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Investigating Usage Potential of *Datura stramonium* L. for Phytoremediation of 2,4-Dichlorophenol

2,4-Diklorofenolün Fitoremediasyonunda *Datura stramonium* L. Bitkisinin Kullanım Potansiyelinin Araştırılması

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ABSTRACT

In this work, the phytoremediation potential of 2,4-Dichlorophenol (2,4-DCP) from soil and wetlands by *Datura stramonium* L. (jimsonweed) was investigated. The medium of seedlings growing in a hydroponic system was adjusted to different concentrations (0.0, 75, 100, 125, 150, 175, 200, 225, 250 and 275 ppm) of 2,4-DCP. Four days later, the remediation rate of 2,4-DCP in the growth medium, and root-stem length, root-stem dry weight, lipid peroxidation (LPO), protein and photosynthetic pigment content of seedlings were evaluated. *D. stramonium* seedlings provided remediation of 2,4-DCP between 52-78% at all concentrations. In addition, the 2,4-DCP treatments inhibited the root-stem lengths and dry weights of seedlings compared to their controls, particularly at high doses such as 200-275 ppm, but not at low doses. The applications generally increased protein and LPO content of roots and leaves slightly, but did not affect chlorophyll. The results show that *D. stramonium* has a high usage potential for phytoremediation of 2,4-DCP.

Key Words

Datura stramonium, 2,4-Dichlorophenol, phytoremediation, lipid peroxidation.

ÖZ

Bu çalışmada, *Datura stramonium* L. (boru çiçeği) bitkisinin toprak ve sulak alanlardan 2,4-Diklorofenolün (2,4-DKF) fitoremediasyon potansiyeli araştırıldı. Hidroponik sistemde büyütülen fidelerin yetişme ortamı 2,4-DKF'nin farklı konsantrasyonlarına (0.0, 75, 100, 125, 150, 175, 200, 225, 250 ve 275 ppm) ayarlandı. Dört gün sonra, yetişme ortamında 2,4-DKF'nin remediasyon oranı, bitkilerin kök-gövde uzunluğu, kök-gövde kuru ağırlığı, lipid peroksidasyonu (LPO), protein ve fotosentetik pigmentlerin içeriği belirlendi. *D. stramonium* fideleri çalışılan tüm konsantrasyonlarda 2,4-DKF'nin remediasyonunu %52-78 oranlarında sağladı. Ayrıca 2,4-DKF uygulamaları, fidelerin kök ve gövde uzunlukları ile kuru ağırlıklarını, kontrollerine göre, özellikle 200-275 ppm gibi yüksek dozlarda genelde hafif derecede inhibe ederken, düşük dozlarda etkilemedi. Uygulamalar, genelde (LPO) ve protein içeriğini hafif derecede artırırken, yaprak klorofil içeriğini etkilememiştir. Bulgular, *D. stramonium*'un 2,4-DKF'nin fitoremediasyonunda kullanılabilme potansiyelinin yüksek olduğunu göstermektedir.

Anahtar Kelimeler

Datura stramonium, 2,4-Dichlorophenol, fitoremediasyon, lipid peroksidasyonu.

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INTRODUCTION

With the increase in global population and parallel rapid changes in the industrialization process, the amount and variety of pollutants distributed to the environment is increasing every day. A result of this is that the majority of organic and inorganic compounds left in the environment cause severe environmental problems. Pollutants with toxic, mutagenic, carcinogenic and/or permanent properties threaten human health, agricultural productivity and the environment [1,2]. Protection of the environment and natural resources from pollution has critical importance in terms of preventing environmental pollution while cleaning polluted areas carries great importance to solve the present environmental pollution. Pollutants mixing with soil and water and accumulating in them has been revealed to cause problems with microbial activity, soil productivity, biological diversity and product losses and even environmental and human health problems through the food chain. The increase in sources of environmental pollution around the world has made the development of techniques to remove them from the natural environment mandatory. Green reclamation (phytoremediation) is considered to have more economic, technical and environmental advantages than traditional physical and chemical treatment methods [2,3,4].

Chlorophenols (CP) generally occur as degradation products of other chlorinated xenobiotics due to their potential for use for general disinfectant aims. Their high tendency for toxicity and accumulation make their presence in soil and aqueous environments worrying. To date, though the permanence of CP in aqueous environments and the aqua toxic effects have been highly studied, there is less information available about the fate and behavior of CP in soil environments [5]. Additionally, these compounds are produced from degradation of pesticides and organic waste incineration [6,7,8]. For example, deadly medications including herbicides and some pesticides containing 2,4-dichlorophnoxycetic acid (2,4-D) are produced from 2,4-dichlorophenol (2,4-DCP) [9]. Additionally, these compounds are used as seed disinfectants and in production of paper pulp [10]. 2,4-DCP, currently used in production of paints, wood preservers, herbicides and other types of pesticide, was used in diet pills in the 1930s. However, after research in 1938 showed the oxidative phosphorylation connection was cut reducing ATP production potential, the use of these medications was banned. According to the Environmental Protection Agency (EPA) and the European Commission (2455/2001/EC), 2,4-DCP is a toxic carcinogenic resistant chemical compound inappropriate for biodegradation with a tendency for bioaccumulation and is classified as a priority environmental pollutant [11]. Additionally, this compound has been identified at high levels in clean water sources, marine environments and industrial water discharges [9,12].

