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Effect of Indole-3-Acetic Acid on Arsenic Translocation in Agricultural Crops

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***Abstract*—**The problem of agricultural-soil pollution is closely linked to the production of ecologically pure foodstuffs and to human health. An important task, therefore, is to rehabilitate agricultural soils with the help of state-of-the-art biotechnologies, based on the use of metal-accumulating plants. In this work, on the basis of literature data and the results of prior research from this laboratory, plants were selected for which the growing technology is well developed and which are widespread locally: sugar sorghum (*Sorghum saccharatum*), sudangrass (*Sorghum sudanense* (Piper.) Stapf.), and sunflower (*Helianthus annuus* L.). I report on laboratory experiments designed to study the influence of synthetic indole-3-acetic acid and the extracellular indole-3-acetic acid released by the plant-growth-promoting rhizobacterium *Azospirillum brasilense* Sp245 on growth of and arsenic accumulation by these plants.

***Keywords*—**Arsenic, bioaccumulation, plant-growth-promoting rhizobacteria, phytohormones.

I. Introduction

ARSENIC (As) is widespread in the environment. Although trace amounts of As occur universally in the lithosphere, its concentration may be substantially higher at specific locations as a result of natural processes and human activities. For many parts of the world, As pollution of agricultural soils, ground water, and surface water constitutes a major hazard to the environment and public health. Pollution sources include natural processes (e.g., in Bangladesh and south India) [1] as well as human activities, such as the wide application of inorganic As herbicides, defoliants, desiccants, insecticides, and fungicides [2], [3]; mining [4]; and destruction of chemical-warfare arsenicals [5]. It has been speculated that systematic application of even low doses of phosphorus fertilizers can lead to accumulation of large amounts of As in soil [6].

The problem of agricultural-soil pollution is closely linked to the production of ecologically pure foodstuffs and to human health. The presence of inorganic As in foodstuffs provokes the development of internal malignancies and other health problems, including skin cancer and diabetes [7]. The cleanup, rehabilitation, and reuse of As-contaminated agricultural soils are, therefore, very important tasks.

The past decade has seen intensive research on and use of higher plants in soil restoration [8], [9]. A major step in the process called phytoremediation is to search for plants typical of specific soil–climatic conditions and types of pollution, plants that are characterized by good germinating ability, high growth rates, relatively large biomasses, tolerance to high pollutant concentrations, and the ability to effectively reduce pollutant content [10].

In most cases, the extent of soil cleanup depends on the availability of the metal to the plant and on the tolerance of the chosen plant. The availability of metals and the level of their bioaccumulation are increased with various chelating agents (e.g., EDTA) [11]; phytotoxicity is reduced and plant tolerance is enhanced by adding organic and mineral fertilizers to soil, treating plants with biopreparations, and inoculating plants with plant-growth-promoting rhizobacteria (PGPR), many of which are active in phytohormone production [12], [13].

To improve plant tolerance, stimulate plant growth, and increase As bioaccumulation, we used and compared the effects of indole-3-acetic acid (IAA, a phytohormone of the auxin series) and a nitrogen-fixing rhizospheric *Azospirillum brasilense* strain, Sp245, able to produce IAA at concentrations physiologically significant for plants [14], [15].

II. Materials and Methods

A. Cultivar Source

Seeds of sorghum (*Sorghum saccharatum* Pers. cvs. Pishchevoye-69 and Volzhsky-4), sudangrass (*Sorghum sudanense* (Piper.) Stapf.), and sunflower (*Helianthus annuus* L. cv. Yubileinyi-60) were obtained from The Sorghum Research Institute, Saratov, Russia.

B. Soil Preparation

Gray forest soil (background As content 2.8 mg As kg^{-1} soil, pH 6.6) was collected in the region of Saratov. Equal portions of soil were taken from 13 sites located at even spacings over a 100 × 100 m area. The level, color, and structure of the soil were the same at all sites. The soil was withdrawn from the top layer (10–20 cm depth) and was mixed after inclusions were removed. Before plants were set out, aqueous sodium arsenite (Na_3AsO_3) was applied to the soil at rates of 25 and 50 mg As kg^{-1} soil (pure-As basis).

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C. Microorganism and Culture Conditions

A. brasilense Sp245 was obtained from the culture collection held at the Russian Academy of Sciences' Institute of Biochemistry and Physiology of Plants and Microorganisms, Saratov, Russia. Solid (1.5% agar) and liquid malate media were used for bacterial cultivation [16].

