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Antioxidative response in leaves, stems and roots of *Phragmites australis* treated with cadmium and nickel

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ABSTRACT

Phytoremédiation is the direct use of green plants to stabilize or reduce contamination in soils, surface water, or ground water. *Phragmites australis* is a plant widely used for treatment of wastewater containing heavy metals. This study aimed to evaluate the antioxidative response of *Phragmites australis* exposed to tow heavy metals, the cadmium chloride ($CdCl_2$) and Nickel chloride ($NiCl_2$). Plants of *Phragmites australis* previously grown in a basic nutrient solution during the 30 days undergoing treatment with three concentrations of $CdCl_2$ (50µML^{-1}, 150µML^{-1}, 300µML^{-1}) and three concentration of $NiCl_2$ (100µML^{-1}, 300µML^{-1}, 500 µML^{-1}). After that, we measured the enzymatic activity of Ascorbate peroxidase (APX), Catalase (CAT), Guaïcol peroxidase (GPOX) and Glutathione peroxides (GST) in the leaves, stems and roots of the plants. The determination of the enzymatic activity (APX, CAT, GPOX and GST) of *Phragmites australis* revealed a varying result depending on the organ of the plant. Our results, showed an increased activity of Ascorbate peroxidase, Catalase, Guaïcol peroxidase and Glutathione peroxides in roots from reliable doses. However in the leaves and stems we found a decreased activity of ascorbate peroxidase and guaiacol peroxidase, the contrary for Catalase and glutathione peroxides activity, we registered a stimulation compared to the results of control plants no treated. This Increased activity of antioxidant enzyme especially in roots probably explains the ability of *Phragmites australis* to tolerate a high concentration of cadmium chloride and Nickel chloride.

KEYWORDS

Heavy metals;
Phytoremédiation;
Enzymatic activity;
Cadmium chloride;
Nickel chloride.

INTRODUCTION

Heavy metal pollution is released in to the environment by various anthropogenic activities, such as industrial manufacturing processes, domestic refuse and waste materials[54,57]. They are ubiquitous in the environment and have long biological life time, their presence in a given ecosystem can lead to accumulation in the food chain with negative effects for living beings[36]. Various technologies have been used for toxic metals removal from industrial efûuents[40]. But, these methods are expensive and not sufûcient to remove heavy metals. Therefore, there is an urgent need to develop an innovative process, which can remove heavy metals

economically[53]. The ability of some plants to tolerate or even to accumulate metals has opened new avenues of research on the treatment of soils and waters whose purpose is phytoremédiation[42,45]. Phytoremédiation is a cheap and eco-friendly technique not just for heavy metals removal but also for various pollutions[51]. This technique is a group of technologies that use plants to reduce, remove, degrade, or immobilize environmental toxins, with the aim of restoring area sites to a condition useable for private or public applications. In this context, this study aims to identify the degree of tolerance and adaptation of *Phragmites australis* placed in water polluted by two heavy metals (separately) one is a trace-metallic element essential to biological processes "nickel" and the other is a toxic heavy metal at very low cell concentrations "cadmium". An analysis of enzymatic activity was studied to evaluate the antioxidative response.

MATERIALS AND METHODS

Plant

Phragmites australis Cav.(Trin.) reeds were collected from BOULHAF DYR (Tébessa, Algeria) in mars 2011, They Were grown in pots and irrigated with nutrient solution for 9 day period of adaptation to new conditions, and then separated into three groups (control and treated with $CdCl_2$ or $NiCl_2$). The reeds of treatment groups were irrigated with different concentrations of cadmium chloride (50µML^{-1} - 150µML^{-1} - 300µML^{-1}) and Nickel chloride (100µML^{-1} - 300µML^{-1} - 500 µML^{-1}). *Phragmites australis* were grown in semi-controlled environment under conditions of a greenhouse with a temperature 8 °C/ 29 °C min/ max in the period from 04-04-2011 to 04-05-2011. Leaves, stems and roots were sampled after 30th days of nickel chloride and cadmium chloride treatment, then they was used for enzyme determination.

