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TOXICOLOGICAL IMPACT ASSESSEMENT OF CADMIUM ON AQUATIC MACROPHYTE: *ELODEA CANADENSIS*

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ABSTRACT: *Elodea canadensis* is a submersed macrophytes, widely distributed in Aquatic environment and able to remove heavy metals from water. This study examines the Cd toxicity on *Elodea canadensis*.The aim of this research was to study damaging effects of prolonged cadmium exposure on leaves of *Elodea canadensis* treated for 7 and 14 days with different concentrations of cadmium (0, 10, 20, 40, 80, 160 µM).These effects have been studied by measuring the plant growth, photosynthetic activities and the stress effects in response to cadmium exposure were also investigated by evaluating the levels of some biomarkers (GSH and GST activity).The results obtained show a dose-related inhibition of the growth and decrease of photosynthetic activities of *Elodea canadensis* compared to the control. We noted also a significant and dose-related increase in GST activity associated with decrease of GSH level .To deal with the cadmium induced oxidative stress, *Elodea canadensis* activated antioxidant enzymes to diminish the effects of Reactive Oxygen Species (ROS).

Keywords: *Elodea Canadensis*, cadmium, Respiratory metabolism, photosynthesis, biomarkers.

INTRODUCTION:

Heavy metals are widely released from human activities, such as agriculture and industries, and considerate high toxic for environment and all living organism, human health included. Effectively, heavy metals are easily transported and accumulated in environment, causing air, ground and water pollutions and the contamination of various organisms (Aksoy *et al.,* 2005).

Cadmium (Cd) is a bivalent, non-essential and highly toxic heavy metal and one of the most studies ones with a well-known phytotoxicity. Indeed, numerous works have indicated that Cd causes nutrient deficiency in plants (Zoghlami *et al*., 2006 ; López-Millán *et al*., 2009) and induces inhibition of chlorophyll biosynthesis and a decline in the photosynthetic rate (Tukaj *et al*., 2007 ; López-Millán *et al*., 2009). In addition, Cd greatly disturbed the cell wall organization and affected membrane-located activities, as ATP production (Dorta *et al*., 2003) and ion uptake (Das *et al*., 1997). Cd toxicity, also, enhanced oxidative stress by increased levels of reactive oxygen species (ROS) (Sharma *et al*.,2009).

Submerged aquatic plants are known to accumulate metals from their environment and affect metal fluxes through those ecosystems (Singh *et al.,* 2011). *Elodea canadensis* is a submerged freshwater macrophyte, which absorbs mineral elements through its wide leaf surfaces directly from the aquatic medium**.** Various studies have demonstrated the potential use of this specie to reduce organic matter or remove heavy metals from polluted water (Dunbabin *et al*., 1992 ; Eugelink ,1998**,** Mishra *et al*., 2009).The Cadmium accumulation in these plants can caused various physiological and biochemical modifications. By this fact, and the aim of this study is the possible use of these changes to evaluate the response of *Elodea canadensis* to cadmium exposure and to understand its mechanisms of resistance and/or adaptations to heavy metal stress, by following the respiratory and photosynthesis metabolism and some stress biomarkers (GSH, GST).

MATERIALS AND METHODS:

Biological material

The biological material chosen for our investigation is an aquatic plant *Elodea Canadensis,* these submerged macrophytes was used as model system to study toxic effects and biological responses upon exposure to cadmium perturbations.

Chemical material

Cadmium (Cd) is a bivalent, non-essential and highly toxic heavy metal, whose concentration in air, soil and waters of the earth is continuously increasing due to industrial and urban activities and agricultural practices. One of the most toxic metals is a persistent contaminant that accumulates in the environment. Large amounts of this metal are released annually in various environmental compartments and may pose a significant threat to the ecosystem (Pacyna *et al*., 1995).

Treatment

Plants of *Elode a Canadensis* are washed with distilled water and cultured in nutriment solution medium, ph = 7.4 (containing 67.32 mg/l de Ca2+ , 10.08mg/l Mg2+, 4.82mg/l K+, 11.96 mg/l Na2+, 27.36 mg/l SO_4^{2+}, 20.82 mg/l Cl^-, 216.07 mg/l HCO3, 2.5 mg/l NO3, 1.00 mg/l NO-2, 2.33 mg/l Si2 and 1000 ml of distilled water) used as control.

In the same medium and after few days of acclimatization to the laboratory condition, the addition of different cadmium concentration (0, 10, 20, 40, 80, 100 µM) was applied for 7 and 14 days of treatment.

