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# Determination of the toxic effects of heavy metals on the morpho-anatomical responses of the leaf of *Typha latifolia* as a biomonitoring tool

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# Abstract

This study aims to assess the water quality in a wetland of Burgas lake (North-East Algerian) subjected to heavy metal contamination and to determine the toxic effects of this polluants on the anatomical responses of the leaf of Typha latifolia as an effective biomonitoring tool. Water samples at the outlet of the constructed open water surface wetland were collected monthly in 2018 over 3 months. The assessment included physical and chemical parameters and the concentrations of Hg, Cd, Cr, and Pb were analyzed using atomic absorption spectrometry, as well as a microscopic study of the tissues and cells that compose the leaf of Typha latifolia. The results showed the physico-chemical parameters of the water of lake Burgas treated by plantations of Typha latifolia. However, despite this The short-term ability of T. latifolia to remediate this lake contaminated causes the presence of low concertations of heavy metals which are harmful pollutants. Regarding the anatomy of the leaves, the results showed anatomical changes such as irregular shapes of the cells of the spongy parenchyma tissue and shrinkage of the size of the conducting vessels, and slight deformations detected on the xylem. In the peridermic sections, no signs of variation in stomatal anatomical features were detected, compared to the plants at the control site. On the other hand, behavioral changes marked by the closure of most stomata may be related to a mechanism of tolerance to toxic conditions, for phytoremediation of metal contaminated environments.


# Introduction

The progress of the demographic population combined with high industrial development causes serious environmental hazards. For a long time, humans have supplemented great quantities of pollutants to the soil, water, and atmosphere biotopes as a consequence of industrial activities, such as mining of ores, gas emission, pesticide application, and municipal waste production (Shah et al. 2020). On the other hand, a wide range of xenobiotic or anthropogenic chemicals in most aquatic environments (Sumpter 2009; Gavrilescu et al. 2015; Sun et al. 2015). Such pollutants alone or in mixtures can have harmful effects on aquatic organisms (Wasi et al. 2013; Arnold et al. 2014; Malaj et al. 2014).

Metallic pollution is a particular problem, as heavy metals are not biodegradable and hazardous (Youbi et al. 2020; He et al. 2005). Furthermore, Heavy metals can accumulate and migrate in the soil environment. Metal pollutants in soil may be absorbed by the plants through their roots and vascular system. Accumulation of metals in soil could affect ecosystem safety and pose a threat to animals, plants, and humans. High concentrations of metals in the plant could inhibit the ability of the plant to produce chlorophyll, increase the plant's oxidative stress and weaken stomata resistance (Ashraf et al. 2011).

Heavy metals, such as cadmium, copper, lead, chromium, and mercury, are important environmental pollutants, particularly in areas with high anthropogenic pressure. Their presence in the atmosphere, soil, and water, even in traces can cause serious problems to all organisms, and heavy metal bioaccumulation in the food chain especially can be highly dangerous to human health. Heavy metals enter the human body mainly through two routes namely: inhalation and ingestion, ingestion being the main route of

exposure to these elements in the human population (damage to the endocrine system, impact on immunity, neurological disorders, and cancer). Heavy metal intake by human populations through the food chain has been reported in many countries (Islam et al. 2007).

Toxic metal ions at the cellular level evoke oxidative stress by generating reactive oxygen species (ROS) (Li et al. 2016). They promote deoxyribonucleic acid alterations in the chemical structure and repair mechanisms, protein functional perturbation, and nutrient homeostasis and also affect the functional activities of membranes and proteins (Tamás et al. 2014).

In Algeria, as in the majority of African countries and countries of the world different studies have been conducted at different scales to evaluate the effectiveness of wastewater treatment of constructed wetlands (Derradji et al. 2015; Mamine et al. 2019; Khaldi et al. 2019; Mamine et al. 2021). Monitoring the health of aquatic ecosystems to reduce negative impacts on the Physico-chemical and biological quality of water relies on the prevention and control of metal pollution, to limit its impact on water resources, biodiversity, food web, and human health (Grara et al. 2017; Mamine et al. 2021) To this end, the introduction of artificial wetlands (CW) between natural aquatic ecosystems is a promising strategy for eco-remediation.

Recently, like any new technology, metallic pollutants are removed by phytoremediation in artificial filter marshes, via a combination of physical, chemical, and biological processes such as sedimentation, precipitation, adsorption to soil particles, uptake by plant tissues, and uptake and transformation by microorganisms (Brix 1993).

Several research works have reported that cattails growing spontaneously in a natural wetland subjected to municipal wastewater and metal contamination could show excellent tolerance to the low and high concentrations of heavy metal. such as *Typha latifolia* (Mamine et al. 2019; Yang et al. 2020), *Typha domingensis* and *Typha angustifolia* (Bonanno et al. 2017; Kumari et al. 2015 ). But monitoring the health of aquatic ecosystems relies mostly on examining the effectiveness of treatment performance of physicochemical magnitudes (Abou-Elela et al. 2014; Mekonnen et al. 2015; Mamine et al. 2019; Haddaji 2020). Despite the numerous studies devoted to the treatment of effluents by cattails, few works have been interested in testing The impact of environmental stressors at the morphological and anatomical levels.

The objective of this work is to evaluate the toxic stressing effect exerted by the pollutants of raw sewage on the anatomical markers of the leaves of the purifying macrophyte-cattails plants of the species *Typha latifolia* of the lake Burgas (Taoura) North-East Algeria, these macrophytes which is considered as a model in ecotoxicology and also it is a tool of biosurveillance in freshwater resources which can be used effectively as biological indicators while determining the environmental pressures. In addition, the physico-chemical parameters of this artificial wetland were determined to evaluate the effectiveness of wastewater treatment.

# Materials And Methods

## Presentation and description of the study area

Our experimental study was carried out at the fragile wetland, threatened by the urban extension of the city Taoura of the city of Souk Ahras (North-east Algerien) which is called lake Burgas (Fig. 1). Lake Burgas is a wetland built with open water surface (FW-CW), it is open water spaces that contain submerged plants or helophytes of the species *Typha latifolia*.

