Morphological, biochemical and quality control analysis of peas genotypes under cadmium toxicity at the seedling stage

ABSTRACT

Cadmium toxicity is an emerging problem as it is the most abundant heavy metal in Pakistani soils. Peas are being grown near cities where an elevated level of cadmium persists. So, this study was conducted for the evaluation of 10 peas genotypes (TRIPLET, MID No. 2, Oskar, LM (OSE), METEOR, P-1, Rohina, PEA-09, LM-POIS, and LAMPO) under cadmium stress at the seedling stage in the greenhouse of Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. There were 3 treatments of cadmium (0, 4, and 8 mg/kg soil) and each treatment was replicated five times in a completely randomized design. Different morphological traits i.e., fresh root weight, fresh seedling weight, root length, seedling length, dry root weight, dry seedling weight and biochemical traits i.e., Cd in the root, shoot, and soil were recorded by atomic absorption spectrometry. Generally, T1 reduced root and shoot parameters while 8 ppm Cd application comparatively enhanced the growth of peas genotypes. LM-POIS and LM (OSE) genotypes performed well under Cd stress and LM-POIS had also an elevated level of Cd in the shoot on the other hand TRIPLET and LAMPO were the least performing genotypes. It is suggested that Cd contamination significantly reduces pea growth; therefore, Cd-tolerant-peas genotypes should be developed or introduced. Further, Cd exposure in the soil should be minimized and already Cd-polluted soils should be remediated.

Keywords: Cd toxicity; Peas genotypes; Quality control analysis; Soil pollution, Pre-breeding evaluation

1. INTRODUCTION

Peas are an important crop in Pakistan, with a significant economic impact on farmers (1). It is the most prevalent crop and, owing to its nutritious value, has a high commercial demand. It is grown on the plains during the winter and in the high lands during the summer (2). The major pea-growing districts in Punjab include Gujranwala, Nankana Sahib, Multan, Sahiwal, Toba Tek Singh, Sialkot, Jhang, and Sheikupura (3). Peas are mostly grown in Punjab during the months of October and November (4). The lack of resistant and high-yielding cultivars, as well as the use of inappropriate peas-growing methods, has resulted in a reduction in the per-acre average yield in Pakistan (5).

Biotic and abiotic stresses have hindered pea production in Pakistan, resulting in decreased yields (6). Abiotic stresses are becoming more severe because of climate change due to global warming (7). Abiotic stress includes drought, salt stress, cold stress, heat stress, and

heavy metals in the soil (8). Heavy metals are accumulated in the soil when crops are irrigated with untreated sewage or industrial water (9). For environmental deterioration it is ranked 7th among most toxic heavy metals (10), due to its carcinogenic properties. Cd polluted the vegetables grown near cities and irrigated with untreated industrial sewage wastewater (11).

Despite having any metabolic function in higher plants, cadmium is a highly toxic and nonessential heavy metal (12). It has the potential to go into the food chain and harm the biota (13) and (14) also found that trace metals (TM) influence public health and the ecological system. Toxic concentrations of Cd in plants obstruct plant growth, and its production and disrupt seedling physiological systems that ultimately decrease crop output in peas (15). The impact of Cd on peas and cowpea seeds was related to a lack of water absorption, limiting the amount of water available for seed embryo development (16).

Cd stress significantly reduces the growth and development of bean seedlings (17). Different peas genotypes have been evaluated under Cd toxicity to evaluate Cd-resistant genotypes (18). Another effort was made to evaluate peas genotypes (Hurst Green and Kelvedon Wonder) against Cd toxicity. It was determined that Kelve-don Wonder was tolerant to Cd toxicity (19). Generally, it has been discovered that peas are significantly affected by Cd toxicity (20), after Cd exposure at germination and seedling stage (21).

