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RESEARCH ARTICLES

Growth and yield responses of soybean (*Glycine max* [L.] Merr.) accessions after exposure to cadmium

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Abstract

Little is known about soybean (*Glycine max* [L.] Merr.) growth and yield responses to cadmium contamination of agricultural soil. Three levels of cadmium ecological screening values (2 $CdCl_2$ ESV, 4 $CdCl_2$ ESV and 6 $CdCl_2$ ESV) were used to contaminate trial soils before sowing viable seeds from ten soybean accessions. The plants were monitored for growth, physiological characteristics and seed yield. Results showed significant growth impairment in *G. max* that was proportional to soil $CdCl_2$ contamination levels. There was 30% yield reduction at $CdCl_2$ 2 ESV and < 50% yield reductions at 6 $CdCl_2$ ESV. Accession TGm-941 had the highest yield in the control but showed a 40% yield reduction upon exposure to cadmium contamination. Morphologically, the shapes and outer appearance of some harvested soybean seeds were distorted, which is likely due to elevated cadmium levels in the soil. The mechanisms attributable to these changes are not known and require further investigation. Soybean is capable of tolerating low levels of cadmium contamination by maintaining growth and limited yield reduction but higher levels of contamination significantly reduced plant growth and yield.

Keywords Cadmium · Soybean (*Glycine max*) · Agronomy · Soil contamination · Protein security

Introduction

Food legumes are important sources of dietary protein for the increasing global human population. However, a growing threat to plant as a source of protein is environmental contamination from heavy metal, which hampers sustainable agricultural practices including growth characteristics, seed yield and genetic variation (Xie et al. 2016). For instance, toxic heavy metal like cadmium (Cd) compete with essential minerals present in legumes like soybean (*Glycine max* (L.) Merr.) and when consumed bind with cellular components thereby altering normal metabolic and systemic functions (Thielecke and Nugent 2018).

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Soil is central to food security and defines the direction of the terrestrial food chain (Toth et al. 2016). Soil contamination by toxic heavy metal mostly result from human activities and sources (Farid et al. 2015; Osawaru et al. 2013a, b; Shi and Ma 2017). The ecology and structure of heavy metal contaminated soil result in reduced soil fertility and productivity. Zwolak et al. (2019) reported that heavy metal threatens human health because the mineral contents of edible plants and plant products that human rely on are influenced by the amount and interaction of trace elements in the soil including heavy metals. Heavy metal accumulation in crops, which result from the uptake of metals in the soil-crop system, can be used to assess the potential health risk associated with heavy metal pollution (Hu et al. 2017). The bioavailability and accumulation of Cd in economic crops have been confirmed and is attributed to soil acidity, excess nitrogen fertilizers, the genetic variation of the crops, atmospheric deposition and pollution (Yang et al. 2016; Chen et al. 2018). Therefore, soil Cd pollution poses a serious threat to soil quality and food security as well as to human health (Ogwu 2019; Ikhajiagbe and Ogwu 2020). Elevated doses of Cd (above 3 µg/L) can result from uncontrolled mining and emissions from combustion engines. In addition, Cd-fertilizers are carcinogenic to humans (Kubier et al. 2019).

Spontaneous degradation of Cd is limited because of the long half-life (~ 13–1100 years) as well as the physicochemical properties of the soil (Šichorová et al. 2004). Although Cd is a non-essential element, it is very toxic and mobile and can replace calcium in minerals due to their similar ionic radius, charge and chemical behaviour, which has potentially devastating effects on soil biota through decreasing soil biological activity as well as plant metabolism even at a low concentration (Sanità and Gabbrielli 1999). According to Kabata-Pendias and Pendias (2001), Cd is 2–20 times more toxic compared to other heavy metals. Apart from geogenic sources of Cd, soil contamination of Cd also results from anthropogenic activities, such as the by-products of smelting and refining of metals (Dudka and Adriano 1997).

The International Institute for Tropical Agriculture and other research institutes are promoting the cultivation of high yielding and stress-resistant soybean cultivars (Ugbabe et al. 2017). However, soybean has been reported (Das et al. 1997) to bioaccumulate Cd in edible plant tissues, resulting in food safety concerns. Cadmium tolerance in the plant is linked to the ability of the plant to regulate entry, intracellular transport, detoxification, exclusion, compartmentalization, precipitation and chelation of cadmium compounds (Zou et al. 2018; Hernandez-Baranda et al. 2019). Soybean growth, development, yield, nodulation and nitrogen-fixing ability is likely to be affected by Cd contamination of soil. This study assesses the performance of soybean genotypes under varying Cd toxicity levels in order to document the effects of Cd stress on the morphological characteristics, growth and seed yield of soybean. We adopted various levels of Cd contamination for the study based on established phytotoxicity benchmarks of Efroymson et al. (1997). The results will determine the effects of environmental contamination by Cd on the growth and seed yield of soybean.

Materials and methods

Seed and soil collection

Viable *G. max* seeds of ten genotypes used in the study were collected from the Genetic Resource Centre of the International Institute for Tropical Agriculture, Ibadan, Nigeria (Supplementary Table 1). Topsoil was collected from the Botanic Garden, University of Benin, Nigeria. The prevailing environmental conditions of the area and the physicochemical and microbial characteristics of the soil have been reported by Osawaru et al. (2014), Ogwu and Osawaru (2015), Ogwu et al. (2016) and Chime et al. (2017). The soil was sundried (22–25 °C) to a constant weight, finely crushed and then sieved through a 2-mm stainless steel sieve and transferred into experimental bags at 20 kg per bag. A sample of the pre-treated soil was analyzed for the presence of N, P, K, Cd, Fe and organic carbon according to Bray and Kurtz (1945a, b), SSSA (1971) and Nasir et al. (2015).