Measurement of Biochemical and Enzymatic Parameters

After treatment of submerged macrophytes, the leaves are collected for analysis of various parameters as respiratory metabolism, photosynthesis and some stress biomarkers (GSH, GST).

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Determination of Glutathione (GSH)

The glutathione was assayed by the method of Weckberker and Cory, 1988, based on measuring the absorbance of the 2-nitro-5 mercapturic resulting from the reduction of the acid 5-5 ′thiol-bis- 2-nitrobenzoic acid (DTNB) by the thiol groups (-SH) glutathione.

Determination of activity Glutathione S-transferase (GST)

The glutathione S-transferase activity is performed by the method of habig *et al.*, 1974. It is based on the conjugation reaction between GST and a substrate, CDNB (1-Chloro-2,4-dinitrobenzene) in the presence of a cofactor: glutathione (GSH). This activity is measured at a wavelength of 340nm by a spectrophotometer visible/UV (Jenway 63000).

Study of Respiratory and photosynthetic metabolism

The apparatus used is an oxygen electrode, HANSATECH type, wich allows the measurement of the production or consumption of oxygen.
The intensity of photosynthesis of *Elodia Canadensis* is measured by the oxygen electrode as for the respiration rate when the sample is hidden by a black box to speed up the metabolic process (Djebar *et al* 2000).

Statistical anlalysis

The statistical analysis is performed by the student "t" test that compares the averages of two populations using data from two independent samples, conducted using a data analysis software: Minitab (version 16.0) (Dagnelie, *et al.*, 1999)

RESULTS AND DISCUSSION:

Glutathione variations (GSH)

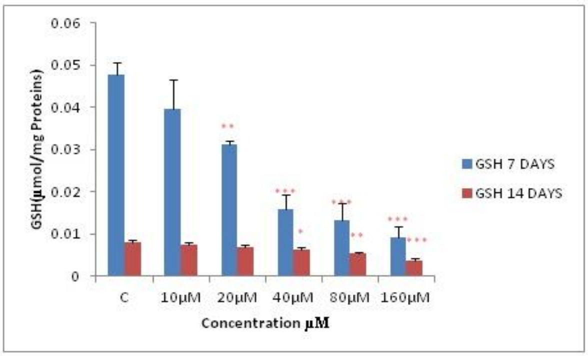

Fig 1. Evolution of GSH level as a function of times and different cadmium concentrations on Elodea Canadensis after 7 and 14 days of treatment.

Figure 01 illustrates the effects of the various concentrations of Cadmium on the GSH rate after 7 and 14 days of treatment. Indeed, the level of GSH decreased from (0.00478 µM/mg of protein) in control to (0.093µM/mg of protein) in cells treated by the strongest concentration of our molecule for the 7 days treatment, and from (0.008µM/mg of protein) in control to (0.003µM/mg of protein) in cells treated by strongest concentration (160µM) of our molecule for 14 days treatment.

GSH is considerate as one of the most frequently used indicators of stress biomarkers preventing damage to important cellular components caused by reactive oxygen species and free radicals (Pompella *et al.*, 2003). Statistical analysis revealed significant differences in the rate of GSH between the control and our 5 concentrations used for 7 and 14 days of treatment.

Glutathione S-transferase activity (GST)

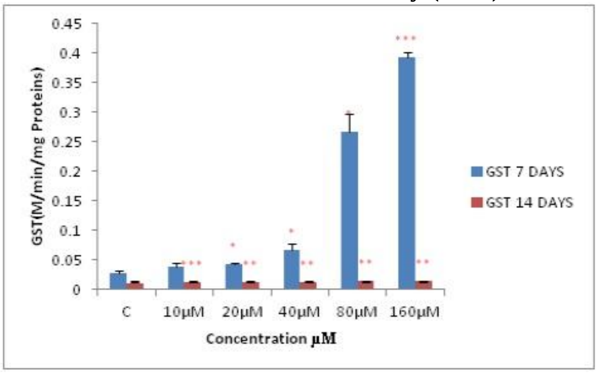

Fig 2. Evolution of glutathione S-transferase activity as a function of different cadmium concentrations on *E. canadensis* after 7 and 14 days of treatment.

The Figure 2 show a dose-dependent increase of GST activity on the *E. Canadensis* treated with different Cadmium concentrations compared to the control after 7 and 14 days of treatment.
The GST increase from (0.0027µM/mg of protein) in the control to (0.393µM/mg of protein) in the *E.Canadensis* treated with the cadmium concentrations of 160µM.

After 14 days of treatment, the GST activity on the *E.Canadensis* was increased by the treatment with the different cadmium concentration. The GST increase from (0.011µM/mg of protein) in the control to (0.015µM/mg of protein) with the strongest concentrations of our molecule.

