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The Effect Of Environmental Factors Of Antioxidant Enzyme Responses Of Phragmites Australis (Cav.) In Bahr Al- Najef Depression Reservoir –Iraq

Methak Abd Muslim Guda1 ,T. K. Merza2 ,Nihad .H.Mutlag3 ,Mohammed J. S. Al-Haidarey

Abstract— Reported in this paper responses of antioxidant. The catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) and peroxidase activity (POD) of Phragmites australis as a scavenger of reactive oxygen species (ROS) for mitigating the environmental conditions. The study was deal with monthly study of eighteen physico-chemical parameters (precipitation, air and water temperature, Turbidity, EC, SAL, TDS, TSS, pH, DO, Total hardness, $HCO_3^{=}$, CO_3^{-} , Cl-, $SO_4^{=}$, BOD, $PO_4^{=}$ and $NO_3^{=}$) during the period from February - June 2015. Four sites were selected in Baher Al-Najef Depression Reservoir (BNDR) at the middle of Iraq. During the course of sampling the physico-chemical factors were found significantly higher in the second site compared to the others, in spring higher pollutants concentration than in winter season, this led to high activity of antioxidant enzymes. Present experiment indicated that in a stressed ecosystem, the Phragmites australis plants overcome the stress by altering their stress enzyme activities, hence suggesting an evidence of adaptive mechanism to thrive in a stressful environment and suitability of *Phragmites australis* for environmental matrices(as indicators), especially under altered climatic conditions

Index Terms— *Phragmites australis* , Environmental stress , Antioxidant enzyme, catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) and peroxidase activity (POD).

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1 INTRODUCTION

The genus Phragmites is one of family Poaceae which comprises the most common perennial, rhizomatous, stoloniferous and tall grasses (2.0–6.0 m) of the temperate and tropical wetlands all over the world. Although the transitional zones (ecotones) of rivers, big lakes and wetlands are the most preferred habitats of the common reed (abdel and al-rawi. 2011). Previous studies show that *Phragmites australis* grows in soils of different ph, salinity, fertility and textures, and attains high productivity under different climatic conditions (uddin et. Al., 2012; al-haidarey, 2002). The genus *Phragmites* has proven ability to mitigate the environmental pollution of its surroundings. Common reed (*phragmites australis* (cav.) Trin.), has been a most preferred unique plant system, where environmental stress is the most limiting factor (uddin et. Al., 2014). To survive the stress, numerous physiological and biochemical changes occur in various plants species (xiong et. Al., 2006). The reactions of the plants to water stress factor differ significantly at various organizational levels depending on intensity and duration of stress as well as plant species and its stage of development (venkatesan and sridevi 2009; chaves et. Al., 2003). Under environmental stress plants resort to many adaptive strategies in response to different abiotic stresses such as high nutrient (aslam et. Al., 2011), low redox potential of sediment (ni, 2001a,b,c), toxic substances, high salt, cold, heat and excessive osmotic pressure, which ultimately affect plant growth , productivity (cao et. Al., 2004). Plant cells and tissues show numerous metabolic responses to environmental stress, some of which may have adaptive significance) srivastava, 2008). The level of reactive oxygen species (ros) in plants is controlled by synchronous action of enzymatic and non enzymatic antioxidants. Among this enzymatic .antioxidants are catalase (cat), ascorbate peroxidase (apx), superoxide dismutase (sod) and peroxidase activity (pod) which are important scavengers of ros (chai and wong, 2012). The physiological effects of environmental stress on this plant have not been well studied (jatin et. Al. ,2014). Baher Al-Najaf depression also have not been well studied too. In the present study, A sub-acute experiment was conducted to examine the combined effects of Environmental stresses on *Phragmites australis* by testing antioxidants defense system of the plant to identify their potential role as biomarkers. To this aim we tested the following hypotheses :

1.Some physical and chemical characters of Baher Al-Najaf water.

2.The climate factors in Baher Al-Najaf.

3.The combined effects of Environmental stresses on Phragmites australis by testing antioxidants defense system of the plant in Baher Al-Najaf depression .

2 PROCEDURE FOR PAPER SUBMISSION

2-1Study site Description:
BNDR is located in south-east of the province of Najaf (figure:1). It is distanced from Euphrates river about fifteen kilometers with a width about (16 km). However, it

extends for 40 km from northwest of Najaf city to the south west of the of Herra city, it narrows 10 km at the middle of depression, while it bounded on the east at the road of Mishkhab -Najaf, and on the west line that connects to the strategic Iraqi oil line from the south to the north-west of Najaf province (Al-Haidarey *et. al*., 2016, Al-Fartusi, 2007).
Four sites were chosen for investigation this study .
1- Site(1) contains various chemicals from the feeder southern tributary (Detergent from households and pesticides from agricultural additions) .
2- Site (2) contains different chemicals from industry and composite municipal waste water .
3- Site(3) water has allot off amount of salts from ground wells.
4- Site(4)is located near the strategic oil line.

