Effect of heavy metals in the cement dust pollution on morphological and anatomical characteristics of *Cenchrus ciliaris* L.

T. Al faifi, A. El-Shabasy

**Biology Department, Faculty of Science, Jazan University, Saudi Arabia**

**Abstract**

This present study illustrated the effect of heavy metals in cement dust pollution on *Cenchrus ciliaris* L. This wild plant species spread surrounding and within the contaminated area. The induced soil by cement dust was changed physically and chemically. There were alterations in morphological characters like chlorotic spot, stem shortening and leaf curling. Similarly, anatomical alterations appeared obviously like rupturing and thickening of cells. The data of control and induced plant species were analyzed statistically separately and combined respectively. The regression equations represented the interaction between control and induced plant species graphically. *Cenchrus ciliaris* L. can be regarded as a standard heavy metal tolerant plant species.

**Keywords:**
- Soil
- Chlorosis
- Leaf curling
- Thickening
- Metaxylem
- Regression

1. Introduction

*Cenchrus ciliaris* L. (buffel grass) (nomenclature according to Albrecht and Pitts, 1997) is a robust, summer growing, deep rooted, C4 perennial tussock halophytic grass (Hall, 2001; Zamin et al., 2016). It’s native to Africa, India, the Middle East and western Asia (Humphreys, 1967). It’s well adapted to a wide range of soils and the climate of arid and semi-arid regions because of its high tolerance to drought, salt stress, capacity to withstand heavy grazing, a deep stabilizing root system and responds quickly to rainfall events (Marshall et al., 2012). It can rehabilitate the industrial, degraded and mined land and support many rural communities and pastoral industries (Grigg et al., 2000) because it is a strong competitor, displace the native flora and fauna, less affected by tropical diseases, produce more biomass than many native perennial grass species, and its high seed yields and light fluffy seed allow it to spread readily via wind and water (Akiyama & Hanna, 2005).

Rapid industrialization and addition of the toxic substances to the environment are responsible for altering the ecosystem. The cement industry also plays a vital role in the imbalances of the environment and produces air pollution hazards (Malakootian et al., 2009).

Air pollution is a social disease which generated primarily from the activities of man adversely affected his health and welfare. Pollution stress can alter plant growth and quality and the effects are often extensive. In relatively recent times, the total amount and complexity of toxic pollutants in the environment are increased day by day (Muhammad and Muhammad, 2001). Air pollutants are responsible for vegetation injury and crop yield losses. Air pollution has become a major threat to the survival of all plants in the industrial areas (Gupta & Mishra, 1994).

Cement industry is one of the 17 most polluting industries listed by the Central Pollution Control Board (CPCB) (Rajajsubramanian et al., 2011). The fly ashes of cement dust comprise incompletely burned coal and a large number of heavy metals (Thangarasu, 2002). They are like cobalt, nickel, lead, silicon and chromium which are well-known as pollutants hazardous to the biotic environment with greatly impact for vegetation, animal and human health (Darweesh & El-Sayed, 2014). The recent scenario of large scale deforestation and damage of several ecosystem components are being recognized as the effect of cement dust pollution (Grantz et al., 2003).
This study evaluates the potential of *Cenchrus ciliaris* L. as a heavy metal tolerant species and focused on its Morpho-Anatomical characterizations as the response to cement dust pollution.

2. Material and methods

2.1. The study area

This study was performed near the cement dust factory in Jazan (originated in 30 October 1981) which is situated at Ahd-El-Masarha 70 km far southwest Jazan city, Kingdom of Saudi Arabia at 16°44′07.5″N latitude and 43°03′03.7″E longitude and is about 132 m above sea level. This area was chosen for the plenty of limestone which is regarded as the main component for cement industry. The cement kiln dusts was made of a complex mixture of heavy metals including F, Mg, Pb, Cd, Zn, Cu, Fe, Mn and Be. The area around the factory is mainly plain, interspersed with several small hills. The soil of the area is sandy loam in texture. The region has a subtropical climate type. The climate is affected according to the position on the Red Sea where the temperature reaches in January to the highest level nearly 31 °C while decreases to the lowest level nearly 22 °C. In July the average temperature reaches nearly 33 °C.

