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Cadmium as an Environmental Pollutant Use of Plant as Bio-Indicator of Pollution (*In vivo* Experimentation) Influence of Cadmium on Chlorophyll Content of Canadian Wonder Beans (*Phaseolus vulgaris*)

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Abstract: Cadmium like other heavy metals is known to be a serious toxic element and one of the most hazardous environmental pollutants not only to soil and plants, but also to humans and animals. Studies carried out in different plant species have revealed that Cd is strongly phytotoxic and causes growth inhibition and even plant death, although the mechanisms involved in its toxicity are still not completely understood. The present investigation was aimed at highlighting some of the problems related to Cd and trying to answer some of the questions that have arisen as a consequence of Cd behavior and its toxic nature to plant species. The culture experiment of beans Phaseolus vulgaris was carried out in a hydroponic solution with variable Cd concentrations to determine the effects of different concentrations of Cd on photosynthesis and chlorophyll content. Chlorosis was the primary symptom caused by Cd toxic levels and this led to the study of its causes by investigating the effect of Cd on Chlorophyll formation and its content in plants. In that respect, one question appeared to be important about the nature of the effect of Cd on chlorophyll and Mg concentration was the first conclusion obtained on the basis of evidence achieved from analysis of the chlorophyll content by atomic absorption and ultra violet spectroscopy. Although some results were obtained indicating the formation of free-Mg chlorophyll suggesting the disturbance of the pigment and probably the removal of Mg caused by the increase of Cd concentrations in the chlorophyll. But more still to be achieved to suggest a possible displacement of Mg by Cd. Meanwhile a speculative view was considered to explain the matter by suggesting that Cd was rather interfering with other divalent elements such Fe and Cu which are known to be involved in the chlorophyll synthesis than Mg directly.

Key words: Toxic, environment, phytotoxic, growth inhibition, chlorosis, spectroscopy

INTRODUCTION

The increasing emission of Cd from a variety of sources because it is commonly used in many fields created a great interest about the possible movement of Cd and other heavy metals into the atmosphere, water and food chain as a result of plant uptake (Wagner, 1993). This led to the great concern shown about the eventual effects of Cd on soil, plants and animals caused by Cd consumption. The application of sewage sludge, city waste and Cd-containing fertilizers causes the increase of Cd content in soils (Williams and David, 1973). Cd is easily taken up by plants and then enters the food chain, resulting in a serious health issue for humans. Therefore, addition of toxic heavy metals into soil and the transfer of these metals into the food chain have been a matter of concern to people in arable soils (Grant *et al*., 1998). The presence of excessive amount of Cd in soil causes many toxic symptoms in plants, such as reduction of growth, especially root growth (Weigel and Jäger, 1980), disturbances in mineral nutrition and carbohydrate metabolism (Moya *et al*., 1993) and may therefore, strongly reduce biomass production. The reduction of biomass by Cd toxicity could be the direct consequence of the inhibition of chlorophyll synthesis (Padmaja *et al*., 1990) and photosynthesis (Bazzaz *et al*., 1975; Baszynski *et al*., 1980).

Some studies reported a marked reduction in photosynthetic rate for different plant species under exposure to Cd stress (Sawhney *et al*., 1990; Sheoran *et al*., 1990a, b). Vegetables growing in medium with high level of Cd showed deleterious effect in photosynthetic processes, such as chlorophyll content and photosynthesis (Baszynski *et al*., 1980; Padmaja *et al*., 1990; Satyakala, 1997). Many other studies have shown and confirmed the inhibitory effect of Cd on

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photosynthesis and chlorophyll content. We can quote, (Greger and Ögren, 1991). Andon *et al.* (1995) and Cheng *et al.* (2002) who found that Cd seriously inhibited plant growth and chlorophyll metabolism and that the addition of Cd in the growth medium also had significant deleterious effect on net photosynthetic rate. Dong Jing *et al.* (2005) also reported that different concentrations of Cd did show effects on photosynthesis and chlorophyll content. The present research was aimed at highlighting some of the problems related to Cd and trying to answer some of the questions that have arisen as a consequence of Cd behavior and its toxic nature to plant species. In that respect, a study was carried out with the main objective to investigate the uptake of Cd and its effects on beans. Two major factors among others known to affect the behavior of Cd were included in this study to give the investigation a more consistent background. pH was shown to affect the solubility, the availability and the transport of Cd. This investigation was mainly dealing with the influence of Cd on photosynthesis and chlorophyll content.

