Research Square

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Copper, Chromium and Nickel Heavy Metal Effects on Total Sugar and Protein Content in Glycine Max.

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Abstract

Background

Metals are one of the micro molecules required by living cell to do their biochemical functions. Out of a list of metals few are very important and useful to cells while few are required in very minute amount and their higher concentration harm or cause adverse effects on living forms. Copper and ferrous are commonly counted as essential metals to plant cells but sometimes their higher concentration is harmful to plant cells. Copper is commonly counted as important metal to plant cells but sometimes their higher concentration is harmful to plant cells.

Results

It was found that higher concentration of copper and ferrous showed reduction in germination percentages and gemination time of investigated plant while chromium shown lethal impacts on seed germination

Conclusions

In the present study effects of copper, chromium and nickel was studied for total sugar and protein content of widely cultivated pulses i.e. *Glycine max*. Collectively present study showed that higher concentration of copper and nickel has adverse effects on *Glycine max* total sugar and protein content while chromium has lethal impacts at lower concentration also.

Background

Nature has about 108 elements (as at the present time frame few more are identified and isolated too) and life has evolved in the presence of these elements only. out of these some elements are recognized as metals. Further, metals groups are proposed depending on their physico-chemical properties and one such group is known as heavy metals [1]. The group that have been identified as heavy metals, trace metals is referred to as elements having relatively high atomic number and mass (>20 and 8 gm/cm^3) [2].

Few members of this heavy metal/trace metal group are copper (Cu), chromium (Cr), iron (Ferrous) (Fe), nickel (Ni), manganese (Mn), zinc (Zn), molybdenum (Mo) etc. they are vital micronutrients as they are required in minute quantities in several biochemical and structural components such as plant growth, redox reactions, electron transport mechanism and many metabolic pathways [3].

At low concentrations, metals can serve as important components in life processes, often serving important functions in enzyme productivity. However, above certain threshold concentrations, metals can become toxic to many species [4] [5]. Some heavy metals such as copper, iron, and zinc are essential

nutritional elements whereas others such as mercury, lead, chromium, and cadmium are not essential for nutrition and are considered to be toxic metals [6][7].

As a rule, in nature anything that is present on this earth should be either degraded out or recycled. Heavy metals cannot be degraded out but they can be recycled by changing their ionic stage. Any kind of biomass can be easily degraded out in nature [8][9].

Surprisingly, there are many examples available from the literature describing plants which synthesize and accumulate secondary metabolites upon treatment with an abiotic stress factor, mainly heavy metal ions [10].

There are about 50 metals that are studied with respect to the toxicological importance to plants, animals and man. Such metals accumulate in soil to reach the plant through roots during water absorption and cause serious adverse effects on plants viz., inhibition of seed germination, growth of seedlings and reduction of yield [11]. From these studies it is revealed that at international level there is awareness about the detrimental effect of various heavy metals on the plants [12] [13].

The toxic threshold level of the metal in the tissue is defined by the “stress point” for metal toxicity, and beyond this level the physiological state of the cell will be irreversibly damaged [14] [15].

Excessive heavy metal pollution can cause a series of physiological and biochemical changes that lead to potential ecosystem risk. Various anthropogenic activities including industries, mining, chemical fertilizers and through transportation vehicles heavy metals enter and accumulate in the natural environment [16] [17]. The metals present in air, water and soil environments have been absorbed by plants via roots as the main source of entrance and even by leaves also and become a cause of dysfunctionality in metabolism and at later stages may create risk to human health [7] [18].

The most pronounced effect of heavy metals on plant development is growth inhibition, which is inseparably connected with cell division. It can alter numerous plant functions such as development and biosynthesis of chloroplast and even photosynthesis also. However, the mechanisms involved in those processes are still not completely understood [19].

In context to copper, it is a natural element having atomic number 29 and atomic mass $1.055\times10^{-22}g$m, found in soil with irregular amounts depending on soil types [20] [21]. Copper is recognized as an essential redox-active transition metal and an essential micronutrient because of multiple oxidation stages *in vivo*, it is involved in many structural and enzymatic activities as it is part of structure in regulatory proteins and is associated in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism and hormone signaling for plant growth and development as and when present in optimal concentration and environmental conditions [20] [21].

