Research article

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The effects of cadmium on growth, some anatomical and physiological parameters of wheat (*Triticum aestiv*um L.)

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ABSTRACT

Nowadays, increased population and traffic density, together with the development of industry, caused increasing levels of heavy metals releasing to the environment, and environmental pollution has reached its highest level worldwide. Chemical products, fertilizers, industrial dyes, construction materials, silver dental fillings and vaccines are some of the well-known sources of heavy metals exposed the environment. Toxic heavy metals can normally be present in body parts of living things at very low levels, but at higher concentrations they can show toxic effects depending on species and duration. Among heavy metals, cadmium is one of the most harmful ones to the environment, humans, animals and plants, and can be toxic even at low concentrations. Thus in this study, Cd was applied to the wheat (*Triticum aestivum* L.) plants grown in Kyrgyzstan in different concentrations (0, 50, 100, 200 and 400 µM for experimental groups) and in addition to accumulations in different plant parts, some growth, development, physiological and anatomic parameters were measured. As a result, it was observed that wheat plants were affected by all Cd concentrations, although they were able to manage lower stress in terms of some parameters. It was also seen that plants were negatively affected by higher levels of Cd stress, although remained alive throughout the experimental period.

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Introduction

Metals, with a relatively higher atomic mass and specific gravity greater than 5 g/cm^3 are called “heavy metals”. More than sixty elements are considered as heavy metals and these metals are located in a part of the periodic table called “transition elements” [1-3]. Heavy metals are important environmental pollutants, many of which can be toxic even at a very low concentrations [4].

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In parallel with the recent increasing world population growth, industrial developments and heavy traffic in cities, heavy metal release into the biosphere caused pollution, and this problem is dramatically increasing day by day [5, 6]. The main factors causing the spread of heavy metals to the environment could be by natural and/or anthropogenic sources such as volcanic activities, industrial activities, motor vehicle exhausts, paints, mineral deposits and mining sites, fertilizers and chemicals used in agriculture [2, 7, 8].

Although mining of heavy metals and their usage in different industrial processes are caused a problem called "heavy metal pollution", they are important raw materials in industry and required for socio-economic development. Nevertheless, they are toxic and therefore a potential threat to human health and ecosystems [9-11]. They are major threats to living organisms, as they tend to be bioaccumulated and do not degrade easily in nature, and as mentioned above, some of them can be toxic even at low concentrations [4, 12].

Uptake of metal ions by plants involves; (1) attachment of metal ions to the root surface, (2) uptake into the roots and (3) translocation to the body through mass flow and diffusion. Uptake of substances from the soil is provided by metal chelating molecules secreted from the roots into the rhizosphere, metal reductase enzyme and proton release [13]. The heavy metals uptaken from soil by the root systems of plants are then transported to the aboveground parts by passing into xylem with the effect of transpiration power [14, 15]. The metals reaching up to the leaf are distributed in leaf cells via apoplastic and symplastic ways, due to the binding of the metals to the chelators such as phytochelatins (PC) and metallothioneins (MT) [16]. These chelators contain large numbers of cysteine sulfhydryl groups, and after binding to the heavy metals, they can form them stable complexes and help for the storage in the vacuole and cell wall [2, 16]. Nevertheless, the success of these mechanisms varies from one plant species to another. While the plants classified as "hyperaccumulator" are more successful in this regard as taking certain heavy metals and accumulate them in their bodies without showing any symptoms, some plant species, which are not successful in these mechanisms are negatively affected from heavy metals biologically, and toxicity symptoms appear in their bodies [17, 18].

In plants exposed to high concentrations of heavy metals, many physiological events such as germination, growth and development, protein synthesis, enzyme activity, transpiration,

stomatal movements, water uptake, photosynthesis, membrane stability are negatively affected [19-21].

Cd, which in the group 2B of the periodic table, is one of the most dangerous heavy metal pollutants in the ecosystem, and a highly toxic element for living things [9, 22]. The importance of Cd as an environmental pollutant has become more evident, especially in recent years. Cd can be found naturally in the world, as well as it can spread to the environment as a result of human activities such as phosphorus fertilizers, sewage wastes and atmospheric deposits [23, 24]. These factors increase the importance of Cd as a pollutant and nowadays, plants, humans and animals, especially living in urban and/or industrial areas are constantly exposed to Cd and are being affected adversely even its small concentrations [2, 4].

The plants exposed to Cd, especially in higher concentrations, show toxicity-related symptoms such as generation of reactive oxygen species (ROS) resulted lipid peroxidation, enzyme inactivation and DNA damage [25, 26], reduced photosynthesis [27], inhibited respiration and gas exchange [28, 29] and cell proliferation [30], diminished water balance [31], and disturbed carbohydrate [32] and nutrient uptake metabolisms [33] resulting in visible symptoms such as chlorosis, necrosis, root blackening, stunting and general reductions in biomass production [34-36]. Due to the above effects, Cd causes a decrease in yield and quality, especially in field crops.