Extraction and assays of antioxidant enzyme activities

The method used to obtain the enzymatic extract of *Phragmites australis* (leaves, stems, roots) is of Loggini et *al*. (1999). Leaves, stems and Root samples were homogenized (each sample separately) and ground with a mortar and pestle in 5 ml of phosphate buffer (50 mM phosphate, pH 7.5) at 4°C. The homogenate was centrifuged at 12000 g for 20 min, the supernatant was utilized for measuring the activity-ascorbate peroxidase (APX), catalase (CAT) and peroxidase-guaiacols (GPOX) and Glutahtione peroxides (GST). For the quantification of different spectrophotometric measurements We used the following formula Act = ΔA. Vt / ε. Δt. L. Ve. p.

All spectrophotometric analyses were conducted in a total volume of 3 ml at 25 C° using a (Jenway 6300) UV spectrophotometer

(a) Assay of ascorbate peroxidase activity (APX)

The spectrophotometric assay of ascorbate peroxidase activity was performed following the protocol adopted by Nakano and Asada (1981). The final reaction volume of 3 ml contains: 100µl of enzyme extract (supernatant), 50µl of 0.3% H_2O_2 and 2850µl NaK phosphate buffer-ascorbate (50mML^{-1} NaK, 0.5 mML^{-1} ascorbate, pH 7, 2). The activity was measured by the decrease in absorbance at 290 nm for one min every 15 seconds. for a linear molar extinction coefficient ε = 2800 µM $^{-1}$.cm^{-1}. the activity is expressed in µmol.min^{-1}.mg^{-1} protein.

(b) Assay of catalase activity (CAT)

The spectrophotometry assay of catalase activity (CAT) is performed Following the method of Cakmak and Horst (1991). The Decrease in absorbance is Recorded for three minutes at 240nm and a linear molar extinction coefficient ε = 39.400 µM^{-1}.cm^{-1}. the reaction mixture contains: 100µl of enzyme extract, 50µl of 0.3% H_2O_2 and 2850µl phosphate buffer (50 mM, pH 7.2). The reaction is Initiated by the addition of hydrogen peroxide.

(c) Assay of guaïcol-peroxidase activity (GPOX)

The assay is based on the use of Guaïcol as a substrate for peroxidase. In the presence of hydrogen peroxide is formed the tetra Guaïcol which has an absorption maximum at 470 nm. The activity (GPOX) is determined using the technique of Fielding and Hall (1978)[21] by measuring the absorbance at 470 nm. GPOX activity is expressed as µmol guaiacol-oxydé.min^{-1}.mg^{-1} protein using the value of extinction coefficient of tetra Guaïcol ε = 26,600 µM^{-1}.cm-1 for a final volume of 3 ml, the reaction mixture contains: 100µl of enzyme extract, 50µl of 0.3% H_2O_2 and 2850µl Guaïcol-phos-

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phate buffer (50mML NaK-1, 8 MML-1 of Guaïcol, pH 7.2). The reaction is initiated by the addition of hydrogen peroxide.

(d) Assay of glutathione-S-transferase activity (GST)

Measuring the activity of Glutathione-S-transferase (GST) is determined by the method of Habig et *al*. (1974)[27]. It is based on the conjugation reaction between GST and a substrate, CDNB (1-chloro 2, 4 dinitrobenzene) in the presence of a cofactor the glutathione (GSH). the combination results in the formation of a new molecule (1 -S-glutathionyl 2-4 - dinitrobenzene) which absorbs light at 340 nm. The samples leaves, stems and roots were homogenized separately in 1 ml of phosphate buffer (0.1 M, pH 6). After that the homogenate was centrifuged at 14,000 round / min for 30 min. the supernatant recovered will serve as a source of enzymes. The assay involves reacting 200 µl of supernatant with 1.2 ml of the mixture CDNB (1 mM) / GSH (5 mM) [20.26 mg CDNB, GSH 153.65 mg, 1 ml ethanol and100 ml phosphate buffer (0.1 M, pH 6)]. The absorbance is carried out for 1 minute for each 15 ' seconds at 340 nm against a blank containing 200 µL of distilled water replacing the quantity of the supernatant.

