academicJournals

Vol. 12(29), pp. 4614-4622, 17 July, 2013
DOI: 10.5897/AJB2013.12387
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Physiological, biochemical and molecular responses of common bean (*Phaseolus vulgaris* L.) plants to heavy metals stress

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Accepted 31 May, 2013

Heavy metals are essential and important for plants growth, and play as key components of many vital compounds. However, when they increase in concentration, heavy metals show symptoms such as growth delay and inhibition of biochemical reactions. The current study focused on the impact of five heavy metals (lead, chromium, nickel, cadmium, zinc) on growth and performance of common bean (*Phaseolus vulgaris* L. cv. Nebraska) plants before and after liming ($CaCO_3$ + $MgCO_3$) as soil correction treatment for the sake of remediation for heavy metal pollution in soil. Chemical analysis of carbohydrates showed significant increases in the contents of reducing sugars in response to lead, cadmium and nickel stress, which were decreased by liming treatments. The contents of total soluble sugars also increased in all heavy metal-treated plants but zinc. All heavy metals significantly lowered the leaf contents of the photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids). The sodium dodecyl sulphate polyacrylamide gel electrophoreseis (SDS-PAGE) of proteins indicated variations in the profile of electrophoretic protein bands in heavy metal-stressed common bean plants before and after liming. The results indicate that the investigated heavy metals were absorbed from the soil solution and then accumulated in the tissues of common bean plants in variable concentrations. The highest accumulation were lead (Pb) and chromium (Cr) then cadmium (Cd), zinc (Zn) and nickel (Ni) while the magnitude of limiting the retarding rate of absorption and accumulation was: Pb > Ni > Cr > Zn > Cd, respectively.

Key words: Heavy metals, common bean, liming, chlorophyll.

INTRODUCTION

Heavy metal stress is one of the major abiotic stresses that cause environmental pollution in recent decades (Gisbert et al., 2003; Castro et al., 2011). These metals are unlike other organic pollutants are not degraded and converted into harmless compounds via biological processes. Heavy metals persist for a long time in the environment. In addition, heavy metals can enter into the food chain. A common feature of environmental stress is their ability of production of toxic oxygen derivatives (Arora et al., 1998; Chiban et al., 2011). Heavy metals make a significant contribution to environmental pollution as a result of human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping and military operations (Nedelkoska and Doran, 2000). However, elevated concentrations of both essential and non-essential heavy metals in the soil can lead to toxicity symptoms and growth inhibition in most plants (Hall, 2002; Li et al., 2010). Toxicity may result from the binding of metals to sulphydryl groups in proteins, leading to

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inhibition of activity or disruption of structure, or from displacement of an essential element, resulting in deficiency effects (van Assche and Clíjsters, 1990; Capuana 2011). In addition, a heavy metal excess may stimulate the formation of free radicals and reactive oxygen species, perhaps resulting in oxidative stress (Dietz et al., 1999; Li et al., 2011). Detailed studies indicate that heavy metals have effects on chlorophyll content in plants. Heavy metals are known to interfere with chlorophyll synthesis either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient (van Assche and Clíjsters, 1990; Meers et al., 2010). The amount of chlorophyll was reduced in *Triticum aestivum* cv. Vergina grown on Cu-enriched soil (Lanaras et al., 1993), and in *Brassica oleracea* var. Botrytis cv. Heavy metals such as Cu and Zn are essential for normal plant growth and development since they are constituents of many enzymes and other proteins.

However, elevated concentrations of both essential and non-essential heavy metals in the soil can lead to toxicity symptoms and growth inhibition in most plants (Hall 2002; Dong et al., 2010). Living organisms require certain metals for their growth and metabolism and so, they have got an appropriate uptake mechanism for metals. Some plant species have capacity to grow in the metal contaminated soil and accumulate elevated amount of heavy metals as an ecophysiological adaptation in metaliferous soil. *Phaseolus vulgaris* has been reported to be a good accumulator of lead and cadmium (Garay et al., 2000; Cannino et al., 2009; Zhang et al., 2010).

The mechanisms involved in heavy metal tolerance may range from exclusion, inclusion and accumulation of heavy metals depending on the plant species (Kaushik et al., 2005). Distinct concentrations of metals induce different biochemical responses in plants. In sensitive plants, high concentration of these metals inhibits enzymes involved in photosynthetic reaction (Smirnoval et al., 2006). *Brassica juncea* (Indian mustard), a high biomass producing plant can accumulate lead, chromium, cadmium, copper, nickel, zinc, boron and selenium (Palmer et al., 2001; Akbaş et al., 2009). Even trace elements have been shown to have toxic effect on different plant traits such as leaf, stem, root flower etc. (Sivakumar et al., 2001). Copper causes injury at cellular level by the formation of free radicals. Cellular injury by this type of mechanism is well documented for copper as well as other metals (Gupta and Kalra, 2006).

