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# Phytoremediation of wastewater by *Phragmites australis* and *Typha angustifolia* in the suburban area of annaba (algeria)

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**Abstract**

Phytoremediation is an environmental rehabilitation technique that uses the ability of plants to remove, contain, or make pollutants less toxic. A phytoremediation study was carried out at Seybouse River (Annaba) Nord-eastern of Algeria. The study focused on assessment of organic pollutants accumulation in certain aquatic macrophytes used as biomonitors in comparison with the station of treatment. The biological treatment of wastewater collected before the entrance to the station of treatment was compared with the purified water by the resort itself. For these test two varieties of marsh plants reeds "*Phragmites australis*" and lesser bulrush "*Typha angustifolia*" was conducted to verify their effectiveness in the treatment of water heavily loaded with pollutant. Results showed that water quality was comparatively better after phytoremediation. The physicochemical analysis after purification with both macrophytes showed a significant reduction of suspended matter (- 75.54 % with reeds and - 71.55% with lesser bulrush), *Chemical Oxygen Demand* COD (- 65.72% with reeds and 52.55 % with lesser bulrush), *Biochemical Oxygen Demand* $BOD_5$ (- 95.14 % with reeds and – 65.63 % with lesser bulrush) and nitrates (- 84.32 % with reeds and - 70.73% with lesser bulrush) and nitrites (- 45.61 % with reeds and -14.03 % with lesser bulrush). Our results confirmed the involvement of enzymes of metabolism in the roots, with the increase in total protein levels and proline reflecting intense root metabolism in a polluted environment.

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## Introduction

Phytoremediation is a solar driven plant based eco sustainable set of techniques. It is relatively inexpensive and environment friendly and hence considered a viable alternative for wastewater purification (Prasad *et al.,* 2010). It has received increasing attention after the discovery of hyper-accumulating plants which are able to accumulate, translocate, and concentrate high amount of certain toxic elements in their above-ground/harvestable parts. These techniques have been used to prevent the generation of hyper-accumulating plants, which are capable of accumulating, translocating, and concentrating high levels of certain toxic elements in their above-ground/harvestable parts.

This technique can be applied to heavy metals, radioelements or organic pollutants in soil or water (Dushenkov *et al*., 1995; Salt *et al*., 1995; Raskin *et al*., 1997; Krämer, 2005). In aquatic systems, where pollutant inputs are discontinuous and pollutants are quickly diluted, analyses of plant components provide time-integrated information about the quality of the system (Baldantoni *et al*., 2005). Constructed Wetlands have been studied for the treatment of rainwater, industrial, agro-industrial, and livestock wastewater, domestic sewage, and already eutrophic lakes and rivers in various regions of the world (Golda *et al*., 2014; Kadlec, 2008). Considering the diversity and the unexplored potential of regional aquatic flora, this study aimed to identify and analyze the potential of native aquatic macrophytes to reduce the organic matter of domestic sewage mainly responsible for water quality deterioration worldwide. Aquatic ecosystems have attracted the attention of researchers worldwide. A definite need exists to develop a low cost and eco-friendly technology to remove pollutants particularly heavy metals, thereby improving water quality. Phytoremediation offers an attractive alternative. They can be considered as ecotones between terrestrial and aquatic environments. They have certain structures and functions characteristic of terrestrial ecosystems, and others typical of aquatic environments.

Several species of aquatic macrophytes have been studied for phytoremediation purposes and *Phragmites* was the species most frequently used in wetland systems followed by *Typha* (Bhatia and Goyal, 2014). *Typha angutifolia* and *Phragmites australis* are emergent macrophyte that widely grows in warm regions and are also commonly found in ponds and streams at different limnological regions of Algeria.

