

## ORIGINAL RESEARCH PAPER

# Evaluation of cement dust effects on soil microbial biomass and chlorophyll content of *Triticum aestivum* L. and *Hordeum vulgare* L.

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Received 14 December 2016; revised 18 February 2017; accepted 20 March 2017; available online 1 April 2017

**ABSTRACT:** Overall plant growth and microbial biomass can be effected by dust accumulation. The chloroform fumigation-extraction method was used to evaluating the effect of cement dust pollution emitted from Kurdistan cement factory on soil microbial biomass carbon. Chlorophyll content (a, b and total) of plants species was measured in different distance from cement factory. Microbial biomass C ( $C_{mic}$ ) amounts ranged from 0.138 to 1.102 mg/g soils in the polluted sites and from 0.104 to 1.283 mg/g soils in the control area. Soils polluted with alkaline cement dust resulted in meaningful reduction in  $C_{mic}$  levels compared to control soils. Pearson correlation coefficients ( $r$ ) show  $C_{mic}$  was positively correlated to soil  $CaCO_3$  content ( $r = 0.09$ ).  $C_{mic}/C_{org}$  ratio was 2.54 and 1.92 in the control and cement polluted sites, respectively. Reduction in this ratio can be resulted from soil degradation in cement polluted soils. A significant decrease in the  $C_{mic}/C_{org}$  ratio in cement dust-polluted soils illustrated that this factor can be applied as a good indicator of soil quality. In the case of chlorophyll content of plant species, maximum reduction of total chlorophyll for *Triticum aestivum* L. was 45% compared to *Hordeum vulgare* L. with 60%. Therefore, results show higher sensitivity of *H.vulgare* than to *T. aestivum*.

**KEYWORDS:** Cement dust; Chlorophyll chloroform fumigation-extraction (CFE);  $C_{mic}/C_{org}$  ratio; *Triticum aestivum* L.

## INTRODUCTION

Microorganisms play vital role in nutrient cycling of carbon, nitrogen, sulfur and phosphor elements in most terrestrial ecosystems. In this way, soil microbial biomass can regulate nutrient availability for terrestrial plants (Sharma *et al.*, 2013, Gougoulas *et al.*, 2014, Mooshammer *et al.*, 2014). Soil condition can affect directly and indirectly on microorganisms respond (Bardgett *et al.*, 2008). For examples, alterations in microbial populations or activity can lead to changes in physical and chemical properties of soil, thereby demonstrating an early indication of soil improvement or an early warning of soil degradation (Crisler *et al.*, 2012). Microbial biomass ratio ( $C_{mic}/C_{org}$ ), and soil

respiration are common indicators soil quality monitoring (Jin and Bethke, 2003). The  $C_{mic}/C_{org}$  ratio demonstrates correlation between microbial biomass and soil organic matter (Li and Chen, 2004). Increasing or decreasing of organic matter stability can change established constant (Westerhoff *et al.*, 2007). Usually, higher amounts of the  $C_{mic}/C_{org}$  ratio determine the equilibrium between microbial activity and the inputs of organic matter in the case of mineralization (Liu *et al.*, 2012, Österreicher-Cunha *et al.*, 2012). Fertility and quality of soil are related to a high amount of microbial biomass. High microbial biomass impact on total organic matter content and resulted in organic matter dynamic of soil (Luo *et al.*, 2016, Dou *et al.*, 2016). Soil pollutants such as cement dust or heavy metal can

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remove microorganisms and decrease substrate availability, lead to a reduction in microbial biomass (Shentu *et al.*, 2008, Dhal *et al.*, 2013). Cement dust particles from the cement factory have alkaline property resulted from the raw and finished material (Bilen, 2010). Other air pollutants of cement manufacturing are sulfur oxides and nitrogen oxides produced from the furnace and drying processes (Pun *et al.*, 2014). The environment pollution with cement dust by alkaline property affects all ingredients of ecosystem (Kara and Bolat, 2007, Paoli *et al.*, 2014, Margesin *et al.*, 2014). In this case, microbial activity utilized to determine the effects of cultivation (Vargas *et al.*, 2015), field management (García-Orenes *et al.*, 2016) or soil contamination (Baemaga *et al.*, 2015). Moreover, there are several reports of cement dust pollution on air and soil, which may leads to a considerable decrease in the soil microbial biomass (Salama *et al.*, 2011).