Keeping in view above scenario the current study aimed at the evaluation of cultivars and breeding lines under cadmium toxicity, at seedling stage in screenhouse. Determination of cadmium concentration helped to identify genotypes with better performance in cadmium toxicity.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1 Experimental Site and Treatments

This experiment was conducted in the greenhouse of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. The experiment was carried out in a completely randomized design with five replicates in winter 2020-21. 500 ml plastic jars were filled with 500 mg sand to sow pea genotypes. Ten peas genotypes (TRIPLET, MID No. 2, Oskar, LM (OSE), METEOR, P-1, Rohina, PEA-09, LM-POIS, and LAMPO) were treated with 3 levels of Cadmium Nitrate (Cd(NO3)2) in solution form after 15 days of sow-ing:

1) 0 mg Cd/kg Soil (T0) (Control)

2) 4 mg Cd/kg Soil (T1)

3) 8 mg Cd/kg Soil (T2)

2.2 Morphological Analysis

The experiment was carried out for 30 days. Five seedlings from each genotype per treatment per replication were selected and the mean was calculated. Data was recorded after two weeks of Cd application. Data were recorded for the following morphological traits: seed length (cm), root length (cm), fresh shoot weight (g), fresh root weight (g), dry shoot weight (g), and dry root weight (g).

2.3 Biochemical Analysis

For this purpose, 0.25g of oven-dried samples were added into 5ml HNO_3 and $HClO_4$ solution (3:1). Prepared material was kept in the chamber overnight and then heated on a hot plate (350℃) until the solution volume remained 1ml. Now, the solution turned from dark yellow color to colorless. The Solution was poured out into a new flask having 50ml volume by filtering the solution with filter paper (What's man No.1). 50ml double distilled water was added to the samples which were ready to be used for Cd analysis in Atomic Ab-sorption Spectrometer.