Soil contamination with Cd, experimental design and sowing of seeds

The chloride form of Cd (cadmium chloride, $CdCl_2$), was used for the study. The phytotoxicity benchmark or ecological screening value (ESV) for Cd is 4 mg/kg of soil (Efroymson et al. 1997). The test accessions were exposed to three different levels (2 ESV, 4 ESV and 6 ESV) of Cd contamination based on the ESV. This implies that for 2 ESV the mass of Cd required for contamination was 8 mg and 24 mg for 6 ESV. Since the molecular mass of $CdCl_2$ is 183.32 g, and Cd is 112.41u, for every 1 mg of $CdCl_2$ weighed, a factor of 1.631 is required to get 1 mg of Cd. Therefore, for the 2 ESV application, $8\ mg \times 1 \times 1.631 = 13.04$ mg of $CdCl_2$ was required per kilogram of soil. For 4ESV, $16\ mg \times 1 \times 1.631\ mg = 26.09$ mg of $CdCl_2$ was required. About 39.14 mg of $CdCl_2$ was required per kilogram of soil for the 6 ESV application. Following the methods of Okoye et al. (2019), $CdCl_2$ solutions were prepared and applied to each soil treatment until the soil reached it water holding capacity at 187.2 ml/kg of soil for each treatment of 20 kg of soil, which required roughly about 3.74 L of water. Therefore, to obtain, each bag was polluted with $CdCl_2$ in solutions as presented below (Supplementary Table 2). The experiment was divided into four groups containing the ten soybean accessions with five replicates in a completely randomized block design. The first group consisted of the control group, which was not contaminated with Cd. The second was contaminated with Cd at twice the ecological screening value of Cd (2 ESV or, 8 mg/kg) in agrarian soils (Efroymson et al. 1997). The third and fourth groups were soils contaminated with Cd at 4 ESV and 6 ESV, respectively. The ESV of Cd was 4 mg/kg (Efroymson et al. 1997). The trial soils were allowed to attenuate for three weeks, after which soybean seeds were planted according to Ohanmu and Ikhajiagbe (2018).

Data collection and statistical analysis

The plants of each accession in each treatment group were observed for morphological and physiological responses until maturity. Plant height, number of leaves per plant, leaf area, dry weight of the plant, number of primary root branches, number of nodules per plant (measured as nodules with a minimum diameter of 5 mm) were recorded. Yield components were measured as the number of pods per plant and total weight of harvested seed per plant. Seed characteristics were determined according to Adewale and Duet (2011) and includes seed shape, seed brilliance, testa texture and basal colour. To understand the biochemical impacts of the treatment on the soybean accessions, nitrogen concentration of leaves were measured according to methods described in Al-Mutawa and El-Katony (2001) while total chlorophyll, tocopherol, lycopene, carotenoids were determined according to the methods described by Ruch et al. (1989). Soybean accession were ranked according to best growth response (αTGm). Percentage reductions in plant seed yield and plant dry weight of metal-exposed plants compared to the control was determined for each accession. These percentage reductions were then ranked (R) from the least reduction to the worst. The mean rank ($\bar{R}$) was then calculated.

$$\bar{R} = \frac{R1 + R2 + R3}{3} \qquad (1)$$

where R1, R2 and R3 were the ranks for individual plant accession exposed to Cd at 2 ESV, 4 ESV and 6 ESV, respectively.

$$\alpha TGm = \text{Plant accession with lowest } \bar{R} \qquad (2)$$

A one-way analysis of variance and *t*-test was performed using SPSS® version 21 for Windows PC. Level of significance was used as $P < 0.05$ for each trait evaluated.

Results

The physicochemical condition of the soil before treatment indicated that the soil was slightly acidic (pH 6.08) (Supplementary Table 3). After 12 weeks, there was 28.41% increase in plant height in TGm-941 at 6 ESV (Table 1). Other than TGm-935 and TGm-941, which had significant increases in the number of leaves per plant upon increased exposure to Cd, general reductions in plant leaves were between 14.63 and 40.82%, respectively (Table 2). Reductions in leaf area at 12 weeks after sowing was a common observation among the various accessions exposed to Cd (5.85–53.33% reduction) (Table 3) and the dry weight of soybean accessions significantly decreased with increased exposure to Cd (Table 4). Accession TGm-934 was the most affected with a 79.48% reduction in whole plant dry weight.

The capacity of any plant species to subsist under stressful environmental condition is also a measure of its antioxidant capacity. To determine antioxidant response to Cd soil levels, tocopherol levels were determined in the leaves. There was a general reduction in leaf tocopherol content due to Cd exposure (Fig. 1). However, increased leaf tocopherol activity was reported in TGm-932 upon exposure to Cd, from 0.228 U/g FW in the control to 2.243 and 2.212 U/g FW in plants exposed to Cd at 4 ESV and 6 ESV, respectively. Total lycopene contents in leaves of the test plants showed differential responses among the accession (Fig. 2). Plants had enhanced its carotenoid activity during the study as the control ranged from 1083.2–3825.6 U/g FW whereas Cd-exposed plants ranged between 3113.4 and 6182.4 U/g FW (Fig. 3). Chlorophyll content showed differential response in the test plants. Increases in chlorophyll content was observed in TGm-932, 934, and 941 in Cd-exposed plants at 6 ESV (Fig. 4). Generally, plant exposure to Cd exhibit impaired nodulation capacity after 12 weeks causing about 40% reduction in root nodules (Fig. 5). Contrary to root nodules, plant exposure to Cd enhanced root development in most of the accessions (Table 5). The foliar concentration of nitrate-nitrogen and nitrogen showed varied patterns in all the soybean accessions assessed due to soil contamination with cadmium (Fig. 6). Total foliar nitrate-nitrogen content in the control ranged from 661.3 to 926.3 ppm (Fig. 6a). Marked increment in foliar nitrate-nitrogen content was

Table 1 Plant height of soybean after 12 weeks of sowing in soil contaminated by cadmium

