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ORIGINAL ARTICLE**Tolerance And Accumulation Of Xenobiotic By *Phragmites Australis* : Worms Of New Methods Of Bio-Depollution****Kleche meriem, Berrebbah Houria, Souiki Lynda, Grara Nedjoud and Djebar Mohamed Reda***Laboratory of Cellular Toxicology, Department of Biology, Faculty of Sciences, Badji Mokhtar University of Annaba, 23000, B.P. 12. Algeria.*Kleche meriem, Berrebbah Houria, Souiki Lynda, Grara Nedjoud and Djebar Mohamed Reda:
Tolerance And Accumulation Of Xenobiotic By *Phragmites Australis* : Worms Of New Methods Of
Bio-Depollution**ABSTRACT**

This work has two parts at first we were interested in variations of physicochemical parameters of waste water loaded with discharges from industrial bins after passing through a purifying E1 and E2. It is a lagoon system designed vertical flow in the laboratory of *Phragmites australis* and compounds. Our results showed a significant reduction of suspended COD and BOD₅ and conductivity. The second part of this study is restricted to the study of biochemical and enzymatic potential changes before and after treatment. Our results confirm the ability of roots of *Phragmites australis* to accumulate and transfer the metals and the involvement of enzymes of metabolism in the roots.

Key words: *Phragmites australis*, roots, Phytoremediation, detoxification, GSH, GST, physicochemical parameters.

Introduction

Water is a source of invaluable life. After oxygen, it is our second vital need. If one can resist 5 weeks the hunger, one cannot remain more than 3-4 days without drinking!

But everywhere on planet, the development of the human, domestic or industrial activities, is dependent on the water resource. The diversity of the uses induces a series of impacts varied on the quality of water.

There is however a common point, intrinsically related to the nature of water: that it is integrated into the agroalimentary die or that it is universal solvent for all kinds of cleanings, water continues its cycle while joining, early or late, the tablecloth, the river, the river. Water carries there that of which one charged it, i.e., symbolically: filth and soap [20].

With the development of town planning and industrialization, as well as the evolution of the modes of consumption, the water discharges known as "worn" evolved/moved considerably in quantity and complexity. The simple domestic rejections grew rich by more complex products (detergents...) and the networks of cleansing collect industrial, commercial or artisanal wastes to the very diverse characteristics. The rainwater, washing increasing bitumen surfaces and roofs, takes care in mineral and organic products and increases by as much flow

polluting to treat. When worn water is not treated, the rivers are exceeded in their natural capacity of purification and find polluted. The treatment or the purification of worn water aims thus to reduce the polluting load which they convey in order to return in the aquatic environment a water of quality, respectful of natural balances and its uses future (fishing, leisure, food, agricultural or industrial use, ...). [1].

Many interest were carried these last decades with the phytoepuration worn water. Developed technologies exploit the capacities of adaptation of the systems racinaires to the strong polluting loads and the conditions of anoxia or hypoxia of the substrate, involving symbiotic relations between the micro-organisms and the roots which support the elimination of the pollutants [21]. The goal of this present study is to evaluate the physicochemical performances épuratoires of an installation of lagunage, exploited successively under the die with macrophytes, (*phragmites australis*) and to see whether these artificial wet mediums or purifying marshes can constitute an adequate system for the domestic water treatment worn, industrial and agricultural.

And this through the study of the capacities of the roots with absorber/adsorber metal particles present in water used, like elucidating the effects of the stress oxidizing induced by worn heavy metal

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water charged on the various bodies (roots and sheets) with the reed *phragmites australis*. To achieve this goal, we set up a system of purification at the laboratory (in vitro simulation) similar to the filter marsh (marsh with vertical flow). This system is composed of two vats: one located in height and the second in against bottom to receive water which runs out of the first vat, in which macrophytes were planted (*Reeds australis*).