Poaceae family member wheat *Triticum aestivum* L. is one of the most important cereal crops in the world as well as in Kyrgyzstan. Wheat and wheat-derived products provide high amounts of energy due to their high carbohydrate content. They also contain substantial levels of protein, lipid, B1 and niacin and therefore together with rice, it is the primer food source for the world [37]. Recent studies conducted with plants in Kyrgyzstan showed that Cd concentrations are higher than normal limits in many plants [38-40]. For this reason, in this study, the effects of Cd on growth, development, some anatomic and physiological parameters were investigated in wheat plants grown under different levels (50, 100, 200 and 400 μM) of Cd exposures.

Materials and Methods

Plant Material

Wheat is a plant belonging to the genus Triticum from the Poaceae family. It is a self-pollinating, one-year monocot plant, with fringed roots and caryopsis type fruit [41]. Wheat can be grouped in three categories, according to the number of chromosomes as; 14 (diploid), 28 (tetraploid) and 42 (hexaploid). The basic set of chromosomes is 7, and the above designations signify 2×7 (2n), 4×7 (4n), and 6×7 (6n) respectively [42]. In this study, Intensivnaya variety belonging to wheat (T. aestivum L.) plant, which has been widely cultivated in Kyrgyzstan was obtained from "Kyrgyzstan State Plant Genetic Resources Center" and used as experiment material.

Plant Sampling and Germination

The seeds of wheat cultivar Intensivnaya were washed with tap water for 1 hours and then germinated in standard pots containing 100 g soils in growth conditions for 14 days and watered with full strength Hoagland nutrient solution [43]. Plants were grown under 5000 μmol m^{-2} s^{-1} fluorescent lights with photoperiod of 16 hours light and 8 hours dark period, at temperature of 24 ± 2°C and relative humidity 45-50%. After 14 days of germination, each of the experimental groups of 10 replicated seedlings were watered with 20 ml Hoagland's solution containing 0, 50, 100, 200 and 400 μM $CdCl_2$ at two-day intervals for 45 days.

Measurements

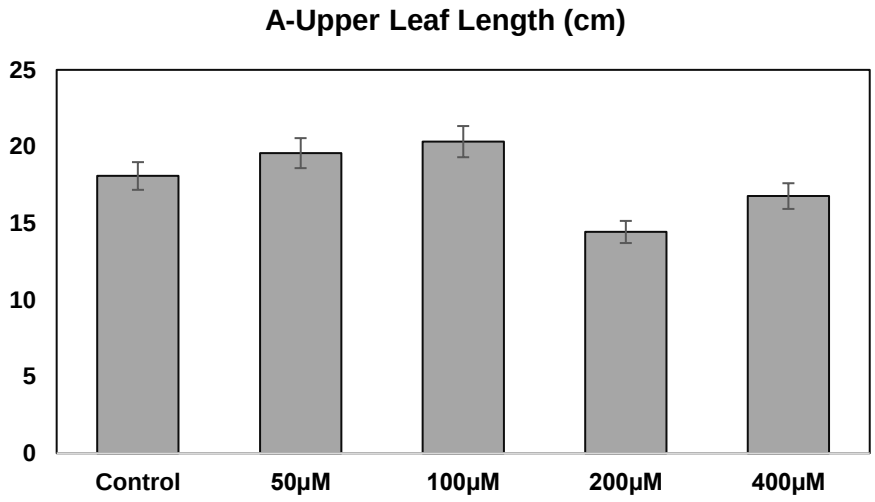
Seedlings were harvested at the end of 45 days experiment period. Parameters such as length and width of the upper and lower leaves, shoot length, and total chlorophyll amounts, were measured using calipers, millimetric rulers and chlorophyll content meter (Opti-Sciences Inc. CCM-300, USA). In addition, stomata and epidermal hair numbers of abaxial leaf surfaces (for each $1mm^2$ area) of control and experimental groups were counted using a digital microscope (Olympus, DSX510). For this purpose, cross sections were taken from the lower surfaces of the leaves using a sharp razor blade and preparations were examined directly under the microscope.

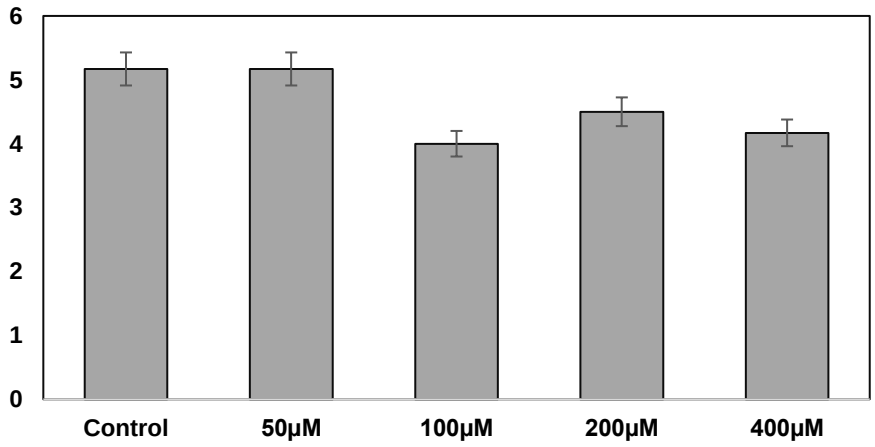
For measurement of Cd accumulation, leaves, shoots and roots were separated and oven-dried for 48 h at 80°C, milled in a micro-hammer cutter and fed through a 1.5-mm sieve. 0.5