GST plays a vital role in detoxification response during oxidative stress, provides a first line of defense after toxic heavy metal insult, and catalyzes the conjugation of the reduced form of GSH to xenobiotic substrates for their elimination (Cherait *et al.*, 2014).
Statistical analysis revealed significant differences in the rate of GST between the control and our 5 concentrations used for 7 and 14 days of treatment.

Effects of cadmium on the respiratory metabolism

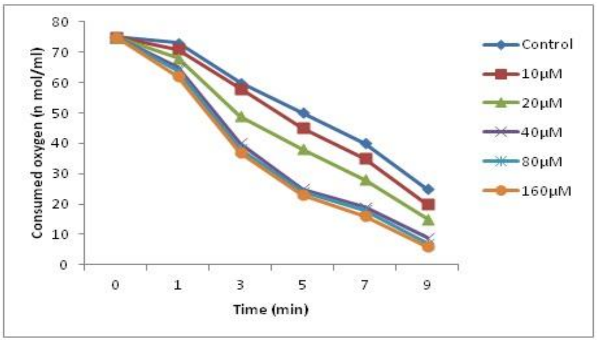

Fig 3. Evolution of oxygen consumption as a function of different cadmium concentrations on *E. canadensis* after 7 days of treatment.

The figure 3 represents the evolution of oxygen consumption after 7 days of cadmium treatment of *E.Canadensis*. The results obtained indicate a dose-dependent decrease on the all *E.Canadensis* treated with different cadmium concentration compared to the control.

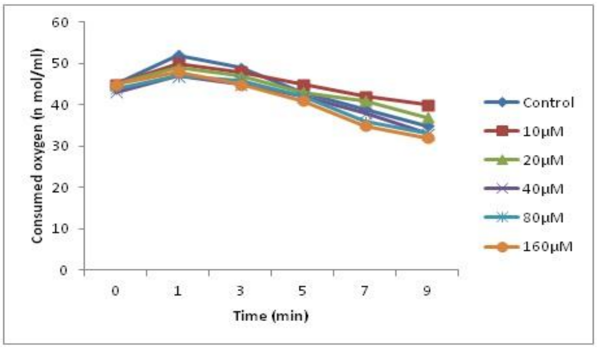

Fig 4. Evolution of oxygen consumption as a function of different cadmium concentrations on *E. canadensis* after 14 days of treatment.

The figure 4 represents the evolution of oxygen consumption after 14 days of cadmium treatment. The results indicated the perturbation on the all *E.Canadensis* treated with different cadmium concentration compared to the control.

Effects of cadmium on the photosynthesic activity

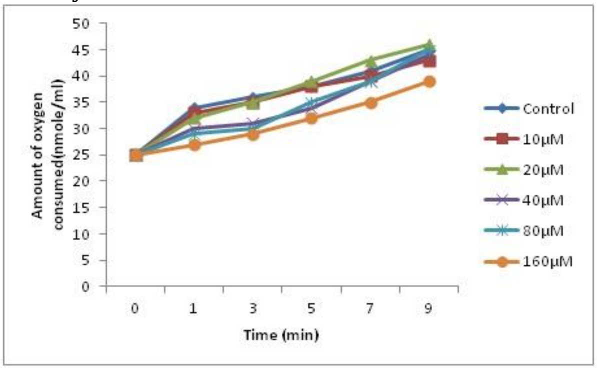

Fig 5. Evolution of photosynthetic activity as a function of different cadmium concentrations on *E. canadensis* after 7 days of treatment.

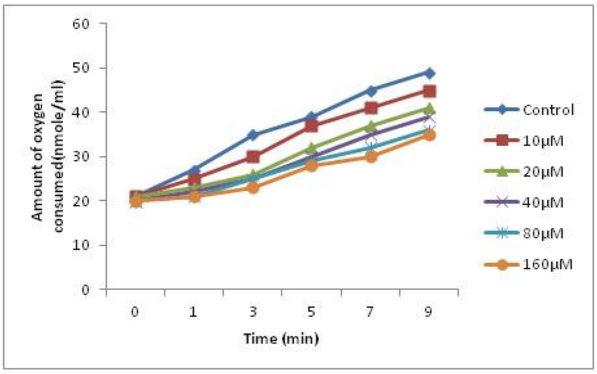

Fig 6. Evolution of photosynthetic activity as a function of different cadmium concentrations on *E. canadensis* after 14 days of treatment.

The figure 5 and 6 below shows a marked increase in the amount of oxygen produced in the middle, dice the second minute of recording for all the concentrations. The maximum amount of oxygen produced is recorded at 9min for the control with (45 and 49 nmol O2/ml).