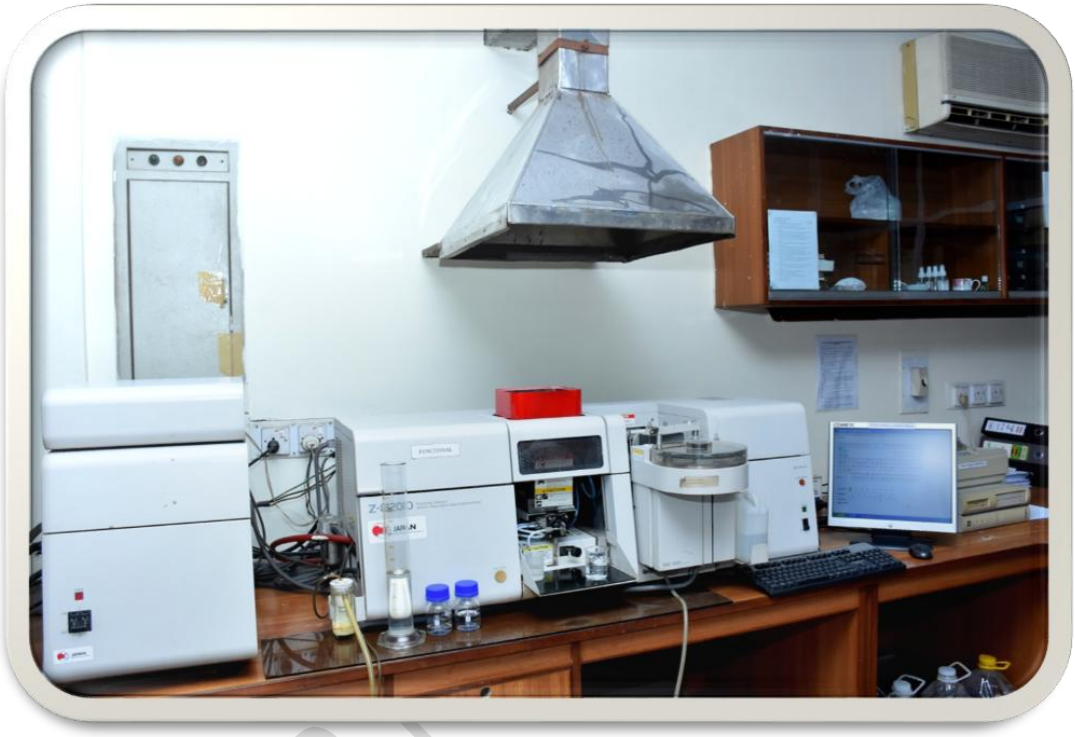

Figure 1: Picture of Hitachi Polarized Zeeman Atomic Absorption Spectrometer, Z-8200, Japan New

2.4 Statistical Analysis

Collected data were subjected to analysis of variance and Tuckey HSD Test for evaluation of significant differences among treatments (22). Analysis of variance and Tukey HSD test were per-formed by using Statistix 8.1 software. Bio-absorption co-efficient (BAC), Bio-concentration Factor (BCF), and Translocation Factor (TF) were calculated by following formulas given by (23).

BAC = (Cd in shoot)/(Cd in soil)

BCF = (Cd in root)/(Cd in soil)

TF = (Cd in shoot)/(Cd in root)

3. RESULTS AND DISCUSSION

Statistical analysis revealed that there were substantial variations among varieties and treatments. An increased level of cadmium was observed in root and seedlings by increasing

the level of Cd in the soil. All genotypes responded differently to Cd application for all measured morphological and biochemical attributes.

3.1 Morphological Analysis

Generally, the fresh root weight of all genotypes was decreased by the application of Cd at 4 ppm, except TRIPLET and MID No. 2. The maximum root weight was recorded in LM (OSE) (2.66 g) when grown under Cd stress at 8 ppm. While METEOR had the lowest root weight (0.40 g) un-der Cd stress at 4 ppm. When Cd stress was increased up to 8 ppm the fresh root weight of almost all genotypes was increased compared to 4 ppm Cd stress. The root length of LM-POIS (27.66 cm) was maximum in Cd stress at 8 ppm, among the root lengths of all genotypes in all treatments. Cd stress at 4 ppm significantly reduced the root length of peas genotypes while Cd stress at 8 ppm comparatively increased the root length. The lowest root length was recorded in LAMPO (7.5 cm) and METEOR (7.67 cm) under 4 ppm Cd stress. There was a significant reduction in the dry root weight of all genotypes by Cd application but a great improvement in the dry root weight of Oskar and LM (OSE) in Cd stress at 8 ppm. LM-POIS (0.93g) had maximum dry root weight and the lowest dry root weight was noted in METEOR (0.12 g). There was a significant difference in fresh seedling weight of all genotypes in all three treatments. The maximum fresh seed-ling weight was recorded in P-1 (3.13 g) in control, on the other hand, the least fresh seedling weight was measured in LAMPO (0.65 cm) in 4 ppm Cd stress. There was also variation in fresh seedling weight in response to Cd stress at 8 ppm for all genotypes. The seedling length of all genotypes was greatly reduced by Cd application as com-pared to the control treatment. The seedling length of Oskar (42.67 cm) was maximum in the control condition than all genotypes in all treatments while the lowest seedling length was measured in LAMPO (8.25 cm) in response to Cd stress at 4 ppm. Dry seedling weight was generally reduced under Cd stress, but 4 ppm application of Cd had more severe effects on dry seedling weight of all genotypes. Maximum dry seedling weight was measured in P-1 (0.91 g) in control conditions than dry seedling weights of all genotypes in all treatments and LAMPO (0.8 g) had the lowest dry seedling weight. Results are summarized in Table 1-6.

