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The determination of Cd and Zn phytoremediation potential of buckwheat (*Fagopyrum esculentum*)

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ABSTRACT

Cadmium (Cd) which is absorbed and stored by the plants is causing too many metabolic changes of the plants. The biological function of cadmium is not known over the plants. The plant is generally taking in small amounts the Cd, this element is competing with the zinc (Zn). Buckwheat has relatively high biomass productivity, is adapted to many areas of the world, therefore buckwheat is widely used for the phytoremediation process. The aim of this study was to evaluate the phytoremediation capacity of the high-yielding plant buckwheat in soils contaminated with Cd and Zn. The soils were applied to different doses Cd (0–12.5–25–50–100 mg Cd kg^{-1} soil) and Zn (0–10–30 mg Zn kg^{-1} soil). Buckwheat seeds (Günes and Aktas cultivars) were sown and grown under greenhouse conditions. After harvest, Cd and Zn concentrations of plant biomass and grain yield (kg d^{-1}) and translocation factors for Zn and Cd were determined. Cadmium accumulation in biomass and grain significantly increased in dose-dependent manner. Günes genotype accumulated higher lead than Aktas genotypes.

Keywords: Buckwheat; Cadmium; Phytoremediation; Zinc; Translocation factor

1. Introduction

Cd is a heavy metal released into the environment as a by-product of power stations, heating systems, metalworking industries, waste incineration furnaces, urban traffic, cement factories and phosphate fertilizers. In low-anthropogenic pressure fields, which can be released as a result of the mineralization process [1], Cd is one of the toxic heavy metals due to its high mobility and toxicity at low concentration [2–4]. It has been reported that Cd pollution in the soil is the main limiting factor for food safety and agricultural land quality [5]. Cd toxicity causes adverse effects on carbon fixation, chlorophyll content and photosynthesis rate, thus preventing plant growth and reducing yield [6,7]. In plants, the water stress occurs due to the decrease in the stomatal conductance, the rate of transpiration and the leaf relative water content (RWC) [8,9].

Cd is not an essential element for plants. However, because of its similar chemical properties to Zn, there is an interaction between Cd and Zn during the intake by the plant, the transport from the roots, or the accumulation of the plant in edible parts of the plant [10–13].

Zn is an essential trace element for plants and animals and is an important micronutrient that can help minimize the accumulation of Cd in plants [6,14].

Cleaning of heavy metals in contaminated soil using plants is an emerging technology. Buckwheat is a plant that produces high-yield biomass and can adapt to large areas in the world [15]. However, recent studies have shown that buckwheat's heavy metal intake is high. It has been determined that lead (Pb) in buckwheat is accumulating at a dry weight of about 4,000 mg kg^{-1} and that Pb is also collected by transporting them to shoots rather than to roots alone [16]. Buckwheat is a very resistant species against aluminium toxicity [17]. Therefore, it has a great potential for use in phytoremediation [18]. Since it is used in human and animal nutrition, it is necessary to investigate the heavy metal intake state of the plant in detail.

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In this study, by applying different doses of Cd and Zn to the buckwheat plant, how the intake and accumulation of these elements happen in these plants, the interaction of these elements and the effect of this interaction on the growth and development of the plant were investigated.

2. Materials and methods

2.1. Experiment

The soil samples used in the experiment were taken from a depth of 0 to 30 cm at Selcuk University Agricultural Faculty Experiment area of Konya. In the experiment, the soil was passed through a 4 mm sieve. Afterwards, soil was weighed (3.6 kg soil pot^{-1}) and filled into pots. The experiment plan is as follows:

Different Zn doses (0(Zn_0)–10(Zn_{10})–30(Zn_{30}) mg Zn kg^{-1}) in the form of $ZnSO_4 \cdot 7H_2O$ and Cd doses (0(Cd_0)–12.5($Cd_{12.5}$)–25(Cd_{25})–50(Cd_{50})–100(Cd_{100}) mg Cd kg^{-1}) in the form of $3CdSO_4 \cdot 8H_2O$ were applied to the soil. Afterwards, the soil was subjected to incubation for 60 d in pots, then 10 seeds per pot were sowed.

