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Arsenic accumulation in *Scutellaria baicalensis* Georgi and its effects on plant growth and pharmaceutical components

Hongbin Cao[a,b,*], Yu Jiang[a,b,1], Jianjiang Chen[a,b], Hui Zhang[a,b], Wei Huang[a,b], Lei Li[a,b], Wensheng Zhang[a,b]

[a] *Beijing Area Major Laboratory of Protection and Utilization of Traditional Chinese Medicine, Beijing 100875, China*
[b] *College of Resource Science & Technology, Beijing Normal University, Beijing 100875, China*

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ABSTRACT

Scutellaria baicalensis Georgi is a traditional Chinese medicinal plant. The effects of arsenic (As) on the growth and the formation of pharmaceutical components of *S. baicalensis*, and the uptake and accumulation of As by *S. baicalensis* were investigated using a field pot-culture experiment. The results show that spiking low concentrations of As ($\leq$100 mg kg^{-1}) into soils can hasten the growth and development of the roots. High levels of As, however, reduced plant growth. The concentrations of five flavone components were not significantly affected by spiking low concentrations of As ($\leq$200 mg kg^{-1}) into soils. High levels of As inhibited the generation of baicalin and wogoninside, but facilitated the generation of baicalein, wogonin and oroxylin A in *S. baicalensis* Georgi. The concentration of As in each part of the plant was proportional to the concentration of As spiked into the soil. The application of phosphorus (P) to the soil promoted the uptake and accumulation of As in the roots of the plant, but this synergistic effect became weaker with the incremental addition of P. Dry biomass did not change in response to low levels of P addition ($\leq$200 mg kg^{-1}) to soils, but it increased significantly under high levels of P. Based on the results of both this pot-culture experiment and human health risk assessments, maximum safety limits of 2.0 mg kg^{-1} of As in the roots of *S. baicalensis* Georgi and 70 mg kg^{-1} of As in cultivated soils are suggested.

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1. Introduction

Arsenic (As) is a naturally occurring toxic element in the environment. It can enter the environment through weathering, biological activity, and volcanic activity. Anthropogenic inputs from agricultural and industrial practices, such as the application of pesticides and chemical fertilizers, wastewater irrigation, precipitation from heavy coal combustion and smelter wastes and residues from metalliferous mining, increase the levels of As contamination in soil, ground water and surface water [1–4]. In China, the concentration of As typically varies from below 5 mg kg^{-1} in non-contaminated soils [5] to as high as 3800 mg kg^{-1} in contaminated soils near the tailing spots of arsenic sulphide mines [6]. Once in the soil, As can be absorbed by plants, including farm crops such as grains, vegetables and fruits, and the ingestion of these contaminated farm crops can have hazardous effects on human health. Chronic exposure to high levels of inorganic As has been found to result in a variety of adverse health effects, including skin and internal cancers and cardiovascular and neurological effects [2,7].

With the boom in the use of natural herbal medicine in the world, traditional Chinese medicine has been increasingly exported. Consequently, the quality and safety of traditional Chinese medicine has drawn more attention in the world. Quality and safety standards for herbal medicine that clearly stipulate the maximum allowable value of heavy metals in herbal medicine have been enacted and put into effect by many countries [8–10]. However, since most of these standards are determined by referring to the quality standards for foods, they are not based on the research on herbal medicine. Unlike nutritional components such as starch, lipids and amino acids, pharmaceutical components in medicinal plants (referring to original plant of traditional Chinese medicine hereafter) are commonly secondary metabolites, and some of them are produced in much higher concentrations under environmental stress [11,12]. The uptake and accumulation of heavy metals may have impacts on medicinal plants that are different from their impacts on farm crops. Moreover, the methods of processing and ingesting medicinal plants are also different from those for farm crops, which should translate into differences in human exposure to and health risks from heavy metals between medicinal plants and

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* Corresponding author at: College of Resource Science & Technology, Beijing Normal University, Beijing 100875, China. Tel.: +86 10 58802280; fax: +86 10 62200669.
E-mail addresses: caohongbin@ires.cn, hongbin_cao@hotmail.com (H. Cao).
[1] Present address: College of Environment & Resources Science, Guangxi Normal University, Guilin 541004, China.

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farm crops. Therefore, it is necessary to improve quality standards for herbal medicines by examining and revising the maximum allowable values of heavy metals in medicinal plants, using research based on medicinal plants.

To date, research on the safety of traditional Chinese medicine has mainly been focused on the development of methods for detecting heavy metals in traditional Chinese medicines [13–15] and the investigation of the level of contamination of traditional Chinese medicines by heavy metals [16,17]. Over 300 species of medicinal plants have been investigated. The results indicate that the concentration of heavy metals in medicinal plants differs significantly depending on the production area and that, even within the same plant, the concentrations of heavy metals may differ among parts of the plant [17]. There has been little research aimed at elucidating the uptake mechanisms, accumulation and partitioning of heavy metals in medicinal plants or at determining the response of medicinal plants to heavy metals in terms of their growth and their production of pharmaceutical components. However, as a reference, we can use the theories, methods and techniques taken for both studies on the hyper-accumulation of heavy metals in plants for soil remediation [18,19] and research on the harmful effects of heavy metals and their mechanisms in farm crops [20,21]. Furthermore, some research has examined the adequacy of environmental quality standards based on the results of risk assessments of human beings exposed to heavy metals in crops [22]. These studies could help inform us for the formulation and revision of quality and safety standards for medicinal plants.