Several studies have been illustrated that the high dust load and its long period can impact on ecosystem components through changing of soil and water characterizations (Langer and Günther, 2001). Reduced microbial biomass C and  $C_{mic}/C_{org}$  ratios were reported as a consequence of soil parameters fluctuation including soil pH levels, carbon amounts and soluble salts (Cheng *et al.*, 2013). Also, by soil parameters alteration, plant physiology can be affected. In this case, dust accumulation on leaves surface can block stomata and resulting in high temperature of leaves (Alavi *et al.*, 2014).

Photosynthesis yield and chlorophyll content as important factors in plant growth may be reduced by high temperature of leaves (Alavi, 2015, Alavi *et al.*, 2016). Therefore, based on above descriptions, our objective was determination of relation between cement dust alters and  $C_{mic}$ . Also, the respond of plant species *Triticum aestivum* L. and *Hordeum vulgare* L. to cement

dust pollution were investigated nearby cement factory in Kurdistan province in in the summer of 2016.

## MATERIALS AND METHODS

### Site description

Kurdistan province is one of the mountainous provinces of Iran and has a generally cold climate. This province is located in northwestern of Iran, bordering Iraq from 34° 44'2" to 36° 30'2" North, and, 45° 31'2" to 48° 16'2" East. Kurdistan province represents about 1.7 % of the area of the entire country and has more than, 450,000 inhabitants. Cement factory is located in Bijar County of Kurdistan Province with geographical profile of 7730-km<sup>2</sup> area with an average altitude of about 1940 m above sea level (Karimi and Alavi, 2016).

### Soil and plant Sampling

Sample sites were located in Bijar County of Kurdistan province including cement dust polluted and control sites. In order to soil sampling, twelve samples from top soil (5-20cm) were collected separately in the summer of 2016. All samples passed through steel sieve (2-mm openings) and were stored at refrigerator with temperature of +4°C before microbial analysis.

In order to physical and chemical measurement of soil, subsamples of the soils were air-dried and ground to pass through steel sieve (2-mm openings), (Kara and Bolat, 2007). As shown in Table 1, plant species were sampled from polluted and control sites with respectively distance of 200-2000 m and 4000-5000 m from cement factory.

### Physicochemical analysis of soil

Firstly, soil/water mixtures with ratio of 1:2.5 and 1:5 were prepared respectively as primary samples for analysis pH measurement and electrical conductivity (EC). Then, pH and EC analysis were carried out

Table 1: Properties of plant species around the cement factory of Kurdistan province

Area	Distance (m)	Plant species
Control	4000-5000	<i>Triticum aestivum</i> L., <i>Salsola kali</i> L., <i>Sophora aleupecuroides</i> L., <i>Hordeum vulgare</i> L., <i>Astragalus</i> , <i>Phragmites australis</i> L., <i>Noaea mucronata</i> L.
Polluted	200-2000	<i>Triticum aestivum</i> L., <i>Salsola kali</i> L., <i>Sophora aleupecuroides</i> L., <i>Hordeum vulgare</i> L., <i>Astragalus</i> , <i>Centaurea iberica</i> L.

respectively by a glass electrode and an electrical conductivity meter. Walkley-Black wet oxidation method was used for indicating of soil organic carbon (Chen *et al.*, 2015). The total carbonate amounts was evaluated by a Scheiber calcimeter (Schindlbacher *et al.*, 2015). Also, total nitrogen was measured by volumetric analysis of ammonia base on method of Kjeldahl (Liu *et al.*, 2014).

#### Soil microbial biomass carbon (SMBC) analysis

SMBC was estimated by chloroform-fumigation extraction method. 35g oven dry equivalent of field moist mineral soil samples extracted in 0.5 M potassium sulfate solution (approximately 1:4 w/v) (Kara *et al.*, 2016). Triplicate subsamples from each soil were placed inside 50ml glass beakers followed by placing of samples in a vacuum desiccator. Chloroform (ethanol-free) with boiling chips was placed in a beaker in the center of the desiccator. In order to maintain moisture during fumigation of soil, it was used water moistened paper towels in each desiccator.

The desiccator was sealed, and chloroform was boiled for 30 s under a laboratory hood. In the dark condition, samples were fumigated at 25°C for 30 h. After removal of chloroform, the samples were transferred to a 250-ml bottle and followed by adding 120 ml of 0.5M potassium sulfate. Samples were shaken for 50 min on a reciprocating shaker followed by filtration of supernatants through a whatman no.40 filter.