RESULTS

Response of antioxidant enzymes

In this study, we examined the activities of some Antioxidant enzymes such as APX, CAT, GPOX, GST which showed varying responses with induction at various concentrations in leaves, stems and roots of the plant where they measured. The effects of metal treatments on enzyme activities, as compared to controls.

Antioxidative response in leaves

Administration of excess cadmium chloride in the nutrient solution was followed by a decrease activity of APX and GPOX except in leaves of plant treated by 50µML^{-1} where we recorded a slight increase in APX activity 0,0029 µmol. min^{-1}. mg^{-1} protein compared to the result in the control group (TABLE 1). However all doses of CdCl2 induce a stimulation of CAT and GST activity, but not significantly for catalase when we treated *Phragmites australis* with 50µML^{-1} of cadmium chloride.

TABLE 1 : Antioxidant enzyme activities measured in leaves of *Phragmites australis* grown hydroponically under controlled conditions and exposed for 30 days to (50 µML^{-1} – 150 µML^{-1} - 300µML^{-1}) of $CdCl_2$ (Data represent the mean of three independent experiments ± ES).

Treatments	Control	$CdCl_2$		
	Dose (0)	50 µM/L	100 µM/L	300 µM/L
APX	0,0029 ± 3,6.10^{-4}	0,0032 ±1,5.10^{-4} NS	0,0019±1,1.10^{-4} ***	0,0008 ± 3.10^{-5} ***
CAT	0,41 ±0,018	0,44 ±0,011 NS	0,50 ±0,017 ***	0,55 ± 0,01 ***
GPOX	12,1 ±0,89	5,73 ± 0,65 ***	4,88 ± 0,33 ***	4,0 ±0,17 ***
GST	0,169 ±0,01	0,34 ±0,025 ***	0,43 ±0,038 ***	0,57 ± 0,02 ***

(NS non significant differences, * very highly significant Pd"0.05 according to Dunnett's test)**

TABLE 2 : Antioxidant enzyme activities measured in leaves of *Phragmites australis* grown hydroponically under controlled conditions and exposed for 30 days to (50 µML^{-1} – 150 µML^{-1} - 300µML^{-1}) of $NiCl_2$ (Data represent the mean of three independent experiments ± ES).

Treatments	Control	$NiCl_2$		
	Dose (0)	100 µM/L	300 µM/L	500 µM/L
APX	0,0021 ± 1,5.10^{-4}	0,0018 ±1,5.10^{-4} NS	0,0016±1,5.10^{-4}***	0,0012 ± 5,3.10^{-5}***
CAT	0,34 ±0,028	0,44 ±0,016***	0,47 ±0,019***	0,47 ±0,023***
GPOX	2,63 ±0,208	3,03 ± 0,32 NS	2,17 ± 0,21 NS	1,89 ± 0,17***
GST	0,09 ± 0,01	0,14 ±0,009***	0,17 ±0,009***	0,21 ±0,011***

(NS non significant differences, * very highly significant Pd"0.05 according to Dunnett's test)**

The effects of nickel chloride on enzymatic activity in leaves are presented in TABLE 2. As what is been found in the leaves of plants treated with cadmium Chloride, an increase activity of CAT and GST, the results

showed that the Most elevated activity of CAT was found in leaves treated with 500 µM/L, while this same treatment Showed the less elevated level for GST activity 0,26 µmol. min^{-1}. mg^{-1} protein. Concerning APX and GPOX activity an inhibition very highly significant was found except for leaves of plants treated with 100µM/L of $NiCl_2$ the difference is not significant

Antioxidative response in stems

In stems We observed that APX and GPOX activity decreased in plants exposed to 50 µM/L -100 µM/L -300 µM/L of $CdCl_2$. In comparison with the control a not signiûcant effect was observed at 50 µM/L for the tow enzyme and also 100 µM/L for GPOX. The CAT and GST activity in the stems of *P.australis* showed the highest activity at 300µm/L respectively 0.44 and 0.22 µmol. min^{-1}. mg^{-1} protein (TABLE 3).

