

Original Research

# ICP-MS Analysis of Cadmium Bioaccumulation and Its Effect on Pea Plants (*Pisum sativum* L.)

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## Abstract

Photosynthesis is one of the main processes involved in plant growth and development. This process is sensitive to environmental stressors, including cadmium environment contamination. Therefore, this study investigated, among other things, the effect of cadmium on the fluorescence of photosynthesis parameters, chlorophyll fluorescence; moreover, as the chlorophyll and carotenoids contents were measured. The toxic effect of cadmium on the growth, development, and photosynthesis process was confirmed. In order to study the cadmium binding mechanism by the plant, the first step was to verify the cadmium quantification method with ICP-Q-MS. It was shown that selecting the *m/z* spectral line: 114; gave a lower background and higher sensitivity for the determination of cadmium in a solution than when selecting 112. The limits of detection and quantification are 2.2 and 7.5 ng/L, respectively, and the method is linear in the investigated concentration range (range up to 100 µg/L). The method of the microwave assisted mineralization in the preparation of a plant sample was shown to meet the requirements of accuracy (recovery with the certified reference material 98%) and repeatability (0.13% in five repetitions). Root and shoot samples of pea were examined and the accumulation of cadmium especially in the roots was found, which proves the excludable properties of the plant.

**Keywords:** Cd bioaccumulation, pea plants, ICP-MS, photosynthesis, chlorophyll

## Introduction

One of the main environmental problems is the contamination with trace metals. The group of trace metals includes cadmium (Cd), which ranks 7<sup>th</sup> among

the 20 most dangerous environmental pollutants [1]. Cd is an extremely dangerous pollutant due to its acute toxicity, high water solubility, non-biodegradability, and persistence in live organisms [2]. The plant root system takes Cd from the soil, which is then transported to other parts of plants [1]. Cd taken up by plants (including cultivated plants) accumulates in them and risks food security [3]. In plants exposed to Cd, the cell membrane

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permeability and ion leakage occur. The toxic effect of Cd leads to the oxidative stress in the plant cells, during which the cells overproduce reactive oxygen species. Cellular homeostasis is essential for the correct growth and development of plants along with physiological and metabolic processes [4]. One of the physiological processes in plants is photosynthesis, which plays an important role in the proper development of plants. This process is significant in plant growth, development, and crop yielding [2]. In addition, photosynthesis plays an essential role in maintaining a balance between light energy and chemical energy [5].

Cadmium is one of the minerals in the soil; the concentration of the soluble fraction was determined in the range of 0.550 to 0.589 mg/kg collected from the vicinity of Toruń at the beginning of the 21<sup>st</sup> century [6]. It belongs to the group of heavy metals due to their toxic properties and high mobility in the soil solution and bioavailability to plants; it is an example of abiotic stress for plants; it is responsible for the toxic effect on plant metabolism and the physiological aspect, and thus on the plant growth [7]. Plants take up mineral elements from the soil through the root system and transport them to various morphological parts thanks to passive and active modes of transport [8-10]. Taking into account the bioaccumulation factor, morphological parts of plants have a different mineral binding capacity, while the roots accumulate the greatest amount of cadmium compared to stems and leaves [11, 12]. Barańkiewicz et al. [13] showed that cadmium was conjugated with phytochelatin: a compound of PC<sub>3</sub> and PC<sub>4</sub> in pea roots.

The analysis of the Cd content of the plant material consists of a sample pretreatment sequence (washing, drying to obtain a constant weight, powdering the sample to obtain smaller particles and homogenizing) [11, 14] and typically, the mineralization of the samples is performed by the microwave assisted digestion [11]. The use of concentrated nitric acid with an additional part of an oxidizing agent such as perchloric acid [11, 15, 16] or a solution of hydrogen peroxide [14] or individual perchloric acid [7] ensures a complete solubilization of the sample and decomposition of the organic matter into elementary compounds.