*Typha latifolia* are plants whose roots develop in a waterlogged substrate while their reproductive and vegetative system remains out of the water. Burgas Lake, located near Taoura, ancient Thagora during antiquity, is a commune of the city of Souk Ahras (North-east Algerian), located about 20 km south-east of Souk Ahras, and is the largest natural lake of the city. It is a preferred stopover for many species of birds during their migrations on the way (cijsa.blogspot.com). This lake is characterized by an arid to semi-arid climate. The average temperature varies between a minimum of 2°C and a maximum of 37°C.

Figure 2 provides a schematic explanation of how they work. In summary, as wastewater flows through the wetland, the wetland undergoes different physical, chemical, and biological processes that remove the pollution. The main processes include sedimentation, filtration, and microbiological degradation. They effectively remove ammonia, organisms, and suspended solids; nitrogen removal efficiency is also high, while phosphorus removal efficiency is low. These processes are used in the tertiary treatment of municipal wastewater and stormwater runoff.

## Water Sampling And Analysis Methods

The experiment was conducted for three months from April to June 2018. Throughout this study period, the functioning of the wetland was monitored by measuring physicochemical parameters. Samples were taken on average at the outlet of the planted filter (at a depth of about 50 cm). They were taken far from natural obstacles as recommended by Rodier (1996). These samples were subject to a measurement of in-situ parameters are the hydrogen potential (pH), temperature (T°C), electrical conductivity (EC), turbidity (Turb) and dissolved oxygen of water that were determined using a multi-parameter analyzer, and the parameters analyzed in the laboratory are the metal composition of discharge (Hg, Cd, Pb and Cr) which were performed by atomic absorption spectrometry (Rodier 2009).

## Botanical Material

In our work, we have chosen the rooted helophyte: the cattails of the species *Typha latifolia* taken from different places and collected from two areas one is from the polluted area of lake Burgase and the other from an unpolluted wetland (Fig. 3). The species *Typha latifolia* is perennial emergent macrophytes often dominating the upper littoral zone of eutrophic lakes and edging rivers in temperate and subtropical areas (Grsntved 1954). This specie produces up to 3 m high linear leaves and an extensive system of horizontal

rhizomes. Cattails can grow on organic, highly reduced sediments, as well as on acid sites with high concentrations of reduced metal ions in the interstitial water (Wenerick et al. 1989). The basis for treatment with these rooted helophytes is particularly based on the fact that:

- They are plants with horizontal and vertical rhizomes that provide support for the growth of bacteria and the filtration of particulate substances (Gesberg et al. 1986).
- The rhizome also provides, together with the roots, high soil permeability and a large soil-wastewater contact area (Radoux and Kemp 1988).
- They can concentrate heavy metals, absorb more nutrients than they need and neutralize extreme pH (Abbisy 2000 ; Hadad et al. 2006).
- They can transmit oxygen from the leaves through the stems into the rhizosphere (Brix 1993 ; Biddlestone et al. 1991).

## Realization of the anatomical sections and staining

We made the sections, at the level of the leaf structure, according to the manual technique, known as freehand. Then we proceeded to the staining of the subjects, according to the double staining technique (carmino-green of Mirande) according to the following steps (Zaffran 1998): - Using a razor blade, we cut the sections of organs studied, in several thin slices. Then we chose among them, the thinnest and most conforming ones.

- The slices obtained are put in a watch glass, containing bleach (to destroy the cellular content and preserve the pecto-cellulosic walls).
- Then, they are transferred to a second watch glass containing distilled water (elimination of excess bleach).
- Then, they are put in a 3rd watch glass in acetic acid (1%), for 2 minutes, to eliminate any trace of bleach and promote staining (cell mordant).
- The sections are then stained in a 4th watch glass containing kongo red (stains dead lignified tissue red) and carmine alum (stains living tissue, and develops the red color with cellulose) in equal parts.
- After staining, the samples are put in alcohol (70%) (replaces the water contained in the cells and thus preserves the sections obtained).

## Visualization And Photography

After putting the samples between the slide and coverslip, in a drop of glycerine, we proceeded to the visualization under the optical microscope. We used a OPPO A53 camera to take photographs of the histological sections at the same time.

## Statistical analysis

Data processing and analysis were performed using statistical data software: Statistica ® 8.0 (Statsoft: www.statsoft.com), The results are expressed as the mean ± standard deviation for each variable studied (physicochemical). The statistical application conditions were checked and respected according to the recommendations of Dagnelie (2013).

# Results And Discussion

## Physico-chemical characterization of burgas lake water

The detailed results of the physico-chemical analyses of the water of lake Burrgas under treatment of hydraulic functioning of the planted filter are grouped in the Table1.

Table 1
General statistics of the measured physico-chemical parameters

| variable physico-chemical | | min | max | moy | Ecart type(±) |
|---|---|---|---|---|---|
| parameters analyzed in the laboratory | Hg (ppm) | 0.001 | 0.060 | 0.015 | 0.01 |
| | Cd (ppm) | 0.004 | 0.07 | 0.02 | 0.02 |
| | Cr (ppm) | 0. 02 | 0. 06 | 0.04 | 0.01 |
| | Pb (ppm) | 0.001 | 0.005 | 0.003 | 0.056 |
| parameters measured in-situ | Turbidity (NTU) | 90,2 | 188 | 73.81 | 12.2 |
| | Oxygen dissolved ($O_2$) (mg/l) | 1.67 | 3.37 | 2.45 | 0.87 |
| | pH | 7.33 | 7.97 | 7 .69 | 1,18 |
| | T°C | 15.3 | 22.56 | 13.43 | 1.54 |
| | EC (µs/cm) | 1236.56 | 1563.23 | 1385.11 | 152.87 |

## Evolution of Temperature : T°C

The temperature of the water in the wetland is between 15.3°C and 22.6°C with an average of 13.43 ± 1.54°C (Table 1). These values are lower than the standard (30°C), considered a limit value for direct discharges to the environment (J.O.R.A 2006), and also an indicative limit value for wastewater used in irrigation (J.O.R.A 2012).

