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Behavior of *Phragmites australis (CAV.) Trin. Ex Steud* used in phytoremediation of wastewater contaminated by cadmium

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ABSTRACT

Direct use of green plants to stabilize or reduce contamination in soils, surface water, or ground water has gained increasing popularity in both academic and practical fields. Phytoremediation is a low-cost and friendly technology to the environment, not only for the elimination of heavy metals but also for the various pollutions. This study investigated the effect of cadmium chloride ($CdCl_2$) on chlorophyll and lipids contents and evaluated the activity of guaiacol peroxidase (GPOX) in *Phragmites australis* aerial and root part. Known as common reed, this plant is widely used for the treatment of wastewater contaminated with heavy metals. The analysis of those selected physiological parameters has allowed understanding cellular behavior in the presence of various concentrations of cadmium. The results showed that low doses of $CdCl_2$ induced a significant increase in total chlorophyll content (A + B) unlike the other two doses, as well it was demonstrated that $CdCl_2$ induced a negative dose-dependent effect on lipid content. However, the decreased level of fat contents was less important in roots than in leaves. The effects of $CdCl_2$ on enzymatic activity in leaves showed a very highly significant inhibition of GPOX activity for all used concentrations; contrarily in roots, an increase in the activity was recorded. Following this study, the increased activity of GPOX in roots partly explains the ability to accumulate $CdCl_2$ in this part of the plant especially if we know that GPOX has a role in cellular protection against oxidative stress imposed by heavy metals and cell wall lignifications where heavy metals adsorbed to minimize penetration inside cells.

Keywords: Cadmium chloride; Phytoremediation; Enzymatic activity; Heavy metals

1. Introduction

The ability of some plants to tolerate or even to accumulate metals has opened new ways of research on the treatment of soils and waters phytoremediation [1]. Phytoremediation is an eco-friendly cost-effective technology not just for heavy metals removal but also for various pollutions [2]. This technology involves a group of methods that use plants to reduce, remove, degrade, or immobilize environmental toxics, with the

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aim of restoring area sites to a condition usable for private or public applications. Rhizofiltration is a subset technique of phytoremediation that refers to the use of plant's roots to uptake, store, and precipitate toxic metals from polluted water [3]. *P. australis* or common reed is a tall, perennial grass that can grow to over six meters grows on level ground in tidal and non-tidal marshes, lakes, swales, and backwater areas of rivers and streams [4]. The essentiality of the biomass of a colony of common reed is especially under the soil surface in roots and rhizomes (60–70%) [4,5], where conditions are suitable, it can spread to 5 m horizontally or more over a year [6]. Effectively, many studies have shown that the presence of common reed causes a significant reduction concentration of metals in water [7–9]. One of the major consequences of exposure of plants to heavy metals is the enhanced accumulation of oxygen free radicals and lipid peroxidation [10,11]. Each plant species has different levels of tolerance toward different contaminants [12]. They have evolved different mechanisms to maintain physiological concentrations of essential metal ions and to minimize exposure to non-essential heavy metals and to minimize the damage caused in plants by heavy metals [13]. For its vigorous growth, tolerance to drought, accumulation, and resistance to heavy metals, and phytoremediation aptitude, this cosmopolitan plant was chosen [4,7].

This study investigated the responses of roots and leaves cells to the presence of cadmium, concerning the contribution of their enzymatic capacities in antioxidant and the comparisons between dose effects on chlorophyll and lipid content.

2. Materials and methods

2.1. Plant material and growth conditions

Reeds *P. australis* Cav. (Trin.) were grown in pots, irrigated with nutrient solution for 9 d period of adaptation, and then separated into two groups (control and treated with $CdCl_2$). Treated groups were superficially irrigated with water contained $CdCl_2$ at different concentrations (10, 27 and 55 ppb). Plant roots were submerged, while leaf and stems remain above the water's surface. They were grown in semi-controlled environment under conditions of a green house; after 30 d, leaves and roots were sampled, and then, they were used for the determination of chlorophyll, lipids contents, and guaiacol peroxidase (GPOX) activity in leaf and root cells.

2.2. Extraction and assays of antioxidant enzyme activity

To obtain the enzymatic extract of *P. australis* (leaves and roots), Loggini et al. method was used [14]. Leaves and root samples were homogenized (each sample separately) in 5 ml of phosphate buffer (50 mM phosphate, pH 7.5) at 4°C. The homogenate was centrifuged at 12,000 *g* for 20 min, and the supernatant was used to measuring the activity of GPOX.

2.2.1. Assay of GPOX activity

The activity (GPOX) is determined according to Fielding and Hall method [15], by measuring the absorbance at 470 nm. GPOX activity is expressed as μmol guaiacol-oxidize $min^{-1} mg^{-1}$ protein using the value of the extinction coefficient of tetra guaiacol $\varepsilon = 26{,}600\ \mu M^{-1} cm^{-1}$.