Table 1: LSD all pairwise comparisons for fresh root weight of different peas genotypes grown under different Cd levels

Genotypes	Control	Cd @ 4 ppm	Cd @ 8 ppm	Mean
TRIPLET	0.750 kl	0.966 g-j	1.150 e-g	0.955 C
MID No. 2	1.266 de	1.866 c	1.866 c	1.666 A
Oskar	1.166 ef	0.900 i-k	2.400 b	1.488 B
LM (OSE)	1.066 f-i	0.966 g-j	2.666 a	1.566 AB
METEOR	1.133 e-h	0.400 m	1.300 de	0.944 CD
P-1	1.133 e-h	0.700 l	0.966 g-j	0.933 CD
Rohina	2.333 b	1.066 f-i	1.366 d	1.588 AB
PEA-09	0.966 g-j	0.633 l	0.900 i-k	0.833 D
LM-POIS	1.800 c	0.966 g-j	1.900 c	1.555 AB
LAMPO	0.966 g-j	0.800 j-l	0.950 h-j	0.905 CD
Mean	1.258 B	0.926 C	1.546	

Lettering shows significant differences among genotypes and treatments

Table 2: LSD all pairwise comparisons for dry root weight of different peas genotypes grown under different Cd levels Lettering shows significant differences among genotypes and treatments

Genotypes	Control	Cd @ 4 ppm	Cd @ 8 ppm	Mean
TRIPLET	0.230 lm	0.133 n	0.260 kl	0.207 G
MID No. 2	0.476 g	0.613 e	0.713 d	0.601 A
Oskar	0.373 h	0.283 jk	0.816 c	0.491 D
LM (OSE)	0.260 kl	0.300 jk	0.883 ab	0.481 D
METEOR	0.280 jk	0.123 n	0.370 h	0.257 F
P-1	0.316 ij	0.206 m	0.360 hi	0.294 E
Rohina	0.870 b	0.256 kl	0.446 g	0.524 C
PEA-09	0.310 j	0.230 lm	0.230 lm	0.256 F
LM-POIS	0.926 a	0.223 lm	0.563 f	0.571 B
LAMPO	0.303 jk	0.200 m	0.193 m	0.232 FG
Mean	0.434 B	0.257 C	0.483 A	

Lettering shows significant differences among genotypes and treatments

Table 3: LSD all pairwise comparisons for root length of different peas genotypes grown under different Cd levels

Genotypes	Control	Cd @ 4 ppm	Cd @ 8 ppm	Mean
TRIPLET	16.500 hi	16.333 hi	14.000 jk	15.611 C
MID No. 2	14.333 j	12.667 lm	18.333 ef	15.111 CD
Oskar	17.333 f-h	12.333 m	17.333 f-h	15.667 C
LM (OSE)	16.667 hi	12.000 mn	24.667 b	17.778 B
METEOR	13.667 j-l	7.667 q	13.000 k-m	11.444 F
P-1	16.667 hi	10.333 o	16.667 hi	14.556 D
Rohina	18.000 e-g	12.667 lm	15.667 i	15.444 C
PEA-09	17.000 gh	9.000 p	11.000 no	12.333 E
LM-POIS	19.667 cd	19.000 de	27.667 a	22.111 A
LAMPO	20.333 c	7.500 q	11.000 no	12.944 E
Mean	17.017 A	11.950 B	16.933 A	

Lettering shows significant differences among genotypes and treatments

Table 4: LSD all pairwise comparisons for the fresh seedling weight of different peas genotypes grown under different Cd levels

Genotypes	Control	Cd @ 4 ppm	Cd @ 8 ppm	Mean
TRIPLET	0.933 mn	1.200 jk	0.866 n	1.000 F
MID No. 2	2.033 cd	1.200 jk	1.433 h	1.555 D

Oskar	1.366 h-j	0.966 l-n	1.133 kl	1.155 E
LM (OSE)	2.300 b	1.166 k	1.666 fg	1.711 C
METEOR	2.333 b	1.166 k	1.433 h	1.644 CD
P-1	3.133 a	1.433 h	1.533 gh	2.033 A
Rohina	2.200 bc	1.766 f	1.766 f	1.911 B
PEA-09	2.166 bc	1.233 i-k	1.400 hi	1.600 D
LM-POIS	1.833 ef	1.966 de	0.900 n	1.566 D
LAMPO	1.166 k	0.650 o	1.100 k-m	0.972 F
Mean	1.946 A	1.323 B	1.275 B	