A popular technique of the elemental analysis based on the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) [17-19] and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) [11, 16, 20, 21] or Atomic Absorption Spectrometry (AAS) [14, 22] with Flame (F-AAS) [23] and Graphite Furnace (GF-AAS) [24] are used in the quantification of Cd. Cadmium has 8 stable isotopes with a mass of 106, 108, 110-114 and 116 atomic mass units, while the most abundant is 114 (28.7% abundance). All masses have noticeable polyatomic interferences, but the cases when there are more than three atoms are very rare [25]. For the emission technique, it is important that cadmium has at least three spectral lines: 214.438, 226.502, 228.802 nm which could be monitored in the Cd quantification

by ICP-OES. The recovery for the Cd quantification was determined at 94.9±4.3% in the ICP-OES studies [16].

The influence of Cd ions on plant development and their overall role in the photosynthetic performance is known. However, depending on the type of plant and the used concentration of Cd, the generated changes in plants can be varied. This study aimed to exploring photosynthetic activity changes in leaves of pea plants (*Pisum sativum* L.) treated with 50 µM of CdSO<sub>4</sub>. Additionally, the condition of the plants was assessed by examining their growth and development. Using the ICP-MS technique with a single quadrupole to determine trace amounts of cadmium in the plant material and the presentation of the distribution of this element in the pea plant, which grew in an environment contaminated with cadmium, were demonstrated.

## Material and Methods

### Plant Material and Growth Conditions

Seeds of pea (*Pisum sativum* L.), Pegaz cultivar, were used in the study. The seeds germinated in the darkness at 25°C for 2 days. Before the germination, the seeds were disinfected by being immersed in isopropanol for 1 minute and 2% sodium hypochlorite for 15 minutes. Next, the seeds were washed in sterile water. The seedlings were transferred to sterile glass containers with 20 ml Hoagland medium at 0.5× concentration and pH values 6. The growth of the seedlings was continued for 6 days at 25/20°C (10/14 hours) day/night temperatures. After this time, the seedlings were transferred to 1-liter plastic containers, and their growth and development continued in hydroponic cultivation with Hoagland's solution (at 0.5× concentration) for 7 days. The growth of the seedlings was continued in the full strength Hoagland solution for the next 7 days. Then, Cd stress was initiated by applying 50 µM of CdSO<sub>4</sub>. At the same time, control plants were prepared without the addition of Cd. After 7 days, the medium solutions were replaced and plants growth continued for another 14 days in the medium without Cd. The nutrients were replaced twice a week. The effect of Cd treatments on root length and shoot height were measured with the ImageJ tool software. Fresh and dry weights were determined.

### Chlorophylls and Carotenoids Concentration

Three weeks after the Cd plants treatment, the content of chlorophylls and carotenoids was determined. Leaves (500 mg) were fragmented and placed in 10 ml 80% acetone for 24 hours at 4°C in the dark. The concentration of chlorophyll a, chlorophyll b and carotenoids were determined spectrophotometrically (Tecan Infinite 200 PRO) and expressed as mg/g fresh

weight. The content of chlorophylls and carotenoids was carried out using the formulas:

$$\text{chlorophyll a} = 12.25 \times A_{663.2} - 2.79 \times A_{646.8} \quad (1)$$

$$\text{chlorophyll b} = 21.5 \times A_{646.8} - 5.1 \times A_{663.2} \quad (2)$$

$$\text{carotenoids} = (1000 \times A_{470}) - (1.82 \times Ch_a) - (85.02 \times Ch_b)/198 \quad (3)$$

#### Chlorophyll Fluorescence Measurements

Chlorophyll fluorescence parameters were measured using a PAR-FluorPen FP 110 (PSI Photon Systems Instruments, the Czech Republic) after 24 h, 1 week and 2 weeks had passed from the Cd plants treatment. The plants were dark-adapted for 20 minutes prior to determination. Chlorophyll fluorescence parameters were measured, such as the minimum fluorescence (F0) and maximum fluorescence (Fm) and fluorescence quantum yield of PS II measured as  $Fv/Fm = (Fm-F0)/Fm$ .