MATERIALS AND METHODS

Growth and germination: *In vivo*, seeds of beans (Canadian wonder) were germinated in vermiculite for 10 to 12 days. The germination took place in a growth chamber under a photoperiod regime of 16 h light and 80% RH. The temperature ranged between 24 and 26°C during light and about 20°C during darkness. After 12 days growth, the seedlings were transferred to a nutrient solution containing different levels of Cd ranging from 0-180 μg total. After 7 days, plants were washed and weighed and half of the samples were analyzed for Cd and Mg using atomic absorption spectrophotometry. The other half was used for chlorophyll extracts, digested with HNO_3 and analyzed for Cd and Mg by atomic absorption spectrometry after being dissolved in diethyl ether and dried with nitrogen. Whereas *in vitro* experiments, Cd was added directly to chlorophyll and samples were kept stored in darkness and analyzed daily for a period of 7 days.

Chlorophyll extraction (Total chlorophyll and chlorophyll a and b): For both *in vivo* and *in vitro* experiments, extraction of chlorophyll from plant beans was carried out using an acetone-hexane solvent (4:1 v v^{-1}) (Vernon, 1960). Five to Ten grams of sodium sulphate were added to remove most of the water from of the organic phase. As a precaution, all extractions were carried out under dim light to minimize the loss of pigments. The solid chlorophyll was dissolved in purified then analyzed using UV spectroscopy. The extraction of chlorophyll a and b was carried the same way as described earlier. Thin Layer Chromatography (TLC) was used for the separation of the different pigments. The absorbent substance silicagel was used to transform the chlorophyll into a solid phase easier to remove. Chlorophyll a and chlorophyll b can be removed, dissolved in diethyl ether, filtered and then analyzed TLC plates were put in a tank containing a mobile phase was selected after preliminary screening using other solvents.

Chlorophyll estimation: For this investigation, the ultra-violet spectrometry was used to estimate the chlorophyll content and determine its UV spectra. For the estimation of chlorophyll, several empirical equations were suggested. The equations were used for calculations those suggested. An extra measurement at 700 nm was carried out for all samples to check their optical clarity. Assuming that the free-Mg chlorophyll was a pheophytin, however, its calculation became important. This may at least give a clearer idea about the extent of the effect of Cd on chlorophyll and lead to greater understanding of the relationship between trace metals and chlorophyll.

RESULTS

Experiment was carried out using thin layer chromatography method to separate the different pigments and to isolate chlorophyll a and chlorophyll b reported. Cd was added on the first day of the transfer of plants to the nutrient solution.

DISCUSSION

The most important observation to be made according to data shown in Table 1-3. This loss in Mg expressed as a loss in chlorophyll content occurring at all added levels of Cd. The opposite result was obtained for the amount of Mg and Cd in the remaining solutions. These observations led to suggest the removal of Mg from the chlorophyll structure and its displacement by Cd as a result of its effect this was suggested on the basis of observations made during the experiments expressed as a changes in the color of the chlorophyll from dark green to olive green and to yellow for samples containing the highest levels of Cd (400-800 μg). The use of TLC method enabled the separation of the different pigments and permitted the determination of the 2 major peaks Chl a and Chl b. From the results presented above, there was a clear indication of a Cd effect on the content of Chl a and Chl b and also on the total chlorophyll. The spectrums obtained by the UV/VIS became increasingly

Table 1: Cd results for chlorophyll extracts

Sample µg Cd	D.W g	Chl a mg L^{-1} (solvent)	Chl b mg L^{-1} (solvent)	µg Cd in plant tissue	µg Cd in rem. Sol
30	2.89±0.01	0.412±0.02	10.000±0.0001	19.05±0.80	8.38±0.65
100	2.64±0.02	0.412±0.01	10.00±0.00010	24.42±0.63	68.28±1.10
150	2.45±0.01	0.405±0.02	10.00±0.00020	30.50±0.63	91.99±2.04
200	1.70±0.03	0.366±0.02	10.00±0.00020	127.40±6.00	34.55±1.07
250	1.57±0.02	0.374±0.01	10.00±0.00030	28.88±1.10	177.10±3.10
300	1.46±0.04	0.336±0.01	10.00±0.00020	26.95±1.06	194.2±6.300
350	1.45±0.03	0.305±0.01	10.00±0.00010	36.27±0.76	265.90±3.34

Table 2: The increasing of Cd concentrations in chlorophyll extracts and the decrease of Mg content in Chl as Cd increases