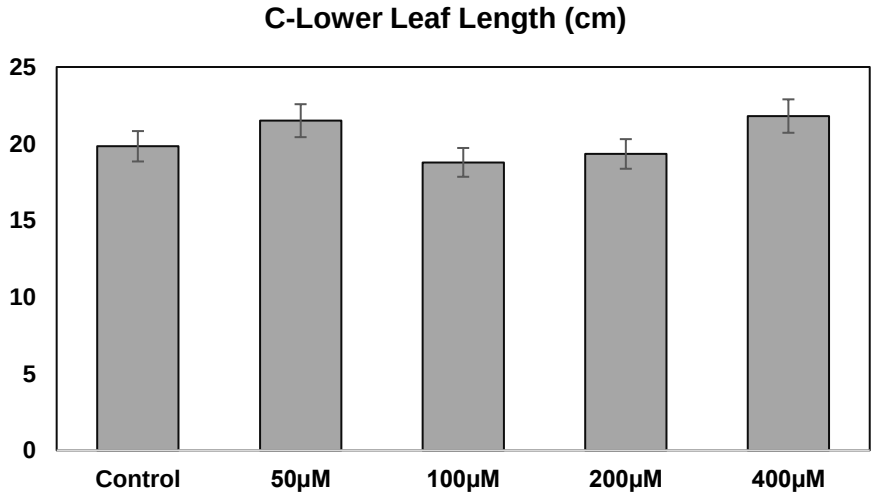
g of plant samples were placed in Teflon vessels and then 8 ml of 65% nitric acid (HNO_3) was added. Samples were mineralized in a microwave oven. After cooling, the samples were filtered with Whatman filters, and diluted to 50 ml with ultra-pure water. Cd concentrations were measured by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; PerkinElmer Optima7000DV).

Result and Discussion

In this study, leaf length, leaf width, shoot length and total chlorophyll amounts (for upper and lower leaves) were analyzed in wheat seedlings in response to different $CdCl_2$ concentrations (0, 50, 100, 200 and 400 μM). According to our results, it was observed that length of upper leaves (in cm) increased up to (19.57 and 20.32) at concentrations of 50 and 100 μM Cd, and decreased down to (14.43 and 16.77) at 200 and 400 μM compared to the control (18.08) (Figure 1-A). Upper leaf width remained the same (5.17) with control group in 50 μM Cd application, whereas decreased (4.00, 4.50 and 4.17) at 100, 200 and 400 μM compared to the control (Figure 1-B). Lower leaf length increased up to (21.50 and 21.80) at 50 and 400 μM Cd applications, and decreased down to (18.78 and 19.33) at 100 and 200 μM compared to the control (19.83) (Figure 1-C). In addition, it was observed that the lower leaf width increased (4.83) at 50 μM Cd application and decreased (3.83, 3.78 and 3.67) at 100, 200 and 400 μM compared to the control (4.33) (Figure 1-D). In this case, it can be said that Cd stress above a certain degree has a reducing effect on the leaf width in wheat. Although there were some fluctuations, it was observed that shoot length was decreased down to (16.57, 12.20, 13.02 and8.52) in all applied Cd concentrations compared to the control (18.95) (Figure 1-E).