It is a constituent element of proteins and enzymes especially those which are involved in redox processes and/or are participating in electron transport reactions [22]. It is also required as a cofactor in

many enzymatic reactions and as an integral part of photosynthetic machinery such as in plastocyanin, a photosynthetic pigment [23]. Many physiological, biochemical and environmental parameters affect copper ion uptake and accumulation in plants [24]. When copper is present in excess amount it alters plant growth and development pattern by inducing oxidative stress and doing changes in plants metabolism [21].

Very little information is available in connection with heavy metal effects such as copper on growth of pulses and though some investigations have been carried out yet such study is not sufficient as impacts of copper on biochemical parameters viz., total sugar and protein content is perfectly not known.

In the present work, impact of copper, chromium and nickel metal on total sugar and protein content was studied in widely cultivated pulses *Glycine max*.

Results

Total sugar content

Sugar is an important molecule necessary for life. It is the reserve source of energy that is required for development and maintenance of life. Presence of heavy metals such as copper in plant cells affects the concentration of sugar.

The total sugar content in *Glycine max* root was 25.69 mg/gm in control. Due to copper treatments of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 mM the proportion of total sugar of root was increased except at 1.4, and 1.6 mM copper concentration. The maximum increase was observed by the treatment of 0.2 mM copper (Table 1). The amount of total sugar content in the stem of *Glycine max* was 15.04 mg/gm in control. The treatment of different concentrations of copper has decreased the total sugar content in stem of *Glycine Max*, Maximum decrease in the sugar content was at 1.6 mM copper treatment (Table 2).

The total sugar content in the root and stem of *Glycine max* was 25.69 and 15.04 mg/gm respectively which was reduced to 17.98 and 10.53 gm by 0.2 mM chromium treatment. Germination was not observed at 0.4 - 1.6 mM chromium concentrations (Table 1,2).

In the root of *Glycine max* the total sugar content was higher than control at the treatment of lower concentrations of nickel. The total sugar content was reduced when concentration of nickel was increased from 0.6 - 1.6 mM in the treatment (Table 1). In the stem of *Glycine max* the total sugar content was lower than control in all concentrations of nickel in the treatment (Table 2).

Protein content

Protein is one of the major molecules necessary for life. Protein works as a tool for almost all cellular activities and is quite susceptible to many materials. Protein content was affected by copper.

In *Glycine max* protein content of root was 36.75 mg/gm and of stem was 29.14 mg/gm in control condition. Protein content was higher than control in root of *Glycine max* in different copper treatments except the higher concentrations (1.2, 1.4, and 1.6 mM copper concentrations) where the amount of protein was reduced (Table 3). The stem of *Glycine max* protein content was lower than control in all treatments of copper. The lowest protein content was found at 1.6 mM copper concentration.

The protein content in the root and stem of *Glycine max* was 36.75 and 29.14 mg/gm respectively which were reduced to 25.71 and 20.59 mg/gm by 0.2 mM chromium treatment (Table 3,4).

In the root of *Glycine max* protein content was higher than control due to the treatment of lower concentrations (0.2 and 0.4 mM) of nickel. At higher concentrations of nickel, the protein content was lower than control. The lowest protein content was found at 1.2 mM nickel concentration. In the stem of *Glycine max* the amount of protein was decreased by increasing concentration of nickel (Table 3,4).

Discussion

When plants are exposed to single heavy metal stress, activity of photosynthesis is readily inhibited [25] [26]. In plant metabolism products, sugars are recognized as essential metabolites as the first complex organic molecule formed as a resultant effect of the photosynthesis occurred in plants and are the main source of energy through respiration. Besides sugars are important as they have a role in protection of plants against infection and wounds and foreign material detoxification [27] [28].

