MINERAL NUTRIENT VARIATIONS IN SOYBEAN (*GLYCINE MAX*) AFTER TREATMENT WITH EXOGENOUS COBALT

K. Jayakumar[1]*, P. Vijayarengan[2]

[1]*Department of Bioscience, Annai College of Arts and Science, Kovilecherry, Kumbakonam- 612 503, Tamilnadu, India*
[2]*Department of Botany, Annamalai University, Tamilnadu, India*

Abstract

In this experiment, we tried to explore the changes that are occurring in soybean plants due to the treatment with exogenous cobalt. A pot culture experiment was conducted and cobalt was given to Soybean (*Glycine max)* plants by soil drenching method. Nitrogen, phosphorus, potassium, copper, iron, manganese and zinc contents were extracted and estimated from both control and treated plant parts (from roots and shoot). The results showed the low concentrations of cobalt (50 mg kg^{-1} Co level) in the soil enhanced these parameters while higher concentration (Co level (100-200 mg kg^{-1}) in the soil) didn't show any significant effect.

Key Words: *Glycine Max*; Nitrogen; Phosphorus; Potassium; Copper; Iron; Manganese; Zinc; Cobalt.

Introduction

Plants species can be selected to extract and assimilate or extract and chemically decompose target organic contaminants. Heavy metals can be taken up and bio accumulated in to plant tissues [1]. Many organic compounds considered being contaminants are, in fact, vital plant nutrients that can be absorbed through the root system for use in growth and development. Heavy metals can be taken up and bio accumulated in plant tissue [2].

With the development of industries, mining activities, application of wastewater and sewage sludge on land, heavy metal pollution of soils is increasingly becoming a serious environmental problem [3]. The concentration of heavy metals in air, water and soil leads to many hazardous effects to living organisms. Excessive metal concentrations in contaminated soils can result in decreased soil microbial activity and soil fertility and yield losses [4]. Accumulation of trace elements, especially heavy metals, in the soil has potential to restrict the soil's function, cause toxicity to plants, and contaminate the food chain [5].

Environmental pollution problems caused by heavy metals cannot be over emphasized. Heavy metals are very toxic and posses a threat to man and the environment [6]. The amount of these heavy metals in our environment increases as a result of industrialization. The anthropogenic sources of heavy metals include waste from the electroplating and metal finishing industries, metallurgical industries, tannery operations, chemical manufacturing, mine drainage, battery manufacturing, leachates from landfills and contaminated ground water from hazardous waste sites [7]. The ever-increasing input of heavy metal in to the environment has become a widely acknowledged ecoproblem. Environmental restoration of metal polluted soils by traditional physical and chemical methods demands large investments of economic and technological resource [8]. Methods based on plants have consequently gained a good deal of attention.

Metals supplied to the environment are transported by water and air ultimately reaching the soil and sediment where they become bound. However, the time taken for them to become bound may be fairly long and it has been shown that the bioavailable fraction of metals in soils is high at the beginning of the binding period, but decreases with time [9]. Thus, there is probably a bigger problem with anthropogenically-supplied metals, with high levels of bioavailable metals, than with high background levels originating from bedrock with slow weathering [10].

_____

* Corresponding Author, Email: drjayakumar@rediffmail.com

One of the main problems of using plant materials and bulk analyses for environmental monitoring of atmospheric deposition of heavy metal emissions is the existence of countless processes that can alter the chemical composition of plant materials. Emissions appear in particular or dissolved form and will usually occur as a mixture of both that changes with distance to source. Uptake of elements into plants can happen via different routes. The elements can be taken up via roots from the soil and transported to the leaves. Elements may be taken up from the air or precipitation directly via the leaves [11].

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.

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.

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.

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.

Nutrient content analysis

Nitrogen, phosphorus, potassium, copper, iron, manganese, zinc and cobalt in plant materials were estimated by the following methods.

