IJAFLS
e-ISSN: 2602-4381

International Journal of Agriculture, Forestry and Life Sciences

Research Article | Open access | Int J Agric For Life Sci (2025) 9(2):73-82

The Impact of Nitrogen Fertilizer Usage on the Uptake of Cadmium and Nutrients by Tobacco (*Nicotiana tabacum* L.) Plants

Hatice Dağhan[1*] **Nurdilek Gülmezoğlu**[1]

[1]*Eskişehir Osmangazi University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, 26160 Eskişehir, Türkiye*

***Correspondence:** hdaghan@ogu.edu.tr

Citation

Dağhan, H., Gülmezoğlu, N. (2025). The Impact of Nitrogen Fertilizer Usage on the Uptake of Cadmium and Nutrients by Tobacco (Nicotiana tabacum L.) Plants. Int J Agric For Life Sci (2025) 9(2):73-82

Received :16 August 2025
Accepted :1 October 2025
Published Online :28 November 2025
Year: :2025
Volume :9
Issue :2 (December)
Pages :73-82

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY-NC) license

https://creativecommons.org/licenses/by-nc/4.0/

International Journal of Agriculture, Forestry and Life Sciences; Edit Publishing, Eskişehir, Türkiye.

Copyright © 2025

Available online https://dergipark.org.tr/ijafls

Abstract

Heavy metal pollution is a significant global issue, primarily caused by industrial and mining activities. Among these pollutants, cadmium (Cd) poses a significant risk to agriculture and food safety. One promising solution is phytoremediation, the use of plants to clean up contaminated soil, which is both cost-effective and environmentally sustainable. Applying nitrogen (N) can enhance the biomass of phytoaccumulator plants such as tobacco, thereby increasing their capacity to accumulate heavy metals from the soil. This study investigates the effects of increasing doses of N (0, 100, 200, 400 mg kg-1) and Cd (0, 1.5, 3, 6 mg kg-1) on Cd uptake and tolerance in tobacco plants grown in pot experiments under controlled climate conditions. Chlorophyll concentration increased with higher N applications, while plant dry weights decreased with increasing doses of Cd. The highest dry matter yield (13.0 g) was recorded in the treatment with 100 mg kg-1 N with 0 mg Cd kg-1. Nitrogen applications significantly increased the N uptake of the tobacco, with the highest N concentration (3.07%) observed at 400 mg kg-1 N. per pot. The effects of Cd applications on the N concentration of the plant were not statistically significant. Combined applications of N and Cd led to increased Cd accumulation in tobacco, with the highest Cd concentration (162 mg kg^{-1}) observed at 6 mg kg^{-1} Cd and 400 mg kg^{-1} N. Nitrogen fertilization also increased the uptake of phosphorus (P), potassium (K), magnesium (Mg), and manganese (Mn) in the tobacco, while reducing the uptake of copper (Cu), iron (Fe), and zinc (Zn) ($p \leq 0.01$). Compared to the control (0 mg Cd kg-1) treatment, Cd treatments decreased the uptake of P, K, Mg, Cu, Mn, and Zn, but increased Fe uptake ($p \leq 0.01$). Further detailed analyses are needed to elucidate the biochemical and physiological response of tobacco plants to enhanced Cd uptake under N fertilizer in future studies.

Key words
Cadmium, fertilizer, nitrogen, phytoremediation, tobacco, plant, soil.

Introduction

The mean Cd concentration in the Earth's crust is approximately 0.10 mg kg-1. In uncontaminated soils, this value is generally below 0.5 mg kg-1. However, depending on the properties of the parent material, Cd content in some soils can exceed 3 mg kg-1 (Özbek et al., 1993; Ballabio et al., 2024). Alarmingly, a gradual increase in Cd levels, by approximately 0.1–0.3 mg kg-1, has been reported in soils compared to pre-industrial periods (Ballabio et al., 2024).

Cadmium is released into the environment through both natural processes (e.g., rock erosion, volcanic activity, forest fires, wastewater discharges) and anthropogenic activities (e.g., mining, paint production, fertilizer and pesticide use, combustion emissions) (Sarwar et al., 2010; Naeem et al., 2019; Bai et al., 2021; Yang et al., 2020; Shaari et al., 2022; Zulfiqar et al., 2022). In agricultural soils, Cd can become mobile and be taken up by plants (Sarwar et al., 2010; Zulfiqar et al., 2022; Shaari et al., 2022; Ballabio et al., 2024). Due to its high toxicity and tendency to bioaccumulate in living tissues, Cd is considered one of the most hazardous heavy metals to living organisms. Consumption of products grown in Cd-contaminated soils can lead to the accumulation of this metal in the human body, potentially causing serious health problems, such as itai-itai disease, neurotoxicity, lung and kidney diseases, osteoporosis, and cancer (Sarwar et al., 2010; Dağhan, 2016; Naeem et al., 2019; Zulfiqar et al.,

2022). According to the 2022 report of the American Agency for Toxic Substances and Disease Registry (ATSDR), Cd is ranked 7th among substances that pose the most significant potential danger to human health (ATSDR, 2025).

Various remediation methods are employed for Cd-contaminated soils, including excavation, covering the contaminated layer with clean soil, vitrification, soil washing, bioremediation, phytoextraction (Naeem et al., 2019; Yang et al., 2020; Balint & Boajă, 2024), and electrokinetics (Delil et al., 2020). Additional strategies such as crop rotation, liming, and the use of organic and inorganic additives are also employed. While physical and chemical treatments offer certain advantages like ease of implementation and short-term effectiveness, they are often costly and environmentally unfavorable due to the generation of secondary pollutants (Sarwar et al., 2010). Consequently, phytoextraction is the most cost-effective and environmentally suitable method for remediating Cd-contaminated soils (Dağhan, 2019; Bai et al., 2021; Shaari et al., 2022).

Phytoextraction relies on the uptake of metals by plant roots and their translocation to aboveground tissues. Plants used in this method must have high metal accumulation in their harvestable parts, tolerance to the accumulated metal, rapid growth, deep rooting, and ease of harvest (Dağhan, 2004, 2007; Dağhan et al., 2012; Dağhan, 2016; Balint & Boajă, 2024).