Effect of copper on total sugar content

The study on the effect of copper on carbohydrates or total sugar content of plant organs is scanty. Presence of heavy metals alter carbohydrate accumulation and distribution in plants. Normally the total sugar content decreases by the treatment of heavy metals [29]. Few workers reported a decrease in total sugar in various plants by cadmium treatment. This was found in *Cajanus cajan* and *Trigonella foenum-graecum* [30], rice [31] and sugar beet [32]. Singh *et al.* [33] observed reduction in total sugar in wheat seedlings in different copper treatments. In the present work the decrease in total sugar content was observed in the root and stem of all three investigated plants except the root of *Glycine max* where slightly higher total sugar content was found at lower concentration of copper. They suggested that copper at lower concentration works as a nutrient in plants but when its concentration increases, it causes toxicity and affects the sugar contents in plants. Ouzounidou *et al.* [34] found that in *Zea mays* due to copper treatment photosynthetic machinery was affected and less amount of photosynthesis was carried out under excess copper treatment. Several other researchers also studied copper toxicity on photo inhibitory and recovery process of PSII and demonstrated that adverse effects of light were enhanced as the concentration of copper increased [23] [35-38]. In thylakoids and PSII-enriched membranes copper induces photoinhibition susceptibility. Copper also increases reactive oxygen species (ROS) as copper is a vital catalyst in formation of hydroxyl radicals [35-38]. Patsikka *et al.* [39] suggested that the decrease in chlorophyll content is due to higher copper concentration in leaves because of Fe-deficiency induced as a cause of copper concentration. Dowidar *et al.* [40] reported decrease in sucrose

and starch content in *Trigonella foenum-graecum* however, they reported increase in monosaccharide content when treated with copper. At higher concentrations copper becomes extremely toxic causing symptoms such as chlorosis and necrosis, stunting, leaf decolorization and inhibition of root growth.

Many changes have been possible by the effect of copper concentration in plant cells. They may be 1) because of copper binding with sulfhydryl groups present on enzymes and/or membrane proteins [41], 2) enhancement of lipid peroxidation rate [42], 3) alteration in essential mineral uptake [43], 4) affected synthesis of chlorophyll, electron transport and thereby reduced photosynthesis due to copper toxicity [23], 5) as ROS and redox element through Fenton and Haber-Weiss reactions [44], 6) cell permeability and/or essential metal binding may be interfered by presence of copper ions [45], 7) reactions with free thiol moieties and thereby dysfunctionality of cell processes [46], 8) copper toxicity may exert in electron transport, ATP production and respiration like subcellular organelle functions [47], 9) as a catalyst copper can do decomposition of H_2O_2 and generate hydroxyl radical (OH^0) that enhanced membrane lipid oxidative deterioration [40] [48].

Zhao *et al*. [49] found that the content of root soluble sugar of *K. obovata* seedlings increased first and then decreased with the increase of a Cu concentration, while the soluble sugar content of leaves decreased with an increasing Cu concentration.

Deef [50] showed that at lower concentrations of copper all carbohydrate fractions increased but as the concentration of copper increased polysaccharides and total carbohydrates decreased greatly. This could be because of reduction of CO_2 fixation in heavy metal treated plants. But for lower concentrations, the photosynthetic measurements were not affected by treatment and thus could be increased in carbohydrate content [51]. Due to reduced photosynthesis total sugar content could also be affected and in turn decreased concentration of sugar results in reduction of growth rate and biomass content has also been reported [12] [13] [52] [53].

Effect of chromium on total sugar

Chromium is known to be an essential element for normal carbohydrate metabolism in animal and human nutrition [54] [55]. Although chromium was found to be a stimulant for plant growth [56], several investigations were carried out to determine its toxic effect on different plant species [57 – 68]. According to Jun *et al*. [69] pulses like *Lablabpurpureus* and *Glycinemax* are most sensitive to chromium.

In the present work plant, *Glycinemax*, was found sensitive to chromium where the seeds could not germinate at higher concentration of chromium and the amount of total sugar was reduced at lower chromium concentration. This was probably due to chromium toxicity which leads to decrease in enzyme activity, causes membrane damage, diminishes photosynthesis and change in chloroplast [59] [70 -73].

Effect of nickel on total sugar

Nickel is an essential micronutrient for higher plants [74-76]. However, nickel at sufficient high levels may be toxic to plants [77 – 79]. Excess nickel can affect physiological or biochemical processes [80] [81].