Accessions	Plant height (cm)				p-value	$\%\Delta_{6ESV}$
	CTR	2 ESV	4 ESV	6 ESV		
TGm-540	$75.3^{A/a}$	$42.2^{B/b}$	$47.6^{B/c}$	$51.2^{B/ab}$	0.013	− 32.01
TGm-932	$63.1^{A/ab}$	$54.9^{AB/b}$	$49.3^{B/c}$	$42.1^{B/b}$	0.041	− 33.28
TGm-933	$55.6^{A/bc}$	$46.7^{A/b}$	$48.1^{A/c}$	$53.5^{A/ab}$	0.623	− 3.78
TGm-934	$69.7^{A/ab}$	$57.2^{A/b}$	$67.3^{A/}$	$62.3^{A/a}$	0.472	− 10.62
TGm-935	$58.3^{A/b}$	$57.2^{AB/b}$	$53.1^{AB/bc}$	$49.7^{B/ab}$	0.183	− 14.75
TGm-941	$53.5^{B/bc}$	$59.9^{B/b}$	$73.5^{A/a}$	$68.7^{AB/a}$	0.038	28.41
TGm-942	$47.9^{A/c}$	$47.5^{A/b}$	$44.8^{A/c}$	$42.2^{A/b}$	0.816	− 11.90
TGm-943	$72.9^{A/a}$	$61.3^{AB/ab}$	$67.1^{AB/ab}$	$59.7^{B/ab}$	0.288	− 18.11
TGm-944	$79.9^{A/a}$	$79.5^{A/a}$	$53.8^{B/bc}$	$52.3^{B/ab}$	0.015	− 34.54
TGm-945	$73.3^{A/a}$	$63.7^{AB/a}$	$67.3^{AB/ab}$	$53.7^{B/ab}$	0.103	− 26.74
p-value	< 0.011	0.181	0.016	0.391		

Means with similar upper case superscripts on the same row do not differ ($p > 0.05$), whereas means with similar lower case alphabets on the same column do not differ from each other ($p > 0.05$). $\%\Delta_{6ESV}$ percentage change in plant parameter at highest Cd concentration (− reduction, + increase)

Table 2 The number of leaves produced by soybean grown on soil contaminated by cadmium

Accessions	No. of leaves per plant				p-value	$\%\Delta_{6ESV}$
	CTR	2 ESV	4 ESV	6 ESV		
TGm-540	$59^{A/ab}$	$41^{B/ab}$	$39^{B/bc}$	$42^{B/bc}$	0.042	– 28.81
TGm-932	$64^{A/ab}$	$57^{B/a}$	$41^{C/bc}$	$41^{C/bc}$	< 0.001	– 35.94
TGm-933	$49^{A/bc}$	$31^{B/b}$	$33^{AB/c}$	$29^{B/c}$	0.391	– 40.82
TGm-934	$77^{A/a}$	$53^{B/a}$	$60^{B/a}$	$61^{B/a}$	0.009	– 20.78
TGm-935	$39^{B/c}$	$29^{B/b}$	$41^{AB/bc}$	$52^{A/ab}$	0.007	33.33
TGm-941	$37^{AB/c}$	$50^{A/a}$	$29^{B/c}$	$44^{AB/b}$	0.13	18.92
TGm-942	$36^{A/c}$	$27^{A/b}$	$33^{A/c}$	$27^{A/c}$	0.592	– 25.00
TGm-943	$41^{A/c}$	$39^{A/b}$	$42^{A/bc}$	$35^{A/c}$	0.429	– 14.63
TGm-944	$52^{A/bc}$	$41^{AB/a}$	$51^{A/ab}$	$31^{B/c}$	0.193	– 40.38
TGm-945	$44^{AB/bc}$	$37^{B/b}$	$52^{A/ab}$	$43^{AB/b}$	0.281	– 2.27
p-value	< 0.001	0.238	0.017	0.027		

Means with similar upper case superscript on the same row are not significant different ($p > 0.05$), whereas means with similar lower case alphabets on the same column are not significantly different from each other ($p > 0.05$). $\%\Delta_{6ESV}$ percentage change in plant parameter at highest Cd concentration (– reduction, + increase)

Table 3 The leaf area of soybean accessions due to soil contamination by cadmium

Accessions	Leaf area (cm^2)				p-value	$\%\Delta_{6ESV}$
	CTR	2 ESV	4 ESV	6 ESV		
TGm-540	$23.1^{A/b}$	$21.3^{A/b}$	$25.3^{A/ab}$	$26.4^{A/bc}$	0.651	14.29
TGm-932	$35.3^{A/ab}$	$25.2^{AB/ab}$	$21.7^{B/b}$	$22.7^{B/bc}$	0.087	– 35.69
TGm-933	$31.7^{A/ab}$	$28.7^{A/ab}$	$31.3^{A/a}$	$29.3^{A/ab}$	0.395	– 7.57
TGm-934	$37.2^{A/a}$	$31.2^{A/ab}$	$20.9^{B/b}$	$23.2^{B/bc}$	0.003	– 37.63
TGm-935	$37.5^{A/a}$	$26.9^{AB/a}$	$23.8^{B/ab}$	$17.5^{C/c}$	< 0.001	– 53.33
TGm-941	$33.5^{A/a}$	$35.5^{A/a}$	$39.6^{A/a}$	$35.4^{A/a}$	0.482	5.67
TGm-942	$41.8^{A/a}$	$33.2^{AB/a}$	$31.7^{BC/a}$	$23.1^{C/bc}$	0.013	– 44.74
TGm-943	$33.5^{A/ab}$	$35.7^{A/a}$	$30.2^{A/ab}$	$37.5^{A/a}$	0.499	11.94
TGm-944	$31.5^{A/ab}$	$31.9^{A/ab}$	$33.7^{A/a}$	$29.6^{A/ab}$	0.381	– 6.03
TGm-945	$34.2^{A/ab}$	$31.3^{A/ab}$	$29.1^{A/ab}$	$32.2^{A/ab}$	0.232	– 5.85
p-value	0.069	0.137	0.095	0.002		

Means with similar upper case superscripts on the same row are not significantly different ($p > 0.05$), whereas means with similar lower case alphabets on the same column are not significantly different from each other ($p > 0.05$). $\%\Delta_{6ESV}$ percentage change in plant parameter at highest Cd concentration (– reduction, + increase)

also reported in TGm-942 from 661.3 ppm to 2001.4 ppm. Similar to nitrate nitrogen, total foliar nitrogen concentrations increased in the Cd-exposed plants (0.41–0.83%) as compared to control plants (0.16–0.57%) (Fig. 6b).

