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Heavy metal accumulation and changes in phenylpropanoid metabolism induced by mining activities in leaves of *Pinus nigra* and *Pinus eldarica*

Akumulacija težkih kovin in spremembe fenilpropanoidne presnove v listih vrst *Pinus nigra* in *Pinus eldarica*

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Abstract: Plants can absorb pollutants produced through industrial activities. In this research, biochemistry and lignin biosynthesis processes are studied in *Pinus nigra* and *P. eldarica* trees, that were exposed to pollutants from Sarcheshmeh copper complex and from the Kentuiyeh as control area (both Iran). Needles were collected in areas adjacent to mining factory, where heavy metals (Cu, Zn, Cd and Pb) are accumulated in higher concentrations than in plants from control areas. Comparison of the two studied pine species reveals that *P. eldarica* needles amass heavy metals 27% more than *P. nigra*. There was a higher content of total phenolic compounds and flavonoids in needles growing in close vicinities of the mining activity. Lignin content was similar in both species of pine and remained unchanged regardless of the pollution level. Higher activities of enzymes in phenylpropanoid pathway in needles from polluted areas were measured for both pine species. Findings of this study suggest involvement of general phenylpropanoid pathway in heavy metal resistance of pine trees. It seems that *P. eldarica* can accumulate more heavy metals in its needles and it has greater resistance to pollutants.

Keywords: flavonoids, heavy metals, lignin, phenolic compounds, pollutants

Izvleček: Rastline lahko absorbirajo onesnažila, ki nastanejo pri različnih industrijskih aktivnostih. V raziskavi smo preučili različne biokemijske in s sintezo lignina povezane procese pri borih *Pinus nigra* in *P. eldarica*, ki so rastli na onesnaženem področju ob industrijskem kompleksu Sarcheshmeh in na kontrolnem področju Kentuiyeh (Iran). V iglicah borov, ki so rastli blizu obrata, se je kopičilo več tečkih kovin (Cu, Zn, Cd in Pb) kot v kontrolnih rastlinah. Iglice vrste *P. eldarica* so kopičile za 27% več kovin kot *P. nigra*. Iglice onesnaženih dreves so vsebovale več fenolnih spojin. Količina lignina je bila podobna pri obeh vrstah bora in ni bila povezana z onesnaženostjo. Pri obeh vrstah bora so bile izmerjene tudi višje aktivnosti encimov fenilpropanoidne presnove. Izsledki raziskave nakazujejo, da so temeljne fenilprpanoidne poti vključene v odpornost borovih dreves proti težkim kovinam. Vrsta *P. eldarica* akumulira več težkih kovin in je bolj odporna proti onesnažilom.

Ključne besede: flavonoidi, težke kovine, lignin, fenolne spojine, onesnažila

Introduction

Mining activities and smelting produce toxic gases and other pollutants, such as hydrochloric acid, sulphuric acid, fluoride, magnesium, and heavy metals on a continuous basis (Salomons 1995). Near the non-ferrous metal smelters, high concentrations of toxic compounds have been detected in soils and vegetation. Recent reports indicate that regardless of the form of anthropogenic trace metals, their availability to plants is significantly higher than those of natural origin (Kabata-Pendias 2010).

Plants growing in industrial areas absorb a variety of pollutants. Toxic particles cause reduced growth and yield, as well as leaf injury in sensitive plants. Before exhibiting visible damage to leaves, plants encounter physiological changes when exposed to pollutants (Seyyednejad et al. 2011). Activation of phenylpropanoid metabolism has been reported for responses to biotic and abiotic stress, such as heavy metals, UV irradiation, and drought stress (Dixon and Paiva 1995). Phenylpropanoids are a diverse group of plant secondary metabolites, including anthocyanin, flavonols, proanthocyanidins (PAs), and lignin that accumulate in wide variety of plant tissues (Deluc et al. 2006).