Resulted samples were at 4 °C for up to seven days. Control soil samples were unfumigated samples. Oxidation of samples was carried out by 0.4 N potassium dichromate ( $K_2Cr_2O_7$ ) at 120°C for 50 min and back-titration with  $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ . Then, SMBC was evaluated in 8 ml aliquots of  $K_2SO_4$  extracts. The difference in extractable organic carbon between the un-fumigated soil samples and fumigated was used to measurement of SMBC based on Eq. 1.

$$SMBC = 2.64 E_c \quad (1)$$

Where  $E_c$  refers to the difference in extractable organic carbon between the fumigated and unfumigated treatments; 2.64 is the proportionality factor biomass carbon released by fumigation extraction.

#### Measurement of chlorophyll content

Chlorophyll content of plants leaves was quantified using Arnon method (Arnon, 1949, Mostajeran *et al.*, 2014). At the end of experiment, four plants from each vessel were collected and cleaned thoroughly by water. Afterwards, 0.2 gr of fresh leaf from each sample was separated, grinded in a mortar with 5 ml of 80% acetone, and 15 ml of acetone (100%). The homogenate was filtered through filter paper (Whatman No.1) and was made a volume of 25 ml with 80% cold acetone. The optical density of each solution was measured at 663 and 645 nm against 80% acetone blank in 1.5 cm cell. The content of the photosynthetic pigments was calculated according to Eqs. 2, 3 and 4.

$$Chl\ a\ (mg/g) = [(12.7 \times A_{663}) - (2.6 \times A_{645})] \times ml\ acetone/mg\ leaf\ tissue \quad (2)$$

$$Chl\ b\ (mg/g) = [(22.9 \times A_{645}) - (4.68 \times A_{663})] \times ml\ acetone/mg\ leaf\ tissue \quad (3)$$

$$ChlT = Chla + Chlb \quad (4)$$

#### Statistical analysis

Comparing the mean characteristics of the control samples with the polluted samples was carried out by a t-test (independent-samples). The association between the different soils properties was measured through Pearson correlation coefficients. SPSS 16 software was used to process the data.

## RESULTS AND DISCUSSION

#### Soil and microbial analysis

Values of soil properties of polluted and control samples are shown in Fig. 1. Microbial biomass C ( $C_{mic}$ ) values ranged from 0.104 to 1.283 mg/g soils in the control area and 0.138 to 1.102 mg/g soils in the polluted area. Significant reductions in  $C_{mic}$  levels compared to control soils caused by alkaline pollution of soils with cement dust ( $P < 0.05$ ). Cement dust can specifically affect the air pollution situation and the soil's chemical characteristics (Salama *et al.*, 2011). As shown in Fig. 1, the area was polluted with high  $CaCO_3$  content ( $18.21 \pm 2.01$ ) than to control area with  $6.12 \pm 1.88$  in the top soil.

Evaluation of cement dust effects on soil microbial biomass and chlorophyll content