Copper being one of the common heavy metals in industrial discharge of aeronautic, metal and metallurgy, and refinery industries shows toxic effects on plants and animals. Previously, the copper concentration in soil and water was usually lower than 5 mg/ml but it has increased during the last decade reaching occasionally 50 mg/ml because of heavy industrialization. Among the pollution-producing metals, lead is a widespread heavy metal in the environment and it is regarded as non-essential elements and have a long half-life which is extremely persistent in the environment (Salt et al., 1998), with high toxicity and easily taken up by plants (Wua et al., 2003) and then enters the food chain, resulting in a serious health issue for animals and humans. Therefore, there is an increasing interest in effects of heavy metals on higher plants and their responses to excessive metal concentrations as stressors (Grant and Loake 2000; Zhang et al., 2010). This study examined the physiological and biochemical responses of common Bean (*Phaseolus vulgaris* L.) plants to heavy metals stress.

MATERIALS AND METHODS

Seeds of *P. vulgaris* L. (cv. Nebraska) were obtained from the Ministry of Agriculture, Kingdom of Saudi Arabia (KSA). The seeds were surface sterilized for 20 min in 1% (v/v) sodium hypochlorite, and then washed several times with distilled water. The sterilized seeds were planted in plastic pots in sand and grit (1:1, v/v) at 27°C temperature. The pots were irrigated daily with 200 ml distilled water. To study the effect of heavy metals (lead, chromium, nickel, cadmium and zinc) on the growth and performance of bean plants before and after adding limestone (calcium carbonate + carbonate magnesium) the following experiment was done. Seeds were germinated at four weeks and then bean seeds were divided into four equal groups; A, B, C and D.

Group A was the control which was irrigated with distilled water throughout the period of the experiment. Group B was irrigated daily with a solution of limestone 0.2 ppm (a mixture of calcium carbonate added to magnesium carbonate by 1:4). Group C was divided into five groups with each of them containing three pots irrigated with a heavy metal salt solution, (lead, chromium, nickel, cadmium, zinc) and concentration 200 ppm and irrigated by 200 ml and next day was irrigated with water and vice versa till the end of the experiment. Group D was as group C but was previously treated with limestone (0.2ppm) instead of using water. After two weeks from heavy metal exposure, plant samples were collected, washed carefully with H_2O, blotted dry and separated into roots, stems and leaves. Leaf area was determined using a moving belt electronic planimeter (Delta. T. Devices, burwell, UK). Fresh weights of different parts were determined and then the same parts were dried in an air oven at 70°C to obtain dry weight.

Measurements of photosynthetic pigments

Photosynthetic pigments, viz, chlorophyll a, chlorophyll b and carotenoids were extracted and determined from expanded young leaves according to the method of Inskeep and Bloom (1998). Known fresh weight (about 0.1 g) of leaves were immersed in 10 ml N, N-dimethylformamide (DMF) and kept overnight at 4°C. After incubation, chlorophyll contents (Chl a and b) and total carotenoids were determined in the extract by UV-spectropho-tometer (LKB, UK). The absorbance of the solution was measured between 400 and 700-nm.

Digestion and assessing of elements

Dry samples of shoot or root were finely ground and assayed for mineral-ions contents according to the method described by Humphries (1956). Metal concentrations (µg gG1 DW) such as Fe, Mn, Mg and N were estimated by atomic absorption spectrometry and Ca, Na and K by flame photometry. The values were expressed as µg gG1 dry weight of root or leaf for each treatment. Electrolyte leakage was measured according to the method des-

cribed by Humphries (1956) (inductive coupled plasma for optical emission spectrometry, ICP-OES).

Determination of carbohydrates

Reducing sugars such as glucose and fructose and total soluble sugars as sucrose and many sugars as starch were used. Extracting sugars operation was performed by separation device centrifugation (3000 rev / min for 5 min) and then filtrate was used in estimating the reducing sugars and total sugars dissolved, the sludge remaining in the tube centrifuge transferred to a petri dish where it was used to estimate the starch after drying at temperature of 80°C.

Determination of reducing sugars

Estimated reducing sugars was measured according to the method of Naguib (1964). Addition of 1 ml of solution Nelson to a certain size (1 ml) of plant extract and encased pipe foil aluminum, and pipes were placed for 15 min in a water bath till boilling, then left to cool; 1 ml solution Erzinomolbydat was added then shaking pipe and left to stop the escalation of bubbles, then eased resulting color after adding a given volume of distilled water. Intensity absorption of extract at wavelength 650 nm been taken using a spectro-photometry.