Phenolic compounds such as phenolic amides are part of the plant cell walls. Overexpression of the enzymes responsible for their biosynthesis and the increase in their incorporation into the cell wall has been proved in biotic and abiotic stress conditions (Moura et al. 2010). Functions of phenylpropanoid compounds in defending the plant range from preformed or inducible physical and chemical barriers against infections to signal molecules involved in local and systemic signaling for defense genes induction (Dixon et al. 2002).

Both deficiency and high concentration of nutrients can cause abnormalities in accumulation of phenolic compounds and the lignin content (Grabber 2005). Lignin renders rigidity and strength to the cell wall and supports standing and plants resistance to pests and microorganisms (Jones et al. 2001). Phenolic precursors are polymerized to lignin through an oxidative mechanism. It is reported that peroxidases (PODs, a H_20_2-dependent hemoprotein) and laccases (an oxygen-dependent oxidase) perform the polymerization of monolignols into lignin (ten Have and Teunissen 2001).

Open pit mining at Sarcheshmeh Copper factory, the largest porphyry copper deposit in Iran, has been active for more than 30 years; and the concentration, melting, and molding plants are currently operating at full capacity. Reverb and converter stacks of the smelting plant respectively release 136 000 and 163 000 m^3/h of gaseous emissions, 24 hours a day, every day. Emissions include NOX, SO_2, CO_2, as well as metal particles (Ardejani et al. 2008). Different plant species have been planted in areas surrounding the factory for ornamental purposes, amongst them two pine species of *Pinus eldarica* and *P. nigra.* These trees grow in vicinity of both reverb and converter stacks and in areas at longer distances from the mining zone.

To determine the probable physiological response of pine trees to anthropogenic mining activities, some of the biochemical compounds and enzymes found in lignin biosynthesis pathways were studied in needles of two pine species (*P. nigra* and *P. eldarica*) collected in the vicinity of the copper mining factory and compared to needles of trees that grow in control areas distant from the factory.

Material and methods

Sarcheshmeh Copper mining factory, the largest porphyry copper deposit in the central region of the Zagros Mountain Range is located 160 km southwest of Kerman and 50 km south of Rafsanjan in Kerman Province, Iran. Kantuiyeh area, 9 km away from the Sarcheshmeh mining factory, was used for comparison. This area was selected because it is located upwind from the factory and the surrounding hills give it some protection from the factory`s pollution. Some plant species such as *P. eldarica* and *P. nigra* were ornamentally planted at different distances from the factory. In this study, three 8 m^2 plots dominated by *P. eldarica* and *P. nigra* were selected as replicates in area adjacent to the factory (as heavily polluted area) and Kentuiyeh (as control area). Needles were collected from the lower branches of the 15 years old tress and compared for their lignin content, total phenolic, flavonoids, and enzymes involved in lignin biosynthesis. The content of some heavy metals such as Cu, Pb, Cd and Zn were also measured in needle samples.

Total phenolic content

Phenolic compounds were determined using the modified method of Singleton and Rossi (1965). 0.5 gr of fresh needles were extracted in 95% ethanol. The extract was mixed with 0.5 ml of Folin–Ciocalteu's reagent (diluted 1:1 with water) and 1 ml of a 5% sodium carbonate solution added. The absorption at 725 nm was measured after 1 h (Singleton and Rossi 1965). Gallic acid was used as a standard and results were expressed as mg g^{-1}dw.

The total phenolic content was expressed as gallic acid equivalents (GAE) in microgram per gram of extract (µg GAE g^{-1} extract).

Flavonoids content

The flavonoids content was analyzed as described by Chang et al. (2002). Based on this method, 0.1 g of each sample was pulverized in 10 ml methanol and centrifuged at 12 000 g. 0.5 ml of supernatant was added to 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water, and left at room temperature for 30 min. Absorbance of the reaction mixture was measured at 415 nm with a double beam UV/Visible spectrophotometer (Perkin Elmer, USA). Quercetin was used for calibration curve (Chang et al. 2002).

