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Effects of different cadmium levels on the growth and yield parameters of wild *Vigna*

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SUMMARY. The assessment of growth and yield parameters of wild *vigna* to to different levels ofcadmium pollution has been investigated in this study. The experimental setup consisted of three (3) treatments namely; 0, 2.5 and 5 ecological screening value (ESV) and parameters recorded were taken 84 days and 20 weeks namely; plant germination factors, plant yield, percentage chlorosis, necrosis and senesced leaves, plant dry matter accumulation as well as plant lifetime morphological changes. Cadmium concentration increased the percentage of foliar chlorosis and necrosis (20.40) in older leaves than younger leaves (4.08) respectively of TVNu-91 sown in 5 ESV cadmium soil and this eventually resulted to an increase in percentage senesced leave with increasing cadmium concentration. Leaf folding and curling symptoms were cadmium-associated in the intermediate partition but same cannot be said for the young plant (YP). However, leaf curling was reported as a prominent morphological feature in this study. With respect to insect foraging, there was total absence of foraging in both control accessions and cadmium polluted accessions. There was also a significant difference ($P>0.05$) in the number of pods per plant as evidenced in TVNu-95 (5ESV) 5.67 when compared to 14.07 in the control of TVNu-95. Thus, there was a gradual decrease with increasing ecological screening value. Generally, there was significant difference in the seed number per pods ($P<0.01$), seed weight per pods ($P<0.01$) plant yield ($P<0.01$), and flower bud size ($P<0.05$). 20 weeks after sowing, plant dry matter accumulation was reduced with increase in metal concentration. There was variability in plant yield response to metal toxicity with a general decrease reported with increased cadmium concentration. However, TVNu-93 had a better yield as compared to the other studied accessions.

Keywords: cadmium, heavy metal, legumes, phytoassessment,Wild *Vigna*.

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Introduction

Metal pollution has developed into one of the utmost severe ecological problems today as a result of increasing ecological pollution from human actions such as mining of metals, gas exhaust, fuel production, electroplating, fertilizer, sewage and use of pesticides (Kabata-Pendias and Pendias, 2001). Heavy metals are branded to have detrimental effects on plant development and soil microflora thus bringing about losses in plant yield. Heavy metals have been shown, particularly in non-tolerant plant species, to affect an extensive range of plant cellular activities such as photosynthesis, mineral nutrition, respiration, membrane structure function, and gene expression (Maksymiec and Baszynski, 1996; Prasad, 1999; Hall, 2002). Cd, Cu, Hg, Pb and Ni denote the most common heavy metal pollutants. Of concern though, is cadmium (Cd).

Cadmium is from a Latin word "cadmia" and Greek word "Kadmeia" which are ancient names for calamine (zinc carbonate). It was discovered in Germany in 1817 by Fredrick Strohmeyer, a German chemist, as an impurity in zinc carbonate (calamine). A typical source of cadmium pollution in the surrounding is the geologic parent material (generally, high concentrations of chromium, manganese, zinc, copper, cadmium, mercury, tin, and lead are found in the geologic plant materials). The organic and inorganic fertilizers are also important sources of heavy metals to agricultural soils. Cadmium however, is of particular concern in plants as it accumulates in leaves at very high levels, and may be consumed by animals or human beings. Measurable amount of cadmium occur in many soils, animals and plant materials, and an increasing attention is been paid to its concentration in these materials for biological, medical, geochemical, and agricultural prospecting (Stewart *et al*., 1974).