Lettering shows significant differences among genotypes and treatments

Table 5: LSD all pairwise comparisons for dry seedling weight of different peas genotypes grown under different Cd levels

Genotypes	Control	Cd @ 4 ppm	Cd @ 8 ppm	Mean
TRIPLET	0.450 gh	0.310 j-m	0.240 m-o	0.333 E
MID No. 2	0.676 cd	0.276 k-n	0.420 hi	0.457 D
Oskar	0.640 d	0.276 k-n	0.460 gh	0.458 D
LM (OSE)	0.686 cd	0.230 mno	0.506 fg	0.474 CD
METEOR	0.543 ef	0.266 lmn	0.470 fgh	0.426 D
P-1	0.913 a	0.283 k-n	0.366 ij	0.521 BC
Rohina	0.770 b	0.640 d	0.500 f-h	0.636 A
PEA-09	0.726 bc	0.350 i-k	0.323 j-l	0.466 D
LM-POIS	0.623 de	0.733 bc	0.216 no	0.524 B
LAMPO	0.290 j-n	0.180 o	0.265 lmn	0.245 F
Mean	0.632 A	0.354 B	0.376 B	

Lettering shows significant differences among genotypes and treatments

Table 6: LSD all pairwise comparisons for seedling length of different peas genotypes grown under different Cd levels

Genotypes	Control	Cd @ 4 ppm	Cd @ 8 ppm	Mean
TRIPLET	29.500 c	14.333 mn	16.500 i-k	20.111 E
MID No. 2	38.667 b	17.333 h-j	15.333 k-m	23.778 B
Oskar	42.667 a	21.333 fg	23.333 e	29.111 A
LM (OSE)	40.000 b	13.667 no	15.000 k-n	22.889 BC
METEOR	27.000 d	17.667 hi	15.333 k-m	20.000 EF
P-1	28.333 cd	20.667 g	17.333 h-j	22.111 CD
Rohina	23.333 e	18.667 h	15.333 k-m	19.111 F
PEA-09	23.000 e	16.000 j-l	14.667 l-n	17.889 G
LM-POIS	28.667 c	12.667 op	22.667 ef	21.333 D
LAMPO	11.667 p	8.250 q	12.500 op	10.806 H
Mean	29.283 A	16.058 C	16.800 B	

Lettering shows significant differences among genotypes and treatments

3.2 Biochemical Analysis

For the mean comparison of genotypes, Cd concentration (5.90 ppm) in the root of Mid No. 2 was the highest while the lowest Cd concentration (4.25 ppm) was observed in the root of LM (OSE). By 4 ppm Cd application the lowest mean value (2.00 ppm) of Cd concentration was in the root of LM (OSE) whereas the highest Cd concentration was observed in the roots of Mid No. 2 (3.96) and LAMPO (3.83). TRIPLET (7.86 ppm) and Mid No. 2 (7.83) accumulated the highest Cd concentration under Cd stress at 8 ppm while the least Cd was observed in the roots of LM-POIS (5.23 ppm). Under Cd stress at 4 ppm Cd concentration in the shoot of LM (OSE) was maximum (4.00 ppm) while the least mean value was observed in Mid No. 2 (2.33 ppm). Under Cd stress at 8 ppm Cd concentration in shoot was maximum in LM-POIS (7.83 ppm). The lowest concentration was observed in Oskar (4.17 ppm) and P-1 (4.17 ppm). When Cd was applied at 4 ppm the average Cd concentration in soil samples was 3.77 ppm while those samples collected from soil treated with Cd at 8 ppm had 7.55 ppm. Results are summarized in Table 7-9.