In this work, we have chosen to work on the wastewater purification capacities of aquatic plants such as the Reeds (*Phragmites australis*) and the lesser bulrush (*Typha angustifolia*). We used the wastewater from the station of treatment of Annaba city (STA), which receives all the wastewater from the Annaba area loaded with effluents from industrial and domestic wastewater and sewage. For example, *Phragmites australis* and *Typha angustifolia* were kept in wastewater bins directly from the STA. Our aim was to compare the wastewater purified by the STA and the waters derived from phytoremediation by the two plant species. The aim is to test the effectiveness of these plants in reducing the pollution rate (physicochemical parameters: $BOD_5$, COD, suspended matter) as well as the study of cellular (biochemical) mechanisms used by *Phragmites australis* and *Typha angustifolia*, to purify polluted water.

## Materials and methods

### *Plant selection*

Two wetland species, the reeds (*Phragmites australis*) and the lesser bulrush (*Typha angustifolia*) were studied for phytoremediation capabilities. *P. australis* is a macrophyte belonging to the group of heliophytes and is characterized particularly by a very active root system able to withstand very difficult conditions even when the aerial part of the plant is desiccated (Pilon Smits, 2005). The height is 1 m to 5 m; this plant contains 2 kinds of stems: one underground, called rhizome, the others erect and simple, bearing long ribbed leaves which end in inflorescences (Den Hartog *et al*., 1989). *T.*

*angustifolia* is a perennial herb that grows in temperate and tropical regions of the northern hemisphere. It includes a submerged rhizome and can withstand brackish water (Machado *et al*., 2007). The plant can be between 1 m and 3 m high, it has narrow alternate leaves.

*Sampling*

The sewage was taken from the station of treatment of Annaba city (STA), located on the edge of the Seybouse river (Northeastern of Algeria). The treated water is collected at the outlet of the treatment station.

*Implementation of experimental devices*

The experiment was carried out at the inlet station under in vitro conditions and using four dark bins, we constructed a purifying system (marsh with vertical flow), the latter consisting of two bins connected together by a pipe. The first is at a height of 53.5 cm (1st floor) and contains three layers of gravel with different particle sizes and thicknesses (filter layer, transition layer and drainage layer).

The second located at a height of 21 cm (second floor) also containing three layers: Two composed of gravels and the third which is the thickest is sand. In the latter, macrophytes were planted (*Phragmites australis*), the first bin is irrigated directly by the sewage and the second receive water from the first stage. In parallel, a third bin irrigated with tap water will serve as a control. An equivalent quantity of reeds is placed in each bin, which stays for at least ten days (Geneviéve, 2002). The same device is made for *Typha angustifolia (*Fig. 1).

*Analysis techniques*

The characterization of wastewater was carried out by analyzing parameters like BOD5, COD, and concentration of nitrite, nitrate and suspended matter (SM). Physicochemical analysis was carried out at the laboratory of the station of treatment of Annaba city (STA) (Table 1).

*Measurement of biochemical parameters*

The two parameters measured are proteins and proline. Total proteins were assayed according to the Bradford method (1976) using beef serum albumin (BSA) as the standard (Merk). The calibration range is made from a stock solution of BSA (1mg / ml). The reading is done at a wavelength of 595 nm. The technique used for the proline assay is Troll and Lindsley (1955) modified by Dreider and Goring (1974). The optical densities of the samples are determined at the wavelength 528 nm.

## Result and discussion

*Physico-chemical parameters*

*Suspended matter (SM)*

The evolution of the suspended matter (SM) is shown in Table 2. Thus, there is a very significant decrease in the suspended matter content for the two macrophytes. This can give an idea of the effectiveness of the purification after the two passages (purification 1 and purification 2), relative to the raw water.

**Table 1.** The main parameters and methods used.

| Parameters | Analysis techniques |
|---|---|
| COD | Rodier, 1996 |
| $BOD_5$ | Rodier, 1996 |
| Nitrite | Molecular absorption spectrometry Method<br>ISO 7890 - 1 – 19840. |
| Nitrate | Dimethyl Phenol Spectrometric Method 2,6<br>ISO-6777: 1984. |
| Suspended Matter (SM) | Rodier, 1996 |

*Chemical Oxygen Demand (COD)*

The values of the chemical oxygen demand are shown in Table 3. After treatment, a reduction in COD is observed, which decreases to 73.7 mg / L after the second purification in the reeds and 102 mg / L in lesser bulrush. These results are very important compared to those recorded per station (STA).

