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Alleviating Effects of Nitric Oxide on Cadmium Toxicity in White Poplar (*Populus alba*)

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Abstract

Cadmium (Cd) is non-essential heavy metal that negatively affects plant metabolism. Nitric oxide (NO) is an increasingly important molecule for plant metabolism that makes signaling. In this study, it was aimed to investigate the alleviating effect of sodium nitroprusside (SNP) application as NO donor in white poplar (*Populus alba*) under Cd stress conditions. SNP and without SNP treatments increased the Cd accumulation in root tissue. While photosynthetic pigments (Chl *a*, Chl *b*, Chl *a*+*b*, and carotenoid) content decreased by only Cd application, SNP+Cd application decreased the rate of photosynthetic pigments reduction. When the results of Cd and Cd+SNP applications were evaluated for mineral (Fe, Zn, Mn and Cu) uptake, it was found that the positive effect of SNP was heterogeneously affected. Depending on SNP application, it was found that malondialdehyde (MDA) amount decreased in leaf in 100 μM Cd applications while hydrogen peroxide (H_2O_2) amount decreased in 100 and 500 μM Cd applications. When antioxidant enzyme activities were examined, it was found that catalase (CAT) and ascorbate peroxidase (APX) enzyme activities increased with 100 μM SNP applications under all Cd applications. As a result, it was found that SNP application under Cd stress generally supports physiological processes positively in white poplar, suggesting that NO molecule plays important alleviating roles in plant metabolism.

Key Words: poplar, cadmium, nitric oxide, sodium nitroprusside, abiotic stress

Introduction

Heavy metal pollution is an important problem in the world and it is estimated that approximately 30,000 tons of cadmium (Cd) is spread to the environment annually, 13,000 tons of which is thought to be caused by human activities (Gallego et al. 2012). Cd, non-essential heavy metal, is a toxic element and negatively affects the growth and development of plants and causes plants to undergo oxidative stress through reactive oxygen species (ROS), such as superoxide dismutase enzymes (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), hydrogen peroxide (H_2O_2) etc. (Romero-Puertas et al. 2006; Chmielowska-Bąk et al. 2014). Cd can be easily taken from the soil with plant roots and transported to the upper parts of the plant and thus, it joins the food chain and adversely affects human

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and animal health (Bernard 2008). The Cd-induced toxicity causes disturbance of the nitrogen (N) and sulfur (S) metabolism, ROS generation, damage to nucleic acid, protein oxidation, inactivation of enzymes in CO_2 fixation, chlorosis etc. (Gill and Tuteja 2011).

Nitric oxide (NO) is a molecule which is of increasing importance for plants and plays a role in many cellular metabolic pathways in plant life and attracts attention with its physical and chemical properties, including short-lived, small size, free radical, no charge, and highly diffusible across biological membranes (Siddiqui et al. 2011). Plants have several enzymes involved in NO production, such as nitric oxide synthase (NOS), plasma-membrane (PM)-nitrite: NO reductase (Ni:NOR), cytosolic nitrate reductase (NR), and xanthine dehydrogenase (XDH) (Gill et al. 2013). Many published studies proved the accumulation of NO level under stress conditions. In addition, exogenous NO applications always enhanced the abiotic stress tolerance with a decrease in H_2O_2 and malondialdehyde (MDA) levels (Groß et al. 2013). The cross-talk between NO and other cell components such as protein kinases, ROS or phytohormones, Ca^{2+}, phosphatidic acid, cyclic guanosine monophosphate (cGMP) supports the molecular basis of NO roles in cell metabolism by indirect regulation (Leitner et al. 2009; Groß et al. 2013). In poplar (*Populus tremula*×*P. alba*), Cd treatment inhibited growth of poplar, whereas zinc (Zn) did not affect any physiological parameters. In addition, both treatments caused significant metal accumulation (Durand et al. 2011). In another study, white poplar (*P. alba*) trees were used as biomonitor for eight trace elements (As, Cd, Cu, Fe, Mn, Ni, Pb and Zn) in leaves and stems of white poplar in contaminated riparian forests (Madejón et al. 2004). In *P. alba* var. *pyramidalis*, phytoextraction potential in Cd contaminated calcareous soils was investigated. Thus, it was found that phytoextraction efficiency of *P. pyramidalis* connected with soil Cd concentration and the tree age (Hu et al. 2014). The aim of this study was to investigate the alleviation effect of sodium nitroprusside (SNP), a nitric oxide donor against Cd toxicity in white poplar (*P. alba*). In this context, Cd and metal nutrient ions accumulations, photosynthetic pigment analyses, malondialdehyde (MDA) and H_2O_2 contents, and scavenger enzymes activities such as catalase (CAT) and ascorbate peroxidase (APX) were analyzed under Cd and Cd+SNP treatments.