## Evolution Of Hydrogen Potential: Ph

The hydrogen potential is an indicator of pollution, it varies as a result of the nature of the effluents basic (cooking, washing ...) or acidic (acetic acids, chlorine derivatives ...) (Grara et al. 2017). The analysis of Table 1 shows that the waters of lake Burgase have a little slightly basic pH that oscillates between 7.33 to 7.97. These values recorded are close to those proposed by the Algerian standards of quality of wastewater intended for irrigation and are in the accepted interval (6.5–8.5) considered as the limit value of liquid effluent discharges into the natural environment (J.O.R.A 2006). Outside this range, the pH has adverse effects on aquatic life and blocks the self-purification processes, a decrease in pH can increase toxicity (Rodier 1996). These results make the biological treatment of raw wastewater very difficult given the conditions of the development of microorganisms (Khoufi et al. 2000). On the other hand, the consequence of the accumulation of $H^+$ ions following the activity of nitrifying bacteria, or the production of $H^+$ ions by the plant (Ndzomo et al. 1994) to compensate for the withdrawal of certain cations (mineral nutrition). On the other hand, the accumulation of $CO_2$ due to plant metabolism or the degradation of organic matter by heterotrophic bacteria (Attionu 1976; Sridhar and Sharma 1985; Jedicke et al. 1989) as well as the secretion of exudates (organic acids) from the plant roots (Bowes and Beer 1987).

## The Electrical Conductivity (Ec)

Allows us to evaluate the global mineralization and estimate the total soluble salts in water (Rodier 1984). The recorded conductivity values vary between 1236.56 µs/cm and 1563.23 µs/cm with an average of 1385.11 ± 152.87µs/cm (Table 1), these values highlight strong mineralization due mainly to the organic load (Chafai 1996). These values are located in the range of permissible values recommended by FAO (1985) which are in the order of 0-3000 µS/cm, and even they are lower than the Algerian standard for irrigation water which requires an electrical conductivity lower than 2000 µS/cm (J.O.R.A 2006). But the existence of planted filters of *Typha latifolia* plantations will be allowed a very strong reduction of this parameter with the increase of the residence time of wastewater (Mamine et al. 2019).

The evolution of the turbidity of water is due to the presence of finely divided suspended matter: clay, silt, silica grains, organic matter, etc. The assessment of the abundance of these materials measures their degree of turbidity (Thayer et al. 2007). The evolution during the study period of the turbidity of the lake Burgase waters is represented in Table 1. The turbidity of the lake Burgase water fluctuates enormously between a minimum of 1.19 NTU and a maximum of 8.5 NTU, it appears that lake Burgase receives more turbid effluents. This is related to the richness of the discharges collected by organic matter, nitrogen compounds, and finely divided suspended particles: clay, silts, silica grains, etc (Grara et al. 2017).

Work conducted by Kumari and Tripathi (2015); Mamine et al (2019); Khaldi et al(2019); Shah Vand Daverey (2020) and Mamine et al (2021) have shown that aquatic plants can adsorb, transfer and metabolize several types of organic and inorganic compounds. Preliminary studies by Roy and Hanninen (1994) have shown that the roots of rooted aquatic plants play a very important role in the removal of organic and inorganic compounds. According to Brix (1996), the stems and leaves of macrophytes

immersed in the open water layer provide an immense surface for biofilm formation. Indeed, the tissues of these plants are colonized by numerous communities of bacteria and protozoa. In addition, the roots and rhizomes that are buried in the soil provide a substrate for the growth of microorganisms.

## Evolution Of The Turbidity

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## Evolution Of Dissolved Oxygen

Dissolved oxygen ($O_2$) plays an important role in the maintenance of aquatic life in self-purification. Its presence in natural waters is determined mainly by the respiration of organisms, by the photosynthetic activity of the flora, by the oxidation and degradation of pollutants, and finally by the air-water exchanges. Dissolved oxygen is an important parameter to take into consideration, as it provides information on the state of the water and also promotes the growth of microorganisms that degrade organic matter. In general, low values of dissolved oxygen favor the development of pathogenic germs (Grara et al. 2017).The evolution during the study period of the dissolved oxygen levels of the Burgase Lake of the treatment plant is represented in Table 1. The waters of this lake present a significant concentration of dissolved oxygen which fluctuates between a minimum of 1.67g/l and a maximum of 3.37 g/l with an average of 2.45 ± 0.87g/l. The concentration of dissolved oxygen in the lake Burgase of the treatment line is superior in comparison to the standard quoted by the Official Journal of the Algerian Republic (J.O.R.A, 2006) and WHO (1989) which is about 5 mg/l. These waters change their infiltration through the planted filters. This oxygenation of the water is due mainly to two processes: the first is due to the intervention of oxygen from the air and the second mechanism is due to the additional oxygen supply from the roots.

## Evolution Of The Inorganic Load

After the analysis of heavy metals in the waters of the wetland of Lake Burgas, the results obtained show the presence of heavy metals (Hg, Cr, Co, and Cd) in low concentrations at the outlet of the waters of the wetland (Table 1). However, the process of phytoextraction will remain a minor factor in the abatement of inorganic pollutants given the short residence time that does not allow this process to extract these pollutants and concentrate them in the root and aerial parts (Amir et al .2020; Mamine et al .2021). The heavy metal contamination of aquatic ecosystems due to the discharge of urban and industrial effluents may pose a serious threat to human health (WHO 2013), in addition, these heavy metals (absorbed by vegetables enter the food chain and cause a phenomenon of bio-concentration at each passage in the upper trophic link (Xiong et al.2005).