Very little research has been conducted on the effect of nickel on sugar contents of plants. Gad *et al*. [82] reported increase in total soluble sugars by increasing nickel in the treatment. Moya *et al*. [83] and Samarakoon and Rauser [84] have found an increase in the amount of carbohydrates in the leaves due to nickel toxicity. In the present study it has been observed that the lower concentration of nickel has no adverse effect on total sugar content in the root and stem of investigated plant but at high concentration the proportion of total sugar was reduced. Few workers reported higher accumulation of carbohydrates in leaves and decrease in root by heavy metals [85 – 87]. Similar accumulation of carbohydrates was found in maize leaves by L'Huillier *et al*. [86] and suggested that this was because of the inhibition of carbohydrate transport from leaves to root due to nickel toxicity. Greger *et al*. [87] proposed a hypothesis whereby heavy metals have a greater effect in reducing carbohydrate transport than reducing photosynthesis.

Effect of copper on protein content

One of the major essential components of the cell is proteins. Environmental stress situations made them easily degradable. One of the indications of oxidative stress is change in proteins [88].

Increased doses of heavy metals reduce protein contents [29]. Guo *et al.* [89] carried out experiments by adding aluminum, copper and cadmium in nutrient solutions on barley plants. They had reported decrease in protein content in roots and leaves by the combined effect of these three metals in Shang 70 - 119 variety of barley. Llorens *et al.* [90] studied copper exposure upon tissue cultured *Vitis vinifera*. They found dramatic changes in nitrogen metabolism, which was reflected in total nitrogen, amino acids and protein contents in both roots and leaves. This change is reflected by an increase in the activity of certain enzymes, and peroxidase induction is a general response of higher plants to uptake of toxic amounts of metals [15] [91].

During the seedling growth of wheat, there was decline in protein content in leaf tissues by the higher copper concentrations [33]. Costa and spitz [51] showed that at lower concentration of some heavy metals, the carbohydrate and amino acid levels increased in shoots but not in roots. At high concentrations all primary metabolites decreased. Reduced growth rate and biomass content due to altered metabolism and modified biochemical parameters has been reported [12] [13] [52] [53].

In wheat also, the reduction in protein was observed in response to copper [92]. Tripathi and Tripathi [93] also found a decrease in protein content in *Albizia lebbeck* and explained that this was either due to reduced de novo synthesis of proteins or increased decomposition of proteins into amino acids. According to Samantaray [94] the decrease in protein content may be due to inhibition of protein synthesis by heavy metal. In this work the protein content of roots and stem was studied where the treatment of higher copper concentration resulted in reduction of protein in these two organs of investigated plants.

Effect of chromium on protein

Plant growth is inhibited by chromium toxicity [68] [95]. This work showed that the seeds failed to germinate by chromium treatment except at 0.2 mM chromium concentration. At this treatment protein content in root and shoot of investigated plants was reduced. Similar decrease in protein content was reported by Sankar *et al*. [96] in *Glycinemax* by increased chromium concentrations.

Chromium can degrade protein [97]. According to Ganesh *et al*. [98] during the transport of heavy metals into the plants, it can act at different sites to inhibit a large number of enzymes having functional sulfhydryl groups. It results in the deleterious effect in the normal protein form [99] by disrupting in the pathways and protein synthesis [100].

Effect of nickel on protein

El - Enany *et al*. [101] studied the nickel toxicity in bean seedlings. According to them nickel inhibits protein contents by about 50 % at low and higher losses. In the roots of *Glycinemax* and *Vignaaconitifolia* protein content was slightly higher than control at lower concentration of nickel. In the root of *Vignaunguiculata* and stem of all three investigated plants protein content was decreased by increasing the nickel concentration in the treatment. Similar results were obtained by Singh and Pandey [102]. They reported an increase in protein content by lower concentration of nickel in the leaves of *Pistiastratiotes* which decreased at higher concentrations. Reduction in protein content in plants by nickel can be attributed to effect on nitrate reductase activity [103].