A-Upper Leaf Length (cm)
25
20
15
10
5
0
Control
50µM
100µM
200µM
400µM


B-Upper Leaf Width (cm)
6
5
4
3
2
1
0
Control
50µM
100µM
200µM
400µM


C-Lower Leaf Length (cm)
25
20
15
10
5
0
Control
50µM
100µM
200µM
400µM

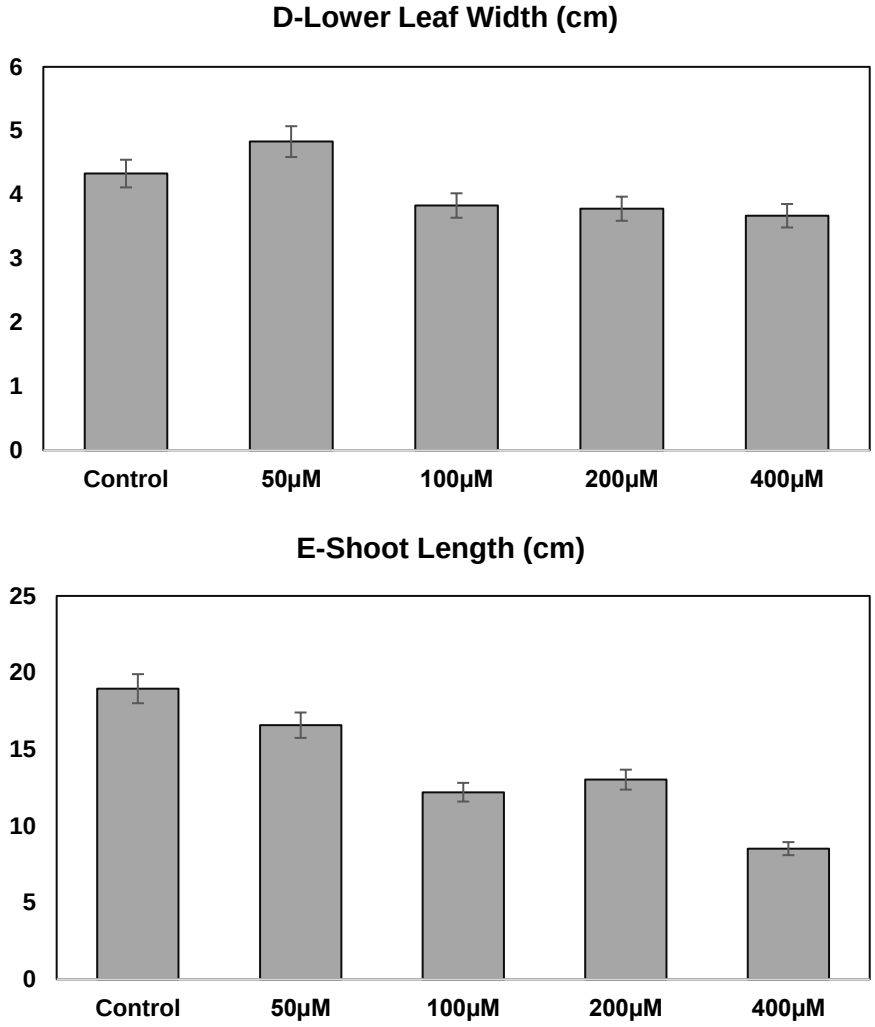


Fig 1 Some growth parameters of $CdCl_2$ treated wheat plants in different concentrations (0, 50, 100, 200 and 400 µM). **(A)** Upper leaf length and **(B)** width, **(C)** Lower leaf length and **(D)** width, and **(E)** Shoot length.

The total chlorophyll contents in the lower and upper leaves were measured using a chlorophyll content meter (CCM-300) (Figures 2-A and B). Accordingly, it was observed that total chlorophyll values (in mg m^{-2}) were increased in both upper (2.09, 2.04 and 1.99) and lower (2.11, 2.58 and 1.82) leaves at 50, 100 and 200 µM Cd applications compared to the control (1.75 for upper and 1.70 for lower leaves), but this value was lower (1.67 for upper and 1.63 for lower leaves) than that of the control at higher Cd concentrations (400 µM).