Table 1. Significant physicochemical parameters of 2,4-DCP [5].

Compound	CAS No	Formula	Density (g / cm³)	Sol. in water	Log Kow	Log Koc	Kb (L.liq/L.gaz)	K_h (atmm³/mol) (atmm³/mol)	pKa	Bo Po. (°C)	Vapor Pre. (mmHg)
2,4-Dichlorophenol	120-83-2	$C_6H_4Cl_2O$	1.38	4.5	3.2	2.42 3.98	1.96E-04	$4.3.10^{-6}$	7.68	210	0.14

Bioaccumulation/soil-water coefficient (K_{ow} , K_{oc}) acidity constant (pK_a), aerobic biodegradation rate (K_b), Henry coefficient (K_h).

Currently efforts to develop removal strategies for 2,4-DCP from aqueous and agricultural areas are gaining increasing importance. In this context, a variety of physicochemical and biological methods are reported in the literature [9,13,21]. Additionally, physicochemical methods (Table 1) mostly have high cost and cannot always be applied. However, when concentrations of pollutants are low, the use of microorganisms for practical amelioration causes some difficulties [14]. Though there are intense studies about the effects of 2,4-DCP on the environment, there is limited information about phytoremediation [9,21]. Instead of using traditional remediation techniques which are expensive and require effort to ameliorate areas polluted with 2,4-DCP, in recent years the use of the lower cost technique of environmentally-friendly phytoremediation technology has been an important approach [21].

Phytoremediation is defined as the process of cleaning polluted areas of pollutants by cultivating plants. Plants uptake organic and inorganic pollutants into their components, accumulate, store or degrade them ensuring cleaning of polluted areas. In this way, the aim is to regulate or stabilize polluted land. Phytoremediation is important as it is sustainable, has appropriate cost and is an environmentally-friendly technique compared to other remediation methods. However, a condition required for phytoremediation to be successful is the development of appropriate plants and determining the tolerance mechanism of plants for pollutants. Some plants have the ability to accumulate 50-500 times the pollutant concentration in soil in their organs above the ground. These plants are called hyperaccumulator plants. They accumulate pollutants in their organs above the ground without any toxicity symptoms.

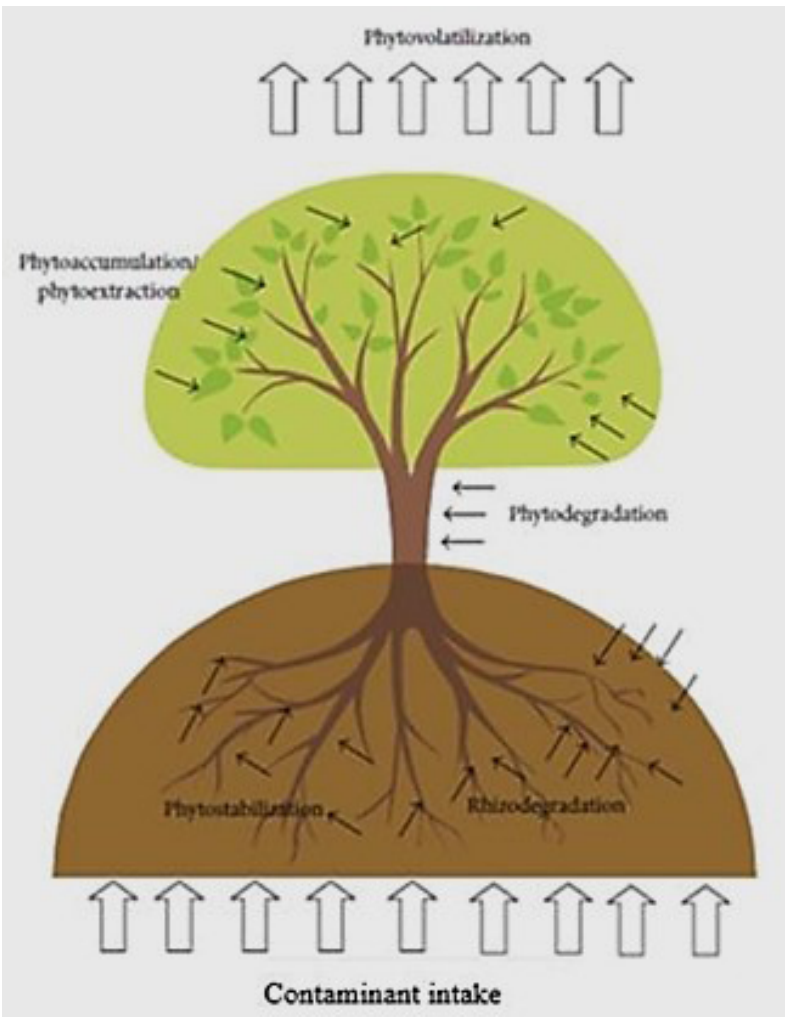

Figure 1. General types of phytoremediation.