The rate of relative humidity reaches in January and August reaches 74% and 66% respectively where the average annual humidity, temperature and rainfall are 68%, 30.4 °C and 397.7 mm respectively. There are some wades which recognized around the factory is mainly plain, interspersed with several small hills. The soil of the area is sandy loam in texture. The region has a subtropical climate type. The climate is affected according to the position on the Red Sea where the temperature reaches in January to the highest level nearly 31 °C while decreases to the lowest level nearly 22 °C. In July the average temperature reaches nearly 33 °C.

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2.2. The study area partition and delimitation

This study was carried out in 9 October 2019 as presence of rain fall at the end of September 2019 to obtain large number of wild plant species. The study area was partitioned and delimited to six different sites; three near to the cement factory which located at the distances of nearly 4000 m (induced sites) and the others far from it as there was no evidence of dust fall on them (control sites). These sites were chosen according to (Khalid, 2020) (Map 1).

2.3. Plant and soil sampling

The plant and soil samples were collected from the same points in all six different sites. At each site, six to nine bushes of *Cenchrus ciliaris* L. were tagged for sampling. They were transported to the laboratory in porous nylon bag with spraying little water to be almost fresh for analyze macro and micro differences between control and induced plant specimens.

Specimens’ morphology and symptoms were investigated by the 7–14 × magnification using an Olympus stereomicroscope. Measurements for length, width and number were done for 8 replicates using a ruler, a microscopic micro-ocular lens, and a digital caliper while anatomical measurements were determined by the ocular micrometer. The area of metaxylem elements was determined by the sum of areas of three larger diameter metaxylem elements in the vascular bundle.

For anatomical characterizations, Plant samples were prepared according to the method as adopted by Johansen (1968). The specimens were dipped into 70% ethanol solution for 15 days after that transverse thin sections (50 μm thick) were done on a Vibratome Zeiss MM France and fixed in Canada balsam. At the fourth node, the stems and leaves were selected while the roots were cut at the distance of 5 cm from the top for section. Double staining dehydration procedure (safarin and fast green) was used for the preparation of permanent slides (Ruzin, 1999) to study various cells and tissues of root, stem and leaf. Measurements and micrographs were made using a digital camera (Nikon FDX-35) equipped with a Nikon stereo-microscope (Nikon 104, Japan).

The soil samples were also collected along with plant samples from all sites up to the depth of (10–20 cm). The soil was sieved, acid washed, distilled water washed and stored in a plastic bag at room temperature being used for analysis.

The soil pH was measured using a digital pHmeter (with a pH reader model (Model Jenway PHM 6) in (1:2.5) soil to water ratio, electrical conductivity (EC), total dissolved salts (TDS), water holding capacity (W.H.C.), organic matter were performed (Wilde et al., 1979).

2.4. Statistical analysis

Each morphological and anatomical data for both induced and control specimens were examined for the descriptive statistical analysis: mean ± SD, geometric mean, harmonic mean, median, mode, range, interquartile range, variance, skewness and kurtosis (Machado et al., 2009).

Coefficient of correlation, coefficient of determination, significance test value, standard error slope value and F-test were used to test the difference between control and induced variables (Shaban, 2005; Tamhane, 2009; Welham et al., 2015; ter Braak and Šmilauer, 2002). The representation of statistical data was done by the linear regression approaches which explored the extent effect for both variables (Maimon, 1992; Miller and Franklin, 2002). P values for significance tests based on degrees of freedom were determined according to Dutilleul’s (1993) approach.

3. Results

3.1. Soil analysis

The induced soil exhibited higher Electric Conductivity (EC) and Total Dissolved Salts (TDS) than control one. This increment leaded to shift pH from 4.8 to 5.94. Water Holding Capacity was also increased but Organic matter (O.M) was decreased due to the high osmotic potential of dust cement particles (Table 1).

3.2. Morphological analysis

The suppressed root was dark in color. There was a reduction in root growth. Number of Root fibers was lesser than control one besides the volume of the soil captured was also decreased. The induced roots were heterogeneous aggregated and scattered on the other hand, the control roots were homogenous aggregated and fluffy texture.

Diameter of the suppressed stem section was wider and the articulation of the stem was lesser than normal one. There were thickening and shortening of stems besides inflorescence lengths. The potential alterations of the induced stem were not assigned widely like root and leaf.

Leaf curling was judged by reduced flexibility of the leaf blade. There was an impairment of leaf development. The emergence of leaves was reduced. Dramatic reduction of leaf surface area and number were observed. Chlorosis at the upper part of shoot system was developed (Photo 1) (Tables 2 and 3).

