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The Effect of Cadmium and Lead Exposure on the Development and Physical Structure of Quinoa (*Chenopodium qinoa* Willd.)

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Abstract: The presence of soil affected by cadmium (Cd) and lead (Pb) and their metals is increasing daily. Quinoa is a plant that can grow in harsh conditions due to being a halophyte plant. This study was planned to examine the effects of lead and cadmium metals, two of the most common metals today, on plant growth, physiology and some biochemical properties of quinoa. Within the scope of the study, heavy metal applications were made as 1 control (no application), 4 doses of Cd (50, 100, 150 and 200 mg/kg), and 4 doses of lead (500, 1000, 1500, 2000 mg/kg). In this study, which was carried out in Atatürk University Plant Production and Application Center greenhouse conditions, it is observed that the metals applied negatively affected the parameters in the plant, and cadmium metal had a more toxic effect than lead metal. It is determined that the fresh weight of the plant lost 62% at the Cd 200 level and 45% at the Pb 2000 level compared to the control group.

Kadmiyum ve Kurşun Uygulamalarının Kinoanın (Chenopodium qinoa Willd.) Gelişimi ve Fiziksel Yapısı Üzerine Etkisi

Anahtar Kelimeler
Kinoa,
Chenopodium quinoa Willd,
Ağır Metal,
Kadmiyum,
Kurşun

Öz: Cd ve Pb metallerinin etkilediği toprak varlığı her geçen gün artmaktadır. Kinoa halofit bir bitki olduğundan tuzlu topraklarda yetişme kabiliyetine sahiptir. Bu çalışma, günümüzde sıkça rastlanan metallerden ikisi olan kurşun ve kadmiyumun, kinoada bitki büyümesi, fizyolojisi ve bazı biyokimyasal özelliklerine olan etkilerini incelemek için planlanmıştır. Çalışma kapsamında 1 kontrol (hiçbir uygulama yapılmayan), Cd 4 doz (50, 100, 150 ve 200 mg/kg), kurşun 4 doz (500, 1000, 1500, 2000 mg/kg) olacak şekilde ağır metal uygulamaları yapılmıştır. Atatürk Üniversitesi Bitkisel Üretim ve Uygulama Merkezi sera koşullarında yapılan bu çalışmada uygulanan metallerin bitkideki parametreleri olumsuz etkilediği, kadmiyum metalinin, kurşun metalinden daha toksik bir etkiye sahip olduğu görülmüştür. Bitki yaş ağırlığının Cd 200 seviyesinde kontrol grubuna göre %62, Pb 2000 seviyesinde ise %45 oranında kayba uğradığı tespit edilmiştir.

1. INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) belongs to the Chenopodiaceae family, growing in the Andes Mountains region of South America [1]. Its extraordinary adaptation to climate and soil conditions increases the spreading worldwide. It is widely grown worldwide, including Europe, North America, North Africa and Asia [2, 3]. The chemical and nutritional compositions of quinoa are greatly affected by genetic diversity, geographical locations and growing environmental conditions [4]. It is reported that the number of countries where quinoa grows was 8 in 1980 and 40 in 2010, which is more than 100 in 2021 [5]. Quinoa cultivation in Turkey started in the 2000s. It was initially a product exported from South American countries and sold in luxury markets at high prices, but today, it has begun to take place at more affordable prices with the spread of domestic production.

Studies on identifying and developing suitable varieties for Turkey among the quinoa species brought from abroad are continuing. Limtar White, the first possible variety for Turkey, received production permission in 2016. According to unofficial figures, it is estimated that it is cultivated in approximately 15 thousand decree areas in our country [6].

Quinoa seeds are used in human nutrition, like bulghur and rice, and since it does not contain gluten, they are known as a safe food source for celiac patients, a genetic disease [7]. The protein content of quinoa seeds is between 7.5% and 22.1%, which means that the plant is a good protein source [8, 9]. Quinoa is also used as a forage plant and in animal nutrition in dry grass and silage form due to its ability to be easily ensiled [10]. The straw and hay of the plant have been included in the nutrition programs of sheep, cattle and horses in South America for many years [11].