As seen above, we can summarize some important concepts in remediation studies using plants as follows;

1. Phytoextraction (phytoaccumulation)

Phytoextraction is the name given to the method of uptaking metal pollutants causing soil pollution aided by plant roots. Plants have different characteristics in terms of accumulating harmful material from soil into their components. As a result, plants should be used that are resistant to the pollutants at high levels. This situation has great importance for locations with high pollution rates.

2. Rhizostabilization

The rhizofiltration method is used more rarely than other methods and requires a well-developed root system acting as a filter. This method is applied to remove heavy metals in much polluted water from the area with soil remediation. Pollutants are either absorbed by the plant roots or absorbed by the roots and transported to other organs in the plant. Hyperaccumulator plants chosen for the rhizofiltration method are gradually adapted to the pollutants in a different environment before being planted directly in the area.

3. Phytostabilization

Phytostabilization with the aim of preventing erosion in land where erosion generally occurs is used to prevent pollutants from seeping into groundwater and to prevent direct contact with soil. For this method, the soil surface is covered with hyperaccumlator plants appropriate to the area.

4. Phytodegradation

The phytodegradation method uses hyperaccumulator plants to degrade the structure of organic pollutants found in the environment and at the same time has separation ability. For this formation to occur, it is necessary for enzymatic reactions to occur. In other words, some pollutants are first absorbed by the plant and later degraded with the aid of enzymes. Organic compounds held in the body of the plant are degraded into smaller particles through metabolic mechanisms.

5. **Rhizodegradation**

The operating mechanism in this method is to make organic pollutants ineffective using plants and soil microorganisms together. Microorganisms producing nutrients required to meet the organism's energy requirements cause changes in chemical structure of pollutant materials with the aid of the root system.

6. **Phytovolatilization**

Vegetative evaporation events occur in trees through the roots with large amounts of water containing organic pollutants and heavy metals. The phytovolatilization method transforms pollutants held in plants into less volatile forms and releases them to nature through transpiration. As is known, water is taken from the roots with the aid of the vascular system and transported to the trunk and leaves. Thus, pollutants mix with the air surrounding the plant in condensation and gas form. Removal processes with methods like drying, burning, gasification, pyrolysis, acid extraction and anaerobic decay of plants containing high pollutant contents used for phytoremediation is another topic requiring care and attention. The usability of phytoremediation technology will increase with the full explanation of molecular, biochemical and physiologic processes characterizing accumulation of the pollutant in the plant body. For plant remediation studies, the size or spread of contaminated areas does not form a disadvantage. In this situation, remediation with plants (phytoremediation) is found to be a cheap choice compared with other methods.

One of the alternative plants used in phytoremediation studies is *Datura* species which is found in many locales in the world and is a plant with some species cultivated in many countries. Annual or perennial species of *Datura* have capsule fruit containing many seeds. Of these, *Datura metel, D. innoxia* and *D. stramonium* grow in our country, especially in the eastern Mediterranean [15]. *D. stramonium* generally flowers throughout the summer. The odorous flowers have trumpet shape, with cream or violet white color and 6 to 9 cm length. The plant grows rapidly in nearly all areas, adapts rapidly and produces many seeds which means it has the potential to be an appropriate plant for phytoremediation studies. For this, in our study we researched the potential to use *Datura stramonium* plant for phytoremediation of soil and wetland areas containing 2,4-DCP.


Figure 2. Images from preliminary trials of *Datura stramonium* in the laboratory.

Table 2. Characteristics of *Datura stramonium* plant.

Type of plant used in research	Physical characteristics of plant	Advantageous aspects of the plant for phytoremediation
Datura Stramonium	1. New shoots or perennial Datura has stem length of 1-1.5 m. 2. 10different types 3. Flower have poor odor and trumpet shape, with cream-violet or white color and 6-9 cm length. 4. Leaves had broad surface area (15-20 cm) 5. Roots are very long and dense.	Plant grows rapidly on all types of land, rapidly adapts to nearly all environments and produces seeds rapidly and in great numbers so it has the potential to be an appropriate plant for phytoremediation studies.

MATERIALS and METHODS

Plant Material and Cultivation Conditions

In the study, seeds collected from healthy plants developing in the natural environment and identified as *Datura stramonium* by an expert systematic botanist were first sterilized on the surface with 10% commercial sodium hypochloride for 10 min, then 75% ethanol for 5 min and then rinsed 5 times in distilled water. Later seeds were planted in a hydroponic environment containing 50% strength Hoagland medium with each basket (5 cm) containing 5 seeds. After germination of seeds, the seedlings were grown under normal conditions in a climate cabinet (22/25°C, 8/16 hour night/day photoperiods) until at least 4 true leaves appeared. Later, seedlings grown in the hydroponic system were cultivated in environments containing different concentrations of 2,4-DCP (0.0, 75, 100, 125, 150, 175, 200, 225, 250 and 275 ppm). Four days after this administration, water samples were collected from the hydroponic environment and the roots and stems of seedlings were collected as experimental material and stored at -80°C in a deep freezer.