Estimation of total nitrogen [12]

Hundred milligrams of dried materials were taken in the Kjeldahl flasks and 5 ml of salicylic – sulphuric acid mixture (5 g salicylic acid in 100ml concentrated sulphuric acid) was added. The flask was, rotated to mix and allowed to stand for 30 minutes. Approximately 0.3g sodium thiosulphate was added and heated gently until fumes appeared. Then 5 ml of concentrated sulphuric acid and approximately 0.1 g of catalyst mixture (Copper sulphate, potassium sulphate and selenium dioxide mixed in the ratio of 1:8:1) were added. Digestion was performed at low heat until frothing stopped and fumes of sulphuric acid were freely evolved. After 5-10 minutes heat was increased, so that the acid is boiled and condensed one third way up to neck of the flask. Digestion was continued for at least 3 hours, till the digest has become colourless. On completion of digestion, the flask was cooled and 20ml of water was added. The flask was again cooled and the content was transferred into a 50 ml volumetric flask and made upto the volume.

Distilled water was boiled in the flask and clips were kept closed and opened respectively when steam passed through the funnel and the funnel was washed twice with 1 ml of water each time. The ground glass stopper was replaced and 8 ml of 40% sodium hydroxide was added in funnel. The lower end of the condenser was kept dipped into 5 ml of 2% boric acid and few drops of mixed indicator (6 ml methyl red solution (0.16% in 95% alcohol) and 12 ml bromocresol green (0.04% in water) were mixed and 6 ml of 95% alcohol was added to the mixture) contained in a 50 ml conical flask. When steam issued freely through the tube, the clip was closed and the ground glass stopper was lifted to allow sodium hydroxide to run into the digest. The stopper was immediately replaced and distillation was continued until 30 ml distillate had been collected. After a few ml of liquid had distilled over, the end of the condenser was raised above the level of boric acid.

Heating was stopped after distillation was completed, so that the liquid in the distillation chamber was automatically sucked into the jacket. A few ml of water was added through the funnel and the stopper was replaced. This liquid in the jacket was allowed to run as waste by opening the clip. Now the apparatus was ready for the next distillation. Then the whole distillate was titrated against standard 1/28 hydrochloric acid solution until the pink colours just reappear. Blank digestion, distillation and titration, were made using all the reagents without plant sample. The percentage of total nitrogen was calculated.

Estimation of phosphorus [13,14]

One gram of dried and ground plant tissue was digested with 10 ml of acid mixture (nitric acid, 750 ml; sulphuric acid, 150 ml; perchloric acid 60%, 300 ml). The digest was cooled and made upto 50 ml and filtered through acid washed Whatman No.1 filter paper. One ml of digest was mixed with 2 ml of 2 N nitric acid and diluted to 8 ml. One ml of molybdovanadate reagent (25 g of ammonium molybdate in 500 ml water, 1.25g ammonium vanadate in 500 ml of 1 N nitric acid; both were mixed in equal volumes) was added, make up to 10 ml, shacked and the absorbance was measured at 420nm in a spectrophotometer, after 20 minutes of standing. Standard graph was prepared using potassium dihydrogen phosphate.

Estimation of potassium [15]

Dried and ground tissues weighing 0.5 g were digested in 100 ml Kjeldahl flasks using 15 ml of concentrated nitric acid, 0.5 ml of 60% perchloric acid and 0.5 ml of concentrated sulphuric acid. Digestion was continued until the nitric and perchloric acids were driven-off. The inorganic residue was cooled and diluted with 15 ml of distilled water and filtered through Whatman No.42 filter paper. The filtrate was made up to 50 ml with distilled water. The filtrate was used for potassium estimation by flame photometer and standards were prepared with potassium chloride.

Estimation of copper, iron, manganese and zinc [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 overnight. 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.