Quinoa is known as one of the most essential grains of the 21st century due to its nutritional and biological properties and its resistance to environmental conditions [12]. There is particular interest in quinoa due to its ability to adapt to harsh conditions, including saline soils, drought and stressful environments. The reason why it is resistant to arid conditions is that it has a tap root system. [13]. Most scientific research on quinoa focuses on its behavior on abiotic stresses such as drought and salinity. In recent years, many studies have been conducted on this plant's harsh temperature conditions and heavy metal stress [14]. Some quinoa varieties can accumulate high amounts of metal in their leaf tissues even if the soil or environment contains a low rate of heavy metal. This situation increases the importance of the plant when assessed with heavy metal pollution increasing [15].

Overuse of chemical fertilizers in agricultural activities causes the accumulation of potassium, nitrogen, phosphorus and some nutrients in the soil. Disproportional use of pesticides and chemical fertilizers, industrial activities and environmental pollution cause heavy metal accumulation. It is reported heavy metal pollution affects approximately 235 million hectares of arable land worldwide [16]. As seen in all kinds of pollution, firstly plants are affected by heavy metal pollution [17]. Heavy metals accumulate in soils on the surface or at depths near the surface [18]. Heavy metal stress conditions negatively affect plant growth, yield and productivity. As a result of these adverse effects, the plants' metabolic, physiological and biochemical properties are involved [19]. These metals, which accumulate in plants, enter the food chain, reach other living organisms, and can increase toxic levels for human health. Each plant has the potential to absorb and accumulate heavy metals. Many studies have shown variations in metal uptake, even among different genotypes of the same plant species [20].
Cadmıım and lead are the most known heavy metals. Cd enters the human body through the food chain and causes serious health problems [21]. The use of cadmium in many industrial branches increases the risk of contamination of foodstuffs with soil, air and water. It is one of the leading metals from agricultural activities [22, 23]. It causes oxidative stress, growth delay and inhibition of plant enzymatic reactions [24, 25, 26]. In addition, it causes chlorosis (yellowing) and color change (browning) at the root tips and that can cause plant death.

Heavy metals prevent the transportation of nutrients from the root to the leaves and branches and negatively affect photosynthesis by suppressing chlorophyll biosynthesis [27, 28]. Also, some lead is present in plants, but that does not mean lead is an essential component in metabolism [29]. Increasing its concentration in the soil suppresses the growth and development of plants and decreases yield [30, 31]. Additionally, Pb negatively affects seed germination and suppresses the plant's root development, even causing the death of the plant [32, 33, 34, 35]. Halophytic plants such as quinoa can be used for cleaning soils contaminated with lead and cadmium metals [19]. Within the scope of this study, the effects of different doses of cadmium and lead metals on plant growth, physiology and some biochemical properties of quinoa were examined.

2. MATERIAL AND METHOD

The study uses the seeds of the Titicaca variety of quinoa (*Chenopodium quinoa* Willd.). The experiment was performed for 9 different applications (control + 4 Cd applications + 4 Pb applications) and three replications for each. There were two pots in each repetition. It was applied in a completely randomized design with 54 (9x3x2=54) pots. The volume of each pot is 2 liters and it was filled with a mixture of garden soil, peat and sand. In the control group, heavy metal application was not made. Heavy metal applications were performed by four different doses of cadmium and lead. Cd dose was performed by using $CdSO_4.8H_2O$ with 50, 100, 150 and 200 mg/kg. Lead doses were applied using $PbNO_3$ with 500, 1000, 1500 and 2000 mg/kg. Heavy metals were added into the soil mixture in each pot by a homogeneous mixture and each incubation period was performed for three weeks. Soil and heavy metal concentration rates were determined from the literature reviews [36, 37, 38]. At the end of the incubation period, 7 seeds were planted in each pot with a depth of 1 to 1.5 cm. After the seedlings were formed, thinning was done and 4 seedlings were left in each pot. The moisture rate of the soil was constantly checked and required watering was applied at appropriate rates. The potting work was completed in 50 days. Afterwards, the following measurements and analyzes were made.

