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Research Article
Growth, Physiological and Anatomical Behaviour of *Cynanchum acutum* in Response to Cement Dust Pollution

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Abstract

Background: Cement industry is one of the most polluting industry, that emits numerous pollutants as dust and heavy metals. Plants growing in the vicinity of cement industry are greatly affected by the pollutants emitted from this industry, so these plants could be used as air pollution monitoring. **Materials and Methods:** *Cynanchum acutum* plant was growing at the vicinity of studied factory and its growth, physiological and anatomical features investigated as biomonitoring for cement factory pollutants. **Results:** All plant growth attributes (shoot length, branches number, root length, leaf area and dry weight of shoot and root) at plant growing in the vicinity of the factory (polluted site) by 54.14, 61.16, 60, 60.01, 43.11 and 70%, respectively as compared to control site (250 m away from the factory). Chlorophyll 'a', 'b' and carotenoids as well as total soluble sugars and carbohydrates were reduced by 39.69, 18.61 and 28.57% as well as 56.03 and 43.48%, respectively at polluted sites as compared to control site. Anatomical investigation revealed that there was a reduction in the palisade and spongy parenchyma, reduction in the xylem vessels and increases in the density of phloem tissues. The estimated heavy metals (zinc, nickel, cadmium and lead) in soil and plant leaves showed a significant increases at polluted site as compared to control site. The dust amount on the plant leaves showed the same trend of heavy metals. **Conclusion:** Cement dust pollution showed a significant alterations in growth, physiological and anatomical features of *Cynanchum acutum* plant, which qualify it to monitor the cement pollution. Plants growing in the vicinity of the pollution is a best tool for monitoring pollution, where these plants exposed enough to the emitted pollutants. On the contrary, the air around the factory move by wind and the pollutants concentrations become poor reprehensive for pollution.

Key words: *Cynanchum acutum*, heavy metals, leaf anatomy, photosynthetic pigments, soil properties

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cement is one of the most widely used materials throughout the globe. Its persistent production plays a hazardous role in production of air pollutants as sulphur dioxide, nitrogen oxides and dust which disturb the environment. Cement industry is considered from the 17 most polluting industries listed by the Central Pollution Control Board (CPCB). The pollutants emitted from cement factories affect the whole ecosystem around the factories including settlement of the pollutants as visible clouds on the soil and vegetation[1,2]. Physiological disorders such as reduction of photosynthesis and respiration rates of leaves in addition to the reduction in growth rate and productivity were recorded as a result of the dust produced from cement industry[3-5].

Environmental pollution with heavy metals is a major problem globally as they are stable and most of them are toxic to living organisms. The most recorded heavy metals with potential hazards and occurrences in soils are cadmium, chromium, lead, zinc, iron and copper[6,7]. Cement industry release such these heavy metals into the surrounding environment, so soils, plants and residents along the polluted areas are subjected to more contamination with heavy metals which could be carried on the dust particles[8,9].

In Egypt, the problem of air pollution is of great concern. The huge increase in population accompanied by increase of urban expansion and industrialization as cement industry lead to substantial increase in air pollution[10].

Assessment of plant behaviour and soil quality nearby the cement factories is a good tool to evaluate the impacts of this industry. This tool is more expressive than estimation of ambient air pollutants only which is transferred by wind to other places and directions, meanwhile vegetation and soil spend more time in the place. The behaviour and responses of plant to air pollution were used in the air pollution monitoring by some researches[11,12]. Several studies were reported on the impact of the cement dust on soil properties and plant[9,13-17].

Cynanchum acutum L. is a climber shrub belonging to family Asclepiadaceae. It is widely distributed in Egypt in the Nile region, Mediterranean coastal region and oases[18]. The Aim of this study is to evaluate the changes in growth, physiological and anatomical features of *Cynanchum acutum* in response to cement dust pollution.

MATERIALS AND METHODS

Sampling: Plant and soil samples were taken at different distances from the factory located at Helwan city. The first distance was at the vicinity of the factory representing the highest degree of pollution (polluted site), the second was at about 100 m away from the factory and the third was at about 250 m away from the factory representing the lowest degree of pollution and called control site which is compared with the first distance.

Soil analysis: Soil samples were collected from a depth of 0-20 cm. The samples were mixed well, spread over sheets of paper and left to dry in the air. Dried soils were passed through a 2 mm sieve and were then packed in paper bags analysis. Organic carbon (OC) was determined by Walkely and Black rapid titration assay. Calcium carbonates ($CaCO_3$) was determined by titration with 0.5 M sodium hydroxide according to Jackson[19].