Studies have shown that cadmium tend to accumulate in plant tissues at concentrations exceeding that of the soil solution (Onweremadu *et al*., 2008). Plants tissues like roots, shoots, trunks and leaves have been studied and cadmium is seen as a cumulative toxicant by most scientists. Previous studies on the effect of cadmium on seeds have been restricted to seedlings that have already germinated. Increase in cadmium also occurs due to the use of sewage sludge, manure and limes (Yanqun *et al.,* 2005). Although the levels of heavy metals in agricultural soils are very small, however constant use of phosphate fertilizer may result in the dangerously high accumulation of some metals (Verkleji 1993). Liming also increases the level of heavy metals in the soil more than the compost refuse, and the nitrate fertilizers. Among heavy metals, cadmium is one of the toxic elements that have no function in living organisms. It has long biological persistence as it causes leaf rolls, chlorosis, growth inhibition, water imbalance, phosphorus and nitrogen deficiency, reduced manganese transport and reduction of root and stem growth (Mishra *et al.,* 2006). It can be found in soils because it is present in insecticides, fungicides, sludge and commercial fertilizers (Ravichandran *et al.,* 2011).

Cadmium treatment with 1μM in 24 hours reduces the root-growth up to 30% and this inhibition has positive correlation with the reduction of root cells viability (Siroka *et al.,* 2004). It is widely recognized that cadmium taken up by plants is the main source of cadmium accumulation in food (Lopez-Millan *et al.,* 2009). Cadmium can be easily absorbed by plant roots and transported to shoots results and this results in the disorder of biochemical and physiological processes, and then affects plant growth and morphology (Sgherri *et al.,* 2002). It has also been suggested that growth inhibition by cadmium is due to a direct effect of cadmium on the nucleus or its interaction with hormones in the aerial parts of the plants.

In Nigeria, legume provides a wide variety of high protein diet. Thus, the choice of wild vigna was informed due to its large cultivation and consumption in most industrial cities of Nigeria, such as Kano and Kaduna, which are also located in regions where significant metallurgic activities are ongoing. Farmers in these regions are also known to depend heavily on fertilizers for improved crop yield. The present study was carried out with the aim to observe impact of different cadmium levels on growth as well as yield parameters of different accessions of wild vigna and to provide a theoretical basis for the risk assessment of cadmium pollution and the maintenance of sustainable agricultural production.

Materials and methods

Experimental site

The experiment was carried out in the Botanica garden of Plant Biology and Biotechnology, University of Benin, Benin city. The experiment lasted between the periods of November, 2016 to March, 2017.

Experimental material

Soils used were collected from 10 random points at a depth of 0 – 15cm using an auger from the botanical garden, University of Benin. The soils were bulked and crushed together to obtain homogeneity. The homogenized soils were placed into polythene bags weighing 15kg and sun dried for 48 hours. Soil samples were sent to the laboratory for physiochemical analysis and generally characterized as sandy loam.

Procurement of seeds

Seeds of five accessions of wild vigna namely TVNu-91, TVNu-93, TVNu-94, TVNu-95 and TVNu-96 were procured from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Treatment preparation

The preparation of treatments is listed in (Table 1) below.

Experimental procedure

The experimental setup consisted of three blocks replicated thrice in a randomized block design making a total of 45 treatments. The seeds obtained from the various accessions were planted directed into each polythene bags. The plants were watered everyday till the end of the experiment. Data was collected weekly and biweekly. Parameters measured are listed below;

Morphological stress responses

Morphological observations of the physical appearances of the plant in response to the experimental conditions were recorded on periodic basis. These observations include the colour, form or the appearance of the leaves and the stem of the plant as well as positioning of flowers and nodes. Care was also taken to ensure that the progression of chlorosis was recorded. In this case, whenever chlorosis was noted, the leaf was immediately tagged so that chlorotic progress would be followed up till when the leaf became entirely chlorotic; in this regard, the progress of chlorosis measured in hours was therefore provided. This same procedure was followed to describe the progression of necrosis.

The rate at which plant lost their leaves as well as which portion of the plant lost a particular leaf was also taken into consideration. Thereupon, every plant was therefore divided into three major partitions according to (Ikhajiagbe and Guobadia 2016), and herein referred to as old partitions which consisted of any plant part comprising its stem and leaves, and other appendages from the soil level measuring up till 45cm above soil level; young partition which also consist of all leaves, stem, flowers and other appendages taking up the portion from the meristem of the plant measuring 45 cm downwards. The intermediate partition therefore was taken in this study as the portion in between the demarcated old partitions and new partitions.