Determination of 2,4-DCP remediation rate

The hydroponic system for the control group (0 ppm) and 75-275 ppm initial pollutant concentrations had equal volumes of water samples taken at the same time every day and the first stage of remaining pollutant analysis after phytoremediation was performed spectrophotometrically. With this aim, the hydroponic environment containing 2,4-DCP had 1 mL sample taken, diluted with distilled water and then set to pH 7.9±0.1 with phosphate buffer solution. Later 1 mL each of 0.098M 4-amino antipyrine solution and 0.243M $K_3(Fe(CN)_6)$ solution were added and the total volume

was set to 100 mL. This solution was mixed for 5 min and then sufficient sample was taken and read with a spectrophotometer at 510 nm. A standard graph previously prepared with known amounts of 2,4-DCP was used to determine the amount of 2,4-DCP as mg/mL in the samples. The initial (control) values were subtracted from the obtained results to calculate removal efficiency [16-17].

Determination of root-stem length and dry weight

When seedlings with 2,4-DCP applied and not applied (control) grown in the hydroponic environment reached the stage of at least 4 leaves, the root and stem length of seedlings was measured and mean root and stem lengths were determined. A total of 10 plants were taken from each group and wrapped in aluminum foil before drying in an oven at 72°C for 72 hours and then were weighed to calculate the dry weights of organs for each application (mg/seedling).

Determination of photosynthetic pigment content

To determine the amounts of chlorophyll a, chlorophyll b and total chlorophyll amounts in plant leaves, leaf samples (0.2 g) were homogenized in cold acetone to a final volume of 10 mL. Later the homogenate was strained through filter paper and the extract was centrifuged at 5000 rpm for 10 min. The supernatants were taken from the tubes and absorbance values were read at 450, 645 and 663 nm. The absorbance values read at three different wavelengths for supernatants were used in the following equations to calculate the chlorophyll a, chlorophyll b and total chlorophyll amounts in plant leaves in mg/tissue [18].

mg chlorophyll a/gr tissue=[(12.7 x OD663) - (2.69 x OD645)] x (V/1000 x W)

mg chlorophyll b/gr tissue=[(22.9 x OD645) - (4.68 x OD663)] x (V/1000 x W)

mg total chlorophyll/gr tissue =[(20.2 x OD645) + (8.02 x OD663)] x (V/1000 x W)

OD : Absorbance value at stated wavelength for chlorophyll extract

V : final volume of 80% acetone

W : Wet weight of extracted tissue in grams.

Determination of amount of soluble protein

From the root or leaves of *D. stramonium* seedlings, 0.2 g samples were randomly taken and ground to a powder with liquid nitrogen in a porcelain mortar. Later, this extract had 2.5 mL phosphate buffer (100 mM KH_2PO_4, pH: 7.0) added and the tissue was homogenized well. The obtained mixture was placed in a centrifuge tube and centrifuged for 15 min at +4°C 12000 rpm and later the supernatant in the tube was transferred to another clean tube. From this tube, 20 µL was pipetted onto a microplate and then 200 µL BCA (Bicinchoninic acid+$CuSO_4$) reactive was added. The contents of the microplate were shaken for a short period and then incubated at 60°C for 15 min. The absorbance of the sample on the microplate was read with a multiscan at 562 nm against a blind. The blind sample contained 20 µL distilled water and 200 µL BCA reactive. Calculation of the protein content of tissues used a standard graph (25-120 mg/mL). Using the standard graph, the protein amounts in leaves and roots are presented as µg/g [19,22].

Determination of lipid peroxidation level (as MDA)

Spectrophotometric measurement of malondialdehyde (MDA) reactives can be used to determine lipid peroxidation levels [20]. Additionally, 0.2 g samples were taken from leaf and root samples and ground with liquid nitrogen. To this extract, 5 mL 5% TCA solution was added and it was homogenized well in a mortar until completely disintegrated. The obtained homogenate was transferred to Eppendorf tubes and centrifuged at 15 min for 13000 rpm. From the supernatant portion, 1.5 mL was taken and 1.5 mL 0.5% TBA reactive (prepared in 20% TCA) was pipetted onto it. After the mixture was vortexed well, it was incubated in a water bath at 95°C for 30 min. Later the tubes were placed in an ice bath to stop the reaction. The obtained solution was centrifuged again at 12000 rpm for 5 min and then the absorbance values of the supernatant were determined at 440, 532 and 600 nm. The amount of malondialdehyde (MDA) per mL of mixture was calculated with the following formula and results are given as mmol/g tissue.

$$MDA\ (nmol/ml) = [[(A_{532}-A_{600}) - [(A_{440}-A_{600}) \times (0.0571)]] / 157000] \times 10^6$$

Statistical Analysis

Data in the study (± SE) were obtained from 6 independent values as 3 samples from each application (3 parallels) and 2 repeats of each sample. Comparison of results used the one-way analysis of variance (ANOVA) in the SPSS 12 program and differences between the groups were determined at $p<0.05$ significance level using Duncan's multiple comparison test.