**Table 2.** Variations of suspended matter (SM) before and after plants of *Phragmites australis* and *Typha angustifolia* in wastewater.

| Plants | Concentration in Suspended Matter (mg/L) | | |
|---|---|---|---|
| | Raw water | Epuration 1 | Epuration 2 |
| *Phragmites Australis* | 276 | 152 | 67.5 |
| *Typha angustifolia* | 276 | 165 | 78.5 |
| STA | 276 | 48 | |

*Biochemical Oxygen Demand ($BOD_5$)*

The biochemical oxygen demand values are shown in Table 4. In the presence of the plants, there is a very significant reduction which affects respectively the 5.4 mg/L in *Phragmites australis*, and 50.2 mg/L in *Typha angustifolia*, and those after the first treatment, $BOD_5$ decreased even further to 5.00 mg/L and 35.4 mg/L after the second treatment, this is a remarkable decrease compared to the same water purified by the station or it reaches 21 mg/L at the exit of the station.

**Table 3.** Variations of COD before and after planting *Phragmites australis* and *Typha angustifolia* in wastewater.

| Plants | Concentration in COD (mg/L) | | |
|---|---|---|---|
| | Raw water | Epuration 1 | Epuration 2 |
| *Phragmites Australis* | 215 | 97 | 73.7 |
| *Typha angustifolia* | 215 | 177 | 102 |
| STA | 215 | 39.7 | |

*The nitrates ($NO^{-3}$)*

The nitrate contents in the raw water before and after the passage of reeds and lesser bulrush are illustrated in Table 5. These data show a high level of nitrogen pollution. However, after the *Phragmites australis* and *Typha angustifolia* plants, we see a decrease in the $NO^{-3}$. The filtration of $NO^{-3}$ is 0.06 mg/L and 0.091 mg/L for purification 1 and the abatement is greater after the second purification; where it reaches respectively 0.045 mg/L and 0.084 mg/L for both plants.

**Table 4.** Variations of $BOD_5$ before and after planting of *Phragmites australisand Typha angustifolia* in wastewater.

| Plants | Concentration in $BOD_5$ (mg/L) | | |
|---|---|---|---|
| | Eau brute | Epuration 1 | Epuration 2 |
| *Phragmites australis* | 103 | 5.4 | 5 |
| *Typha angustifolia* | 103 | 50.2 | 35.4 |
| STA | 103 | 21 | |

*The nitrites ($NO^{-2}$)*

Table 6 shows the nitrite levels after successive passages in reed beds and reeds. We note discounts of this parameter after the second purification for the two plants, comparing to the treatment plant or this result remains the same. The nitrite level is 0.057

mg/L for the raw water and 0.031 mg/L for the reed and 0.049 mg/L for the lesser bulrush after the two purifications.

*Biochemical parameters*

Dosage of proteins in Phragmites australis and Typha angustifolia. Effect of sewage on the protein content in the roots of Phragmites australis

Fig. 2 shows the variations in protein content in the roots of Phragmites australis. We find that the protein level increases to 11.91 µg compared to the control plants which are of the order of 8.532 µg and those after passages through the first purification. This rate decreases during the second purification to reach 5.419 µg.

**Table 5.** Nitrate variations before and after plants of *Phragmites australis* and *Typha angustifolia* in wastewater.

| Plants | Concentration en $NO^{-3}$ en (mg/L) | | |
|---|---|---|---|
| | Eau brute | Epuration 1 | Epuration 2 |
| *Phragmites australis* | 0.287 | 0.06 | 0.045 |
| Typha *angustifolia* | 0.287 | 0.091 | 0.084 |
| STA | 0.287 | 0.08 | |

*Effect of sewage on the protein content in the roots of Typha angustifolia*

Fig. 3 shows the variations in protein levels at the roots of *Typha angustifolia*. We note for the plants passed by the first purification (E1) that this rate increases to reach 9.131 µg/g compared to the control plants which are of the order of 7.571 µg/g, but after the second purification this rate decreases to reach 6.211µg.