2-2 Physiochemical parameters: Sub-surface water samples were collected monthly (February - June / 2015), air-water temperature(C◦), pH, Electrical Conductivity (EC) , Salinity and Dissolved Oxygen(DO), were measured by using portable Multimeter (WTW-350i), turbidity by using a turb-meter (WTW-Turb-550), biochemical oxygen demand (BOD), total suspended solids (TSS), total hardness (TH), chlorides(Cl^{-1}), sulfate (SO_4^{-2}), carbonates(CO_3^{-3}), bicarbonates (HCO_3^{-2}), nitrate(NO_3^{-2}), phosphate (PO_4^{-2}) using adopted methods of American Public Health Association (APHA,2003) .The climatological features of the sites included mean annual precipitation (mm/m2), mean annual temperature (C◦) using portable weather station JRI.

2-3 Plant material: Plant materials were harvested twice time from sites of study . The samples were collected during winter and spring in ice bucket in the shortest possible time in order brought to the laboratory. Plant samples were cleaned with tap water then distilled water, fresh plant material (fully expanded and undamaged leaves) was separated from whole plants and kept in cool place -20C◦.
Leaf samples (0.5 g) were homogenized in ice cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 ml EDTA with prechilled pestle and mortar. The homogenate was transferred to centrifuge tubes and was centrifuged at 4C◦ in Beckman refrigerated centrifuge with 15000 r.p.m. for 15 min. The supernatant was transferred to tubes of 30 ml and referred to enzyme extract (Esfandiari et. al., 2007).

2-4 Enzyme assays: Superoxide Dismutase (SOD) activity determination according to Marklund and Marklund (1974), catalase(CAT) activity was measured according to Beer and Sizer (1952), ascorbate peroxidase (APX) activity was determined according to Nakano and Asada (1981), and peroxidase (POD) activity was estimated according to Hemeda and Klein (1990) .

2-5 Statistical analysis :Analysis of variance was performed using SAS version 9.1(SAS Institute Inc,. Cary, NC, USA). The data were presented as the means for each treatment. Means were compared using the LSD test at the 0.05 probability level.

3-Results and Discussion

The nature and extent of physico-chemical stress on the Phragmites australis macrophytes, eighteen parameters of water and climate were compared among the four stations. The spatial and temporal changes of the physico-chemical parameters of BNDR water are illustrated in Table (1). Households detergents and agricultural pesticides were runoff from the feeder southern tributary to Site-1 contains various chemicals. The composite municipal waste water used in Site 2, also contains different chemicals from industry.Site-3 water has a lot of amount of salts from ground wells .site- 4 is located near the strategic oil line, thus the trend Site 2 > Site 3 > Site 4 > Site 1 , were found for all physio-chemical parameters (Kad ̓im, 2015; Kad ̓im et. al., 2016). Due to high temperature and evaporation the DO, EC, TDS,TSS and SAL were the highest in the spring in all of four sites, due to heavy rainfall in winter the water got diluted also, so all those components were lowered down. The same pattern was also seen in $HCO_3^{=}$, CO_3^{-} , Cl^-,$SO_4^{=}$, and pH concentrations (Table 1), while the concentration of BOD, $PO_4^{=}$, and NO_3= were increased in winter and decreases in spring due to thrive of plants in spring. the physio-chemical parameters were analyzed by one way ANOVA method in order to reveal the differences among four sites. The EC, TDS, TSS, SAL, HCO_3 =, CO_3^{-} ,Cl^-, SO_4= and pH were higher in Site 2 than the other sites (Table 1). The results showed that positive correlation between annual precipitation with DO, EC, TDS, TSS , SAL, HCO_3 =, CO_3^{-} , Cl^-, SO_4= and pH , while BOD, PO_4 = and NO_3= showed negative correlation. In contrast to four sites, the activity of SOD , CAT, APX and POD in Phragmites australis were significantly ($P < 0.05$) high in Site 2 and Site 3 than other sites (Figs: 1, 2, 3, and 4), The SOD activity didn't change significantly ($P < 0.05$) in winter and spring but increased significantly in different sites, also SOD activity was higher in Site 2 and Site 3 than site 4 and site 1 (Fig.1).
The activity of CAT increased significantly ($P < 0.05$) in spring than in winter and increased significantly in different sites, also observed that CAT activity was higher in Site 2 (site 2 > Site 3 > site 4 > site 1). However, the CAT activity was higher in cold stress than in the pollutant stress (Fig.2).
Figure 3, showed that the highest activity of stress enzyme APX in the contaminated sites at spring than in winter .The APX activity was increased significantly ($P < 0.05$) in site 2 followed by site 3 and site 4 then site 1.
The POD activity was highest in site 2 followed by 3 and 4 at last 1.but not change significantly ($p< 0.05$). Figure 4 showed that the highest activity of stress enzyme POD in the contaminated sites at spring seasons than in winter. The value of CAT was much higher than that of POD under all conditions that is agree with Xiang et. al. (2012). The activity of studies antioxidant enzymes.