Determination of total sugars dissolved

A given volume (1.5 ml) of the enzyme invertase (0.1%) was added to a given volume of 1.5 ml of filtrate and left at room temperature for 30 min. Then three tubes were prepared and placed in each tube (1 ml) of the filtrate (1 ml) of Nelson. Pipes were encased by aluminum paper and placed in a water bath for 15 min, then cooled in a cold water bath. Then 1 ml Erzinomolbydat was added, and then left aside until bubbles stop rising. The resulting color was eased by adding a given volume of distilled water, and the amount of reducing sugars (sucrose) calculated by subtracting the amount of reducing sugars from reading total sugars dissolved.

Determination of numerous sugars (starch)

The quantity of many sugars as (starch) were taken by taking a constant weight which is known as the remaining sludge which was dried to 0.01 g and then 0.2 ml of enzyme Aldeastaz (0.1%) + (0.1 ml) of acetate solution organizer was added. 3 ml of distilled water was added and the mixture was left at 28°C for 24 h and 1 ml of toluene was added and then the amount of starch in a given volume was estimated by taking the same steps listed earlier for the estimation of reducing sugars.

Protein analysis by SDS-PAGE

Total proteins of fresh leaves were analyzed by SDS-PAGE. Leaves were ground on liquid nitrogen in 0.2 M Tris pH 8, 2% (w/v) SDS, 10% sucrose and 1% BME. Proteins were separated by SDS-PAGE according to (Laemmli, 1970).

Statistical analysis

Differences in the plants' physiological parameters under heavy metal effects were compared using ANOVA with means separation by Duncan's test using SPSS 15 software at a significance level of $P \leq 0.05$. Correlations between the metal concentrations and the physiological parameters were analyzed by a bivariate correlation test with Pearson correlation coefficient and a two-tailed test of significance using SPSS 15 software at significance levels of $P \leq 0.05$ and 0.001. ANOVA at 5% level of significance ($p \geq 0.5$) and the separation of averages worth less significant difference LSD (Steel and Torrie, 1980).

RESULTS

Soil and water are the fate of most chemical substances produced by humans, where most plants uptake all their macro and micronutrients essential for their growth and development. These nutrients exist in the soil in natural balance and acceptable levels. Excessive levels of essential elements lead to polluted soils and could possibly cause phytotoxicity to crop plants - that is the objective of the current research study.

Growth responses to heavy metals before and after liming

The statistical data analyses of the results declared non-significant ($P \geq 0.05$) variations in shoot and root lengths, shoot and root fresh weights, shoot and root dry weights, number of leaves of common bean plants before and after liming (Table 1); while the results were significant for the leaf fresh weight and the leaf area. The number of flowers was significantly reduced in response to heavy metal treatments only before liming. This indicates the high sensitivity of these developmental stages to heavy metal stress and liming treatment as well. Cadmium (Cd) was most inhibitory to leaves and flowers development and most responded to liming treatments (Table 1).

Chemical analysis of carbohydrates

Chemical analysis of carbohydrates showed significant increases in the contents of reducing sugars in response to lead, cadmium and nickel stress, which were decreased by liming treatments (Figure 1). The contents of total soluble sugars also increased in all heavy metal-treated plants expect for zinc (Figure 2). Moreover, the contents of polysaccharides increased under all heavy metal stress, and then decreased by liming treatment in all heavy metal-treated plants except for lead-treated plants (Figure 3).

The contents of non-reducing sugars decreased in lead and cadmium-treated plants, while it increased in zinc-treated plant (Figure 4). The contents of non-reducing sugars increased by liming except for zinc which equaled their contents in controlled plants (Figure 4). In general, the contents of total available sugars increased in all heavy metal-treated common bean plants except for lead. These increases were hindered by liming treatment in lead, chromium and zinc stressed plants.

Table 1. The impact of heavy metal stress measurements vegetative and reproductive growth of a Beans plant before and after adding limestone each value represents the average 3 replicates ± standard error coefficient.