Buckwheat seeds (*Fagopyrum esculentum*) (Günes and Aktas species) were taken from Bahri Dagdas International Agricultural Research. Buckwheat seeds were sown and grown for 180 d under greenhouse conditions. The test plants were irrigated by using pure water after the planting process.

2.2. Soil analysis

To test some initial characteristics of the soil, soil sample was vertically collected from 0 to 20 cm depth, dried in air, sieved to pass a 2 mm screen, analysed physically and chemically (sand, silt, clay and textural class) [19]; pH (H_2O,1:2.5) and EC (H_2O;1:2.5) [20]; organic matter (%) [21]; calcium carbonate by Scheibler calcimeter method as described by Ref. [20]; available P [22]; available Fe, Cu, Zn, Mn, Cd as Ref. [23]. Samples worked to be defined and characterized as soil classification that is shown in Table 1.

Table 1
Physical and chemical properties of the soil used in experiment

Textural class	Loam sand
Organic matter (%)	1.98
$CaCO_3$ (%)	12.94
pH (1/2.5)	7.75
EC (µS/cm)	249
Field capacity (%)	27.34
Ca (mg kg^{-1})	1,940
Mg (mg kg^{-1})	108.9
P (mg kg^{-1})	5.15
K (mg kg^{-1})	832
Cu (mg kg^{-1})	1.85
Cd (mg kg^{-1})	0.07
Mn (mg kg^{-1})	14.73
Zn (mg kg^{-1})	0.74
Fe (mg kg^{-1})	3.69
Na (mg kg^{-1})	214.8

2.3. Observation the plant growth and harvesting

The following data were taken in all pots to obtain the effect of Zn and Cd on plant growth.

2.3.1. Chlorophyll content in leaf

Chlorophyll values were determined on the 80th day and their averages were obtained with three readings from each leaf with a chlorophyll meter (SPAD (Soil Plant Analytical Division) 502 chlorophyll meter), which is a sufficient and appropriate device for measuring the chlorophyll amount in plants [24].

2.3.2. Relative leaf water content

Fresh weights were taken to determine the proportional water content of six leaf samples taken from each pot. Leaf samples were weighed fresh (FW) and placed in distilled water for 24 h to rehydrate. Leaf turgid weight (TW) was measured and then leaves were dried at 65°C for 48 h to determine dry weight (DW).

Relative leaf water content (RLWC) was calculated as RLWC = [(FW – TW)/(FW – DW)] × 100 [25].

2.3.3. Biomass content (kg d^{-1})

The stem weights of the harvested buckwheat plants are taken and calculated using formula of the Biomass content = ((250,000 × stem weights)/soil weight)/1,000.

2.3.4. Grain yield (kg d^{-1})

Matured grains were collected. The grain harvested from each pot were weighed and calculated in kg d^{-1}.

2.3.5. Zn and Cd contents of leaf, grain and stem

Plants were harvested by cutting the shoots from the soil surface and washed with deionized water. Plant leafs and stems were dried for 48 h at 65°C and were ground. Harvested plants have been ashed in a microwave oven (CEM Mars 5) by using 5 mL of 65% HNO_3. Following the digestions Zn and Cd have been analysed by using ICP-AES (Vista AX). To verify the mineral element analysis, we have used plant samples with STD CDV-1 with a standard certificate, amount of which elements have been specified.

2.3.6. Transport factor

Transport factor (TF) is a criterion for the ability of the plant to take metal concentrations in soil solution and are used to evaluate hyperaccumulator plants in terms of their properties [26].

$TF = (M_g + M_s)/T_{SM}$

M_g = Grain metal concentration (mg kg^{-1});

M_s = Stem metal concentration (mg kg^{-1});

T_{SM} = Total metal concentration of harvested soil (mg kg^{-1}).

