-MS.

PFOA concentration was measured by HPLC-MS-MS, Waters 2690 (Waters, Milford, MA). The column was the ascentis C18 reversed phase column (250 × 4 mm, 5 μm particle, Supelco, St. Louis, MO), injection volume being 10 μL, mobile phase being the HPLC grade methanol and water at a flow rate of 0.3 mL min^{-1}, the measurement time being 30 min, and mobile phase composition being: 0 min, 40% methanol; 5 min 80% methanol; 10 min 100% methanol, 25 min, 40% methanol.

2.4. Statistical analysis

All of the experiments were conducted at least thrice. Data were expressed with arithmetic means ±standard deviation (SD). Significant test of data in confidence $p < 0.05$ were one-way ANOVA with SPSS18.0.

3. Results and discussion

3.1. Effect of PFOA on wheat germination

PFOA inhibited wheat germination in a dose-dependent manner. Germination rate increased rapidly in the first 5 d for all doses were applied except 1600 mg kg^{-1} PFOA (Fig. 1). This pattern was observed in the remaining incubation periods. PFOA <20 mg kg^{-1} did not significantly affect germination rate; by