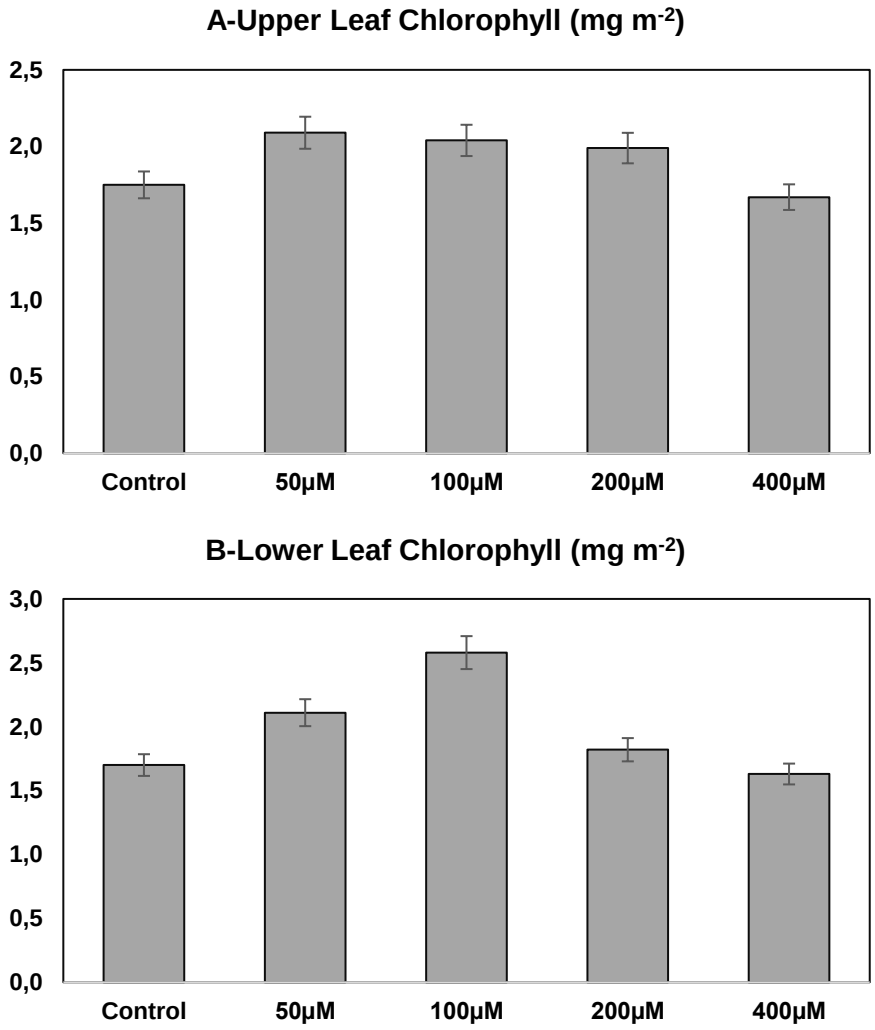


Fig 2 Total chlorophyll amounts of $CdCl_2$ treated wheat plants in different concentrations (0, 50, 100, 200 and 400 µM). **(A)** Upper leaf and **(B)** Lower leaf.

Cross sections were taken from the lower surfaces of the leaves, and stomata and epidermal hair numbers of abaxial leaf surfaces (for each $1mm^2$ area) of control and experimental groups were counted (Figure 3).

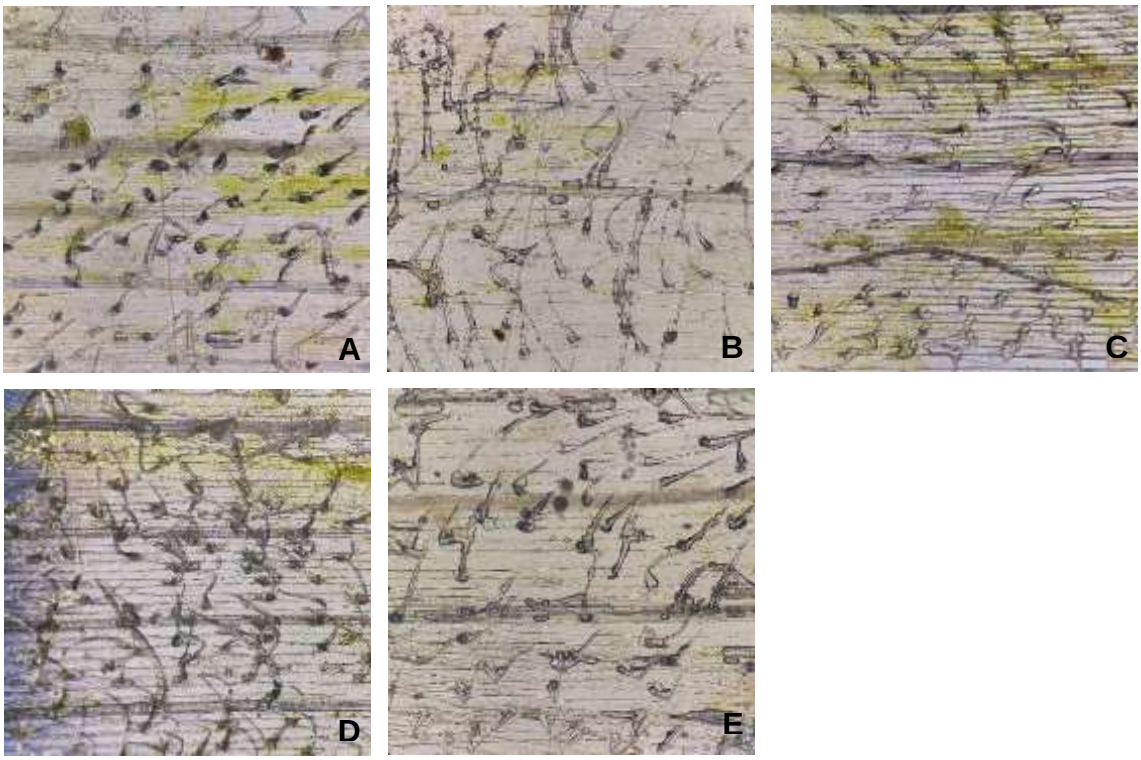


Fig 3 Anatomical view of abaxial leaf surfaces (1mm^2) treated with four different concentrations of $CdCl_2$. **(A)** 0 for control, **(B)** 50, **(C)** 100, **(D)** 200 and **(E)** 400 µM.

It was observed that stomata of wheat plants were negatively affected by Cd stress, and the stomata numbers on 1mm^2 surface area was 15.75 in control, and this value decreased to 13.00, 12.63, 7.75 and 3.20 at 50, 100, 200 and 400 µM Cd applications, respectively (Figure 4-A). However, the reduction was the lowest in 50 µM Cd application. Changes in the number of epidermal hairs on a 1 mm^2 surface area of leaves under Cd stress showed that, as a response of plants, the number of epidermal hairs were increased parallel to increasing Cd stress as; 83.00 for 50 µM, 103.14 for 100 µM, 99.60 for 200 µM. Conversely, the number of epidermal hairs was quite lower compared to the control (57.29) at 400 µM Cd application (Figure 4-B).