Table 7: LSD all pairwise comparisons for Cd concentration in root of different peas genotypes grown under different levels of Cd stress

Genotypes	Cd @ 4 ppm	Cd @ 8 ppm	Mean
TRIPLET	3.67 fg	7.87 a	5.76 AB
MID No. 2	3.97 f	7.83 a	5.90 A
Oskar	2.67 h	7.70 a	5.18 CD
LM (OSE)	2.00 i	6.50 cd	4.25 E
METEOR	3.47 g	6.96 bc	5.21 CD
P-1	3.53 fg	7.66 a	5.60 AB
Rohina	3.56 fg	5.37 e	4.46 E
PEA-09	3.73 fg	6.16 d	4.95 D
LM-POIS	3.36 g	5.23 e	4.30 E
LAMPO	3.83 fg	7.16 b	5.50 BC
Mean	3.38 B	6.8467 A	

Lettering shows significant differences among genotypes and treatments

Table 8: LSD all pairwise comparisons for Cd concentration in shoot of different peas genotypes grown under different levels of Cd toxicity

Genotypes	Cd @ 4 ppm	Cd @ 8 ppm	Mean
TRIPLET	3.67 g	4.23 ef	3.95 DE
MID No. 2	2.33 h	6.17 bc	4.25 D

Oskar	3.67 g	4.16 ef	3.91 E
LM (OSE)	4.03 fg	4.30 ef	4.16 DE
METEOR	3.67 g	6.46 b	5.06 B
P-1	3.63 g	4.17 ef	3.90 E
Rohina	3.93 fg	6.00 c	4.97 B
PEA-09	3.93 fg	4.53 e	4.23 D
LM-POIS	3.67 g	7.83 a	5.75 A
LAMPO	3.86 fg	5.43 d	4.65 C
Mean	3.64 B	5.33 A	

Lettering shows significant differences among genotypes and treatments

Table 9: LSD all-pairwise comparisons test of Cd concentration in soil

Trt	**Mean**	**Homogeneous Groups**
T1	3.7778	B
T2	7.5556	A

Lettering shows significant differences among genotypes and treatments

3.3 Quality Control Analysis

The quality control analysis of peas genotypes was observed by calculating BAC, BCF, and TF. The BAC in LM (OSE) (1.07), Rohina (1.04), PEA-9 (1.04), and LAMPO (1.02) was increased than 1 under Cd stress at 4 ppm, and BAC of Cd in LM-POIS increased than 1 under Cd stress at 8 ppm. BCF for Cd in MID-No 2 (1.05) and LAMPO (1.01) was also elevated than 1 under 4 ppm Cd stress while BCF in TRIPLET (1.04), MID-No 2 (1.04), Oskar (1.02) P-1 (1.01) was the indicator of Cd uptake from soil to root in Cd stress at 8 ppm. TF under Cd stress at 4 ppm increased than 1 in almost genotypes except for MID-No.2. Surprisingly TF for Cd in LM(OSE) was 2.02, which was the highest value of TF for Cd in all genotypes under both treatments. Results are summarized in Table 10.

Table 10: Bio-absorption Coefficient, Bio-concentration factor and translocation factor for Cd in different peas genotypes grown under different levels of Cd stress

	Bio-absorption Coefficient		Bio-concentration factor		Translocation factor	
Genotypes	T1	T2	T1	T2	T1	T2
TRIPLET	0.97	0.56	0.97	1.04	1.00	0.53
MID No.2	0.61	0.81	1.05	1.03	0.58	0.78
Oskar	0.97	0.55	0.70	1.01	1.37	0.54

LM(OSE)	1.06	0.56	0.52	0.86	2.01	0.66
METEOR	0.97	0.85	0.91	0.92	1.05	0.92
P-1	0.96	0.55	0.93	1.01	1.02	0.54
Rohina	1.04	0.79	0.94	0.71	1.1	1.11
PEA-09	1.04	0.60	0.98	0.81	1.05	0.73
LM-POIS	0.97	1.03	0.89	0.69	1.08	1.49
LAMPO	1.02	0.71	1.01	0.94	1.00	0.75