Lignin content

Lignin content was assayed based on acetyl bromide protocol (Fukushima and Hatfield 2001). For that, 100 mg of air-dried samples were pulverized in 12.0 ml distilled water. The reagent then centrifuged at 10,000 g for 10 min and the resulting pellet was washed twice, using pure ethanol, each time for 30 min after filtration and then incubated in mixture of choloroform and ethanol (2:1 V/V) overnight. The filtered solution was centrifuged at 10 000 g for 20 min. The supernatant was removed and the obtained pellet was air dried. The dried sample was weighed, grinded and stored in desiccator.

6 ml of stored sample was digested in 2.5 mL of a 25% acetyl bromide in acetic acid reagent containing 0.1 ml 70% perchloric acid at 71 ºC for 30 min, with occasional mixing. After cooling in cold water, 6.0 ml glacial acetic acid and 5 ml 0.3 M NaOH was added to the volume. After bleaching, the volume was brought up to 25 ml using glacial acetic acid and centrifuged (3 000 g, 15 min). Optical density was measured at 280 nm and concentration was determined using the following equation:

$$\text{Lignin (\%)} = \frac{100\,(\text{As} - \text{Ab}) \times \text{V}}{a \times w}$$

where, As is the sample absorbance at 280 nm, Ab is blank absorbance at 280 nm, V is final volume, w is dry weigh of pellet and a is extinction coefficient (20.09 nm^{-1} cm^{-1}). A blank was included to correct for reagent background absorbance (Lacerda et al. 2006).

Preparation of extracts for enzyme activity

0.5 g of frozen leaves were homogenized in 50 mM phosphate buffer (pH 7.2) containing 1 mM EDTA, 1 mM PMSF and 1% PVP. The homogenate was centrifuged at 14 000 g for 15 min at 4 °C. The supernatant was used directly for the assay of enzyme activity and Bradford protein content assay using bovine serum albumin as the standard.

Coniferyl alcohol dehydrogenase (CAD, EC 1.1.1.195)

CAD activity was measured following the oxidation of appropriate hydroxycinnamyl alcohol at 30 ºC. The assay mixture (1 mL) contained 50 µl of 2M coniferyl alcohol, 100 mM Tris-HCl buffer (pH 9.3), 100 µl NADPH, and 10 µl of enzyme extract. The formation of coniferyl aldehyde was assayed at 390 nm (Halpin et al. 1998).

Phenylalanine ammonia lyase (PAL, EC 4.3.1.5)

Enzyme activity was assayed radio-chemically using L-Phe as the substrate at 35 ºC, based on the method of Thorpe and Hall (1984). The reaction mixture consisted of 0.5 ml 10 mM L-phenylalanine, 0.4 ml deionized distilled water and 100 µl of supernatant. The reaction mixture was incubated at 37 ºC for 1 h. The reaction was terminated by the addition of 0.5 ml 6M chloridric acid (Thorpe and Hall 1984). PAL activity in the buffer was determined from production of cinnamate during 1 h as measured from absorbance change at 260 nm. A unit of enzyme activity was defined as the amount of enzyme required for the formation of 1 mol of product in 1 min under assay conditions. Cinnamic acid standard curve was used for determination of product content.

Polyphenol oxidase (PPO, EC 1.14.18.1)

The reaction mixture for polyphenoloxidase activity consisted of 2800 µl 0.2 M tris buffer, pH 6.8 in 0.02 M pyrogallol, and 100 µl enzyme extract. The absorbency of the purpurogallin formed was taken at 420 nm. The content of the rest of pyrogallol in reaction mixture was calculated using extinction coefficient of 6.2 $mM^{-1}cm^{-1}$ (Kar and Mishra 1976). PPO activity was expressed as units per mg of protein. A unit of enzyme activity was defined as the enzyme required for formation of 1 M pyrogallol to purpurogallin**.**

Guiacol peroxidase (GPX, EC 1.11.1.7)

Peroxidase activity was measured using the method of Plewa et al. (1991). Guaiacol oxidation (tetraguaiacol formation) was monitored by reading the absorbance at 470 nm. The increase in absorbance was recorded until constant which was estimated about 3 min in this study. Activity was calculated using extinction coefficient 25.5 $mM^{-1}cm^{-1}$ at 470 nm for tetraguaiacol and expressed in units per gram of protein. The enzyme unit in the extract was calculated for the formation of 1 mM tetraguaiacol for 1 minute.