Materials and Methods

Plant material and growing conditions

Woody cuttings of white poplar (*P. alba*) were obtained from the Poplar and Fast Growing Forest Trees Research Institute, Izmit, Turkey. Later, 18 cuttings (~25 cm length×1 cm diameter) were selected with a uniformity of height and were rooted in perlite medium. After 10 weeks, the cuttings were transferred to pots filled with three liters of perlite. To support the acclimation in root zone, modified Hoagland solution (six-day quarter-strength; four-day half-strength; four-day full-strength) was used for watering the poplar cuttings. For Cd applications, acclimatized 18 cuttings showing homogeneous morphological characteristics were divided into two main groups, SNP (Sigma Aldrich St. Lois, MO, USA), a nitric oxide donor, and without SNP. Poplar cuttings were treated to six treatments during 21 days, containing control (full-strength modified Hoagland solution), 100 μM $CdCl_2$ treatment, 500 μM $CdCl_2$ treatment, 100 μM SNP treatment, 100 μM $CdCl_2$ and 100 μM SNP treatment, and 500 μM $CdCl_2$ and 100 μM SNP treatment dissolved in full-strength modified Hoagland solution. The full-strength modified Hoagland solution consisted of 5 mM $Ca(NO_3)_2 \times 4H_2O$, 5 mM KNO_3, 2 mM $MgSO_4 \times 7H_2O$, 1 mM KH_2PO_4, 45.5 μM H_3BO_3, 44.7 μM $FeSO_4 \times 7H_2O$, 30.0 μM NaCl, 9.1 μM $MnSO_4 \times H_2O$, 0.77 μM $ZnSO_4 \times 7H_2O$, 0.32 μM $CuSO_4 \times 5H_2O$, 0.10 μM $(NH_4)_2Mo_7O_{24} \times 4H_2O$ and 54.8 μM EDTA-$Na_2 \times 2H_2O$ adjusted to pH 6.0. Poplar cuttings were watered with 150 mL of the full-strength modified Hoagland solution which prepared according to six applications every day during the experiment. The experiment was performed as a complete randomized factorial design with three replications. In 21 days', treatment, leaves in poplar cuttings were carefully harvested and washed in tap water. Subsequently, these samples were rinsed three times with de-ionized water. Similarly, root and bark in poplar cuttings were removed and washed. All leaf, bark and root samples were oven-dried at 70°C for three days, and dry weight (DW) was immediately calculated.

Extraction and estimation of chlorophyll and carotenoid

Fresh leaf samples were collected from the youngest fully

expanded leaves before harvest. Then, they were homogenized using 500 mg fresh leaves with added 10 mL of acetone (90% v v^{-1}). The absorbencies were determined at 663, 645 and 470 nm using spectrophotometer (Shimadzu UV-1201; Tokyo). Concentration of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), chlorophyll *a*+*b* (Chl *a*+*b*), and carotenoids (Car) were estimated by the equation of Lichtenthaler (1987).

Cadmium and metal nutrient ions content

For mineral and Cd analyses, leaf, bark and root tissues were harvested and digested using the dry-ashing method in a muffle furnace at 500°C for 6 hours (Miller 1998). The obtained ashes were dissolved in 2 mL of 10 M nitric acid (HNO_3) and diluted to a final volume of 50 mL with distilled water. Subsequently, the concentrations of Cd and metal nutrient ions such as iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) in leaf, bark and root tissues were measured by using inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer Optima 2100 DV, Waltham, MA).

Hydrogen peroxide and malondialdehyde detection

For analysis of hydrogen peroxide (H_2O_2) content, fresh leaf samples were extracted with cold acetone. An aliquot of extracted solution (3 mL) was mixed with 1 mL 0.1% titanium dioxide (TiO_2) in 20% (v:v) sulphuric acid (H_2SO_4). Subsequently, the mixture was centrifuged at 6,000×g for 15 min. The intensity of yellow supernatant was measured at 415 nm. The concentration of H_2O_2 was calculated from a standard curve plotted with the range of 100-1,000 nmol H_2O_2 and expressed as μmol g^{-1}, fresh weight (Mukherjee and Choudhuri 1983). The level of lipid peroxidation in fresh leaves was determined with measuring malondialdehyde (MDA), a major thiobarbituric acid reactive species (TBARS) and product of lipid peroxidation (Hodges et al. 1999). MDA concentration was found using means of an extinction coefficient of (ε=155 mM^{-1} cm^{-1}).