*Determination of proline in Phragmites australis and Typha angustifolia*

*Effect of sewage on proline content in roots of Phragmites australis*

Fig. 4 illustrates the effect of wastewater on the change in proline level at the roots of Phragmites australis. We find that in the treated patients, the proline level increases to 27.78µg/g for the purification 1 compared to the control which reaches 7.78 µg, this tendency has increased even more to reach 81.12 µg/g in the second purification.

**Table 6.** Variations of nitrites before and after plants of *Phragmites australis* and *Typha angustifolia* in sewage.

| Plantes | Concentration en $NO^{-2}$ en (mg/L) | | |
|---|---|---|---|
| | Eau brute | Epuration 1 | Epuration 2 |
| *Phragmites Australis* | 0.057 | 0.042 | 0.031 |
| Typha *angustifolia* | 0.057 | 0.051 | 0.049 |
| STA | 0.057 | 0.570 | |

*Effect of sewage on the proline content in the roots of Typha angustifolia*

Fig. 5 illustrates the effect of phytoremediation on proline levels at the roots of Typha angustifolia. We find that in the treated plants the proline level increases to 9.44 µg/g during the treatment, and this is higher than the control which is 7.22 µg/g after the second treatment by plants this rate tends to increase to reach 10.213 µg/g.

The results obtained in the various physicochemical analyzes revealed a significant decrease in these parameters after passing through the natural filters of *Phragmites australis* and *Tyha angustifolia* compared to the raw sewage of the treatment station. We have seen a reduction in the parameters of organic pollution which are the COD and $BOD_5$. We note the same effects to suspended solids, where the values are well above the standard of 35 mg/L (Rodier *et al.*, 2005). They are caused by sewer rejects and urban domestic rejects that are rich in mineral or organic colloidal matter (Bowes and Beer, 1987). We noted a decrease after passage through the two stages of purification. This result is visible to both plants by comparing with the station.