Results

Nitrogen

Nitrogen content of root of soybean plants is represented in Table 1. Nitrogen content of cobalt treated soybean root increased considerably at 50 mg kg^{-1} of soil level (viz., 31.63, 44.21 and 49.32) in all the sampling days. Cobalt beyond this level showed a gradual decrease in nitrogen content. The lowest nitrogen content

was recorded at 250 mg kg^{-1} of cobalt level (viz., 13.69, 25.86 and 29.32) in all the sampling days.

Nitrogen content of shoot of soybean plants is represented in Table 1. Nitrogen content of soybean shoot was found to be the highest at 50 mg kg^{-1} cobalt level (viz., 38.11, 69.42 and 88.62) in all the sampling days. With further increase in cobalt level there was a gradual decrease in the nitrogen content in all the sampling days.

Phosphorus

Phosphorus content of root of soybean plants is compiled in Table 2. Phosphorus content of cobalt treated soybean root increased considerably at 50 mg kg^{-1} of soil level (viz., 3.7, 4.8 and 5.4) in all the sampling days. Cobalt beyond this level showed a gradual decrease in phosphorus content. The lowest phosphorus content was recorded at 250 mg kg^{-1} of cobalt level (viz., 1.4, 1.9 and 2.1) in all the sampling days.

Phosphorus content of shoot of soybean plants is represented in Table 2. There was a gradual decrease in phosphorus content with increasing cobalt level in the soil. Maximum decrease of phosphorus content was recorded at 250 mg kg^{-1} cobalt level (viz., 1.8, 2.2 and 2.6) in all the sampling days. However, the phosphorus content increased at 50 mg kg^{-1} cobalt level (viz., 3.6, 4.8 and 5.6) in all the sampling days.

Potassium

Potassium content of root of soybean plants is represented in Table 3. Potassium content of cobalt treated soybean root increased considerably at 50 mg kg^{-1} of soil level (viz., 17.12, 19.46 and 22.19) in all the sampling days. Cobalt beyond this level showed a gradual decrease in potassium content. The lowest potassium content was recorded at 250 mg kg^{-1} of cobalt level (viz., 8.12, 9.77 and 11.41) in all the sampling days.

Potassium content of shoot of soybean plants is represented in Table 3. Potassium content of soybean shoot was found to be the highest at 50 mg kg^{-1} cobalt level (viz., 26.32, 29.12 and 33.37) in all the sampling days. With further increase in cobalt level there was a gradual decrease in the potassium content in all the sampling days.

Copper

Copper content of root of soybean plants is represented in Table 4. There was a gradual decrease in copper content with increasing cobalt level. Maximum decrease of copper content was recorded at 250 mg kg^{-1} cobalt level (viz., 7.86, 9.51 and 11.17) in all the sampling days. However, the copper content

003

increased at 50 mg kg⁻¹ cobalt level (viz., 17.43, 20.64 and 22.53) in all the sampling days.

Table 1 & 2. Effect of cobalt on nitrogen and phosphorus contents (mg g⁻¹ dry weight) of *Glycine max* (L.) Merr.

Cobalt added in the soil (mg kg⁻¹)	ROOT			SHOOT		
	Sampling days					
	30	60	90	30	60	90
Control	28.37	39.43	43.15	31.22	57.30	71.19
50	31.63 (+11.49)	44.21 (+12.12)	49.32 (+14.29)	38.11 (+22.06)	69.42 (+21.15)	88.62 (+24.48)
100	25.44 (-10.32)	36.54 (-7.075)	39.58 (-8.273)	26.37 (-16.72)	47.61 (-16.91)	59.71 (-16.12)
150	22.72 (-19.91)	31.73 (-19.52)	36.19 (-16.12)	21.41 (-31.42)	41.74 (-27.15)	48.23 (-32.25)
200	17.45 (-38.37)	28.92 (-26.65)	32.46 (-24.77)	18.64 (-40.29)	36.19 (-36.84)	42.44 (-40.38)
250	13.69 (-51.74)	25.86 (-34.41)	29.32 (-32.05)	15.98 (-48.81)	32.92 (-42.54)	36.41 (-48.85)