Our choice it is related to water of Meboudja Wadi because of their strong pollution by effluents of the iron and steel complex, waste waters, water of valves and water of agricultural irrigation.

Material and Methods

1-1 Description of the system of purification:



Fig. 1: System of purification (two stages of purifications). (*Laboratory of Cellular Toxicology*)

1-2 biological Material:

In this work, we used a *macrophyte* considered for its strong purifying capacity the common reed (*phragmites australis*). it is one of the invading vegetable species (Jodoin and Al 2008). The reed is probably one of the vascular plants most widespread in the world (Badly & Narine, 2004). The reeds are able to absorb and to concentrate significant quantities of pollutants and they contribute to the self-purification of water. Their development accelerates in calm water and the rivers (Dajoz,

In our work we built a standard system of purification filter marsh (marsh with vertical flow), this last are composed of two vats connected to each other by a pipe, the first located at height a 53.5 cm (1st stage) and contain three layers of gravels of different granulometry and thickness.

The second located at a 21 cm height (second stage) also containing three layers: two made up of gravels and the third which is thickest consists of sand, in the latter of the macrophytes were planted (*phragmites australis*), the first vat is irrigated directly by the water of Wadi Meboudja and the second receives the water of the first stage. In parallel, a third vat irrigated by water of tap will be used as witness. One places in each vat an equivalent quantity of reeds which remain ten days at least [12]. (figure1).

1985) Once established in the vat, the plant develops a very significant system racinaire.

1-3 Methods of analyses of water:

For the various physico-chemical analyses, we took worn water samples of the various planted vats of reeds (after purification), as well as rough worn water of Meboudja Wadi.

The analyses were carried out on the level of the analysis laboratory " horizon " Annaba. The table (1) gathers the principal parameters and methods used.

Table 1: Principal studied parameters and methods of analysis for each water sample.

	Elements	Method of analysis
01	COD	Rodier, 1996
02	BOD5	Rodier, 1996
03	MIS	Rodier, 1996
04	Conductivity	Rodier, 1996

2. biochemical parameters:

2-1 Proportioning of total proteins:

The total proteins of *phragmites australis* are proportioned according to the method of Bradford, (1976) using the albumin of ox serum (BSA) like standard (Merk). The range calibration is carried out starting from a solution mother of BSA (1mg/ml). The reading is done with at ($\lambda = 595$ nm).

2-2 Proportioning of the prolin:

The assay technique of the proline used is that of Troll and Lindsley, [37], modified by Dreider and Goring [9]. The determination of the optical densities of the samples is carried out with the at ($\lambda = 528$ nm).

3. Proportioning of Biomarkes:

3.1. Preparation of the enzymatic extract:

The enzymatic extract from the roots of Reeds australis is obtained according to the method of Loggini *et al.*, [24]. the extract will be used for the measurement of the activity ascorbate-peroxidase (APX), guaiacol-peroxidase (GPX) and Glutathione transferase (GST).

3-1. Activity Glutathione S-Transferase (GST):

The proportioning of the activity glutathione S-transferase is carried out according to the method of [14].

3-2 Glutathione (GSH):

The enzymatic extract is homogenized in a solution of (E.D.T.A) with 0,02M and undergoes a deproteinisation by the acid sulfo-salycilic 0,25%. After centrifugation to (2000 tours/mn) during 10 minutes the supernatant is used for proportioning spectrophotometric with reagent DTNB 0,01M at 412nm. The concentrations of the GSH are proportioned by the method of Weckbecker and Cory, [39] and are expressed in μM / mg of protein.

Results

1 Analysis of the variations of the physicochemical parameters of worn water:

1-1 Variation of the chemical demand for oxygen DCO:

The table (02) illustrates the values of the DCO before and after purification. We note that these last are very high. After purification, we note a reduction of the DCO on the level of the two stages of purifications. Thus the rate of the DCO decreases by approximately 80 A 90 % after the second purification for the three other seasons, however, in spring, the value of the DCO remains higher a norme(160mg/l).