In nature, species such as Minuartia verna, Alyssum murale, Sedum alfredii, Thlaspi caerulescens, Arabidopsis halleri, and Noccaea caerulescens are classified as hyperaccumulators (Muszynska & Hanus Fajerska, 2015; Reeves et al., 2018). These plants can accumulate significantly higher concentrations of metals in their green parts in comparison to non-hyperaccumulator species. Their accumulation capacity varies: typically, they can accumulate 10,000 mg kg-1 Mn and Zn, 1,000 mg kg-1 Se, As, Ni, Cu, and Co, and 100 mg kg-1 Cd in dry mass (Rungruang et al., 2011). The relatively low Cd threshold (100 mg kg-1) reflects its high toxicity. However, hyperaccumulator plants are often slow-growing and produce limited biomass, and they usually accumulate only one specific metal, which restricts their practical application (Salt et al., 1995).

One promising strategy to improve the efficiency of Cd phytoextraction is to optimize plant biomass and accumulation. Recent studies have shown that tobacco (Nicotiana tabacum L.), a high-biomass species, is a candidate for cleaning heavy metal-contaminated soils (Dağhan, 2004; Ehsan et al., 2014; Yang et al., 2016; Dağhan, 2019). Tobacco can accumulate substantial amounts of Cd in its aerial tissues due to its characteristics, such as wide ecological tolerance, rapid growth, a deep root system, abundant green parts, and as well as its non-food production (Dağhan, 2007; Dağhan, 2019). It is an annual plant with minimal climate and soil selectivity, fast growth, high shoot biomass, and ease of harvest.

Since biomass production is critical for successful phytoremediation and phytoextraction, fertilization is considered one of the most effective interventions (Wang et al., 2020). Certain nutrients influence Cd bioavailability and uptake in the soil-plant system, either directly or indirectly. One indirect mechanism is biomass dilution, which reduces Cd concentration and alleviates physiological stress (Sarwar et al., 2010).

After carbon, N is the most abundant essential element for plant growth. It plays a vital role in helping plants tolerate Cd stress (Bai et al., 2021). Nitrogen fertilizers, which promote vegetative growth, are the most commonly used inorganic plant nutrients (Rodríguez Ortiz et al., 2006). Nitrogen is indispensable for key metabolic processes such as photosynthesis, nutrient transport, biomass formation, and yield (Yang et al., 2020). Additionally, as a structural component of some biomolecules, N contributes to signalling pathways involved in defence responses of plants under stress conditions (Durner & Klessig, 1999). Numerous studies have examined defense responses triggered by nitrogen deficiency or by applications of ammonium or nitrate forms (Chaffei et al., 2008; Zhao et al., 2005; Polesskaya et al., 2004). However, some nitrogenous fertilizers can acidify the soil, changing the mobility and uptake of heavy metals by plants. For instance, nitrate nutrition has been demonstrated to enhance Cd and Zn phytoextraction in the hyperaccumulator Thlaspi caerulescens (Xie et al., 2009). Moreover, increased nitrogen application aimed at boosting cereal protein content has been associated with elevated grain Cd concentrations in contaminated soils (Wangstrand et al., 2007). Rodríguez-Ortiz et al. (2006) investigated the uptake and accumulation of Cd and Pb in tobacco shoots by applying different N forms (ammonium nitrate (NH4NO3) and urea (CO (NH2)2)) at increasing doses. They reported that higher doses of ammonium nitrate enhanced plant dry weight and metal accumulation. Diatta and Grzebisz (2006) demonstrated that the effects of different N sources [NH_4NO_3, CO $(NH_2)_2$ (urea), NH_4NO_3 + $CaCO_3$] and doses on soil Cd, Cu, Pb, and Zn contents were closely linked to the buffering capacity of the soil. Nitrogen application is capable of increasing the amount of heavy metals removed per unit area by enhancing plant biomass. Nevertheless, studies examining the effects of N fertilizers on heavy metal uptake remain limited.

This study investigates the impacts of varying concentrations of N and Cd on Cd bioaccumulation, leaf chlorophyll content, biomass productivity, and the nutrient element composition in tobacco plants used for phytoextraction under controlled climate chamber conditions.

Materials and Method

Plant Material

The Akhisar-97 tobacco variety (Nicotiana tabacum L.) was used as the plant material for this study.

Soil Material

The soil sample used for the experiment was taken from a depth of 0–30 cm in the field of the Faculty of Agriculture at Eskişehir Osmangazi University. The soil was air-dried and passed through a 4 mm sieve for the experiment and a 2 mm sieve for analyzing some physical and chemical properties. The soil texture was analyzed using the Bouyoucos hydrometer method (Bouyoucos, 1951). The pH and electrical conductivity (EC) were measured in a 1:2.5 soil: water extract (Richards, 1954). Walkley and Black's rapid titration method (Nelson & Sommers, 1996) was used to estimate the soil's organic matter content. Available P was determined according to the method described by Olsen et al. (1954). The K concentration was determined using the ammonium acetate extraction method (Richards, 1954). The concentrations of Zn, Fe, Cu, Mn, and Cd were determined by Lindsay and Norvell (1978), while the total Cd concentration was determined by the EPA 3051A microwave method (USEPA, 1995).

The experimental soil was loam-textured and slightly alkaline (pH 7.67). It was non-saline (0.026%) and contained a moderate amount of $CaCO_3$ (11.8%), along with a very low organic matter content (0.69%). The concentrations of available P and K were also low, measured at 4.13 mg/kg and 136 mg/kg, respectively. The available micronutrient concentrations in the soil were as follows: Mn very low (3.54 mg kg-1), Zn low (0.21 mg kg-1), Cu sufficient (1.38 mg kg-1), and Fe moderate (1.49 mg kg-1). The concentration of Cd could not be determined.

Experiment setup and plant growth

The experiment was conducted in a climate chamber with a 16/8-hour light/dark cycle, maintaining humidity at 60-70% and a temperature between 22-25°C. Air-dried soil samples weighing 2 kg were filled into plastic pots. Increasing doses of N (0, 100, 200, and 400 mg N kg-1 of soil) in the form of NH_4NO_3 and Cd (0, 1.5, 3, and 6 mg kg-1 of soil) in the form of $CdSO_4 \cdot 8H_2O$ were applied to the pots and incubated for three weeks. The experimental design followed a split-block layout with three replicates.

