

The Effect of Heavy Metals in Potato (*Solanum tuberosum* L.) Genotypes for Some Physiological Parameters


Patates (*Solanum tuberosum* L.) Genotiplerinde Ağır Metallerin Bazı Fizyolojik Parametrelere Etkisi


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Abstract


The main aim of this study is to identify physiological characteristics, including Chl a, b, total Chl "a+b" and carotenoids, in potato genotypes under the influence of lead (Pb+2) and cadmium (Cd+2) heavy metals. Two potato genotypes from the Netherlands, Riviera and Agria, were used in the study. Potato tubers were transferred to 2 kg compost pots and placed in a controlled environment with a 12 photoperiod, a day/night temperature of 25/19°C and a relative humidity of 75%. Since Pb+2 and Cd+2 poisoning was greater than in the control and different fractions of heavy metal residues in the substrate were transferred to plant organs, the amounts of these two metals in each treatment (outside the control) were measured. Plant pigments were extracted from fresh leaves in amounts ranging from 60 to 100 mg and these were then extracted into samples containing 80/20% (v/v) acetone/water with 0.5% w/v MgCO₃ at room temperature for a full day. Photosynthetic pigments of each sample were extracted three times. The absorbances obtained at 663 nm, 644 nm and 452.5 nm for the maximum absorption of Chl a, Chl b and carotenoids, respectively, were used to measure the amount of chlorophyll and carotenoids. Differences between Pb+2 and Cd+2 and physiological markers were examined with the Duncan Multiple Range test. Information on Pb+2 and Cd+2 content in applications revealed wide variability. When potato genotype seedlings were exposed to varying levels of Pb+2 and Cd+2, the amount of chlorophyll and carotenoids in their leaves was lower than the control group. The results showed that there were significant and statistically significant changes in carotenoid and chlorophyll concentration at the LSD p = 0.01 level. Similar to Pb+2 and Cd+2 inhibiting plant growth, it had a negative effect on photosynthesis as well as chlorophyll and carotenoid contents. Moreover, these effects became more pronounced when the concentrations of two stress factors (Pb+2 and Cd+2) increased.

Keywords: Potato, Cd⁺², Pb⁺², Chlorophyll, Carotenoids, Heavy metals

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Öz

Bu çalışmanın temel amacı, kurşun (Pb⁺²) ve kadmiyum (Cd⁺²) ağır metallerinin etkisi altındaki patates genotiplerindeki Chl a, b, toplam Chl "a+b" ve karotenoidler dahil olmak üzere fizyolojik özelliklerin tanımlanmasıdır. Hollanda'dan iki patates genotipi, Riviera ve Agria, çalışmada kullanılmıştır. Patates yumruları 2 kg'lık kompost saksılarına aktarılarak 12 fotoperiyodu, 25/19°C gündüz/gece sıcaklığı ve %75 bağıl nemi olan kontrollü bir ortama yerleştirildi. Pb⁺² ve Cd⁺² zehirlenmesi kontrolden daha fazla olduğundan ve substrattaki ağır metal kalıntılarının farklı kısımları bitki organlarına aktarıldığından, bu iki metalin her bir uygulamadaki (kontrol dışında) miktarları ölçülmüştür. Bitki pigmentleri, 60 ila 100 mg arasında değişen miktarlarda taze yapraklardan ekstrakte edildi ve bunlar daha sonra, tam bir gün boyunca oda sıcaklığında %0,5 w/v MgCO₃ ile %80/20 (h/h) aseton/su içeren örneklerle ekstrakte edildi. Her örneğin fotosentetik pigmentleri üç kez ekstrakte edildi. Chl a, Chl b ve karotenoidlerin maksimum emilimi için sırasıyla 663 nm, 644 nm ve 452,5 nm'de elde edilen absorbanslar, klorofil ve karotenoid miktarını ölçmek için kullanılmıştır. Pb⁺² ve Cd⁺² ile fizyolojik belirteçler arasındaki farklar Duncan Çoklu Aralık testi ile incelenmiştir. Uygulamalardaki Pb⁺² ve Cd⁺² içeriğine ilişkin bilgiler geniş bir değişkenlik ortaya koymuştur. Patates genotipi fideleri değişen seviyelerde Pb⁺² ve Cd⁺² 'ye maruz bırakıldığında, yapraklarındaki klorofil ve karotenoid miktarı kontrol grubuna göre daha düşük olmuştur. Elde edilen sonuçlar, karotenoid ve klorofil konsantrasyonunda LSD p = 0.01 düzeyinde belirgin ve istatistiksel olarak önemli düzeyde değişiklikler olduğunu göstermiştir. Pb⁺² ve Cd⁺²'nin bitki büyümesini engellemesine benzer şekilde, klorofil ve karotenoid içeriklerinin yanı sıra fotosentez üzerinde de olumsuz bir etkisi olmuştur. Ayrıca, bu etkiler iki stres faktörünün (Pb⁺² ve Cd⁺²) konsantrasyonları arttığında daha belirgin hale gelmiştir.