Cobalt added in the soil (mg kg⁻¹)	ROOT			SHOOT		
	Sampling days					
	30	60	90	30	60	90
Control	2.8	3.7	4.2	3.1	4.1	4.7
50	3.7 (+32.14)	4.8 (+17.07)	5.4 (+28.57)	3.6 (+16.12)	4.8 (+17.07)	5.6 (+19.14)
100	2.5 (-10.71)	3.2 (-13.51)	3.7 (-11.90)	2.8 (-9.677)	3.6 (-12.19)	4.2 (-10.63)
150	2.1 (-25.00)	2.7 (-27.02)	3.2 (-23.80)	2.4 (-22.58)	2.9 (-29.26)	3.5 (-25.53)
200	1.8 (-35.71)	2.3 (-37.83)	2.6 (-38.09)	2.1 (-32.25)	2.6 (-36.58)	2.9 (-38.29)
250	1.4 (-50.00)	1.9 (-48.64)	2.1 (-50.00)	1.8 (-41.93)	2.2 (-46.34)	2.6 (-44.68)

(Per cent over control values are given in parentheses)

Table 3 & 4. Effect of cobalt on potassium and copper contents (mg g-1 dry weight) of *Glycine max* (L.) Merr

Cobalt added in the soil (mg kg⁻¹)	ROOT			SHOOT		
	Sampling days					
	30	60	90	30	60	90
Control	16.41	18.57	21.32	21.71	24.65	28.18
50	17.12 (+4.326)	19.46 (+4.231)	22.19 (+4.080)	26.32 (+21.23)	29.12 (+18.13)	33.37 (+18.41)
100	14.34 (-12.61)	16.72 (-10.44)	19.14 (-10.22)	18.41 (-15.20)	21.43 (-13.06)	25.43 (-9.758)
150	12.67 (-22.79)	13.70 (-26.62)	16.35 (-23.31)	17.64 (-18.74)	19.27 (-21.82)	22.15 (-21.39)
200	10.43 (-36.44)	11.22 (-39.90)	13.24 (-37.89)	15.17 (-23.30)	17.62 (-28.51)	21.61 (-23.31)
250	8.12 (-50.51)	9.77 (-47.57)	11.41 (-46.48)	12.32 (-43.25)	14.46 (-41.33)	19.31 (-31.47)

Cobalt added in the soil (mg kg⁻¹)	ROOT			SHOOT		
	Sampling days					
	30	60	90	30	60	90
Control	15.21	18.32	20.17	22.84	23.64	28.17
50	17.43 (+14.59)	20.64 (+12.66)	22.53 (+11.70)	25.67 (+12.39)	27.81 (+17.63)	33.63 (+19.38)
100	13.12 (-13.74)	16.14 (-11.89)	18.12 (-10.16)	19.35 (-15.28)	22.17 (-6.218)	26.41 (-6.247)
150	11.63 (-23.53)	14.19 (-22.45)	15.64 (-22.45)	17.41 (-23.77)	21.41 (-9.433)	23.74 (-15.72)
200	9.41 (-38.13)	12.63 (-31.05)	13.22 (-34.45)	15.67 (-31.39)	17.35 (-26.60)	21.61 (-23.28)
250	7.86 (-48.32)	9.51 (-48.08)	11.17 (-34.37)	12.92 (-43.43)	14.19 (-39.97)	18.37 (-34.37)

Table 5 & 6. Effect of cobalt on iron and manganese contents (µg g-1 dry weight) of *Glycine max* (L.) Merr.