Metal concentrations (zinc, nickel, cadmium and lead) in soil and plant leaves were estimated according to Allens[20] as follow, the samples were dried at 60°C till constant dry weight. The dried samples were ground to fine powder and stored in a tightly closed paper bag until metals measurement. About 0.1 g of each dried powder was digested by using concentrated HNO_3 and heated gently until the solution turned quite clear. The clear samples were made up to a known volume using distilled water. Then the samples measured by a flame atomic absorption spectrophotometer (A Perkin-Elmer, Model 2380, USA).

Photosynthetic pigment: About 1 g of fresh leaves was ground in a mortar with 10 mL of absolute acetone using a quartz sand pinch. A little volume of acetone was initially added to start grinding process and then more solvent added in small amounts while continuing to grind the leaves. The extract was centrifuged for 3-5 min using bench top centrifuge at 5000 rpm. The extract was transferred into a 10 mL graduated cylinder using Pasteur pipette. An aliquot of the supernatant was transferred to a 1 cm cuvette and the absorbance readings were recorded against a solvent blank using UV-visible spectrophotometer at wavelengths of 662, 645 and 470 nm for determination of the photosynthetic pigments concentrations like chlorophyll a, b and carotenoids using the formula described by Lichtenthaler[21].

Pigments quantification (in case using 100% acetone):

- Chl-a ($\mu g\ mL^{-1}$) = $11.24\ A_{662}-2.04\ A_{645}$
- Chl-b ($\mu g\ mL^{-1}$) = $20.13\ A_{645}-4.19\ A_{662}$
- Carotenoids = $(1000\ A_{470}-1.90\ C_a-63.14\ C_b)/214$

Estimation of total soluble sugars and total carbohydrates content: About 0.5 g of the air dried plant material was soaked in 10 mL of 80% (v/v) ethanol and left overnight at room temperature with periodic shaking. The mixture was filtered and the filtrate was kept in refrigerator for further analysis. For estimation of the non-soluble carbohydrates content, the residue was washed several times using 80% methanol to remove any soluble sugars. After that, 5 mL distilled water and 6.5 mL perchloric acid (52%) were added to the residue, then the mixture was centrifuged at 5000 rpm and the supernatant filtered using Whatman filter paper No. 5 m. These procedures were repeated several times and the resulted supernatant was completed up to 100 mL with distilled water[22]. About 0.1 mL of the ethanolic extract was mixed with 3 mL of the freshly prepared anthrone reagent (150 mg anthrone reagent+100 mL sulphuric acid (98%), boiling in water bath and cooling. The absorbance was measured at 625 nm using UV-vis. Spectrophotometer (Model 21 D). The total soluble sugars and total soluble carbohydrates were estimated using standard curve of glucose[23].

Analysis of plants anatomical features: The anatomical description of the studied plants was done according to the methods described by Johansen[24] and modified by Peacock and Bradbury[25]. Examination and photographing step as follow; perfect cross sections were selected for recording observation. The microscope used was a full automatic Olympus microscope. Sections were photographed at 40X, 100X and 400X.

Dust deposition: The plant leaves were washed then the water containing dust was filtered in a weighted filter paper (w1). The filter paper with dust was dried in oven until constant weight (w2). The dust amount equal the difference between w1 and w2 (gram per plant).

Statistical analysis: One way analysis of variance (ANOVA) was applied to assess the significance of variation between means by Turkey's HSD at $p \leq 0.05$ using SPSS version 20.

RESULTS AND DISCUSSION

Effect of cement dust pollution on plant growth attributes: The results in Table 1 showed the plant growth attributes of *Cynanchum acutum* at different distances from cement factories.