Heavy metal	Root length (cm) (before)	Root length (cm) (after)	Stem length (cm) (before)	Stem length (cm) (after)	Number of leaves (before)	Number of leaves (after)	Number of flowers (before)	Number of flowers (after)	Wet weight of root in g (before)	Wet weight of root in g (after)	Dry weight of root in g (before)	Area of leaves cm^2 (after)
Pb	23.67±3.84	19.00±1.52	23.33±1.20	24.33±1.85	3.00±0.57	3.33±0.33	0.67±0.67	1.67±1.20	0.217±0.07	0.310±0.02	0.127±0.04	47.41±9.98
Cr	22.00±5.03	24.00±6.50	22.00±1.52	24.00±1.52	2.33±0.33	3.00±0.57	0.33±0.33	1.67±1.20	0.340±0.01	0.303±0.07	0.100±0.02	27.15±1.39
Ni	19.00±5.13	16.00±2.88	21.33±3.17	25.33±5.17	3.33±0.88	3.67±0.88	0.67±0.33	3.33±1.66	0.417±0.17	0.453±0.13	0.120±0.04	33.70±1.91
Cd	15.66±0.88	21.33±5.92	21.00±1.52	22.33±3.17	2.67±0.33	2.67±0.33	0.00±0.00	1.67±0.88	0.336±0.05	0.423±0.16	0.196±0.01	28.16±3.87
Zn	19.33±2.84	22.00±3.05	24.67±2.91	26.00±2.08	3.67±0.66	3.67±0.66	0.67±0.33	4.67±1.45	0.630±0.13	0.340±0.03	0.227±0.01	52.10±3.66
Control	20.33±3.71	21.00±3.21	23.67±2.02	22.33±2.33	4.00±0.57	4.00±1.15	3.66±0.88	3.33±1.76	0.483±0.16	0.293±0.03	0.180±0.06	40.41±3.37
LSD (P≥0.05)	5.22	5.11	2.91	3.98	1.86	1.57	2.11	2.82	1.64	0.161	0.136	6.51
Significance	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	*

**, Highly significant; *, significant; NS, non-significant.

Table 1. Contd.

Heavy metal	Dry weight of root in g (after)	Wet weight of stem in g (before)	Wet weight of stem in g (after)	Dry weight of stem in g (before)	Dry weight of stem in g (after)	Wet weight of leaves in g (before)	Wet weight of leaves in g (after)	Areas of leaves cm^2 (before)
Pb	0.180±0.01	3.851±0.02	5.58±0.96	0.380±0.17	0.627±0.16	1.81±0.54	3.02±0.63	17.74±3.73
Cr	0.177±0.02	3.750±0.96	4.24±1.12	0.527±0.14	0.560±0.26	1.17±0.27	2.65±0.19	14.46±2.14
Ni	0.240±0.08	4.041±0.66	10.01±5.81	0.617±0.18	0.987±0.93	0.416±0.08	3.18±0.61	5.328±1.84
Cd	0.203±0.04	3.37±0.57	4.09±1.31	0.500±0.02	0.673±0.15	1.103±0.22	2.58±0.27	5.328±1.84
Zn	0.193±0.06	4.03 ±0.71	8.51±1.13	0.850±0.41	0.847±0.22	1.41±0.09	5.31±0.37	13.90±0.91
Control	0.167±0.03	5.42±2.29	5.86±2.16	0.870±0.26	0.677±0.19	1.34±0.13	4.63±0.38	12.58±0.89
LSD (P≥0.05)	0.089	1.77	6.16	0.499	4.42	0.380	1.11	4.14
Significance	NS	NS	NS	NS	NS	*	*	*

**, Highly significant; *, significant; NS, non-significant.

Determination of chlorophylls content

All heavy metals (lead, chromium, nickel, cadmium, zinc) significantly lowered the leaf contents of the photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids). Chlorophyll a showed high sensitivity to lead, nickel, zinc and then cadmium, but low sensitivity to chro-mium. Chlorophyll b was less sensitive to heavy metal stress, but more sensitive to nickel (Table 2). Carotenoid contents were severely decreased by lead, but fairly affected by chromium. As an interesting result, compared to the controlled plants, the results of pigment analysis in common bean leaves were non-significant under liming (Table 2).

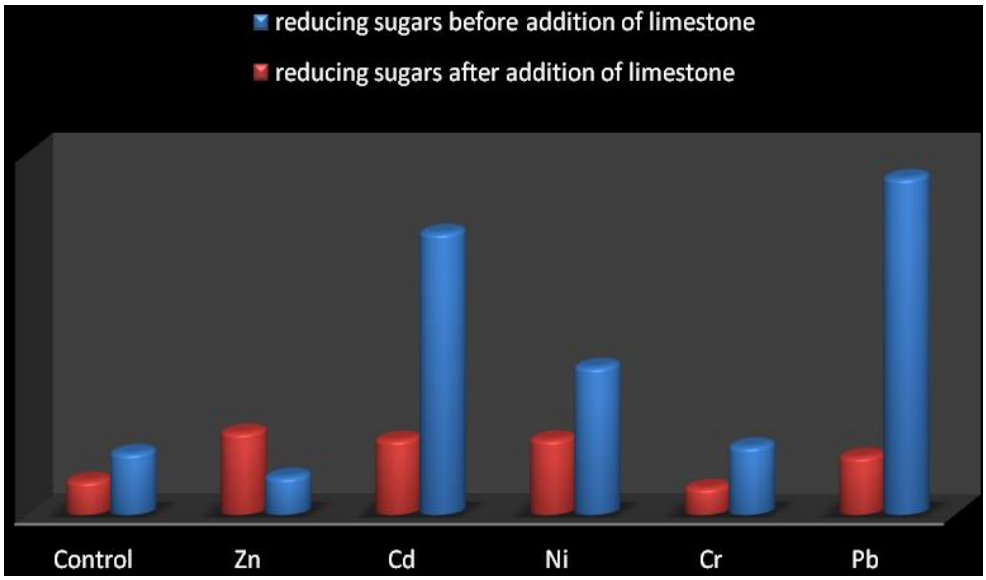

Figure 1. Effect of heavy metals on reducing sugars before and after)addition of limestone.