Antioxidant enzyme activity measurement

Fresh leaf samples were homogenized in 5 mL of 100 mM sodium phosphate (Na_2HPO_4) buffer (pH 7.5) with 1 mM EDTA-Na_2 and 0.5 mM ascorbate. Later, homogenate was centrifuged at 10,000×g for 5 minutes. Supernatant was used for crude enzyme extract to analyze the activities of ascorbate peroxidase (APX, EC 1.11.1.11) and catalase (CAT, EC 1.11.1.6). All enzymatic measurements were made at between 0-4°C. Also, all colorimetric measurements for enzyme activities were done at 25°C using a spectrophotometer (Shimadzu UV/VIS1201). Enzyme activities were estimated as units per gram fresh weight of tissue. The APX activity was identified by measuring the decrease for ascorbic acid in absorbance at 290 nm for 1 minute in reaction mixture (3 mL per 0.1 mL supernatant), containing 50 mM potassium dihydrogen phosphate (KH_2PO_4) buffer (pH 7.0), 0.1 mM EDTA-Na_2, 0.05 mM ascorbic acid, and 1.5 mM H_2O_2 (Nakano and Asada 1981). The APX activity was calculated using extinction coefficient of (2.8 mM^{-1} cm^{-1}). The CAT activity was determined by measuring the decrease for H_2O_2 in absorbance at 240 nm for 1 minute (Cakmak et al. 1993). The reaction mixture (3 mL per 0.2 mL supernatant) contained 50 mM KH_2PO_4 buffer (pH 7.0), and 1.5 mM H_2O_2. The CAT activity was calculated using extinction coefficient (40 mM^{-1} cm^{-1}) for H_2O_2.

Data analyses

The experiment was performed as a randomized plots trail design with three replications. Statistical analyses were subjected with ONE-WAY ANOVA using the MINITAB Statistical Software (Minitab Corp., State College, PA). The comparisons of multiple means between Cd treatments were evaluated by Tukey's honestly significant difference (Tukey's HSD) test at significance level (α=0.05). The levels of significance were indicated as *at $p < 0.05$, **at $p < 0.01$, ***at $p < 0.001$, and ns: non-significant.

Results

Cd concentration and biomass

In 100 and 500-μM $CdCl_2$ treatment, visual toxicity symptom of Cd occurred as reduction of shoots and root growth, chlorosis and color clarification in young leaves, and discernible browning in main roots in experimental plants. With 100 or 500 μM $CdCl_2$ and 100 μM SNP treatment, however; it was observed that visible chlorosis in young leaves due to Cd toxicity did not occur, and discernible browning decreased in main roots.

Within the scope of the study, firstly, Cd accumulations due to SNP application in leaves, bark, and root tissues was examined. In addition, changes in dry weight were investigated. It was determined that Cd concentrations increased significantly in leaves, bark and root tissues under 100 and 500 μM Cd applications (Table 1). In the application of 100 and 500 μM Cd exposure compare to the control, Cd accumulation increased approximately 58- and 74-fold in the root tissue, 16- and 11-fold in the bark tissue, and 13- and 17-fold in the leaf tissue, respectively. Remarkably, depending on SNP application, it was seen that Cd accumulation increased 119- and 155-fold in the root tissue, 26- and 16-fold in the leaf tissue, and 23- and 16-fold in the bark tissue in 100 and 500 μM Cd applications in comparison with SNP application, respectively. As a result, SNP treatment has been found to further increase Cd accumulation at all tissues except for bark tissues.

When the dry weight results were examined, it was found that there was a decrease of 25.0% and 26.3% in leaves, 32.7% and 24.5% in bark, and 12.8% and 26.9% in roots with the application of 100 and 500 μM Cd, respectively (Table 1). However, changes in dry weights of bark and root tissues were not found statistically significant. SNP application remarkably increased DWs in leaves, bark and roots. For example, the increments in DWs of leaves, bark and roots was found by 50.0%, 90.9%, and 47.0% with 100 μM Cd+SNP treatment according to 100 μM Cd, respectively.