2.4. Statistical analyses

The data attained as a result of the study have been subjected to analysis of variance (ANOVA) variance analysis with the use of Minitab 16 Statistical Software package program. The smallest differences between the applications have been determined in a way that they will be $P < 0.05$ with Tukey test.

3. Results and discussions

3.1. Leaf proportional water content (%) and chlorophyll content of buckwheat cultivars

The effect of different Zn and Cd doses and Cd–Zn interactions on leaf proportional water content (%) and chlorophyll content of buckwheat cultivars are presented in Figs. 1 and 2. The RLWC (%) of Aktas cultivars at 10 and 30 mg Zn kg^{-1} doses are generally higher than in Günes cultivar. The effects of Zn and Cd doses *applications on* RLWC *are statistically insignificant.* Results of Ref. [27] showed that leaf RWC did not change under 180 mg Cd kg^{-1} compared with the control of tolerance threshold of centipede grass. In another research RWC in water hyacinth increased slightly, up to 400 mg L^{-1} Pb concentration, and decreased slightly at higher concentrations compared with the control [28].

When the effect of Cd doses, Zn doses and Cd–Zn interactions on the chlorophyll content of buckwheat cultivars was examined, there was no statistically significant difference among the cultivars, Zn doses and Cd doses. However, there were statistically significant differences observed between the cultivars × Zn doses ($P < 0.01$) and the cultivars × Zn × Cd doses ($P < 0.05$).

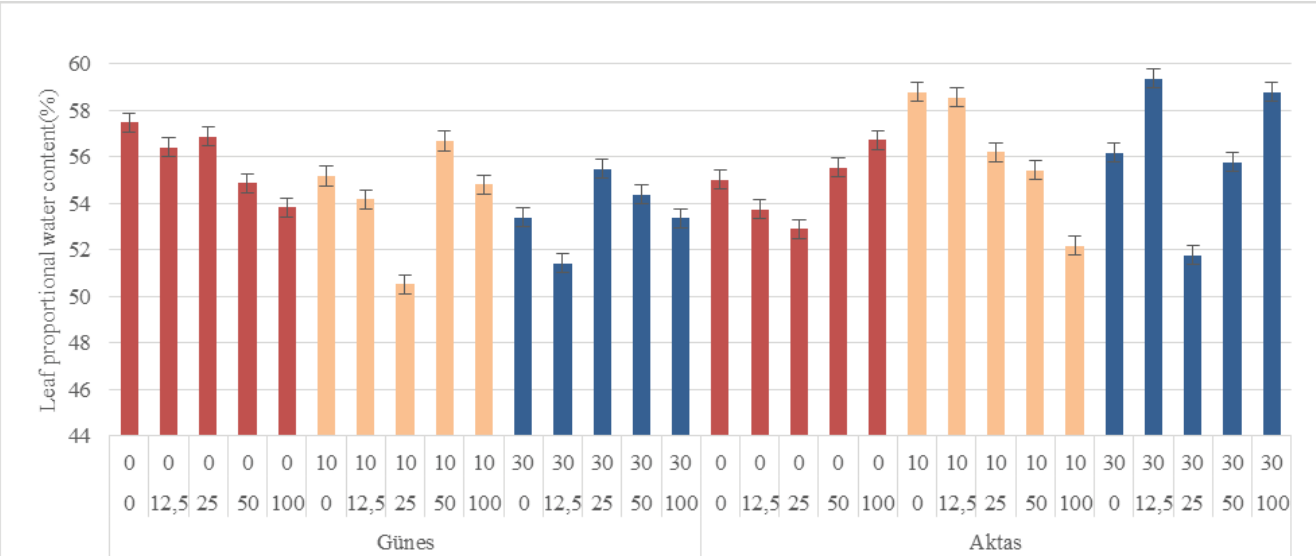

Fig. 1. The effect of different doses of cadmium and zinc on buckwheat cultivars on RLWC (%) (Zn doses: 0–10–30 mg kg^{-1}; Cd doses; 0–12.5–25–50–100 mg kg^{-1}doses).