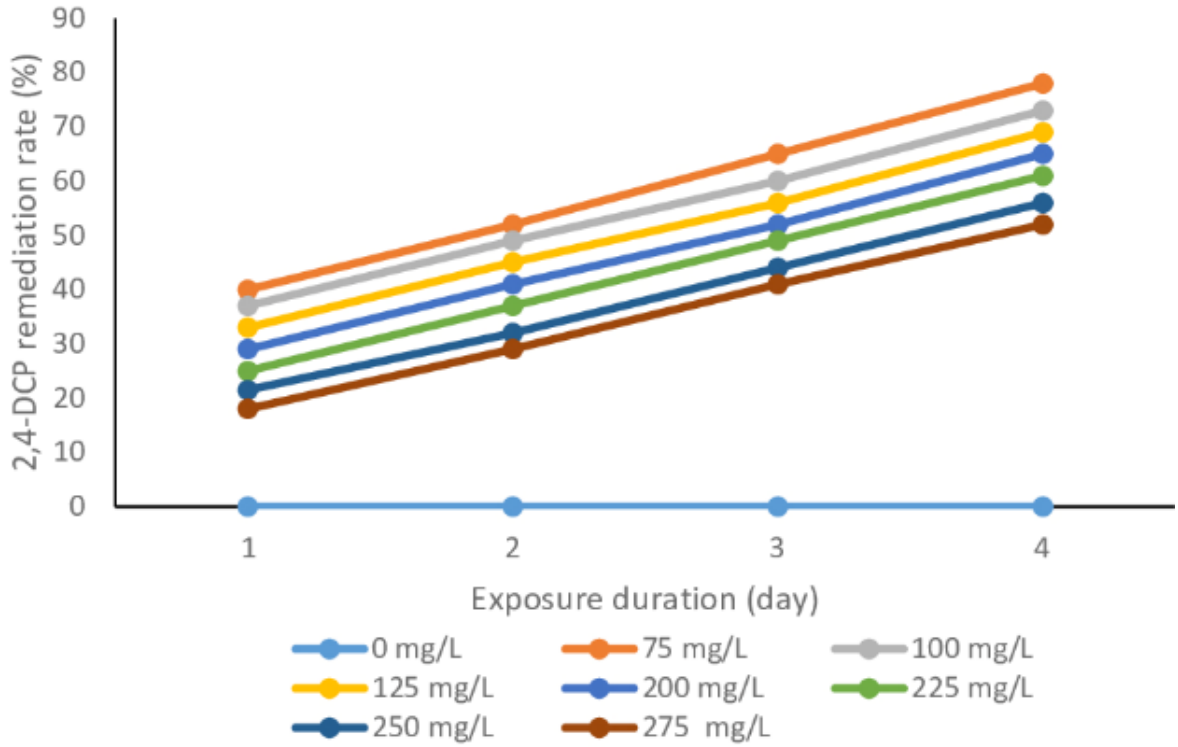

Figure 3. Remediation rates of 2,4-DCP according to day in plant hydroponic system.

RESULTS and DISCUSSION

The present research aimed to use *Datura stramonium* L. (jimsonweed) plant for phytoremediation of 2,4-dichlorophenol (2,4-DCP), which is highly toxic for environmental and human health, in soil and wetland areas. As a result, seedlings grown in a hydroponic system were exposed to different concentrations of 2,4-DCP (0.0, 75, 100, 125, 150, 175, 200, 225, 250 and 275 ppm). After this application, the remediation rate of 2,4-DCP in the hydroponic environment in which the plants were cultivated, the root-stem length of the plants, root-stem dry weights, soluble protein, lipid peroxidation (LPO) and photosynthetic contents were determined. These parameters are generally used to evaluate the potential use of *D. stramonium* for cleaning and remediation studies of areas polluted with 2,4-DCP [21].

In our study, *D. stramonium* was cultivated in a hydroponic environment and plants developed at the end of experiment and samples of the hydroponic medium containing 2,4-DCP were taken and the remediation rates for 2,4-DCP were evaluated compared to initial concentrations. With this aim, preliminary studies used the 4-amino antipyrine method stated in standard methods for spectrophotometry [16-17]. Firstly, the calibration curve for 2,4-DCP was drawn (Figure 1), and analyses were performed according to the direct photometric method. Removal efficiency for all concentrations of 2,4-DCP studied were 18-40% at the end of the 1st day, 29-52% at the end of the 2nd day, 41-65% at the end of the 3rd day and 52-78% at the end of the 4th day. The highest efficiency was provided for 75 ppm at the end of the 4th day (~78%), with the lowest efficiency for 275 ppm at the end of the 4th day (~52%) (Figure 1).

Variation in plant height and dry weight parameters lead the response mechanisms of a plant to negative conditions (stress). If the plant does not show tolerance to the negative conditions, very significant falls are determined in these two parameters [22]. In our study, exposure of *D. stramonium* seedlings to different doses of 2,4-DCP only caused a reduction in seedling height at the highest doses of 2,4-DCP (Table 3). For example, for root length compared to control, only doses of 175 ppm and above caused significant levels of reduction ($p<0.05$). At other doses, significant differences were not determined compared to control. Stem length sig-

Table 3. Effect of 2,4-DCP applications on root-stem length and dry weight of *D. stramonium* seedlings.