Atomic absorption spectroscopy

To determine the accumulation of Cu, Zn, Pb and Cd in plants' needles, atomic absorption spectroscopy analysis was conducted. 5 ml of 65% nitric acid and 1 ml 75% hydrochloric acid were added to 1 g of dried needle sample. The mixture was heated for 1 hour at 80 ºC in water bath. After digestion, the final total volume of 50 ml was reached using ion-free water. The obtained solution was analyzed using GFA spectra-220 atomic adsorption spectrometer. GTA-110 graphite tube atomizer was used for determination of copper, zinc, lead and cadmium.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's test. Mean values of three replicates were calculated and the differences were considered significant for $p<0.05$.

Results and discussion

Heavy metals accumulation in pine needles

Cd, Pb, Cu and Zn contents were significantly higher in needles collected from trees in polluted areas adjacent to the mining factory relative to amounts found in needles collected from control trees in Kentuiyeh (Tab. 1). *P. eldarica* accumulated higher amounts of Cu, Zn and Cd, while *P. nigra* trees had higher amounts of Pb in their needles (Tab. 1). Both species collected high amounts of Cd in needles. *P. eldarica* accumulated much more contents of Cd collecting 310.10 µg g^{-1} DW compared to Cu with 240.06 µg g^{-1} DW.

It's been confirmed that copper smelting and toxic emissions at Sarcheshmeh Copper Complex have polluted the surrounding soil. Sarcheshmeh Copper Complex surface soil was shown an area polluted with potentially toxic metals such as As, Cu, Pb, Zn, Mo, and Cd (Rastmanesh et al. 2011). Our results indicate higher contents of heavy metals, such as copper, zinc, cadmium and lead accumulated in plants grown in vicinity of Sarcheshmeh copper mining factory, as compared to plants at Kentuiyeh area, located 9 km upwind from the factory surrounded by hills. Results also indicate that *P. eldarica* trees have greater capacity for amassing heavy metals from polluted areas than *P. nigra* trees. The result of other researchers show that majority of contaminated areas are in the prevailing wind directions coming from North and North-East (Rastmanesh et al. 2011).

Table 1: Content of Cu, Zn, Cd and Pb in needles of *P. eldarica* and *P. nigra* near Sarcheshmeh copper mining factory (polluted area) and in Kantuiyehas (control area). Mean value ± SE is shown (n = 3). Different letters in superscript indicate statistical significance between both areas and pine species (ANOVA and Duncan test, p< 0.05).

Tabela 1: Vsebnost Cu, Zn, Cd in Pb v iglicah *P. eldarica* in *P. nigra* na onesnaženem področju blizu bakrovega rudnika Sarcheshmeh in na kontrolnem področju Kantuiyehas. Prikazane so povprečne vrednosti ± SN (n = 3). Različne črke v nadpisu prikazujejo statistično značilne razlike med področjema in borovima vrstama (ANOVA in Duncan-ov test, p< 0,05).