Sample µg Cd	Total µg Cd in Chl extract	Total µg Cd In rem sol	µg Mg plant tissue	µg Mg in rem.sol
30	10.14±1.264	9.27±1.535	879.6±4.30	1019.0±160.2
100	15.41±0.926	66.54±2.783	887.1±3.60	727.0±127.2
150	22.09±0.931	99.25±4.229	798.9±3.20	966.8±32.40
200	25.34±0.931	141.00±5.108	650.8±5.20	1220.0±62.60
250	20.07±1.610	192.40±4.893	148.0±6.60	1693.0±23.11
300	30.20±0.926	233.00±7.994	371.0±3.60	1398.0±94.42
350	32.83±0.610	312.36±3.470	348.6±15.8	1592.0±19.20

Table 3: Mg results for chlorophyll extract. Chlorophyll of samples of the second lot was extracted, dried, digested and analyzed with atomic absorption. Levels of Cd were added at a range of 30-350 µg

Sample (µg Cd)	Total µg Mg In Chl ext	Total µg Mg In rem sol	Recovery (%)
30	774.3±35.43	942.8±71.890	87.52
100	466.8±16.73	1316.0±72.730	89.16
150	401.6±9.737	1471.0±40.570	93.62
200	322.9±6.246	1575.0±35.201	94.89
250	148.0±6.595	1639.0±23.110	89.34
300	371.0±3.593	1398.0±97.420	88.46
350	348.6±15.80	1592.0±19.200	97.03
Control	868.76±7.38	1097.19±22.34	96.91

clearer when Cd levels were increased; thus, its similarity to Mg-free chlorophyll also increased. Concerning the results of chlorophyll b and its constant evolution, one may suggest that chlorophyll b was less affected by Cd than chlorophyll a because the latter is considered to be the major pigment occurring in all plants that produce oxygen by photosynthesis. Through this investigation, one major hypothesis can be drawn is that, the synthesis of chlorophyll requires iron for its formation and any iron deficiency causes a loss in chlorophyll which is expressed as chlorosis. This may explain that Cd could disturb the biosynthesis of chlorophyll by possibly interfering with intermediate elements (or precursors) involved in chlorophyll formation. Iron-protein such as, ferredoxin and cytochrome are the best known for their role in the biological system including chlorophyll formation. They both contain Fe with which Cd can compete and displace by binding to the -SH group.

Prospects for future research: *In vivo* experiment: In addition to several effects due to Cd-toxicity such as decrease in growth and inhibition of the photosynthesis process, chlorosis is considered the most apparent symptom of Cd-toxicity. After investigating the behavior of Cd, its interaction with Zn an its effects on beans, the main results and suggestions converged towards one aspect of Cd-toxicity and this was chlorosis . In that respect, attempts were made to find out about the origins of chlorosis and assess possible mechanisms involved. Cd may interfere with Fe by preventing it from fulfilling its role as an electron carrier or causing its deficiency, therefore impeding the formation of chlorophyll. The displacement of Fe^{2+} by Cd^{2+} in the ferredoxin chain possible and could be enhanced by the presence of an -SH group present in cystein and favorable to bind with Cd. Alongside the evidence already obtained during this investigation, extensive experiments on chlorophyll using the same techniques (i.e., U.V. and A.A. Spectrophotometry) should be carried out in order to obtain more results. These results will be backed up by the use of Grignard reagent on a regular basis to confirm the removal of Mg^{2+} from the chlorophyll structure. Thus one can have more material in hands to suggest a replacement of Mg^{2+} by Cd^{2+} by suggesting a more valid chemical approach to explain the mechanism involved. However, this chemical approach to explain the mechanism responsible for the substitution of Mg^{2+} by a divalent metal is far from simple because of the complicated biological process of chlorophyll synthesis and the biochemistry involved. Nevertheless, one can still consider the possibility of a displacement of Mg^{2+} by a divalent metal by using the coordination chemistry and the notion of the ionic radius to determine the distance of the N-M^{2+} (N = Nitrogen of the pyroll ring) and also to determine whether a given divalent metal can fit in the center of the porphyrin ring or not as suggested by with emphasis on the nature of the N-M^{2}.