Table 1. Changes in biomass accumulation and total Cd content in leaf, bark and roots of white poplar plants exposed to individual Cd, sodium nitroprusside (SNP) and combined Cd+SNP applications for 21 days

SNP (μM)	Cd (μM)	Cd concentration (μg g^{-1} DW)			Dry weight (g $plant^{-1}$)		
		Leaf	Bark	Root	Leaf	Bark	Root
0	0	1.5±0.2^{e}	2.2±0.1^{b}	6.6±0.08^{c}	2.24±0.13^{a}	0.49±0.05^{bc}	0.78±0.06^{abc}
	100	19.0±0.4^{d}	35.1±0.5^{a}	384.6±24.3^{b}	1.68±0.09^{b}	0.33±0.02^{c}	0.68±0.08^{bc}
	500	25.5±0.3^{c}	24.5±3.0^{a}	491.5±51.8^{b}	1.65±0.05^{b}	0.37±0.02^{c}	0.57±0.05^{c}
100	0	2.0±0.1^{e}	1.4±0.1^{b}	4.1±0.24^{c}	2.45±0.13^{a}	0.53±0.04^{ab}	0.94±0.06^{ab}
	100	51.0±2.0^{a}	32.2±4.1^{a}	490.2±14.4^{b}	2.52±0.09^{a}	0.63±0.03^{ab}	1.00±0.09^{a}
	500	30.0±0.4^{b}	21.7±1.7^{a}	637.4±30.3^{a}	2.36±0.11^{a}	0.65±0.03^{a}	0.83±0.03^{abc}
F-test		457.7***	22.8***	98.1***	14.2***	15.2***	6.9**

Values are the mean of three replicates (means±SE, n=3). Different letters in the same column are significantly different according to the Tukey's HSD test ($\alpha < 0.05$).
**$p < 0.01$, ***$p < 0.001$.

Table 2. Changes in contents of photosynthetic pigments in leaves of white poplar plants exposed to individual Cd, sodium nitroprusside (SNP) and combined Cd+SNP treatments for 21 days

SNP (μM)	Cd (μM)	Chl *a*	Chl *b*	Chl *a*+*b*	Car	Chl *a*/*b* ratio
		(μg g^{-1} FW)				
0	0	899±6.8^{a}	233±2.9^{a}	1,132±9.6^{a}	575±5.7^{a}	3.86±0.02
	100	414±27.7^{b}	105±11.0^{b}	519±38.8^{b}	266±16.9^{b}	3.94±0.17
	500	376±10.1^{b}	104±3.5^{b}	480±10.3^{b}	241±5.5^{b}	3.62±0.16
100	0	921±11.7^{a}	248±6.4^{a}	1,169±17.7^{a}	598±14.4^{a}	3.71±0.07
	100	912±30.8^{a}	244±19.2^{a}	1,157±47.5^{a}	580±23.5^{a}	3.74±0.22
	500	860±16.0^{a}	216±2.2^{a}	1,076±17.1^{a}	552±5.8^{a}	3.98±0.07
F-test		180.3***	50.7***	142.5***	148.3***	1.1^{ns}

Values are the mean of three replicates (means±SE, n=3). Different letters in the same column are significantly different according to the Tukey's HSD test ($\alpha < 0.05$).
***$p < 0.001$; ns: non-significant.

Photosynthetic pigments

It was found that the amount of photosynthetic pigments decreased for both doses (100 and 500 μM) of Cd applications. The contents of Chl *a*, Chl *b*, Chl *a*+*b*, and Car decreased by about 54%, 55%, 54%, and 54% under 100 μM Cd exposure, respectively (Table 2). Under 500 μM Cd application, Chl *a*, Chl *b*, Chl *a*+*b*, and Car contents decreased by about 58%, 55%, 57%, and 58%, respectively. SNP application remarkably increased amount of photosynthetic pigments in leaves. For instance, the increases in the contents of Chl *a*, Chl *b*, Chl *a*+*b*, and Car of leaves was found by 2.28-, 2.08-, 2.24-, and 2.29-fold with 500 μM Cd+SNP treatment in comparison with 500 μM Cd treatment, respectively.