1-2 Variation of the biochemical Demand for oxygen (DBO5):

The values of the biochemical demand for oxygen are represented in the table (03). In spring and in summer, the values of the DBO5 are higher than those obtained during the winter and the autumn with respectively 805 mg/l and 250 mg/l. In the presence of the seedlings of *phragmites australis* the DBO5 tends to decrease by approximately 5 to 10%, in autumn, after the first purification, and by approximately 35% after the second purification with an abatement from approximately 95% in spring to reach 43 mg/l (near to the Algerian standard); a third basin of purification can give a better output.

1-3 Variation of the Matter in suspension (MIS):

The evolution of the matter in suspension, is represented in table (04). Thus we note a very significant reduction in the content of suspended matter after the first and the second purification. The difference is about 80% and 90% respectively for the water used after purification 1 and purification 2 and this some are the season.

1-4 Variation of electric conductivity (EC):

The table (05) highlights the variations of the conductivity of water in the vats at purification. We note a very apparent reduction in the electric conductivity of water after passage daN the two vats. Thus after the first purification conductivity decreases by approximately 10% compared to water not having been in contact with the plants. After the second passage (the second purification), conductivity decreases by approximately 60% in Autumn and 15% in spring.

Table 2: Variations of the contents of DCO before and after stay of the plants of reeds *Phragmites australis* in worn water.

Seasons	Concentrations of COD (mg/l)		
	Raw water	Eputation 1	Eputation 2
Spring	1000	500	160
Summer	750	143	35
Autumn	605	250	60
Winter	96,66	53	23,23

Table 3: Variations of the contents BOD 5 before and after stay of the plants of reeds *Phragmites australis* in worn water.

Seasons	Concentrations of BOD ₅ (mg/l)		
	Raw water	Eputation 1	Eputation 2
Spring	805	550	43
Summer	250	160	80
Autumn	140	120	94
Winter	99,01	71,03	40

Table 4: Variations of MIS before and after stay of the plants of reeds *Phragmites australis* in worn water.

Seasons	MIS (mg/l)		
	Raw Water	Eputation 1	Eputation 2
Spring	125	80	20
Summer	111	67,33	32
Autumn	95,33	19,95	10,11
Winter	75,22	8,99	4,65

Table 5: Variations of conductivity before and after stay of the plants of reeds *Phragmites australis* in worn water

Seasons	Conductivity (µs/cm)		
	Raw Water	Eputation 1	Eputation 2
Spring	825	289	156
Summer	1020	850	350
Autumn	1580	1100	500
Winter	1490	1150	950

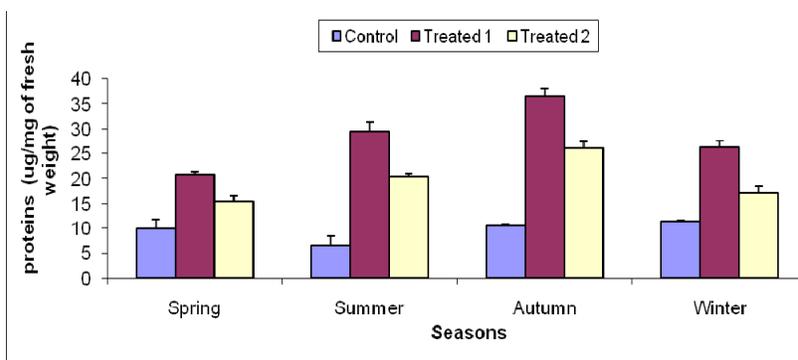
Biochemical Parameters:

Variation of the content of total proteins on the level of the roots of *Phragmites australis*:

Figure (02) represents the variations of the contents of proteins on the level of the roots of *Reeds australis*. We note for the plants placed in the first stage of purification (E1) that the protein rate increases of more than 100% (in a highly significant way with $p = 0,001$) compared to the pilot plants and this during the four seasons. Indeed the protein rate is about 29.45 µg at the roots of the plants of E1 in summer, whereas at the witnesses the rate does not exceed 06.42 µg. In winter this rate is of 36.650 µg for E1 with witnesses not exceeding the 10.503 µg. (an increase of more than 100%). This rate decreases up to 26 µg for the stage E2 but remains always higher than the witness. This observation is the same one for all four season.