#### ICP-MS Method Verification to Cd Quantification

The ICP-MS (Shimadzu ICP-MS 2030, Shimadzu, Kyoto, Japan) spectrometer was used to quantify cadmium in pea roots and shoots after the sample decomposition for the microwave-assisted digestion. However, the first step was to verify the method for determining cadmium in the plant material. The linearity of the analytical method was determined. The limits of detection, quantification and method range were established. Series of standard solutions were prepared, the 1000 mg/L cadmium solution (SPEX CertiPrep, New York, US) was used to prepare the Cd analytical standards in range 0.1 µg/L to 100 µg/L in 1% HNO<sub>3</sub> matrix (Suprapur, Merck, Darmstad, Germany). The <sup>103</sup>Rh (1µg/L) as an internal standard was used; the solution with was mixed the standard sample by a peristaltic pump in the volume ratio 1:10. The signals at *m/z*: <sup>112</sup>Cd and <sup>114</sup>Cd were monitored. RF power 1.20 KW was applied. The plasma gas, auxiliary and cooling gas flows were: 8 L/min, 1.10 L/min and 0.7 L/min, respectively. The temperature of the chamber was 5°C. The sampling depth was 5 mm. The collision gas was applied in this study, the flow of helium was 6 mL/min; the cell voltage was -21 V and energy filter was +7.0 V. The obtained dependence of the increase in the signal intensity with the given *m/z* changes in the cadmium concentration was modeled by the linear regression, determining the parameters of the calibration curve (slope, intercept, correlation coefficient *r*) and analytical parameters: limit of detection, quantification, and BEC (background equivalent concentration), which was calculated by the original software of the manufacturer; the scope of the analytical method was given.

#### The Accuracy and Repeatability of Cd Quantification in Plant Material

The accuracy and repeatability were assessed by the determination of the Cd content in ERM® Certified Reference Material - RYE GRASS (CD281) (European Commission, Joint Research Centre (JRC), Geel, Belgium). The 200 mg of the sample was weighed on the analytical balance directly to the glass microwave-assisted digestion vessel. The sample (five repetitions along with the blank method were carried out) was digested in the reagent mixture: HNO<sub>3</sub>:H<sub>2</sub>O<sub>2</sub> (5 mL : 2 mL) by the microwave-assisted digestion apparatus (Novawave SA, SCP Science, Canada) at 180°C (holding time was 20 min). After the solution was cooled to room temperature, an aliquot was diluted 100 times in high-quality water (Merck Millipore, Merck, Darmstadt, Germany). The solution was analyzed by the ICP-MS method applying the standard addition calibration method, where the spike volume was 100 µL of 100 µg/L of the Cd standard solution.

#### The Cadmium Content in Roots and Shoots Parts of Pea Plant

The plant material after cultivation was divided and prepared as described in "Plant material and growth conditions" section. Due to the limited amount of plant material, the mass of the sample was about 50 mg of roots and shoots. The material was prepared to analysis in the same way as described in "The accuracy and repeatability of Cd quantification in plant material" section and analyzed by the ICP-MS method described in "ICP-MS method verification to Cd quantification" section. According to Pachura et al, the bioconcentration factor (BCF) and translocation index (Ti) were calculated as follows [26]:

$$BCF = \frac{Cd_{in\ plant} \left[ \frac{mg}{kg} \right]}{1000 \times Cd_{in\ culture\ medium} \left[ \frac{mg}{kg} \right]} \quad (4)$$

$$Ti = \frac{Cd_{in\ above\ ground\ tissues} \left[ \frac{mg}{kg} \right]}{Cd_{in\ roots} \left[ \frac{mg}{kg} \right]} 100 \quad (5)$$

#### Results and Discussion

The impact of Cd on plants of pea was examined. The longest shoots and roots were observed in the control plants which were 20.4 cm and 28.5 cm long (Fig. 1A), respectively. The shoots and roots which had grown in Hoagland medium with 50 µM of CdSO<sub>4</sub> were on average shorter than the shoots and roots of the control plants by 31% and 6%, respectively. The length of the shoots was related to the number and length of the internodes (Fig. 1B). The Cd that inhibited the plant growth reduced the number of internodes and their length in plants. The study noticed that the number