Discussion

The root weight of peas genotypes greatly de-creases due to Cd stress (24-26) because Cd hinders the availability of other nutrients which were important for root growth (27). Surprisingly, it was observed in our study that the root growth of peas genotypes increased by increasing the Cd level by more than 4 ppm in the soil. Hence, Cd was applied in combination with nitrate, this could also be the reason for enhanced root growth due to a sufficient supply of nitrogen (28). The other reason could be the struggle of peas plants for survival under elevated Cd stress. As a result of high level of cadmium stress, roots triggered itself under mechanism of stress resistance to get nutrients from soil that were hindered due to high application of Cd. Opposite results to our study were observed from other researchers as recently reduced peas root length was reported under Cd stress (29). A similar phenomenon was observed in the root length of almost all genotypes that Cd stress up to 4 ppm greatly reduced root length, but it was comparatively increased at 8 ppm Cd stress. (30) found reduced seedling weight when they subjected peas elevated Cd levels. The current study also supported the previous ones for decreased seedling weight with increased Cd level. (31) also reported decreased root and shoot dry weight in peas upon Cd application. In line with our findings, Cd also reduced the growth of other plant species (32-35). In this regard, Cd stress inhibits root and stem cell growth by inhibiting cell elongation and cell division via an inhibition activity of the glycolysis pathway. Peas genotypes whose seedling weight was less affected by Cd stress can be selected for further studies to include in the next breeding programs to make Cd tolerant genotypes.

When the soil was polluted with Cd (36-37) then grown vegetables had a higher accumulation of Cd in their parts (38). (39-42) reported elevated level of Cd in shoot of those plants grown under Cd rich soil. In the case of peas, a considerable reduction in the growth was also observed associated with increased Cd accumulation in the root and shoot (43). Peas mutants and *Brassica juncea* accumulated higher Cd in their shoot when grown under elevated levels (44). Those peas genotypes that enhanced their growth by stimulating more response to nitrogen availability could also be used for phytoremediation if they could be harvested at the seedling stage. The increased values of quality control indicators of more than 1 could suggest the higher uptake of Cd in respective parts of the plant. BAC indicated the transfer of Cd from the soil to the shoot of peas genotypes while BCF determined the transference of Cd from soil to root. TF explained the transfer of Cd from root to the shoot of peas genotypes. These are the quality control indicators and determine if the plant is storing

metal in root or shoot. Those genotypes which have higher TF than 1, are considered to accumulate higher Cd in their shoot while those that have TF less than 1, have stored Cd in their roots.

Previously it has been determined that Cd adversely affects plant health (45) so, this study is the extension to suggest the negative effects of Cd on peas growth. Variation in response to Cd stress by all genotypes is also an important aspect of the study to suggest the genotypes for further studies. So, those genotypes which accumulated higher Cd and transferred to their shoot can be considered very important in the next studies to develop a breeding program in consideration of phytoremediation of Cd polluted soils.

4. CONCLUSION

10 peas genotypes were evaluated under two Cd stressed treatments (4 ppm and 8 ppm) and one control condition (0 ppm). There was a great variation in the data for all observed traits as every genotype differently responded to Cd application. Biochemical analysis of root, shoot and soil samples revealed that there was an elevated level of Cd in all samples when Cd applied in the soil but there was also variation in the data of Cd concentration in plant parts of each genotype because some genotypes might have tolerance at seedling stage against elevated Cd level. All morphological and biochemical analysis revealed the negative impact of Cd on both soil and peas genotypes. So, it is suggested to remediate Cd polluted soils and also develop Cd tolerant peas genotypes in aspect of increasing heavy metal stress in soils.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki

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