2.3. Chlorophyll content measurement

Chlorophyll extraction was done according to the methodology developed by Holden [16]. Chlorophyll was extracted from *P. australis* leaves with acetone diluted to 80%, and the amount of chlorophyll was determined at 663 and 645 nm.

2.4. Extraction and assay of total lipids

Total lipids were evaluated according to the method of Goldsworthy et al. [17]. Extraction method is according to Shibko et al. [18]. In brief, each sample of fresh leaves and roots (0.5 g) was individually homogenized in 10 ml of trichloroacetic acid at (20%); homogenate was submitted to a differential centrifugation at 5,000 g.

2.5. Statistical analyses

All results were expressed as the mean of three replicates ± standard deviation, each measured parameter was the subject of an analysis of variance with $\alpha \leq 0.05$ (ANOVA), and mean comparisons were made using DUNNETT test for comparing two samples (control and treated).

3. Results and discussion

3.1. Chlorophyll content

The chlorophyll content is often used to evaluate the impact of many environmental stresses on plants. The results show that the low dose of $CdCl_2$ induces a

significant increase on total chlorophyll content (A + B). In the leaves of *P. australis*, an increase of 50% in the presence of 10 ppb of $CdCl_2$ was shown unlike the other two doses where there is a decrease in dose-dependent manner (Table 1).

In addition, a chlorosis and a yellowing very pronounced compared to what was observed in control plants. At low concentrations of heavy metals, many species showed chlorophyll content equal to or greater than that of untreated plants including *Sesuvium portulacastrum* treated with 50 μM of Cd^{2+} [19]. The giant reed *Arundo donax* belongs to the same family of grasses that of *P. australis* where Cd and Ni do not significantly affect its chlorophyll content [20]. It has been revealed also that in *P. australis* under salt alkali-stress in the presence of 15 mM of nitrogen, the content on chlorophyll increased by 20% more than controls [21]. In these studies, hypotheses have been suggested, that almost all metallic trace elements are stored in root cells, that minimize the translocation of these contaminants to leaf cells and the impact on chlorophyll, there is no damage to the function of photosynthetic process if a small quantity was penetrated into leaf cells.

Lichtenthaler [22] suggested that there was a formation of chloroplasts type of shadow where they develop high contact surface granum to increase their intake of light energy; this increase was observed in the presence of substances inhibiting electron transport during photosynthesis. Also, we can suggest that *P. australis* under metal stress (below the critical concentration) increases the number of chloroplasts which increases the synthesis of carbohydrates, a glycolysis, and cellular respiration given citric acid involved in chelating and inhibiting the mobility and translocation of cadmium to the aerial parts.

Indeed, a study on *Halimione portulacoides* in the presence of cadmium, adding citric acid exogenous to the plant reduced the concentration of cadmium in the aerial part [23] which is a mechanism of tolerance [24]. In this way, Davis and Carlton-Smith [25] showed that the phytotoxic effects of heavy metals were significant only when the concentration exceeds the upper critical concentration. Fediuc and Erdei [26] reported that *P. australis* have not only an intrinsic ability to protect chloroplasts against the toxicity caused by nanomolar amounts of Cd but this ability was higher in leaf development than in mature leaves, which probably explains the decrease in total chlorophyll content (A + B) under high concentrations of $CdCl_2$. This result could be interpreted as effect of oxidative stress due to the high presence of Cd in the photochemical device or the reduction of density and size of chloroplasts [27] probably due to the interference of heavy metals with Fe^{2+} root uptake [28]. Cadmium has a direct effect on chlorophyll biosynthesis enzymes, by inhibiting the synthesis of aminolevulinic acid and reducing the conversion of protochlorophyllide into chlorophyllide [29].

3.2. Lipids content

It is known that heavy metals cause oxidative stress on the cell, which generates reactive oxygen species "ROS" (O_2^-, H_2O_2) that can react with cellular components and cause oxidation of lipids, carbohydrates, proteins, pigments, and DNA.

In this study, the inhibitory effect of $CdCl_2$ on lipid content was found from the low concentrations in leaves also in roots (Table 2), this certainly due to the cadmium accumulation in plants tissues. There was a very highly significant decrease of total lipids content compared with the control plants, probably due to a damage of cellular and chloroplast lipids or may be due to the reactive oxygen species generated by the metal causing the degradation of lipids. However, $CdCl_2$ can also inhibit the biosynthesis of these molecules. Lipids and membrane polyunsaturated fatty acids are the main target of ROS. However, in this study, it was less important in roots than in leaves although the rhizosphere is the first area of contact with $CdCl_2$ [30].

This shows that the root is less sensitive than leaves to the detrimental effects of $CdCl_2$. Treatment with 50 μM l^{-1} of $CdCl_2$ causes a sharp drop in levels of lipids, polyunsaturated fatty acids in rapeseed

Table 1
Measured chlorophyll content in leaves of *P. australis* grown hydroponically under controlled conditions and exposed to (10, 27 and 55 ppb) of $CdCl_2$

Treatments	Control	$CdCl_2$		
	Dose (0)	10 ppb	27 ppb	55 ppb
Leaves	157.2 ± 16.3	239.4 ± 3.1*	93.63 ± 6.0*	79.1 ± 3.03*

*Differences very highly significant $p \leq 0.05$ according to Dennett's test.