According to the statistical analysis of morphological traits, the variance of induced parameters were less than 1 except stem...
length (4.411) while the variance of control ones were more than 1 except inflorescence length, leaf wide, number of root fibers and root length which measured 0.496, 0.018, 0.857 and 0.517 respectively. Kurtosis analysis for control parameters showed negative values except leaf number and root length which measured 0.222 and 0.18 respectively while induced parameters showed also negative ones except leaf wide (1.872). (Tables 2 and 3).

Coefficient of correlation between control and induced parameters showed negative values except stem length and leaf wide which measured 0.358 and 0.084 respectively. The highest coefficient of determination was expressed by leaf length (33.097%) while the lowest one was 0.714% for leaf wide. Each two variables had more or less the same $F$-test; inflorescence length and number of root fibers were 1.7619 and 1.5484; stem length and leaf number were 2.6194 and 2.4359; leaf length and leaf wide were 5.2500 and 5.0506. Simple linear regression (SLR) equations represented the data analysis as a scattered plot graph which was regarded as exponential curves for stem length and leaf wide while inversely exponential for other parameters (Table 4) (Fig. 1).

3.3. Anatomical analysis

In induced roots, epidermis and cortex were easy to be ruptured due to cell wall damage. Aggregation of xylem vessel elements was more obvious. There were more vascular bundles and layers of collenchyma. The deformed and shrunken epidermis was considerably compensated by widening and increasing of

| Table 1 | Characterization of control and induced soil. |
|---------|---------------------------------------------|
| Parameters | Control soil | Induced soil |
| pH     | 4.8 ± 0.25 | 5.94 ± 0.30 |
| EC (ms cm$^{-1}$) | 4.93 ± 3.50 | 123.7 ± 4.10 |
| TDS (ppm) | 4.65 ± 0.05 | 92.1 ± 0.09 |
| Organic matter (%) | 25 ± 0.03 | 5 ± 0.08 |
| W.H.C. (%) | 18 ± 1.01 | 45 ± 0.85 |

| Table 2 | Statistical analysis for morphological parameters of control Cenchrus ciliaris L. measured in cm. |
|---------|---------------------------------------------|
| Control | Mean ± SD | Geometric mean | Harmonic mean | Median | Mode | Range | Interquartile range | Variance | Skewness | Kurtosis |
| Stem length | 84.125 ± 3.399 | 84.065 | 84.006 | 84.0 | 0 | 80–90 | 5.75 | 11.554 | 0.411 | −0.166 |
| Leaf length | 22.875 ± 2.031 | 22.794 | 22.71 | 23.5 | 0 | 20–25 | 4.25 | 4.125 | −0.595 | −1.249 |
| Inflorescence length | 8.188 ± 0.704 | 8.16 | 8.133 | 8.25 | 9 | 7–9 | 1.25 | 0.496 | −0.48 | −0.56 |
| Leaf wide | 1.069 ± 0.133 | 1.062 | 1.055 | 1.05 | 1.1 | 0.9–1.3 | 0.21 | 0.018 | 0.606 | −0.348 |
| Leaf number | 55.625 ± 1.302 | 55.612 | 55.599 | 55 | 55 | 54–58 | 1.75 | 1.696 | 0.929 | 0.222 |
| Number of root fibers | 26 ± 0.936 | 25.986 | 25.971 | 26 | 0 | 25–27 | 2 | 0.857 | 0 | −2.1 |
| Root length | 19.344 ± 0.719 | 19.332 | 19.32 | 19.5 | 20 | 18–20 | 1.19 | 0.517 | −0.934 | 0.18 |
Statistical analysis interaction for morphological parameters between both control and induced plant species.