Anahtar Kelimeler: Patates, Cd⁺², Pb⁺², Klorofil, Karotenoid, Ağır metaller

1. Introduction

Potato (*Solanum tuberosum* L.), a member of the *Solanaceae* family, is one of the most widespread and used agricultural crops in the whole world and which has a great importance in reducing the growing hunger (Özdemir, 2023). The potato as an agricultural crop was introduced to Europe during the year 1570, and during the 17th century it spread to other countries of the world (Bradshaw and Ramsay, 2006). Pointed out that there was a need to improve the properties of the potato to fight hunger and various diseases at that time, so a greater interest in breeding wild potatoes began over 150 years ago. Nowadays, the potato plant is cultivated for human consumption and is the fourth most important food crop in the world, after rice, wheat, and corn (Farooq et al., 2008). Global potato production has increased significantly in recent years, particularly due to increased production in developing countries. Genetic variability offers plant breeders greater opportunities to discover new traits, through selection and breeding, as well as to improve various traits such as increased resistance to biotic and abiotic stresses (Shilpa et al., 2021). Kosovo has a land area of 10.887 km² or 1.1 million ha of this surface, about 430.000 ha are forested or 39% and 577.000 ha are agricultural land (52%), which is 31% is pasture and about 69% arable land. Potato is an important food source of Kosovo (Rusinovci et al., 2010; 2012 and 2015). However, the amount of heavy metals (HM's) affects environmental pollution due to various human activities such as: intensive agriculture, mining, energy transmission, etc. (Nedelkoska and Doran., 2000). Also, heavy metal pollution affects the biosphere in many countries around the world (Meagher, 2000). This type of heavy metal pollution is one of the most important environmental problems. This comes as a result of various agricultural and industrial activities including industrial fertilizers and pesticides, organic manure, emissions from smelters and incinerators, traffic, the distribution of mining waste, the use of contaminated sewage sludge, etc. (Aliu et al., 2013). Lead (Pb⁺²) and cadmium (Cd⁺²) can be considered to be the most dangerous in environmental pollution (Malkowski et al., 2005). In addition to having an adverse effect on plant growth and development, excessive plant absorption of Cd⁺² can build up in various plant sections and subsequently enter human bodies through the food chain. Some results indicate that among the most dangerous heavy metals in the environment is cadmium (Jaishankar et al., 2014). Growing amounts of soil pollution from mining, sewage irrigation, air pollution, and fertilizer application harm crops and the health of people and animals. According to Cd⁺² inhibits the process of photosynthesis and has an impact on the activity of the organism's protection enzymes or antioxidants in plants. Because agricultural soil surfaces contain amounts of heavy metals, they tend to bio-accumulate in the food chain (Quartacci et al., 2006). The main sources of agricultural land pollution are agronomic practices, the application of sludge as organic fertilizer, as well as the application of industrial effluents as a source of water for irrigation of agricultural plants (Singh and Sinha., 2005). Mining also plays an important role in contamination (Nedelkoska and Doran., 2000). Pollution with heavy metals affects the biosphere in different countries of the world (Meagher, 2000). The effect of Cd⁺² and Pb⁺² has also been observed in inhibiting the activity of photosynthesis (Baszyński et al., 1980), transpiration and carbohydrate metabolism (Moya et al., 1993; Pal et al., 2006; Paunov et al., 2018).

Plants show different patterns of heavy metal accumulation which would affect the biosynthesis of photosynthetic pigments (reduction of photosynthetic pigments such as chlorophylls and carotenoid content) (Mobin and Khan., 2007). However, there are also plants with heavy metal tolerance mechanisms, which accumulate and bind them to amino acids, peptides, and proteins (Horváth et al., 2007). Therefore, Pb⁺² and Cd⁺² cause inhibition in plant growth and development, ion absorption and transport disorders, inhibition of the photosynthesis process (Fargašová, 2001). The Chlorophyll a and b pigments usually use photosystem II to absorb light, which is followed by electron transport (Taiz and Zeiger., 2002). However, the mechanisms of heavy metal toxicity on photosynthetic activity cannot yet be considered a settled issue, due to variations in experimental design (Giardi et al., 1997). High concentrations of heavy metals (Cd⁺², Pb⁺²) in agricultural areas have been identified as toxic and the situation is defined as heavy metal stress (Yıldırım et al., 2019). Similarly, heavy metal pollution is present in Kosovo, which is caused by various sources, including the Obiliq power plant, the Zvečan smelter and the ferronickel factory. In addition to soil surface pollution, they also affect the growth and development of plants during their vegetation (Aliu et al., 2013). In this experiment, we evaluated the effect of some heavy metals (Pb⁺² and Cd⁺²) in potato genotypes on different physiological traits.