Haven followed this demarcation based on partitioning, changes with regards to necrosis, or any other physical observation was made and reported on time basis. Care was taken to ensure that the total number of leaves that folded, curled or showed signs of foraging were taken into notes and as such were counted and presented as percentage of the total number of leaves that appeared in the plant at any given time. These were therefore presented in the results sections as percentage of folded leaves.

Plant yield

The plant yield (kg/m^2) was calculated using the formula;

$$\frac{S \times P \times 120}{1000 \times 22}$$

where S = seed weight per pod; P = number of pod per plant

Data analysis

Data was subjected to analysis of variance (ANOVA) using SPSS 16.0 version. Least significant difference (LSD) was used to separate the means at 5% level of significance.

Results and discussion

The study was undertaken to investigate the impact of different cadmium levels on the growth and yield parameters of different accessions of wild vigna and some morphological as well as yield parameters were on hand to provide necessary interpretations of the research outcome. (Table 1) shows the treatment designations required for the study.

Table 1.

Treatment designations for metal concentrations

Designations	Description
0 ESV	Control (unpolluted soil)
2.5 ESV	0.15 g of cadmium chloride diluted in 2L of water and mixed in 15 kg soil
5 ESV	0.30 g of cadmium chloride diluted in 2L of water and mixed in 15 kg soil

ESV –Ecological screening value (4mg/kg) (Efroymson, 1997)

Germination factors

The effects of cadmium treatment on emergent time of different accessions of wild vigna at 7days after sowing have been presented on Table 2. The lowest (fastest) average emergence value was 2.30 observed in TVNu-95 as opposed to the highest (slowest) average emergence value of 3.70 obtained in TVNu-93. First day of emergence in TVNu-91 was 3.00 which increased to 4.60 with increasing ecological screening value of 2.5 ESV (4.20) and 5 ESV (4.60) respectively. In TVNu-93 it took an average of 3.70 days which eventually increased to 4.00 with increasing ESV. Similar trend was observed for TVNu-94, TVNu-95 and TVNu-96. Over all, the number of days it took to emergence of the various accessions of wild vigna was not significantly different ($P > 0.05$) hence heavy metal incidence did not significantly affect first day of emergence of the plant. Similarly, final emergent percent in the metal treated accessions was 66.67% except in 2.5 and 5 ESV of TVNu-96 ESV which gave a final emergence percent of 33.33%; again this was not significantly different ($P = 0.00$). Table 2 also shows an average mean daily emergent of 4.76 to 9.53 days in the various accessions of wild vigna ($P < 0.01$).

Percentage foliar chlorosis, necrosis and senesced leaves

The percentage of total foliar chlorosis and necrosis of wild vigna have been shown in (Table 3a and 3b). Cadmium pollution on the incident of foliar chlorosis was more predominant in the older leaves than in the fresh leaves of the plants. TVNu accessions sown in control soils had no chlorosis in the fresh leaves as compared with those sown in cadmium polluted soils (Table 3a). However, there was no significant difference (P>0.05) in cadmium pollution of the various accessions studied. With respect to foliar necrosis (Table 3b), necrosis was more prevalent in the older leaves than the fresh leaves of the plants in both control and cadmium polluted soils of the various accessions. It was suggested by (Ohanmu *et al.,* 2018) that cadmium may have been transported to older leaves as a survival strategy in order to overcome the stress exerted by the metal. Although there was no significant difference (P>0.05) in both control and cadmium pollution of the various accessions. It has been reported by Ghoshroy and Nadakavuharen (1990) that within few hours of its supply, cadmium is readily absorbed by roots and then transported to other parts of the plant. Cadmium pollution also significantly affected the percentage of senesced leaves 84 days after sowing (DAS). TVNu-91 sown in control soils had a percentage senesced leaf of 18.6 % as compared to TVNu-91 (47.8 %) sown in the 5 ESV (Fig. 1). Plant responds to environmental stresses by a variety of means. It is noted frequently that many crop species tolerance to prevailing stress conditions increases with the advancing age of the plant (Ohanmu *et al.,* 2018).