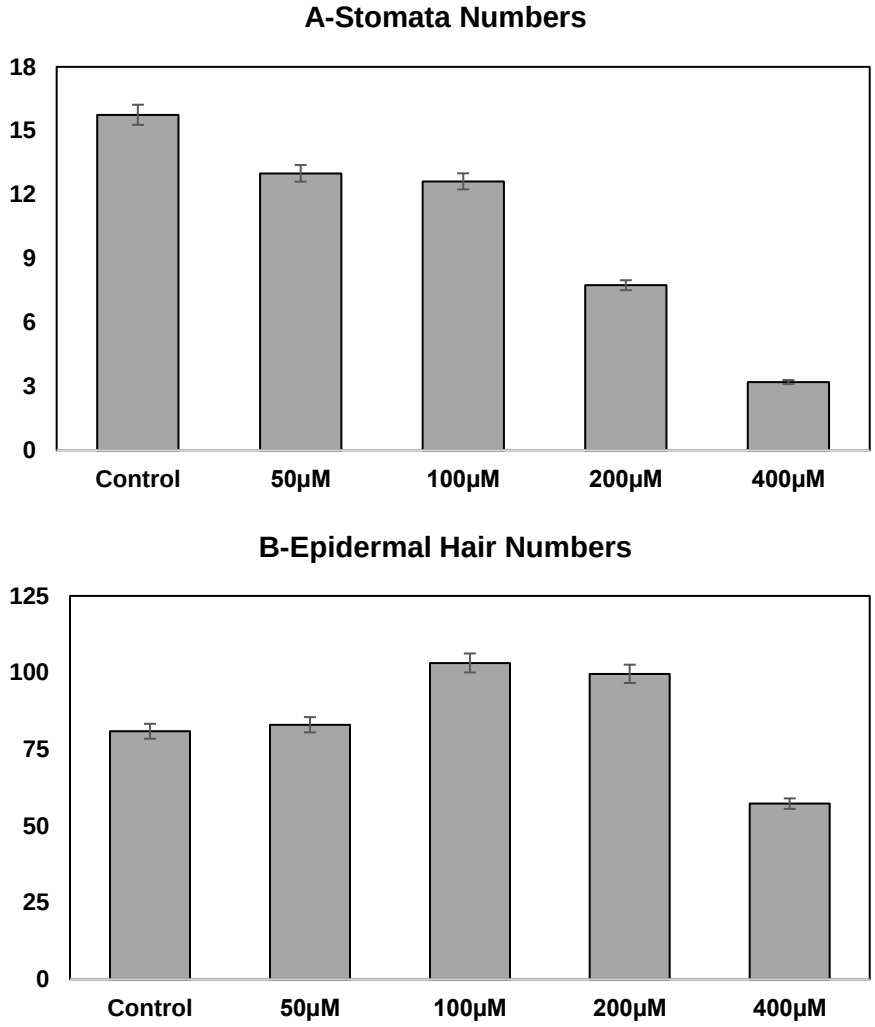


Fig 4 (A) Stomata and **(B)** Epidermal hair numbers of $CdCl_2$ treated wheat plants in different concentrations (0, 50, 100, 200 and 400 µM).

In a similar study, inhibited biomass production, decreased chlorophyll and carotenoid concentrations were observed in sunflower (*Helianthus annuus*) seedlings exposed to 20 µM Cd [44]. Another study indicated a decrease in root/shoot length in 7 days stressed (0, 50 or 200 µM Cd) Indian mustard (*Brassica juncea*) [45]. Also, reduced chlorophyll and carotenoid contents were observed in the same study. Inhibited relative growth and significant reduction in biomass and leaf area were observed in an aquatic fern (*Ceratopteris pteridoides*) at 20 and 40 µM Cd applications [46]. In another study conducted with cotton (*Gossypium*

hirsutum), growth inhibition, decreased plant height, reduced chlorophyll content, decreased biomass and leaf area were observed at 25, 50, and 100 μM Cd applications [47].

In *Groenlandia densa*, a gradual decrease in photosynethic pigmentation, chlorophyll *a* and *b*, and total chlorophyll ratios were observed in concentrations of 0-20 mg L^{-1} Cd [48]. 20-120 μM Cd were applied to two kenaf (*Hibiscus cannabinus* L.) varieties and reduction in the root and shoot lengths, root and shoot biomass were observed, while cv. Fuhong 1991 was essentially unaffected under the 20 μM treatment compared with control [49]. In 10-50 μM Cd treated *Brassica napus*, a reduction was observed in plant height, root length, leaf area and number of leaves, root diameter, root surface area, number of root tips, and root volume especially in higher concentrations [50]. 10, 25, and 50 μM Cd stress was applied to *Tagetes patula* and reductions in fresh and dry weights of roots/shoots, in biomass, growth rate, and chlorophyll content were observed [51]. In *Urtica pilulifera*, root and shoot lengths were gradually decreased from 13.83 cm (control) to 10.24 cm and 7.48 cm in roots and from 7.3 (control) to 4.8 cm and 3.7 cm in shoots after the application of 100 and 200 μM Cd [52]. Ozyigit et al., (2016) observed considerable reductions in chlorophyll concentrations of kalanchoe (*Kalanchoe daigremontiana*) plants after 60 days of Cd application at different levels (0, 50, 100, 200 and 400 μM) in comparison with control group. The reduction rates were ~40.57% for chlorophyll *a*, ~37.63% for chlorophyll *b*, ~20.58% for chlorophyll *a*/*b* and ~36.27% for total chlorophyll [34].