2,4-DCP (ppm)	Root (cm/plant)	Stem (cm/plant)	Dry weight (mg/plant)
Control	$15.93^{a}\pm1.23$	$18.70^{ab}\pm3.12$	$2.332^{ab}\pm0.10$
25	$13.75^{ab}\pm2.14$	$18.68^{a}\pm4.13$	$2.368^{ab}\pm0.12$
100	$12.06^{ab}\pm0.98$	$17.12^{a}\pm3.12$	$2.215^{ab}\pm0.13$
125	$12.62^{ab}\pm1.17$	$18.25^{a}\pm2.9$	$2.651^{a}\pm0.09$
150	$15.75^{a}\pm2.31$	$20.93^{a}\pm3.25$	$3.107^{a}\pm0.15$
175	$12.68^{ab}\pm2.43$	$15.43^{b}\pm4.33$	$2.434^{a}\pm0.09$
200	$11.43^{b}\pm1.19$	$18.05^{a}\pm2.90$	$2.999^{a}\pm0.078$
225	$11.72^{b}\pm1.41$	$15.31^{b}\pm3.33$	$2.717^{a}\pm0.21$
250	$11.25^{cb}\pm2.01$	$17.78^{a}\pm3.56$	$2.057^{ab}\pm0.05$
275	$8.62^{c}\pm0.78$	$15.31^{b}\pm2.89$	$1.837^{b}\pm0.02$

The difference between the values indicated by the same letters in a column is insignificant according to Duncan's Multiple Range Test ($p> 0.05$). ± refers to standard deviation.

nificantly fell compared to control at doses of 200 ppm and above, while at other doses no difference was observed compared to control. When the dry weights of seedlings developing in the same conditions are investigated (Table 3), apart from 275 ppm, there were no significant differences determined compared to control at all other concentrations. These findings show that at high dose conditions of 25-200 ppm 2,4-DCP *D. stramonium* did not show significant changes in root-stem length and dry weights ($p<0.05$) indicating the plant is resistant to high doses of 2,4-DCP. Similarly, a study with *Typha latifolia* L. studied 2,4-DCP at doses from 1.5-300 ppm and determined the plant had significant falls in growth rates after 50 ppm [21]. A study of *Salix matsudana* determined it had significant reductions in root-stem lengths at low doses like 30 ppm [14]. In our study, there were significant falls in seedling root length and stem length of *D. stramonium* after 175 ppm and 200 ppm doses, respectively (Table 3). This result supports the view that *D. stramonium* has strong tolerance for 2,4-DCP.

Though the chlorophyll amount in a plant does not directly show photosynthetic efficiency, it is a parameter directly linked to photosynthetic efficiency. As a result, chlorophyll amount is among the most commonly evaluated parameters in plants exposed to toxic agents [23]. In our study, when the chlorophyll a, chlorophyll b and total chlorophyll amounts in *D. stramonium* seedlings exposed to low and high doses of 2,4-DCP are compared to control plants, even at the highest doses of 2,4-DCP, chlorophyll content was not determined to change significantly ($p<0.05$) (Table 4). This situation is one of the most important proofs that the plant shows tolerance to toxic doses of 2,4-DCP because chlorophyll biosynthesis is one of the important parameters affected most rapidly by toxic agents and negative environments [23,24]. Studies of 2,4-DCP phytoremediation with *Salix matsudana* and *Typha latifolia* plants determined significant reduction in chlorophyll content at doses of 2,4-DCP higher than 30 ppm [14,21]. In this study, even at high doses of 275 ppm 2,4-DCP *D. stramonium* seedlings were not determined to have significant differences in chlorophyll a, chlorophyll b and total chlorophyll compared to controls (Table 4). This result shows the photosynthetic mechanism of *D. stramonium* produces a strong response to 2,4-DCP and indicates the plant is protected from the toxicity of the compound.

The soluble protein amount in plants is among the first parameters assessed for the plant stress response. A significant fall was determined in the protein content of plant cells sensitive to toxic or negative environmental conditions [22]. However, protein amounts are generally preserved in plants with tolerance. In this study, at low doses of 2,4-DCP (25-150 ppm) the protein content of roots and leaves of *D. stramonium* slightly increased, while they generally fell at high doses (200-275 ppm) (Table 5). These falls were 27% in roots and 23% in leaves at the highest dose of 275 ppm. In terms of protein content, the plant showed tolerance of 2,4-DCP at a dose of 150 ppm at higher levels than other plants, which is an important finding indicating it can be used for the remediation of 2,4-DCP.

Table 4. Effect of 2,4-DCP applications on chlorophyll content in the leaves of *D. stramonium* seedlings.