The roots of *Scutellaria baicalensis* Georgi (Labiatae) are an important Chinese medicine that is used as a diuretic, laxative, febrifuge, an antipyretic and for hemoptysis, bloody stool, and nasal haemorrhaging when prescribed together with other herbs [10]. A major component of this plant, baicalein, has antibacterial, antiviral and lipoxygenase inhibitory activities [23,24]. Many Chinese medical preparations containing mainly *S. baicalensis* Georgi, such as “San Huang Pian”, “Ku Gan Chong Ji” and “Yin Huang Kou Fu Ye”, have been developed and used widely in China as drugs with antibacterial and antiviral functions, partly substituting for antibiotics. Over 300 species of plants belonging to *Scutellaria* Linn. grow throughout the world, except for the tropical areas of Africa. *S. baicalensis* Georgi is one species of *Scutellaria* Linn., and it is widely distributed in most provinces of China. Chengde in Hebei province and Chifeng in Inner Mongolia municipality are two of the largest areas of production of natural *S. baicalensis* Georgi. Shandong province also has major plantations of *S. baicalensis* Georgi [25].

The objectives of this paper are: to clarify the accumulation and partitioning of As in *S. baicalensis* Georgi and the influence of P in soil; to elucidate the responses of *S. baicalensis* Georgi to As in terms of both its growth and its production of five major active flavone components, baicalin, wogoninside, baicalein, wogonin and oroxylin A; and furthermore, to provide suggestions for the establishment of environmental safety standards for As in *S. baicalensis* Georgi.

2. Materials and methods

2.1. Field site and soil characterization

The field pot-culture experiment was conducted at the Fangshan Experimental Station (39°41′N and 116°03′E), which belongs to Beijing Normal University and is located in the suburb of Beijing. The altitude is 38 m. The experimental station is in the temperate zone with a semi-moist continental climate, an average annual temperature of 11.6 °C and an average annual precipitation of 611 mm.

Most soil samples were collected from the surface (0–20 cm) of a field in the station. This soil was a yellow sandy loam; total N, total P, total K and pH were 0.055%, 0.043%, 2.02% and 7.34, respectively. The concentrations of exchangeable Ca, exchangeable Mg, Al, S, Fe and Mn were 0.45%, 0.015%, 6.22%, 0.019%, 2.92% and 0.059%, respectively. The concentration of As in the soil was 12.2 mg kg^{-1}.

2.2. Experimental procedure

Eighty-five plastic pots (27.5 cm in height and 30 cm in diameter) were used. The same quantity of soil (20 kg) was collected from a field in the Fangshan Experimental Station and placed into each pot. Two-year old striking roots of *S. baicalensis* Georgi were purchased from the plantation in the Wafangdian, Liaoning province. In April, three seedlings of striking roots of *S. baicalensis* Georgi of similar sizes were transplanted into each pot before turning green. To simulate field conditions, the plants were grown under open field conditions and no fertilizer was added. Any loss in water was alleviated by using tap water to sustain the water holding capacity at 16%.

In this study, disodium hydrogen arsenate [$Na_2HAsO_4{\cdot}7H_2O$] and sodium dihydrogen phosphate (sodium phosphate) [NaH_2PO_4] were used without further purification for the As and P treatments. The arsenic and phosphate solutions were prepared by mixing the appropriate amount of disodium hydrogen arsenate and sodium dihydrogen phosphate into the suitable volume of purified water (no As detected) for each treatment. Preliminary tests were performed to determine the appropriate range of As concentrations to use for testing the plants sensitivity. The As addition only (nine levels of As at 0 (CK1), 10, 20, 40, 100, 160, 200, 400 and 600 mg kg^{-1}, no P addition) and the As and P addition (eight levels of P at 0 (CK2), 10, 20, 40, 100, 200, 400 and 600 mg kg^{-1}, all with As at 40 mg kg^{-1}) experiments were arranged to investigate the uptake and accumulation of As by *S. baicalensis* Georgi and the influence of the soil P concentration on the accumulation of As. The solutions were carefully irrigated into the soil to avoid trickling any onto the leaf. All treatments were replicated five times in the experiments.

The experimental treatments were conducted three and a half months after the seedling transplantation. Sixty days later at the end of September, the plants were harvested, when both the aboveground parts and the roots experienced their peak growth periods and reached their physiological maturity. The whole plant was scooped out. The attached soil was gently removed from the roots by a soft brushing. The roots and the aboveground parts were dried in an oven at 60 °C for 72 h until completely dry. The fresh weight and dry weight (after 72 h at 60 °C) of *S. baicalensis* Georgi were measured.

2.3. Plant and soil analysis

The dried plant samples were ground in a mill. After subsampling for the analysis of flavone components, the ground plant samples were sifted through a 50-mesh (0.30 mm) sieve. 0.1 g of plant samples were then digested in 4 ml of pure HNO_3, using a microwave digestion system (WX-8000). The soil samples were air-dried and ground to sift through a 100-mesh (0.15 mm) sieve. 0.2 g of soil samples were then digested with an 8 ml solution of 1:3 HNO_3: HCl, using the WX-8000 microwave digestion system. The As concentrations were determined by using hydrogen generation atomic fluorescence spectroscopy (AFS-830). The subsample of the dried plant samples were sieved through a 40-mesh (0.42 mm) sieve. 0.1 g of plant subsamples were ultrasonically extracted in a conical breaker with 25 ml of 70% ethanol for 40 min, and then the extracts were separated by filtering them through 0.45 μm membrane filters. The concentrations of five flavone components (baicalin, wogoninside, baicalein, wogonin and oroxylin A) in *S. baicalensis* Georgi were determined by using high performance liquid chro-

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