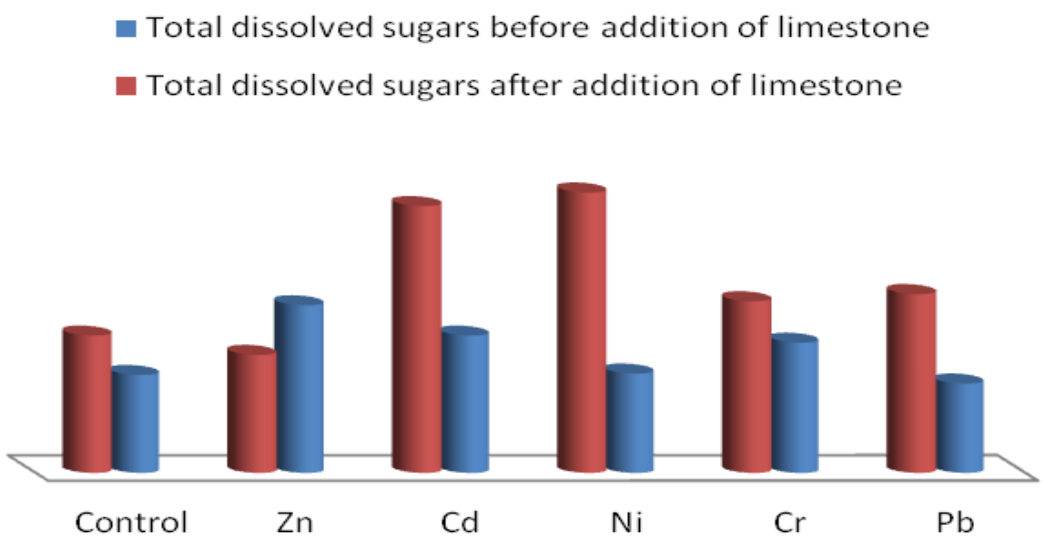

Figure 2. Effect of heavy metals on total dissolved sugars before and afteraddition of limestone.

The SDS-PAGE of proteins indicated both quantitative variations in the profile of electro- phoretic protein bands in heavy metal-stressed common bean plants before and after liming. The total number of protein bands and the band intensity (band %) increasedin all plants after liming. Moreover, liming treatments restored the synthesis of six protein polypeptides of molecular weights 295, 97, 84, 47, 30 and 23 KDa. Chromium and nickel stress induced a 71 KDa protein which was not restored by liming; while the synthesis of a 20 KDa protein in nickel and cadmium treated plants was completely inhibited by soil liming (Figure 5). In conclusion, the results indicate that the investigated heavy metals were absorbed from the soil solution, and then accumulated in the tissues of common bean plants in variable concentrations. The highest in accumulation were lead (Pb) and chromium (Cr) then cadmium (Cd), zinc and nickel (Ni) in the order: Pb > Cr > Cd > Zn > Ni; while the magnitude of liming in retarding the rate of absorption and accumulation was in the order: Pb > Ni > Cr > Zn > Cd (Figure 6).

DISCUSSION

Heavy metals are essential and important for normal growth and development of plants being an essential