Table 2
Lipids content measured in leaves and roots of *P. australis* grown hydroponically under controlled conditions and exposed to (10, 27, and 55 ppb) of $CdCl_2$

Treatments	Control	$CdCl_2$		
	Dose (0)	10 ppb	27 ppb	55 ppb
Leaves	339.1 ± 20.1	300.2 ± 11.85*	183.2 ± 6.0**	62.9 ± 6.27**
Roots	102.5 ± 20.1	87.26 ± 1.9**	72.3 ± 6.0**	60.9 ± 1.41**

*Differences highly significant
**Very highly significant $p \leq 0.05$ according to Dennett's test.

Table 3
GPOX activity measured in leaves and roots of *P. australis* grown hydroponically under controlled conditions and exposed to (10, 27, and 55 ppb) of $CdCl_2$

Treatments	Control	$CdCl_2$		
	Dose (0)	10 ppb	27 ppb	55 ppb
Leaves	12.1 ± 0.89	5.73 ± 0.65*	4.88 ± 0.33*	4.0 ± 0.17*
Roots	18.74 ± 0.85	20.13 ± 1.18^{NS}	38.1 ± 1.21*	41.06 ± 0.81*

^{NS}Non significant differences
*Very highly significant $p \leq 0.05$ according to Dennett's test.

leaves, and reduced levels of phospholipids and neutral lipids in the roots [31]. Thus, it appears that the decline in content is the cause of inhibition of lipid biosynthesis. In addition, research of Cillard and Cillard [31] showed that low doses of cadmium cause peroxidative degradation of lipids in treated plants. Other similar results in other species are Vigna Mungo treated with nickel [32], in *Lycopersicon esculentum* treated with copper [33].

3.3. Guaiacol peroxidase activity

The results of GPOX in the organs of *P. australis* showed a lower rate in the leaves, an inhibition increased with increasing doses of $CdCl_2$. These results corroborate those on *Vicia faba* treated with lead [34]. Heavy metals are known to increase the formation of reactive oxygen species producing oxidative stress, including impairment of secondary structure of proteins by oxidation of the thiol groups [35]; in contrast to what was found in the leaves, a stimulation of GPOX activity was observed in roots for all used concentrations. A sharp increase in GPOX activity is induced in roots by treatment with $CdCl_2$ (Table 3); this suggests a role of this enzyme in the elimination of excess H_2O_2 produced in the roots, which could be an adaptation mechanism in response to heavy metals effects, especially in plants who accumulate heavy metals in roots more than in leaves like *P. australis* (Table 4).

Many studies have suggested the involvement of GPOX in response of oxidative stress imposed by heavy metals, in *Arabidopsis thaliana* [36], also for rice [37].

In this study, a hypothesis may partly explain the decreased activity of GPOX in the leaves and contrarily in roots is the role of GPOX in the synthesis of cell walls, particularly the biosynthesis of lignin and pectin which adsorb heavy metals. The sugar beet pectin has a high affinity for Cu^{2+} and Pb^{2+}, apple pectin for Co^{2+}, and citrus pectin for Ni^{2+} ions [39]. Therefore, the lignin and pectin limit the presence of heavy metals intracellularly, and this form of phytostabilization is called phytolignification [40]. Ederli et al. [41] showed that root cells of *P. australis* treated

Table 4
Concentrations (mg kg^{-1} Dw) of some heavy metals in leaves and roots of *P. australis*

Element	Leaves	Roots and rhizomes	References
Ni	0.92	14.4	[38]
Pb	0.22	7.4	[38]
Cd	0.090	1.35	[11]
Cd	0.077	3.69	[27]

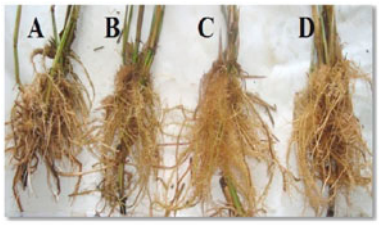

Fig. 1. Roots of *P. australis* treated with $CdCl_2$: (A) control, (B) 10 ppb, (C) 27 ppb, and (D) 55 ppb.

by cadmium have a lignified wall contrarily to untreated plants [42]. This is what has been observed in this work (Fig. 1).

4. Conclusion

The results of this research work indicated that common reed could tolerate a high concentration of $CdCl_2$. However, it appears that cadmium induces a disturbance on chlorophyll and lipid metabolic, but it is clear that the antioxidative response of GPOX is different in leaves and roots, area of first contact with cadmium chloride. The increased activity of GPOX probably is an adaptation mechanism or a response to cadmium toxicity for preserving leaves and aerial part of the adverse effect of cadmium. These findings may contribute to a better understanding the response mechanism of common reed to heavy metals and the proper use as a phytoremediator in aquatic ecosystems with cadmium pollution.

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