## The effect of environmental pollution on the anatomical responses of *Typha latifolia*

Helophytes are relevant ecological indicators of metal pollution of the aquatic ecosystem through the use of biomarkers that constitute a natural biological tool that can signal the pollution of an environment as well as the bioavailability of toxic substances (Bonanno 2013; Gavrilescu et al 2015; Bonanno and Cirelli 2017). The use of the macrophyte *Typha latifolia*, as a model for studying the toxicity of pollutants in raw wastewater, finds a first argument, the semi-aquatic plant *Typha latifolia*, known for its ability to accumulate pollutants (Zn, Cu, Pb.change ...) at significant concentrations in its subterranean and aerial tissues (Amir et al. 2020 ; Mamine et al 2021). Examination of cross sections of control and macrophyte *Typha latifolia* leaves exposed to raw sewage first identified the control histological organization (Fig. 4). This shows that the leaf tissue of *Typha latifolia* is formed by two cuticles: one on the upper surface and the other on the lower surface. The cuticle of the leaf of *Typha latifolia*: is a very thin protective film covering the whole epidermis of the leaf. Then the microscopic observation of the epidermis which is a single layer of cells that are generally more elongated that covers the leaf presents dorsoventral anatomy: the upper (adaxial) and lower (abaxial) surfaces. The epidermal cells are closely related to each other and provide a boundary between the plant and the external environment (Fig. 4A-B).

We thus find epidermal structures found only on the abaxial face: the epistomatic stomata: These structures have only one stomatal type found, it is: anomocytic type, it is constituted by two guard cells, bean-shaped, are thicker on the internal side and framing an opening called ostiole, they are often accompanied by companion cells, lacking chloroplasts, with which they are intimately in contact by their external face (Fig. 4A; Fig. 5). We observe then, between the inferior and superior epidermis, a tissue is composed the biggest part of the interior of a leaf called the mesophyll (Fig. 4A**).** This tissue is non-homogeneous, it consists of two parts:

Towards the upper leaf surface: the chlorophyllous palisade parenchyma is located under the epidermis, it is formed by a layer of elongated cells and joined to each other, without meats. The cells located on the side of the upper leaf surface have a large number of chloroplasts. (Fig. 4C).

- Towards the lower leaf surface: lacunar (spongy) chlorophyll parenchyma: more or less rounded cells, characterized by a reduced number of chloroplasts, between which are large lacunae.
- However, the inner leaf tissue of monocots is characterized by parallel veins reinforced with bundles of fibers made of elongated sclerenchyma cells giving a high tensile strength. The veins are formed by conducting bundles: xylem and phloem. The xylem is usually found on the upper side of the veins and the phloem on the lower side. The veins are surrounded by a set of cells called the fascicular sheath, around which are the mesophyll cells.

Microscopic observation of *Typha latifolia* leaves exposed to raw sewage toxicity underwent some anatomical alterations of the leaves compared to that of *Typha latifolia* leaves of the control site, such as the presence of structural alterations of irregular shapes of the cells of the spongy parenchyma tissue (Fig. 6).

These results would testify to the cytotoxic power of heavy metals present in raw wastewater (Cr, Hg, Cd, and Pb), these compounds cause oxidative damage by the destruction of membrane lipids. The works of Amir et al (2020); Mamine et al 2021 reported that these hyperaccumulating and tolerant plants to heavy metals are exploited for their bioremediation property. Our histological study also revealed some changes in the conductive vessels such as shrinkage of the cell size of the conductive vessels and slight deformations detected on the xylem in the stressed leaves compared to the control leaves. This is probably related to an initial biological response due to the stress of heavy metals found in raw wastewater during the hydraulic operation of these planted systems. According to Iqbal et al (2020), the anatomical changes in the xylem vessel elements of *Typha latifolia* leaves may be due to the deposition of wall materials. This may be a possible strategy to restrict water flow to overcome the rapid mobility and transport of the amount of metals to protect the excess cellular components from the unpleasant consequences of metals. The work of Kong et al.(2020) demonstrated increased Cd concentration at the xylem level of hydroponically grown Salix matsudana Koidz in response to increased production of phytochelatins (e.g., proline) that form Cd chelates and help prevent toxic morphological impacts caused by Cd-induced ROS. Heavy metals can pose a threat to aquatic ecosystem health. Unlike organic chemicals, heavy metals cannot be removed by natural processes such as degradation into less toxic compounds (Nguyen et al. 2021). This creates unique challenges for their removal from water. The use of cattail macrophytes (*Typha latifolia*) in phytoremediation of aquatic ecosystems represents a unique tool to remediate Burgase lake, as they can accumulate heavy metals from contaminated water via roots (Mamine et al. 2021) and their transfer to rhizomes and aerial parts (stems, leaves) (Ben Salem et al. 2017). Cattails of the species *Typha latifolia* may play in mitigating metal toxicity in the aquatic environment.

## The Para-dermal Sections Of The Leaves

Regarding the peridermic sections of the leaves of the polluted site, the same epidermal structures are found only on the abaxial face: the epistomatic stomata: These structures have only one stomatal type

found, it is the anomocytic type (Fig. 4A and Fig. 5).

Because of the results found, no sign of a variety of anatomical features of the stomata was detected, this can be explained, by the stomatal characteristics of the leaves of the cattail aquatic macrophytes which are the result of an adaptation to the acquisition of carbon in the ambient environment. But we reported behavioral changes by the closure of most stomata compared to controls (Fig. 7), this means that the macrophyte *Typha latifolia* tolerates the presence of heavy metals by controlling its losses, this effect may result from a stomatal regulation (Brunet 2008). Stomata are epidermal cells specialized for $CO_2$ absorption, therefore they release $O_2$ and water. These structures play an important role in photosynthesis (Castro et al. 2009).

Doheny-Adams et al. 2012 associated this structural change in stomata with genetic and environmental changes. Work by Brodribb et al. (2017) showed plants exhibit a degree of homeostasis in leaf water content to protect against damage to photosynthetic and xylem tissues and to maintain an efficient allocation of resources. This is achieved by strong coordination between the systems regulating water delivery (xylem) and water loss (stomata).

# Conclusion

In conclusion, macrophytes in the wetland of lake Burgas subjected to municipal wastewater contamination create the first biological response due to heavy metal stress during hydraulic functioning. The impact of the environmental stressor was found to be manifested at the morphological and anatomical level and was identified by anatomical changes such as irregular shapes of the cells of the spongy parenchyma tissue and shrinkage of the size of the conducting vessels and slight deformations were detected on xylem, Concerning peridermic sections no signs of variations in anatomical features of the stomata detected, compared to the plants of the control site. It is hypothesized that the morphological and anatomical changes identified to contribute to the tolerance to heavy metal stress of plants when exposed to anthropogenic pollution. Therefore, cattails can be effectively used as biological indicators while determining environmental pressures. This study is important as a reference for future monitoring studies. Further studies on the bioavailability of metals to other aquatic macrophytes are recommended.