There was a significant reduction in the number of pods due to Cd exposure as the number of pods per plant in the control for each accession was 17–24 pods per plant but 12–19 at 2ESV, 11–19 at 4ESV and 9–16 pods per plant at 6ESV (Fig. 7a–d). Significant yield reductions were observed due to Cd exposure ($p < 0.05$) (Table 6). Results showed minimal changes in seed characteristics including seed shape, seed brilliance, testa texture and basal colour (Table 7a–d). Seed shape of the majority of the accessions were oblong both for the control and when exposed to Cd. However, in TGm-933, 934 and 945, seed shape was rhomboid.

An attempt was made to indicate plant accessions that were least affected by the Cd polluted soil compared to respective controls (Table 8). Accessions TGm-933 and TGm-943 can be considered for breeding and cultivation due to their better performance in Cd polluted soil compared to the other accessions evaluated in the study. However, owing to poor response in the Cd contaminated soil, TGn-932, TGm-934, and TGm-942 were not good candidate for growth and yield performance in a Cd polluted soil. Table 9 shows the bivariate correlation between selected growth parameters and either foliar nitrate or foliar nitrogen contents, respectively. There was a significant positive

Table 4 Dry weight of soybean accessions after 12 weeks with increasing soil cadmium concentration

Accessions	Plant dry wt. (g)				p-value	$\%\Delta_{6ESV}$
	CTR	2 ESV	4 ESV	6 ESV		
TGm-540	$2.89^{A/a}$	$0.75^{A/c}$	$0.72^{A/b}$	$1.01^{A/b}$	< 0.001	− 65.05
TGm-932	$3.29^{A/ab}$	$2.37^{B/b}$	$0.81^{C/b}$	$1.81^{C/ab}$	< 0.001	− 44.98
TGm-933	$0.59^{A/c}$	$0.78^{A/c}$	$0.49^{A/b}$	$0.56^{A/b}$	0.629	− 5.08
TGm-934	$4.24^{A/a}$	$2.33^{B/b}$	$0.51^{C/b}$	$0.87^{C/b}$	< 0.001	− 79.48
TGm-935	$1.77^{A/bc}$	$1.35^{A/bc}$	$1.31^{A/b}$	$1.61^{A/ab}$	0.489	− 9.04
TGm-941	$3.12^{B/ab}$	$4.94^{A/a}$	$3.15^{B/a}$	$2.71^{B/a}$	0.037	− 13.14
TGm-942	$3.16^{A/ab}$	$1.62^{B/bc}$	$1.61^{B/ab}$	$1.32^{B/ab}$	0.004	− 58.23
TGm-943	$1.99^{AB/bc}$	$2.51^{A/b}$	$1.54^{B/b}$	$1.58^{B/ab}$	0.138	− 20.60
TGm-944	$2.46^{A/b}$	$2.38^{A/b}$	$2.27^{A/a}$	$1.94^{A/ab}$	0.572	− 21.14
TGm-945	$2.36^{A/b}$	$1.61^{AB/bc}$	$1.09^{B/b}$	$1.11^{B/b}$	< 0.001	− 52.97
p-value	< 0.001	< 0.001	< 0.001	0.015		

Means with similar upper case superscripts on the same row are not significantly different (p > 0.05), whereas means with similar lower case alphabets on the same column are not significantly different from each other (p > 0.05). $\%\Delta_{6ESV}$ percentage change in plant parameter at highest Cd concentration (− reduction, + increase)

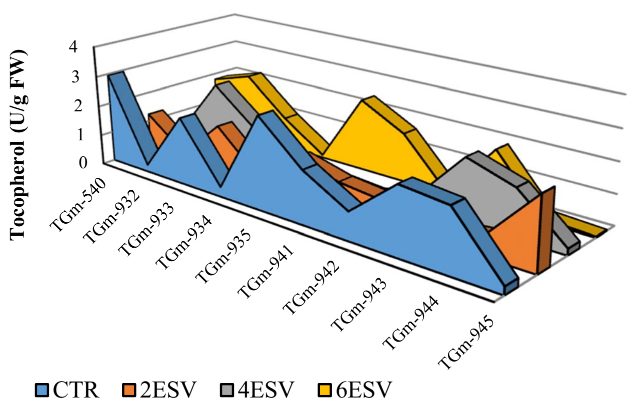


Fig. 1 Total foliar tocopherol concentration of the various soybean accessions after exposure to different cadmium contamination levels

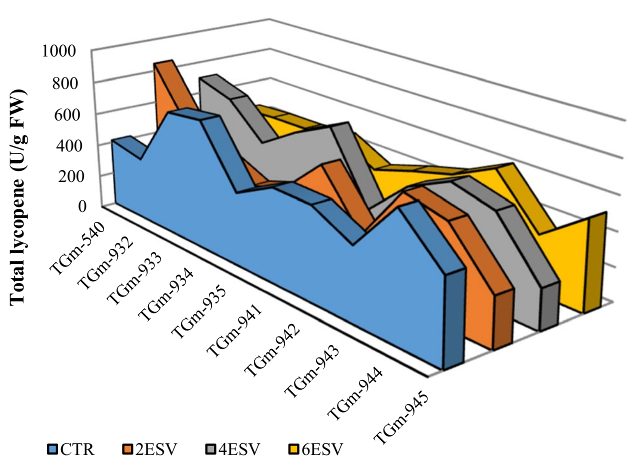


Fig. 2 Total foliar lycopene concentration of the various soybean accessions after exposure to different cadmium contamination levels