Cobalt added in the soil (mg kg⁻¹)	ROOT			SHOOT		
	Sampling days					
	30	60	90	30	60	90
Control	126	135	241	198	203	241
50	133 (+5.555)	149 (+10.37)	273 (+13.278)	231 (+7.575)	221 (+8.866)	273 (+13.278)
100	112 (-11.11)	123 (-8.888)	228 (-5.394)	191 (-3.535)	194 (-4.433)	228 (-5.394)
150	94 (-25.39)	106 (-21.48)	219 (-9.128)	173 (-12.62)	186 (-8.374)	219 (-9.128)
200	87 (-30.95)	98 (-27.40)	185 (-23.23)	136 (-31.31)	147 (-27.58)	185 (-23.23)
250	72 (-42.85)	84 (-37.74)	163 (-32.36)	118 (-39.89)	132 (-34.97)	163 (-32.36)

Cobalt added in the soil (mg kg⁻¹)	ROOT			SHOOT		
	Sampling days					
	30	60	90	30	60	90
Control	35.11	41.19	64.76	59.47	61.19	64.76
50	38.63 (+10.02)	45.74 (+11.04)	73.31 (+13.20)	66.41 (+11.66)	69.41 (+13.43)	73.31 (+13.20)
100	29.73 (-15.32)	36.53 (-11.31)	62.18 (-3.983)	55.15 (-7.264)	58.32 (-4.690)	62.18 (-3.983)
150	26.13 (-25.57)	31.65 (-23.16)	55.41 (-14.43)	48.61 (-18.26)	53.43 (-12.68)	55.41 (-14.43)
200	22.41 (-36.41)	26.37 (-35.97)	52.21 (-19.37)	44.67 (-24.88)	49.31 (-19.41)	52.21 (-19.37)
250	18.65 (-46.88)	21.87 (-46.87)	43.63 (-32.62)	32.32 (-45.65)	37.19 (-37.19)	43.63 (-32.62)

(Per cent over control values are given in parentheses)

Copper content of shoot of soybean plants is presented in Table 4. Copper content of soybean shoot was found to be the highest at 50 mg kg⁻¹ cobalt level (viz., 25.67, 27.81 and 33.63) in all the sampling days. With further increase in cobalt level there was a gradual decrease in the copper content in all the sampling days.

Iron

Iron content of root of soybean increased at 50 mg kg^{-1} and decreased further with an increase in the cobalt level in the soil (Table 5). There was a gradual decrease in iron content with increasing cobalt level. Maximum decrease of iron content was recorded at 250 mg kg^{-1} cobalt level (viz., 72, 84 and 163) in all the sampling days. However, the iron content increased at 50 mg kg^{-1} cobalt level (viz., 133, 149 and 273) in all the sampling days.

Iron content of shoot of soybean increased at 50 mg kg^{-1} and decreased further with an increase in the cobalt level in the soil (Table 5). Iron content of soybean shoot was found to be the highest at 50 mg kg^{-1} cobalt level (viz., 231, 221 and 273) in all the sampling days. With further increase in cobalt level there was a gradual decrease in the iron content in all the sampling days.

Table 7. Effect of cobalt on zinc content (µg g^{-1} dry weight) of *Glycine max* (L.) Merr

Cobalt added in the soil (mg kg^{-1})	ROOT			SHOOT		
	Sampling days					
	30	60	90	30	60	90
Control	20.31	23.64	25.74	35.15	37.73	44.15
50	23.17 (+14.09)	26.21 (+10.87)	28.93 (+12.39)	40.41 (+14.96)	43.32 (+14.81)	47.37 (+15.03)
100	18.45 (-6.15)	21.42 (-8.39)	22.34 (-13.20)	31.72 (-4.75)	34.41 (-4.78)	38.62 (-4.22)
150	16.87 (-18.21)	18.38 (-22.33)	19.81 (-23.01)	29.14 (-17.09)	31.57 (-16.32)	36.17 (-12.18)
200	14.27 (-28.73)	15.41 (-34.81)	17.34 (-32.34)	26.18 (-25.03)	28.59 (-24.22)	32.31 (-21.55)
250	12.11 (-40.37)	13.72 (-41.95)	15.42 (-40.05)	20.43 (-41.94)	23.15 (-38.64)	27.19 (-33.98)

(Per cent over control values are given in parentheses)

Manganese

Manganese content of root of soybean plants is represented in Table 6. Manganese content of soybean shoot was found to be the highest at 50 mg kg^{-1} cobalt level (viz., 38.63, 45.74 and 73.31) in all the sampling days. With further increase in cobalt level there was a gradual decrease in the manganese content in all the sampling days.