Tobacco seeds were germinated in a mixture (1:1) of peat: perlite in multi-cell trays. Three seedlings, each with two to three leaves, were transferred into each pot. Prior to planting, the soil was supplemented with basal fertilizers (KH_2PO_4 at 100 mg kg-1 P and 125 mg kg-1 K, and Fe-EDTA at 2.5 mg kg-1 Fe in solution form).

The plants were grown until they reached maximum biomass before the flowering stage (approximately six weeks). Throughout the experiment, the morphological characteristics of the plants were tracked and their developmental stages monitored. Treatments and the detectable changes were documented photographically. Chlorophyll concentration was measured on the fifth and sixth leaves from the top of each plant using a Konica Minolta SPAD-502 chlorophyll meter before harvest.

Plant Harvest and Analysis

At the end of the experiment, the plants were harvested 1 cm above the soil surface, washed in deionised water, dried, and stored in a drying cabinet at 65°C for 48 hours until they reached a constant weight. The dry weights of the plants were recorded, and the dried samples were ground in a mill (Retsch MM301 Mixer Mill, Retsch GmbH, North Rhine-Westphalia, Germany) for further analysis.

Approximately 0.2 g of the ground sample was weighed precisely, then solubilized with 3 mL of deionized water, 2 mL of 35% H_2O_2, and 5 mL of 65% HNO_3 in a microwave digestion system (MarsXpress, CEM, Matthews, USA). The digested samples were diluted with water to obtain a final volume of 20 mL. Blank samples were prepared following the same procedure.

The total concentrations of Cd and selected micronutrients (Zn, Fe, Cu, Mn, K and Mg) were determined by using atomic absorption spectrometry (AAS; Analytic Jena GmbH, Berlin, Germany). The amount of P in plant leaves was analyzed using the yellow color method and quantified with a spectrophotometer (Kacar, 1995). The N content was measured using the Kjeldahl method (Bremner, 1965). To ensure accuracy, plant nutrients and Cd analyses were validated using a certified reference material (Virginia Tobacco Leaves, CTA-VTL-2) with known elemental concentrations.

Statistical analysis

The experimental data were analyzed using the analysis of variance (ANOVA) in the SPSS software. The differences between treatment means were evaluated using the least significant difference (LSD) test.

Results

Morphological Observations

During the experiment, the effect of increasing doses of N application on Cd accumulation in tobacco plants was evaluated. The plants grown without N (0 mg N kg^{-1}) and exposed to increasing Cd doses (0, 1.5, 3, and 6 mg Cd kg^{-1}) showed stunted growth and smaller leaves. The old leaves exhibited homogeneous yellowing in the absence of both N and Cd. However, Cd application caused severe necrosis in older leaves, while younger leaves and shoots remained light green (Figure 1A).

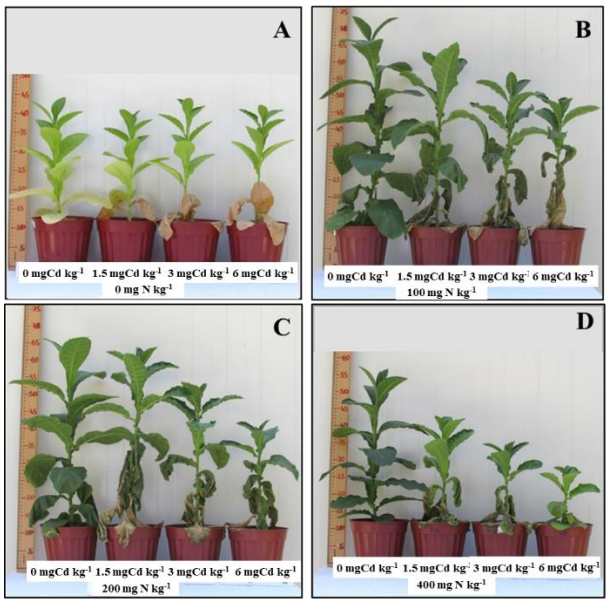


Figure 1. Morphological changes of tobacco with the increasing dose of N and Cd applications for 6 weeks.

In contrast, plants not treated with Cd but supplied with increasing N doses (100-200 and 400 mg N kg^{-1}) showed vigorous shoot development and large green leaves. Nevertheless, when both N and Cd doses were increased simultaneously, severe chlorosis appeared in older leaves, accompanied by reductions in biomass and leaf number (Figs. 1B-C and D). Pronounced necrosis was observed in the lower, older leaves, whereas the younger leaves remained green and exhibited doming on the leaf surface.

Chlorophyll Concentration

Chlorophyll concentration of the tobacco leaves is shown in Table 1. Based on mean values, the highest chlorophyll concentration (47.2 SPAD units) was determined at 100 mg kg^{-1} N application. Nitrogen doses had a statistically significant effect on chlorophyll concentration ($p \leq 0.01$), whereas the effects of Cd application and NxCd interactions were not statistically significant.

Plant Dry Weight

The highest dry weight (13.0 g $plant^{-1}$) was obtained from 100 mg N kg^{-1} with 0 mg Cd kg^{-1} (Table 1). Conversely, the lowest dry weight (1.70 g $plant^{-1}$) was recorded under 400 mg N kg^{-1} with 6 mg Cd kg^{-1}. Cadmium treatments caused a decrease in the dry weight of the plants. The effects of N applications, Cd applications, and NxCd interaction on the dry weight were all statistically significant ($p \leq 0.01$).

Nitrogen and Cadmium Concentrations

The effects of N application on the N concentration of tobacco plants were found to be statistically significant ($p \leq 0.01$). However, the impact of Cd application and NxCd interaction on the N concentration was not significant (Table 1). Evaluating the means revealed that the highest N concentration (3.07%) in tobacco plants was recorded with a 400 mg kg^{-1} N application.

The application of N and Cd, as well as the interaction, had a very significant effect ($p \leq 0.01$) on the concentration of Cd in tobacco plants (Table 1). There was an increase in Cd concentration in the shoots with increasing N and Cd treatments. The highest concentration of Cd (162 mg kg^{-1}) was observed under the combined application of 6 mg Cd kg^{-1} and 400 mg N kg^{-1}.