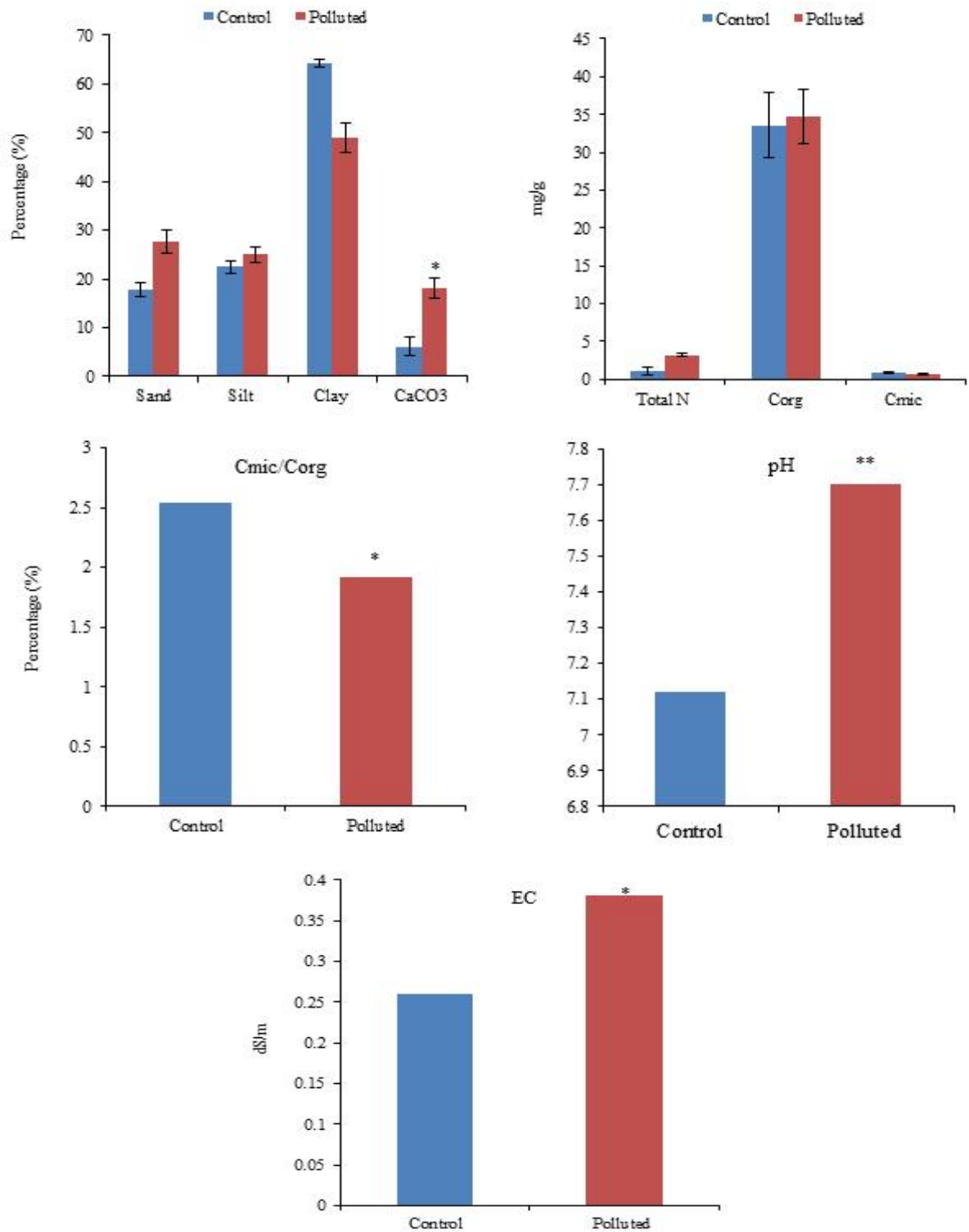


Fig. 1: Physicochemical and biological properties of soil. Values are means of three replicates. Asterisks indicate significant differences between control and polluted soil; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$

The calcium carbonate content of polluted soils was meaningfully higher than control soils ( $P < 0.01$ ). Also, a value of soil pH was about 7.7 in polluted area compared to control area by 7.12 (Fig. 1). In this case, lime can alter soil microbial biomass (Felsmann *et al.*, 2015).

Electrical conductivity (EC) of the soil samples ranged from 0.15 to 0.41 dS/m in the control site and 0.21 to 0.63 dS/m in the polluted site. There was no definite difference in soil electrical conductivity between the studied areas (Fig. 1). Pearson correlation coefficients ( $r$ ) show  $C_{mic}$  was positively correlated with soil  $CaCO_3$  content ( $r = 0.09$ ) (Table 2). It can be suggested that differences in  $C_{mic}$  between the polluted soils and controls appeared to be resulted from the higher amounts of  $CaCO_3$ . In spite of the soil pH, temperature, water, substrate quantity and availability have been illustrated to affect the biological response to lime content of soil (Felsmann *et al.*, 2015). Also, texture of soils in polluted area had more clay content than to sand and silt (Fig. 1). Soil texture has major role in indicating of microbial biomass through changing soil organic C content and soil microclimatic conditions (Kooch *et al.*, 2016). Soils with high clay content results in higher microbial biomass and more stabilization of soil organic C (Pun *et al.*, 2014). Results of this study illustrate that the effect of clay amount within the narrow range of soil textures was not well understood. In this case, changes in soil chemical properties, such as pH and  $CaCO_3$  content, may influence on clay content on soil  $C_{mic}$ . Table 2 shows the clear relationship between  $C_{mic}$  with  $C_{org}$  (0.73). A linear relationship was indicated between soil organic carbon and soil microbial biomass. Moorhead and coworkers (2013) were reported the association between biomass and  $C_{org}$ . Results of their study did show that  $C_{mic}$  augmentation were linear up to about 2.5% soil carbon (Moorhead *et al.*, 2013). However,  $C_{org}$  did not show significant difference between control soils (33.59 mg/g) and polluted soils (34.7 mg/g). These results suggest similarity of  $C_{org}$  availability for soil microorganisms in the 2 sites. Total N ( $N_{tot}$ ) was lower in the polluted site (1.06 mg/g) compared with the control site (3.21 mg/g) (Fig. 1). Reduced microbial abundance and activity may be reason of the low  $N_{tot}$  values in the polluted soils. Similarly, Koschke *et al.* (2011)