Manganese content of shoot of soybean plants is represented in Table 6. There was a gradual decrease in manganese content with increasing cobalt level. Maximum decrease of manganese content was recorded at 250 mg kg^{-1} cobalt level (viz., 32.32, 37.19 and 43.63) in all the sampling days. However, the manganese content increased at 50 mg kg^{-1} cobalt level (viz., 66.41, 69.41 and 73.31) in all the sampling days.

Zinc

Zinc content of root of soybean plants is recorded in Table 7. Zinc content of soybean shoot was found to be the highest at 50 mg kg^{-1} cobalt level (viz., 23.17, 26.21 and 28.93) in all the sampling days. With further increase in cobalt level there was a gradual decrease in the zinc content in all the sampling days.

Zinc content of root of soybean plants is represented in Table 7. There was a gradual decrease in zinc content with increasing cobalt level. Maximum decrease of zinc content was recorded at 250 mg kg^{-1} cobalt level (viz., 20.43, 23.15 and 27.19) in all the sampling days. However, the zinc content increased at 50 mg kg^{-1} cobalt level (viz., 40.41, 43.32 and 47.37) in all the sampling days.

Discussion

Cobalt treated soybean plants showed an increase in micro and macro element contents under low concentration (50mg/kg of soil). But the higher concentrations seem to be deleterious to the mineral status of this plant. Since heavy metals may cause essential nutrient deficiency and even change the concentrations of basic nutrients such as N and P in plant tissues [17], better understanding of how heavy metal toxicity may be reversed by elevated macronutrient supply is needed. The deficiency of plant nutrients causes different changes in the physiological and biochemical processes within the plant cell resulting in a reduction of growth, delay of development and qualitative and quantitative decrease of yield [18-23].

The nitrogen content of soybean showed a progressive decline with increase in cobalt level. However 50 mg kg^{-1} cobalt level produced positive effect on the nitrogen content of soybean. The reduction in nitrogen content under cobalt treatments was comparable with the results [21-24]. Heavy metals sharply decrease the NO$_3$ uptake by roots, incorporation of nitrogen into organic compounds and translocation of nitrogen to leaves. The uptake of nitrogen from soil is also inhibited by the presence of heavy metals [23]. Decrease in nitrogen content of leaves of soybean due to cobalt could be attributed to decreased uptake of nitrogen from the soil.

Phosphorus content of leaves of soybean plants decreased with an increase in the cobalt content (except 50 mg kg^{-1}) in the soil. Reduction in uptake and distribution of phosphate from the soil into the roots and shoots of barely due to Cr, Ni, Cu, Zn, Cd, Hg and Pb treatments. Excess amount of trace elements usually affect the mineral nutrition of plants [25]. Metal toxicity may affect certain elements more than others and interactions among elements may occur [26]. Here the phosphorus content of leaves of soybean plants decreased by cobalt stress.

Cobalt level above 50 mg kg^{-1} significantly reduced the potassium content in leaves of soybean plants. Many

investigators have reported that heavy meal toxicity in general was associated with reduced absorption and accumulation of potassium [21-27]. Potassium is one of the essential macronutrients, taken up by the roots is generally transported to shoot through the xylem and this transport seems to be controlled by the shoots growth. Decrease in potassium content of soybean due to cobalt may be due to the toxic effect of cobalt on plant growth or competition by other ions, which in turn exercised a regulatory control on potassium uptake. The mineral nutritional status of plant can strongly affect the "quality" of crop plants and its organs. If potassium is inadequate, the synthesis of protein is inhibited [26]. The decreased potassium and protein content in soybean plants under cobalt stress in the present study justified the above statement.