Furthermore, the threshold for Cd hyperaccumulation (>100 mg Cd kg^{-1}) was exceeded in the control and N doses at 6 mg Cd kg^{-1} (Cd concentrations of 130, 117, 149, and 162 mg kg^{-1}, respectively). At lower Cd doses, N application may reduce Cd uptake, resulting in concentrations below the hyperaccumulation threshold. Another

possible explanation is that the increase in shoot and root biomass after N applications may be due to the dilution of Cd in the tissues.

Table 1. The impact of N and Cd applications on chlorophyll concentration, dry weight, and N and Cd concentrations in shoots.

Treatments (mg kg^{-1})		Chlorophyll (SPAD Unit)	Dry Weight (g plant^{-1})	N (%)	Cd (mg kg^{-1})
N0	**Cd0**	31.9	4.36ef	0.74	0.002h
	Cd1.5	28.5	3.83ef	0.65	56.7f
	Cd3	28.5	3.53fg	0.71	113cd
	Cd6	31.0	3.14fg	0.8	130bc
N100	**Cd0**	46.5	13.0a	2.46	0.001h
	Cd1.5	46.6	8.15c	2.44	24.5gf
	Cd3	48.2	6.82cd	2.71	63.4f
	Cd6	47.3	4.67ef	2.84	117cd
N200	**Cd0**	46.9	10.6b	2.33	0.001h
	Cd1.5	47.2	7.92c	3.04	25.8gf
	Cd3	46.2	5.80de	2.57	66.3ef
	Cd6	46.6	3.24fg	3.09	149ab
N400	**Cd0**	48.8	7.14cd	3.20	0.001h
	Cd1.5	49.8	4.44ef	3.27	47.5fg
	Cd3	47.8	2.75fg	2.59	93.3de
	Cd6	44.3	1.70g	3.21	162a
N mean	**N0**	30.0b	3.72c	0.73b	74.6a
	N100	47.2a	8.15a	2.61a	51.2b
	N200	46.7a	6.90b	2.76a	60.2b
	N400	46.2a	4.01c	3.07a	75.6a
Cd mean	**Cd0**	43.7	8.78a	2.18	0.001d
	Cd1.5	43.0	6.09b	2.35	38.6c
	Cd3	41.4	4.73c	2.15	83.9b
	Cd6	42.0	3.19d	2.49	139a
ANOVA	**N**	**	**	**	**
	Cd	n.s.	**	n.s.	**
	NxCd	n.s.	**	n.s.	**

**: $p \leq 0.01$, *: $p \leq 0.05$, n.s: non-significant

Mineral Nutrient Element Concentrations

The changes in the content of mineral nutrients (P, K, Mg, Cu, Fe, Mn, and Zn) of tobacco plants in response to increasing N and Cd applications are presented in Table 2. The highest P concentration (0.62%) was observed in plants treated with 400 mg N kg^{-1}, without Cd treatment. When the mean values of the applications were analysed, the highest P concentration (0.51%) was recorded in the 200 mg N kg^{-1} application. In contrast, it was found that Cd applications reduced the P concentration in tobacco. The lowest P concentration (0.34 %) was obtained at a dose of 6 mg Cd kg^{-1}. The impact of N and Cd applications on the P concentration of plants was found to be statistically significant ($p \leq 0.01$). However, the effect of NxCd interaction on P uptake of tobacco plants was found to be insignificant.

The highest K concentration in tobacco plants (2.77%) was obtained from a treatment with 100 mg N kg^{-1} and 1.5 mg Cd kg^{-1}. When the results were evaluated in terms of means, the highest K concentration (2.48% and 2.43%, respectively) was obtained from treatment doses of 100 and 200 mg N kg^{-1}. As the N dose increased, a decrease in the K concentration was observed. However, except for the 6 mg Cd kg^{-1} dose, K concentration values were found to be similar and high at all doses, including the control. The effects of N and Cd applications, as well as the NxCd interaction, on the K concentration of tobacco plants were found to be statistically significant ($p \leq 0.01$).

The Mg concentration in plants ranged from 0.37% to 1.59%. Compared to the control, Mg concentration was found to be similar and high in all doses of N application. The lowest Mg concentration (0.68%) was observed in the control plant that did not receive N application. The effect of increasing N application doses and NxCd interaction on Mg uptake by tobacco plants was found to be statistically significant at $p \leq 0.01$. However, it was found that the effect of Cd applications on the plant's Mg concentration was not significant.

The effect of N and Cd applications and NxCd interaction on plant uptake of Cu, Fe, Mn, and Zn was found to be statistically significant ($p \leq 0.01$). The highest Cu concentration in tobacco plants was obtained at application doses of 100 mg N kg^{-1} (3.71 mg kg^{-1}) and 0 mg Cd kg^{-1} (7.17 mg kg^{-1}), based on the means of N and Cd applications. The highest Fe concentration (146 mg kg^{-1}) was obtained from the application of 0 mg N kg^{-1} with 6 mg Cd kg^{-1}. The highest Mn concentration (100 mg kg^{-1}) was achieved with 400 mg N kg^{-1} and no Cd applied, while the highest Zn concentration (14.0 mg kg^{-1}) was found in control plants without N and Cd application. In N application averages, the highest Fe and Zn concentrations (75 mg Fe kg^{-1} and 8.31 mg Zn kg^{-1}) were observed when no N was applied, while the highest Mn concentration (60.4 mg kg^{-1}) was determined in the 400 mg N kg^{-1} application.

Regarding Cd averages, the highest Fe concentration (95.6 mg kg^{-1}) was observed at the 6 mg Cd kg^{-1} dose, while the highest Mn and Zn concentrations (58.3 mg kg^{-1} and 10.1 mg kg^{-1}, respectively) were found in plants without Cd.

