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Effect of Cadmium Pollution on the Germination and Growth of *Phaseolus vulgaris* L.

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ABSTRACT: *Phaseolus vulgaris* L. seeds were grown in soil treated with different concentrations of cadmium to investigate its effect on germination and growth. The seeds were sown in three replicates of 5 kg soils treated with 25, 50, 75 and 100 mg/kg of cadmium and control. Toxicity effects increased with increasing concentration. Germination was significantly affected as data recorded for the various treatment showed great inhibition along the concentration gradient. Cadmium treated soil of 100 mg/kg caused death to experimental plants before 28 days. The effects of cadmium on the growth (number of leaves, height, leaf area, flower production and root length) showed a similar trend.

Keywords: *Phaseolus vulgaris* L., Toxicity, Inhibition,

Introduction

The accumulation of heavy metals in agricultural soils is of increasing concern due to the food safety and potential health risks as well as its detrimental effects on soil ecosystems. The dangers this pose are even more relevant to us owing to our indecent and indiscriminate disposals of heavy metal containing materials and equipment. The increasing influx of heavy metals into water bodies from industrial, agricultural, and domestic activities is of global concern because of their well documented negative effects on human and the ecosystem (Mataka *et al*., 2006).

The term heavy metal includes transition metal, metalloids, lanthanides and actinides, which have been proposed based on their density, atomic number and their chemical properties or toxicity (John, 2002). Cadmium, copper, lead and zinc are among the most common heavy metals in agricultural soils (Forstner, 1995). Metal toxicity in plants has been reported by various authors (Vassilev and Yordanov, 1997; Jiang and Liu, 2000; Anoliefo and Osubor 1998; Vwioko *et al.,* 2006**).** Metals like Pb, Hg, Cd, Ar, and Cr have no known biological function and are toxic to life even at very low concentration (Salt *et al*., 1995).

Cadmium, in its purest form, is a soft silver white metal that is found naturally in the earth's crust. However, the most common forms of cadmium found in the environment exist in combinations with other elements. For example, cadmium oxide (a mixture of cadmium and oxygen), cadmium chloride (a combination of cadmium and chlorine) and cadmium sulfide (a mixture of cadmium and sulfur) are commonly found in the environment. It gains entry into the agricultural lands through the use of irrigation water mixed with untreated industrial effluent, sewage water and also through application of phosphate fertilizer. Among heavy metals, cadmium appears to be one of the most dangerous elements to all kinds of organisms (Wojcik and Tukiendorf, 2005). Although it is considered to be a

non-essential element for metabolic processes, it is easily absorbed by plants and even in small amount, it causes toxicity symptoms (Wojcik and Tukiendorf, 2005; Benavides *et al.,* 2005). It is a highly toxic contaminant that affects many plant metabolic processes (Li *et al*., 2008). Cadmium has been shown to inhibit enzymatic activities in plants (Babich and Stotzy, 1978). It also inhibits mitosis by interfering with the formation of mitotic apparatus (Brachet and Mirsky, 1981). Cadmium has many uses in industry and consumer products like batteries, pigments, metal coatings, and plastics.

Humans are exposed to cadmium by intake of contaminated food or polluted air (Järup *et al*., 1998). High concentrations of cadmium in soils represent a potential threat to human health because it is incorporated in the food chain mainly by plant uptake (Alvarez-Ayuso, 2008). The consumption of cadmium has been reported to cause gastro intestinal, haematological, musculoskeletal, renal, neurological and reproductive adverse health effects (ATSDR, 1999).

The presence of excessive amount of Cd in soils causes many toxic symptoms in plants, such as reduction of growth, especially root growth (Weigel and Jager, 1980), disturbances in mineral nutrition and carbohydrate metabolism (Moya *et al.,* 1993) and may therefore reduce biomass production.

Phaseolus vulgaris (common bean) represents the most important source of protein for low-income populations in Latin America and in Africa. It was rumoured sometime in the past that poisonous beans was in circulation. The use of common beans in this study is both out of curiosity and its habit. This study investigated the effect of different concentrations of cadmium on the germination and growth of common beans (*P. vulgaris)*.

Materials and Methods

Study Area: The study was carried out behind the departmental building of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria.