2-2 Variation of the content of prolin on the level of the roots of *Pragmites australis*:

Figure (03) illustrates the effect of biological purification on the rate in proline on the level of the roots of *Reeds australis*. We note that this rate is high at the reeds of the first basin during the four seasons. This increase is very highly significant for the seasons of spring and the summer, for spring this rate is 5,33 Mg / G for the witness and 29,25 Mg / G after a passage in the first basin to reach 18,87 Mg / G after the second passage. This difference is highly significant with $p = 0.001$. In autumn the rate of proline is 3,34 Mg / G for the witness this rate reaches 18,34 Mg / G for the first purification this difference is highly significant with a $p = 0.001$. Ce rate decreases after the second passage to reach 7.80 Mg / G with a non significant difference.

**Fig. 2:** Variations of the contents of total proteins at the roots of *Phragmites australis* placed in worn water (E1, E2) and control

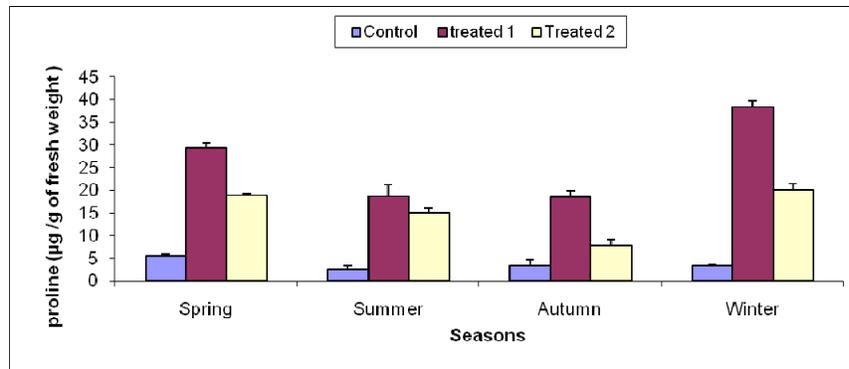


Fig. 3: Variations of the contents of proline at the roots of *Phragmites australis* placed in worn water (E1, E2) and control

Variation of rate GSH on the level of the roots of Phragmites australis:

The figure (04) highlights the evolution of the rate of GSH at the level of are two stages E1 and E2 and of the witness. One observes a very significant evolution of the rate of GSH on the level of the roots of the seedlings from the two basins from purification compared to the pilot roots. This result is valid for the four seasons, particularly for E1. This increase is more significant in autumn where the rate of GSH is practically ten times superior with that of the witnesses, Indeed the rate of GSH is 63,155 µmole/min/mg proteins for E1, and of 6,713 protein µmole/min/mg for the witnesses as in summer when the rate of **GSH is of 74,17** protein µmole/mg for E1, and of 13,37 protein µmole/min/mg at the witnesses. This rate tends to decrease during the second purification to reach 20 µmole/mg proteins for the autumn and 27,75 protein µmole/mg for the summer. The analysis of the variance between the witness E1 and E2, reveals differences very highly significant

(***) $p = 0.001$ during the first purification and a highly significant difference (* *) has very highly significant for the second purification with a nonsignificant difference for the second purification in winter.

3-2 Variation of activity GST on the level of the roots of Phragmites australis:

The data obtained after proportioning of activity specific GST expressed in protein nmoles/min/µg measured to the level of the roots are gathered in figure 05. It is noticed that activity GST increases on the level of the first basin, this increase persists for the second basin and this result is recorded for the four seasons of study. This was confirmed by the study of variance has two controlled factors which shows a difference very highly significant (***) between the activity of the GST on the level of the two basins of purification and the witnesses and this for the measurements taken in summer.