The enzyme activity in the stems treated by nickel chloride showed a trend similar to that observed in the stems of plants treated with cadmium chloride. TABLE 4 demonstrates a dose-dependent inhibition of both APX and GPOX activity except in stems treated by 100 µM/L of NiCl2 where we obtained a slight stimulation while for CAT and GST activity the maximum level was obtained at 500 µM/L an increase very highly significantly for all doses.

TABLE 3 : Antioxidant enzyme activities measured in stems of *Phragmites australis* grown hydroponically under controlled conditions and exposed for 30 days to (50 µML^{-1} – 150 µML^{-1} - 300µML^{-1}) of $CdCl_2$ (Data represent the mean of three independent experiments ± ES).

Treatments	Control	$CdCl_2$		
	Dose (0)	50 µM/L	100 µM/L	300 µM/L
APX	0,0021 ± 1,5.10 $^{-4}$	0,002 ± 6,5.10 $^{-5}$ NS	0,0017± 9,5.10 $^{-5}$ **	0,0011±6.10 $^{-5}$***
CAT	0,34 ±0,028	0,41 ±0,006 **	0,38 ±0,025 NS	0.44 ± 0,01 ***
GPOX	2,63 ±0,208	2,60 ±0,095 NS	2,19 ±0,116 NS	1,80 ± 0,09 ***
GST	0,09 ± 0,01	0,16 ±0,005 ***	0,18 ±0,007 ***	0,22 ±0,013 ***

TABLE 4 : Antioxidant enzyme activities measured in stems of *Phragmites australis* grown hydroponically under controlled conditions and exposed for 30 days to (50 µML^{-1} – 150 µML^{-1} - 300µML^{-1}) of $NiCl_2$ (Data represent the mean of three independent experiments ± ES).

Treatments	Control	$NiCl_2$		
	Dose (0)	100 µM/L	300 µM/L	500 µM/L
APX	0,0021 ± 1,5.10 $^{-4}$	0,0018 ±1,5.10 $^{-4}$ NS	0,0016±1,5.10 $^{-4}$***	0,0012 ± 5,3.10 $^{-5}$***
CAT	0,34 ±0,028	0,44 ±0,016***	0,47 ±0,019***	0,47 ±0,023***
GPOX	2,63 ±0,208	3,03 ± 0,32 NS	2,17 ± 0,21 NS	1,89 ± 0,17***
GST	0,09 ± 0,01	0,14 ±0,009***	0,17 ±0,009***	0,21 ±0,011***

(NS non significant differences, * very highly significant Pd"0.05 according to Dunnett's test)**

TABLE 5 : Antioxidant enzyme activities measured in roots of *Phragmites australis* grown hydroponically under controlled conditions and exposed for 30 days to (50 µML^{-1} – 150 µML^{-1} - 300µML^{-1}) of $CdCl_2$ (Data represent the mean of three independent experiments ± ES).

Treatments	Control	$NiCl_2$		
	Dose (0)	100 µM/L	300 µM/L	500 µM/L
APX	0,0016 ± 10 $^{-4}$	0,0018 ± 9,5.10^{-5}NS	0,0028 ± 8,5.10^{-5}***	0,00469 ± 10^{-4}***
CAT	0,25±0,026	0,34 ± 0,01**	0,42±0,022***	0,67 ± 0,03***
GPOX	18,74±0,85	18,36±0,61 NS	36,46±0,61***	41,7 ± 0,87***
GST	0,15 ± 0,02	0,27 ± 0,02***	0,50±0,041***	0,33 ± 0,02***

(NS non significant differences, * very highly significant Pd"0.05 according to Dunnett's test)**

Antioxidative response in roots

The results of enzymatic activity obtained showed a dose-dependent stimulation for all enzymes (TABLE 5) $CdCl_2$ stress resulted in increased enzymatic activity. APX and GST was enhanced 3-fold in 500 µM/L of $CdCl_2$ treatment, the same for CAT and GPOX the

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high level of activity is observed in this concentration.