# Declarations

**Ethical Approval:** All authors : [**Nedjma Mamine**] ; [**Nedjoud Grara**] and [**Fadila Khaldi**] approved Ethical Approval and the rules of good scientific practice.

**Consent to Participate** (all authors : [**Nedjma Mamine**] ; [**Nedjoud Grara**] and [**Fadila Khaldi**] approved the version to be Participated)

**Consent to Publish** (all authors : [**Nedjma Mamine**]; [**Nedjoud Grara**] and [**Fadila Khaldi**] approved the version to be published )

-**Authors Contributions** :

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [**Nedjoud Grara**] and [**Fadila Khaldi**]. The first draft of the manuscript was written by [**Nedjma Mamine**] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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-**Competing Interests** :

- all authors : [**Nedjma Mamine**] ; [
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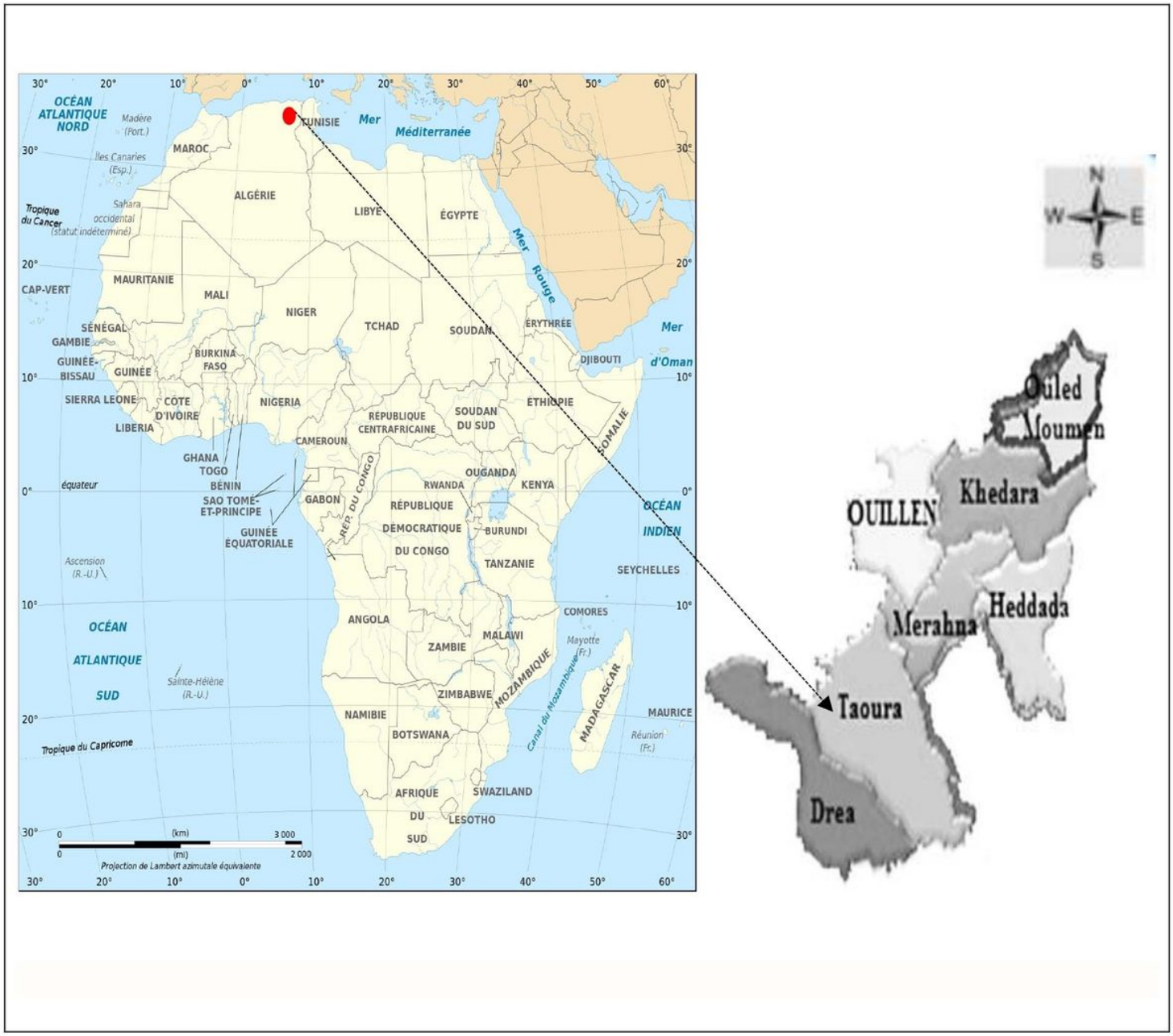
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# Figures




## Figure 1

The geographical location of the study area: Bourgas lake (Taoura city of Souk Ahras) North-eastern Algeria