D. Plant Inoculation

Seeds were size-calibrated, washed with a detergent for 5 min to remove contaminants, and rinsed thoroughly in tap water. The seeds then were placed in petri dishes on tap-water-moistened filter paper and were left to germinate for 24 h. After that time, the seeds were inoculated with bacteria harvested at the end of logarithmic-phase growth. Cells to be used for inoculation were grown in the malate medium for 18 h, washed free of medium under sterile conditions, and pelleted by centrifugation (11000 × g for 5 min). The pellet was suspended in a nitrogen-free mineral medium. The germinated seeds were placed in a bacterial suspension (10^7 cells), after which they were planted out.

E. Climate-Chamber Experiments

Plants were grown in a KTLK 1250 growth chamber (NEMA, Germany) for 30 days. As typical short-day plants, they were provided with a 13-h light period and an 11-h dark period. The plants received continuous illumination from a set of 250-W lamps (1-m distance). The relative humidity was 60%. After the plants were grown for 30 days, the soil was sampled and the plant biomass was harvested for As analyses.

To study the phytoremediation effect of IAA, we treated 3-day-old seedlings of sunflower, sudangrass, and sorghum with water solutions of IAA for 24 h. IAA was used at 10^{-5}g l^{-1}.

At the end of the experiments, we estimated the degree of pollutant extraction as a ratio between the initial and the final (after plant cultivation) pollutant concentration in the soil.

F. Determination of Indole Derivatives

The culture liquid was analyzed by gas–liquid chromatography by using an HPLC HP 1090 apparatus (Hewlett Packard, USA). The eluent flow rate was 0.5 ml min^{-1}. The synthetic indole derivatives tryptophan and IAA (both from Fluka) were used as standards.

G. Determination of As in Soil and Plant Samples

Soil and plant sampling and sample preparation for As analyses were done by standard procedures adopted in agriculture for the determination of macro- and micronutrients. The soil and plant-biomass samples were analyzed for total As by using the color reaction of ammonium molybdate with As [5].

H. Statistics

Data from each experiment are the arithmetic means of three replicated samples, each sample consisting of 20 plants. The data were processed by using Fisher's least significant difference at $P = 0.05$ or the confidence interval. Calculations were done with Excel 2003 software (Microsoft Corp., USA).

III. Results and Discussion

Auxin phytohormones are widely used in plant husbandry to increase the growth rate and to regulate plant-root formation [14]. Prior work from this laboratory has shown the possibility of using IAA to intensify sunflower remediation of As-polluted soils and has chosen a suitable IAA concentration [5]. This paper reports on laboratory experiments designed to study the influence of synthetic IAA and the extracellular IAA released by the PGPR *A. brasilense* Sp245 on the growth of sunflower, sugar sorghum, and sudangrass and on the accumulation of As by these plants. Before study, plant seeds were soaked in a phytohormone solution, dried a little, and set out in a sodium arsenite-polluted soil.

Previous work has demonstrated that the optimal range of sodium arsenite concentrations for the plants used, in terms of pure As, does not exceed 100 mg kg^{-1} dry soil, with higher concentrations strongly inhibiting plant growth [5]. In this work, we used a soil containing 50 mg kg^{-1} As. After the plants were grown on the polluted soil for 30 days, we measured the final As concentration in plant-biomass and soil samples, and we determined the degree of pollutant extraction in different treatments with IAA. The results are shown in Table I.

Morphogenetically, the plants whose seeds were phytohormone-treated did not fall behind the control plants, grown without the pollutant. The cleanup effect was the most significant (degree of extraction 4.2) with sunflower treated with 10^{-5} g l^{-1} IAA. Some PGPR can produce phytohormones. *Azospirillum* bacteria are well known as rhizosphere nitrogen fixers that can improve cereal-crop performance [17]. The wide occurrence of azospirilla in soil and their ability to produce phytohormones suggest that these bacteria have a role in metal uptake by plants. Moreover, it is believed that the ability of microorganisms to synthesize phytohormones is a critical factor underlying the improvement of plant growth and development.

TABLE I
THE DEGREE OF AS EXTRACTION FROM SOIL IN DIFFERENT TREATMENTS WITH IAA

IAA (g l^{-1})	Plants		
	Sunflower	Sorghum	Sudangrass
0	1.5	1.8	1.6
10^{-9}	2.0	1.8	1.9
10^{-7}	2.4	2.8	2.8
10^{-5}	4.2	4.0	4.1
10^{-3}	1.6	1.9	1.8

A. brasilense strain Sp245 was highly tolerant to As. The minimum inhibitory concentration of sodium arsenite was 1 g l^{-1}, and the maximum tolerant concentration was 0.18 g l^{-1}. We also determined the ability of the bacteria to excrete IAA at various concentrations of sodium arsenite in the culture medium. Because our data, along with the data of others [18], show that maximum production of IAA occurs at the stationary phase of bacterial growth, we examined IAA

production by *A. brasilense* Sp245 after 3 days of culture growth.