reported that alkaline fly ash reduced N concentration through alteration of specific microbial and enzymatic processes in the soil decomposition (Koschke *et al.*, 2011).  $C_{mic}/C_{org}$  ratio was 2.54 and 1.92 in the control and polluted soil, respectively (Fig. 1).  $C_{mic}/C_{org}$  ratio of soil samples in the polluted site was prominently lower than that of soil samples in the control site. In this case, efficiency of organic carbon into microbial carbon and losses of soil carbon during microbial decomposition can be described by this ratio as a soil health indicator (Lü *et al.*, 2015). Commonly, the microbial carbon sources as the  $C_{mic}/C_{org}$  ratio will reduce at a faster rate than the organic carbon (Llorente and Turrión, 2010, Janssens *et al.*, 2010).

Microbial biomass as a presentation of soil organic matter is one of the important factors that can be applied to measurement the change of natural ecosystem under soil pollutions such as cement dust pollution (Shentu *et al.*, 2008). An important soil microbial ratio for describing the change in the artificial ecosystem is parameter  $C_{mic}/C_{org}$  ratio (Cheng *et al.*, 2013). Results of Pearson correlation coefficients ( $r$ ) illustrate linear, and positive relationship between organic carbon and the biomass carbon amounts in control site (Liu *et al.*, 2012). In contrast, there was not such relationship in polluted sites (Shentu *et al.*, 2008). Based on previous studies, an equilibrium threshold for cultivated soil is a value of 2.2 for  $C_{mic}/C_{org}$  ratio which in our investigation was lower than this threshold (2.08%) (Cheng *et al.*, 2013).

#### Measurement of chlorophyll content

Chlorophyll *a*, *b* and total chlorophyll content (mg/g fresh weight (FW)) and reduction percentage each samples than to control for *Triticum aestivum* and *Hordeum vulgare* plant species are illustrated in Figs. 2 and 3. Also, plant age (20, 40, 60, 80 and 100 days) was other factors that were analyzed for this case. Both plant species have demonstrated increasing of chlorophyll content response to rising of distance (200, 800 and 2000m).

As shown in in Figs. 2 and 3, it is obvious that the exposure to cement dust pollution resulted in a decrease in chlorophyll content close to distance

200m compared with control. However, statistical analysis (ANOVA) indicated no significant difference for all treatments. After 20 and 100 days of plant age, these reductions for *Triticum aestivum* were respectively lower (55% at 2000m distance) and higher (76% at 200m distance) (Fig. 4). Similarly, *Hordeum vulgare* had higher (75% at 200m distance) and lower (40% at 2000m distance) in plants by 100 and 20 days old respectively (Fig. 4). Also, percentage of total chlorophyll of polluted samples than to control in *T. aestivum* and *H. vulgare* and Comparing of minimum and maximum percentage of total chlorophyll for plant species are shown in Figs. 3a, b and c. As shown in Fig. 3c, maximum reduction of total chlorophyll for *T. aestivum* was 45% compared to *H. vulgare* with

60%. Therefore, results show higher sensitivity of *H. vulgare* than to *T. aestivum*.

Overall plant growth can be effected by dust accumulation on the leaf surface (Prajapati and Tripathi, 2008). Plant biomass and photosynthesis yield of plants with dust stress illustrate reduction result in photosynthesis function that can be resulted from less light covering (Alavi et al., 2014; Zia-Khan et al., 2014).

In previous study, we reported reduction in photosynthesis yield parameters including  $\Delta F/F_m'$ , ETR and Fv/Fm by increasing the dust concentrations (Alavi, 2015). In similar studies, there was significant difference between C<sub>3</sub> (*Triticum aestivum*) and C<sub>4</sub> (*Zea mays*) plant species at biomass, chlorophyll *a*, and *b* (Alavi et al., 2016).