Table 2.

Effects of Treatment on emergent time of wild *Vigna* at 7 days after sowing

Plant Accessions	Cd. Conc. (ESV)	First day of emergent (days)	Final Emergent percentage(%)	Mean daily emergent (%/day)	Mean emergent time
TVNu-91	0	3.00	66.67	9.53	5.33
	2.5	4.20	66.67	9.53	6.00
	5	4.60	33.33	4.76	6.00
TVNu-93	0	3.70	66.67	9.53	5.33
	2.5	3.90	66.67	9.53	6.00
	5	4.00	66.67	9.53	6.00
TVNu-94	0	3.30	33.33	4.76	5.33
	2.5	3.60	66.67	9.53	5.33
	5	3.90	33.33	4.76	5.33
TVNu-95	0	2.30	66.67	9.53	5.00
	2.5	2.80	33.33	4.76	5.00
	5	2.87	66.67	9.53	5.00
TVNu-96	0	2.34	33.33	4.76	4.67
	2.5	2.56	33.33	4.76	4.67
	5	2.77	33.33	4.76	5.00
P-value		0.147	0.000	0.012	0.034
Sig*		P>0.05	P=0.000	P<0.01	P<0.05

Table 3a.

Percentage of total foliar chlorosis of wild *Vigna* at 84 days after sowing

Plant Accessions	Cd. Conc. (ESV)	No. of affected leaves per Plant partition			Total
		Younger	**Intermediate**	**Older**	
TVNu-91	0	0	0	4.30	4.30
	2.5	0	4.22	16.90	21.12
	5	4.08	4.08	20.40	28.56
TVNu-93	0	0	2.05	2.05	4.10
	2.5	4.43	4.43	13.29	22.14
	5	5.39	5.39	21.57	32.36
TVNu-94	0	0	2.24	4.48	6.72
	2.5	0	7.96	11.94	19.90
	5	4.53	9.07	18.13	31.73
TVNu-95	0	1.65	1.65	3.30	6.60
	2.5	5.43	2.72	13.58	21.72
	5	6.55	6.55	19.65	32.74
TVNu-96	0	0	1.73	3.46	5.19
	2.5	2.58	5.17	10.33	18.08
	5	6.55	6.55	16.37	29.47
P-value		0.614	0.553	0.984	0.999
Sig*		P>0.05	P>0.05	P>0.05	P>0.05

Table 3b.

Percentage of total foliar necrosis of wild *Vigna* at 84 days after sowing

Plant Accessions	Cd. Conc. (ESV)	No. of affected leaves per Plant partition			Total
		Younger	**Intermediate**	**Older**	
TVNu-91	0	2.15	2.15	6.44	10.74
	2.5	4.22	12.67	16.90	33.80
	5	4.08	16.32	20.40	40.80
TVNu-93	0	0	4.10	4.10	8.21
	2.5	8.86	8.86	17.71	35.43
	5	10.79	16.18	21.57	48.54
TVNu-94	0	2.24	2.24	4.48	8.96
	2.5	7.96	7.96	19.90	35.81
	5	9.07	13.60	27.20	49.86
TVNu-95	0	1.65	3.30	4.95	9.89
	2.5	2.72	8.15	16.29	27.15
	5	3.27	9.82	22.92	36.02
TVNu-96	0	1.73	1.73	6.93	10.39
	2.5	5.17	5.17	15.50	25.83
	5	6.55	6.55	22.92	36.02
P-value		0.520	0.685	0.995	0.969
Sig*		P>0.05	P>0.05	P>0.05	P>0.05