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Table1 Temporal variation of physio-chemical parameters

Sequence	Season / Station / parameter	The mean value in Winter				The mean value in Spring				unit
		Station (1)	Station (2)	Station (3)	Station (4)	Station (1)	Station (2)	Station (3)	Station (4)	
1	precipitation	2	2	2	2	0	0	0	0	(mm/m2)
2	air temperature	29	30	27	27	39	40	38	37	(Silesia)
3	Water temperature	24	25	20	21	32	33	31	30	(Silesia)
4	pH	8.3	8.7	8.6	8.1	8.9	9.5	9	10	
5	Electrical conductivity (EC)	1007	3302	2903	1202	1708	3930	3540	1770	(µS / cm)
6	Total Dissolved Solids (TDS)	462	549	843	856	572	787	1.019	1.340	(mg / L)
7	Suspended solids (TSS)	1.654	1.270	0.876	0.912	2.325	1.530	1.210	1.721	(mg / kg)
8	Nitrate($NO3^{-2}$)	0.492	0.589	0.325	0.302	0.432	0.356	0.345	0.231	(mg / L)
9	Phosphate(PO_4^{-2})	2.540	3.100	3.021	4.654	2.211	1.762	1.342	0.865	(mg / L)
10	Water hardness	375.4	241.6	354.7	345.4	386.7	260.2	342.6	337.7	(mg / L)
11	Chloride(Cl^{-1})	167.67	1704.7	506.92	864.01	178.2	2490.2	633.43	953.63	(mg / L)
12	Carbonate(CO_3^{-3})	114.5	116.1	117.4	115.5	118.8	123.5	123.2	121.3	(mg / L)
13	Bicarbonate (HCO_3^{-2})	97.6	112.5	98.6	98.2	100.6	114.4	102.3	97.9	(mg / L)
14	Sulfate(SO_4^{-2})	265.8	850.3	771.8	331.5	296.2	959.3	654.5	413.5	(mg / L)
15	Dissolved Oxygen (DO)	5.9	5.25	6.83	5.65	2.09	1.15	2.18	2.70	(mg / L)
16	Bio-oxygen demand(BOD)	0.68	0.84	1.013	1.019	0.60	1.09	1.48	1.50	(mg / L)
17	turbidity	3.36	5.84	4.5	3.4	7.21	5.33	5.91	6.74	(cm)
18	Salinity	6	23,8	17..6	7.2	10.2	28.8	21.7	12.2	(ppt.)

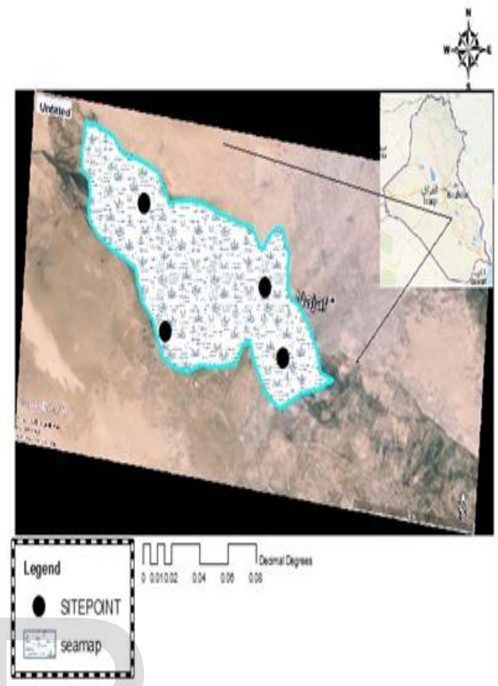

Fig. 1. Study area map

r=o.92 at (P < 0.05) of air temperature and water temperature. (positive correlation), r=o.95 at (P < 0.05) of DO, EC, TDS,TSS , SAL, HCO3=, CO3- , Cl-,SO4= , pH and annual precipitation. (positive correlation), and r= -0.84 at (P<0.05) BOD,PO4= and NO3=
with annual precipitation (negative correlation) .