### Table 3

Statistical analysis for morphological parameters of induced *Cenchrus ciliaris* L. measured in cm.

| Parameter               | Mean ± SD | Geometric mean | Harmonic mean | Median | Mode | Range | Interquartile range | Variance | Skewness | Kurtosis |
|-------------------------|-----------|----------------|---------------|--------|------|-------|---------------------|----------|----------|----------|
| Stem length             | 78.125 ± 2.1 | 78.1           | 78.075        | 78.5   | 80   | 75–80 | 3.75                | 4.411    | -0.341   | -1.932   |
| Leaf length             | 15.75 ± 0.886 | 15.729         | 15.707        | 15.5   | 0    | 15–17 | 1.75                | 0.786    | 0.615    | -1.481   |
| Inflorescence length    | 6.188 ± 0.53 | 6.167          | 6.147         | 6.25   | 6.5  | 5.5–7 | 0.88                | 0.281    | -0.045   | -0.94    |
| Leaf wide               | 0.319 ± 0.059 | 0.313          | 0.307         | 0.325  | 0.35 | 0.2–0.4 | 0.05                | 0.004    | -0.97    | 1.872    |
| Leaf number             | 25.875 ± 0.835 | 25.863         | 25.852        | 26     | 0    | 25–27 | 1.75                | 0.696    | 0.277    | -1.392   |
| Number of root fibers   | 15.625 ± 0.744 | 15.61          | 15.595        | 15.5   | 0    | 15–17 | 1                  | 0.554    | 0.824    | -0.152   |
| Root length             | 15.563 ± 0.395 | 15.558         | 15.554        | 15.625 | 0    | 15–16 | 0.81                | 0.156    | -0.542   | 1.024    |

**Table 4**

Statistical analysis interaction for morphological parameters between both control and induced plant species.

| Parameter               | Coefficient of correlation | Coefficient of Determination | Significance test value | Standard error slope value | F-test | P-value |
|-------------------------|----------------------------|------------------------------|-------------------------|---------------------------|--------|---------|
| Stem length             | 0.358                      | 12.796%                      | 0.938                   | 0.236                     | 2.619  | 0.227   |
| Leaf length             | -0.575                     | 33.097%                      | -1.723                  | 0.146                     | 5.250  | 0.0439  |
| Inflorescence length    | -0.490                     | 24.038%                      | -1.378                  | 0.268                     | 1.7619 | 0.4724  |
| Leaf wide               | 0.084                      | 0.714%                       | 0.208                   | 0.181                     | 5.0506 | 0.0486  |
| Leaf number             | -0.312                     | 9.744%                       | -0.805                  | 0.249                     | 2.4359 | 0.2630  |
| Number of root fibers   | -0.316                     | 10.008%                      | -0.817                  | 0.345                     | 1.5468 | 0.5782  |
| Root length             | -0.212                     | 4.489%                       | -0.532                  | 0.219                     | 3.3071 | 0.1372  |

For coefficient of correlation, all values were negative except for epidermis of root, metaxylem of stem, mesophyll and metaxylem of leaf. Moreover, the highest coefficient of determination was 47.98% for cortex of root while the lowest one was 0.119% for epidermis of leaf. According to F-test, metaxylem of root revealed the greatest value 136.1780 so metaxylem of root can be assumed to be the most remarkable efficient parameter because it was more repetitive effluent statistical factor in this study. All graphs of plotted points for the relationships between control and induced anatomical variables were assigned by the regression lines (Table 7) (Fig. 2).

### 4. Discussion

Ion exchange and retention processes were established accurately in induced soil. Enrichment in mineral content encouraged more ion competition, combination and reaction through enforces physiological and metabolic changes. The heavy metals may be regarded as analogues with the same ion radius or electric charges with iron and magnesium (Seyed et al., 2016). This interference and competition between ions may affect on iron and magnesium chelation and metabolism during root uptake (Zeng et al., 2011). Similar studies on cement dust pollution showed elevated levels of soil pH (Lerman, 1972; Mandre, 1997; Mandre et al., 1999). In addition, the heavy metal concentrations of cement dust can be enhanced by rhizosphere of *Cenchrus ciliaris* through causes more mineralization. Bioavailability of metals is enriched by chelators that are released in the rhizosphere where facilitates plant’s uptake (Pankaj and Madhusudan, 2018, Nazir et al. 2011) reported significant accumulation of different heavy metals in *Cenchrus* sp. growing in industrial contaminated zones according to the high potential of metabolic root exudates of sterols, such as phytol, cycloergost and β-tocopherol (Singariya et al., 2012; Khurshid et al., 2016).

The smaller volume of the soil which is captured by induced root reflects the lower phytomass production besides nutrition and water balance in the plant (Jiang et al., 2007). Higher susceptibility of root growth as compared with shoot growth has also been reported by other authors (Camacho and González, 1999).

