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Biochemical Changes Observed in Isolated Roots of *Phragmites australis* Treated with Industrial Wastewater

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Abstract: The objective of this study is to highlight the behavior of isolated roots of a plant known for this potential bio-purification (sewage plant): *Phragmites australis* treated with wastewater discharges from the steel complex of El Hadjar (Annaba). The analysis of the dust released from the complex shows that the majority of the rejected elements are heavy metals with very high concentrations. The evolution of the total protein levels of isolated roots shows that they are much higher than in the roots treated especially during the month of June. Concerning, the content of Proline it varies between 4 and 20 mg/g in roots PF witnesses. In the presence of wastewater rates are 6 times higher; indicating that treatment with wastewater strongly stimulates the synthesis of proline (indicator parameter of stress). Monitoring of the peroxidase enzyme activity (enzyme oxidative stress) shows that the values obtained in isolated roots of *Phragmites australis* controls tend to decrease as a function of time (less than 50%). Parallel in the presence of water, these values increase to reach a max level. This increase is by 20% during the month of March, 40% and 60% for the months of June and October. Finally, Polarographic monitoring of the respiration of isolated roots showed a high consumption of oxygen at the roots witnesses (nearly 90%). The respiratory activity is strongly reduced in the presence of sewage; the reduction is 50% /10 minutes. *Phragmites australis* is a plant that adapts well to oxidative stress generated by wastewater from industrial sources resulted in high levels of proline. This stress causes the activation of detoxification involving the use of anti-oxidant enzyme activities, confirmed by stimulating the synthesis of total protein and by inhibition of respiratory metabolism of the plant.

Key words: *Phragmites australis* · Oxidative stress · Respiratory metabolism · Detoxification

INTRODUCTION

Environmental pollution is a major problem in the world. Water pollution attracts the attention of all decision makers and subject to all policies in both developed countries and developing countries [1]. Given this situation, it becomes essential to find techniques that are effective cleansing and yet inexpensive. Among them, technical treatment plants helophytic plays a very important particularly those using the *Phragmites australis* which is the case in our study.

The potential of this plant between plants is rather old [2] Indeed, it is reported that plants play an important role in the treatment of effluents from industrial chemical compounds such as pesticides or heavy metals. Numerous research reports the use of this plant in various phytopurification of xenobiotics [3-6].

The reed is a rhizome plant essential for the multiplication of bacteria on roots that gives the soil a high permeability as well as an important interface between wastewater and soil [7, 8].

Most of the works conducted so far have concerned the role and potential of the plant in phytopurification; however, very few studies have focused on the biochemical and physiological changes brought out in the plant during its treatment by the wastewater.

This aspect is the major aim of our research work. Thus we sought to identify the physiological and biochemical changes resulting from treatment of *Phragmites australis* by sewage polluted by industrial wastewater generated by steel mills complex of Annaba (East-Algeria).

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MATERIALS AND METHODS

The plant model used is represented by isolated roots of reed (*Phragmites australis*) from plants have reached maturity harvested in November 2008 from a sampling site considered unpolluted (SERAIDI). The wastewater used for treating plants from Wadi Meboudja near the steel complex and two steel mills.

Analysis of Discharges Steel: The analysis of the quantitative and qualitative composition of the compound released by the mills of the steel complex is carried out by mass spectrophotometry.

Treatment Plants: Each pilot test consists of 10 plants; the plants are arranged in trays of 100 liters of capacity as the case with tap water (control plants) or sewage (treated plants) and for a period of 10 to 15 days [9].

Determination of Total Protein: The determination of total protein was done by the method of [10] using bovine serum albumin (BSA) as standard.

Determination of Proline: The assay of proline was carried out by the method of [1] modified by [12].

Determination of Peroxidase Activity GPX: The peroxidase activity was measured spectrophotometric at 470 nm by the method of [13] based on measuring the intensity of the color of the oxidation product of guaiacol obtained by the action of peroxidase after intake of hydrogen peroxide.

Measurement of Respiratory Activity: The measurement of respiratory metabolism of isolated roots of *Phragmites australis* is achieved through oxygen electrode type Hansatech. The method used is that of [14] in which the environment is constantly stirred by a magnetic stirrer.

RESULTS

Composition of Discharges from Steel Mills Complex: The results of discharges analysis from the mills of the steel complex of El Hadjar are summarized by Table 1.