Other observable morphological parameters

Other observeable morphological changes of wild vigna during the plant lifetime such as leaf folding, leaf curling and insect foraging have been showed on (Table 4). Leaf folding was totally absent in all control plants of the various accessions. With respect to leaf curling, only the older portions of TVNu-91, TVNu-94, and TVNu-95 in the control accessions experienced leaf curling. Although, the cadmium polluted accessions showed presence of leave folding and curling which progressed from the younger portion to the older portion. However, there was total absence of insect foraging in both control and cadmium polluted accessions. Leaf curling was reported as a prominent morphological feature in the study. From (Table 4), leaf folding and curling symptoms were cadmium-associated in the intermediate partition but same cannot be said for the young plant (YP). The mechanism behind this could not be explained. It is therefore suggested that this may just be an indicative characteristic that Plant Biologists might use to suggest cadmium toxicity. With respect to insect foraging, there was total absence of foraging in both control accessions and cadmium polluted accessions.

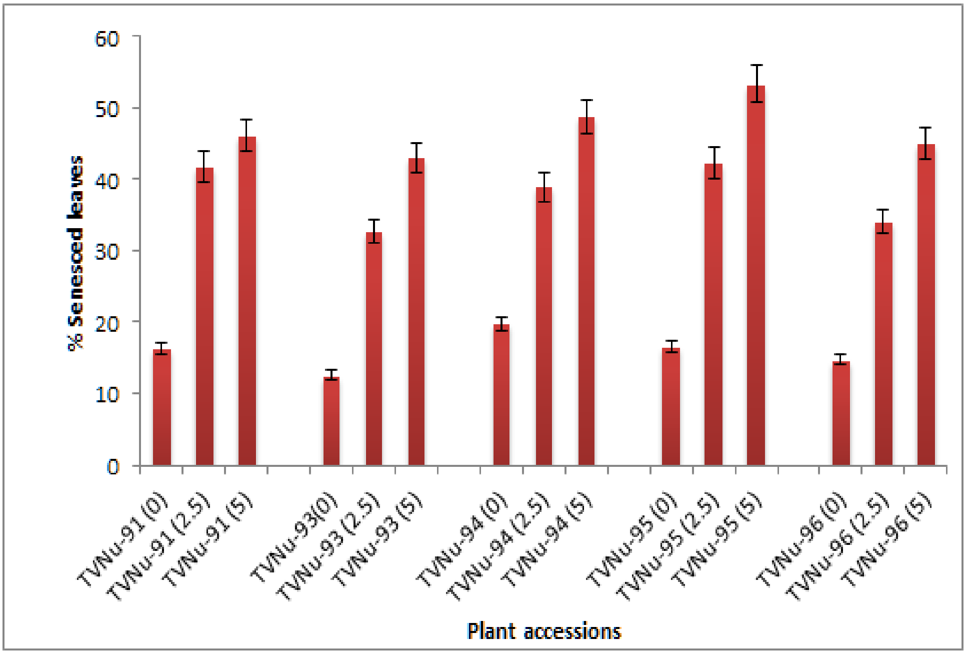

Figure 1. Percentage senesced leaves of wild vigna accessions 84 days after sowing

Table 4.

Other observable morphological presentations of wild *Vigna*.
Results represent total observation during the lifetime of the test plant

Plant Accessions	Cd. Conc. (ESV)	Folded leaves			Curled leaves			Leaves with sign of foraging by insects		
	Control	YP	IP	OP	YP	IP	OP	YP	IP	OP
TVNu-91	0	0	0	0	0	0	2.15	0	0	0
	2.5	0	4.22	12.67	8.45	8.45	0	0	0	0
	5	0	4.08	16.32	12.24	12.24	0	0	0	0
TVNu-93	0	0	0	0	0	0	0	0	0	0
	2.5	0	0	0	8.856	8.86	4.43	0	0	0
	5	0	0	0	21.57	21.57	5.39	0	0	0
TVNu-94	0	0	0	0	0	0	2.24	0	0	0
	2.5	0	15.9	11.94	7.96	7.96	11.94	0	0	0
	5	0	22.7	27.2	13.6	13.6	22.67	0	0	0
TVNu-95	0	0	0	0	0	0	3.3	0	0	0
	2.5	5.43	8.15	5.43	2.72	2.72	10.86	0	0	0
	5	6.55	26.2	16.37	6.55	6.55	22.92	0	0	0
TVNu-96	0	0	0	3.46	0	0	0	0	0	0
	2.5	0	10.3	5.17	0	0	5.17	0	0	0
	5	3.27	19.7	16.37	0	0	6.55	0	0	0