All the induced stem changes were much less expressed compared to the induced root changes due to the biological filter of heavy metal ions (Gomes et al., 2011) resulting in better survival stem against subsequent acute stress (Krishnamurthy et al., 2011). Several authors had reported that the heavy metals have an effect on plants resulting in reduction of growth and phytomass accumulation (Marques et al., 2000; Sandalio et al., 2001).

Leaf curling can be a strategy to reduce the transpiration area on the surface, keeping stomata in a humid microclimate and thus preventing dryness (Turner and Jones, 1980). Emergence of leaves was reduced. It may be the result of the malformation of young developing leaves (El-shintinawy 1999; Hajboland and Farhanghi 2010). Chlorosis is a clear symptom appeared at shoot system under sever environmental condition. It is developed due to low photosynthetic process (Chugh and Sawhney, 1999; Prasad et al., 2001) where heavy metals are able to contribute to chlorophyll synthesis (Stobart et al., 1985), photosynthetic efficiency (Chugh and Sawhney, 1999), water balance (Zhou and Qiu, 2005) and Calvin cycle enzymes activity (Singh et al., 2006). The thickened epidermis of the adaxial and abaxial sides, absence of trichomes, decreasing in number of leaf vascular bundles and absence of krans anatomy minimized the water loss as an adaptive strategy to secure water flow (Melo et al., 2007; Marcelo et al., 2011).
It is worth noting that Variance reflects the general variation of each parameter (Sokal and Rohlf, 1995). According to morphological traits, majority of induced parameters had variance less than 1 that didn’t like control ones. Kurtosis is the degree of relative flattening or elevation of a distribution of different traits. A distribution is called leptokurtic if the curve has a relative high peak, with negative excess, i.e. a kurtosis coefficient <0.263; it is platykurtic if the curve has the top more flattened, with positive excess, i.e. a kurtosis coefficient >0.263; The intermediate curve is called mesokurtic, with kurtosis coefficient = 0.263 Ruppert (2011). In our morphological study, all control traits showed leptokurtic more than induced ones (Tables 2 and 3).

Correlation describes the co-variation between control and induced parameters (Maindonald, 1992). The stem length was
Table 5
Statistical analysis for anatomical parameters of control Cenchrus ciliaris L. measured in μm.

| Control       | Mean ± SD | Geometric mean | Harmonic mean | Median | Mode | Range | Interguartile range | Variance | Skewness | Kurtosis |
|---------------|----------|----------------|--------------|--------|------|-------|---------------------|----------|-----------|----------|
| Root Epidermis| 2.143 ± 0.283 | 2.127          | 2.112        | 2.033  | 1.965| 1.834–2.62 | 0.47    | 0.08      | 0.68     | −0.985   |
| Cortex        | 0.283 ± 0.250  | 4.529          | 4.522        | 4.588  | 4.585| 3.9–4.712 | 0.09    | 0.063     | −2.566   | 6.983    |
| Metaxylem     | 1.36 ± 0.080   | 1.313          | 1.311        | 1.31   | 1.31 | 1.179–1.441| 0.11    | 0.006     | −0.232   | 0.438    |
| Pith          | 4.938 ± 0.262  | 4.932          | 4.926        | 4.978  | 0   | 4.585–5.24 | 0.57   | 0.069   | −0.334   | −1.345   |
| Stem          | 21.626 ± 1.53  | 21.578         | 21.53        | 21.635 | 19.65| 23.58    | 3.00   | 2.341     | −0.191   | −1.66    |
| Ground tissues| 0.528 ± 0.114  | 0.517          | 0.506        | 0.512  | 0.655| 0.393–0.655| 0.25   | 0.013     | 0.088    | −1.94    |
| Leaf Epidermis| 0.599 ± 0.065  | 0.596          | 0.593        | 0.628  | 0.655| 0.524–0.655| 0.13   | 0.004     | −0.394   | −2.294   |
| Mesophyll     | 2.416 ± 0.283  | 2.407          | 2.383        | 2.56   | 2.62 | 1.965–2.62 | 0.53   | 0.08      | −1.241   | −0.332   |
| Metaxylem     | 0.209 ± 0.025  | 0.207          | 0.206        | 0.205  | 0   | 0.18–0.25 | 0.05   | 0.001     | 0.538    | −0.936   |

Table 6
Statistical analysis for anatomical parameters of induced Cenchrus ciliaris L. measured in μm.

