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Uptake and Accumulation of Cobalt in Plants: a Study Based on Exogenous Cobalt in Soybean

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Abstract: An investigation was carried out with the heavy metal Cobalt and legume plant soybean, in order to test the heavy metal accumulating ability of soybean. Cobalt was given to Soybean (*Glycine max)* plants in pot culture by soil drenching method. The accumulation and uptake of cobalt was tested from root, stem and leaves of treated plants. The results showed higher concentration (Co level (100-200 mg kg^{-1}) in the soil) resulted in maximum accumulation in all parts of soybean plants, while the low concentrations of cobalt (50 mg kg^{-1} Co level) in the soil didn't show any significant effect.

Key words: *Glycine max* · Uptake · Accumulation · Cobalt

INTRODUCTION

Estimation of the migration ability of any pollutant in the natural environment is considered to be a necessary stage for predicting the ecological situation. The process of heavy metals accumulation in agricultural crops is especially interesting because they contribute toxic elements into the human food chain [1]. Special attention should be paid to the dangerous health elements and to most consume agricultural crops [2].

Chemical elements accumulation in plants depends not only on their absolute content in a soil, but also on the level of soil fertility, acidic–alkaline and reductive–oxidative conditions and content of organic matter [3]. By the data of previous studies the content of mobile forms of elements in soils of light granulometric composition with low content of clay minerals, humus and acidic reaction of soil solution, is bigger than the one in heavy fertile neutral soils [4-8]. The dynamic equilibrium in the soil is determined by the cation exchange complex, the soil components and heavy metals, which are sorbed specifically [9]. The pattern of heavy metals accumulation in plants, regarding the type of soil, the biological peculiarities of plants, the elements nature and concentration, is discussed by other investigators [8-11]. Some of them trend to a linear dependence between metal content in the soil and in the crop. However, the influence of heavy metals concentration on their accumulation in various crops and on the yield of these for different types of soil [12].

Heavy metals are continuously released into the terrestrial environment by natural sources and human activities. The uptake and accumulation of heavy metals by plants promotes a mechanistic understanding of the biological significance of particular metal concentrations and distributions in biota [13]. The toxicity of chromium, zinc, copper and cobalt ions and their binary mixtures are studied at varying test levels using duckweed as the test organism. The accumulations of metal ions are determined by atomic absorption spectroscopy. The type of toxic interactions in binary mixtures is assessed as `synergistic', `antagonistic' and `additive' by a statistical approach.

Investigation carried out on agricultural chemical enterprises territories has been found out its soil was characterized by significant accumulation of Cd, Co, Cu, Ni, Zn and other heavy metals in the superficial stratum with depth to 20 sm [14]. Migration of the elements in soil-plant system has some peculiarities: its solid mass accumulates by root system of the plant [9]. Mechanic composition and agrochemical structure of the soil, species assortment of the plants prevailing in the grass influence on this processes too. The most informative parameter is metal biological accumulation index (MBAI). In condition soil contamination by heavy metals root system metal biological accumulation index was higher than over ground organs. High accumulation ability of the cereals grass manifestoes on early stage of its development and then constantly grows [11]. Thus, metals accumulative ability of cereals grasses is indicator

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of level of environment contamination by heavy metals and it allows using these parameters for passive monitoring [15].

MATERIALS AND METHODS

The present investigation has been carried out to find out the effect of cobalt a heavy metal pollutant on growth, biochemicals, enzyme activities and nutrient content of soybean (*Glycine max* (L.) Merr.) cultivar CO-1.

Materials

Seed: The experimental plant, the soybean (*Glycine max* (L.) Merr.) cultivar CO-1 belongs to the family Fabaceae is one of the important pulses of India. Seeds used in the experiments were obtained from the Pulses Division, Tamil Nadu Agricultural University, Coimbatore. Seeds with uniform size, colour and weight were chosen for experimental purpose.

Seeds were surface sterilized with 0.1 per cent mercuric chloride solution and washed thoroughly with tap water and then with distilled water.

Pot Culture Experiments: The experiments were conducted during January-April 2007. Soybean (*Glycine max* (L.) Merr.) cultivar CO-1 plants were grown in pots in untreated soil (control) and in soil to which cobalt had been applied (50, 100, 150, 200 and 250 mg kg^{-1} soil). The inner surfaces of pots were lined with a polythene sheet. Each pot contained 3 kg of air dried soil. The cobalt as finely powdered ($CoCl_2$) was applied to the surface soil and thoroughly mixed with the soil. Ten seeds were sown in each pot. All pots were watered to field capacity daily. Plants were thinned to a maximum of six per pot, after a week of germination. Each treatment including the control was replicated five times.