DISCUSSION:

Cadmium (Cd) is a non-essential and toxic element, without any metabolic significance. Cd2+ ions are known to affect the structure and function of chloroplasts in many plant systems such as *Triticum aestivum* (Neelima,1991 ; Loggini,1999; and Prasad, 1995). The primary site of action by any heavy metal has been reported to be the photosynthetic pigments especially the biosynthesis of chlorophyll and carotenoids (Baszynski 1980 and Prasad, 1995). Cd inhibiting chlorophyll biosynthesis and the proper development of chloroplast structure have been reported in *Pennisetum typhoideum* (Prasad, 1987) and *T. aestivum* (Malik, 1992).

The treatment with cadmium significantly affects the growth of plants "*Elodea candensis*", similar in vitro studies show that cadmium tends to significantly affect the growth parameters (Chugh *et al*., 1995; *Herrieche, 2004).*Treatment with cadmium at low concentrations appears to positively affect all the morphological parameters of *elodea* plants. Cadmium at low concentrations rather seems to activate some metabolic processes involved in the growth and development of *E. canadensis*.

Respiratory metabolism, we found that the treatment of *Elodea* plants greatly reduced respiratory activity, this inhibition is firstly due to the presence of ROS, which are known as disruptive as well as elements of the respiratory metabolism of photosynthesis (Kiss *et al., 2003*; Kuciel *et al*.,2004) and secondly it could be explained by the rapid passage of cadmium in roots. Indeed , it has been shown that the roots of some durum voltage dependent calcium channels in the plasma membrane, known as the appellation (RCA), are permeable to cadmium , and those in the absence of Ca++ (White, *2000).*

This rapid penetration of cadmium causes a strong disturbance of the respiratory chain specifically at the site responsible for substrate oxidation from the Krebs cycle. Cadmium can thus inhibit certain enzymes dehydrogenases and NADPH-dependent. While the photosynthetic metabolism following fluctuations in function of the different concentrations of the metal. At low concentrations of cadmium, photosynthesis remains active but still smaller than the control in contrast to high concentrations where it is inhibited. Our results are consistent with those obtained by (Israr *et al.*, 2006).

The use of these modifications for the evaluation of toxicity of these polluants was largely discussed in various organisms (Prasad *et al*., 2001; Perry *et al*., 2002*;* Dhir *et al*., 2004; Pavlĭková *et al*., 2008). Indeed, this species was shown to accumulate heavy metals such as Cd, Se, and Cu in their tissues (Thiébaut *et al*., 2010; Qian *et al.,* 1999). These plants were therefore used for the evaluation and monitoring of metals in water (Wahaab et *al., 1995;* Kähkönen *et al., 1999;* Cardwell *et al*., 2002). In addition, aquatic plants could be used in phytoremediation to reduce organic matter or remove metallic pollutants from water. (Dunbabin et al.,1992; Mishra *et al*., 2009). Some of this research was conducted using *Elodea* sp. (Miretzky *et al*., 2004; Olette *et al*., 2008).

The induction of detoxifying enzymes of plants under stress conditions is often reported (Nimptsch, *et al*., 2007). Plant cells are able to protect their lives through the use of enzyme mechanisms GST (Apel *et al*.,2004). The treatment with different concentrations of cadmium induce a high activity of GST, this could be explained by the entry of xenobiotics in plant cells (*Elodea Canadensis*) and induction of detoxification system (Lagadic *et al*., 1997).

Our results agree with those reported by dazy *et al*.,2009, where they found maximal activities of GST and significant decrease of GSH in samples from polluted area.

Glutathione plays a central role in the process of intracellular defense and exists in two forms, oxidized GSSG and reduced GSH. GSH deficiency exposes the cell to a risk of oxidative damage (Sies,1991), through its ability to bind to heavy metal ions (Adam *et al*.,2005). The glutathione-enzymes include glutathione peroxidase (GSH-Px) and glutathione S-transferase (GST) involved in the detoxification reaction intermediates and oxygen radicals (Yu, 1994).The level of GSH decreased in cells treated by different concentration of cadmium. Several studies confirm the results and help to better explain the relationship between the decrease in GSH and an increase in GST activity.

CONCLUSION:

In conclusion, our results showed that cadmium is toxic in concentration dependent manner to the submersed macrophytes *Elodea canadensis*. The physiological and biochemical process in plants was significantly affected by stress of cadmium. To deal with the cadmium induced oxidative stress, *Elodea canadensis* adopt a defense strategies by activation of some stress biomarkers (GSH and GST activity) to diminish the effects of reactive oxygen species.

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