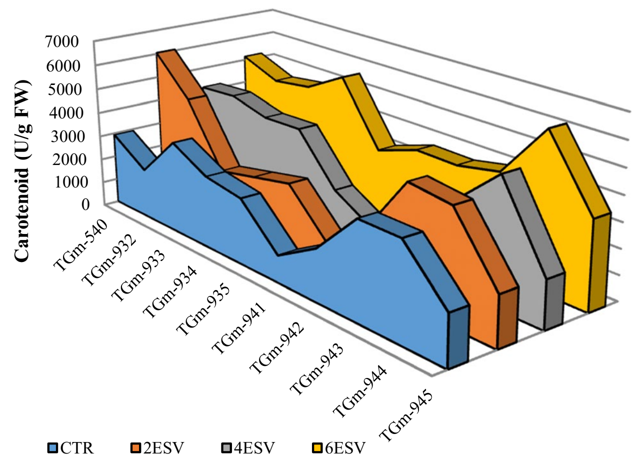


Fig. 3 Foliar carotenoid concentration of the various soybean accessions after exposure to different cadmium contamination levels

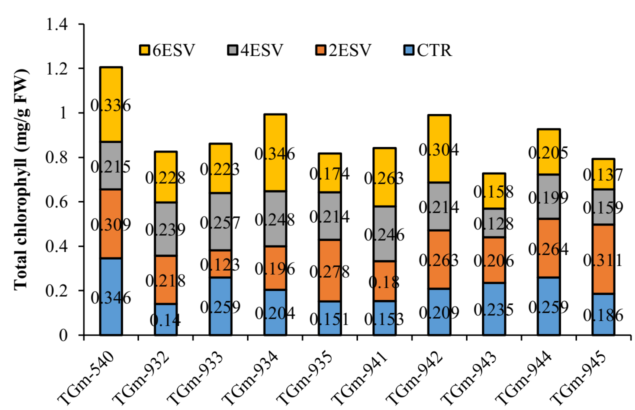


Fig. 4 Total chlorophyll content of leaves of the various soybean accessions after exposure to different cadmium contamination levels

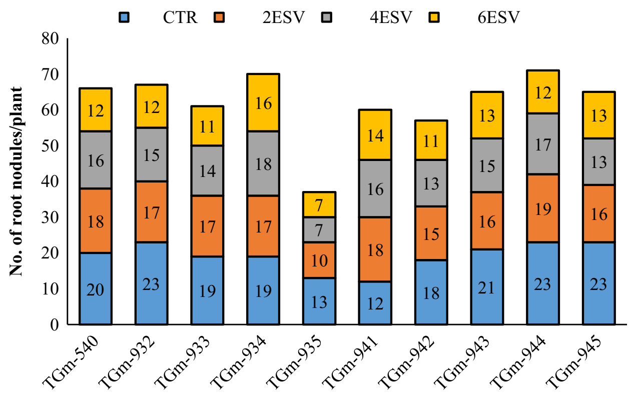


Fig. 5 Effects of different cadmium contamination levels on nodule number per plant at 12 weeks after sowing

correction between seed yield and total foliar nitrogen (R = 0.700, p = 0.024) in plants without metal exposure. No bivariate correlation between the growth parameters and either total or nitrate-nitrogen existed when plants were exposed to Cd at higher ESV.

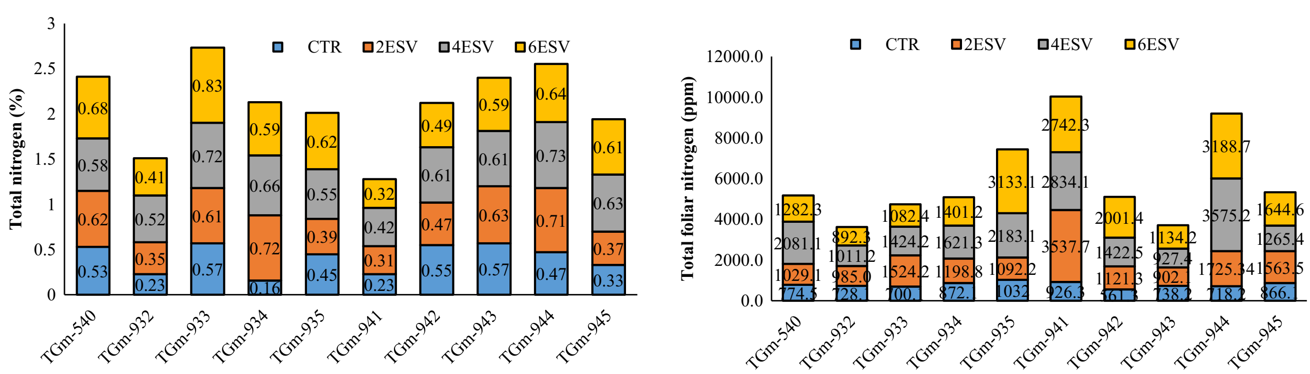
Discussion

The effects of three levels of soil contamination by Cd on the growth, development and yield of ten soybean accessions was investigated. Our findings were in conformity with de Souza Silva et al. (2014) that Cd toxicity is characterized by growth impairments, reduction in plant growth and poor yield. Higher Cd concentrations in the soil had more inhibitory effects on the growth, development and seed yield characteristics of the soybean accession evaluated. These effects could be linked to the mobility of Cd in the soil–plant