Sample Collection: The plant samples were collected at thirty days interval, upto harvest stage viz., 30, 60 and 90th day for the measurement of various morphological growth parameters. The biochemicals, enzymes, nutrients and cobalt content of the plants were estimated at all the three sampling periods. Six plants from each replicate of a pot was analysed for it's various parameters and the average was calculated. These mean values were used for statistical analysis.

Estimation of Cobalt [16]: One ml of sulphuric acid and 15 ml of double distilled water were added to a Kjeldahl flask containing 0.5 g of dried and powdered material and incubated at 80°C for over night. After that 5 ml of acid mixture (nitric acid, 3: perchloric acid, 1) was added and digested until the nitric acid and perchloric acid were driven off. The digest was cooled, diluted, filtered through Whatman No.42 filter paper and made up to 50 ml.

The solution was directly, aspirated to an Atomic Absorption Spectrophotometer (Perkin – Elmer – 2280), with air/acetylene flame for estimating copper, iron, manganese, zinc and cobalt.

Statistical analysis was performed using two way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). P values $\leq$ 0.05 were considered as significant.

RESULTS

Uptake and Accumulation of Cobalt in Root: Cobalt content of root of soybean plants is recorded in Table 1. Maximum cobalt content of soybean root (1.19) was observed at 250 mg kg^{-1} cobalt level in the soil. The minimum cobalt content of soybean root (0.85) was observed in control plants.

Uptake and Accumulation of Cobalt in Nodules: Cobalt content of nodules of soybean plants is recorded in Table 1. Maximum cobalt content of soybean nodules (1.22) was observed at 250 mg kg^{-1} cobalt level in the soil. The minimum cobalt content of soybean nodules (0.71) was observed in control plants.

Uptake and Accumulation of Cobalt in Stem: Cobalt content of stem of soybean plants is recorded in Table 1. Maximum cobalt content of soybean stem (0.88) was observed at 250 mg kg^{-1} cobalt level in the soil. The minimum cobalt content of soybean stem (0.71) was observed in control plants.

Uptake and Accumulation of Cobalt in Leaves: Cobalt content of leaves of soybean plants is recorded in Table 1. Maximum cobalt content of soybean leaves (1.16) was observed at 250 mg kg^{-1} cobalt level in the soil. The minimum cobalt content of soybean leaves (0.83) was observed in control plants.

Uptake and Accumulation of Cobalt in Seeds: Cobalt content of seeds of soybean plants is recorded in Table 1. Maximum cobalt content of soybean seeds (0.23) was observed at 250 mg kg^{-1} cobalt level in the soil. The minimum cobalt content of soybean seeds (0.10) was observed in control plants.

Table 1: Uptake and accumulation of cobalt (µg g^{-1} dry weight) on plant parts *Glycine max* (L.) Merr

	30 DAS				60 DAS				90 DAS				
Cobalt added in the soil (mg kg^{-1})	Root	Nodules	Stem	Leaf	Root	Nodules	Stem	Leaf	Root	Nodules	Stem	Leaf	Seed
Control	-	-	-	-	-	-	-	-	-	-	-	-	-
50	0.68	0.71	0.58	0.63	0.81	0.84	0.65	0.77	0.85	0.89	0.71	0.83	0.10
100	0.76	0.78	0.62	0.74	0.88	0.92	0.69	0.85	0.92	0.97	0.75	0.89	0.14
150	0.81	0.85	0.68	0.78	0.96	0.99	0.73	0.93	0.98	1.08	0.79	0.96	0.18
200	0.84	0.90	0.74	0.82	1.05	1.09	0.77	0.98	1.13	1.15	0.83	1.07	0.21
250	0.89	0.96	0.79	0.87	1.11	1.16	0.81	1.03	1.19	1.22	0.88	1.16	0.23

(Per cent over control values are given in parentheses)

Comparison of significant effectsF test

Cobalt levels**   Sampling days**

Plate 1: Photographs showing the effect of cobalt on the growth of soybean

DISCUSSION

Heavy metal contamination of soils has markedly increased in the past few decades. Many factors such as metal-enriched parent materials, mining or industrial activities, non-point sources of metals, especially automotive emission and use of metal-enriched materials, including chemical fertilizer, farm manures, sewage sludge and wastewater irrigation, can contribute to this contamination, [17-19].