2.1. Physical Methods

Within the scope of the study, seedling height (cm) and number of leaves are measured. Additionally, fresh and dry weights (g) of roots and above-ground plant parts were determined. The samples were kept at 68 °C to determine the dry weights until they reached constant weight. LICOR, LI-3100 model (Lincoln, NE, USA) device was used for leaf area measurement and SPAD-502 model chlorophyllometer (Konica Minolta Sensing, Inc., Japan brand) was used for chlorophyll determination.

2.2. Electrical Conductivity (EC)

When plants are exposed to stress, the damage usually occurs in leaf tissue or cell membranes. The damage rate is measured by measuring the electrical conductivity in fresh leaf tissues. In this study, two plants were randomly selected from each replicate and the youngest leaf on the plant was used for electrical conductivity measurement. Samples taken from the leaves with a diameter of 1 cm were put in 20 ml of pure water and shaken for 24 hours and then electrical conductivity was measured [39]. In this way, permeability, which also indicates damage in the cell membranes, was determined (EC1). Afterwards, the samples were put in an autoclave at 121 ^{0}C for 20 minutes to ensure the complete disintegration of cells and tissues and then the second measurement was performed (EC2). Finally, the required calculation determines the relative electrical conductivity ratio (EC1/ EC2) [39].

2.3. Leaf relative water content (LRWC)

Leaf relative water content (LRWC) was determined according to [39].

2.4. Hydrogen Peroxide (H_2O_2) Analysis

For this analysis, the methods and applications mentioned in the studies of Sahin et al. and Caşka Kılıçaslan et al. were preferred [40, 41]. The procedures and sequences mentioned here were applied to quinoa with minor modifications. In this context, firstly, leaf tissues were homogenized in 0.1% (w/v) TCA solution on ice and the homogenate was centrifuged at 12,000 g for 15 minutes. The resulting supernatant was added to the mixture of 10 mmol l^{-1} potassium phosphate buffer (pH 7.0) and 1 mol l^{-1} potassium iodide. Following this process, measurements were made at a 390 nm absorbance level. Measures made at different intervals were evaluated using a standard curve created under appropriate conditions.

2.5. Lipid Peroxidation (Malondialdehit-MDA) Analysis

For MDA analysis, the methods mentioned in the studies of Sahin et al. and Caşka Kılıçaslan et al. were used [40, 41]. The leaves were put into the fine powder using liquid nitrogen and then leaf extracts were extracted using cold ethanol. The obtained crude extracts were centrifuged at 12,000 g for 20 minutes with heated trichloroacetic acid, thiobarbituric acid, butylated hydroxytoluene and a supernatant mixture. Then, the mixture was cooled in an ice bath and centrifuged again. Finally, measurements were made at 400, 500 and 600 nm absorbance values. Finally, the MDA concentration is defined with the help of a coefficient of 155 mmol L^{-1} cm^{-1}.

2.6. Statistical Assessment

Within the scope of the study, a random parcel trial design was preferred and three repetitions were made. SPSS 18 package program was used to evaluate the results, analysis of variance and Duncan's multiple comparison test was applied.

3. RESULTS

The effects of cadmium and lead metals at different concentrations on quinoa development are presented in Table 3.1. The plant height is negatively affected by Cd and Pb applications. The results for both metals are statistically in the same group. The lowest values for fresh and dry weights of plants and roots were determined at the highest metal concentrations. For the Cd 200 level, fresh plant weight decreases by 62% compared to the control, and for the Pb 2000 level, it reduces by 45%. Heavy metals are known to prevent the plant from absorbing nutrients from the soil. This situation restricts plant growth parameters such as plant and root lengths, number of leaves and leaf area [42, 43]. Similar results were obtained in our study when compared to previous studies. Fresh root weight decreases by 64% and 50% in Cd 200 and Pb 2000 exposures. Heavy metal deconstructs the structure of cell membranes and damages the root surfaces, negatively affecting root length [44]. In a similar study made on quinoa, root dry weight decreased by 66-63% in Cd 90 and Pb 150 applications [19]. In another study, where the heavy metal concentrations were applied to annual grass (Lolium multiflorum Lam.) at the same rate, even seed emergence was not achieved with the last two doses of cadmium metal, while all seeds emerged ultimately in quinoa [45].