Preparation of Seeds: The seeds of *Phaseolus vulgaris* were obtained from Uselu market, Benin City, Edo state. The viability test was carried out using the floatation test. The seeds were soaked in water, stirred and allowed to stay for 2-3 minutes. After this time lag, those seeds found floating were discarded and the ones that settled at the base were used for the experiment.

Experimental Setup: Each bucket with a capacity of 10 kg was filled with 5 kg of soil and the seeds were planted at a depth of 3 cm. The placement of the buckets was random in order to get good sampling result. There were five treatments, with 5 replicates and 10 seeds were planted per bucket in order to carry out germination study. The treatments were 25, 50, 75 and 100 mg/kg with cadmium. Untreated soil served as control. Watering was done at regular intervals, and germination was studied till 10 days. Two replicates were used for root elongation studies. Root length, stem height, leaf area and number of leaves were taken at regular intervals of 14 DAT (Days After Thinning) for 70 days. At the end of the experiment, the whole surviving plants were analysed using AAS (Atomic Absorption Spectrometer) after drying and grounded to powdery form.

Source of Cadmium: The cadmium used was provided in the form of $[Cd(SO_4).8(H_2O)]$.

Germination Study: Germination percentage is an estimate of the viability of seeds and it is calculated as:

$$GP = \frac{\text{seeds germinated}}{\text{Total seeds}} \times 100$$

It is determined by calculating the Germination Percent (GP) daily after planting and then plotting these data. Sprouting was observed 3 days after planting and the germination was studied for 10 days i.e. from day 3 to day10.

Thinning: The reduction of number of plants was done to avoid overcrowding.

Number of Leaves: The number of leaves on the plant was counted and recorded on 14 day intervals. It was done by visual counting.

Measurement of Shoot Height: The height was measured from the soil level to the terminal bud. Measurements were taken on a 14 day interval, up to 10 weeks.

Measurement of Leaf Area: Leaf area was determined by the proportional method of weighing a cut-out of traced area with standard paper of known weight to area ratio (Eze, 1965).

Root Elongation: Root measurements were taken during thinning and at the end of the experiment.

Number of Flower Buds: The numbers of flower buds were counted after the first flower buds were observed.

Statistical Analysis: The values obtained as shown on the tables are those of mean (x) and standard error (S_x) of each replicate taken at 2 weeks (14 days) interval for 70 days as well as the standard error of each mean.

Analyses

Both plant and soil samples were ground into fine powder. Two (2) g portions of the samples were weighed accurately and 10.0ml of concentrated HNO_3 was added to each. The samples were digested on a hot plate for 15 minutes. The digest was cooled and 5 ml of concentrated nitric acid was added and heated for additional 30 minutes. The later step was repeated and the solution was reduced to about 5 ml without boiling. The sample was cooled again and 5ml of concentrated hydrochloric acid and 10 ml of distilled water was added and the sample was heated for additional 15 minutes without boiling. The sample was cooled and filtered through a whatman No.42 ashless filter paper and diluted to 60 ml with distilled water. Metal content in the digested samples were analyzed for Cd using the Atomic Absorption Spectrophotometer.

Results

The effect of different concentrations of cadmium on the percent germination of *Phaseolus vulgaris* is presented in Figure 1. Results show a progression from 16% germination at day 3 to 98% at day 10 for control, while 100mg/kg concentration progressed from 0 % at day 3 to 32 % at day 10. Germination declined with increasing concentration.