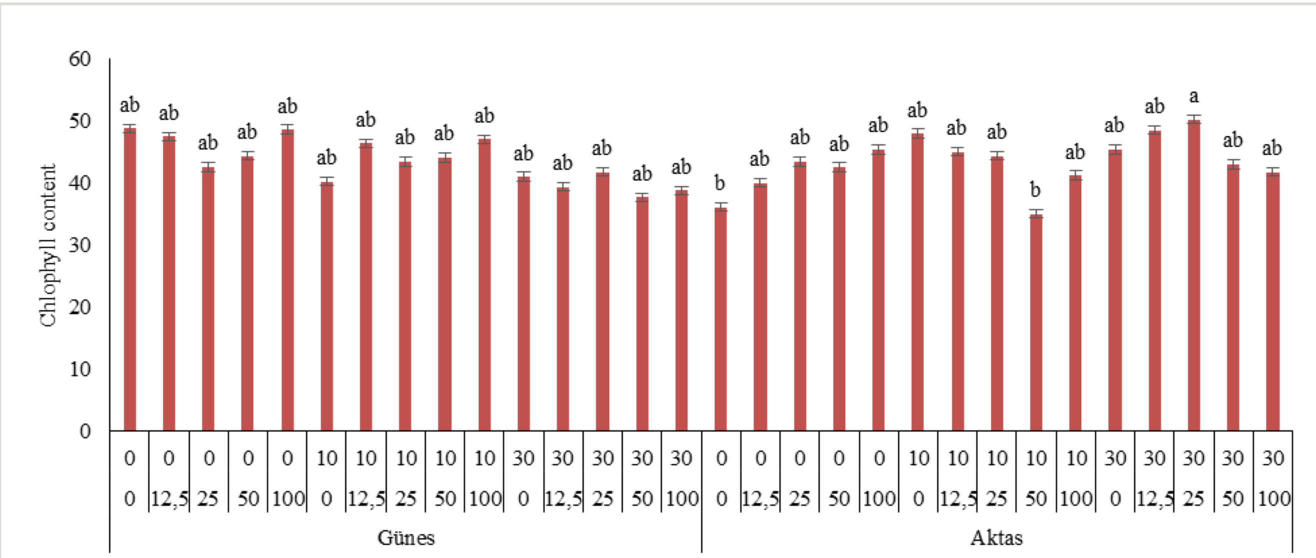

Fig. 2. The effect of different doses of cadmium and zinc on buckwheat cultivars on leaf chlorophyll content (Zn doses: 0–10–30 mg kg^{-1}; Cd doses; 0–12.5–25–50–100 mg kg^{-1}doses).

The highest content of chlorophyll was found in the application of $Cd_{25}Zn_{30}$ at Aktas cultivar and the lowest content of chlorophyll in $Cd_{25}Zn_{30}$ application. The amount of chlorophyll was reduced with the increasing Zn doses in the Günes cultivar. The amount of chlorophyll increased with increasing doses of Cd at doses of Zn_0 in the Aktas variety; but chlorophyll content reduced with increasing doses of Cd at Zn_{10} and Zn_{30} doses. In Günes variety, chlorophyll content decreased with increasing doses of Cd at plants treated with Zn. In this case, chlorophyll content was reduced due to the negative interaction between Cd and Zn at the presence of increased Zn. Chlorophyll a and total Chl contents in the mature leaves of *Helianthus annuus* were significantly decreased in plants grown with both Cd concentrations in the nutrient solution [29].