The municipal wastewater is affluent to site 2, site 3 has a lot of mount of salt waters come from salt spring, site 4 is located near the strategic oil line. EC, TDS,TSS, SAL were significantly high in site 2 > site 3 > site 4 > site1, that mean the composite municipal wastewater was higher effect than other factors, site 3 has higher concentration of salts, oil pollutant from site 4, then agricultural pollutant in site 1, so we can address the response of antioxidant enzymes to extreme of water quality and quantity (Das and Rychoudhury, 2014). SOD, CAT,APX and POD provide the first defense line against oxygen toxicity in individuals (Abedi and Pakniyat, 2010). Generally in oxidative stress condition the SOD catalyzes dismutation of superoxide radical which in turn forms water and hydrogen peroxide (Gill and Tuteja 2010). After that hydrogen peroxide being the substrate, it gets detoxified by CAT,APX and POD. The present study indicates that higher activity of CAT in *Phragmites australis* collected from contaminated sites was higher in winter due to cold stress of *Phragmites australis*. The higher activity of APX and POD in spring due to higher concentration of pollutants than in winter. (Figs. 1,2,3,4). This clearly indicates that the enhanced production of superoxide radicals create an oxidative stress to the *Phragmites australis* as pollutants indicator (Karuppanapandian *et al* 2011).

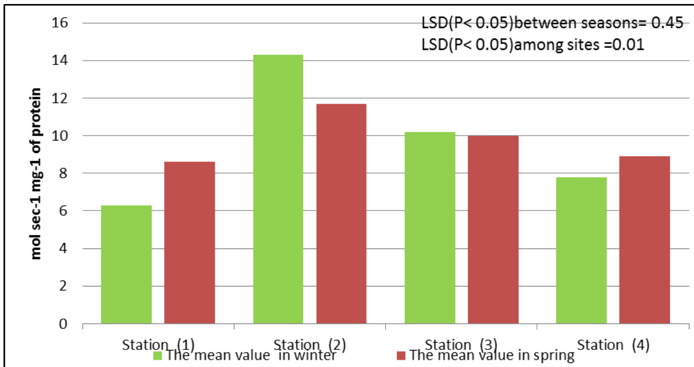

Fig. 4 POD responce indifrent station

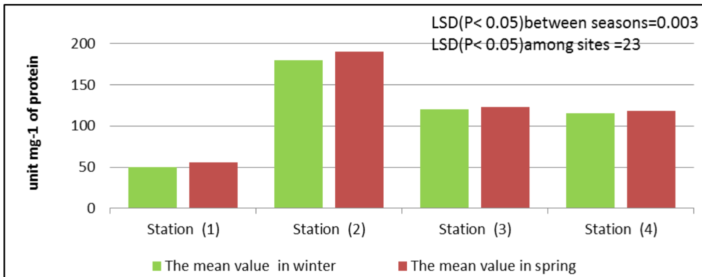

Fig. 1 SOD responce in diffrent stations

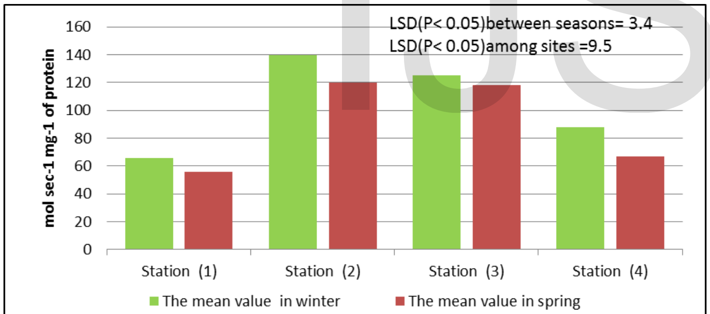

Fig. 2 CAT responce in diffrent stations

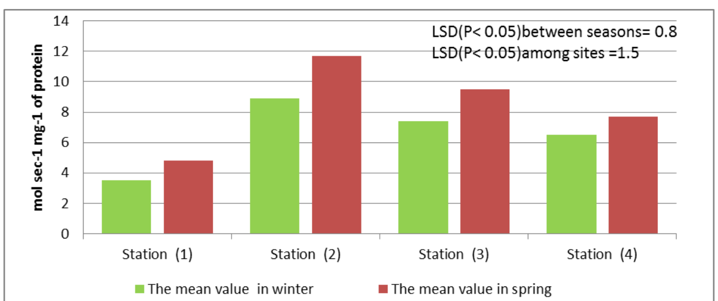

Fig. 3 APX responce in diffrent stations

4-ACKNOWLEDGEMENT

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5-COMPETING INTERESTS

.Authors have declared that no competing interests exist

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