Table 3.1. Effect of Cd and Pb exposure on quinoa development

Exposure	Plant length (cm)	Plant fresh weight (g)	Plant Dry Weight (g)	Root Fresh Weight (g)	Plant Dry Weight (g)
Control	9,350±1,343a	1,910±0,495a	0,300±0,141a	0,145±0,007bc	0,100±0,014b
Cd 50	9,433±1,101a	1,600 ±0,150ab	0,227±0,028ab	0,120±0,017de	0,097±0,006b
Cd 100	9,233±1,692a	1,300±0,236bc	0,153±0,025bc	0,110 ±0,100e	0,083±0,006b
Cd 150	10,400±2,007a	1,397±0,506abc	0,163±060bc	0,087±0,006f	0,067±0,006bc
Cd 200	9,133±0,924a	0,723 ±0,171d	0,117±0,028c	0,053±0,006g	0,027±0,006d
Pb 500	9,667±1,069a	1,583± 0,231ab	0,167± 0,035bc	0,170±0,010a	0,103±0,006b
Pb 1000	10,500±0,854a	1,677±0,140ab	0,200±0,017bc	0,157 ±0,012ab	0,867±0,057a
Pb 1500	9,833±0,416a	1,407±0,086abc	0,177±0,006bc	0,134±0,006cd	0,080±0,010b
Pb 2000	9,734±0,321a	1,057±0,206cd	0,140 ±0,026bc	0,073±0,023fg	0,040±0,010cd

There is no statistical difference between the means shown with the same letter given. The effects of lead and cadmium applications on the number of leaves in quinoa are shown in Table 3.2. The leaf area of the control group, which is 12,750, decreased to 9,667 for cadmium 200 (24% decrease). It is reported that heavy metals decompose the chloroplast structure and thus reduce the chlorophyll content of plants. However, it also negatively affects stomatal conductance in plants, preventing the continuity of the photosynthesis process and reducing water consumption. As a result, yield and product quality are also negatively affected [46]. Although the amount of chlorophyll in some applications decreases in this study compared to the control group, it is included in the same statistical group (Table 3.2). Leaf area decreases with the increase in heavy metal. At the highest dose of cadmium, the leaf area had the lowest value among all samples (42,570 cm^2/plant). Lead applications negatively affect the leaf area, and the results of Pb 1500 and 2000 applications were in the same statistical group. Electrical conductivity increases with heavy metal applications, and LRWC value decreases. The decrease in LRWC value may be due to heavy metal-induced reductions in hydraulic conductivity [47].

Table 3.2. Effects of Cd and Pb exposure on physical properties

Exposure	Leaf Number (for each plant)	Chlorophyll (SPAD)	Leaf area (cm^2/plant)	EC (%)	LRWC (%)
Control	12,750±0,636ab	43,400±1,697a	98,640±13,641a	54,815±4,772c	82,745±4,433a
Cd 50	13,533±0,585a	42,367±3,564a	76,163±19,247abc	65,153±5,505b	80,837±1,385a
Cd 100	10,800±2,066bc	39,00±6,090a	67,090±12,588abc	72,050±1,516a	72,923±1,414bc
Cd 150	11,600±1,558abc	42,20±1,509a	74,907±21,010abc	72,170±1,607a	68,760±1,711d
Cd 200	9,667±1,242c	40,767±5,670a	42,570±3,712c	73,843 ±3,112a	63,837±2,169e
Pb 500	10,900±1,015bc	41,90±3,650a	77,503±16,840abc	65,593±0,115b	76,790±2,931b
Pb 1000	12,300±1,277ab	43,734±2,040a	89,510 ±18,312ab	71,970±1,573a	69,260±1,905cd
Pb 1500	11,267±0,058abc	40,334±6,407a	58,313±11,553bc	73,827±0,769a	66,537 ±2,188de
Pb 2000	12,734±1,021ab	42,267±2,532a	63,397±28,080bc	74,736±0,788a	63,863±1,824e