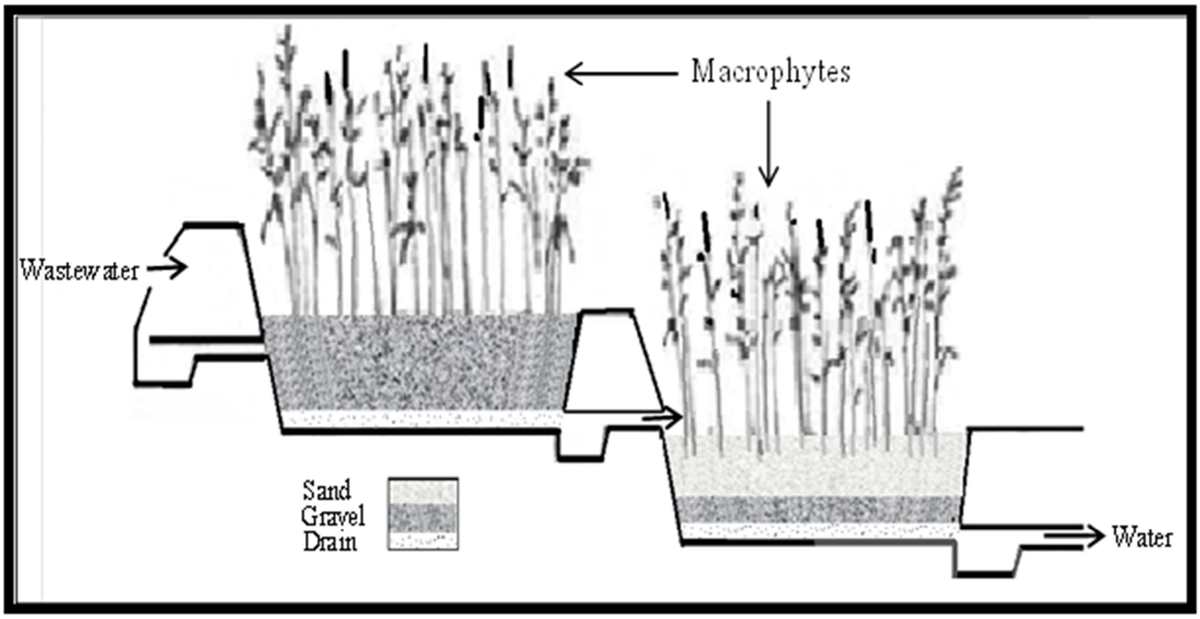


**Fig. 1.**Purifying system.

This decrease can be explained by the maintenance of a shaded surface due to emerging plants, thus alleviating algae growth and maintaining good removal yields for Suspended matter (Hancock *et al.,* 1991). Horizontal or vertical flow systems offer very good Suspended matter removal efficiencies ranging from 90% to 95% when the filter is chosen (Cooper, 1990).

The results obtained concerning the DOC, offers a complete representation of the oxidizable materials present in the water. We noted a significant reduction in reeds relative to the second plant tested and those after passing through the two stages of purification; this could be explained by a heavy load of pollutants given the lack of precipitation for this year.

According to Tchobanouglos (1987) the depletion of DOC in the two plants studied is mainly due to the sedimentation of pollutants in the two experimental purification basins.

The physicochemical characterization of the raw sewage revealed that this liquid discharge is very loaded with organic matter in terms of $BOD_5$ (103 mg/L), despite the fact that these wastewaters have a high organic load, they have a satisfactory biodegradability (Baumont and Camard, 2005).

However, this value decreases after the stay of reeds and *Typha angustifolia* in polluted water. This is consistent with the results of Sankar (2000) and Kadlec and Knight (1996) which show that these plants have root zones that promote bacterial growth and thus allow degradation of organic matter and thus decrease $BOD_5$. *Phragmites australis* has the particularity of creating an oxidizing environment in the root zone and of supplying carbon and energy to microorganisms (which explains the reduction of these parameters in water purified by *Phragmites* *australis* compared to *Typha angustifolia*). Mizi A. (2006) add that the decrease in $BOD_5$ after plant stays may be due to the temperature which favors the growth of bacteria, this was also observed in the work of Copper (1998) on the treatment of domestic wastewater.