According to the Table, we find that the composition of discharges from steel complex is predominantly rich in heavy metals with concentrations higher than those allowed by WHO [15] particularly for iron.

Table 1: Composition of discards of complex steel mills

Compounds	Tenors (ppm)
Cr	10.7
Zn	720.0
Pb	86.4
Cr	22.0
Ni	2.5
Mn	860.0
Fe	6600.0

Changes in Total Protein Level: The Figure 1 shows the changes in protein level obtained from isolated roots of *Phragmites australis*, Controls and in treated wastewater.

We notice that the average content of total protein is much higher in treated plant roots. However, the difference between the roots of control plants and treated is growing more and more during the months of June and October. Indeed, it was during these two months that the recorded rates of total protein higher. At the roots of control plants these rates remain close whatever the season.

Changes in Proline Levels: Figure 2 represents changes in the average recorded levels of proline isolated from the roots of *Phragmites australis* controls and treated by wastewater.

This figure shows that the average rates of proline are high in roots isolated from plants which have remained in the wastewater. These rates are much higher in the roots of plants treated. We note that the difference between the rates of proline isolated from roots of treated plants also increases depending on the season and reached the peak of observable during the months of June and October.

Variations of Peroxidase Activity (GPX): The activity of peroxidase in nmol/min /mg of protein is shown in Figure3.

In Figure 3 we observed that the average recorded values of GPX activity are between 0.46 and 0.22 (50% reduction). Among isolated from roots of control plants the highest values are obtained during the months of January and March. In isolated roots of plants treated with wastewater, it is noted that these values increase with time to reach almost a max from March with a peak of apparent during the month of October. This value is 3 times higher than in roots isolated from plants.

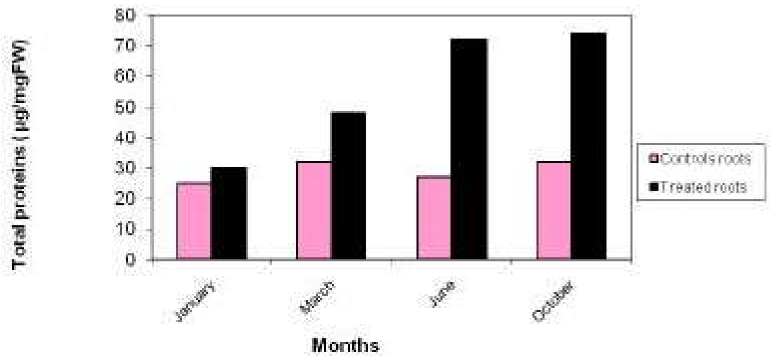

Fig. 1: Changes in total protein isolated from the roots of *Phragmites australis*

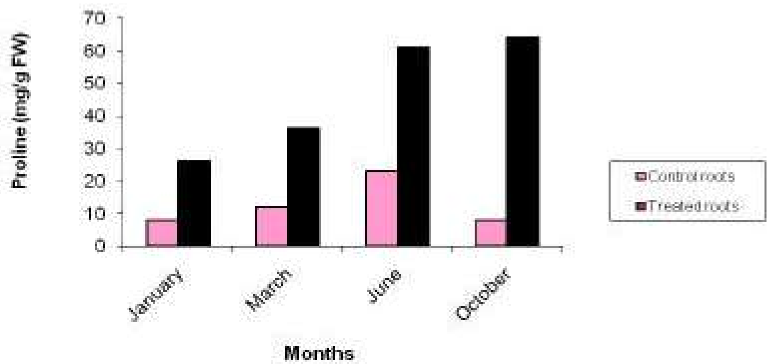

Fig. 2: Variations in average grades of proline isolated from the roots of *Phragmites australis*

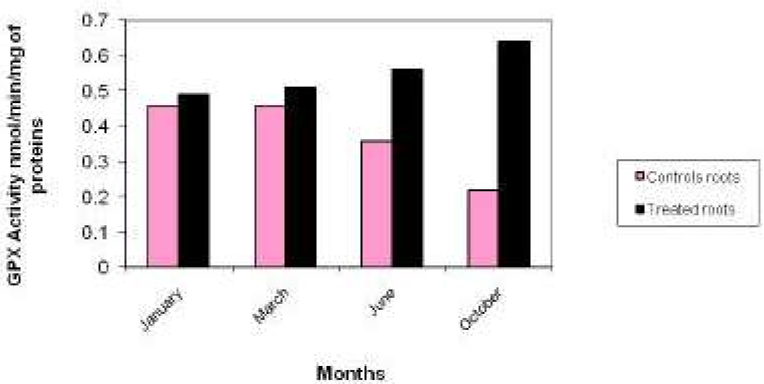

Fig. 3: Variations of the GPX activity of *Phragmites australis* isolated roots according to the treatment of wastewater