Increased cobalt content of soil significantly decreased the copper content of leaves of soybean. However low level of cobalt (50 mg kg^{-1}) increased the copper content of soybean plants. Similar decrease in copper content with concomitant increase with heavy metals were also reported by other scientists [24-28].

The decrease in copper content of the plant tissues studied can be explained to some extent by the close association of copper with nitrogen ligands. It has been reported that there was a close parallel relation in the movement of copper and nitrogen out of the old leaves. In the present investigation, the decrease in the copper content was parallel with the decrease in nitrogen and potassium. The strong binding of copper by nitrogen ligands may be involved in the relationship of copper and nitrogen movement out of the leaves. Zn and Cu are essential for human diet and both elements can also cause toxicity to human and plants if exceeded [28].

Iron content of root and shoot of soybean plants decreased with increase in cobalt content in the soil. The inhibition of iron absorption was due to occupation of interfering ions in iron absorbing sites. It is becoming a generally accepted fact that iron and probably other micronutrients, do not more in the plant as simple ions. Their efficient translocation appears to depend on chelation or complexing by organic acids in the plant. Cobalt may be dependent on a similar type of mechanism with in plant and may well compete with iron in the translocation of iron from leaves. The decrease in iron content with increase of cobalt level suggests the heavy metal induced iron deficiency. Industrialization has brought in water pollution, which is one of the major hazards facing environment today. Since many industries discharge their effluent on to open lards because of high cost of dilution and inadequate treatment facilities, effective and profitable utilization of the effluent of the industries needs greater attention. Phytoremidation (such as phytoextraction, phytostablization and rhizofilitration) of soils contaminated by effluents has been widely accepted as a cost-effective and environmental - friendly cleanup technology [1]. However, the progress in this field is hindered by lack of understanding of complex interactions in the rhizosphere and plant – based mechanisms which allow metal translocation and accumulation in plant [2].

References

1. Otabong, E., 1989. Chemistry of Cr in some Swedish soils. 4. Influence of CrO_3 and $KH_2 PO_4$ on uptake and translocation of Mn, Cu, Zn, Fe and Al by rye grass (*Lolium perenne*). Acta Agric. Scand, 39: 149-157.
2. Ouzoundidou, G., 1994. Copper reduced changes on growth, metal content of *Alyssum montanum* (L.) Plants. Environ. Exp. Botany, 34(2): 165-172.
3. Parmar, G. and V. Chanda, 2005. Effects of mercury and chromium on peroxidase and IAA oxidase enzymes in the seedlings of *Phaseolus vulgaris*. Turk. J. Biol., 29-21.
4. Peach, K. and M.V. Tracey, 1956. Modern Methods of Plant Analysis, Vol. I. Springer Verlog, Berlin, pp. 477.
5. Peters, J.L., F.J. Castillo and R.L. Heat, 1989. Alteration of extracellular enzymes in pinto bean leaves upon exposure to air pollutant, ozone and sulfur dioxide. Plant Physiol., 89: 159-164.
6. Phor, S.K., U.C. Pandey and U. Verma, 1995. Effect of zinc on the growth and yield of garlic (*Allium sativum* L.) Crop Res., 9(2): 286-291.
7. Piccini, P.F. and E. Malvolta, 1992. Effect of nickel on two common bean cultivars. J. Plant Nutr., 15(11): 2343-2350.
8. Pitman, M.G. and W.J. Cram, 1977. Regulation of ion content in whole plants. In: D.H. Jennings (ed.) Integration of activity in the higher plant. Symp. Soc. Exp. Biol., 31: 391-424.
9. Pokhrel, A. and H.D, Leahak, 2001. Effects of lead on germination and seedling growth of some vegetable crops. Environment and Agriculture: Biodiversity, Agriculture and Pollution in South Asia, pp. 519-524.
10. Prakash, S.M., M.S. Yogeetha, V.R. Ramakrishna parama and Ramegowda, 2004. Effect of irrigation water with chromium on germination and growth of radish (*Raphanus sativus* L.). Nature Environ. Pollut. Tech., 3(1): 121-124.
11. Prasad, D.D.K. and A.R.K. Prasad, 1987. Effect of lead and mercury on chlorophyll synthesis in mung bean seedlings. Phytochem., 26: 881-883.