3.2. The grain yield and biomass

When the grain yield was examined, statistical differences were observed between buckwheat cultivars ($P < 0.01$), between the Zn doses ($P < 0.01$), the cultivars × Zn interactions ($P < 0.01$) and the cultivars × Zn × Cd interactions ($P < 0.05$) (Figs. 3 and 4). Grain yield has reduced with increasing Cd doses in the Günes cultivar. Generally, the increasing doses of Cd in the Günes cultivar caused the grain yield (kg d^{-1}) to decrease. The grain yield (kg d^{-1}) of the Aktas cultivar

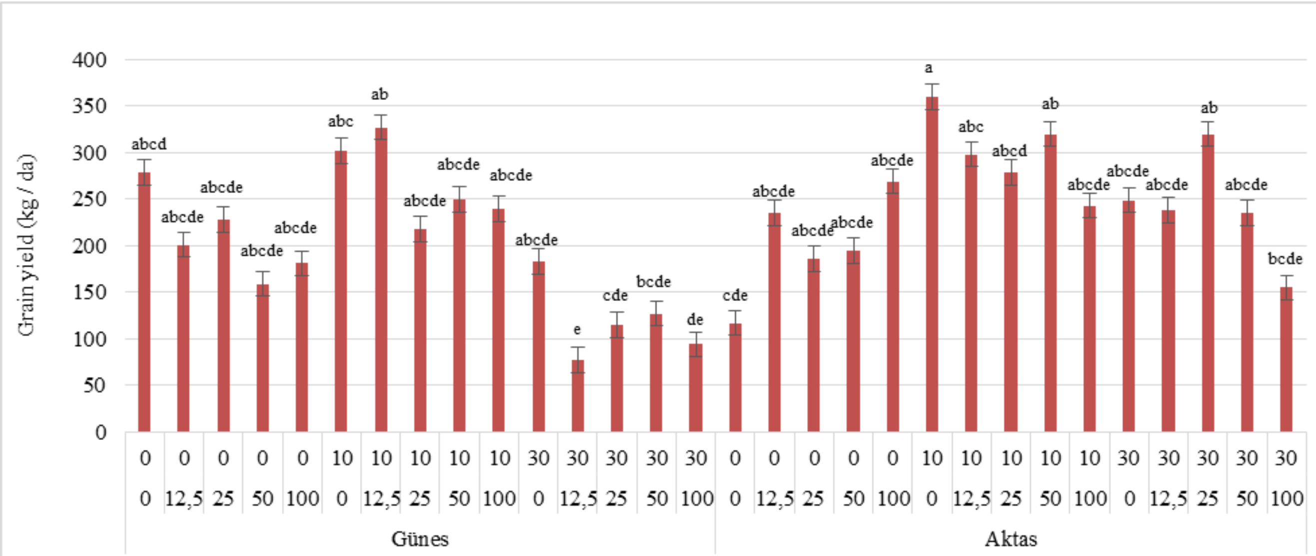

Fig. 3. The effect of different doses of cadmium and zinc on buckwheat cultivars on grain yield (kg d^{-1}) (Zn doses: 0–10–30 mg kg^{-1}; Cd doses; 0–12.5–25–50–100 mg kg^{-1}doses).