2. Materials and Methods

2.1 Plant material and growth conditions

The plant materials included in our research were 2 different genotypes of potato (*Solanum tuberosum* L.); Potato genotypes (PG's) Riviera and Agria of Dutch origin. During this period, potting compost (minimum 1 kg/replications) was prepared for PG's cultivars and each treatment. In our research there were a total of 8 pots for heavy metals which were PbCl₂, CdCl₂ and control. Potato tubers were transferred to compost in pots weighing 2 kg in a controlled environment or controlled rooms with a photoperiod of 12 and a temperature of 25/19°C day/night and a relative humidity of 75%. The compost consisted of pH (CaCl₂) = 5.8; moisture= 65.11%, Humus= 30.9%, Organic Matter= 62.14%, Nitrogen(N)= 2.64mg100⁻¹, Phosphorus (P₂O₅)= 116 mg100⁻¹, Calcium (Ca)= 36.40 mg100⁻¹, Magnesium (Mg)= 15.58 mg100⁻¹, Zinc (Zn)= 0.12 mg100⁻¹, Copper (Cu)= 0.17 mg100⁻¹, Iron (Fe)= 2.73 mg100⁻¹, Molybdenum (Mb)= 0.34 mg100⁻¹.

In this experiment, during the growth period, were prepared different concentrations of heavy metals: Control T0-dH₂O, T1- 55.62 mg kg⁻¹ PbCl₂, T2-111.24 mg kg⁻¹ PbCl₂, T3-166.86 mg kg⁻¹ PbCl₂, T4-11 mg kg⁻¹ CdCl₂, T5-22 mg kg⁻¹ CdCl₂, T6-23 mg kg⁻¹ CdCl₂.

2.2 Chl (a, b, and total) and carotenoid analysis

Plant pigments were extracted in amounts ranging from 60 to 100 mg of fresh leaves, which were then extracted into samples containing 80/20% (v/v) acetone/water with 0.5% w/v MgCO₃ at room temperature for a full day without knowledge. Each sample's photosynthetic pigments were extracted three times. Using the absorbance measured at 663 nm, 644 nm, and 452.5 nm for the maximum absorption of Chl a, Chl b, and carotenoids, respectively, in the UV-Vis spectrophotometer, BK-UV-10, the concentration of chlorophyll and carotenoid content was computed (Turfan, 2022). By using the absorption coefficient calculations outlined by Lichtenthaler(1986), Aliu et al. (2013) and Aliu et al. (2014) pigment content was determined in mg g⁻¹ leaf dry weight. After 20 days of exposure, the following parameters were determined in different part of plants include heavy metal contents (HMC), Chl a, b, and carotenoid concentrations. All analyzes for the determination of HMs, including Pb²⁺ and Cd²⁺, were achieved using a Spectrophotometer of atomic absorber (SAA) Thermo Elemental M, by U.S. EPA Method 245.5 Cold Vapor Atomic Absorption Spectroscopy.

2.3 Statistical analyses

The experimental design was a completely randomized design with three (3) replications. All changes for the investigated physiological parameters for Pb²⁺ and Cd²⁺ were tested through the statistical package MINITAB-14. Statistical differences were performed through Duncan's multiple tests.