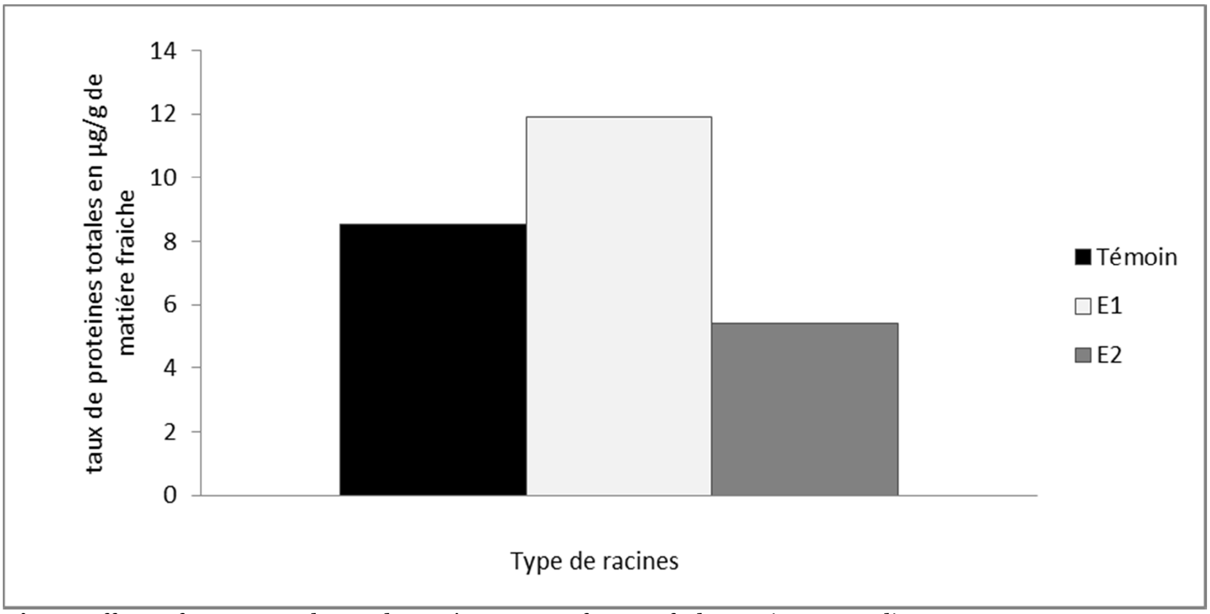


**Fig. 2.** Effects of sewage on the total protein content of roots of *Phragmites australis.*

We also demonstrated a decrease in nitrates after passage through the two stages of purification for the two plants tested. Thus, the main mechanisms for removing nitrogen in a wet environment are nitrification and denitrification (Pride *et al.,* 1990; Martin and Moshiri 1994). Macrophytes absorb mainly inorganic nitrogen in the form of nitrates ($NO3^-$) and ammonium ($NH4^+$) (Engelaar *et al.,* 1995; Reddy and D'Angelo, 1997). Similarly, high levels of nitrates have been observed compared to nitrites and this is explained by the reduction of nitrite ions to nitrates by bacterial action, according to (Aminot and Chaussepied, 1983) the concentrations detected are very high in the entrance of the which can be explained by the incomplete degradation of organic matter.