| Induced       | Mean ± SD | Geometric mean | Harmonic mean | Median | Mode | Range | Interguartile range | Variance | Skewness | Kurtosis |
|---------------|----------|----------------|--------------|--------|------|-------|---------------------|----------|-----------|----------|
| Root Epidermis| 0.502 ± 0.099 | 0.493          | 0.485        | 0.524  | 0.524| 0.393–0.655 | 0.19    | 0.01      | 0.192    | −0.104   |
| Cortex        | 0.788 ± 0.070  | 0.785          | 0.783        | 0.786  | 0.786| 0.655–0.917 | 0.01    | 0.005      | −0.127   | −1.216   |
| Metaxylem     | 3.806 ± 0.934  | 3.705          | 3.603        | 3.93   | 0   | 2.62–5.42 | 1.55    | 0.873   | 0.27     | 0.049    |
| Pith          | 2.464 ± 0.186  | 2.458          | 2.446        | 2.495  | 2.62 | 2.2–2.7  | 0.36    | 0.035      | −0.361   | −1.372   |
| Stem          | 3.7 ± 0.223    | 3.694          | 3.688        | 3.75   | 3.93 | 3.275–3.93 | 0.36    | 0.05      | −1.013   | 0.684    |
| Ground tissues| 2.479 ± 0.125  | 2.476          | 2.474        | 2.45   | 0   | 2.358–2.62 | 0.26    | 0.016      | 0.244    | −2.322   |
| Leaf Epidermis| 1.354 ± 0.153  | 1.346          | 1.337        | 1.355  | 1.31 | 1.048–1.572| 0.13    | 0.023      | −0.908   | 2.05     |
| Mesophyll     | 4.155 ± 0.294  | 4.146          | 4.137        | 3.975  | 0   | 3.93–4.59 | 0.58    | 0.087      | 0.927    | −1.215   |
| Metaxylem     | 0.752 ± 0.106  | 0.74           | 0.695        | 0.695  | 0   | 0.655–0.917| 0.21    | 0.011      | 0.913    | −1.094   |

Table 7
Statistical analysis interaction for anatomical parameters between both control and induced plant species.

| Coefficient of correlation | Coefficient of Determination | Significance test value | Standard error slope value | F-test | P-value |
|---------------------------|------------------------------|-------------------------|----------------------------|--------|---------|
| Root Epidermis            | 0.114                        | 1.298%                  | 0.281                      | 0.142  | 8.1531  | 0.0129  |
| Cortex                    | −0.693                       | 47.980%                 | −2.352                     | 0.083  | 12.7323 | 0.0034  |
| Metaxylem                 | −0.346                       | 11.979%                 | −0.904                     | 4.470  | 136.178 | <0.0001 |
| Pith                      | −0.493                       | 24.290%                 | −1.804                     | 0.253  | 1.9726  | 0.3901  |
| Stem Ground tissues       | −0.331                       | 10.980%                 | −0.860                     | 0.056  | 47.1948 | <0.0001 |
| Metaxylem                 | 0.237                        | 5.604%                  | 0.597                      | 0.435  | 1.2020  | 0.8144  |
| Leaf Epidermis            | −0.035                       | 0.119%                  | −0.085                     | 0.964  | 5.5820  | 0.0372  |
| Mesophyll                 | 0.122                        | 1.483%                  | 0.301                      | 0.421  | 1.0788  | 0.9229  |
| Metaxylem                 | 0.670                        | 44.831%                 | 2.208                      | 1.272  | 17.0607 | 0.0012  |

Table 5
Statistical analysis for anatomical parameters of control Cenchrus ciliaris L. measured in μm.

Table 6
Statistical analysis for anatomical parameters of induced Cenchrus ciliaris L. measured in μm.

Table 7
Statistical analysis interaction for anatomical parameters between both control and induced plant species.