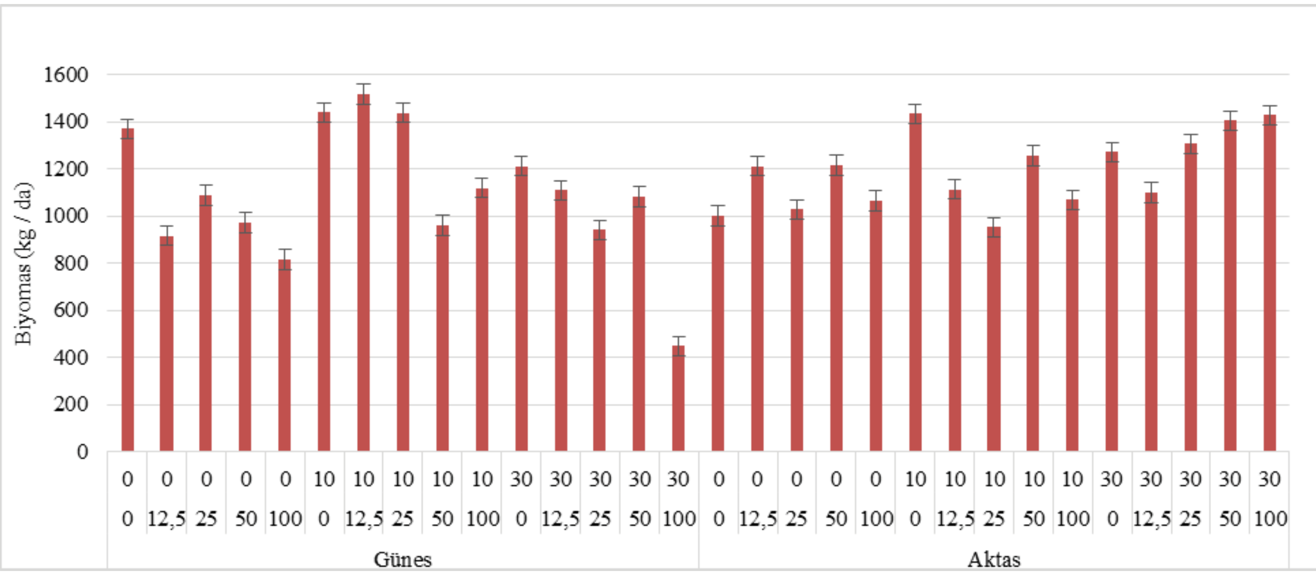

Fig. 4. The effect of different doses of cadmium and zinc on buckwheat cultivars on biomass (kg d^{-1}) (Zn doses: 0–10–30 mg kg^{-1}; Cd doses; 0–12.5–25–50–100 mg kg^{-1}doses).

was not affected as badly as the Günes cultivar from the Cd application. The highest yield was 327 kg d^{-1} in the $Cd_{12.5}Zn_{10}$ application in the Günes cultivar and 360 kg d^{-1} in the Cd_0Zn_{10} application in the Aktas cultivar (Fig. 3). Ref. [30] has reported that the grain yield, Zn and Cd contents in leaves and grains of Kızıltan and Dagdas wheat varieties varied significantly ($P < 0.01$) by applications of Zn and Cd, and was found especially an interaction relationships between the Zn and Cd in term of Cd content in grain.

When biomass was examined, statistically significant differences were observed between the Zn doses ($P < 0.01$) and the cultivars × Zn interaction ($P < 0.01$) (Fig. 4). The biomass decreased with increasing doses of Cd in the Günes cultivar and increased with the increasing Zn doses. In the Aktas cultivar, the biomass did not change with the increasing doses of Cd, and the biomass increased with the increase of Zn doses. The highest yield is 1,517 kg d^{-1} in the application of $Cd_{12.5}Zn_{10}$ in the Günes cultivar, whereas it is 1,434 kg d^{-1} in the application of Cd_0Zn_{10} in Aktas cultivar (Fig. 4).

3.3. TF for Zn and Cd

TF is a parameter used for the ability of plants to transfer metal from the root region to the top part of the root or from the body tissue to the seed or the fruit. It is also a parameter that serves to evaluate the transfer potentials of plants