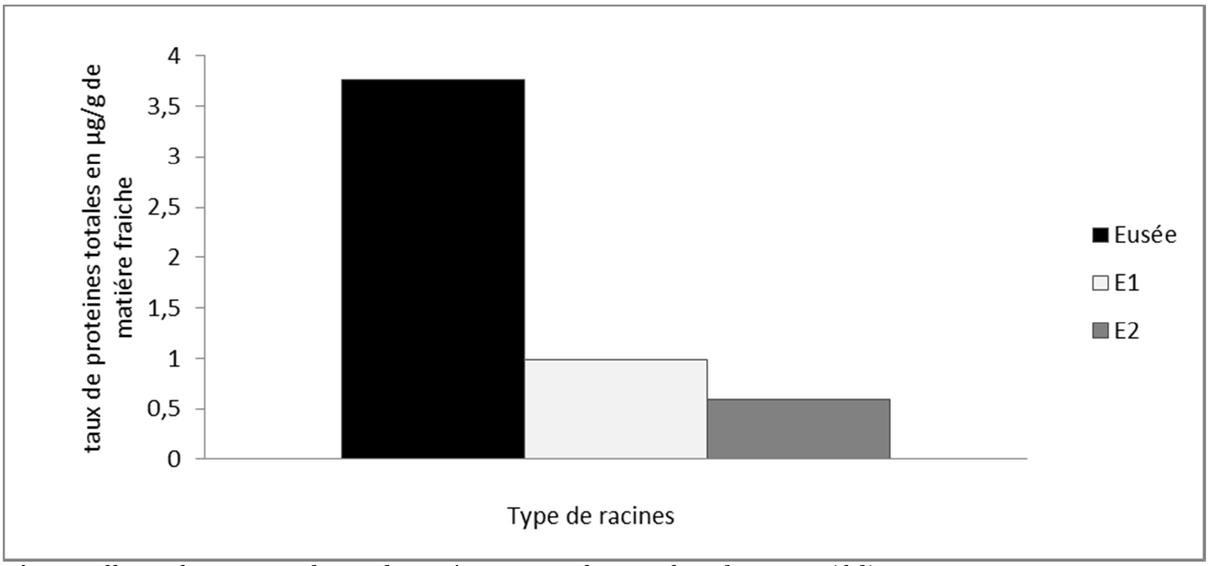


**Fig. 3.** Effects of sewage on the total protein content of roots of *Typha angustifolia.*

In order to confirm the stress in the presence of polluted water, we first focused on changes in the protein content in the roots of the two species. Indeed, according to Sandermann (1992) and Solt *et al.* (2003) in the presence of xenobiotics, the plant increases the protein synthesis; in particular of the phytochelatins whose role is the detoxification of xenobiotics.

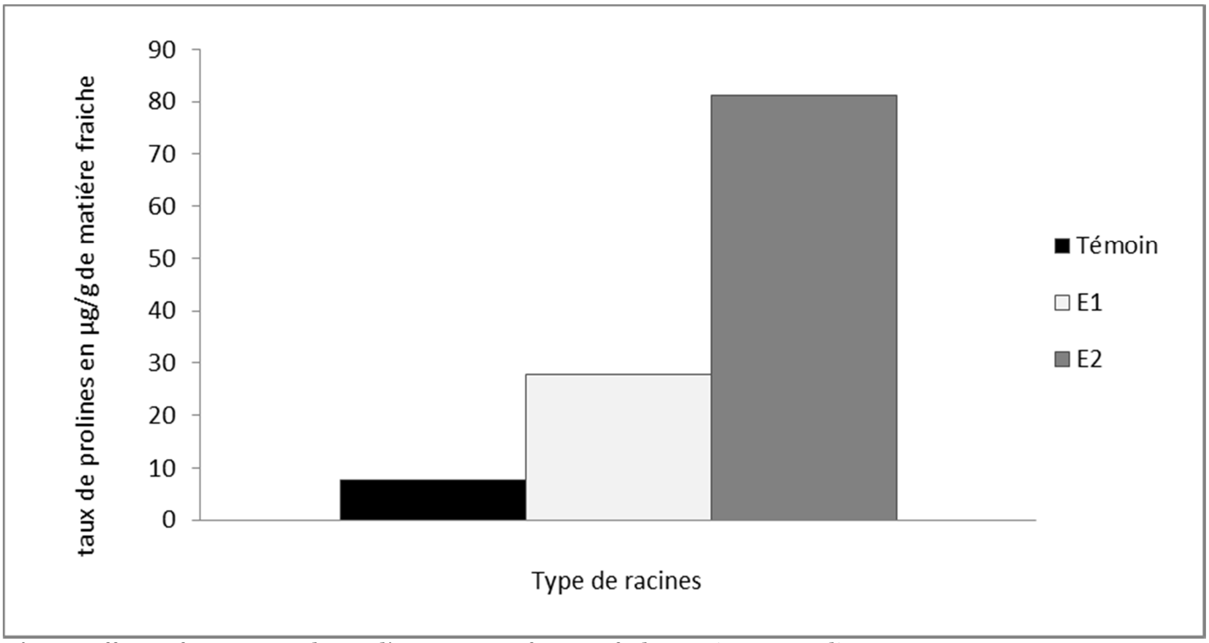


**Fig. 4.** Effects of sewage on the proline content of roots of *Phragmites australis.*

Indeed, the results obtained show an increase in total proteins in the two plants treated after the first purification compared to those irrigated with tap water, this rate tends to decrease in the second purification. This can be explained by the presence of xenobiotics in the tissues which stimulate the protein synthesis of many enzymes, such as those involved in detoxification and in particular phytochelatins and to neutralize substances toxic to plants (Solt *et al.*, 2003; Shraddha *et al.*, 2004).