The effects of nickel chloride on enzymatic activity in roots are presented in TABLE 6. the results showed that the Most elevated activity of APX,CAT and GPOX was found in roots treated with 500 µM/L, while for GST the high stimulation are observed in roots of plants treated with 300 µM/L. Concerning the low concentration (100 µM/L) no significant effect of APX and GPOX activity compared to controls.

TABLE 6 : Antioxidant enzyme activities measured in roots of *Phragmites australis* grown hydroponically under controlled conditions and exposed for 30 days to (50 μML^{-1} – 150 μML^{-1} - 300μML^{-1}) of $NiCl_2$ (Data represent the mean of three independent experiments ± ES).

Treatments	Control	$CdCl_2$		
	Dose (0)	100 µM/L	300 µM/L	500 µM/L
APX	$0,0016 \pm 10^{-4}$	$0,0020 \pm 10^{-4}$ NS	$0,0028 \pm 3.10^{-4}$***	$0,0046 \pm 2.10^{-4}$***
CAT	0,25±0,026	0,35±0,014***	0,43 ± 0,04***	0,65 ±0,019***
GPOX	18,74±0,85	20,13±1,18 NS	38,1 ± 1,21***	41,06 ±0,81***
GST	0,15 ± 0,02	0,27 ± 0,07***	0,44±0,055***	0,48 ±0,035***

(NS non significant differences, * very highly significant Pd"0.05 according to Dunnett's test)**

DISCUSSION

In this paper, we investigated the antioxydantes response and capacities of enzymes involved in ROS detoxification in leaves, stems and roots of *Phragmites australis* under CD and Ni stress condition. We chose to study these biomarkers of stress regularly used to characterize the physiological state of plants. Our results showed varying profiles according to the organ where activity was measured.

Ascorbate peroxidase activity

Our results clearly show a reduction of Ascorbate peroxidase activity in the presence of $NiCl_2$ or $CdCl_2$ in the leaves and stems unlike the roots where the activity increases with increasing dose applied, these results are in agreement with that of Iannelli and *al.* (2002)[24] where the application of $CdSO_4$ on *Phragmites australis* induced stimulation of APX activity this response was higher in roots than in leaves, similar results were found by Ederli and *al.* (2004) when *Phragmites australis* istreated with cadmium also in *Vigna Mungo* irrigated by $NiCl_2$[18], sunflower under stress induced by cadmium[32], in *Helianthus annuus*[25]. According to these studies the increase in APX activity is a response of plants to oxidative stress caused by cadmium and nickel stress due to increased rate of H_2O_2 in cells and particularly in the chloroplast, it reduces hydrogen peroxide in water using the reducing power of ascorbate (vitamin C) which shows the importance of APX in the defense system against oxidative damage. This hypothesis was proposed by Foyer and Halliwell (1977)[22] they found that ascorbate peroxidase is the reduction of hydrogen peroxide (H_2O_2) in chloroplasts reaffirmed by Asada (1992)[4] where he showed that the main function of APX is the rapid removal of H_2O_2 on the site of his generation.

The stimulation of APX activity in roots in our study may suggest that *Phragmites australis* exposed to $NiCl_2$ or $CdCl_2$ accumulates a large amount of these enzymes in their roots as related to tissue that cause cellular stress and adapt by activation of glutathione-ascorbate cycle to neutralize reactive oxygen species. while the Inhibition of APX activity in the aerial part can be proposed that there is a deficiency in trace elements (copper, zinc) are essential for the activity of antioxidant enzymes due to the antagonism exerted by cadmium and nickel on entry of these elements nutritivs, mainly for copper required for several important physiological and biochemical processes and an essential component for plant metalloenzymes, such as ascorbate oxidase and superoxide dismutase[6,13] or perhaps the possibility of redistribution of ascorbate peroxidase in leaves, stems and roots as the organ most affected by oxidative stress.