The highest shoot length of *C. acutum* was recorded at the control sites (67.60±7.44 cm), while the lowest value was recorded at the polluted sites (31.00±3.94 cm). The shoot length of *C. acutum* showed a significant reduction ($p \leq 0.01$) at the polluted sites by 54.14% of the control sites. The number of branches of *C. acutum* exhibited the highest value of 24.20±4.44 at the control sites, meanwhile the lowest number of branches was recorded at the polluted sites (9.4±1.67). Furthermore, there was a significant difference ($p \leq 0.001$) between the number of branches at polluted sites and control sites, where there was a reduction by 61.16% in the number of branches between polluted and control site. Concerning the root length, it recorded the highest value of 14.00±1.41 cm at control sites, whereas the plants growing at the polluted sites have the lowest root length of 5.60±0.89 cm. The root length was significantly ($p \leq 0.05$) reduced by 60% in plants nearest to the cement factory. Regarding the leaf area of *C. acutum* plant at polluted sites it was 5.18±1.33 cm^2, while it was 9.30±3.24 cm^2 at 100 m distance from polluted site and 12.97±2.94 cm^2 at the control site. Also, it is observed that, there was a significant difference ($p \leq 0.05$) between the *C. acutum* plant growing in polluted sites and control site, where it reduced by 60.01% at the polluted sites.

In addition, the obtained data revealed that the dry weight of *C. acutum* shoot ranged from the maximum value of 5.08±0.26 g recorded at control sites to minimum value of 2.89±0.18 g at polluted site. It was observed that there was a significant reduction by 43.11% in polluted site from the control site ($p \leq 0.05$). On the other side, the highest dry weight of root was exhibited in control plants with a value of 2.06±0.23 g, while the lowest value of 0.60±0.25 g was recorded at the polluted sites. It is clear that there was a

Table 1: Growth attributes of *Cynanchum acutum* at different distances from the cement factory

Distance		Shoot length	No. of branch	Root length	Leaf area	Shoot dry weight	Root dry weight
Vicinity	Mean	31.00^a	9.40^a	5.60^a	5.184^a	2.89^a	0.60^a
	Standard deviation	3.94	1.67	0.89	1.33	0.18	0.25
100 m	Mean	48.20^b	17.00^b	10.00^b	9.3^b	3.82^b	0.92^b
	Standard deviation	7.56	3.16	1.87	3.24	0.45	0.32
250 m	Mean	67.60^c	24.20^c	14.00^c	12.97^c	5.08^c	2.06^b
	Standard deviation	7.44	4.44	1.41	2.94	0.26	0.23
	F-value	39.30***	25.30***	42.00***	10.9**	60.10***	41.60***

**Significant at $p \leq 0.01$, ***Significant at $p \leq 0.001$, bars labelled with different letters are significantly different (Turkey's HSD, $p \leq 0.05$)

significant difference ($p \leq 0.05$) between the studied sites where plant of the polluted site was reduced by 70% comparing to plant of control site.

The present results of studied growth attributes were confirmed by some researchers who studied effect of cement dust on growth and morphology of some pans as Prasad and Inamdar[26] on *Vigna mungo*, Iqbal and Shafiq[13] on *Carissa carandas* and *Azadirachta indica* and Chaurasia *et al.*[17] on *Arachis hypogaea*. This may be attributed to alkaline properties of cement particles that deposits on the neighbouring soils changing its features which intern cause significant changes in vegetation growth[13,27-29].

Effect of cement dust pollution on photosynthetic pigments: The illustrated data in Fig. 1 revealed that Chlorophyll 'a' content in *C. acutum* plant recorded the highest value of 1.94 ± 0.23 mg g^{-1} fresh weight at the control sites, while it was 1.32 ± 0.24 mg g^{-1} at 100 m distance from factory site and 1.17 ± 0.15 mg g^{-1} fresh weight at the polluted sites. The Chl 'a' content increases as the distance from industry increases, where there was a significant difference between the different distances and a significant reduction of 39.69% in chlorophyll 'a' was recorded in the samples from the polluted sites in comparison to control.

The content of chlorophyll 'b' was 0.70 ± 0.05 mg g^{-1} in the cement factories polluted sites, while it was 0.82 ± 0.09 mg g^{-1} at 100 m distance from polluted site and 0.86 ± 0.12 mg g^{-1} fresh weight at the control site. It is clear that there was a significant difference between the studied sites, whereas the distance increase the concentration of Chl 'b' increase and a significant reduction of 18.61% in chlorophyll 'b' in polluted sites from the control was observed.

The content of total carotenoids in *C. acutum* cement factory polluted sites was 0.30 ± 0.01 mg g^{-1} fresh weight while it was 0.34 ± 0.05 mg g^{-1} at 100 m distance from polluted site and 0.42 ± 0.10 mg g^{-1} fresh weight at the control site. Thus a significant reduction of 28.57% in carotenoids was estimated in the plants of the polluted sites from those of the control.