Table 2. Effect of N and Cd treatment on mineral nutrient concentrations of shoot parts

Treatments (mg kg^{-1})		P	K	Mg	Cu	Fe	Mn	Zn
			(%)			(mg kg^{-1})		
N0	**Cd0**	0.36	2.40a-c	0.37c	5.44c	47.5d-f	13.6f	14.0a
	Cd1.5	0.28	1.79c-f	0.64bc	1.83d-f	67.7b-f	21.6d-f	8.76bc
	Cd3	0.28	1.37ef	0.90b	5.14c	38.9f	24.3d-f	5.60d
	Cd6	0.24	1.245f	0.80b	1.39d-g	146a	15.7ef	4.84d
N100	**Cd0**	0.60	2.00b-e	1.59a	6.28bc	47.8d-f	53.4bc	10.2b
	Cd1.5	0.50	2.77a	1.39a	5.09c	59.0c-f	37.5c-f	7.00cd
	Cd3	0.45	2.57ab	1.43a	2.84d	82.5bc	42.4b-d	5.06d
	Cd6	0.36	2.57ab	1.38a	0.61fg	78.3bc	40.7b-e	4.66d
N200	**Cd0**	0.58	2.23a-d	1.52a	7.89ab	46.5d-f	65.9b	8.78bc
	Cd1.5	0.54	2.57ab	1.38a	2.68de	43.0ef	42.7b-d	5.56d
	Cd3	0.51	2.70ab	1.36a	2.90d	46.6d-f	42.1b-e	5.06d
	Cd6	0.40	2.20a-d	1.47a	1.09e-g	89.41b	52.4bc	6.90cd
N400	**Cd0**	0.62	2.14a-d	1.42a	9.08a	56.7c-f	100a	7.31b-d
	Cd1.5	0.51	2.02b-e	1.34a	1.06efg	43.3ef	54.0bc	5.83d
	Cd3	0.42	2.13a-d	1.45a	0.08g	74.8b-d	45.6b-d	5.17d
	Cd6	0.35	1.61d-f	1.45a	0.001g	68.9b-e	41.7b-e	7.51b-d
N mean	**N0**	0.29b	1.70b	0.68b	3.45a	75.0a	18.8c	8.31a
	N100	0.48a	2.48a	1.45a	3.71a	67.0ab	43.5b	6.73b
	N200	0.51a	2.43a	1.43a	3.64a	56.4b	50.8b	6.57b
	N400	0.48a	1.98b	1.42a	2.55b	61.0b	60.4a	6.46b
Cd mean	**Cd0**	0.54a	2.19a	1.22	7.17a	50.0c	58.3a	10.1a
	Cd1.5	0.46b	2.29a	1.19	2.67b	53.2bc	39.0b	6.79b
	Cd3	0.41b	2.19a	1.29	2.74b	60.7b	38.6b	5.22c
	Cd6	0.34c	1.91b	1.28	0.77c	95.6a	37.7b	5.98bc
ANOVA	**N**	**	**	**	**	**	**	**
	Cd	**	**	n.s	**	**	**	**
	NxCd	n.s	**	**	**	**	**	**

**: $p \leq 0.01$, *: $p \leq 0.05$, n.s: not significant

Discussions

Globally, N fertilizers are the most widely used basic fertilizer to increase crop yields and quality (Yang et al., 2020; Naeem et al., 2019). Nitrogen fertilizers are essential for producing abundant green plant biomass (Rodríguez-Ortiz et al., 2006).

Nitrogen is a crucial macronutrient for plant growth and development. Plants need N to produce essential amino acids, nucleic acids, proteins, enzymes, and chlorophyll (Yang et al., 2020; Naeem et al., 2019). Therefore, N deficiency exhibits symptoms such as low chlorophyll levels, reduced biomass, and decreased grain yield and quality. Leaf chlorophyll concentration is closely related to N content (Wood et al., 1992). This relationship may vary depending on plant species, diversity, crop growth stages, and different applications to the plant (Esfahani et al., 2008). Leaf chlorophyll concentration can be expressed using SPAD readings. In this study, we observed that leaf chlorophyll concentration increased with higher doses of N compared to control plants. Conversely, Cd applications decreased the chlorophyll concentration relative to the control group. Since leaf chlorophyll concentration is crucial for photosynthesis, low levels can lead to issues such as chlorosis, slower growth, and lower yields (Arjenaki et al., 2012).

In general, the utilization of nitrogenous fertilizers such as ammonium nitrate or urea has been demonstrated to promote the yield of shoot dry matter (DM) in tobacco plants (Rodriguez-Ortiz et al., 2006). In cases of N deficiency, however, stunted growth, leaf yellowing, and reduced yield are observed (Yang et al., 2020).

The highest dry weight of tobacco plants (13.0 g $plant^{-1}$) was obtained at a dose of 100 mg N kg^{-1} in this study. However, higher doses of N reduced dry matter production. Bailey (2014) stated that increasing N doses increased yield; however, excessive N application did not increase yield and could reduce leaf quality. According to research by Chouteau and Fauconnier (1988), the N content of tobacco leaves ranges from 2% to 5%. Deficiency symptoms, like reduced biomass and yield loss, appear when N levels drop below 1.5%. We found that the amount of N in the control plants without N treatment ranged from 0.65% to 0.80%. These amounts were found to be well below the plants' N requirements. This was also observed morphologically (Figure 1A).

The amount of Cd uptake by plants depends on the solubility and concentration of Cd in the soil (Eriksson, 1990). The long-term and high-concentration uptake of Cd can lead to chronic toxicity in plants (Jackson & Alloway, 1992). Data indicate that N applications reduce the uptake of Cd in tobacco plants. This situation may result from the dilution of Cd in the plant's shoots and roots as it develops in conjunction with N uptake. Conversely, Cd hyperaccumulation levels (>100 mg Cd kg^{-1}) were exceeded in plants without N application at 3 and 6 mg kg^{-1} Cd doses (113–162 mg kg^{-1}, respectively).

The application of nitrogen reduced the uptake of Cd in tobacco plants at low Cd concentrations, leading to uptake levels below the threshold for Cd hyperaccumulation. Multiple studies have documented varying effects of nitrogen fertilization on Cd uptake from soil. Ru et al. (2004) determined that increasing doses of Cd applied to the soil increased the amount of Cd uptake by canola plants (543 mg Cd kg^{-1}). These amounts are well above the Cd hyperaccumulation threshold of 100 mg Cd kg-1 on a dry weight basis. They concluded that canola is an excellent hyperaccumulator plant for Cd.

It has been reported that the use of nitrogenous fertilizers enhances the phytoextraction of various metals and metalloids, such as Cd (Gülmezoğlu and Dağhan, 2022; Yi et al., 2022; Liu et al., 2023), Cd and Pb (Rodriguez-Ortiz et al., 2006), and Cd and Zn (Jacobs et al., 2018). Gülmezoğlu and Dağhan (2022) stated that N application may increase Cd uptake, but excessive N may not contribute to an increase in biomass. In contrast, Liu et al. (2023) found that N application may reduce the adverse effects of Cd on plant growth and increase total biomass. Jacobs et al. (2018) reported that although N fertilization increased biomass in *Noccaea caerulescens*, total metal uptake was affected differently by fertilization because Cd and Zn concentrations were lower in fertilized plants.