Catalase activity

The study of catalase activity shows a simulation in the three organs at low doses of $NiCl_2$ and $CdCl_2$, especially in stems and roots unlike what was found for

ascorbate peroxidase activity when low concentration doesn't significantly affect the activity. This increase in catalase activity under metal stress has been shown by Iannelli and *al*. (2002)[24] in leaves, stolons and roots of *Phragmites australis* treated with 50 µM of CdSO4 where they showed that the activity was greater in the stolon and root than in the leaf, as Pietrini and *al*. (2003)[37] on the same species showed that the presence of cadmium in hydroponic solution stimulates the activity of catalase in the leaves, this stimulation is dose-dependent, this is not the case in chloroplasts where catalase was not detected, which suggests that the action of this enzyme is extra chloroplast. Also Arora and *al*. (2002)[2] and apel and Hirt (2004)[2] showed that catalases are enzymes predominantly peroxisomal eliminate H_2O_2 directly in peroxisomes, for Smirnoff (1998)[50] The role of catalase is to detoxify the hydrogen peroxide produced close by cytochrome chloroplast and especially processes β - oxidation and photorespiration in the same context Fediuc and Erdei (2002)[20] found that the presence of Cd^{2+} inhibit the activity of catalase in the aerial part of *Phragmites australis* in the root the activity is slightly higher compared with the untreated plants. It has been proposed that the mechanism of protection involves the transcription of genes encoding proteins with key roles in antioxidant defense, including POD, SOD and CAT[29]. In our study we can consider the stimulation of CAT activity as a normal response of *Phragmites australis* to oxidative stress caused by $CdCl_2$ and $NiCl_2$ thus resulting in an increase in support for ROS mainly in roots.

Del Rio et *al*. (2003)[14] showed that superoxide radicals formed in the cells are transformed into H_2O_2 by superoxide dismutase (SOD). These molecules (H_2O_2) are then degraded by catalase (CAT) and ascorbate peroxidase (APX) in peroxisomes by the peroxiredoxin and glutathione-ascorbate cycle (Halliwell-Asada cycle) in chloroplasts and cytosol, and only by the ascorbate-glutathione cycle in mitochondria[34]. The increase in catalase content in oxidative stress was also found in other species such as Vetiveria zizanioides[28], Pisum sativum[17], Sesbania drummondii[43], Brassica juncea treated with $NiCl_2$[1].

Guaïcol peroxidase activity

The results of Guaïcol peroxidase activity (GPOX) in the organs of *Phragmites australis* showed a lower rate in the leaves, an inhibition increases with increasing doses of $NiCl_2$ and $CdCl_2$. These results corroborate those of Pourrut and *al*. (2008)[38] in Vicia faba treated with lead also in Brassica napus treated with 1 mM of ZnSO4[5]. According to Siedlecka and Krupa (2002)[47] Heavy metals are known to increase the formation of ROS. Producing oxidative stress, including impairment of secondary structure of proteins by oxidation of the thiol groups[15,16], leading to a decrease in activity GPOX. ROS are indeed capable of oxidizing the thiol groups of proteins, thus affecting the activity of many enzymes[16]. The decrease in activity was observed GPOX also in stems, however, it is significant only in plants exposed to higher doses of $NiCl_2$ or $CdCl_2$ which probably indicates the tolerance of the shaft at low concentrations unlike leaf of *Phragmites australis* they seem more sensitive, in contrast to what was found in the leaves and stems, GPOX activity in the roots showed a stimulation from the low concentrations of $CdCl_2$ and NiCl2. A sharp increase in guaiacol peroxidase activity is induced in roots by treatment with $CdCl_2$ and $NiCl_2$, this suggests a role of this enzyme in the elimination of excess H_2O_2 produced in the roots of *Phragmites australis*, which could be an adaptation mechanism in response to heavy metals effects, or the priority is to protect cells against the effects of ROS especially in plants that accumulate large amounts of metal trace elements in roots, this is was revealed in *Phragmites australis* under stress induced by Cd^{2+} also Wang and *al*. (2012)[58] in *Agrostis stolonifera*. Number of studies have suggested the involvement of guaiacol peroxidase in response of oxidative stress imposed by heavy metals, in *Phaseolus vulgaris*[11], *Bacopa monnieri*[33], *Arabidopsis thaliana*[45], in rice[46].