Table 5 Root length of different soybean accessions at 12 weeks after planting

Accessions	Root length (cm)				p-value	$\%\Delta_{6ESV}$
	CTR	2ESV	4ESV	6ESV		
TGm-540	$9.7^{A}/^{a}$	$8.6^{A}/^{b}$	$13.5^{A}/^{ab}$	$11.8^{A}/^{b}$	0.421	21.65
TGm-932	$8.2^{B}/^{a}$	$11.2^{B}/^{ab}$	$10.6^{B}/^{b}$	$18.4^{A}/^{a}$	< 0.001	124.39
TGm-933	$8.5^{B}/^{a}$	$11.3^{B}/^{ab}$	$17.4^{A}/^{a}$	$13.1^{AB}/^{ab}$	0.015	54.12
TGm-934	$9.2^{A}/^{a}$	$8.5^{A}/^{b}$	$11.0^{A}/^{b}$	$10.5^{A}/^{bb}$	0.481	14.13
TGm-935	$11.5^{B}/^{a}$	$15.5^{A}/^{a}$	$15.1^{A}/^{ab}$	$12.5^{B}/^{ab}$	0.002	8.70
TGm-941	$13.5^{A}/^{a}$	$11.2^{A}/^{ab}$	$11.5^{A}/^{b}$	$12.2^{A}/^{ab}$	0.517	− 9.63
TGm-942	$10.1^{B}/^{a}$	$11.5^{B}/^{ab}$	$17.2^{A}/^{a}$	$14.5^{AB}/^{ab}$	0.093	43.56
TGm-943	$11.5^{A}/^{a}$	$15.3^{A}/^{a}$	$13.5^{A}/^{ab}$	$12.6^{A}/^{ab}$	0.318	9.57
TGm-944	$11.2^{A}/^{a}$	$14.6^{A}/^{a}$	$12.1^{A}/^{ab}$	$10.2^{A}/^{b}$	0.662	− 8.93
TGm-945	$9.6^{A}/^{a}$	$12.4^{A}/^{ab}$	$11.2^{A}/^{b}$	$14.5^{A}/^{ab}$	0.292	51.04
p-value	0.418	0.013	0.118	0.136		

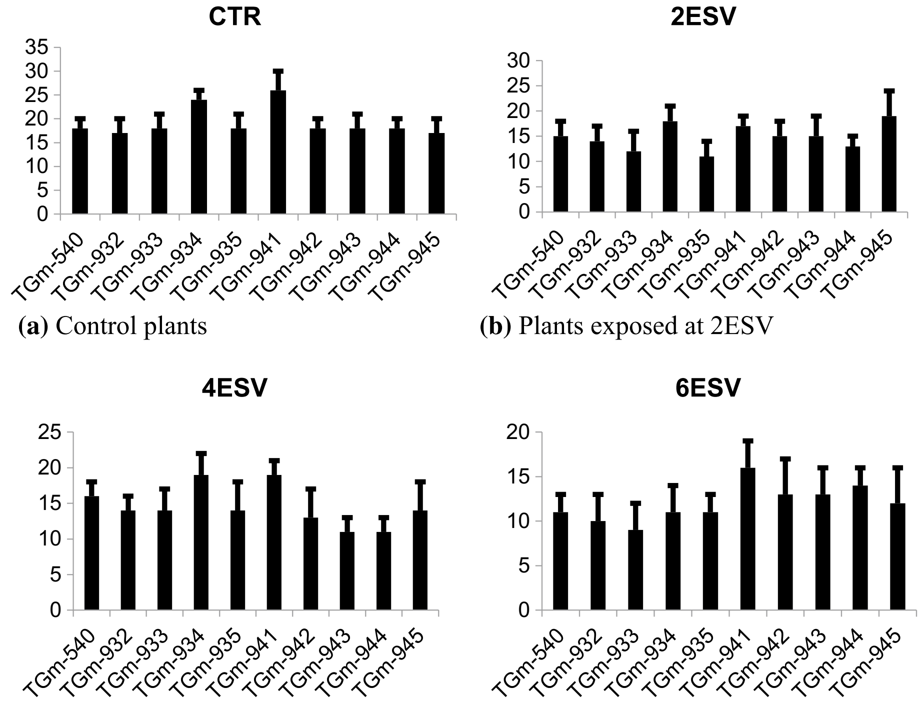
Means with similar upper case superscripts on the same row are not significantly different (p > 0.05), whereas means with similar lower case alphabets on the same column are not significantly different from each other (p > 0.05). $\%\Delta_{6ESV}$ percentage change in plant parameter at highest Cd concentration (− reduction, + increase)



(a) Foliar nitrate-nitrogen

(b) Total foliar nitrogen

Fig. 6 Foliar concentration of (**a**) nitrate-nitrogen and (**b**) total nitrogen in cadmium-exposed plants at 12 weeks after sowing



(a) Control plants (b) Plants exposed at 2ESV (c) Plants exposed at 4ESV (d) Plants exposed at 6ESV

Fig. 7 Number of pods per plant in soybean accessions in the (**a**) control as well as after exposure to cadmium at different ecological screening values (**b**, **c** and **d**)

Table 6 Plant yield as measured by dry weight (g) of seeds per plant at harvest

	Seed wt. per plant (g)				p-value	$\%\Delta_{6ESV}$
	CTR	2ESV	4ESV	6ESV		
TGm-540	124.54$^{A/b}$	99.31$^{B/a}$	90.24$^{B/a}$	59.32$^{C/bc}$	< 0.001	− 52.37
TGm-932	114.24$^{A/b}$	79.82$^{B/ab}$	65.26$^{BC/b}$	41.9$^{C/bc}$	< 0.001	− 63.32
TGm-933	75.23$^{A/c}$	64.64$^{A/b}$	61.35$^{A/b}$	36.04$^{B/c}$	0.062	− 52.09
TGm-934	165.03$^{A/a}$	103.53$^{B/a}$	98.53$^{B/a}$	51.53$^{C/bc}$	< 0.001	− 68.78
TGm-935	104.22$^{A/bc}$	65.35$^{B/b}$	59.46$^{B/b}$	53.01$^{B/bc}$	0.028	− 49.14
TGm-941	181.45$^{A/a}$	108.37$^{B/a}$	95.71$^{B/a}$	90.31$^{B/a}$	0.013	− 50.23
TGm-942	121.53$^{A/b}$	86.95$^{B/ab}$	57.59$^{C/b}$	61.46$^{BC/bc}$	0.047	− 49.43
TGm-943	98.47$^{A/bc}$	81.47$^{A/ab}$	48.19$^{B/b}$	60.47$^{AB/bc}$	0.103	− 38.59
TGm-944	124.54$^{A/b}$	72.69$^{B/b}$	46.99$^{C/b}$	66.66$^{BC/ab}$	< 0.001	− 46.48
TGm-945	111.49$^{A/b}$	109.42$^{A/a}$	65.26$^{B/b}$	51.48$^{B/bc}$	< 0.001	− 53.83
p-value	< 0.001	0.093	0.318	0.293		