The P concentration in tobacco plants was found to range from 0.24% to 0.62%. Compared to the control plants, the nitrogen doses increased the P concentration. Ammonium-based nitrogen fertilizers can decrease soil alkalinity and lower the pH of the rhizosphere (Mitchell et al., 2000; Rodriguez-Ortiz et al., 2006; Huo et al., 2018). Additionally, the ammonium ion (NH_4^+) can cause metals to be released from exchange sites or soil colloids through ion exchange (Mitchell et al., 2000; Rodriguez-Ortiz et al., 2006). These findings reinforce our results. The Cd applied to tobacco had a decreasing effect compared to the control plants. The effect of Cd on phosphorus content varies depending on the plant variety and the plant organ. Ciecko et al. (2004a) reported that high Cd concentrations in soil increased the P concentrations in the grain and roots of oats, maize, and the green parts of lupins.

The K concentration in the tobacco plant varied with N applications, reaching its highest level at 100 mg N kg^{-1}. Conversely, Cd applications reduced the K concentration in the plant. The primary reason for decreased water and ion uptake in plants under cadmium stress is damage to root cells, which inhibits root growth and development. Additionally, stomatal closure decreases water loss through transpiration in plants under Cd stress. This may also interfere with the transport of other nutrients to the upper parts of the plant (Dağhan et al., 2012). This finding aligns with our results on K uptake. The impact of Cd on K levels differed among plants, as reported by Ciecko et al. (2004b). Chouteau and Fauconnier (1988) found that K levels in tobacco leaves ranged from 1.66% to 6.64%.

The Mg concentrations, which are an essential component of chlorophyll and play a significant role in plant development (Pinkerton, 1972), were determined to be between 0.37% and 1.59% in the test plants. The concentrations of microelements in the tobacco plants were found to be as follows: Cu 0.001 to 9.08 mg kg^{-1}, Fe 38.9 to 146 mg kg^{-1}, Mn 13.6 to 100 mg kg^{-1}, and Zn 4.66 to 14.0 mg kg^{-1}. The microelement deficiency observed in plants aligns with the results of the soil analysis. Iskander et al. (1986) noted that tobacco leaves contained 325–520 mg kg^{-1} of Fe, 16.8–30.5 mg kg^{-1} of Zn, and 155–400 mg kg^{-1} of Mn. Dias et al. (2012) reported a range of Cu concentrations from 0.15 to 0.52 mg kg^{-1}. Similar findings were also reported by Gülmezoğlu and Dağhan (2022). Researchers have reported that the concentration of microelements in *Brassica napus* L. plants increases with Cd applications, as well as N applications ranging from 100 to 200 mg/kg. They have also noted that this situation may cause deficiencies by affecting the uptake of microelements in plants grown in soils contaminated with Cd and deficient in N.

Conclusions

The effects of nitrogen fertilization on the growth, nutrient uptake, and Cd phytoremediation capacity of tobacco plants in cadmium-contaminated soils were investigated in this study. While the applied nitrogen doses led to an increase in leaf chlorophyll concentration and dry matter production, high doses may have negatively impacted yield. Furthermore, nitrogen applications reduced Cd uptake, thereby mitigating phytotoxicity. The effects of Cd and N on certain micro and macronutrients were found to vary. Cadmium applications decreased the concentrations of P, K, Mg, and some micronutrients in the plants.

In conclusion, N fertilization can enhance the Cd uptake capacity and tolerance of tobacco plants. However, the biochemical and physiological mechanisms underlying this process require further study. In addition, the data obtained show that the addition of N fertilizer improves phytoextraction only in soils with very low N content.

Fertilization is known to be the most effective way to increase biomass production, which is necessary for the phytoextraction of heavy metals. However, soils contaminated with heavy metals might often be deficient in other macro and micronutrients as well. Therefore, in addition to nitrogen, other nutrients can be supplied to the plant as needed to increase biomass and promote Cd uptake. Additionally, the physical and chemical properties of the soil play a significant role in the remediation of Cd-contaminated soils and the plant's uptake of heavy metals.

Acknowledgment

We would like to thank the Eskişehir Osmangazi University Scientific Research Projects Coordination Unit for their support of our research (Project No: 2016-23026). We would also like to thank the anonymous reviewers for their valuable comments.

Author Contributions

All authors contributed to the emergence of the manuscript and approved the final version

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Arjenaki F.G., Jabbari R., Morshedi A. (2012). Evaluation of drought stress on relative water content, chlorophyll content, and mineral elements of wheat (*Triticum aestivum* L.) varieties. *Int. J. Agric. Crop*. Sci., 4, 726–729.

ATSDR (2025). The Agency for Toxic Substances and Disease Registry (ATSDR) 2022 Substance Priority List. https://www.atsdr.cdc.gov/programs/substance-priority-list.html

Bai Z., Li D., Zhu L., Tang X., Wang Y., Mao R., Wu J. (2021). Nitrate increases cadmium accumulation in sweet sorghum for improving phytoextraction efficiency rather than ammonium. *Front. Plant Sci.*, 12, 643116. DOI:10.3389/fpls.2021.643116

Bailey W.A. (2014). Effect of nitrogen rate on growth, yield, quality, and leaf chemistry of dark tobacco. *Tob. Sci.*, 51(51),13–22. DOI: 10.3381/14-035R.1

Balint R., Boajă I. P. (2024). Assisted phytoextraction as a nature-based solution for the sustainable remediation of metal(loid)-contaminated soils. *Integr. Environ. Assess. Manag*., 20(6), 2003–2022.

Ballabio C., Jones A., Panagos P. (2024). Cadmium in topsoils of the European Union–an analysis based on LUCAS topsoil database. *Sci. Total Environ*., 912, 168710.

Bouyoucos G. J. (1951). A recalibration of hydrometer for making mechanical analysis of soils. *Agron. J.*, 43, 434–438.

Bremner J.M. (1965). Total nitrogen. In: Methods of soil analysis: part 2 chemical and microbiological properties, 9:1149-1178.