		Heavy metal content (μg g^{-1} DW)			
		Cu	Zn	Cd	Pb
P. eldarica	Polluted	240.06±8.21^{a}	34.61±2.08^{a}	310.10±12.16^{a}	14.47±2.90^{b}
	Control	48.32±3.65^{c}	18.35±0.57^{b}	11.72±1.82^{c}	3.39±0.68^{c}
P. nigra	Polluted	175.41±6.40^{b}	38.66±2.01^{a}	176.39±4.68^{b}	49.64±7.61^{a}
	Control	46.18±3.10^{c}	19.44±.06^{b}	8.12±2.90^{c}	3.81±1.14^{c}

DW – dry weight

Phenylpropanoids compounds content

The contents of the total phenolic compounds (p=0.22) and total flavonoids (p=0.01) (Fig. 1) in needles collected from the polluted site were significantly higher than amounts found in Kentuiyeh area; for both pine species studied here. On the other hand, there was no significant difference (p=0.165) between lignin contents in needles collected from either of the two areas for both species (Fig. 1).

Based on the results from current project, mining activities influenced the phenylpropanoid pathway, suggesting a possible role of phenolic in response to environmental pollutants. Enhanced accumulation of phenolic is considered to be one of the most common reactions of plants to stressful conditions (Wild and Schmitt 1995). It has been demonstrated that long-term exposure to air pollutants leads to enhanced accumulation of phenolic compounds (Gostin 2009a, Gostin 2009b). Based on our results that show higher phenolic and flavonoid contents in pine trees in area close to mining activity, it seems that chronic exposure to toxic emissions of Sarcheshmeh Copper Complex stimulates carbon flux in the secondary metabolic pathways; hence, inducing a shift of the available resources towards the synthesis of secondary products. Researchers proposed specification of the total and simple phenolic compounds and also flavonoid contents in the needles as biological indicators of pollution and air quality in Aleppo pine (*Pinus halepensis*) (Pasqualini et al. 2003, Robles et al. 2003). In vitro studies have shown that flavonoids can directly scavenge molecular species of active oxygen: superoxide, hydrogen peroxide, hydroxyl radical, singlet oxygen or peroxyl radical. Their antioxidant activity is due to their ability in donating electrons or hydrogen atoms (Michalak 2006).

It has been proposed that heavy metals such as Al or Pb may suppress metabolic pathways for lignin biosynthesis in plants. In this study, no significant difference in lignin content was found in either of the pine species. However, higher lignin biosynthesis in presence of excess Cu concentrations has been reported (Michalak 2006). It has been demonstrated that cellulose and lignin biosynthesis activities (enzymes and transcripts) are reduced by the ozone in hybrid poplar wood; as a consequence of decrease in cambial activity and cell wall production (Richet et al. 2011). From the results of current study, it seems that pine trees and with more specification, *P. elderica*, raise phenolic and flavonoids production throughout activation of phenylpropanoid pathway to tolerate stressful condition in area close to mining activities. While little amounts of phenylpropanoid compounds go through lignin biosynthesis in pine needles.