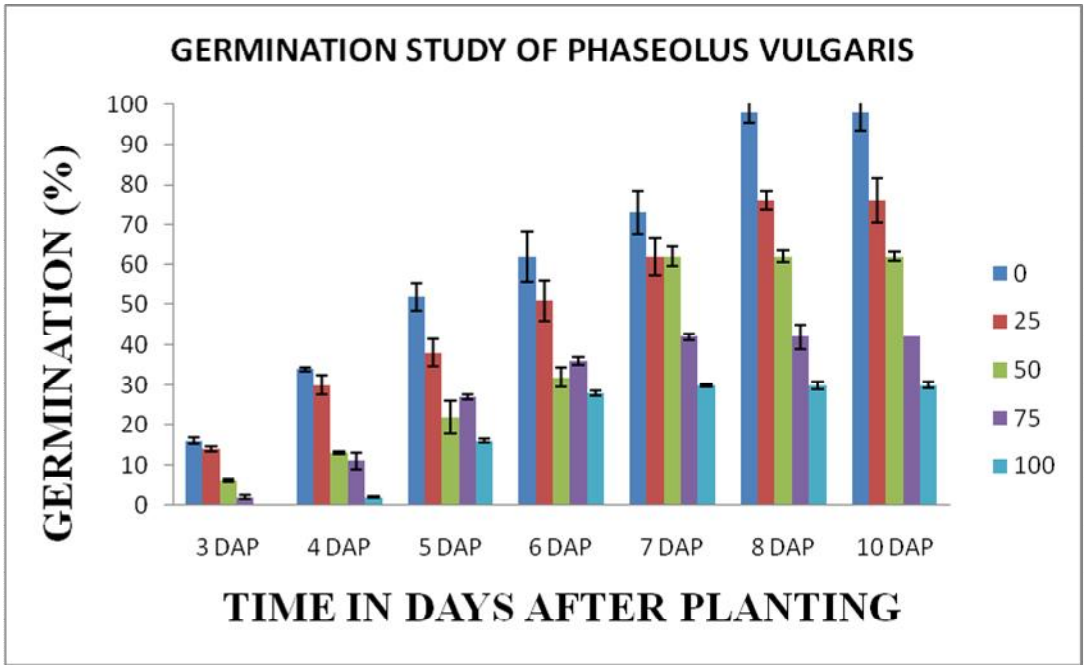

Figure 1: Percent germination of *Phaseolus vulgaris*

The results obtained for leaf count (Table 1) in the various treatments show that there was significant difference between the control plants (56.00 ± 0.47) and the 25mg/kg treated plants (37.67 ± 1.5**2).** Above this level the plants grown in 50, 75 and 100 mg/kg all showed toxic effect on *Phaseolus vulgaris,* and subsequently withered.

Table 1: Effect of different concentrations of cadmium on the number of leaves of *Phaseolus vulgaris.*

Concentration (mg/kg)	Time in days					
	0	14	28	42	56	70
Control	2.00±0.39	15.33±0.72	19.66±0.27	31.00±1.25	43.00±0.47	56.00±0.47
25	1.60±0.22	11.00±0.47	17.33±0.27	25.33±1.36	32.33±1.19	37.67±1.52
50	1.60±0.22	5.67±0.72	13.67±1.52	9.33±0.27	-	-
75	1.20±0.44	4.35±0.82	1.20 ±0.31	-	-	-
100	1.20±0.44	1.12 ±0.27	-		-	-

- Dead plants

Table 2 shows the results recorded for height of *Phaseolus vulgaris*. A marked retardation in the height of *Phaseolus vulgaris* was observed between control and treatments. At 70 DAT, the 25 mg/kg had a height of 75.33±2.37cm showing a depression in height compared to control with a height of 138.31±1.12cm. With increase in concentration there was greater depression in height and subsequently death was observed with the 75 mg / kg and 100 mg / kg treatments

Table 2: Effect of different concentrations of cadmium on the stem height (cm) of *Phaseolus vulgaris*

Concentration (mg/kg)	Time in days after thinning					
	0	14	28	42	56	70
Control	15.44±0.33	30.06±0.44	42.70±0.84	79.33±1.19	104.33±2.12	138.31±1.12
25	14.50±0.20	24.33±0.27	29.10±0.54	44.56±1.12	64.33±1.96	75.33±2.37
50	12.12±0.73	18.83±0.49	21.20±0.91	22.73±1.01	24.33±1.33	24.33±1.33
75	8.70±0.44	16.80±0.41	14.70±0.46	-	-	
100	4.14±0.95	5.50±5.37	-	-	-	--

- Dead plants

Data recorded for leaf area (Table 3) of *Phaseolus vulgaris* reveal that, at the end the study there was significant difference between control and cadmium treated plants, with control having a mean leaf area value of 137.19±1.12 cm^2 and 25 mg/kg with 50.31± 4.37cm^2. Above this level all other plant withered.