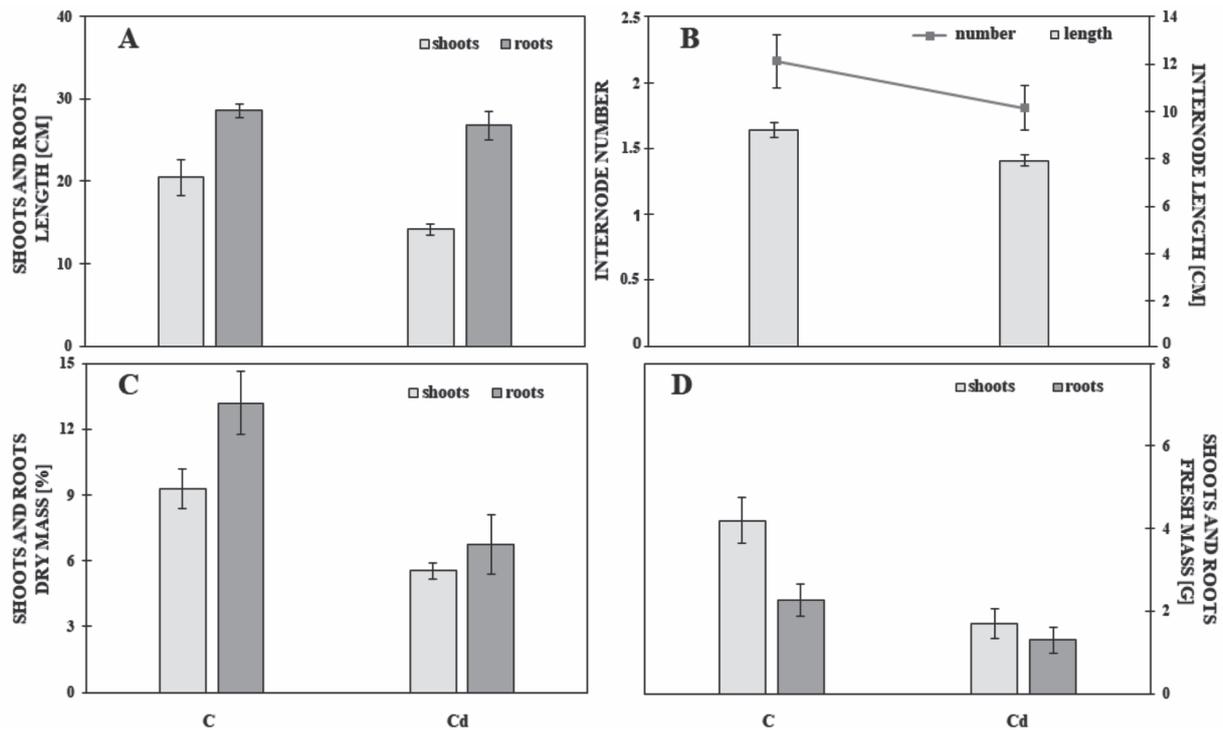


Fig. 1. Shoots and roots length [cm] A), internode length [cm] and number B), shoots and roots dry mass [%] C) and shoots and roots fresh mass [g] (D).

of internodes was 16% lower in the plants' shoots treated with Cd. Their length was also shorter than that of the internodes in the control plants. The length and number of internodes contribute to the height of the soybean [27]. Cd affects the morphogenesis of the stem through abnormal cellular division, too [28].

Moreover, the addition of Cd treatment decreased the fresh mass of shoots by 46% and roots by 24% compared to control plants (Fig. 1D). The seedling dry ratio (%) was also analyzed (Fig. 1C). The shoots and roots dry mass ratio of plants grown in the nutrient solution with Cd was greater by 30% and by 18% in the case of the control plants. These results suggest that Cd inhibited the growth of the plants. The negative effect of Cd on plant development was proven in many plant species [29]. Cd inhibited the development of plants, which was determined by examining the length of shoots and roots, along with the plant biomass [11].