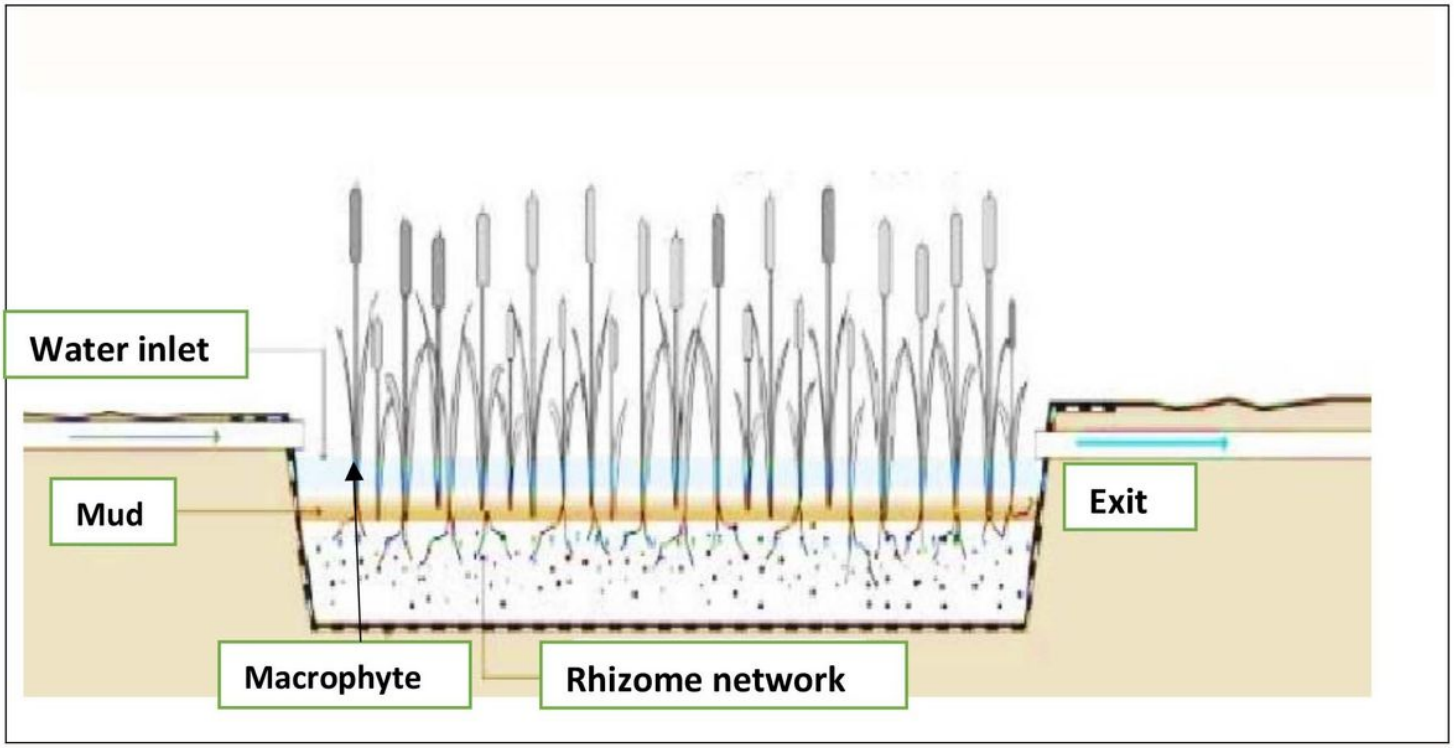



**Figure 2**

Diagram of operation constructed wetlands with open water surface (FW-CW)