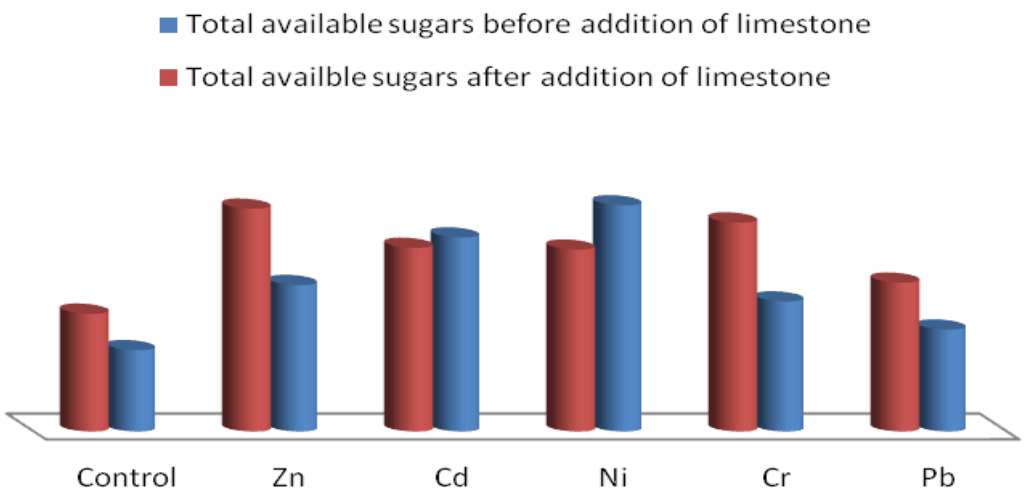

Figure 3. Effect of heavy metals on total available sugars before and after addition of limestone.

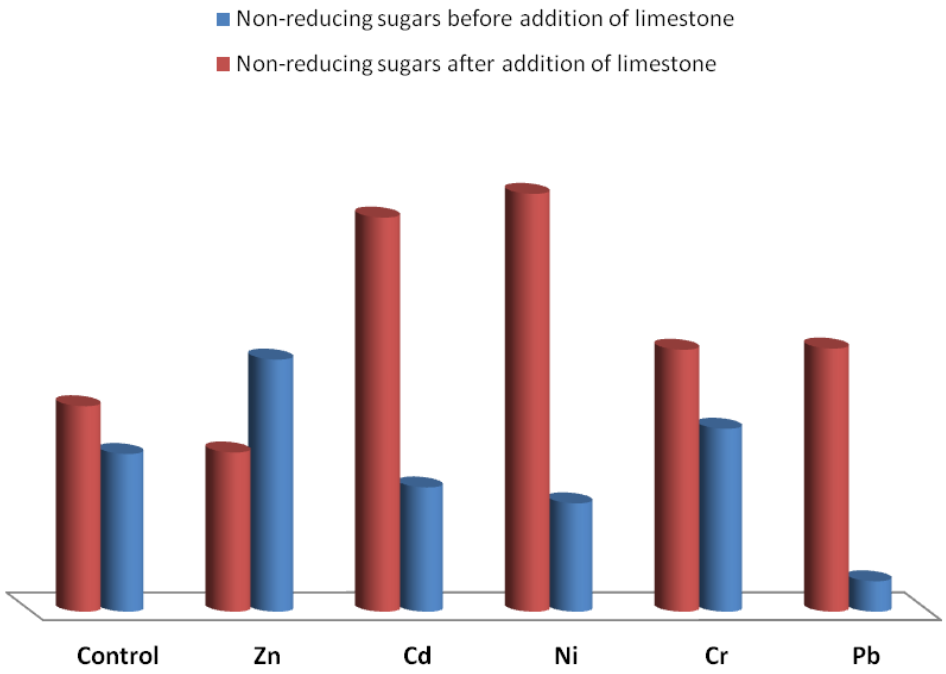

Figure 4. Effect of heavy metals on non-reducing sugars before and after addition of limestone.

component of many enzymes and proteins. On the other hand, it has been found that increasing heavy metals concentrations have led to the emergence of symptoms of poisoning such as inhibiting plant growth (Hall, 2002). Plants vary in their ability to absorb and accumulate minerals from the soil solution. Gülser and Erdogan (2008) found that low soil content of heavy metals, lead to a significant increase in the activity of enzymes. Several studies demonstrated that heavy metals can function as stressor, causing some physiological constrains that decrease plant vigor and inhibit plant growth (Schutzendubel et al., 2001). In this study, phototoxic symptom such as reducing number of flowers leaf fresh weight and area was observed as result of heavy metal treatment, and leaf water content were among the most sensitive responses to heavy metal exposure and faster than most of the otherphysiological reactions analyzed (Table 1). Ouzounidou et al. (1998)

Table 2. Effect of heavy metal content (mg/g wet weight) pigments construction on photosynthesis (chlorophyll a, chlorophyll b, carotene) in beans plant before and after adding limestone.