Mean with similar upper case superscripts on the same row are not significantly different ($p > 0.05$), whereas means with similar lower case alphabets on the same column are not significantly different from each other ($p > 0.05$). %Δ6ESV percentage change in plant parameter at highest Cd concentration (− reduction, + increase)

system. The cadmium contamination affected soybean plant height causing a significant reduction, which agrees with previous reports (Epelde et al. 2010; Per et al. 2016), which linked the effects to reduced cell growth and division, photosynthesis, translocation and transpiration. The reduction of Cd solubility and bioavailability is vital to mitigate some of the effects on plant growth, development and yield (Qi et al. 2018).

Plant roots are vulnerable to soil contamination by heavy metals. Soybean roots are the first plant tissue to interact with soil Cd where it gains entry into the plant and begin inhibitory interactions targeting vital physiological and metabolic processes (Zou et al. 2018). We observed that Cd had a stimulatory effect on soybean root growth, which was similar to the observation of Zou et al. (2018) in *Salix* species. However, this effect may be linked to the concentration of cadmium in the soil and rate of Cd transformation

Table 7 Presentation of the most prominent seed characteristics measured for (a) seed shape (b) seed brilliance (c) testa texture and (d) basal colour

(a) Seed shape (1: oblong, 2: rhomboid)	CTR	2ESV	4ESV	6ESV
TGm-540	1	1	1	1
TGm-932	1	1	1	1
TGm-933	2	1	1	1
TGm-934	2	2	1	1
TGm-935	1	1	1	1
TGm-941	1	1	1	1
TGm-942	1	1	1	1
TGm-943	1	1	1	1
TGm-944	1	1	1	1
TGm-945	2	1	1	1
(b) Seed brilliance (1: Shiny, 2: Medium, 3: Matt)	CTR	2ESV	4ESV	6ESV
TGm-540	3	3	2	2
TGm-932	1	1	2	1
TGm-933	3	3	2	2
TGm-934	3	2	2	3
TGm-935	3	2	3	3
TGm-941	3	1	1	1
TGm-942	1	2	3	2
TGm-943	2	1	1	2
TGm-944	2	3	1	3
TGm-945	2	1	1	3
(c) Seed testa texture (1: smooth, 2: rough, 3: wrinkled (folds on testa)	CTR	2ESV	4ESV	6ESV
TGm-540	3	2	1	3
TGm-932	1	1	1	1
TGm-933	3	1	3	2
TGm-934	3	3	1	1
TGm-935	1	2	3	1
TGm-941	3	1	1	1
TGm-942	3	3	3	3
TGm-943	3	2	3	1
TGm-944	3	3	1	1
TGm-945	2	1	1	1
(d) Seed basal colour (1: cream, 2: grey, 3: light brown, 4: reddish brown, 5: dark brown)	CTR	2ESV	4ESV	6ESV
TGm-540	3	3	3	3
TGm-932	5	5	5	5
TGm-933	5	3	3	2
TGm-934	5	5	3	3
TGm-935	3	5	3	5
TGm-941	3	3	3	5
TGm-942	5	5	5	5
TGm-943	4	5	5	4
TGm-944	3	3	5	1
TGm-945	5	5	5	5

Table 8 Selection of best and worst responsive plant accessions upon exposure to cadmium for at least 12 weeks using plant dry weight and seed yield

Accessions	Percentage change (%) (− reduction, + increase)[a]						R̄ (B)	R̄ (A + B)	Remark[b]
	Plant dry wt. at 12 weeks (A)			Seed yield (B)					
	2ESV	4ESV	6ESV	2ESV	4ESV	6ESV			
TGm-540	− 74 (10)	− 75.1 (8)	− 65.1 (9)	− 20 (4)	− 28 (2)	− 52.4 (7)	4	7	+
TGm-932	− 28 (6)	− 75.4 (9)	− 45 (6)	− 30 (6)	− 43 (5)	− 63.3 (9)	7	7	−
TGm-933	32.2 (2)	− 16.9 (3)	− 5.1 (1)	− 14 (2)	− 19 (1)	− 52.1 (6)	3	3	+++
TGm-934	− 45 (8)	− 88.1 (10)	− 79.5 (10)	− 37 (7)	− 40 (3)	− 68.8 10)	7	8	−
TGm-935	− 23.7 (5)	− 26.1 (5)	− 9 (2)	− 37 (7)	− 43 (5)	− 49.1 (3)	5	5	+
TGm-941	58.3 (1)	1.1 (1)	− 13.1 (3)	− 40 (9)	− 47 (7)	− 50.2 (5)	7	4	+
TGm-942	− 48.7 (9)	− 49.1 (6)	− 58.2 (8)	− 29 (10)	− 53 (9)	− 49.4 (4)	8	8	−
TGm-943	26.1 (3)	− 22.6 (4)	− 20.6 (4)	− 17 (3)	− 51 (8)	− 38.6 (1)	4	4	++
TGm-944	− 3.3 (4)	− 7.7 (2)	− 21.1 (5)	− 42 (5)	− 62 (10)	− 46.5 (2)	6	5	+
TGm-945	− 31.8 (7)	− 53.8 (7)	− 53 (7)	− 1.9 (1)	− 42 (4)	− 53.8 (8)	4	6	+

[a]Values in parentheses are corresponding ranks; R—mean rank; accessions with least mean selected as most tolerant