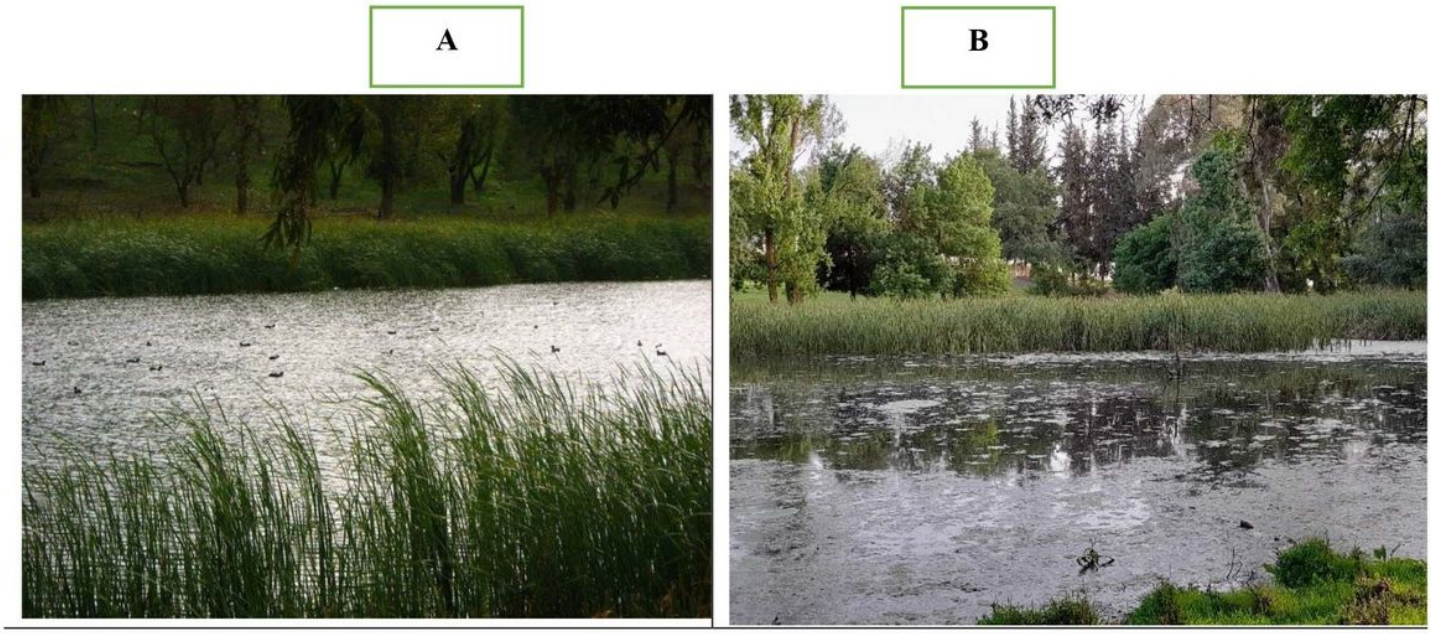
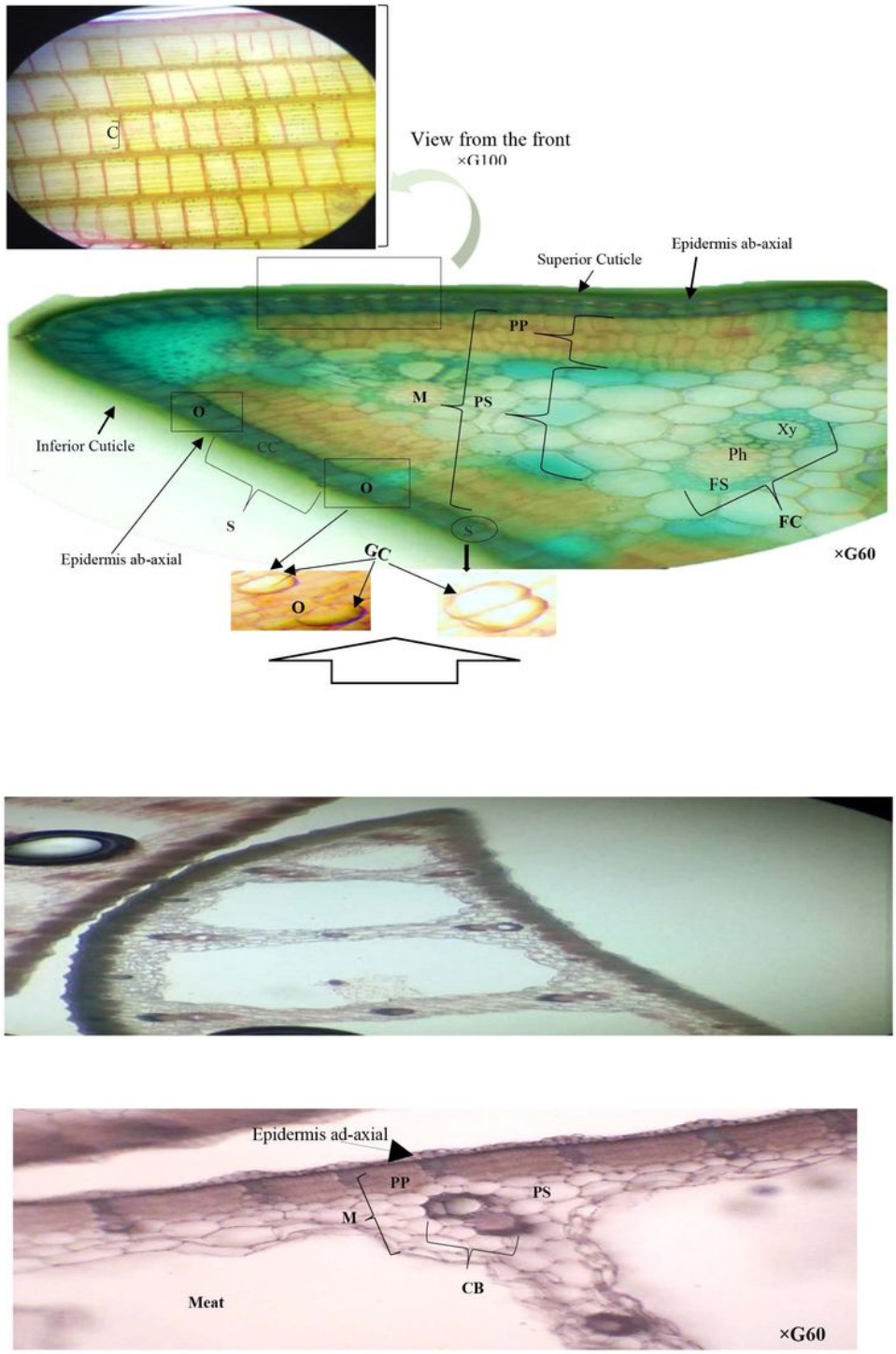



**Figure 3**

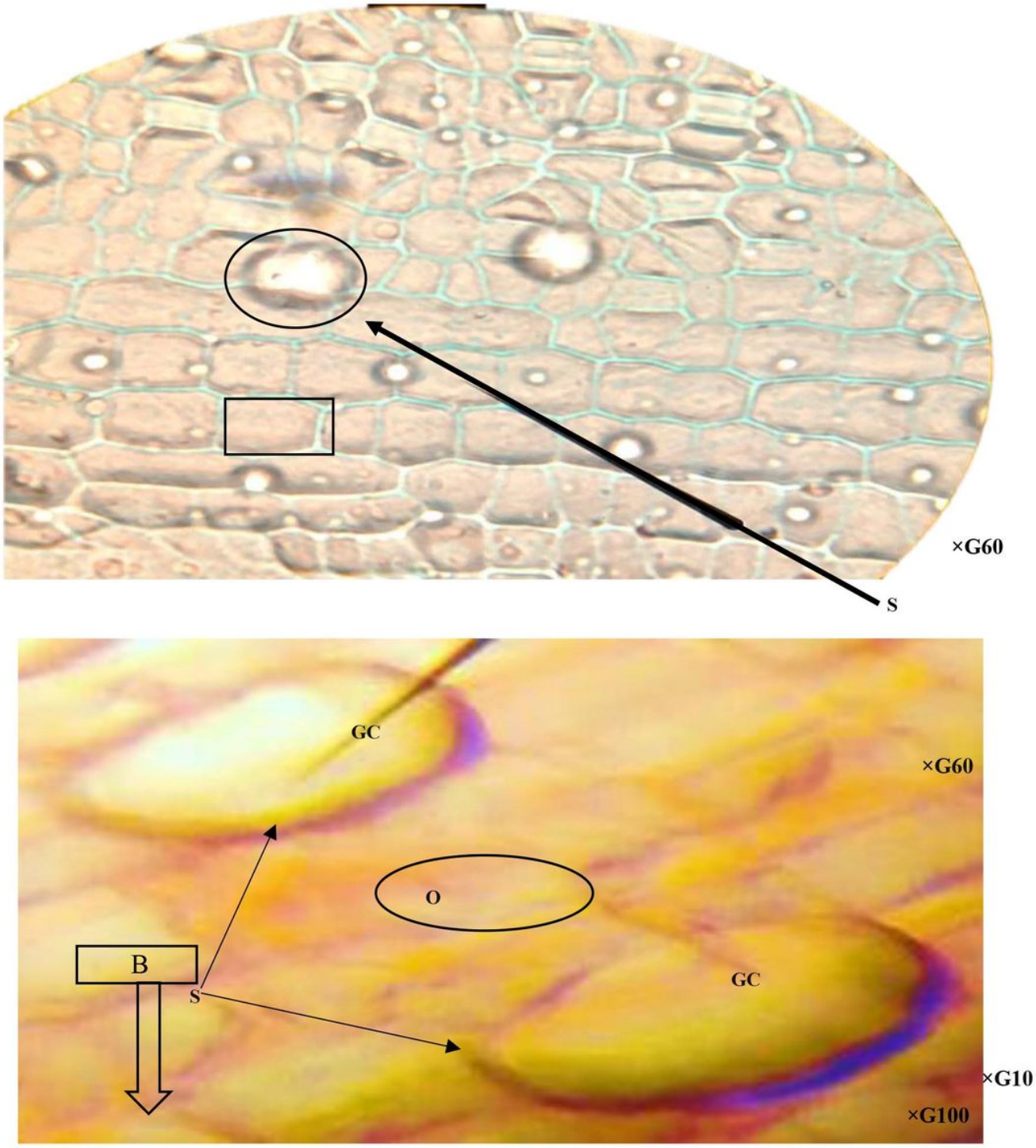
Location of the collection site the cattails *Typha latifolia* collected from the region of taoura (City of Souk Ahras) North-East of Algeria (A: unpolluted area, B: polluted area)