Furthermore, reduced seed germination [53], reduced growth and photosynthesis parameters [54], negatively induced oxidative stress [54], reduced photosynthesis and chlorophyll contents [56], detrimental effects on growth and physiological process [57, 58], reduction in growth and antioxidant system activity of seedlings [59], negative effect on photosynthesis and chlorophyll fluorescence [60], disturbance in roots proteins [61], negative effects in growth, oxidative stress and antioxidant system in seedlings [62], reduced glutathione reductase activity and isoforms in leaves and roots [63], were observed in wheat plants, which were treated to Cd in different concentrations.

According to similar studies on stoma and epidermal hairs (for 1 mm^2 leaf surface); in *Trigonella foenum graecum* Linn., similarly to our results, number of stomata was reduced from 33.20 (control) to 29.21, 27.20, 25.19, 22.17 and in contrary to the results we obtained

in our study, number of epidermal hair was reduced from 10.07 (control) to 9.92, 8.80, 7.80, 6.20 after application of 5, 15, 30, 50 μg/g Cd respectively [64]. In another study, stomata numbers of lemon balm (*Melissa officinalis*) were reduced from 3.5 to 2.6, 1.6, 0.8 in abaxial leaves and from 5.8 to 4.5, 3.3, 1.6 in adaxial leaves in 10, 20, 30 mg/kg Cd applications [65]. In *Cenchrus ciliaris* number of stomata reduced from 80.25 to 69.12 and 60.23 in 30 and 60 mg/kg Cd applications, respectively [66]. Furthermore, in *Cajanus cajan*, stomata numbers were reduced from 26.40 to 20.64, 19.04, 18.60, 18.40, 17.20 in 5, 10, 15, 25, 50 μg/g Cd applications respectively [67]. Lately, in *Biscutella auriculata*, stomata numbers were increased from 189.86 to 291, and epidermal numbers (like our study) from 22.37den 34.64, after the applications of 0 and 125 μM Cd [68]. This could be a response for survival of the plant in applied Cd concentrations, with increased stomata and epidermal hair numbers even when the plant is under stress. Also, related to species, and applied Cd concentrations the numbers of both stomata and epidermal hairs could be increased and decreased.

It is obviously seen from above studies that, there are different responses in plants depending on the adequacy of the defense system. Exact plant response may change from one species to another depending on the duration and concentration of the applied Cd stress. As seen in our study, it has been observed that plants can manage with Cd stress in lower concentrations, but cannot achieve this after a certain concentration level. After a certain stress level, decreases have been started in growth and development parameters.

When the average Cd accumulation values (μg/kg) that gave the above obtained changes were examined it can be seen that the Cd accumulation value of 21 in control groups of the roots, reached to 171, 322, 1076 and 1447 in 50, 100, 200 and 400 μM Cd applications, respectively; 11 in the control groups of the shoots became 74, 177, 560 and 952 in the experimental groups, and 15 in the leaves of control groups became 135, 282, 812 and 1094 in the experimental groups, respectively (Figure 5 A-B and C). Accordingly, the accumulations were seen as root>leaf>shoot.