Table 2: Pearson correlation coefficients (r) among measured variables in polluted site

Variable	Clay (%)	CaCO <sub>3</sub> (%)	pH	EC (dS/m)	N <sub>tot</sub> (mg/g)	C <sub>org</sub> (mg/g)	C <sub>mic</sub> (mg/g)
Clay	1.00	0.05	-0.47	0.35	-0.002	-0.4	-0.47
CaCO <sub>3</sub>		1.00	-0.81	0.51	0.06	0.65	0.09
pH			1.00	-0.48	0.32	-0.14	0.46
EC				1.00	0.002	0.56	0.1
N <sub>tot</sub>					1.00	0.44	0.83
C <sub>org</sub>						1.00	0.73
C <sub>mic</sub>							1.00

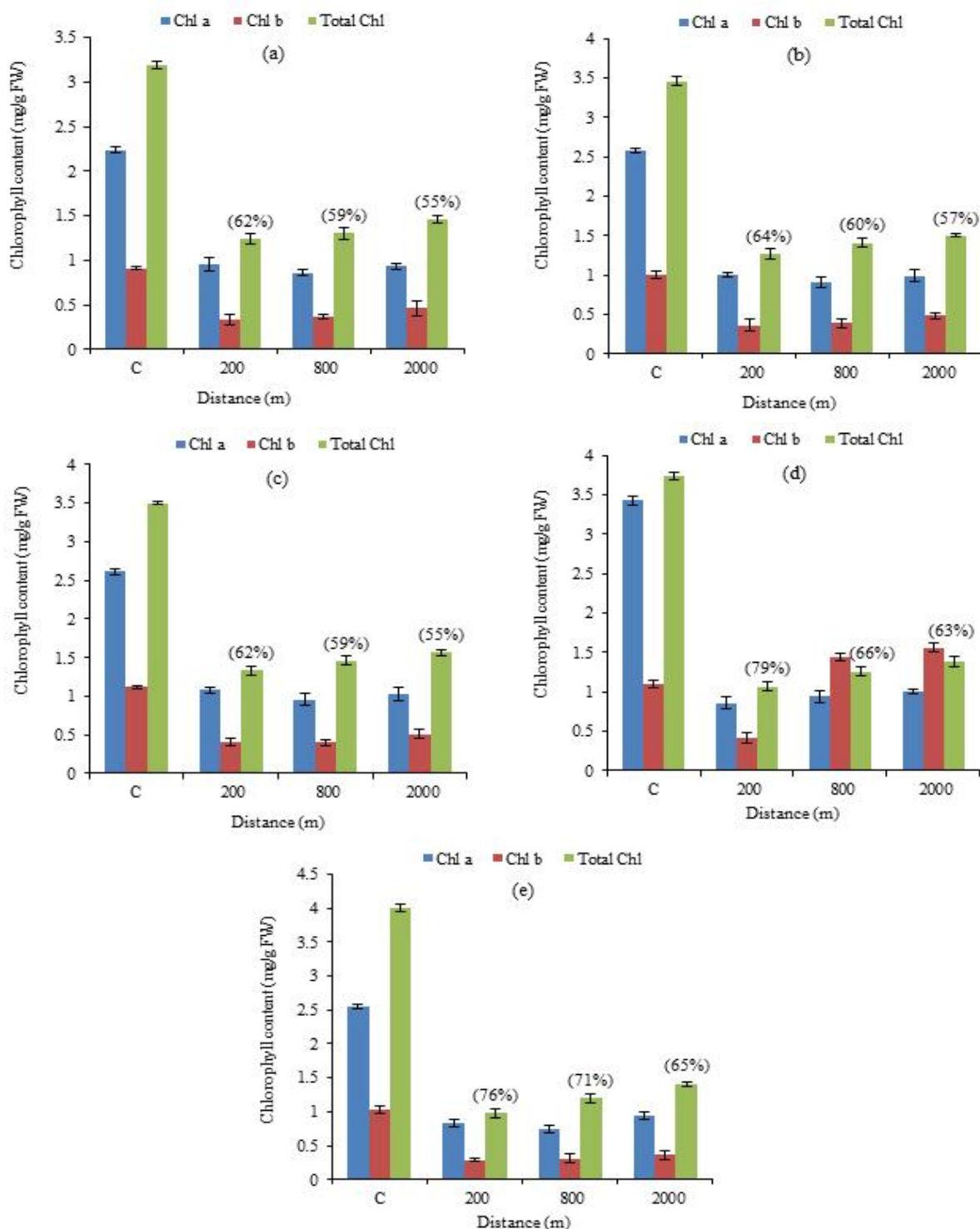


Fig. 2: Chlorophyll content (a, b and total) (mg g<sup>-1</sup> FW) and percentage decrease over control in study area's *Triticum aestivum* L in the age of 20 (a), 40 (b), 60 (c), 80 (d) and 100 days (e)