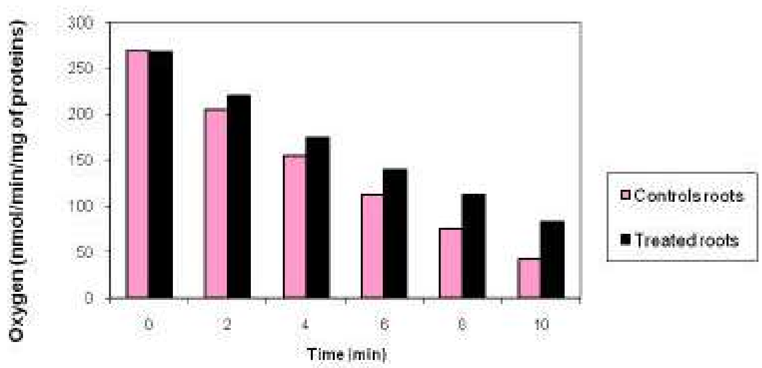

Fig. 4: Changes in respiratory activity of isolated roots of *Phragmites australis*

Variations of Respiratory Activity: Variations in the respiratory activity of isolated roots of treated plants and witnesses are presented by Figure 4.

This figure shows that the consumption of oxygen in the isolated witnesses roots of *Phragmites australis* is important in function of time. It goes from 270 nmol of O_2/ml to 31 nmol/ml, representing consumption of about 90% O_2 environment measurement and for 10 minutes of recording. In isolated roots of treated plants, we find that the consumption of O_2 initially significant at 2 and 4 min of recording at a slow trend from 6 min of recording. Concerning the roots of plants treated by sewage this reduction reached 90% of the respiratory time equal to 10 minutes.

DISCUSSION

The objective of this study is to highlight one part of the quantitative and qualitative composition of industrial waste generated by the units of the complex steel mills and the other to monitor physiological and biochemical changes observed in *Phragmites australis* in the treatment of wastewater from industrial sources.

The results obtained show that the discharges generated by the steel complex units are loaded with heavy metal concentrations far exceeding those permitted by WHO. In qualitative terms, these discharges are composed of heavy metals such as Zn, Cu, Pd, etc…, with the addition of a very large amount of iron (close to the complex).

Alongside the model plant used in our study "*Phragmites australis*" treated sewage loaded metal compounds present a rate of total protein that increases depending on the season especially during the months of June and October. This protein synthesis could be explained by the activation of detoxification systems of *Phragmites australis*.

Indeed, the work of [16] and [17] support our observations in this work the stimulation of protein synthesis was due to the synthesis of phytochelatin whose role in the detoxification is well known. According to [18] these particular proteins PCS (phytochelatin synthesis) plays a key role in the mechanism to capture heavy metals; the catalyzes glutathione enzyme from phytochelatin production of heavy metal chelators promote their sequestration in the vacuoles.

In our case, the stimulation of protein in the isolated roots of *Phragmites australis* explained by the fact that these bodies are real sites of resistance to oxidative stress. This confirms the results reported by [18]. In addition, high levels of proline in the roots observed in plants treated by sewage and just confirm the fact that the roots are indeed in the presence of oxidative stress. Thus the role of proline could affect the root osmoprotection [19, 20]. Which would stabilize the protein levels [21], inhibitor of heavy metals [22] and reduce peroxidation [23]?

The values of peroxidase activity showed a strong stimulation observed during the months of June and October. These values indicate a high antioxidant capacity of *Phragmites australis* in the presence of oxidative stress produced in conditions of heavy metal pollution. This supports the observations of [24, 25].

Finally, the respiratory metabolism of isolated roots of *Phragmites australis* treated with wastewater is highly disturbed. This result could be explained by a slowing of the respiratory activity of isolated roots of *Phragmites australis* and processed to the activation of detoxification systems/sequestration of heavy metals contained in wastewater. This regulatory mechanism of the plant is a strategy adapted it to better withstand xenobiotics and continue its growth. In this option the work of [26, 27] and [28] support this hypothesis in plants.

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