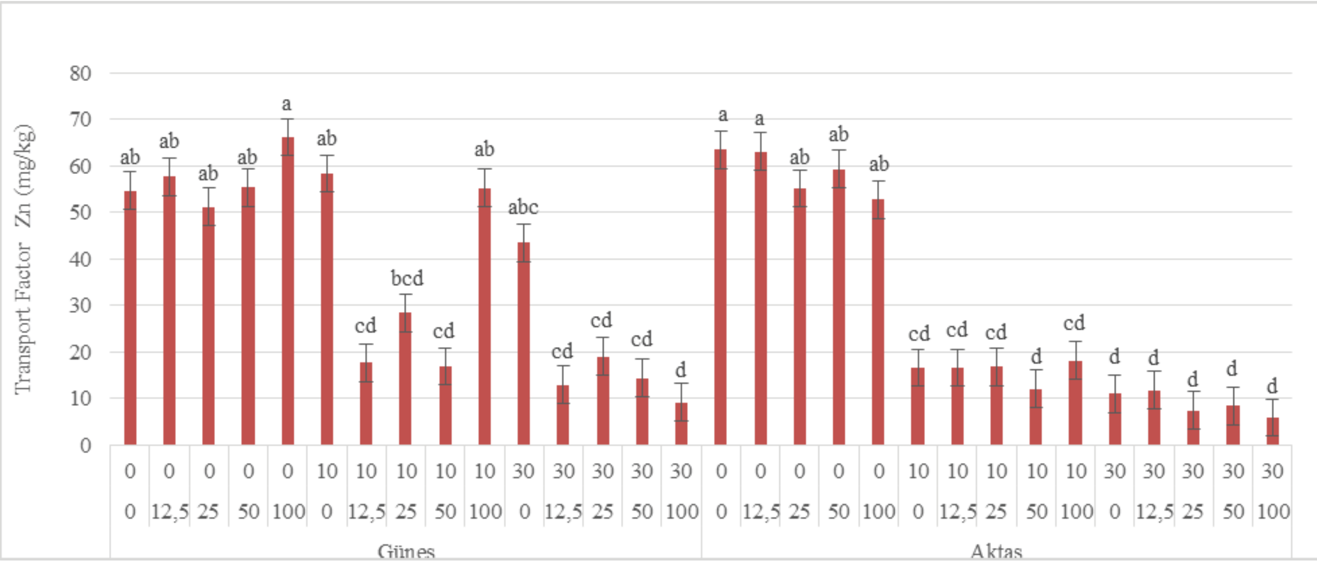

Fig. 5. The effect of different doses of cadmium and zinc on buckwheat cultivars on transport factor (Zn) (Zn doses: 0–10–30 mg kg^{-1}; Cd doses; 0–12.5–25–50–100 mg kg^{-1}doses).