Hypothesis may explain the decreased activity of GPOX in the leaves and the stimulation in root of *Phragmites australis*, is the transfer leaf - root which has a role in the synthesis of cell walls, particularly the biosynthesis of lignin and pectin which adsorb heavy metals, Kartel and *al*. (1999)[28] found that sugar beet pectin has a high affinity for Cu^{2+} and Pb^{2+}, an apple pectin for Co^{2+} and citrus pectin for Ni^{2+} ions. This limits the presence of heavy metals intracellularly. This form of phytostabilisation is called phytolignification[10]. Ederli and *al*. (2003) found that root cells of *Phragmites*

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australis treated by Cd^{2+} have wall lignification in roots a contrast of untreated plants, according to the study of Soukup and *al*. (2007)[48] The deposition of lignin and suberin on the cell wall of the root of *Phragmites australis* is also attributed to minimized the loss of oxygen to the rhizosphere.

Glutathione-S-transferase activity (GST)

The effect of $CdCl_2$ and $NiCl_2$ on Glutathione-S-transferase (GST) shows a stimulation of activity in the three organs. All the results of treated plants are higher than those of control plants. Our results are in agreement with those of Iannelli and *al*. (2002)[24] where they caused a metal stress using cadmium in *Phragmites australis*, according to their study leaves, stolons and roots of plants treated with 50 µM of $CdSO_4$ stimulate GST activity more than plants Untreated, the same result was found by Pietrini and *al*. (2003) in the leaves of *Phragmites australis* under 50 µM and 100 µM of CdSO4, also in roots (Ederli and *al*., 2003), even in organic pollution Schroder and *al*. (2005)[49] showed a stimulation of GST activity in *Phragmites australis*.

Based on our results and those appearing with other results found in the literature we can explain the stimulation of GST activity in different organs of *Phragmites australis* as a reaction to an increase of ROS, antioxidant enzyme activity that reflects indirectly where ROS production levels are increased. According to Iannelli and *al*. (2002) the increase in the content of Glutathione-S-transferase and glutathione (GSH) simultaneous in *Phragmites australis* appeared to be associated with induction of the detoxification process in response to a high concentration of heavy metals. Marrs (1996)[31] in his serche prove that Glutathione is a substrate for glutathione-S-transferase (GST) which catalyzes the conjugation of xenobiotics, thus contributing to their detoxification. Or glutathione conjugation to protect transient metabolites such as oxylipins[12].

Stimulation of GST activity in metal stress has been found in other species as *Typha angustifolia*[56], and in *Macrotyloma uniflorum* and *Cicer arietinum*[41], *Hordeum vulgare*[52], rice[8] Catalyzing the glutathione conjugation with certain substrates represents a step in the formation of compounds that are less toxic than the starting molecules[9].

However, glutathione-S-transferases are a family of multifunctional enzymes mainly cytosolic with various operations involved in transport and intracellular biosynthesis[26]. other activities associated with the GST have been postulated by[12], including intracellular transport of small molecules such as flavonoids, the introduction of sulfur secondary metabolites such as glucosinolates. According to Francis (2002)[23] the glucosinolates become an important source of sulfur when sulfur deficiency.

CONCLUSION

Our ûndings indicate that Phragmites australis can tolerate a high concentration of CdCl2 and NiCL2. The Increased APX, CAT, GPOX and GST activity might play a role in the defense response to cadmium and nickel toxicity, it was a similar enzymatic antioxidant response, however, it is clear that the reaction is different in the roots, stems and leaves. Thus, these findings may contribute to a better understanding of the antioxidant response mechanisms in Phragmites australis. Thus, seems suitable for use as a phytoremediator in aquatic ecosystems with CD and Ni pollution.

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