Heavy metal micronutrient concentrations

The concentrations of Fe, Zn, Mn, and Cu were investigated under Cd and Cd+SNP combination in white poplar (Table 3). The Fe and Zn concentrations in leaves, bark, and root of white poplar was decreased by Cd treatment, but these decreases were found significant in bark and root for Fe concentrations and in leaves and bark for Zn concentrations under 500 μM Cd treatment. The Fe concentrations in leaves, bark and root decreased by 14.3%, 30.8% and 46.3% under 500 μM Cd application, respectively. The Zn concentrations in leaves, bark and root decreased by 11.9%, 23.7% and 29.1% with 500 μM Cd treatment,

Table 3. Changes in concentrations of Fe, Zn, Mn and Cu in leaf, bark and roots of black white plants exposed to individual Cd, sodium nitroprusside (SNP) and combined Cd+SNP treatments for 21 days

SNP (μM)	Cd (μM)	Concentrations (μg g^{-1})			
		Fe	Zn	Mn	Cu
Leaves					
	0	80.10 ± 0.55^{b}	26.13 ± 0.63^{a}	31.23 ± 0.65^{c}	4.57 ± 0.04
0	100	67.07 ± 1.42^{c}	24.67 ± 0.40^{ab}	36.67 ± 0.79^{b}	4.88 ± 0.54
	500	68.63 ± 1.23^{bc}	23.02 ± 0.53^{bc}	35.89 ± 0.52^{b}	4.70 ± 0.08
100	0	98.26 ± 4.59^{a}	21.12 ± 0.71^{c}	45.01 ± 1.28^{a}	5.71 ± 0.58
	100	97.64 ± 2.90^{a}	17.79 ± 0.19^{d}	45.50 ± 0.44^{a}	5.73 ± 0.78
	500	109.36 ± 3.13^{a}	16.89 ± 0.41^{d}	35.83 ± 0.93^{b}	4.23 ± 0.11
F-test		42.4***	53.4***	42.3*	1.80^{ns}
Bark					
0	0	28.33 ± 0.84^{b}	33.91 ± 1.09^{a}	11.25 ± 0.17^{abc}	5.27 ± 0.41^{a}
	100	27.13 ± 0.90^{b}	30.24 ± 1.94^{ab}	10.09 ± 0.35^{bcd}	3.73 ± 0.19^{b}
	500	19.60 ± 0.76^{c}	25.87 ± 0.39^{bc}	8.47 ± 0.82^{d}	4.65 ± 0.08^{ab}
100	0	37.03 ± 4.76^{a}	24.48 ± 0.66^{c}	12.93 ± 0.51^{a}	4.39 ± 0.27^{ab}
	100	31.27 ± 1.07^{ab}	14.01 ± 0.57^{d}	11.59 ± 0.29^{ab}	4.29 ± 0.36^{ab}
	500	30.70 ± 1.62^{ab}	16.88 ± 1.24^{d}	9.33 ± 0.37^{cd}	3.68 ± 0.13^{b}
F-test		6.9**	47.4***	12.1***	4.98*
Root					
0	0	680.9 ± 50.1^{c}	31.57 ± 0.36^{a}	43.01 ± 9.65^{b}	13.15 ± 0.70^{a}
	100	634.5 ± 53.0^{c}	25.72 ± 0.27^{b}	36.44 ± 2.81^{b}	14.64 ± 1.24^{a}
	500	365.4 ± 14.4^{d}	22.38 ± 0.46^{c}	34.31 ± 1.83^{b}	12.93 ± 0.75^{a}
100	0	$1,075.9\pm44.3^{a}$	25.66 ± 0.24^{b}	65.01 ± 2.26^{a}	8.52 ± 0.81^{b}
	100	$1,000.3\pm90.9^{ab}$	22.70 ± 0.87^{c}	45.12 ± 1.96^{ab}	12.38 ± 1.06^{ab}
	500	753.1 ± 29.0^{bc}	21.09 ± 0.31^{c}	46.74 ± 1.02^{ab}	14.85 ± 0.91^{a}
F-test		24.2***	65.4***	6.2**	6.1**

Values are the mean of three replicates (means±SE, n=3). Different letters in the same column are significantly different according to the Tukey's HSD test ($\alpha < 0.05$).

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; ns: non-significant.

respectively. Moreover, SNP application notably increased the Fe concentrations in leaves, bark, and root, but not Zn concentrations. The increments in the Fe concentrations in leaves, bark, and root was found by 1.59-, 1.57-, and 2.06-fold with 500 μM Cd+SNP treatment in comparison with 500 μM Cd treatment, respectively. Contrarily, Cd+SNP treatment according to Cd application was remarkably decreased Zn concentrations in leaves, bark, and root. The Mn concentrations was remarkably decreased in bark and root with Cd treatment, whereas it was notably increased in leaves of white poplar. The Mn concentrations in leaves significantly increased 100 μM Cd+SNP treatment in comparison with 100 μM Cd treatment, but these increments in bark and root was not significant. It was found that significant changes in the Cu concentrations in bark and root; however, these changes were not significant in leaves. Cd exposure decreased the Cu concentrations in bark, but it was seen that these decreases was significant in 100 μM Cd treatment. Cd+SNP exposures according to Cd exposures was not significant changes in bark and root of white poplar. As a result, it was understood that the positive effect of SNP application on the contents of heavy metal micronutrient showed a heterogeneous distribution.