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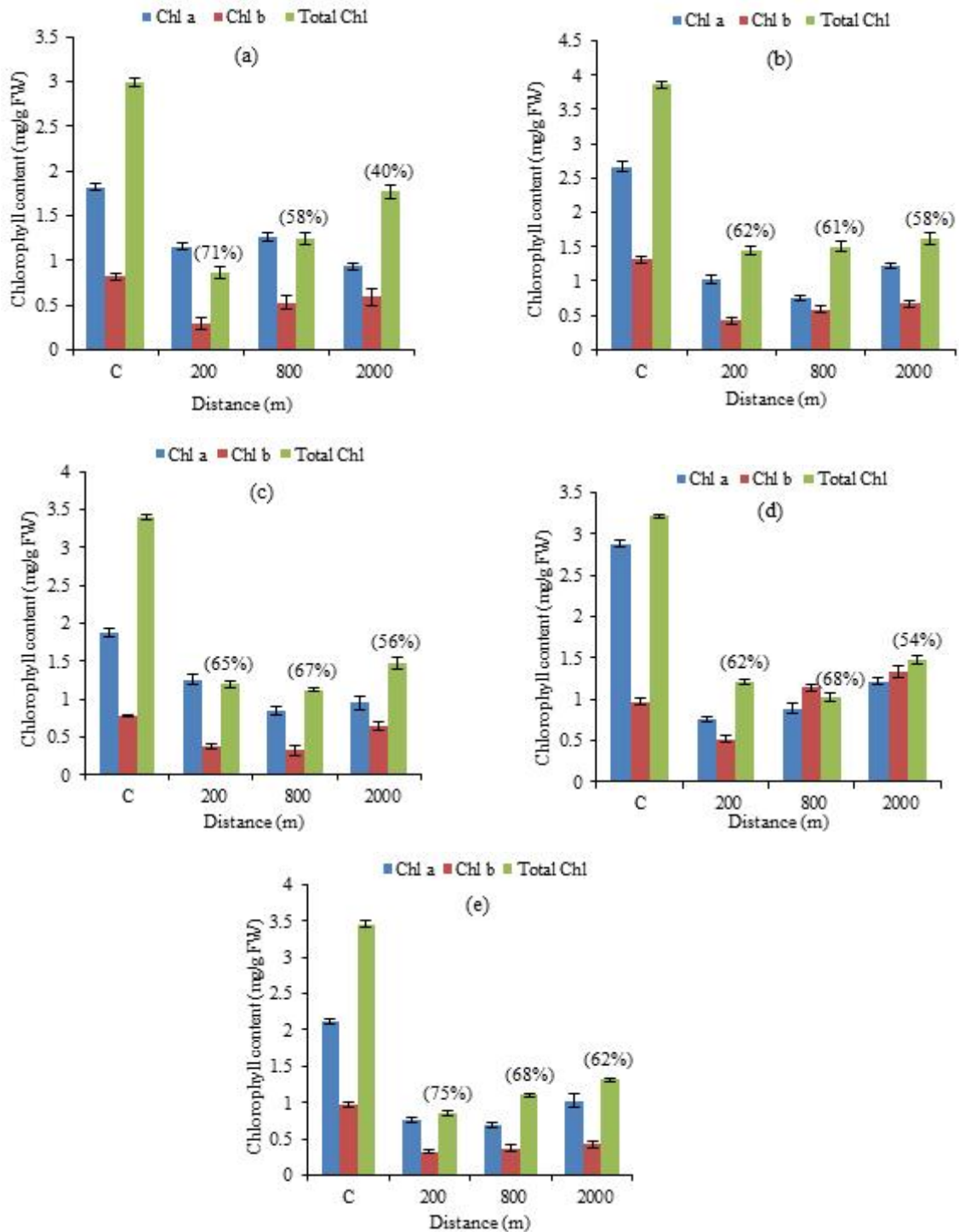


Fig. 3: Chlorophyll content (a, b and total) (mg g<sup>-1</sup> FW.) and percentage decrease over control in study area's *Hordeum vulgare* L in the age of 20 (a), 40 (b), 60 (c), 80 (d) and 100 days (e)