[b]Key: + + + Most likely selected when considering plant performance in Cd polluted soil; + + Likely selected when considering plant performance in Cd polluted soil; + Intermediate; − Not likely considered when considering plant performance in Cd polluted soil

Table 9 Correlation between selected growth parameters and foliar nitrate and/or foliar nitrogen contents

	Pearson's correlation coefficient (R)							
	Control		2ESV		4ESV		6ESV	
	R	p-value	R	p-value	R	p-value	R	p-value
Nitrate nitrogen								
Br	0.063	0.863	− 0.317	0.372	− 0.091	0.803	− 0.497	0.144
PlHt	0.082	0.822	− 0.218	0.545	0.333	0.347	− 0.129	0.722
RtNo	0.083	0.819	0.371	0.292	0.179	0.621	− 0.266	0.457
LfAr	− 0.22	0.541	− 0.182	0.0614	0.593	0.071	− 0.452	0.19
Nod	− 0.533	0.113	− 0.371	0.292	− 0.003	0.992	− 0.194	− 0.591
Yield	0.33	0.352	− 0.191	0.574	− 0.433	0.211	− 0.591	0.072
Total nitrogen								
Br	0.012	0.974	− 0.028	0.939	− 0.36	0.307	0.239	0.507
PlHt	− 0.013	0.971	1.000**	**< 0.001**	− 0.084	0.817	0.473	0.168
RtNo	− 0.075	0.837	0.079	0.828	0.266	0.458	0.585	0.076
LfAr	0.019	0.96	− 0.193	0.594	0.024	0.947	− 0.08	0.826
Nod	0.093	0.797	0.632*	**0.050**	0.063	0.862	0.14	0.7
Yield	**0.700**	**0.024**	0.254	0.479	0.357	0.311	0.537	0.109

Significant correlation are indicated in bold and asterisks

and mobilization in the plant tissues that are affected by the concentration. Moreover, Cd effects on soybean roots could be attributed to the excessive production of monolignols forming lignin, which causes the hardening of root cell wall and restricts root growth (Finger-Teixeira et al. 2010). Our findings on root development contradict those reported by Konotop et al. (2012) where cadmium treatment resulted in stunted root growth and blackening of the apexes signalling metal-induced oxidation of phenol compounds and increased level of lipid peroxidation. However, they noted that the effect could be remedied by nitrogen fertilization. The study of Shi and Ma (2017) highlighted the capacity of Cd contamination to affect soil microbial activities thereby influencing the nodulating capacity of leguminous crops like soybean. This observation was confirmed in our study through reduced nodulation. Therefore, even though cadmium contamination results in adverse effects on plant growth, and soil microbial activities, it encourages the evolution of Cd tolerance, which develops over time (Xie et al. 2016).

The effects of Cd contamination on the number of leaves have implications for plant survival as this is the major photosynthetic apparatus of the plant. Moreover, the reduction in dry weight could be due to cadmium effects on water absorption and translocation capacity. Other workers (Grifferty and Barrington 2000; Adegoke and Owoyokun 2009; Konotop et al. 2012) have reported that high cadmium concentration has a negative influence on physiological activities of plants associated with water translocation which could impact plant growth, dry matter accumulation, photosynthesis and yield, whereas the reduction in dry mass due to Cd might be attributed to reduced lipids, protein and carbohydrates in soybean (Malan and Farrant 1998).

We recorded significant yield reduction especially at the highest concentration used in this study. Thakur (2014) also reported a significant decrease in soybean yield, specifically in the number of pods per plant, total seeds per plant and seed weight due to different levels of Cd in the soil. Miura et al. (2016) suggested that liming can be used to mitigate the effects of Cd toxicity on soybean yield. However, liming has no effects on the bioaccumulation of Cd by soybean seeds, causing safety concerns associated with eating soybean cultivated on Cd contaminated soils. Results from the study suggest that TGm-933 and TGm-943 were the most tolerant among the soybean accessions due to its plant performance in Cd polluted soil. Therefore, we recommend TGm-933 and TGm-943 for cultivation and genetic improvement for tolerance to Cd. The varietal difference in the uptake, distribution and accumulation of Cd by soybean could be exploited to produce improved Cd-tolerant cultivars (Arao et al. 2003). The mechanism of Cd toxicity in soybean is linked to cellular Cd homeostasis, phytochelatin-based sequestration and compartmentalization, oxidative stress, increasing the affinity for sulfhydryl groups in peptides, lipid peroxidation, and promoting the inhibition of enzyme activities leading to protein denaturation (Benavides et al. 2005; Garg and Bhandari 2013). Nonetheless, Bagheri et al. (2014) acknowledged that little is known regarding the coordination of cellular sequestration of Cd that ultimately results in reduced growth, development and yield in soybean. To elucidate this effect will likely require the identification of Cd ligands present in the cytosol and vascular tissues of soybean (Hasan et al. 2009).

The harvested soybean seeds in this study were distorted and irregularly shaped due to elevated cadmium levels in the soil. This might be attributed to changes in the interphase stage of cells causing the micronuclei to produce irregularly shaped nuclei and nuclei with decomposed nuclear material under the heavy metal stress (Wierzbicka 1994). Cadmium stress is known to induce membrane damage, impair food reserve mobilization, nutrient loss, and increased lipid peroxidation in seeds as well as reduced seed viability, embryo growth and distribution of biomass (Sfaxi-Bousbih et al. 2010; Sethy and Ghosh 2013).

Conclusion

In conclusion, we presented evidence that cadmium toxicity affects soybean morphological growth and reduced seed yield. However, soybean in uncontaminated soil differed in their growth and yield characteristics. The capacities of selected soybean accessions to maintain yield levels under further elevated Cd conditions suggest possible Cd tolerance for those accessions. Rice yields were reduced by over 50% in the susceptible crop accessions, depending on the severity of toxicity. Therefore, differences in plant growth and yield responses to Cd toxicity may be genetic.

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