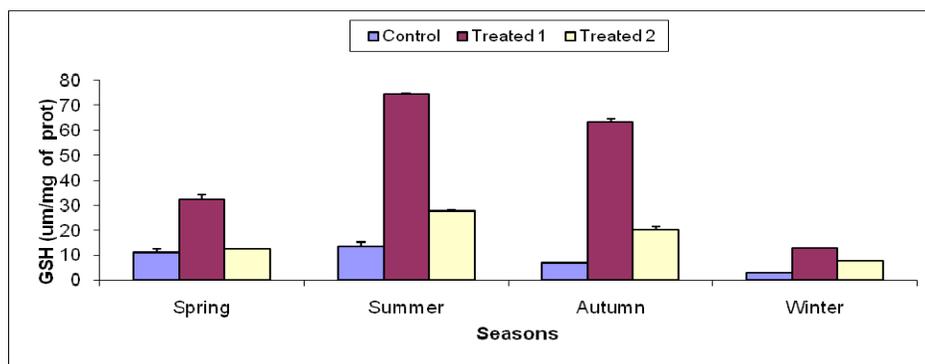


Fig. 4: Variations of the contents of GSH at the roots of *Phragmites australis* placed in worn water (E1, E2) and Control

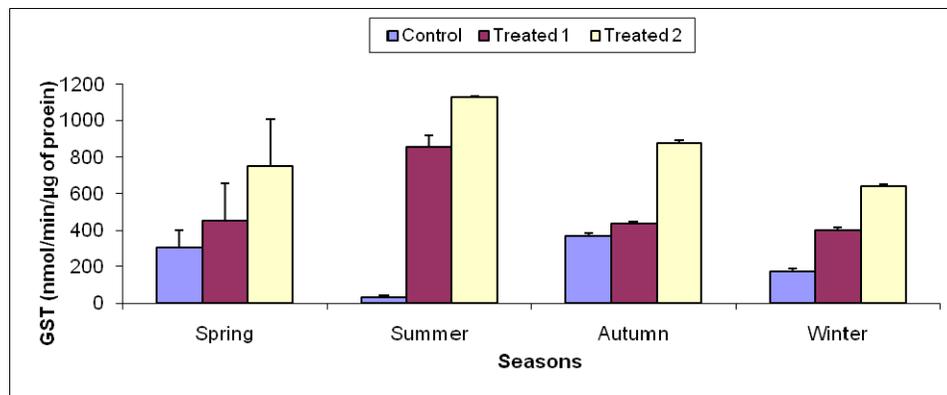


Fig. 5: Variations of the contents of GST at the roots of *Phragmites australis* placed in worn water (E1, E2) and Control.

Discussion:

In this work we highlighted a strong presence of xenobiotic at the level of used water, like on the level of the roots of seedlings of *Phragmites australis* placed in worn metal mais water charged this pollution has tendency decreased after both purification.

According to Polprasert and khatiwada, [29] the elimination of the organic matter in the macrophyte basins is based on a symbiotic relation plant-bacteria, in which the bacteria use the oxygen provided to the medium by the plants during photosynthesis to degrade organic carbon.

It is what explains the reduction in this parameter after passage of water of Wadi through the two stages of purification planted of reeds.

This agrees with the results of [32,18] which show that *Phragmites australis* has sites in their zones racinaires which support the bacterial growth and thus allow the degradation of the organic matter and thus the reduction in the DBO₅. Thus our results are in agreement with those of [23] which used planted filters of reeds for the dehydration of the liquid manure and confirmed a reduction in the DBO₅ after the stay of *Phragmites australis*.

The abatement of the DCO is obviously á the physical retention of the organic matter of the water used in the filters and the oxidation of this one by the microbial flora.