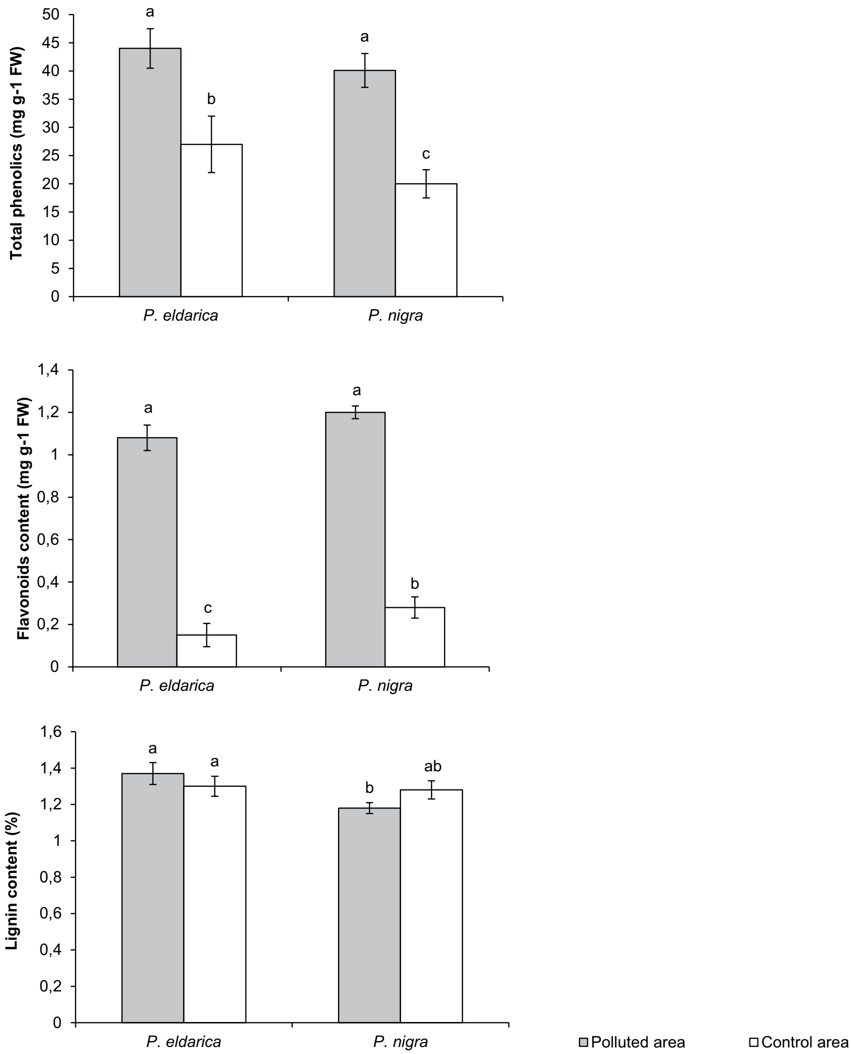

Figure 1: The content of total phenolic compounds, total flavonoids and lignin in needles of *P. eldarica* and *P. nigra* near Sarcheshmeh copper mining factory (polluted area) and in Kantuiyehas (control area). Mean value ± SE is shown (n = 3). Different letters in superscript indicate statistical significance (ANOVA and Duncan test, $p< 0.05$).

Slika 1: Vsebnost fenolnih spojin, flavonoidov in lignina v iglicah *P. eldarica* in *P. nigra* na onesnaženem področju blizu bakrovega rudnika Sarcheshmeh in na kontrolnem področju Kantuiyehas. Prikazane so povprečne vrednosti ± SN (n = 3). Različne črke v nadpisu prikazujejo statistično značilne razlike (ANOVA in Duncan-ov test, p< 0,05).

Activity of enzymes involved in phenylpropanoid pathway

In this study, activities of CAD and PAL were higher in *P. eldarica* needles in areas adjacent to the factory. In *P. nigra,* CAD activity was similar in both sites and PAL was higher in the control site in *P. nigra* needles (Fig. 2).