Conclusions

From the results obtained in the present study it revealed that low levels of copper and nickel concentration have little positive impact in *Glycine max* root total sugar and protein content. However, as concentration increased, reduction in total sugar and protein content in the root and stem of *Glycinemax* reported indicating that higher doses of these metals have a negative impact on total sugar and protein content. Chromium showed negative impact even at low concentration as no growth in *Glycinemax* was recorded and proved that chromium is lethal to *Glycinemax* even at lower concentration.

Methods

Design and establishment of experiment

The experimental work was accomplished in the area of Rajkot city (22^0 17’ Lat. and 70^0 49’ Lon.).

Coarse loam soil was collected from the natural habitats used for the study of seedling growth and emergence where selected plants were cultured by seed germination. The soil after collection, firstly air dried and then passed through a 2 mm sieve to get uniform particle size throughout the experimental set.

To detect the effect of copper metal on total sugar and protein content, copper metal salt was added and mixed with the soil in the form of copper sulphate salt ($CuSO_4$ $5H_2O$), potassium dichromate salt ($K_2Cr_2O_7$) and nickel sulphate salt ($NiSO_4H_2O$). Soil sets of 10 kgs. each with eight different copper concentrations were prepared for experimental plant cultivation. The concentrations prepared were 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 mM copper respectively. To get these concentrations 1.95, 3.90, 6.00, 7.80, 9.74, 11.70, 13.60, and 15.60 grams copper sulphate; 3.30, 6.60, 10.00, 13.30, 16.60, 19.90, 23.20, and 26.60 grams potassium dichromate salt and 2.40, 4.70, 7.10, 9.40, 11.80, 14.20, 16.50, and 18.90 nickel sulphate salt was used respectively. One additional set of soil was arranged without metal salt addition used as control, considering heavy metal concentration zero as it was counted negligible.

Polyethylene bags were filled with 1 kg metal salt mixed soil. For each concentration 10 bags of 1 kg soil each were stacked. Up to the field capacity of the soil, tap water was added and then allowed to dry for six days.

Seed sowing and plant harvesting

From Sanjiv Agro Center, Rajkot, seeds of *Glycine max* were obtained. The seeds were surface sterilized with H_2O_2 and sown in bags of copper metal treated soil. In each bag 10 seeds were sown at the depth of about 8-10 mm in evening with immediate watering and then after on alternate days. Seeds were allowed to germinate.

Overall experiment was conducted twice and the results obtained are average of two sets of experimentations.

Plants harvested after a specific duration of time in such a manner that tap root and root hairs were not damaged or minimal damage will be there. By gentle washing soil particles were removed from roots. Plants brought to the laboratory, washed again and blot dried to remove moisture and any other particles from the surface. The roots and stem were analyzed for total sugar and protein content. Biochemical analyses were done by using Nelson and Somogyi technique for total sugar estimation and protein estimation was carried out by using Folin-phenol technique.

Total sugar estimation

To estimate total sugar content freshly harvested and cleaned plant parts were used. The estimation was done by Nelson [104] [105] technique.

Extraction and determination of total sugar

100 mg of plant material was taken; homogenized and the volume was made up to 10.0 ml with 80 % ethanol. It was then kept in a boiling water bath for 20 minutes. The sample was centrifuged and the supernatant consisted of ethanol soluble free sugars. From this solution 1.0 ml of solution was taken and 1 ml of reagent C was added in this solution. This solution was kept in a water bath for 10-15 minutes. 1.0 ml of arsenic molybdate was added in this solution after cooling. The solution was kept for 15

minutes for color development and optical density was measured with the help of spectrophotometer using 620 nm wavelengths.

Regression equation for sugar concentration and optical density was evaluated and the trend line was determined from the following regression equation.

$Y = 0.2257 (X) - 0.25$

$R^2 = 0.9966$

Where, Y = Concentration

X = Optical density

Protein content estimation

The roots and stem were analyzed for protein contents. Protein content was estimated by Folin Phenol technique [106].