We found that leaf length and wide had the highest difference between values within each group while stem length, leaf number and root length had more or less difference but inflorescence length and number of root fibers had the lowest one. Simple linear regression (SLR) is suitable for representation of data analysis and describes the relationship between the response and the predictor variables in terms of control and induced ones. This is accomplished by applying the regression equation where presence/absence of an object is transformed into a continuous probability ranging from 0 to 1. The scattered probability points were discrete into a line graph between induced values on X axis and control values on Y axis (Miller and Franklin, 2002) (Table 4) (Fig. 1).
It has been found that the metal sequestration through the epidermis leaded to loss the elasticity of its cell walls which consequently were damaged or broken down because the cell wall was reported as a site of heavy metal allocation (Wójcik et al., 2005). Presence of more than one layer of collenchymatous tissue helped the hydraulic capacity and accumulated the heavy metals as an apoplastic barrier to flow in the opposite direction of vascular cylinder (Gonçalves et al., 2011). The cell degradation in the root accelerated the thickening of epidermis and endodermis through maturation of cell wall due to the oxidative effect of heavy metals (Raven et al., 2001). The stele diameter and its cell numbers were more decreased and thickened. This thickening could be regarded as a plant strategy to tolerate the translocation of heavy metals to the shoot part (Lux et al., 2004). The collapse of the vascularization elements and formation of narrower xylem vessel elements were present due to decline in expansion of precursor cells (Roghieh et al., 2012). This collapse may also be done according to reduction in cell division which induced by the heavy metals (Hossain et al. 2012). Any changing in cell shape, degeneration, induction or organization probably was likely because of the heavy metal disruption on the hormonal balance (Barceló et al., 1990; Sandalio et al., 2001) (Plate 1 and Plate 2).

Absence of motor cells and collenchymatous tissue in the induced leaves were present close to the epidermis to reduce the heavy metal ion translocation to chlorophyll parenchyma to save primary CO$_2$ fixation system. Number of mesophyll layers was lesser, so the photosynthetic area would be reduced. This agreed with (Srighar et al., 2005; Zhao et al., 2000) who noticed a reduction in

Fig. 2. Simple Linear Regression of the significant relationships found in anatomical parameters between control and induced plant species.
the size of mesophyll cells which collapsed after exposure to heavy metals. This reduction seemed to be from induced deficiency of some ions with the same electrical charge or ion radius such as magnesium and iron (Marschner, 1995). It had also been known
for many years that interference of Zn with Fe metabolism can lead to chlorosis due to competition between Zn and Fe and/or interference with the chelation processes of Fe during root uptake (Zeng et al., 2011). Inhibition of cell elongation and reduction of metaxylem area changed the hydraulic capacity to be more safe and efficient through reforming and loosening of cross-links in the cell wall (Wolf et al. 2012). Furthermore, the heavy metals altered the physical properties (plasticity and extensibility) of the cell wall so; they impaired cellular elongation (Broadley et al., 2012).

The induced anatomical traits expressed heterogeneous repetitive data where all variance of induced parameters estimated values with less than 1 except control ones. In our anatomical study, major of control traits showed leptokurtic more than induced ones. As a result of correlation analyses between control and induced variables, metaxylem of leaf had the highly positive correlated while pith, metaxylem and cortex root, ground tissues and epidermis of leaf had the negative correlated but epidermis root, metaxylem and cortex root, ground tissues and epidermis of leaf had weakly positive correlated. The coefficient of determination showed that only cortex root and metaxylem leaf had the largest difference between control and induced values while pith and metaxylem root and ground tissues had more or less difference but epidermis root, metaxylem of stem and mesophyll leaf had weakly positive correlated. The Further analysis F-test, which revealed that Metaxylem root and ground tissues had the lowest difference between values within each group while cortex and epidermis root, epidermis and metaxylem leaf had more or less difference between within each group but metaxylem of stem and mesophyll leaf had the highest difference between within each group. All graphs of plotted points for the relationships between control and induced anatomical variables were assigned by the regression lines.

Morphological and anatomical changes could be likely affected by the impairment of phytohormone balance. These hormones are important for cell stretching, lateral growth control and cell formation (Marchi et al., 2008). They may also stimulate the synthesis of proteins, DNA, RNA, promote accelerated cell division and provide plant growth. The phytohormones: auxin, cytokinin, ethylene, and gibberellins are the major regulators of cell specification, proliferation, and expansion by extensive crosstalk between them can ensure rapid responses to external and internal cues. (Stahl and Simon, 2010).

Finally, all morphological and anatomical alterations were related to heavy metal-induced imbalances in physiological and metabolic pathways in plant responses (Barceló et al., 1990).

5. Conclusion

Phenotypic plasticity is the key which keeps the plant more adaptive to survive in soil contaminated by heavy metals under specific environmental conditions through changing its morphological and anatomical distinctive characteristics. Cenchrus ciliaris can be investigated as a standard heavy metal tolerant species which can accumulate and restore areas contaminated by these metals.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Environmental pollution on plant biology.

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