In leaves of plants treated with Cd, a slight degradation of chlorophyll and carotenoids was observed (Fig. 2A). The Cd caused a 3%, 6%, and 12% drop in chlorophyll a, chlorophyll b, and carotenoids content, respectively. The content of photosynthetic pigments in leaves of plants treated with Cd was similar to the control plants. The maximum quantum yield of photosystem II (PSII) was highest for the control plants (Fig. 2B). In the Cd treated plants, a reduction in the Qy of PSII photochemistry was observed. Leaves of plants with Cd showed a decrease in Fv/Fm in the 24 hours, first and second weeks from adding Cd. The reduction in the value of the Qy parameter in the first week from

the Cd treatment of the plants was over 4%. Xue et al. [30] showed that Cd inhibited the photosynthesis apparatus in plants. The course of photosynthesis in soybean leaves stressed with Cd was influenced by the concentration of Cd and the development phase of the leaves. The negative effect of Cd on the photosynthesis process in *Hybrid Pennisetum* was also shown by Song et al. [2]. They determined that Cd accumulated in the leaves restricted photosynthesis and inhibited non-stomatal in regulating the photosynthetic performance. The soil contamination of Cd (at 50-100 mg/kg) also affected the chlorophyll content by significantly reducing the concentration while maintaining the structure and quantity of chloroplasts. In various plant species, a reduction in the rate of photosynthesis was observed. However, the sensitivity of plants to Cd toxicity depends on the plant species, cultivars, ecotypes, and plant tissues [31]. It is considered that the changes in Fv/Fm are a reliable parameter of stress in plants [32].

Fig. 3 shows the graphs of the linear dependence of the signal intensities at  $m/z$ : 112 and 114 on the cadmium concentration in the solution. On the basis of the performed linear regression, the determination of cadmium for both signals meets the requirements of the method as to the linearity of signal changes from concentration. The linear determination coefficients are 1 (while correlation coefficients are: 1.000 and 0.999 for 112 and 114, respectively) in the studied range of the concentration. However, at  $m/z$  114 the analysis is more sensitive (slope around 329 in comparison

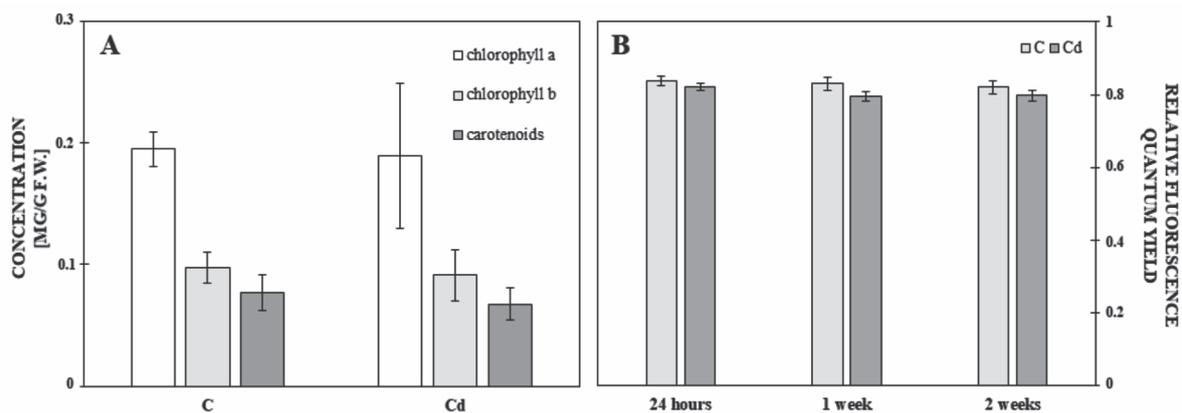


Fig. 2. Concentration of photosynthetic pigments in leaves of plants treated with Cd A), maximum quantum yield of PSII in dark-adapted state (Fv/Fm) B).

to 257 for  $m/z$  112). It is known that the sensitivity of the ICP-MS analysis is the result of the ionization properties of the element and the abundance of the isotope in the element isotope mixture [33]. Taking into account the parameters of LOD, LOQ and BEC (Table 1) both spectral lines allow for the analysis at a very similar level of concentrations; while the background level for 112 was recorded at a much higher level (BEC more than two times higher for 112 than 114). Cadmium at 112 and 114 suffers from the polyatomic interference from oxides of atoms rather poorly distributed in the environment such as Ru, Mo for 114, as well as for 112 tetra atomic clusters from  $^{40}\text{Ca}$  and  $^{40}\text{Ar}$  [25, 34]. The use of a collision cell minimizes the penetration of the interferent into the mass analyzer,

however, the recorded background value may prove that there is no complete reduction of interferences in the blank sample (higher BEC). However, line 111 was shown to be a better choice for the matrix of chocolate [35].