3. Results and Discussion

Data regarding content of heavy metals (Pb²⁺ and Cd²⁺) showed a large range of variability among the treatments. Results are given in *Table 1*. Based on the results of ANOVA (*Table 1*), the highest value for lead content for both Riviera and Agria potato genotypes was reached in the third treatment (T3-166.86 mg kg⁻¹PbCl₂). Values for the Riviera potato genotype at content of Leaf/Stem (L/S) were 0.091 mg kg⁻¹PbCl₂, while Root Lead Content (RLC) was 0.112mgkg⁻¹PbCl₂. While in the Agria potato genotype, the L/S values were 0.157 mgkg⁻¹PbCl₂ and RLC = 0.099mgkg⁻¹PbCl₂. Differences between genotypes for L/S content were D= +0.066mgkg⁻¹ PbCl₂, and RLC= +0.022 mgkg⁻¹ PbCl₂, highly significant differences at the 0.05 and 0.01 probability level. Also, the cadmium content for different treatments was different for the two potato genotypes that were in our research. The highest cadmium content for both genotypes Riviera and Agria was found in the sixth treatment (T6-23 mg kg⁻¹CdCl₂). The content of cadmium in the Riviera potato genotype for L/S was 0.007mg kg⁻¹CdCl₂, while in Agria 0.0079mg kg⁻¹CdCl₂. The differences between them were D= -0.0009mg kg⁻¹CdCl₂. While the root cadmium content (RCC) in the Riviera potato genotype was 0.0079mg kg⁻¹CdCl₂ and Agria 0.0089mg kg⁻¹CdCl₂. The differences between them were D= -0.001mg kg⁻¹CdCl₂. Results are given in *Table 1*. Heavy metals (HM's) were mostly observed accumulate in the roots. The content of Cd²⁺ and Pb²⁺ increased with increasing doses of these elements in the root (Kabata-Pendias, 2000; Cannata et al., 2013).

Table 1. The content of heavy metals in potato genotypes

Genotype	Treatments	Leaf/Stem	Roots
Riviera	Control	0.025 ^{cd}	0.071 ^b
	T1	0.040 ^c	0.078 ^b
	T2	0.083 ^{ab}	0.057 ^c
	T3	0.091 ^a	0.112 ^a
	T4	0.002 ^f	0.009 ^d
	T5	0.003 ^e	0.004 ^f
	T6	0.007 ^d	0.007 ^e
Agria	T1	0.032 ^c	0.087 ^b
	T2	0.080 ^b	0.014 ^c
	T3	0.157 ^a	0.099 ^a
	T4	0.001 ^f	0.0036 ^f
	T5	0.0029 ^e	0.0038 ^e
	T6	0.0079 ^d	0.0089 ^d

* – values within individual marked by at least one same letter are not significantly different at 0.05 probability level

The reaction of the potato genotypes to Pb⁺² and Cd⁺² resulted in a difference (reduction) in the content of the physiological parameters of chlorophyll and carotene in the leaves, which were significantly different compared to the control. The levels with different concentrations of heavy metals consisted in their differences in the content of chlorophyll and carotenoids, which were different and significantly higher at the probability level of LSDp = 0.0. For both potato genotypes Riviera and Agria the effect with a high reduction in the content of Chla was found to treatment of third content (T3-166.86 mg kg⁻¹ PbCl₂) on values 0.04 mgg⁻¹ FW (Riviera) and 0.26 mgg⁻¹ FW (Agria). The differences between potato genotypes were D=-0.22 mgg⁻¹ FW. The average value for control was 0.86 mgg⁻¹ FW, compared to values (0.04 and 0.26) for content of Chla. The differences were 0.82 mgg⁻¹ FW and 0.60 mgg⁻¹ FW. Also, content for Chla affected by Cd⁺² with a higher reduction for both potato genotypes Riviera and Agria was in T6- 23 mg kg⁻¹ CdCl₂ (0.26 and 0.59 respectively). For the content of Chlb affected by Pb⁺² was found a significant difference between potato genotypes. The potato genotypes Riviera compared to control (0.32 mgg⁻¹ FW) was with higher reduction in T3-166.86 mg kg⁻¹ PbCl₂ on value 0.02 mgg⁻¹ FW. While the Agria on value 0.19 mgg⁻¹ FW. The differences between genotypes were - 0.17 mgg⁻¹ FW. Also, for the Cd⁺² content, significant changes were determined for both potato genotypes. Thus, the lowest value for content of Chl b for both potato genotypes was found in T6-23 mg kg⁻¹ CdCl₂ with values of 0.12 mgg⁻¹ FW and 0.23 mgg⁻¹ FW. The differences between them were statistically significant. Results are presented in *Table 2*. Also, the results for the content of carotenoids depending on the concentration of the content of HM's had different results in relation to the control (1.28 mgg⁻¹ FW). The lowest value of the Riviera and Agria genotype for Pb⁺² and Cd⁺² content was reached in T3 (0.18 mgg⁻¹ FW), while the highest was T4 (0.95 mgg⁻¹ FW). While in the Agria genotype, the highest value for Pb⁺² content was in T1 (1.11 mgg⁻¹ FW), while the lowest value was T3 (0.59 mgg⁻¹ FW). For Cd⁺² concentration level content, T4 had a high value (1.01 mgg⁻¹ FW) while T6 had a low value (0.84 mgg⁻¹ FW). The differences between them were very significant. Results are presented in *Table 2*. Various studies in relation to the presence of heavy metals have shown that the ability of plants to absorb and accumulate metals can vary, because this can depend to a significant extent on genotypes but also between cultivars of the same species. In this direction, significant changes in Cd⁺² levels were found from one genotype to another and from one part to another of the same plant. Then it was found that some genotypes could accumulate less Cd⁺² in the tuber, but more in the stem and leaves, results presented according to Dong et al. (2020). But other plants could also show a different change in the presence of heavy metals between different genotypes; for example, rice (Zhou et al., 2015).