*There is no statistical difference between the means shown with the same letter given

The effects of Cd and Pb on H_2O_2 and MDA values are presented in Table 3.3. As can be seen from the table, an increase in H_2O_2 and MDA enzyme activities is observed as a result of heavy metal applications. H_2O_2 activity, which is 89,400 in the control group, increased to 136.,00 mmol/kg with Cd application. Higher values were obtained in Pb applications than in the control, except for the Pb 500 application. Pb 1000 and Pb 2000 applications were stayed in the same statistical group. The type of stress factor varies depending on many factors such as the processes occurring in the plant and the type of plant [48]. As the ratio of applied Cd and Pb elements increases, the MDA content in quinoa rises steadily. The enzyme activity known as malondialdehyde (MDA) varies depending on the type and severity of stress [49]. In a study investigating the effects of drought stress on quinoa, it is reported that, due to the increase in the stress factor, root length, root fresh-dry weight and chlorophyll amount in plants decreases compared to the control group, the MDA value increases by 82% [50]. Researchers attributed these results to the severe damage caused by heavy metal stress in plant cells [51].

Table 3.3 Effect of Cd and Pb on enzymes of quinoa

Exposure	H_2O_2 (mmol kg^{-1})	MDA (nmol g^{-1})
Control	89,400± 23,416bc	5,876± 0,655ab
Cd 50	122,533± 11,582ab	6,367± 1,787ab
Cd 100	122,700± 13,323ab	6,334± 0,631ab
Cd 150	135,933± 22,982a	6,627± 0,340ab
Cd 200	136,00± 24,093a	7,107 ± 0,304a
Pb 500	75,166± 20,004c	5,467 ± 0,093b
Pb 1000	109,133± 28,916c	5,573± 0,466b
Pb 1500	125,300± 4,479ab	6,474± 0,271ab
Pb 2000	115,833± 11,202a	6,850± 0,685ab

*There is no statistical difference between the means shown with the same letter given

As it is known, plants exposed to some environmental effects or stresses are treated differently. The most common of these is some reactive oxygen species accumulation in plant tissues [41]. Some free radicals resulting from oxidative stress provoke lipid peroxidation in the cell membrane, leading to malondialdehyde (MDA) formation. Many scientific studies address this issue and investigate possible harms and treatments [52, 53, 40, 55]. This study observed that both H_2O_2 and MDA levels increased significantly due to the Cd and Pb heavy metal stress applied. This situation can be expressed as an effort to adapt quinoa to current conditions to reduce the harmful effects of relevant heavy metals or eliminate these effects. Some studies have reported that quinoa plants exposed to heavy metals or different stresses have effective mechanisms in these and similar ways [56, 57, 58].

4. CONCLUSION

The results showed that quinoa was negatively affected by Pb and Cd metals in all parameters, and its toxic effect increased depending on the increase in concentration. Seed germination occurred at all concentrations of both metals. Cd had a more toxic effect compared to Pb metal. Quinoa has a defense mechanism against heavy metals by increasing enzyme activity. More detailed studies are needed to understand the defense mechanism of quinoa, which is known to be resistant to abiotic stresses.

Ethics Statement
The authors of this article declare that the materials and methods used in this study do not require ethics committee approval and legal-specific permission.

Conflicts of interest
As the authors of this study, we declare that there is no financial conflict of interest with any institution, organization, or person related to this article and no conflict of interest between the authors.

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