Changes in MDA and H_2O_2 contents

The MDA content in leaves of white poplar was increased by 10.9% and 11.9% with 100 and 500 μM Cd exposures according to the control, but not significant (Fig. 1A). Besides, The MDA content in leaves was remarkably decreased by 23.8% with 500 μM Cd+SNP treatment in comparison with 500 μM Cd exposures. The H_2O_2 content in leaves of white poplar was enhanced by 17.8% and 58.9% with 100 and 500 μM Cd treatment in comparison with control; however, these changes were not found significant (Fig. 1B). Also, the H_2O_2 content in leaves was remarkably increased by 2.81- and 3.84-fold with Cd+SNP treatments in comparison with 100 and 500 μM Cd exposures.

Changes in antioxidant enzyme activities

The activities of antioxidant enzymes such as CAT (Fig. 2A) and APX (Fig. 2B) were evaluated under Cd and Cd+SNP applications. While CAT activity in leaves of white poplar was increased by 22.9% with 100 μM Cd application compared to the control, it was decreased by 16.4% with 500 μM Cd application. However, these changes were not statistically significant. The CAT activity in leaves was

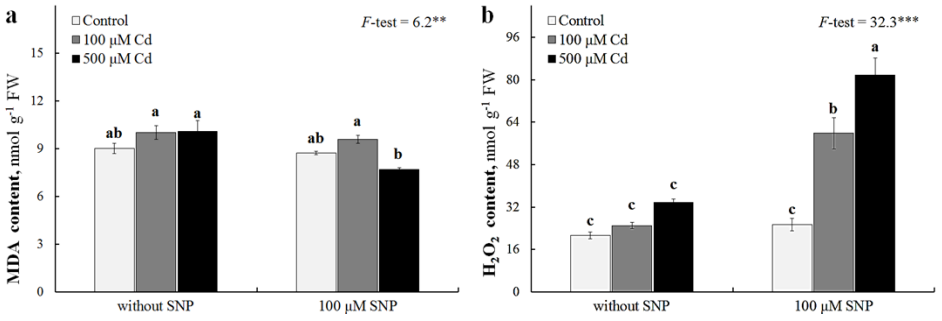

Fig. 1. MDA (a) and H_2O_2 (b) contents in leaves of white poplar exposed to individual Cd, sodium nitroprusside (SNP) and combined Cd+SNP treatments for 21 days. Bars indicate means of three replicates±SE. Different letters on the bars indicates significant differences according to the Tukey's HSD test ($\alpha < 0.05$).

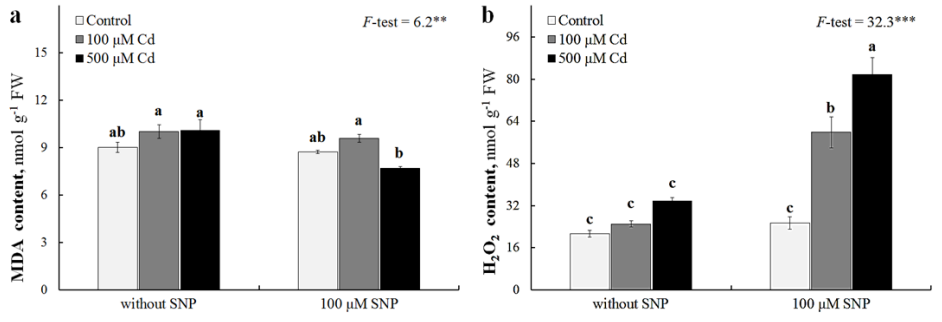

Fig. 2. CAT (a) and APX (b) activities in leaves of white poplar exposed to individual Cd (0, 100, and 500 μM), sodium nitroprusside (SNP) and combined Cd+SNP (100 μM+100 μM or 500 μM+100 μM) for 21 days. Bars indicate means of three replicates±SE. Different letters on the bars indicates significant differences according to the Tukey's HSD test ($\alpha < 0.05$).

also increased with Cd+SNP applications compared to Cd applications, but these changes were not found significant. When APX activity was examined, it was found that APX activity significantly increased by 15.0% in 100 μM Cd application. In comparison with 100 and 500 μM Cd exposures, it was found that APX activity notably increased by 22.9% and 20.1% in 100 and 500 μM Cd+100 μM SNP applications. According to the findings, it was clearly seen that SNP applications increase CAT and APX activities in both Cd dose exposures in leaves.