2,4-DCP (ppm)	Chl a (mg/FV)	Chl b (mg/FW)	Total Chl (mg/FW)
Control	3.1^{b}±0.23	3.8^{a}±0.04	7.1^{a}
25	3.1^{a}±0.12	2.4^{b}±0.11	5.5^{ab}
100	2.3^{a}±0.06	2.1^{b}±0.06	4.4^{b}
125	2.5^{a}±0.32	2.7^{b}±0.02	5.3^{ab}
150	3.5^{a}±0.28	3.7^{a}±0.06	7.2^{a}
175	2.5^{b}±0.11	2.8^{a}±0.08	4.9^{b}
200	2.7^{a}±0.05	2.5^{b}±0.03	5.2^{ab}
225	3.1^{a}±0.10	3.3^{a}±0.09	6.4^{a}
250	3.1^{a}±0.26	3.3^{a}±0.04	6.4^{a}
275	3.4^{a}±0.15	3.3^{a}±0.04	6.7^{a}

The difference between the values indicated by the same letters in a column is insignificant according to Duncan's Multiple Range Test ($p > 0.05$). ± refers to standard deviation. Chl: Chlorophyll; FW: Fresh weight.

Lipid peroxidation (LPO) is known as the oxidative destruction of unsaturated (polyunsaturated) fatty acids. When plants are exposed to toxic compounds, at cellular level reactive oxygen species (ROS) are produced. ROS enter rapid reaction with unsaturated fat acids in cell membranes and cholesterol's unsaturated bonds causing formation of LPO products.

As a result, membrane permeability and structure are disrupted and electrolyte leakage from membranes increases. In this situation, due to degradation of unsaturated fatty acids, the end products of toxic aldehydes are formed and the most well-known is malondialdehyde (MDA). The increase in MDA does not show the amount of LPO quantitatively but provides information about the level of LPO. As a result, increased MDA is used as an important indicator of LPO levels [25]. In this study, seedlings exposed to 2,4-DCP doses were not found to have significant differences ($p<0.05$) in MDA content compared to controls from lowest dose to highest dose (Table 5). The MDA results show that *D. stramonium* suppresses ROS production even at the highest dose of 2,4-DCP studied and can control LPO level.

Table 5. Effect of 2,4-DCP applications on soluble protein and MDA content in roots and stems of *D. stramonium* seedlings.

	Soluble Protein (mg/g FW)		MDA (µmol/g FW)	
2,4-DCP (ppm)	Root	Leaf	Root	Leaf
Control	1.66^{b}±0.02	7.20^{ab}±0.7	0.11^{ab}±0.03	0.12^{c}±0.01
25	2.23^{a}±0.09	8.74^{a}±0.6	0.13^{a}±0.02	0.15^{b}±0.02
100	2.11^{a}±0.01	9.12^{a}±0.1	0.09^{b}±0.01	0.19^{a}±0.01
125	1.58^{b}±0.01	7.87^{a}±0.3	0.13^{a}±0.02	0.17^{ab}±0.08
150	2.08^{a}±0.02	7.89^{a}±0.3	0.11^{ab}±0.02	0.13^{c}±0.03
175	1.23^{b}±0.01	7.46^{a}±0.1	0.12^{a}±0.02	0.18^{a}±0.01
200	1.41^{b}±0.11	5.87^{b}±0.1	0.12^{a}±0.01	0.12^{c}±0.02
225	1.48^{b}±0.03	5.86^{b}±0.5	0.14^{a}±0.02	0.19^{a}±0.04
250	1.55^{b}±0.02	6.70^{b}±0.4	0.12^{a}±0.03	0.16^{ab}±0.05
275	1.21^{b}±0.01	5.50^{b}±0.6	0.11^{ba}±0.03	0.14^{cb}±0.03

The difference between the values indicated by the same letters in a column is insignificant according to Duncan's Multiple Range Test ($p > 0.05$). ± refers to standard deviation. Chl: Chlorophyll; FW: Fresh weight.

CONCLUSION

In this research, according to the results obtained from the studied parameters, *Datura stramonium* seedlings exposed to doses of 2,4-DCP from 25-275 ppm were tolerant of 2,4-DCP and show that it is a plant with evaluation potential for the phytoremediation of 2,4-DCP that has been found to have 4th Grade High Acute Toxicity [26].