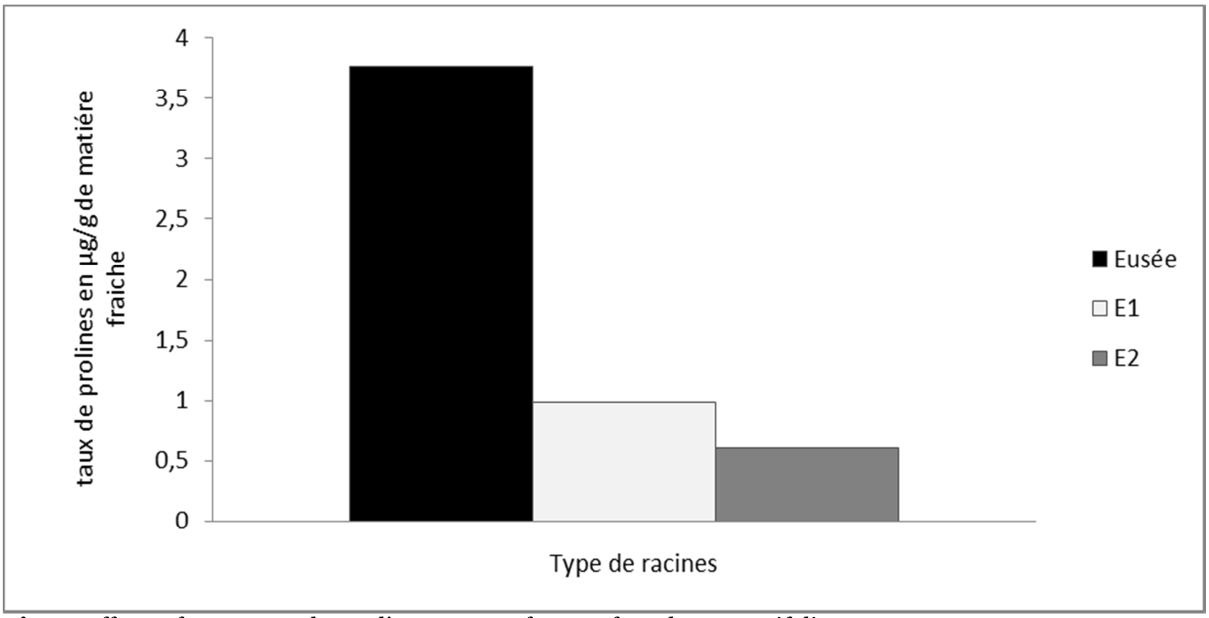


**Fig. 5.** Effects of sewage on the proline content of roots of *Typha angustifolia.*

The other indicator of stress in the plant is the increase in proline levels. Proline is known as a biomarker (Lagadic *et al.,* 1997). Its content varies according to plant species and can accumulate in large quantities in tolerant species (Driouich *et al.,* 2001). Our results show an increase in proline in plants placed in wastewater.

This increase in the proline level can be explained according to Adjeb and Khezane (1998), by a stress effect in the plant. During our experiment, we noted an increase in proline content in the roots of both plants. This increase can play an osmoprotective and protein stabilizing role (Shah and Dubey, 1998). According to Monneveux and Nermann (1989), proline accumulation is associated with plant resistance to stress, which may be one of the factors which explain the adaptation strategy of plants.

It is clear that the treatment of wastewater by the macrophyte or phyto-purification process can be both inexpensive and effective. Indeed, the water once purified, can be profitable both for irrigation and for the industry. These two species, *Phragmites australis* and *Typha angustifloia*, have thus shown efficiencies in the treatment of wastewater, compared to the system of purification of the station of Annaba.

## Conclusion

*Phragmites australis* and *Typha angustiflolia* develop a root system that can withstand the most polluted environments, as well as an increase in biochemical parameters (proteins, proline). This result has been observed both in the roots of the reeds and the lesser bulrush which shows that the roots of both species have excellent purifying power and remain the number one in the purification of wastewater.

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