Total chlorophylls and carotenoids are considered as the main core of energy production in green plants and their amounts are significantly altered because of the environmental impacts on plant metabolism[30]. Air pollution is considered as one of the main factors that impose damaging effect on the photosynthetic pigments. Under environmental stress chlorophyll pigments might undergo several photochemical reactions such as oxidation, reduction and reversible bleaching[31,32].

The significant reduction in chlorophyll 'a', chlorophyll 'b' and carotenoids in *C. acutum* growing in the vicinity of cement factory comparing to that growing at control sites. be interpreted as follow: (1) In the deposition of fly ash and dust on the surface of plant leaves act as sunscreen and reduce interception of incident light as well as clogging the stomatal opening[33], which cause gaseous exchange interfering Borka[34]. This might lead to increase in leaf temperatures that might retard the synthesis of chlorophyll[35] and (2) The incorporation of cement dust into foliar tissue damages the chloroplast and consequently, reduced the chlorophyll "a" and "b" as well as total chlorophyll content in the plants nears the industry[36,37]. The same behaviour was observed by Pandey *et al.*[38], Abdel-Rahman and Ibrahim[39] and Kumar and Thambavani[2]. In addition, a reduction in carotenoid content of plants growing in polluted locations was reported by Joshi and Swami[40], Joshi *et al.*[41] and Giri *et al.*[32].

Effect of cement dust pollution on total soluble sugars and total carbohydrates: The data in Fig. 2 showed that the total soluble sugars of *C. acutum* at the control site recorded the highest value of 184.54 mg g^{-1} dry weight, while it was 131.24 mg g^{-1} at 60 m distance from polluted site and the

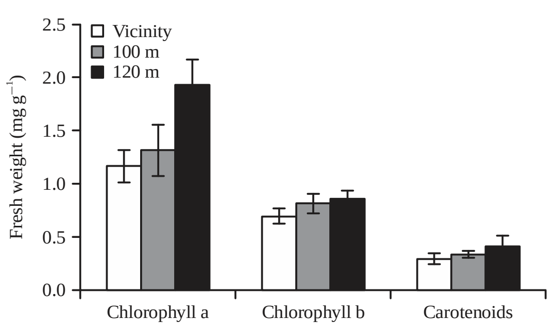

Fig. 1: Effect of cement dust pollution on photosynthetic pigments content of *Cynanchum acutum* at different distances

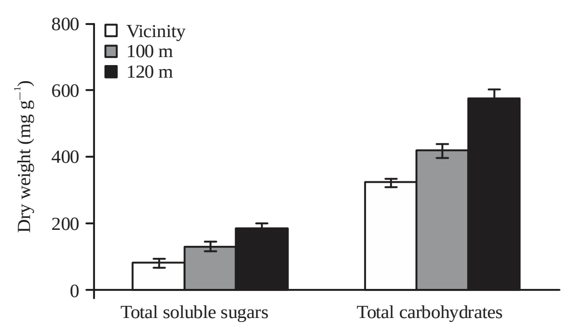

Fig. 2: Effect of cement dust pollution on total soluble sugars and carbohydrates content of *Cynanchum acutum* at different distances

lowest value was 81.15 mg g^{-1} dry weight at the polluted site. It is clear from the results that, there was a significant reduction of 56.03% in total soluble sugars of *C. acutum* of the polluted sites comparing to that of the control site. On the other hand, the total carbohydrates content (Fig. 2) was 325 mg g^{-1} dry weight in *C. acutum* of the polluted sites while it was 575 mg g^{-1} dry weight at the control site . It is obvious from the obtained data that total carbohydrates content showed a significant reduction of 43.48% in the plant of the control site comparing to the polluted sites.

Soluble sugars play important roles in plants, where they are compartments of the plant structure and sources of energy in all organisms[42-44]. In this study the significant reduction in soluble sugars content of *C. acutum* plant at the polluted site may be due to the increase in respiration rate and decrease in fixation of CO_2 due to chlorophyll deterioration, as well as pollutants from cement industry like sulphur oxides and nitrogen oxides might cause more depletion of total soluble sugars in plants grown in polluted areas[45,32].