References

1. B.M. Greenberg, X.-D. Huang, K. Gerhardt, B.R. Glick, J. Gurska, W. Wang, M. Lampi, A. Khalid, D. Isherwood, P. Chang, H. Wang, S.S. Wu, X.-M. Yu, D.G. Dixon, P. Gerwing, Field and laboratory tests of a multi-process phytoremediation system for decontamination of petroleum and salt impacted soils, In: Proceedings of the Ninth International in Situ and On-Site Remediation Symposium. A.R. Gavaskar, C.F. Silver, eds., Battelle Press, Columbus, 2017.
2. T. Kösesakal, Tatlı Su Eğreltisi *Azolla filiculoides* Lam. kullanılarak petrol hidrokarbonlarının fitoremediasyonu. Doktora Tezi. İstanbul Üniversitesi, Fen Bilimleri Enstitüsü, 2011.
3. J. Chappell, Phytoremediation of TCE using Populus: Status Report, J. Chappell, Washington: US Environmental Protection Agency, Technology Innovation Office, 1997. (Electronic resource). Mode of access http://www.clu-in.com/phytoTCE. Htm, 1997.
4. T. McIntyre, Phytoremediation of heavy metals from soils. Phytoremediation, Springer, Berlin, Heidelberg, pp. 97-123, 2003.
5. A.O. Olaniran, E.O. Igbinosa, Chlorophenols and other related derivatives of environmental concern: Properties, distribution and microbial degradation processes, Chemosphere, 83 (2011) 1297-1306.
6. G. Favaro, D. De Leo, P. Pastore, F. Magno, A. Ballardin, Quantitative determination of chlorophenols in leather by pressurized liquid extraction and liquid chromatography with diode-array detection, J. Chromatogr. A, 1177 (2008) 36-42.
7. U.G. Ahlborg, T.M. Thunberg, Chlorinated phenols: Occurrence, toxicity, metabolism, and environmental impact, Crit. Rev. Toxicol., 7 (1980) 1-35.
8. E.J. Hoekstra, H. de Weerd, E.W.B. de Leer, U.A.T.H. Brinkman, Natural formation of chlorinated phenols, dibenzo-p-dioxins, and dibenzofurans in soils of a Douglas fir forest, Environ. Sci. Technol., 33 (1999) 2543-2549.
9. M.A. Talano, D.C. Busso, C.E. Paisio, P.S. Gonzalez, S.A. Purro, M.I. Medina, E. Agostini, Phytoremediation of 2,4-dichlorophenol using wild type and transgenic tobacco plants, Environ. Sci. Pollut. Res., 19 (2012) 2202-2211.
10. Stoilova, A. Krastanov, V. Stanchev, D. Daniel, M. Gerginova, Z. Alexieva, Z. Biodegradation of high amounts of phenol, catechol, 2,4-dichlorophenol and 2,6-dimethoxyphenol by Aspergillus awamori cells, Enz. Microb. Tech., 39 (2006) 1036-1041.
11. P., Jeffrey, M. Koplan, Toxicological Profile for chlorophenols. US Department of Health and Human Services. Public Health Service Agency for Toxic Substances and Disease Registry (ATSDR), 1999.
12. A.D., Dimou, T.M., Sakellarides, F.K., Vosniakos, N., Giannoulis, E., Leneti, T. Albanis, Determination of phenolic compounds in the marine environment of Thermaikos Gulf, Northern Greece. Intern. J. Environ. Anal. Chem., 86 (2006) 119-130.
13. F.W. Shaarani, B.H. Hameed, Ammonia-modified activated carbon for the adsorption of 2, 4-dichlorophenol, Chem. Enginer. J., 169 (2011) 180-185.
14. X. Shi, H. Leng, Y. Hu, Y. Liu, H. Duan, H. Sun, Y. Chen, Removal of 2,4-dichlorophenol in hydroponic solution by four *Salix matsudana* clones, Ecotoxicol. Environ. Saf., 86 (2012) 125-131.
15. Kariñho-Betancourt, A.A. Agrawal, R. Halitschke, J. Núñez-arfán, Phylogenetic correlations among chemical and physical plant defenses change with ontogeny, New Phytol., 206 (2014) 796-806.
16. APHA, AWWA, WEF, Standart methods for the examination of water and wastewater.American Public Health Association. Washington District of Columbia, (1998) 50-40.
17. C.E. La Rotta, E. D'Elia, E.P.S. Bon, Choloroperoxidase mediated oxidation of chlorinated phenols using electrogenerated hydrogen peroxide, Elec. J. Biotechnol., 10 (2007) 24-36.
18. F.H. Witham, D.F. Blayles, R.M. Devlin, Experiments in Plant Physiology. Van Nostrand Reinhold Company, New York. Pp, 55-56, 1971.
19. P.K. Smith, R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H., Gartner, M.D. Provenzano, E.K., Fujimoto, N.M., Goeke, B.J. Olson, D.C., Klenk, Measurement of protein using bicinchoninic acid, Anal. Biochem., 150 (1985) 76-85. 1985.
20. H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem., 95 (1979) 351-8.
21. M.C. Rodriguez-Hernandez, R.G. De la-Cruz, E. Leyva, G. Navarro-Tovar, *Typha latifolia* as potential phytoremediator of 2, 4-dichlorophenol: Analysis of tolerance, uptake and possible transformation processes, Chemosphere, 173 (2017) 190-198.
22. E. Genç, Ö. Atıcı, Chicken feather protein hydrolysate as a biostimulant improves the growth of wheat seedlings by affecting biochemical and physiological parameters, Turk. J. Bot., 43 (2019) 67-79.
23. K. Buonasera, M. Lambreva, G. Rea, E. Touloupakis, M.T. Giardi, Technological applications of chlorophyll a fluorescence for the assessment of environmental pollutants, Anal. Bioanal. Chem., 401 (2011) 1139-1151.
24. O.O. Ayeni, P.A. Ndakidemi, R.G. Snyman, J.P. Odendaal, Chemical, biological and physiological indicators of metal pollution in wetlands, Sci. Res. Essays, 5 (2010) 1938-1949.
25. G. Noctor, C.H. Foyer, Ascorbate and glutathione: keeping active oxygen under control, Annual Rev. Plant Biol., 49 (1998) 249-279.
26. Z. Ceylan, Ş.D. Aydın, Investigation of the effect of substituent species/positions and numbers on removal of toxicity from chloro and nitro phenol compounds with fenton and fenton-like processes, J. Chem. Soc. Pak., 42 (2020) 639-657.