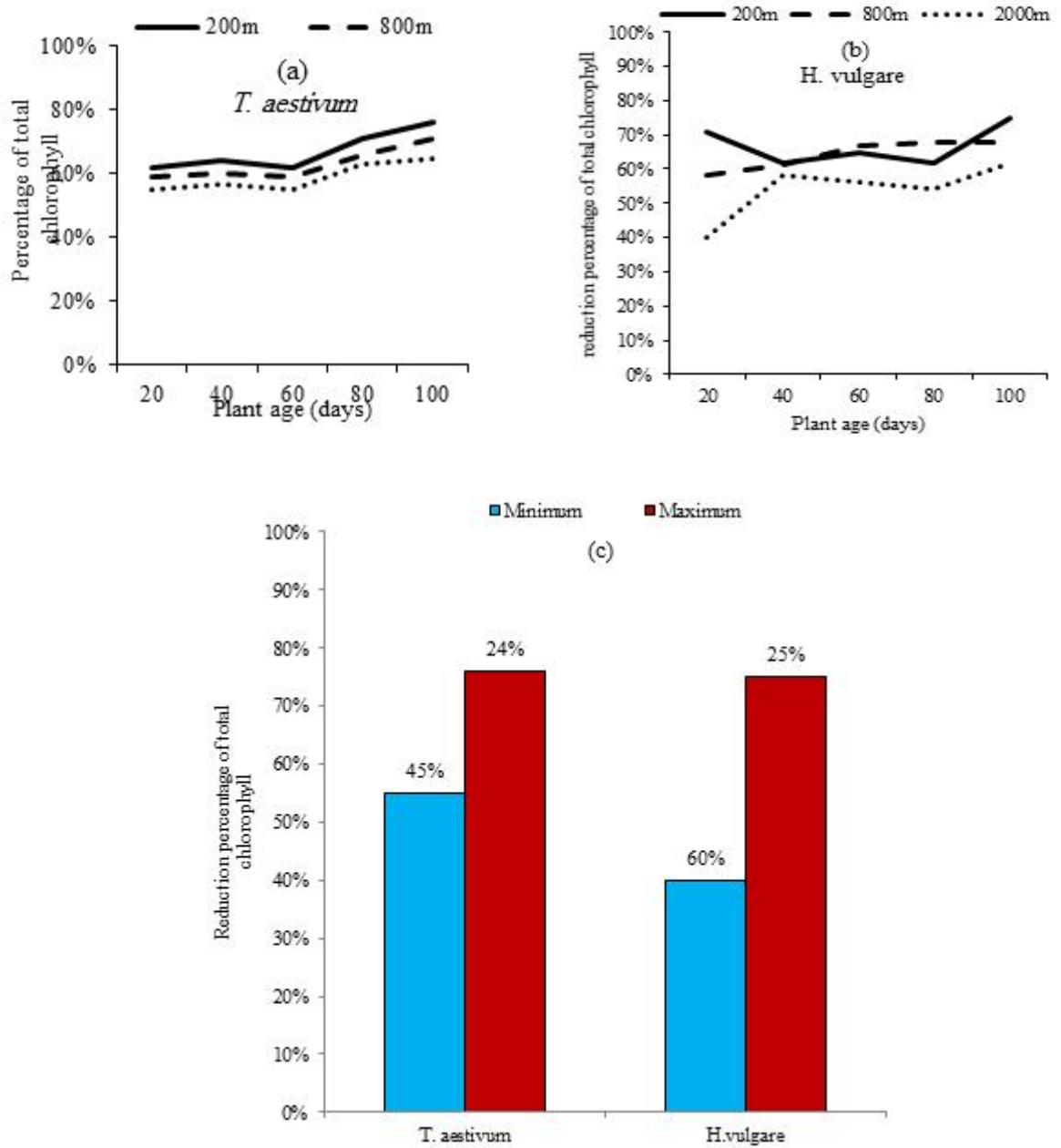


Fig. 4: Percentage of total chlorophyll of polluted samples than to control in *T. aestivum* (a) and *H. vulgare* (b). Comparing of minimum and maximum percentage of two plant species (c)

### CONCLUSION

In order to serious effects of cement dust on ecosystem, increasing of this dust type from cement

factory has drawn much attention. In this case, this study proved that soil chemical properties were

changed through microbial biomass reduction under cement dust pollution. Measurement of the critical level of  $C_{mic}/C_{org}$  ratio in polluted soil is difficult because there are the ranges of values of this parameter in the literatures. In order to the dust accumulation in cement polluted soil with alkaline property, augmentation of soil pH values will reduce microbial biomass and consequently will decrease organic matter decomposition and element cycling such as C and N in cement dust-affected areas in Bijar County. Also, this study illustrated a reduction in chlorophyll content (*a*, *b* and total) close to distance 200m of cement factory compared with control. In the end, more investigations related to other aspect of plant physiology and microbial communities are needed due to better understand of dust effect on plants and microbes.

#### ACKNOWLEDGMENT

The authors would like to appreciate Razi University for providing the financial supports for the study.

#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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