Ciecko Z., Kalembasa S., Wyszkowski M., Rolka E. (2004a). The effect of soil contamination with cadmium on the phosphorus content in plants. *Electronic J. of Polish Agricultural Univ.*, 7(1), 05. http://www.ejpau.media.pl/volume7/issue1/environment/art-05.html

Ciecko Z., Kalembasa S., Wyszkowski M., Rolka E. (2004b). Effect of soil contamination by cadmium on potassium uptake by plants. *Polish J. of Env. Studies*, 13(3), 333–337.

Court W.A., Elliot J.M., Hendel J.G. (1984). Influence of applied nitrogen fertilization on certain lipids, terpenes, and other characteristics of flue-cured tobacco. *Tob Sci*., 28, 69–72.

Chaffei C., Gouia H., Debouba M. Ghorbel M.H. (2008). Differential toxicological response to cadmium stress of bean seedlings grown with NO_3^- or NH_4^+ as nitrogen source. *Int. J. Bot.* 4, 14–23.

Chouteau J. Fauconnier D. (1988). Fertilizing for quality and yield tobacco. *IPI Bulletin*.11, 11–17. https://www.ipipotash.org/uploads/udocs/ipi_bulletin_11_fertilizing_for_high_yield_and_quality_tobacco.pdf

Dağhan H. (2004). Phytoextraction of heavy metal from contaminated soils using genetically modified plants. RWTH-Aachen Fakultät für Mathematik, Informatik und Naturwissenschaften, Institut für Umweltforschung (Biology V), Doktorarbeit, Aachen, Germany.

Dağhan H. (2007). Fitoremediasyon: bitki kullanılarak kirlenmiş alanların temizlenmesi. GAP V. Tarım Kongresi Bildiri Kitabı, 362–367, 17-19 Ekim, Şanlıurfa.

Dağhan H. (2016). *Tagetes patula* L. bitkisinin fitoremediasyon amaçlı kullanım potansiyelinin su kültürü koşullarında araştırılması. *Toprak Su Dergisi*, 5(2), 25–31.

Dağhan H. (2019). Transgenic tobacco for phytoremediation of metals and metalloids. In: *Transgenic plant technology for remediation of toxic metals and metalloids* (pp. 279–297). Academic Press.

Dağhan H., Köleli N., Uygur V., Arslan M., Önder D., Göksun V., Ağca N. (2012). Kadmiyum ile kirlenmiş toprakların fitoekstraksiyonla arıtımında transgenik tütün bitkisinin kullanımının araştırılması. *Toprak Su Dergisi*, 1:1–6.

Delil A.D., Köleli N., Dağhan H., Bahçeci G. (2020). Recovery of heavy metals from canola (*Brassica napus*) and soybean (*Glycine max*) biomasses using electrochemical process. *Environ. Technol. Innov.*, 17, 100559.

Diatta J.B. Grzebisz W. (2006). Influence of mineral fertilizer nitrogen forms on heavy metals mobility in two soils. *Pol. J. Environ. Stud*., 15(2a), 56–62.

Dias F.S., Bonsucessoa J.S., Oliveiraa L.C. Santos W.N.L. (2012). Preconcentration and determination of copper in tobacco leaves samples by using a minicolumn of sisal fiber (*Agave sisalana*) loaded with Alizarin fluorine blue by FAAS. *Talanta*, 89, 276–279. DOI: 10.1016/j.talanta.2011.12.027

Durner J., & Klessig D. F. (1999). Nitric oxide as a signal in plants. *Curr. Opin. Plant Biol.*, *2*(5), 369–374.

Ehsan S., Ali S., Noureen S., Mahmood K., Farid M., Ishaque W., Mohammad B.S., Rizwan M. (2014). Citric acid assisted phytoremediation of cadmium by *Brassica napus* L.. *Ecotoxicol. Environ. Saf.*, 106, 164–172.

Eriksson J.E. (1990). A field study of the factors influencing Cd levels in soils and in grain of oats and winter wheat. Water Air Soil Pollut., 53, 69–81.

Esfahani M., Ali Abbasi H.R., Rabiei B. Kavousi M. (2008). Improvement of nitrogen management in rice paddy fields using chlorophyll meter (SPAD). Paddy Water Environ., 6, 181–188.

Gülmezoğlu, N., Dağhan, H. (2022). The potential of nitrogen applied rapeseed (*Brassica napus* L) in remediation of cadmium polluted soil. *Res. J. Biotechnol*, 17(7), 16–22.

Iskander F.Y., Bauer T.L. Klein, D.E. (1986). Determination of 28 elements in American cigarette tobacco by neutron-activation analysis. Analyst, 111(1), 107–109.

Huo W., Zou R., Wang L., Guo W., Zhang D. Fan H. (2018). Effect of different forms of N fertilizers on the hyperaccumulator *Solanum nigrum* L. and maize in intercropping mode under Cd stress. *RSC advances*, *8*(70), 40210–40218. DOI: 10.1039/c8ra07151a

Jackson A.P. Alloway B.J. (1992). The transfer of cadmium from agricultural soils to the human food chain. In: Adriano, D.C. (Ed.), Biogeochemistry of Trace Metals. Lewis Publishers, London. pp. 109–158.

Jacobs A., De Brabandere L., Drouet T., Sterckeman T., Noret N. (2018). Phytoextraction of Cd and Zn with *Noccaea caerulescens* for urban soil remediation: influence of nitrogen fertilization and planting density. *Ecol. Eng.*, 116, 178–187. https://doi.org/10.1016/j.ecoleng.2018.03.007

Kacar B. (1995). Bitki ve toprağın kimyasal analizleri, III. toprak analizleri. A.Ü. Ziraat Fak. Eğitim, Araştırma ve Geliştirme Vakfı Yayınları No:3, Ankara, 704 s. (In Turkish).

Lindsay W.L. Norvell W. (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Am. J.*, *42*(3), 421–428.

Liu M., Shi M., Gao H., Zheng Y., Yi L. (2023). Nitrogen addition alleviates cadmium toxicity in *Eleocarpus glabripetalus* seedlings. Forests, 14(6), 1264. https://doi.org/10.3390/f14061264

Mitchell L.G., Grant C.A. Racz G.J. (2000). Effect of nitrogen application on concentration of cadmium and nutrient ions in soil solution and in durum wheat. *Can. J. Soil Sci.*, 80, 107–115.