Extraction and determination of protein

100 mg of plant material was crushed in 10.0 ml of 80 % ethanol. The crushed material was kept for 15 minutes in a boiling water bath. It was then centrifuged. The residue was suspended in water and 10 ml 10 % perchloric acid was added to remove sugars and soluble nitrogen fractions. The residue was washed with distilled water and digested in a water bath with 1.0 ml 0.1 N NaOH for 10 minutes. The mixture was cooled and volume was made up to 10.0 ml with distilled water. It was then centrifuged and residue was discarded. From the supernatant, 1.0 ml was taken and to this solution 5.0 ml of reagent C was added, both were mixed well and allowed to stand for 10 minutes at room temperature. 0.5 ml of reagent D was added rapidly and mixed well. The solution was allowed to stand for 30 minutes. The developed blue color was measured as optical density in spectrophotometer at 680 nm and the content of protein in terms of mg/gm was calculated by using the following regression formula.

$y = 0.004x - 0.0041$
$R^2 = 0.997$

Where, Y = Concentration

X = Optical density

Abbreviations

N.G.: No Growth

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contribution

Yongtao Duan[1] and Kunjal V. Soni[2*] proposed scientific ideas and did manuscript preparation, Chetan B. Sangani[2] and Mohd. Muddassir[3] did practical work. All authors equally contribute to manuscript preparation.

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Tables

Table 1: Change in root total sugar in *Glycine max* due to effect of heavy metals

Concentration (mM)	Copper	Chromium	Nickel
Control	25.69	25.69	25.69
0.2	30.35 + 0.22	17.98 + 0.01	29.43 + 0.09
0.4	29.79 + 0.22	N. G.	28.16 + 0.09
0.6	29.51 + 0.22	N. G.	24.84 + 0.09
0.8	28.98 + 0.22	N. G.	22.74 + 0.09
1.0	28.44 + 0.22	N. G.	21.44 + 0.09
1.2	27.89 + 0.22	N. G.	19.19 + 0.09
1.4	24.72 + 0.22	N. G.	N.G.
1.6	22.70 + 0.22	N. G.	N.G.

Table 2: Change in stem total sugar in *Glycine max* due to effect of heavy metals

Concentration (mM)	Copper	Chromium	Nickel
Control	15.04	15.04	15.04
0.2	14.00 + 0.05	10.53 + 0.01	13.08 + 0.01
0.4	13.44 + 0.05	N. G.	11.81 + 0.01
0.6	13.16 + 0.05	N. G.	10.49 + 0.01
0.8	12.63 + 0.05	N. G.	9.39 + 0.01
1.0	12.09 + 0.05	N. G.	8.09 + 0.01
1.2	11.54 + 0.05	N. G.	6.84 + 0.01
1.4	11.37 + 0.05	N. G.	N. G.
1.6	10.35 + 0.05	N. G.	N. G.

Table 3: Change in root protein in *Glycine max* due to effect of heavy metals

Concentration (mM)	Copper	Chromium	Nickel
Control	36.75	36.75	36.75
0.2	41.40 + 0.19	25.71 ± 0.01	40.49 ± 0.31
0.4	40.85 + 0. 19	N. G.	39.21 ± 0.31
0.6	40.56 + 0. 19	N. G.	35.90 ± 0.31
0.8	40.04 + 0. 19	N. G.	33.80 ± 0.31
1.0	39.50 + 0. 19	N. G.	32.49 ± 0.31
1.2	35.94 + 0. 19	N. G.	30.24 ± 0.31
1.4	34.78 + 0. 19	N. G.	N. G.
1.6	33.75 0. 19	N. G.	N. G.

Table 4: Change in stem protein in *Glycine max* due to effect of heavy metals

Concentration (mM)	Copper	Chromium	Nickel
Control	29.14	29.14	29.14
0.2	28.46 + 0.05	20.59 ± 0.01	27.18 ± 0.06
0.4	27.91 + 0.05	N. G.	25.90 ± 0.06
0.6	27.62 + 0.05	N. G.	22.59 ± 0.06
0.8	27.10 + 0.05	N. G.	20.49 ± 0.06
1.0	26.56 + 0.05	N. G.	19.18 ± 0.06
1.2	26.00 + 0.05	N. G.	16.93 ± 0.06
1.4	22.84 + 0.05	N. G.	N. G.
1.6	20.81 + 0.05	N. G.	N. G.