Table 3: Effect of different concentrations of cadmium on the leaf area (cm^2) of *Phaseolus vulgaris.*

Concentration (mg/kg)	Time in days					
	0	14	28	42	56	70
Control	29.29±0.46	46.47±0.44	68.69±2.34	97.19±2.50	109.17±2.12	137.19±1.12
25	21.39±0.72	22.96±0.27	34.01±3.15	39.93±3.12	44.86±3.96	50.31±4.37
50	8.11±0.73	21.59±2.49	27.22±0.91	24.14±1.01	-	-
75	6.36±0.44	16.72±0.41	11.86±0.46	-	-	-
100	3.39±0.95	7.85±0.37	-	-	-	-

- Dead plants

Data recorded for root length of *Phaseolus vulgaris* during thinning (0 DAT) and 70 days after thinning are presented in Table 4. There was significant difference between treatments and control plants. Cadmium adversely affected the root growth of the plants with the 25 mg / kg treatment having root length of 16.20±0.79cm and control with 19.50±0.20cm.

Table 4: Root length (cm) of *Phaseolus vulgaris* grown in soil treated with different concentrations of cadmium

Concentration (mg/kg)	Time in days	
	0	70
Control	3.11 ± 0.19	19.50±0.20
25	2.80 ± 0.19	16.20±0.79
50	2.80 ± 0.08	4.61±0.37
75	2.61 ± 0.28	-
100	2.70 ± 0.12	-

- Dead plants

Table 5 shows flower production of *Phaseolus vulgaris* which was recorded from day 56 till day 70. It was observed that only control plants produced flowers while all the plants treated with cadmium did not flower.

Table 5: Effect of different concentrations of cadmium on flower production of *Phaseolus vulgaris*

Concentration (mg/kg)	Time in days	
	56	70
Control	2.33±0.27	5.00±0.47
25	-	-
50	-	-
75	-	-
100	-	-

- Dead plants/No flower

The average concentration of cadmium recovered from whole plant of *Phaseolus vulgaris* 70 days after thinning is presented in Table 6. The quantity recovered from control was 0.05 ± 0.02 mg/kg while the 25 and 50mg/kg treated plants recorded 4.51 ± 0.25 and 8.61± 0.23 mg/kg respectively.

Table 6: Average concentration (mg/kg) of cadmium recovered from whole plant of *Phaseolus vulgaris* 70 days after thinning

Concentration (mg/kg)	Concentration (mg/kg)
Control	0.05 ± 0.02
25	4.51 ± 0.25
50	8.61 ± 0.23
75	–
100	–

– Dead plants

The data for cadmium content of soil samples at the end of the study (Table 7) shows that there is a correlation between the quantities of cadmium bioaccumulated in the plants and that lost by the soil.

Table 7: Average concentration (mg/kg) of cadmium present in soil at 20 days and 70 days after thinning of *Phaseolus vulgaris*

Concentration (mg/kg)	Time in days after thinning.	
	20	70
Control	-	1.14 ± 0.07
25	4.33 ± 0.27	11.97 ± 0.18
50	± 0.94	20.04 ± 2.34
75	35.00 ± 1.25	22.67 ± 3.55
100	41.33 ± 5.67	28.42 ± 2.55
	72.23 ± 15.55	

- Not detected

Discussion

The results for germination (Figure 1) shows that there was a marked difference between control (98% germination) and other treatments, with the 100 mg/kg (32% germination) treatment with cadmium being the most affected. The effect increased with increase in pollution concentration. Cadmium has a profound effect on the germination of common beans (*Phaseolus vulgaris)*. In the present study cadmium caused decrease in seed germination. This decrease in seed germination is in conformity with the findings of Iqbal and Mehmood (1991) who found that higher concentrations of cadmium and chromium (100-250 ppm) affected germination and early growth performance of *Allium cepa*.

Investigation by Subramani *et al*. (1997) revealed that the germination and seedling growth of black grain [*Vigna mungo* (L) Hepper] showed a gradual decline with increase in the concentration of cadmium. Claire *et al.* (1991) observed that heavy metals inhibited germination in a study using nickel and other heavy metals on cabbage, lettuce, millet, radish, turnip, and wheat. Kalimuthu and Siva (1990) also found reduction in seed germination in maize treated with 20, 50, 100 and 200 µg/ml lead acetate.