12. Peach, K. and M.V. Tracey, 1956. Modern Methods of Plant Analysis, Vol. I. Springer Verlog, Berlin, pp. 477.

13. Yoshida, S., D.A. Forno, J. Cock and K.A. Gomez, 1972. Laboratory Manual for Physiological Studies of Rice, IRRI, Philippines.

14. Black, C.A., 1965. Method for soil analysis. Part 2. Chemical and microbiological properties. American Society of Agronomy Inc., Madison, Wisconcin, pp. 242.

15. Williams, C.H. and V. Twine, 1960. In: Modern Methods of Plant Analysis (Eds.). K. Peach and M.V. Tracey. Vol. V. Springer Verlog, Berlin, pp. 3-5.

16. De Vries, M.P.C. and K.G. Tiller, 1980. Routine procedures for determining Cu, Zn, Mn and Fe in plant materials. Commonwealth Scientific and Industrial Research Organisation, Australia.

17. Dinew, N. and Stancheva, 1993. Effect of aluminium on growth of wheat, rye and triticale. J. Plant Nutr., 16(3): 461-467.

18. Quang D., T.V. Hai., E.T. Kanyama and J.E. Dufey, 1996. Combined effects of alumimium, iron and phosphorus on uptake and yield of rice plants (*Orysa sativa* L.) in nutrient solution. Agronomic (Paris), 16(3): 175-186.

19. Rauno, A., C.H. Poschenrieder and J. Barcelo, 1988. Growth and biomass partritioning in zinc toxicity of bush beans. J. Plant. Nutr., 11(5): 577-588.

20. Dang, Y.P. and K.S. Verma, 1990. Effect of Cd, Ni, Pb and Zn on growth and chemical composition of onion and fenugreek. Commun Soil Sci. Plant Anal., (9-10): 717-735.

21. Ren, F.C., T.C. Liu, H.Q. Liu, and B.Y. Hu. 1993. Influence of zinc on the growth, distribution of elements and metabolism of one year old American ginseng plants. J. Plant Nutr., 16: 393-405.

22. Robson, A.D. and M.G. Pitman, 1983. Interactions between nutrients in higher plants. In: A. Lauchli and R.L. Bieleski (eds.), Inorganic Plant Physiology, N.S., Vol. 15A, Springer Verlog, Berlin, pp. 147-180.

23. Rohr and Baughinger, 1976. Chromosome analysis in all cultivates in Chinese hamster after application of cadmium sulfate. Mutat. Res., 40: 125-126.

24. Ross, S. 1994. Toxic metals in soil plant systems. Wiley, Chichester, p. 469.

25. Roth, J.A., E.F. Wallihan and R.G. Sharpless, 1971. Uptake by oats and soybeans of copper and nickel added to a peat soil. Soil Sci., 112: 338-342.

26. Rother, J.A., J.W. Millbank and I. Thronton, 1983. Nitrogen fixation by white clover (*Trifolium repens*) in grassland on soils contaminated with Cd, Pb and Zn. Soil Sci., 34: 127-136.

27. Rout, G.R. and P. Das, 2003. Effect of metal toxicity on plant growth and metabolism: 1 Zinc, INRA, EDP Sciences. Agronomic, 23: 3-11.

28. Samantaray, S. and B. Deo. 2004. Studies on chromium toxicity in mungbean (*Vigna radaita* L.). Ad. Plant Sci., 17(1): 189-194.