YP= Young plant, IP= Intermediate Plant, OP= Older Plant

Plant Dry Matter Accumulation

Cadmium pollution significantly reduced ($P<0.01$) the overall foliar yield of TVNu-91 (5ESV) to 29.15 as compared to TVNu-93 (44.20) sown in the control soil (Table 5). There was also significant reduction ($P>0.01$) in the plant dry weight of the different accessions studied with increasing cadmium concentration. TVNu-93 (5ESV) had a reduced dry plant weight of 23.79 when compared to 37.34 obtained in the control soil of TVNu-93 and this is supported by earlier reports showing that legumes were sensitive to increased cadmium levels (Van Assche *et al.,* 1998). The effect of cadmium pollution on the root dry weight as well as the shoot to root ratio was also reported. There was significance difference ($P=0.00$) in the root dry weight as well as shoot to root ratio of both control and cadmium polluted accessions. This could be attributed to the fact that increased cadmium concentration in the root environment resulted in reduction of absorption of water and nutrients, reduction of water transpiration and disturbance in water balance, inhibition of enzymes activities and of cell metabolism (Ohanmu *et al.,* 2018).

Table 5.

Effects of cadmium pollution on Plant Dry Matter Accumulation of wild *Vigna* at 20 weeks after sowing

Plant Accessions	Cd Conc.	Overall foliar yield (g)	Plant dry weight(g)	Root dry weight (g)	Shoot: Root Ratio
TVNu-91	0	44.2	19.45	1.45	12.41
	2.5	34.11	12.62	1.02	11.37
	5	29.15	7.44	0.97	6.67
TVNu-93	0	114.17	37.34	13.82	1.7
	2.5	85.38	29.09	11.77	1.47
	5	59.34	23.79	10.49	1.27
TVNu-94	0	52.07	17.89	0.87	19.56
	2.5	33.93	12.87	0.78	15.5
	5	28.79	8.5	0.58	13.66
TVNu-95	0	28.66	3.67	0.49	6.49
	2.5	17.01	1.92	0.16	11
	5	13.45	1.54	0.15	9.27
TVNu-96	0	86.93	37.5	3.88	8.66
	2.5	45.84	19.07	3.74	4.1
	5	42.75	17.17	3.52	3.88
P-value		0.01	0.004	<0.001	<0.001
LSD(0.05)		21.7	13.2	12.35	16.03

Plant yield

The effects of cadmium on the reproductive capacity of wild vigna have been presented (Table 6a and 6b). There was a significant difference ($P>0.05$) in the number of peduncle per plant as evidenced in TVNu-95 (5ESV) 5.67 when compared to 14.07 in the control of TVNu-95. Generally, there was significant difference in the length of peduncle ($P<0.01$), days to flower bud initiation ($P<0.01$), days to 50% flower bud initiation ($P<0.01$), flower bud size ($P<0.05$), number of flowers per plant ($P<0.05$), flowering duration ($P<0.05$) as well as days to maturity ($P<0.01$). The number of peduncle per plant, length of peduncle, days to flower bud initiation, flower bud size, number of flowers per plant, flowering duration as well as days to maturity were absent for TVNu-93, TVNu-94 and TVNu-96. However, 2.5 ESV and 5 ESV of TVNu-91 also showed absence of the above named parameters. Days to flowering, duration of flowering and the time required for physiological maturity each increased with the degree of climbing ability of the plants (Hernandez *et al*., 1979). It has been suggested that one of the first traits observed by the ancient seed gatherers of wild legume species could have been earliness in flowering and formation of fruit and seeds (pod), which, along with uniformity of these periods, continue to be used as criteria for cultivar selection (Hernandez *et al*., 1979). Thus, control soil of TVNu-95 (8.05) had a faster maturity rate when compared to TVNu-91 (8.13).