The growth parameters investigated in this study namely, number of leaves, height, leaf area, root length and number of flowers revealed that the treatment with cadmium had an intense adverse effect on all these parameters. The degree of effect was along the concentration gradient. Higher pollution levels resulted in death of plants. Growth in any living organism is a function of cell division in a process known as mitosis. Heavy metals affect the cell division of plants (Brachet and Mirsky, 1981), and the effects are different and depend on the concentration. Mo and Li (1992) studied the effects of Cd on the cell division of root tips in beans and observed similar results. Zhang (1997) investigated the effects of Cd, Hg and Pb on the cell division of barley *(Hordeum vulgaris* Linn.) where he observed that the cell cycle was extended (delayed mitosis) under the 0.01 mol / L treatment concentration. Cd, Hg and Pb also combined with nucleic acid and damaged the structure of the nucleolus after 24 hours of treatment with a 0.005-0.0005 mol/L dosage, inhibited the DNase and RNase activities (Duan and Wang, 1992), thus resulting in the interruptive synthesis of DNA (Yang and He,1995a) to affect cell division. Brown *et al.,* (1983) reported loss of leaf tugor by *Thlapsi cacsulescens* due to Cd uptake.

Anoliefo and Osubor (1998) reported decrease of 50% in the vine length of *Cucumeropsis manni* plants grown in cadmium treated soils. They also reported significant decrease in leaf area in the same experiment. The reduction in root length in heavy metal treatment could be due to reduced mitotic cells in meristematic zone of roots. Lerda (1992) made similar observations in root of *Allium cepa*. The findings by Lerda confirmed that heavy metal treatment reduced the frequency of mitotic cell in meristematic zone and are responsible for inhibition in root growth. The presence of excessive amount of Cd in the soil causes many toxic symptoms in plants, such as reduction of growth, especially root growth (Weigel and Jager, 1980).

In this study, *P. vulgaris* treated with cadmium did not produce any flower bud. This means that cadmium even at low concentration inhibits the production of flowers and fruits. Though plants in 25 mg/kg soil survived till the end of the experiment, no flowering was observed.

In the results of plants analyses, it was observed that control plant recorded 0.05 ± 0.02 mg/kg while the 25 mg/kg treatment plant had 4.51 ± 0.25 mg/kg of cadmium. There was increase in uptake along the concentration gradient. These plants may have survived due to the low amount of cadmium absorbed which is in proportion to what was available in the soil. The 75 and 100 mg/kg treated plants did not survive; though the 50 mg/kg treated plants survived, although they had stunted growth, very weak stem and no leaves. The early death of the plants treated with higher concentration of cadmium can be attributed to high uptake of cadmium in the plant tissue. Thus, higher soil Cd concentration can result in higher levels of its uptake by plants (John *et al.,*1972) and resultant bioaccumulation in the plant tissue. Kramer and Konig, 1993 recorded high Cd content in the grain and vegetative parts of wheat and oat while Brown *et al*. (1983) reported high uptake of Cd by *Cynodon dactylon*. There is a correlation between data recorded in Tables 6 and 7 as the cadmium bioaccumulated in the plants is accounted for by the depletion of the metal from the soil. At the end of the study, control soil had 1.14 ± 0.07 mg/kg left in the soil while the 25, 50, 75 and 100 mg/kg treated soils had 11.97 ± 0.18, 20.04 ± 2.34, 22.67 ± 3.55 and 28.42 ± 2.55 mg/kg respectively.

In this experiment, no symptom of phyto-toxicity in the above ground parts of plants was observed with control. The death and withering of plants observed with higher concentrations of cadmium (75 and 100 mg/kg) may be due to the higher concentration of cadmium taken up by the plant. According to Nasu *et al*., (1984) the degree of the Cd effect depends on the concentration absorbed. Cadmium left in soil 70 days after treatment (Table 7) shows that at higher concentration of cadmium (100 mg/kg), the plant absorbed more amounts (28.42 ± 2.55) of cadmium, and this led to the early death of the plant. This shows that cadmium has a lethal effect on *P. vulgaris* at higher concentration. Haghiri (1973), reported that toxicity of some metals may be so severe that plant growth is reduced before large quantities of the element can be translocated. Also, Panda (2007) stated that metal toxicity reduces vigour and growth of plants, causes death and in extreme cases interferes with photosynthesis, respiration, water relation, reproduction and causes changes in certain organelles, disruption of membrane structure and functions of different plant species.

Conclusion

Cadmium is found to be highly toxic to germination and growth of common beans at higher concentration. Results of these findings can be useful indicator of cadmium toxicity. *Phaseolus vulgaris* could not tolerate cadmium.

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