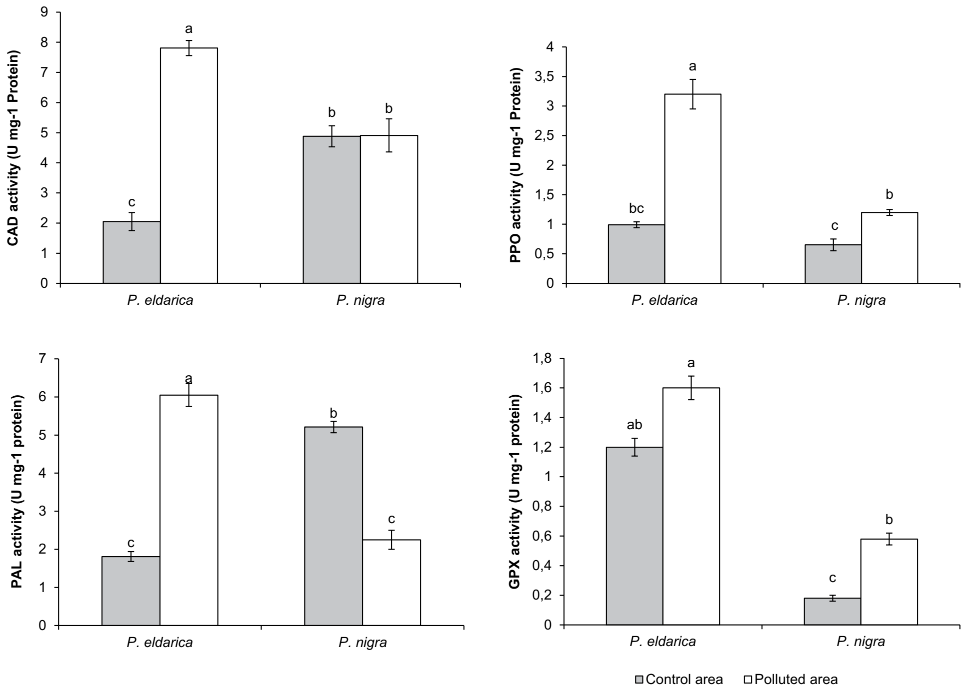

Figure 2: The enzyme activity of coniferyl alcohol dehydrogenase (CAD), phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO) and guiacol peroxidase (GPX) in needles of *P. eldarica* and *P. nigra* near Sarcheshmeh copper mining factory (polluted area) and in Kantuiyehas (control area). Mean value ± SE is shown (n = 3). Different letters in superscript indicate statistical significance (ANOVA and Duncan test, $p < 0.05$).

Slika 2: Vsebnost koniferil alkohol dehidrogeneze (CAD), fenilalanin amonij liaze (PAL), polifenol oksidaze (PPO) in gvajakol peroksidaze (GPX) v iglicah *P. eldarica* in *P. nigra* na onesnaženem področju blizu bakrovega rudnika Sarcheshmeh in na kontrolnem področju Kantuiyehas. Prikazane so povprečne vrednosti ± SN (n = 3). Različne črke v nadpisu prikazujejo statistično značilne razlike (ANOVA in Duncan-ov test, $p < 0,05$).

CAD is an indicator of lignin biosynthesis due to its specific role at the monolignol biosynthesis (Boerjan et al. 2003) which catalyzes the reduction of hydroxycinnamaldehyde to hydroxycinnamyl alcohols. CAD is expressed under stressful conditions (Galliano et al. 1993). In poplar, it has been proposed that stimulation of CAD was correlated to the increase in the first enzyme of the general phenylpropanoid pathway. PAL which catalyzes deamination of phenylalanine to cinnamate is one of the enzymes that has a key role in phenylpropanoid metabolic pathway (Iriti and Faoro 2009). It was reported that high concentrations of Cu causes accumulation of phenolic compounds and lignin along with an enhancement of polyphenol oxidase, glucose-6-phosphate dehydrogenase, shikimate dehydrogenase, PAL and CAD activities in *Panax ginseng* root suspension culture (Ali et al. 2006). Cu has indirect impact on oxidation and polymerization of monolignols (Moura et al. 2010). These finding supports our results on *P. eldarica* having higher CAD and PAL activities on areas polluted by heavy metals. However, CAD activity was the same in P. *nigra* in both areas. There was a contradiction in our findings where there were no significant changes in lignin content of pine species in each area, while CAD and PAL activities increased in polluted areas. However, from the results achieved by phenolic and flavonoids measurements, it seems that activation of phenylpropanoids pathway caused increment of phenolic compounds not involved in lignin biosynthesis. Since gravimetric and spectrophotometric methods may suffer from interference caused by non-lignin components, there is no ideal method for measuring the lignin content (Cabané et al. 2004); hence, it is proposed that lignin structure, content and composition in different parts of the tree should be studied using other more relevant techniques.