## Figure 4

Paradermal sections of *Typha latifolia* leaves (Witness)

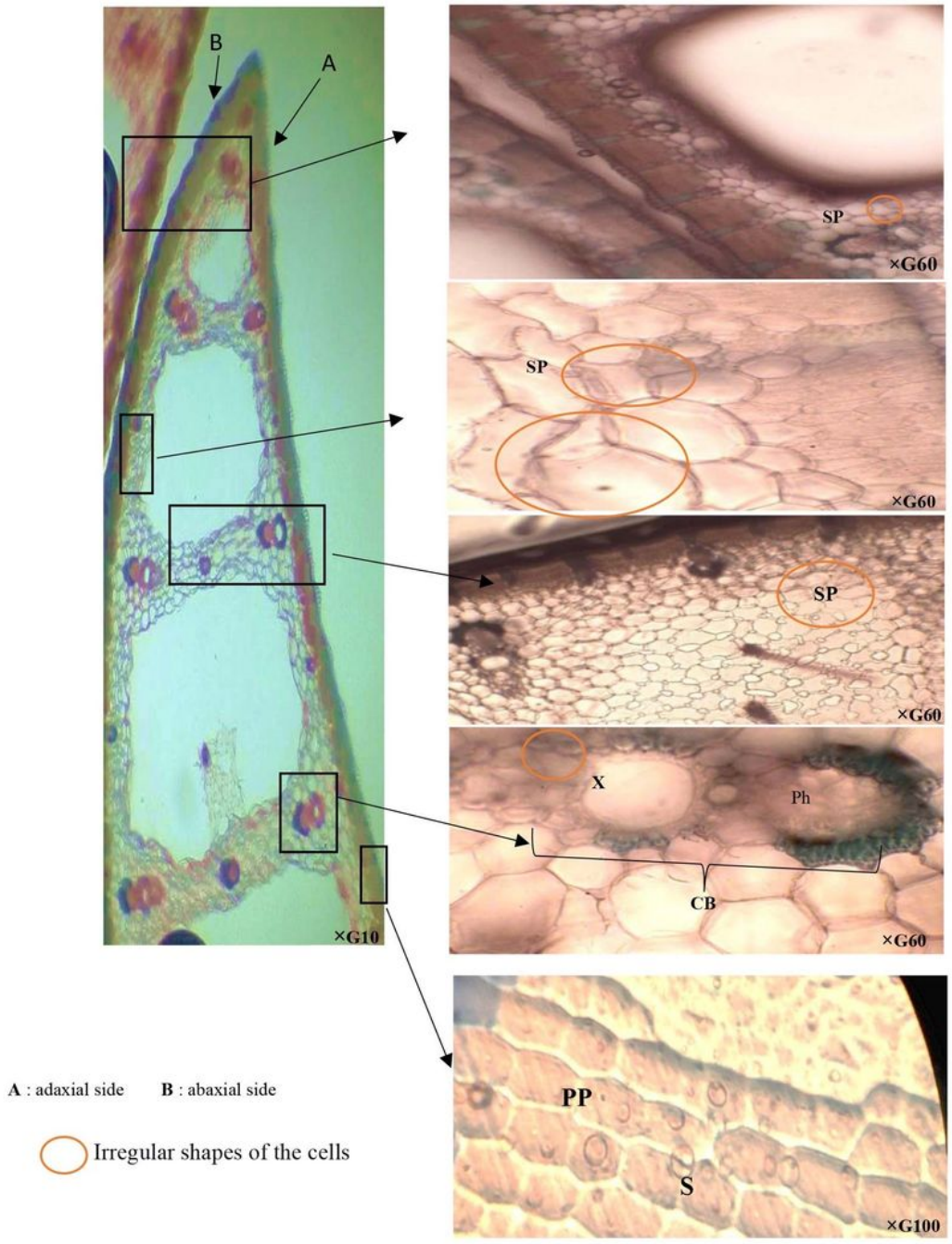
**C** : Chloroplasts ; **CB** : Conducting Bundles ; **CC** : Companion Cells ; **FS** : Fascicular Sheath ; **GC** : Guard Cell ; **IC** : Inferior Cuticle ; **M** : Mesophyll ; **Me** : Meat ; **SC** : Superior Cuticle ; **S** : Stomata; **SP** : Spongy Parenchyma ; **PP** : Palisade Parenchyma ; **O** : Ostiole ; **P** : Phloem; **X** : Xylem




## Figure 5

Stomatal characteristics of *Typha latifolia* leavs (Witness)

**GC**: Guard Cell; **S** : Stomata; **O**: Ostiole




## Figure 6

Paradermal sections of *Typha latifolia* leaves (Under wastewater stress)