The limit of detection, LOD and quantification LOQ, values for  $^{114}\text{Cd}$  are: 2.2 and 7.5 ng/kg (determined in Cd concentration range between 0.01  $\mu\text{g/L}$  to 100  $\mu\text{g/L}$ ). Reported values are within the scope presented in other source works; LOD was 4.22  $\mu\text{g/kg}$  [36]; LOQ around 5  $\mu\text{g/kg}$  depending on the calibration methods [37]. However, by using the sector-field ICP-MS, the LOD for Cd quantification was 0.6  $\mu\text{g/kg}$  [38]. de Oliveira et al. [39] observed the matrix effect during the cadmium analysis by the ICP-MS analysis, which can be reduced

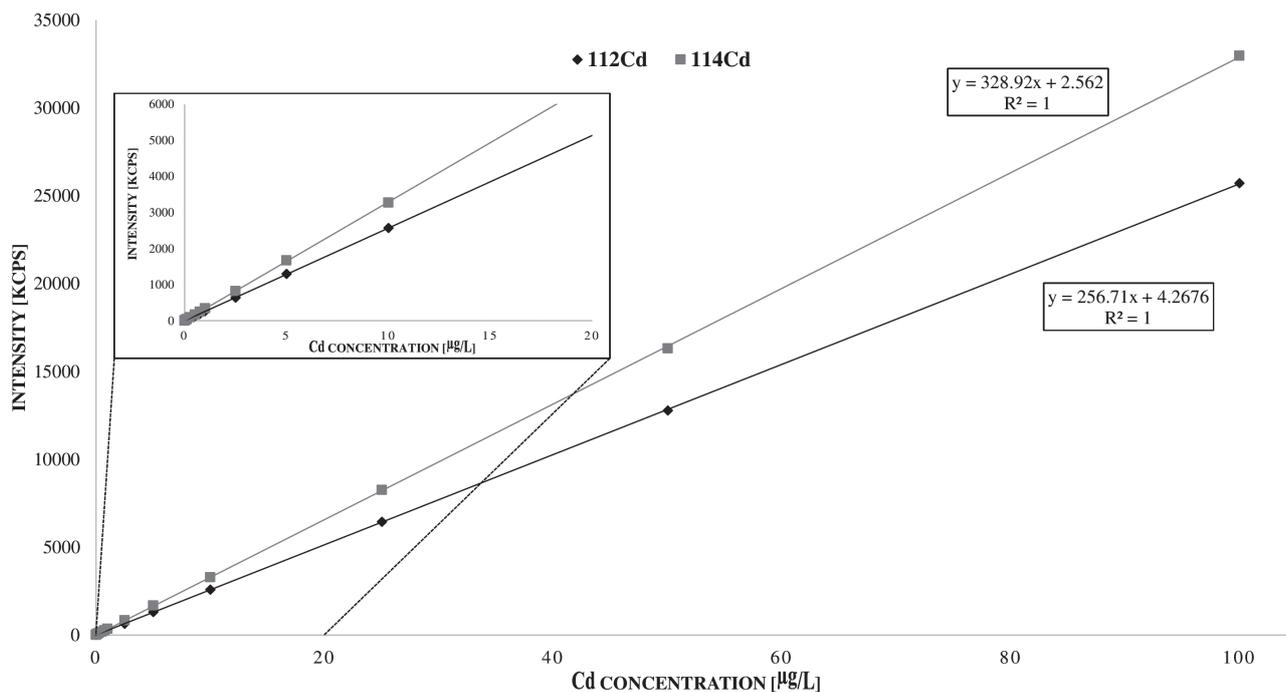


Fig. 3. The plot of the dependence of the signal intensity for  $^{112}\text{Cd}$  and  $^{114}\text{Cd}$  on the cadmium concentration in the solution.