PPO and GPX activities were higher in *P. eldarica* needles in areas adjacent to the factory. In *P. nigra* the same changes were seen for PPO and GPX activities (Fig. 2). Peroxidases are oxidoreductases that catalyze the oxidation of a diverse group of organic compounds using hydrogen peroxide as the ultimate electron acceptor (Dawson 1988). The last step in the synthesis of lignin and suberin has been proposed to be catalyzed by peroxidases (Quiroga et al. 2000). There are some evidence suggesting the role of specific peroxidases in lignin polymerization (Marjamaa et al. 2009). In soybean plants, cadmium has raised GPX and laccase activity as well as the lignin content (Finger-Teixeira et al. 2010). Peroxidases are effective quenchers of reactive oxygen species (ROS) and play an important role in the adaptation and ultimate survival of plants during periods of stress (Verma and Chandra 2014). Quiroga et al. (2000) have reported that TPX1 overexpression in tomato significantly increased the lignin content in transgenic tomato plants. However, determining the activity of peroxidases exclusively as involved in biosynthesis of lignin peroxidase is a more appropriate approach to identifying their role in phenylpropanoids pathway metabolism.

In this project, PPO showed higher activity in trees grown on polluted area. Increase in enzymes involved in phenolic compound biosynthesis has also been reported under other stress conditions such as ozone. In poplar (*Populus maximorwizzii*×*Populus trichocarpa*), higher levels of PAL activity were found to be associated with a greater ozone tolerance. Researchers also illustrated cadmium-induced increases in phenols, peroxidase and polyphenol oxidase activities from plant extracts (Lavid et al. 2001b). It is suggested that polymerization of polyphenols by peroxidases, enhanced after heavy-metal uptake and detoxification, is responsible for the binding of heavy metals in *Nymphaea* epidermal glands (Lavid et al. 2001a). Increase in PPO and PAL activities could be considered as the main reason for production of phenolic compounds, and flavonoids content in pine trees grown close to Sarcheshmeh Copper Complex. These changes convey the plants the ability to withstand stressful condition resulted from mining activities.

Conclusions

In summary, *P. eldarica* needles accumulated significant amounts of Cu, Zn and Cd while the accumulated contents in *P. nigra* were lower. However, Pb content was higher in *P. nigra* needles.

Our findings indicate that phenolic compounds and activities of enzymes, involved in phenylpropanid metabolism, increased in both pine species because of exposure to pollutants from the Sarcheshmeh Copper Complex. Higher content of phenolic compounds, flavonoids and higher activity of enzymes PPO, PAL and CAD in needles of both pine species suggest potential tolerance to pollution induced by stressful conditions in areas adjacent to mining activities. However, no difference in the lignin content was recorded.

Although concentrations of total phenolic, flavonoids and lignin were similar in both species, the activity of CAD, PPO and PAL was higher in *Pinus eldarica*. These findings suggest higher potential for *P. eldarica* plants to tolerate stressful conditions as created in polluted areas of Sarcheshmeh copper mining factory as compared to *P. nigra* species.

Povzetek

Iglice vrste *P. eldarica* so akumulirale značilno več Cu, Zn in CD kot iglice *P. nigra*, medtem, ko je bila vsebnost Pb višja v iglicah *P. nigra*. Rezultati so pokazali, da so se pri obeh vrstah bora povišale vsebnost fenolnih spojin in aktivnosti encimov fenilpropanoidne presnove, če so bile rastline izpostavljene onesnaženju iz

bakrovega industrijskega obrata Sarcheshmeh. Višja vsebnost fenolnih spojin, flavonoidov in višja encimska aktivnost polifenol oksidaze (PPO), fenilalanin amonij liaze (PAL) in koniferil alkohol dehidrogeneze (CAD) v borovih iglicah kaže na potencialno tolerance za stresne dejavnike, ki jih na bližnjih območjih povzroča onesnaženje. Vsebnost lignina se ni spremenila.

Kljub temu, da je bila celokupna koncentracija fenolov, flavonoidov in lignina podobna, so bile encimske aktivnosti višje pri *P. eldarica*, kar kaže, da vrsta lažje prenaša stresne razmere, ki nastanejo zaradi onesnaženja z bakrovim rudniškim obratom kot *P. nigra*.

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