Heavy metal	Chlorophyll a (before)	Chlorophyll a (after)	Chlorophyll b (before)	Chlorophyll b (after)	Carotene (before)	Carotene (after)	Chlorophyll a/ chlorophyll b (before)	Chlorophyll a/ chlorophyll b (after)	Total contents of pigments (before)	Total contents of pigments (after)
Pb	1.493±0.39	1.499±0.14	0.509±0.12	0.499±0.06	0.418±0.13	0.435±0.08	2.827	3.004	2.366±0.64	2.433±0.28
Cr	2.072±0.14	1.56±0.24	0.58±0.06	0.485±0.10	0.805±0.05	0.755±0.07	3.572	3.216	3.457±0.25	2.8±0.41
Ni	1.405±0.29	1.06±0.16	0.464±0.108	0.337±0.06	0.552±0.12	0.424±0.05	3.028	3.157	2.421±0.51	1.825±0.27
Cd	1.717±0.08	0.921±0.18	0.558±0.02	0.256±0.07	0.654±0.03	0.354±0.06	3.077	3.597	2.929±0.13	1.531±0.31
Zn	1.23±0.04	1.272±0.05	0.526±0.03	0.404±0.02	0.588±0.02	0.499±0.02	2.752	3.148	2.562±0.09	2.175±0.09
Control	2.497±0.24	1.454±0.22	0.796±0.107	0.612±0.12	0.914±0.07	0.705±0.09	3.136	2.375	4.207±0.41	2.771±0.43
LSD(P≥0.05)	0.794	0.364	0.288	0.167	0.361	0.259	0.112	0.873	0.801	0.718
Significance	NS	*	NS	*	NS	*	*	*	*	*

Each value represents the average of 3 replicates ± standard error coefficient.

suggested that the inhibitory action of heavy metals on root length, shoot height and leaf area seems principally to be due to chromosomal aberrations and abnormal cell divisions and may also be correlated with the metal-induced inhibition of photosynthetic process and the respiration in the shoot system and protein synthesis in the root, or due to the reduction in cell proliferation and growth (Maria and Tadeusz, 2005). In this study, heavy metals were absorbed from soil solutions and then accumulated within common beans plant tissues in varying ratio and highest accumulation was for lead, chromium, zinc and nickel, respectively. The efficiency of limestone in reducing the rate of accumulation was as follows: lead, nickel, chromium, zinc and cadmium, respectively. As is clear from the results of this research, limestone resulted in an increase in protein bands of the protein profile as well as an increase in the optical density of proteins that is, the ability to resynthesize proteins affected by the negative impact of heavy metal stresses. The above mentioned data agreed with those of Kiekens (1983) who found that, the presence of some cations (positive ions) in the soil solution such as Ca^{2+} and Mg^{2+} compete with cations of heavy metals efficiently and prevent It from adhering with plasma of plant tissues and subsequently their accumulation decrease. On the other hand, the addition of limestone to the soil works to reduce soil acidity and increase alkalinity and high pH of the soil solution and thus make heavy metals in the form that they are non-available for absorption in the root and accumulation within plant tissues (McGrath et al., 1988). The contents of total available sugars increased in all heavy metal-treated common bean plants except for lead. These increases were hindered by liming treatment in lead, chromium and zinc stressed plants. Soluble sugar, is an important constituent manufactured during photosynthesis and breakdown during respiration by plants. All metals have decreased the content with increasing concentration as reported in agricultural crops (Hemalatha et al., 1997; Rascio and Navari-Izzo, 2011). Such inhibition of photosynthesis in higher plants by heavy metals has been reported (Bazzaz et al., 1975). The low sugar levels may be due to lowered synthesis or diversion of the metabolites to other synthesis processes. The decrease of chlorophylls contents as observed in this study showed high sensitivity to lead, nickel, zinc then cadmium, but low sensitivity to chromium. Chlorophyll b was less sensitive to heavy metal stress, but more sensitive to nickel (Table 2) and this agrees with the results of Appenroth et al. (2010). Carotenoid contents were severely decreased by lead, but fairly affected by chromium. As an interesting result, compared to the controlled plants, the results of pigment analysis in common bean leaves were non-significant under liming. Somashekaraiah (1992) found that the treatment of bean seeds with different concentrations of cadmium led to reduced levels of chlorophyll and iron in plants, and this contributed to the inhibition of its biosynthesis (Fabrizo et al., 2003). Proteins are important constituents of the cell that are easily damage in environmental stress condition (Prasad, 1996; Wu et al., 2010). Hence, any change in these compounds can be considered as an important indicator of oxidative stress in plants. The results of this study show variable changes in insoluble protein content in different metal treat-