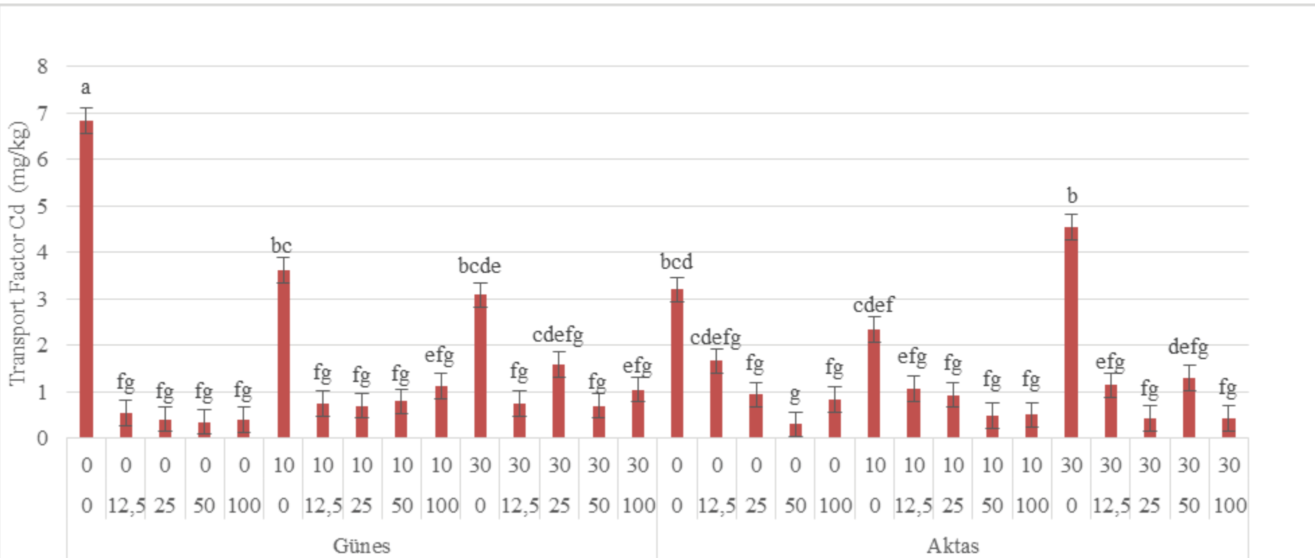

Fig. 6. The effect of different doses of cadmium and zinc on buckwheat cultivars on transport factor (Cd) (Zn doses: 0–10–30 mg kg^{-1}; Cd doses; 0–12.5–25–50–100 mg kg^{-1}doses).

[31,32]. When TF was examined, statistically significant differences were observed in all applications, that is, between Zn, Cd doses and buckwheat cultivars ($P < 0.01$) and in the cultivar × Zn, cultivar × Cd, Zn × Cd, cultivar × Zn × Cd interaction ($P < 0.01$). With increasing doses of Cd in the Günes cultivar, TF (Zn) decreased especially with Zn_{30} treatment; these reductions in Aktas cultivars are notable (Fig. 5). A similar phenomenon was also observed in the Cd TF. Significant differences in Cd doses, cultivar × Cd, Zn × Cd and cultivar × Zn × Cd interactions ($P < 0.01$) were observed (Fig. 6). The TF (Cd) value is higher when there is no Zn in the medium. As the Cd dose increases, the TF (Cd) value decreases.

As a result of the research, TLF < 1 indicates that the pollutant element Zn and Cd in the plant root is not transported to the body and seed parts with high efficiency. The data obtained as a result of the research are in accordance with many studies on this subject.

The Pb TLF values were determined in the range of 0.2–3 in the study performed with *Thlaspi caerulescens* plant collected from five different regions [33]. Usman and Mohamed [34] conducted the study using a chelator on sunflowers (*H. annuus*) and corns (*Zea mays*), the Pb TLF values of the chelators used were 0.14, 0.69, 0.18, 0.15 and the Cd TLF values of the chelators used were 0.38, 0.98, 0.52, 0.40, respectively. In a study using canola (*Brassica napus* L.), root and shoot yield of plants decreased with increasing ethylenediaminetetraacetic acid (EDTA) doses [35]. In another research, the yield of the roots and shoots of patience dock decreased with increased application of EDTA, however, the mass of cadmium in the roots and shoots increased with EDTA application [36].

4. Conclusions

As a result, the increasing Cd and Zn doses have affected the Günes cultivar more negatively compared with Aktas cultivar. The yield and biomass values of Aktas cultivar are higher than that of the Günes cultivar. According to Grant and Bailey, Cd accumulation in plants can be affected or not affected by increased Zn. There can be large differences between species and even between different cultivars of the same species [37]. Franzaring et al. [38] reported that common buckwheat is a suited candidate for cultivation on metal polluted soils. Also, when Cd is present in the nutrient medium, it causes a change in different metabolic processes and a strong inhibition of growth [39]. Some researchers report that the interaction between Cd and Zn is antagonistic [40,41], but some researchers report that it is actually synergistic [42–44]; the Zn content can inhibit Cd adsorption and thus cause a low Cd concentration in plants [2,44]. In this study, it is observed that there is a negative interaction between Cd and Zn at high doses of application, and even when the Cd is not in the medium, the grain yield, chlorophyll and biomass are better in the Günes cultivar, but the increasing Cd doses negatively affect these values. In the Aktas cultivar, it was found that Cd application had a less adverse effect on the plant and the plant was more resistant to Cd toxicity.

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