CONCLUSION

Chlorosis was the primary symptom caused by Cd toxic levels and this led to the study of its causes by investigating the effect of Cd on Chlorophyll formation and its content in plants. Suggestions were obtained concerning the role of pH in the uptake and availability of Cd, its interactions with Zn and its interference in chlorophyll synthesis. It was shown from the results obtained that pH played a great role in affecting the behavior of Cd. It was also found that Cd uptake by beans increased at low pH value (3.0-5.2) The conclusions of this investigation confirmed the fact that chlorosis is a loss of chlorophyll due to the loss of Mg caused by Cd as shown in the results. In that respect, one question appeared to be important and this was about the nature of the effect of Cd on chlorophyll and Mg concentration was the first conclusion obtained on the basis of evidence achieved from analysis by atomic absorption and ultra violet spectroscopy. Although some results were obtained indicating the formation of a free-Mg chlorophyll suggesting the removal of Mg and the increase of Cd concentration in the chlorophyll, but more still to be achieved to suggest a possible displacement of Mg by Cd. Meanwhile a speculative view was taken to explain the matter by suggesting that Cd was rather interfering with other divalent elements such Fe which is known to be involved in the chlorophyll synthesis than Mg directly. Although some investigations confirmed that Cd suppresses chlorophyll content, this investigation went one step further and attempted to explain the mechanism involved on the basis of the evidence. This research lays the foundations for further research aimed at investigating whether Cd can directly displace the Mg atom from its central position in the chlorophyll structure.

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REFERENCES

Andon, V., I. Iordanov, E. Chakalova and Vassil Kerin, 1995. Agricultural University, 12 Mendeleev Str., Plovdiv 4000, Bulgaria 2Acad. M. Popov Institute of Plant Physiology, Acad.

Baszynski, T., L. Wajda, M. Krol, D. Wolinska, Z. Krupa and A. Tuken-Dorf, 1980. Photosynthetic activities of cadmium-treated tomato plants. Physiol. Plant, 48: 365-370.

Bazzaz, F.A., R.W. Carlson and G.L. Rolfe, 1975. Inhibition of corn and sunflower photosynthesis by lead. Physiol. Plant, 34: 326-329.

Cheng, Shuiping, Ren, Feng, Grosse, Wolfgong and Wu Zhenbin, 2002. Effects of cadmium on chlorophyll content, photochemical efficiency and photosynthetic intensity of canna indica linn. Int. J. Phytoremediation, Vol. 4, No. 3.

Dong Jing, Wu Fei-Bo and Zhang Guo-Ping, 2005. Effect of cadmium on growth and photosynthesis of tomato seedling. J. Zhejiang Univ. Sci., 6 (10): 974.

Greger, M. and E. Ögren, 1991. Direct and indirect effects of Cd^{2+} on photosynthesis in sugar beet (*Beta vulgaris*). Physiologia Plantarum, 83: 129-135.

Grant, C.A., W.T. Buckley, L.D. Bailey and F. Selles, 1998. Cadmium accumulation in crops. Can. J. Plant Sci., 78: 1-17.

Moya, J.L., R. Ros and I. Picazo, 1993. Influence of cadmium and nickel on growth, net photosynthesis and carbohydrate distribution in rice plants. Photosynthesis Res., 36: 75-80.

Padmaja, K. Padmaja, D.D.K. Prasad and A.R.K. Prasad, 1900. Inhibition of chlorophyll synthesis in *Phaseolus vulgaris* Seedlings by cadmium acetate. Photosynthetica, 24: 399-405.

Satyakala, G., 1997. Studies on the effect of heavy metal pollution on *Pistia stratiotes* (water lettuce). Ind. J. Environ. Health, 39: 1-7.

Sheoran, I.S., H.R. Singal and R. Singh, 1990. Effect of cadmium and nickel on photosynthesis and the enzymes of the photosynthetic carbon reduction cycle in pigeonpea (*Cajanus cajan* L.). Photosynth Res., 23: 345-351.

Sawhney, V., I.S. Sheoran and R. Singh, 1990. Nitrogen fixation, photosynthesis and enzymes of ammonia assimilation and ureide biogenesis in nodules of mungbean (*Vigna radiata*) grown in presence of cadmium. Ind. J. Exp. Biol., 28: 883-886.

Wagner, G.J., 1993. Accumulation of cadmium in crop plants and its consequences to human health. Adv. Agron., 51: 173-212.

Weigel, H.J. and H.J. Jäger, 1980. Subcellular distribution and chemical forms of cadmium in bean plants. Plant Physiol., 65: 480-482.

Williams, C.H. and D.J. David, 1973. The effect of superphosphate on the cadmium content of soils and plants. Aust. J. Soil Res., 11: 43-56.