Effect of cement dust pollution on the anatomy of *C. acutum*: Anatomical characteristics of *C. acutum* leaf were investigated at polluted sites and control site. Leaf cross- section (Fig. 3a) is composed of adaxial and abaxial epidermis of cubical and some conical shaped cells. The lower epidermis of the midrib is projecting form the blade. This projection consists of a group of collenchymatic cells and represent characteristic feature of the leaf. The mesophyll is bifacial, differentiated into palisade and spongy parenchyma. The palisade tissue is bi-layered and lies beneath the upper epidermis, consists of vertically elongated cells and possesses relatively few intercellular spaces. Spongy tissue situated below the palisade parenchyma and is loosely arranged. The vascular bundle of the midrib composed of lignified radiating xylem vessels arranged in vertical rows.

Owing to cement dust emitted from cement factory, the cross-section in *C. acutum* leaf (Fig. 3b) showed that, there were collapsed cells appeared in the mesophyll tissues; reduction in the palisade and spongy parenchyma as well as in the size of chloroplasts. Concerning the vascular vessels, there was a reduction in the xylem vessels and increases in the density of phloem tissues. These results confirmed by those obtained by Gostin[46], El-Khatib *et al.*[47] and Sukumaran[48].

Effect of cement dust pollution on soil properties and heavy metal content: Analysis of soil properties (Table 2) showed that the Organic Carbon (OC) content was increased by increase the distance from the factory which varied from 0.32% at the polluted sites to 0.55% at control sites. Meanwhile calcium carbonate ($CaCO_3$) content showed the reverse trend of OC and ranged from 10.2% at the polluted site to 6.8% at control site. The pH was alkaline to slightly alkaline and fluctuated from 9.53% at the polluted site to 7.86 at control site. Electric Conductivity (EC) was increased at the polluted site and varied from 2190 µS cm^{-1} at polluted site to 1590 µS cm^{-1} at control site. Cement dust analysis indicated that it composed mainly of limestone (80%) and characterized

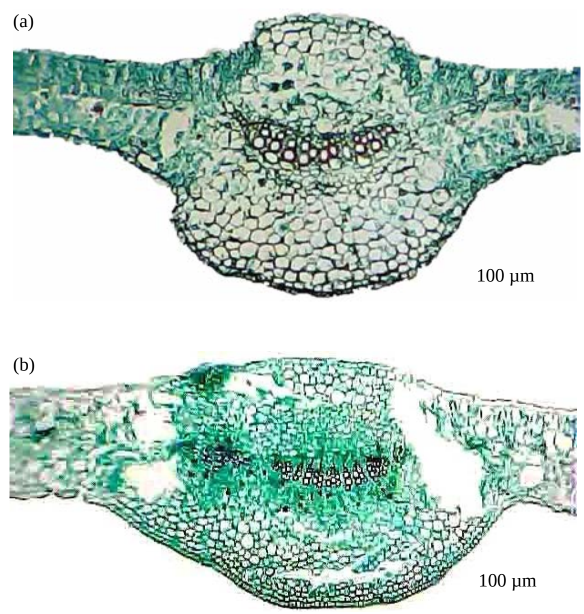

Fig. 3(a-b): Cross section in leaf of *Cynanchum acutum* growing at (a) Control site and (b) Polluted site

Table 2: Properties of soil samples at different distances from the cement factory

Parameters		OC (%)	$CaCO_3$ (%)	pH	EC (µS cm^{-1})
Vicinity	Mean	0.32	10.20	9.53	2190
	Standard deviation	0.13	3.18	0.23	95
100 m	Mean	0.49	9.50	8.03	2010
	Standard deviation	0.15	2.53	0.58	165
250 m	Mean	0.55	6.80	7.86	1590
	Standard deviation	0.19	2.29	0.25	41
	F-value	2.2^{ns}	0.7^{ns}	27.45***	0.38^{ns}

***Significant at p$\leq$0.001, ns: Not significant (p>0.05) by alkaline pH and elevated salinity[16]. So the soil analysis in the vicinity of cement factory attained these properties which had adverse impacts on the plant growth[1,39,49].

The obtained results in Fig. 4a showed that the levels of Zn, Ni, Cd and Pb in the soil in the vicinity of cement factory at various distances exhibited a significant variation. The lowest values of heavy metals concentrations were recorded at the control site (27.45, 18.52, 0.15 and 8.56 μg g^{-1}, respectively); while the highest ones were recorded at the polluted cement factories site (84.09, 39.81, 0.42 and 18.5 μg g^{-1}, respectively). The heavy metals content in the soil significantly decreases as the distance from industry increases.