Muszynska E., Hanus-Fajerska E. (2015). Why are heavy metal hyperaccumulating plants so amazing? *BioTechnologia. Journal of Biotechnology Computational Biology and Bionanotechnology*, 96(4).

Naeem A., Zafar M., Khalid H., Zia-ur-Rehman M., Ahmad Z., Ayub M.A. Qayyum M.F. (2019). Cadmium-induced imbalance in nutrient and water uptake by plants. In: *Cadmium toxicity and tolerance in plants* (pp. 299–326). Academic Press.

Nelson D.W. Sommers L.E. (1996). Total carbon, organic carbon, and organic matter. In: Methods of soil analysis: Part 3 Chemical methods, 5, 961–1010.

Olsen, S.R., C.V. Cole, F.S. Watanabe, L.A. Dean, (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate.* US Dept. of Agric. Cric. 939.

Özbek H., Kaya Z.,Gök M, Kaptan H. (1993). Toprak bilimi. Ç.Ü. Ziraat Fakültesi Ders Kitabı, Yayın No:16, 73s.

Pinkerton A. (1972). Recovery of flue-cured tobacco from magnesium deficiency: changes in leaf magnesium content and effects on leaf quality. *Aust. J. Agric. Res.*, *23*(4), 641–649.

Polesskaya O.G., Kashirina E.I. Alekhina N.D. (2006). Effect of salt stress on antioxidant system of plants as related to nitrogen nutrition. *Russ. J. Plant Physiol*. 53, 186–192.

Reeves R.D., Baker A.J.M., Jaffré T., Erskine P.D., Echevarria G. Van Der Ent A. (2018). A global database for plants that hyperaccumulate metal and metalloid trace elements. *New Phytol*, 218: 407–411.

Richards L.A. (Ed.). (1954). *Diagnosis and improvement of saline and alkali soils* (No. 60). US Government Printing Office.

Rodríguez-Ortíz J.C., Valdez-Cepeda R.D., Lara-Mireles J.L., Rodríguez-Fuentes H., Vázquez-Alvarado R.E., Magallanes-Quintanar R., García-Hernández J.L. (2006). Soil nitrogen fertilization effects on phytoextraction of cadmium and lead by tobacco (*Nicotiana tabacum* L.). *Bioremediation J.*, 10(3), 105–114.

Ru S.H., Wang J.Q. Su D.C. (2004). Characteristics of Cd uptake and accumulation in two Cd accumulator oilseed rape species. *J Environ Sci* (China), 16(4):594–8.

Rungruang, N., Babel, S., Parkpian, P. (2011). Screening of potential hyperaccumulator for cadmium from contaminated soil. *Desalin. Water Treat*., 32(1–3), 19–26.

Salt D., Price R., Pickering I. Raskin I. (1995). Mechanisms of cadmium mobility and accumulation in Indian mustard. *Plant Physiol*., 109, 1427–1433.

Sarwar N., Saifullah Malhi S.S., Zia M.H., Naeem A., Bibi S., Farid G. (2010). Role of mineral nutrition in minimizing cadmium accumulation by plants. *J. Sci. Food Agric.*, *90*(6), 925–937.

Shaari N.E.M., Tajudin M.T.F.M., Khandaker M.M., Majrashi A., Alenazi M.M., Abdullahi U.A., Mohd K.S. (2022). Cadmium toxicity symptoms and uptake mechanism in plants: a review. *Braz. J. Biol.*, *84*, e252143. https://www.scielo.br/j/bjb/a/Jz3BCcHF7fXNfzg5Prdt4SH/?lang=en

USEPA (1995). Microwave-assisted acid digestion of sediments, sludges, soils, and oils, in Test Methods for Evaluating Solid Waste, 3rd Ed., US Environmental Protection Agency, Washington, DC.

Wang L., Hou D., Shen Z., Zhu J., Jia X., Ok Y.S., Tack F.M.G., Rinklebe J. (2020). Field trials of phytomining and phytoremediation: A critical review of influencing factors and effects of additives. *Crit. Rev. Environ. Sci. Technol.*, *50*(24), 2724–2774. https://doi.org/10.1080/10643389.2019.1705724

Wangstrand H., Eriksson J. & Öborn I. (2007). Cadmium concentration in winter wheat as affected by nitrogen fertilization. *Eur. J. Agron.*, *26*(3), 209-214.

Wood C.W., Tracy P.W., Reeves D.W., Edmisten K.L. (1992). Determination of cotton nitrogen status with a hand-held chlorophyll meter. J. Plant Nutr., 15, 1435–1448.

Xie H.L., Jiang R.F., Zhang F.S., McGrath S.P. Zhao F.J. (2009). Effect of nitrogen form on the rhizosphere dynamics and uptake of cadmium and zinc by the hyperaccumulator *Thlaspi caerulescens*. *Plant Soil, 318*, 205–215.

Yang L., Sung H.Y., Mao Z., Hu T.W. Rao K. (2016). Economic costs attributable to smoking in China: update and an 8-year comparison, 2000–2008. In *Economics of Tobacco Control in China: From Policy Research to Practice* (pp. 9-28).

Yang Y., Xiong J., Tao L., Cao Z., Tang W., Zhang J. Lu Y. (2020). Regulatory mechanisms of nitrogen (N) on cadmium (Cd) uptake and accumulation in plants: a review. *Sci. Total Environ.t*, 708, 135186.

Yi L., Wu M., Yu F., Song Q., Zhao Z., Liao, L. Tong J. (2022). Enhanced cadmium phytoremediation capacity of poplar is associated with increased biomass and Cd accumulation under nitrogen deposition conditions. *Ecotoxicol. Environ. Saf.*, 246, 114154.

Zhao D.L., Reddy K.R., Kakani V.G. Reddy V.R. (2005). Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum. *Eur. J. Agron*. 22:391–403.

Zulfiqar U., Jiang W., Xiukang W., Hussain S., Ahmad M., Maqsood M.F., Ali N., Ishfaq M., Kaleem M., Haider F.U., Farooq N., Naveed M., Kucerik J., Brtnicky M., Mustafa, A. (2022). Cadmium phytotoxicity, tolerance, and advanced remediation approaches in agricultural soils: a comprehensive review. *Front. Plant Sci.*, 13, 773815.