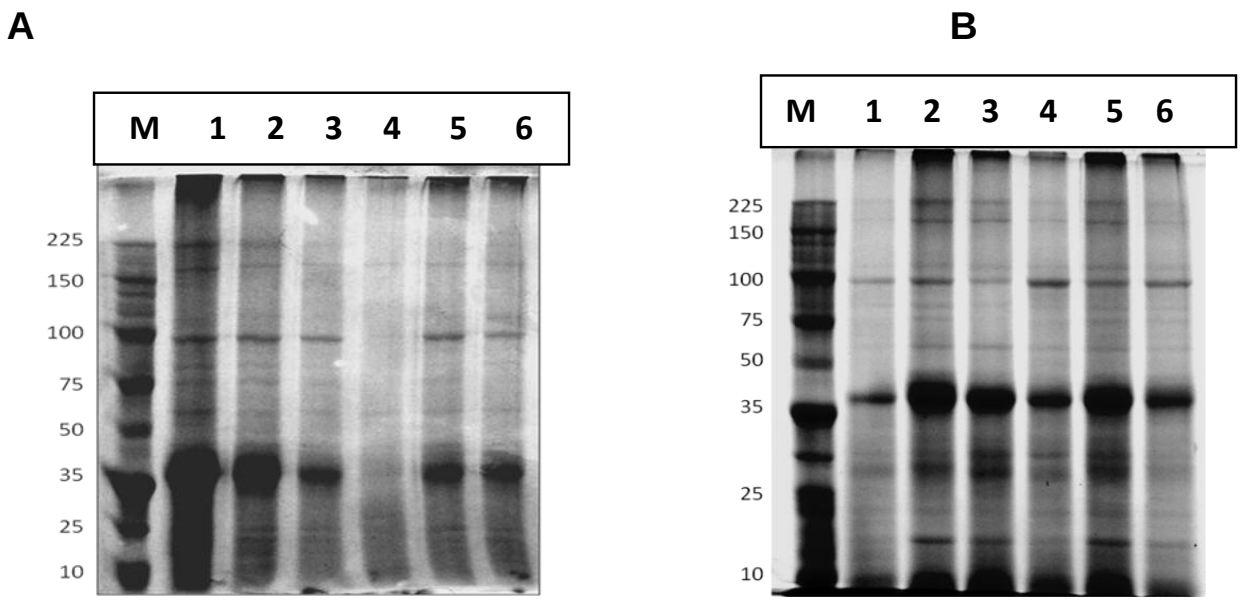

Figure 5. (Figure 5. SDS-PAGE of total protein of common bean. **A** , Lane 1, broad range marker; lane 2, control; lanes 2, 3, 4, 5 and 6 are heavy metals (Pb, Cr, Ni, Cd and Zn respectively) before liming. **B**, Lane 1, broad range marker; lane 2, control; lanes 2,3,4 ,5 and 6 are heavy metals (Pb, Cr, Ni, Cd and Zn respectively) after liming.

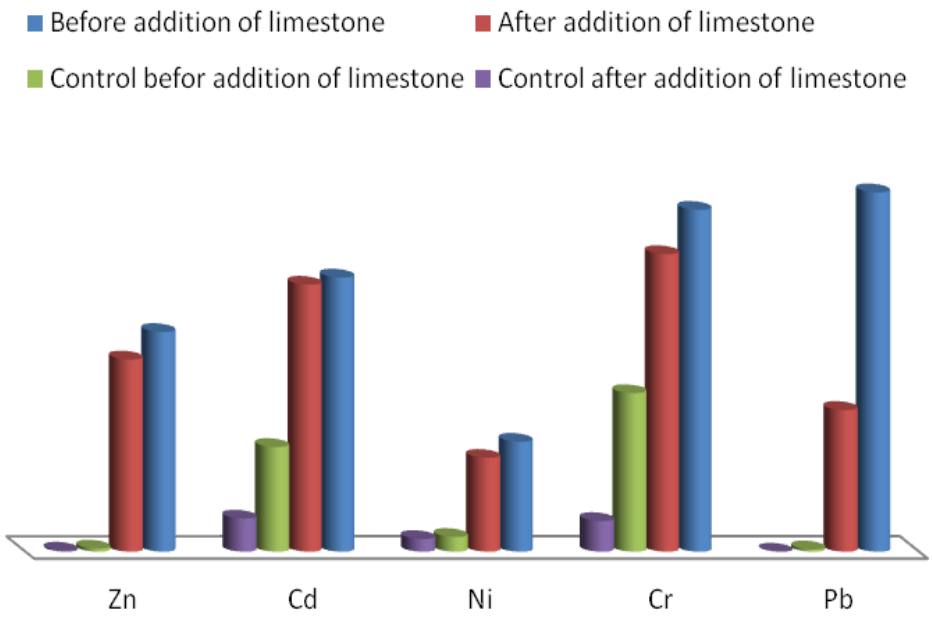

Figure 6. Heavy metal content (mg/L) in tissues of common bean, before and after the addition of limestone.

ments that might reflect different levels of antioxidant defense. The increase in total soluble protein content under heavy metal stress may be related to the induced synthesis of stress proteins such as enzymes involved in Krebs cycle, glutathione and phytochelatin biosynthesis and some heat shock proteins (Mishra et al., 2006). Based on the results and physiological and biochemical responses of beans plants to stresses of heavy metals in the study, it is recommended that limestone be used in some heavy metals polluted agricultural soils to maintain crop productivity and the overall health of humans and animals. It is worth to mention that limestone natural ingredients is environmentally safe, cheap, and added to some agricultural soils routinely to correct some of their

physical and chemical properties.

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