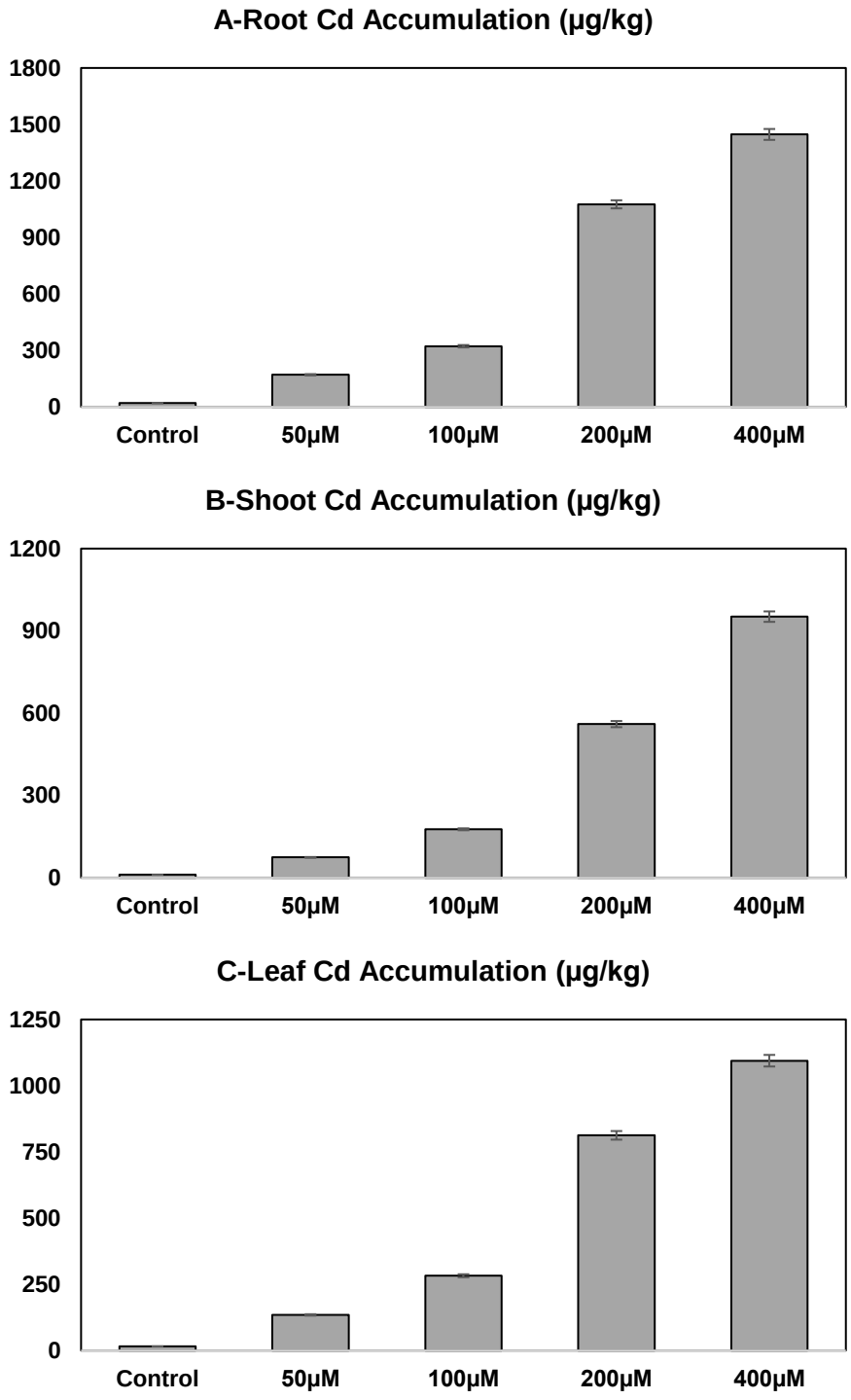


Fig 5 Cd accumulation values (µg/kg) in **(A)** roots, **(B)** shoots and **(C)** leaves of $CdCl_2$ treated wheat plants in different concentrations (0, 50, 100, 200 and 400 µM).

In a similar study, Di Baccio et al., (2014) applied lower (54.3 mg/kg) and higher (163 mg/kg) Cd concentrations to poplar (*Populus* x *canadensis*) clones and their Cd accumulations in plant parts (in mg/kg) were 7.6 (leaves), 4.6 (shoots), 57 (roots) for lower and 12.7 (leaves), 15 (shoots), 80 (roots) for higher Cd applications [69]. In *Apium graveolens*, Cd accumulations (in mg/kg) were between 220 and 300 (roots) and 16 and 40 (shoots) in 40-120 mg/kg Cd applications for 35-50 days [70], while in *Paspalum atratum* cv. Reyan accumulations (in mg/kg) were 349 (roots) 46 (shoots) in 8 mg/kg Cd applications [71]. In another study conducted with kalanchoe plants, Cd accumulations (in mg/ml) were increased from 0.629 to 3.164 in leaves, 0.460 to 2.890 in shoots, and 1.327 to 5.178 in roots after 0 to 400 μM Cd applications. This means that the increment levels were found to be ~5.03 fold in leaves, ~6.28 fold in shoots and ~3.90 fold in roots [34].

Roots' uptake ability, xylem sap's transporting efficiency, and ultimate re-translocation within plant seeds are main factors for Cd accumulation efficiency in plants [72, 73]. Plant roots play a key role for the uptake and accumulation of mineral nutrients together with water and heavy metals as well as Cd. Also, NRAMPs (natural resistance associated macrophage proteins) play an important role in Cd transport across the cell membrane [74, 75]. Translocation of Cd from roots to aerial parts, occurs both passive (transpiration) and active (ion channels) mechanisms. On the contrary, the ability of Cd uptake and accumulation also depend on plant type, species and genotype [2, 73, 76].

In this study, the wheat plant showed an increasing Cd accumulation parallel with the increasing Cd applications in its organs. Cd accumulation was the highest in the roots and then in the leaves in all applied concentrations. In addition, the plant was affected by all concentrations and represented different responses. Also, there have been positive changes in some parameters, especially in the 50 μM application. This has shown that the plant can manage lower level of Cd stress by activating its defense mechanisms in order to survive, growth and development. Nevertheless, this situation did not work in higher Cd concentrations, especially in applications of 400 μM. Almost all parameters were adversely affected by the application of 400 μM.

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