Table 1. The validation parameters and determined cadmium content in the Certified Reference Material - RYE GRASS as well as in the roots and shoots of pea plant sample with calculated BCF and Ti parameters.

Cd analysis parameters				
Parameter	112		114	
LOD [ng/L]	2.1		2.2	
LOQ [ng/L]	7.1		7.5	
BEC [ng/L]	16.5		7.5	
Cd content in ERM® (CD281) RYE GRASS				
Content [mg/kg]	118±0,010			
Recovery [%]	98±0,13			
Pea plant				
	Control [mg/kg]	Cd- medium [mg/kg]	BCF	Ti [%]
Roots	5.55	281.27	0.05	22.5
Shoots	0.80	63.37	0.01	

by a sample dilution, an appropriate calibration method. Due to the lower background level, lines  $^{114}\text{Cd}$  were selected for later research.

After the verification process of the cadmium determination method, the results concerning the determination of accuracy and repeatability were presented (Table 1). The recovery of the cadmium content is 98 % with repeatability equal to 0,13 %. On this basis, it can be concluded that the method meets the accuracy requirements (it is within the recommended limits  $\pm 20$  % [40]) and is repeatable.

The obtained results provide an overview of the phenomenon of cadmium bioaccumulation by pea plant grown in controlled hydroponic cultivation at pH 6. The process of Cd accumulation depends on the amount of Cd in the environment and on the bioavailability of the form of this element in the medium, at studied pH, Cd occurs in the forms of  $\text{CdCl}_2$ ,  $\text{CdHCO}_3$  and  $\text{CdCO}_3$  which are bioavailable for plants [41]. The content of cadmium in the roots and shoots of peas was investigated using the verified sample preparation and determination methods (Table 1). It was determined that cadmium in ultra-trace amounts (5.55 mg/kg) accumulated mainly in the roots of the control plant, while the content in the shoots was measurable. A plant that grows in an environment contaminated with cadmium, accumulates this element in parts of the roots. Cadmium uptake by plant roots can follow two paths: apoplastic adsorption and then symplastic uptake [42]. First step is pH controlled due to ionic exchange properties of cell wall of root and its nature is passive uptake; however, the symplastic step is active transport of ions through cytoplasm and plasmodesmata (using specific transporters) and it's controlled by metabolic activity of plant [43]. On this basis, it is possible to postulate possible mechanisms of the accumulation and transport of cadmium in the plant. It was previously proved that cadmium had a strong affinity for sulfur atoms in chemical compounds; it

turns out that cadmium can be effectively chelated with phytochelatin in the roots [44, 45]. Other mechanisms of cadmium accumulation in roots are also possible, including binding to compounds of the (mainly pectin) cell wall membrane or roots exudates outside the outer layer of roots [46]. Referring to a four-point scale of



Fig. 4. Control plants A) and B) plants three weeks after the cadmium treatment. Scale bar: 1 cm.

BCF, accumulation in both roots and shoots could be classified as low [26].

Cadmium transport into the root xylem and phloem can be realized via both pathways, which are controlled by phytochelatins, vacuolar sequestration, and apoplastic barriers [43]. According to the Baker classification and the translocation coefficient, pea plant in these studies is classified to excluders [47]. This means that the most adsorbed Cd content is localized in roots than upper parts of plants. Most of plants exhibits this characteristic [48].

Fig. 4 shows a photo of a plant grown in a non-contaminated medium and in an environment contaminated with cadmium. By comparing the two samples, you can see macroscopic differences in the under-ground and above-ground parts of the plant.

### Conclusions

This study proved that the 50  $\mu\text{M}$  of  $\text{CdSO}_4$  concentration in medium solution caused the decrease of photosynthesis parameters. Under the impact of the stressor in pea leaves, the content of photosynthetic pigments, especially chlorophyll a, was reduced. Additionally, a negative role of Cd in plant growth and development has been shown. This means that the inhibition of the growth of plants was caused not only by the occurrence of oxidative stress but also by a decrease in the efficiency of the photosynthesis process. Research has shown that pea accumulates the majority of cadmium in the roots, which may be due to the binding of this metal in a complex with phytochelatin.

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### Conflict of Interest

The authors declare no conflict of interest.

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