Discussion

Environmental fluctuations affect many pathways in plant metabolism, growth, and yield. Thus, abiotic stresses factors such as salinity, drought, heat, heavy metals etc. cause plant stress (Durand et al. 2011). Cd is one of the most dangerous heavy metal elements in the soil and toxic for plant metabolism (Kumar Rai and Kumar 2010). NO molecule is a very good agent to combat to adverse environmental conditions as a signaling messenger (Siddiqui et al. 2011). In this study, it was found that Cd concentrations increased in white poplar compared to control plants according to Cd and Cd+SNP applications. Cd amounts increased in *Populus*× *canescens* roots, wood, bark and leaves under 50 μM $CdSO_4$ application for 1, 10 or 20 days (He et al. 2013). Cd contents increased were identified as roos > wood > bark > leaves using different Cd exposures in *Populus*× *canescens* plantlets (Dai et al. 2013). These data supported our findings about Cd accumulation. Cikili et al. (2019) reported that although Cd application increased Cd concentrations, it was seen that SNP application generally decreased Cd concentration in black poplar. Interestingly, SNP results were not parallel with our findings. Plants reduce the effects of toxicity by preventing the uptake of metal ions, complexing them in the extracellular space, complexing and chelating them in the cytoplasm, and potentially sequestering them in the vacuole and tolerance to heavy metals is created (Ovečka and Takáč 2014). The cell wall, is the first barrier for plants to cope with Cd stress, has a fixation effect on Cd and provides a very important Cd resistance mechanism for plants to minimize the damage caused by Cd to their cells (Lux et al. 2011). Xiong et al. (2009) reported that it was able to increase Cd accumulation in root and stem cell walls by increasing the pectin and hemicellulose content, and reduces Cd distribution in the soluble components of the leaves under Cd stress exogenous NO, and thus enhancing Cd tolerance in rice. In this study, it can be suggested that the Cd concentration increases in the *P. alba* plant because Cd is retained and stored in different cell components to combat Cd.

Cd application is decreased dry weights of leaves, bark and root tissues of white poplar; in spite of the increases Cd concentrations in these organs, the decreases in dry weights of these organs are improved by Cd+SNP treatments (Table 1). The increments in the amount of dry matter under Cd stress and exogenous NO may be due to the decreases in Cd distribution in the soluble components of the leaves and bark, although Cd accumulation increased in the root and stem cell walls by increasing the pectin and hemicellulose content. Significant growth inhibition in lobelia is induced by Cd stress, whereas exogenous GSNO (NO donor) increased Cd uptake by lobelia but also enhanced its SOD and CAT activities, increased Pro content, and improved membrane integrity, thus alleviating the toxic effects of Cd on lobelia (Xu et al. 2011). Meng et al. (2022) explained that the resistance of plants to Cd stress by exogenous NO might be enhanced regulating the movement and distribution of Cd in plants by regulating the content of plant cell wall components. Our findings are parallel to these data.

The content of photosynthetic pigments was another indicator of the Cd toxicity. The Cd stress effect activates oxidative stress by increasing the production of reactive oxygen species, which affects the integrity of some biomolecules such as lipids and proteins (Gill and Tuteja 2010). The enzymatic and non-enzymatic system cannot eliminate all ROS produced at the cellular level, and then the remaining ROS promote oxidative stress, which results in greater damage to the thylakoid membranes and decreasing in photosynthetic pigment content (Choudhury et al. 2017). In our study, the content of photosynthetic pigments of leaves of white poplar decreased by Cd applications compare to control; however, Cd+SNP treatments considerably increased these parameters compare to Cd stress (Table 2). Similar results showing improved photosynthetic pigments content in black poplar (Cikili et al. 2019) and bamboo plants (Emamverdian et al. 2021) secured by exogenously

SNP under Cd stress have also been reported.