Table 6a.

Effects of cadmium pollution on reproductive capacity of wild *Vigna*

Plant Accessions	Cd Conc.	No. of peduncle/ plant	Length of peduncle (cm)	Days to flower bud initiation (DAS)	Days to 50% flower bud initiation (DAS)	Flower bud size (mm)	No. of Flowers/ plant	Flowering duration (days)	Days to maturity (days)
TVNu-91	0	12.1	1.78	99.04	109.21	1.69	14.05	12.03	8.13
	2.5	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0
TVNu-93	0	0	0	0	0	0	0	0	0
	2.5	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0
TVNu-94	0	0	0	0	0	0	0	0	0
	2.5	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0
TVNu-95	0	14.07	1.92	98.07	107.21	1.57	13.07	12.92	8.05
	2.5	8.31	1.74	101.02	111.07	1.33	9.37	9.08	10.1
	5	5.67	1.31	107.5	112.65	1.17	8.09	7.14	11.72
TVNu-96	0	0	0	0	0	0	0	0	0
	2.5	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0
P-value		0.04	0.007	0.002	0.003	0.014	0.03	0.02	0.001
LSD(0.05)		11.01	1.14	57.95	67.21	0.98	7.63	6.38	6.09

There was a significant difference ($P>0.05$) in the number of pods per plant (Table 6b) as evidenced in TVNu-95 (5ESV) 5.67 when compared to 14.07 in the control of TVNu-95. Thus, there was a gradual decrease with increasing ecological screening value. Generally, there was significant difference in the seed number per pods ($P<0.01$), seed weight per pods ($P<0.01$), the plant yield ($P<0.01$), and flower bud size ($P<0.05$). However, the above parameters were absent for TVNu-93, TVNu-94, TVNu-96 as well as 2.5 ESV and 5 ESV of TVNu-91.

Table 6b.

Effects of cadmium pollution on reproductive capacity of wild *Vigna*

Plant Accessions	Cd Conc.	No. of pods/plant	Seed No./ pods	Seed wt./ pods (g)	Plant yield (g/m^2)
TVNu-91	0	1.76	4	0.19	2.68
	2.5	0	0	0	0
	5	0	0	0	0
TVNu-93	0	0	0	0	0
	2.5	0	0	0	0
	5	0	0	0	0
TVNu-94	0	0	0	0	0
	2.5	0	0	0	0
	5	0	0	0	0
TVNu-95	0	2.48	2	0.19	3.55
	2.5	1.23	2.67	0.15	1.93
	5	1.09	2.94	0.13	1.48
TVNu-96	0	0	0	0	0
	2.5	0	0	0	0
	5	0	0	0	0
P-value		0.024	0.032	0.014	0.0072
LSD(0.05)		1.01	1.68	0.11	0.67

Conclusion

Due to the incidence of cadmium pollution, there was reduced plant growth with increase in chlorosis, necrosis, curled as well as folded leaves. Thus, the plant had to senesced older leaves in order to remove most of the cadmium-toxicant. With respect to the reproductive capacity as well as foliar chlorosis and necrosis, TVNu-95 showed a high tolerance to cadmium stress than other accessions, which makes TVNu-95 suitable to cadmium contaminated soil. Although cadmium significantly reduced the yields of wild vigna accessions, wild vigna accessions can serve as a good indicator to quantify the presence of cadmium toxicity in soils. However, further work is recommended in area of mechanisms of action in the inhibition of enzymes as well as interaction of the root nodule with rhizospheric bacterial and if this also had a determining effect on the plant yield.

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