This result is probably related to the presence of the plants which create physico-chemical conditions favorable to the oxidation of the organic matter by the microbial flora. This one brings oxygen in the filter solid mass via the roots and the rhizomes [3]. According to Woodland and *et al.*, [39] the abatement observed on this parameter can be explained by the presence of the reeds which oxygenate the filter solid mass by the intermediary of the rhizomes.

Concerning eclectic conductivity, its reduction in the four seasons can be explained according to

Coulibaly and *et al.*, [6] by a salt retention in the filter solid mass. These salts can be retained by various physico-chemical and biochemical reactions (absorption, exchanges ionic, oxidation, neutralization).

Our results concerning the suspended matter, highlighted a reduction in these last after passage by the two stages of purification. This reduction can be explained by the maintenance of a shaded surface thanks to the emergent plants attenuating the growth of the algae thus and making it possible to maintain good outputs of removal for MIS (80 to 90%) [16].

According to Cooper [5] the systems with horizontal or vertical flow, offer very good outputs of removal as of MY which can vary from 90 to 95% when the filter medium is well chosen and that there is no short-circuiting. In general, the use of a sandy medium ensures a very good filtration, [22].

Concerning the biochemical parameters Our results show an increase in the protein rate in the roots of *Phragmites australis* this increase is significant on the level of E1 what shows a strong level of pollution waters used, on the other hand in E2 we highlighted a nonsignificant increase compared to the witnesses that confirms that the water of the second stage after passage by the first purification was filtered.

According to Zienk [40] the increase in the protein rate in the roots and the sheets of the reeds placed in a polluted water is due to the fact that on the cellular level of the reactions of detoxification take place thanks to the phytochélatines. This induces the formation of a complex protein / metal. The phytochélatines trap the xénobiotique one and / or metal in association with a group HS (sulfhydrile), it is formed a complex thiolate metal which becomes immediately inactive. This reaction makes metal inactive and allows its storage in vacuoles [28,38].

The other indicating element of an effect of stress at the plant is the increase in the rate of the proline. In our work, we highlighted a highly significant increase in the rate of proline at the roots

of the treated reeds. The proline can play a role osmoprotector [36], stabilizer of the proteins [33], inhibitor of metals [10] and inhibitor of the peroxidation [26]. This increase in the rate of proline can be explained according to Ober and sharp [27], by an effect of stress at the plant.

In order to highlight the intervention of the systems of detoxification, in the adaptation of the plant to the variations of the medium, in particular in the presence of pollutant, we followed the evolution of enzymes of detoxification (GSH, GST). This experiment highlighted an increase in the rate of GSH for the plants placed in polluted medium, this result agrees with those of Fabrizio and *et al.*, [10] as Grara [13] which showed an increase in the rate of glutathion at the time of a stress to Cadmium at the reeds *Phragmites australis* like those of Kamara and Pflugmacher [19], out of two species *Phragmites australis* and *Quercus suber* planted in polluted water. Concerning the rates of GST (glutathion S-transférase), our results translate a phenomenon of resistance to heavy metals, which suggests that this variability should be due partly to the degree according to which the organizations can detoxify these metals at the various stages of their vital cycle. In addition the reactions of metals with the glutathion are translated is by the formation of complexes [metal GSH] or by the oxidation of the GSH [30], According to the study of Christie and Costa [4] metals which involves the oxidation of the GSH are Cu, Co, the mn, Fe and Cr while the stable complexes with the GSH are formed by Zn, Cd, Hg, Pb and Ni, these two reactions could explain the reduction in the glutathion in the second basin of purification [15,35]. It appears however that xenobiotic what exists in worn water induces an answer different from the antioxydant system existing in the roots and the sheets of *Phragmites australis* This type of observation was already brought back at *Pisum sativum* [8], which showed that the sheets present mainly a stimulation of the enzymes of the antioxydant system.

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