Effect of cement dust pollution on the heavy metals content of *C. acutum*: The average heavy metals content in *C. acutum* leaves at the control sites exhibited the lowest levels of Zn, Ni, Cd and Pb (9.44, 12.81, 0.09 and 0.94 μg g^{-1}, respectively), meanwhile at the polluted site attained the highest values were recorded (30.65, 35.7, 0.26 and 3.02 μg g^{-1}, respectively). It is obvious from the obtained data that, there were significant differences in the heavy metals content in the leaves of the studied plants at control and polluted site (Fig. 4b).

The cement dust contain mixture of metals that impose many impacts on the plants includes yield reduction and adversely affect leaf area, seed germination and water content[50-52]. These metals cause several effects including reduction in plant growth, seed yield, pollen fertility and total protein levels in addition to other cytogenetic effects[39,50-55]. Wild plants can be used as biomonitors for the metals in the air due to their ability for heavy metals accumulation[11,12,56-58].

Dust deposits: It is clear from the illustrated results in Fig. 5 that there was a highly significant difference ($p \leq 0.001$) in the amount of dust on the *C. acutum* leaves. Where, the highest amount of dust was on plant leaves of polluted site (1.48 g per plant), meanwhile the lowest amount was recorded on the plant leaves of control site (0.45 g per plant).

In this study, the estimated amount of dust on *C. acutum* leaf surface showed significant increase with decrease of the distances from the cement factory. Van Jaarsveld[59] reported that the atmosphere gained approximately 30 million tons of dust each year. The most widespread sources of dust are cement and brick factories, power plants, agricultural activities and heavy vehicles[60]. Dust particles have harmful effects on plants where as mentioned before it act as sun screen which intern cause reduction in photosynthesis and decline in plant growth[61]. In addition Farmer[62] revealed that dust deposition

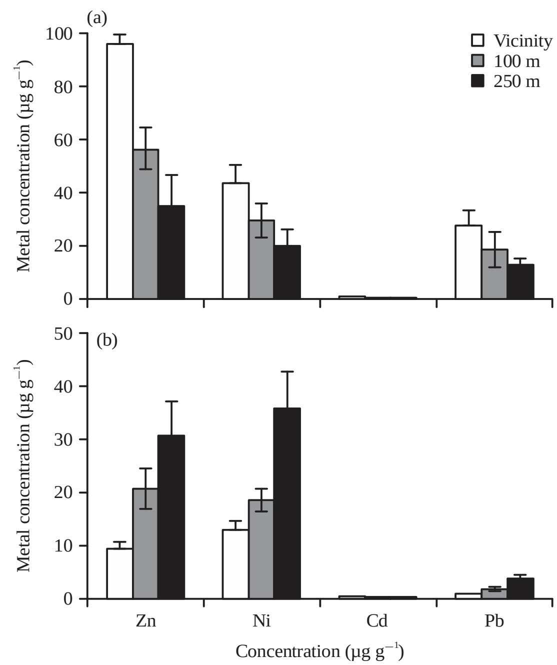

Fig. 4(a-b): Effect of cement dust pollution on heavy metals content (μg g^{-1}) of (a) Soil and (b) *Cynanchum acutum* leaves at different distances

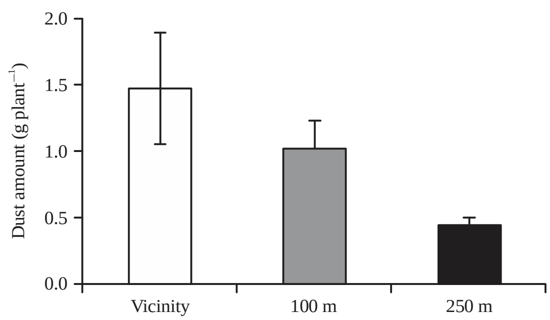

Fig. 5: Effect of cement dust pollution on the dust amount on *Cynanchum acutum* leaves at different distances

on the plant leaf increase their temperature which, in turn, increase the susceptibility of plant to drought.

CONCLUSION

Cement dust pollution showed a significant alterations in growth, physiological and anatomical features of *Cynanchum acutum* plant which qualify it to monitor the cement pollution. The soil properties in the vicinity of cement factories were changed and acquired the properties of cement dust which intern change the plant features. In addition, the heavy metals concentrations increased in plant leaves and soil nearby the cement factories.

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