Iron and trace elements such as Cu, Mn and Zn are crucial for plant nutrition, and they are needed for catalytic activity of various types of enzymes (Madejon et al. 2004). In this study, it has been determined that there is a general decrease in micronutrient contents due to Cd application. However, it was found that the alleviating effect of SNP+Cd treatment showed a complex interaction depending on mineral and tissue types (Table 3). Cd is absorbed by the root through bivalent cation transporters found in the plasmatic membrane, like Zn and Fe transporters and ZIP (Zrt/Irt-like protein) (Parmar et al. 2013) and also it can compete with Mn^{2+}, Cu^{2+}, Ca^{2+}, and Mg^{2+} transporters (Clemens 2006). Our findings indicate that this competition might be reduced the absorption and translocation of Zn and Fe in white poplar plants. In *P. tremula*×*P. alba* genotype 717-1B4, distribution of Zn^{2+}, Ca^{2+}, Mg^{2+}, K^{+}, and Fe^{2+} in different organs altered under Cd treatment (Durand et al. 2011). In *P. nigra*, decreases in Zn^{2+}, Fe^{2+} and Mn^{2+} levels in leaves, bark and roots were observed under Cd exposure (Cikili et al. 2019). These findings were in agreement with our data.

MDA indicates level of membrane lipid oxidation and it is commonly used to assess plant tolerance to abiotic stresses. Hydrogen peroxide (H_2O_2) is a form of reactive oxygen species and plays important roles in cell signaling with various biochemical and physiological processes in plants (Barba-Espín et al. 2011). In *P. nigra*, both the MDA and H_2O_2 levels decreased with 500 μM Cd+SNP treatments (Cikili et al. 2019). MDA levels were not significantly affected by Cd treatment but some species-specific differences were found in six poplar species. In the same study, the highest MDA concentrations were also measured in bark and leaves tissues of *P. nigra* (He et al. 2013). H_2O_2 and MDA amounts were significantly increased in root, followed by wood, bark and leaf in plantlets of *Populus*× *canescens* (*P. tremula*×*P. alba*) (Dai et al. 2013). Recent studies showed that Cd stress modulates NO generation in plants. NO is a signaling molecule that plays many important roles involved in responses to biotic and abiotic stress conditions, plant growth and development processes by binding to critical Cys residues, heme or iron-sulfur centers (Arasimowicz-Jelonek and Floryszak-Wieczorek 2011). In this study, MDA level decreased using SNP application, whereas H_2O_2 content increased in both Cd applications with SNP. The presence of the NO donor (i.e. SNP) negatively correlated with reactive oxygen species, such as $O_2^{\bullet-}$ and H_2O_2, production and oxidative damage mainly caused by lipid peroxidation (Terrón-Camero et al. 2019). The increase in H_2O_2 content of leaves may be due to the increase in concentration, and uptake of Cd in leaves, and/or SNP level for white poplar may have been high and thus it may have had a cytotoxic effect. Although exogenous NO protects against heavy metal stress and alleviates oxidative stress (Terrón-Camero et al. 2019), it has shown that high NO concentrations may have cytotoxic properties (Beligni and Lamattina 2001). For example, it has reported that the response of the antioxidant system to arsenic in rice more sensitive to NO donors, such as SNP (Terrón-Camero et al. 2019). It can be suggested that NO application may induce gene expression network alleviating ROS damages in *P. alba* under Cd stress condition.

Reactive oxygen species (ROS) are required for many important signaling reactions and cellular proliferation and differentiation. Moreover, ROS is unavoidable toxic by-products of aerobic metabolism (Mittler 2017). Plants have two efficient systems to combat ROS, (i) enzymatic components such as ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (GPX) etc. (ii) non-enzymatic antioxidants like glutathione (GSH), ascorbic acid (AA), carotenoids, α-tocopherol, flavonoids, and proline (Das and Roychoudhury 2014). In current study, SNP exposures increase CAT and APX activities in both Cd dose exposures in leaves of *P. alba*. In black poplar (*P. nigra*), it was found increases in APX activity in leaves by SNP treatments but not observed any increases in CAT activity by SNP applications (Cikili et al. 2019). He et al. (2013) stated that APX activity increased in *P. deltoides* wood by 59%, whereas APX activity decreased by 43-49% in leaves of four poplar species (*Populus*×*euramericana*, *P. alba*×*P. glandulosa*, *P. nigra* and *P. popularis*) under Cd stress. Yang et al. (2015) showed that significant increases in APX, CAT, and SOD activities under increases of Cd application time (0, 4, 8, and 12 days) in *P. yunnanensis*. In this study, increased APX and CAT activities with SNP applications may have helped to provide adequate cellular responses of cell metabolism due to signal potential of NO molecule under Cd stress condition. As a result, in this study, it was seen that

SNP application generally has a positive effect on the formation of molecular response of cells under Cd stress. We believe that the findings of this study will contribute to the understanding that NO molecule has a positive role in response to Cd stress for white poplar (*P. alba*). In addition, the results of this study will provide a scientific basis for future studies on plant physiology related to NO molecule, especially in woody species.

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