For both potato genotypes, Chl a concentration was positively correlated with Chl b, carotenoids, total chlorophyll a+b. There was no correlation of Chla with total Roots and Leaf/ stem. Carotenoids were (next to Chl a) positively correlated with total Chla + b. Total chlorophyll (a and b) was (next to Chl b) positively correlated with Chla and carotenoids but negatively with roots and leaf / stem. The root biomass was (next to already mentioned parameters) positively correlated with the Leaf/stem. Results are presented in *Table 3*.

Table 2. The different concentration of lead (Pb²⁺), and cadmium (Cd²⁺) on photosynthetic pigment contents of different potato genotypes

Genotype	Treatments	Chl a (mgg ⁻¹ FW)	Chl b (mgg ⁻¹ FW)	Carotenoid (mgg ⁻¹ FW)	Total Chla+b (mgg ⁻¹ FW)
Riviera	Control	0.86 ^a	0.32 ^a	1.28 ^a	1.18 ^a
	T1	0.29 ^{bc}	0.14 ^b	0.41 ^c	0.43 ^b
	T2	0.08 ^c	0.06 ^c	0.19 ^c	0.14 ^d
	T3	0.04 ^d	0.02 ^d	0.18 ^d	0.06 ^e
	T4	0.62 ^b	0.25 ^{ab}	0.95 ^{ab}	0.87 ^b
	T5	0.52 ^b	0.20 ^b	0.82 ^{bc}	0.72 ^{bc}
Agria	T6	0.20 ^{cd}	0.12 ^b	0.79 ^d	0.32 ^c
	T1	0.65 ^b	0.29 ^{ab}	1.11 ^a	0.94 ^b
	T2	0.33 ^{bc}	0.18 ^b	1.01 ^{ab}	0.51 ^{cd}
	T3	0.26 ^{bc}	0.19 ^b	0.59 ^d	0.45
	T4	0.84 ^a	0.34 ^a	1.01 ^{ab}	1.18 ^a
	T5	0.78 ^{ab}	0.30 ^{ab}	0.95 ^b	1.08 ^{ab}
	T6	0.59 ^b	0.23 ^{ab}	0.84 ^{bc}	0.82 ^b

* – values with in individual marked by at least one equal letter are not significantly different at 0.05 probability level

Table 3. Pearson correlation coefficients for potato genotypes across different treatments

Traits	Chl-a	Chl b	Carotenoids	Total Chl (a+b)	Roots
Chl b	0.96**				
Carotenoids	0.84**	0.88**			
Total Chla+b	0.99**	0.98**	0.86**		
Roots	-0.41	-0.36	-0.44	-0.40	
Leaf/Steam	-0.63	-0.50	-0.52	-0.60	0.69**

Correlation is significant at $p < 0.05$ (*) or $p < 0.01$ (**).

4. Conclusions

The level of different concentrations for heavy metals including Pb²⁺ and Cd²⁺ in the two potato genotypes Agria and Riviera resulted in high effects and differences for the investigated parameters. The content of the quantity of chlorophyll in the control was the highest, while the lowest value of the content of the quantity of chlorophyll in the two genotypes of potato was present in the third (T3) and sixth (T6) treatment with lead and cadmium. From this we can see that the presence of Cd²⁺ and Pb²⁺ had a bad (negative) effect on plant growth, photosynthetic activity, chlorophyll and carotenoid content, and these effects were more apparent when the concentration of two stress factors (Pb²⁺ and Cd²⁺). We evaluated this effect in laboratory conditions and for future studies we recommend that this stress be evaluated in potato fields in Kosovo

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept:Aliu S., Demirbas S.; Methodology:Aliu S.,Jakupi M.; Formal analysis and investigation:Aliu S., Rusinovci I.,Zeka D.; Statistical Analyses:Aliu S.; Writing: Aliu S., Jakupi M.;Reviewand Editing: Aliu S., Demirbas S.All the authors have read and agreed to the published version of the manuscript.

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