Analysis by high-performance liquid chromatography showed that in the control, the amount of IAA in the culture liquid was 25 mg l^{-1}. Increasing the As concentration in the medium from 10 to 100 mg l^{-1} brought about no significant decline in auxin synthesis. Further increases in the As concentration reduced IAA synthesis by 30 to 50%, as compared with the control (Fig. 1). The chromatographic data show that there was a relationship between tryptophan utilization and IAA formation, suggesting that the *A. brasilense* bacteria would catabolize the tryptophan from the plant-root exudates to IAA in the presence of As as well. Because accumulation depends on the state and level of development of the plant-root system, we compared sodium arsenite accumulation by plants treated with 10^{-5} IAA with that by plants inoculated with *A. brasilense* Sp245. Untreated plants served as controls. The initial concentration of As was 25 mg kg^{-1} soil.

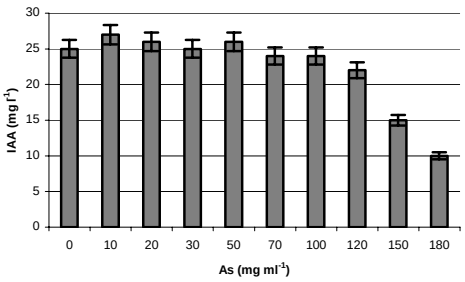

Fig. 1 IAA synthesis by *A. brasilense* Sp245 versus the concentration of As

Arsenic pollution inhibited slightly the germination ability and vigor of the seeds and, in some cases, the survivability of the plants growing on polluted soil. However, all auxin-treated and bacteria-inoculated plants showed good germination ability and vigor.

Inoculation also enhances the initiation of lateral roots, which give plants better access to soil nutrient resources [19]. Such an effect on root morphology is characteristic of auxins, including IAA. Auxin synthesis by *Azospirillum* spp. plays a major role in plant-growth promotion, and strain Sp245 is a known producer of appreciable quantities of extracellular IAA in culture media supplemented with the IAA precursor tryptophan [20], [21].

In evaluating As accumulation in the plant biomass, we found that As was accumulated the most by plants whose seeds had been treated with an auxin solution. The results are presented in Table II.

Sunflower plants treated with strain Sp245 were characterized by good biomass, and they accumulated 25% more As in their tissues than did the untreated control plants but less As than did the auxin-treated plants. The most decrease in total-As content in the soil (70%) was observed in the sunflower-plus-IAA treatment.

Inoculation of sudangrass decreased As content in the plant tissue by 30%, as compared with the control. In sorghum, the decrease in bioaccumulation during treatment with *A. brasilense* Sp245 was 14%. However, soil-As content in these two treatments was reduced by 50% of the initial content, with this value being larger than those observed in the control. Most probably, such a decline in the pollution level, with a concurrent decrease in plant-tissue accumulation, is associated with the large plant biomass in these treatments and with the inoculation-induced higher tolerance of the plants to the pollution. Earlier, Lyubun *et al.* [16], using spring wheat as an example, showed that *Azospirillum*-inoculated plants accumulate less As than do surface-sterilized uninoculated plants.

TABLE II
CONCENTRATIONS OF AS IN THE SOIL AND IN DRY PLANT BIOMASS OBTAINED IN DIFFERENT TREATMENTS

Plants	Experiment	As (mg kg^{-1} soil)	As (mg kg^{-1} dry plant biomass)
Sorghum	No treatment	14.91	13.45
	IAA	9.94	21.75
	A. brasilense Sp245	13.03	11.47
Sudangrass	No treatment	16.93	15.64
	IAA	11.12	19.37
	A. brasilense Sp245	12.86	10.42
Sunflower	No treatment	17.87	14.28
	IAA	7.58	22.5
	A. brasilense Sp245	10.5	17.64

In summary, this work has demonstrated that PGPR and their secondary metabolic products, such as the phytohormone IAA, can influence the level of As accumulation in biomass and the development of sorghum, sudangrass, and sunflower. Treatment with IAA at 10^{-5} mg l^{-1} increases As content in plant biomass. Inoculation with the PGPR *A. brasilense* Sp245 has a positive effect on plant development, increases biomass, but does not enhance As translocation by the plants.

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