Mineral rock weathering and anthropogenic sources provide two of the main types of metal inputs to soils. The anthropogenic sources of metal contamination can be divided to five main groups: metalliferous mining and smelting (arsenic, cadmium, lead and mercury); industry (arsenic, cadmium, chromium, cobalt, copper, mercury, nickel, zinc); atmospheric deposition (arsenic, cadmium, chromium, copper, lead, mercury, uranium); agriculture (arsenic, cadmium, copper, lead, selenium, uranium, zinc); and waste disposal (arsenic, cadmium, chromium, copper, lead, mercury, zinc). In Finland, most cases of soil metal contamination have been caused by waste treatment plants, sawmills and wood impregnation plants, shooting ranges, garages and scrap yards [20]. In 2001, a total of 20,000 metal contaminated sites were identified. Because 38 % of these metal contaminated sites are located in groundwater areas or close to settled areas, metal contaminated soil sites are of great concern [21].

Metals play an integral role in the life processes of microorganisms. Some metals, such as calcium, cobalt, chromium, copper, iron, potassium, magnesium, manganese, sodium, nickel and zinc, are essential, serve as micronutrients and are used for redox-processes; to stabilize molecules through electrostatic interactions; as components of various enzymes; and for regulation of osmotic pressure [22]. Many other metals have no biological role (e.g. silver, aluminium, cadmium, gold, lead and mercury) and are nonessential and potentially toxic to microorganisms.

Toxicity of nonessential metals occurs through the displacement of essential metals from their native binding sites or through ligand interactions [18-20]. For example, Hg^{2+}, Cd^{2+} and Ag $^{2+}$tend to bind to SH groups and thus inhibit the activity of sensitive enzymes. In addition, at high levels, both essential and nonessential metals can damage cell membranes; alter enzyme specificity; disrupt cellular functions; and damage the stucture of DNA. To have a physiological or toxic effect, most metal ions have to enter the microbial cell. Many divalent metal cations (e.g. Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}) are structurally very similar. Also, the structure of oxyanions such as chromate resembles that of sulfate and the same is true for arsenate and phosphate.

Plants possess homeostatic mechanisms to maintain the correct concentration of essential metals like Cu and Mn in different cell compartments. A regulated network of metal transport, chelation, trafficking and sequestration activities functions and provides the uptake, distribution and detoxification of excess metal ions. Heavy metal-contaminated waste can be disposed in landfills, but this solution negatively affects human health, wild flora and fauna, as well as productivity of crop plants and livestock. The management of heavy metal-contaminated water or biosolids is becoming more challenging as stricter regulations to improve water quality and soil fertility are imposed. In Italy, many paint factories and tanneries need a sustainable alternative to expensive technologies, such as treatment plants or large landfills, for treating and/or immobilising heavy metal-enriched organic waste [23]. Phytoremediation is an emerging cleanup technology, which uses grasses or higher plants for treating environmental contaminants such as heavy metals, trace elements, organic or radioactive compounds in soils, groundwater and industrial waste. Phytoremediation includes the overall biological, chemical and physical processes that enable the uptake, sequestration, degradation and metabolisation of contaminants, either by plants or by organisms that constitute the plant's rhizosphere. Compared to current remediation technologies landfill disposal or in situ chemical and (physical treatments) phytoremediation provides an in situ solution at a relative low level of fi- nancial and technical input [19-23]. The early phytoremediation studies used hyperaccumulator species, which are plants able to accumulate unusually high levels of metals in their tissues. In addition to hyperaccumulators, plants such as trees and grasses are now being actively evaluated, though their metal bioconcentrating capability is well below that of hyperaccumulator plants. Fast-growing tree species, such as poplar, could be a suitable candidate to treat heavy metal-polluted soils and to produce economically valuable non-food biomass exploitable for energy production [23-24].

Application of cobalt resulted in significant increase in cobalt uptake by soybean plants over control. Cobalt increased the concentration and uptake of cobalt in plants. Cobalt treatment at 50 mg kg-1 soil level proved to be favourable for the overall growth of soybean plants. Under lower cobalt application improved root system helped the plants in better absorption of water and other nutrients dissolved in it and consequently improved the growth of different organs and the entire plants [19]. The improvement in the growth efficiency of plant organ might also be due to beneficial effects of cobalt treatment on the physiological activities of plants which was responsible in improving the growth of plant and its component organs ultimately influencing the relative development of plant parts and their growth efficiency.

A complex metal homeostasis network system has evolved in plants to regulate heavy metal uptake and distribution, thus protecting the metabolic process. Several mechanisms have been proposed to describe how plants tolerate heavy metals in the soil growing media environment. These include exclusion, restricting heavy metal uptake, inclusion (i.e. sequestrating and compartmentalizing metal in organs and organelles) and phytochelatins binding [24].

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