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Anti-oxidative Potential Survival of *E. crassipes* Under Oxidative Stress

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Abstract- The present study evaluates the kinetic constants of enzymatic assays in crude extracts of roots and shoots of *E. crassipes* growing in wastewater bodies situated in the *trans*-Ganges/Yamuna regions of Allahabad. The increased kinetic constants in crude extracts of shoots and roots of *E. crassipes* showing the ability to protect plants from heavy metal induced oxidative stress by activating multi-defense mechanisms and for better growth in polluted environments. This study demonstrates the phytoremediation potential of *E. crassipes* for the removal of polluted effluents and their ability to survive under oxidative stress.

Keywords: *Phytoremediation, kinetic constants, oxidative stress, wastewater, heavy metal, multi-defense mechanisms*

I. INTRODUCTION

Water is an important substance on earth for the survival of living organism *i.e*. human, plants and animals. Speedy population growth and increasing use of water is likely to become an issue in near future due to limited sources of drinking water [1].

Water pollution is jeopardizing our health. Our drinkable water sources are finite. Less than 1 percent of the earth's freshwater is actually accessible. Challenges only increases by 2050, when global demand for freshwater is expected to be one-third greater by now. Municipal and industrial waste discharges contribute their fair share of toxins contaminating stream, river, lake, ocean, aquifer or other bodies of water, degrading the water quality [2].

Although nature possesses abundant capacity to deal with waste water and even pollution *via* macrophyte which possess a well-developed fibrous root system and large biomass and has been successfully used in wastewater treatment systems to improve water quality by reducing the levels of organic and inorganic nutrients and help in re-establishing and preserving biological, chemical and physical integrity of water. Conserved wastewater can be applied in the restoration of natural ecosystems, industries and for human consumption [3]. The term "phytoremediation" is derived from the Greek word Φυτο (phyto) = plant and Latin word "*remedium*" = restoring balance, or remediation; involves justifying contaminant concentrations in polluted soils, water or air with naturally occurring plants that have ability to accumulate, crude oil, degrade metals, explosives, pesticides, solvents, , and its derivatives [4] and [5]. *Eicchornia crassipes* growing in wetlands and flooded fields, their roots play significant role in improving the physico-chemical properties of the wastewater [6], [7] and [8].

The study reports that the increased kinetic constants in crude extracts of shoots and roots of *E. crassipes* showing the ability to protect plants from heavy metal induced oxidative stress by activating multi-defense mechanisms and for better growth in polluted environments. This study demonstrates the phytoremediation potential of *E. crassipes* for the removal of polluted effluents and their ability to survive under oxidative stress.

II. MATERIALS AND METHODS

- **Study area and Sampling Points**

Prayagraj (Allahabad) is situated at 25.45°N 81.84°E in the southern part of Uttar Pradesh at an elevation of 98 metres (322 ft) and stands at the confluence of two rivers, the Ganges and Yamuna. The study covers the urban area as well as the adjoining areas of Allahabad district along with industrial areas. In Allahabad, after an initial survey of waste water bodies were selected as sampling sites from the *trans*-Ganges/Yamuna regions where *E. crassipes* was present. Waste water bodies without *E. crassipes* served as control sampling sites present on *trans*-Ganges region of Allahabad. The present study also examine the changes occurred in fresh aquatic *E. crassipes* plants surviving under oxidative stress by estimating the enzymatic kinetic constants and the biochemical parameters in roots and shoots of *E. crassipes* growing in the polluted aquatic environment of Allahabad city region.

The sample collection and experimental analysis was done from three sampling sites of *trans*-Ganges/Yamuna areas.
The experimental work was performed at the Department of Biochemistry and Biochemical Engineering, SHUATS, Prayagraj (Allahabad) and at the Department of Biochemistry of Allahabad University, Prayagraj (Allahabad).

- **Sample Collection:**

The free floating *E. crassipes* (Fig. 1) plants were collected from the sampling sites for further biochemical estimation. The samples were labelled, stored in clean and dry polyethylene bags and transported to the laboratory. The plants were washed in running tap water blotted dry with filter paper. Damage to root and leaf apices were avoided. The samples were refrigerated at 4^oC until used.

Fig. 1. Image of water hyacinth *(E.crassipe*)

- **Methodology:**

All assays were carried out at room temperature (25-30 °C).The method followed for enzyme extraction was given by **[9]**.Enzyme extraction was carried out at between $4\text{-}6^0C$**.** The extracts were prepared by grinding the plant sample (5.0g fresh wt) in 10 ml 0.1M phosphate buffer pH 7. The extracts were centrifuged at 15,000g for 30min at 4^0C in refrigerated centrifuge and the supernatant used for enzyme analysis.
Experiments were conducted to determine the effect of substrate concentration on the enzymatic activity of roots and shoots of *E. Crassipes.* K_m and V_{max} value for each enzyme was calculated using the Lineweaver and Burk (1/V *vs* 1/[S]) plot.

Catalase (CAT): The enzyme assay was done at five concentrations of H_2O_2 substrate ranging from 0.2mM to 0.8 mM according to the method described by **[10]**.

Guaiacol peroxidase (POD): K_m and V_{max} for POD was done according to the method given by **[11]**. The enzyme activity was assayed at five concentrations of guaiacol ranging from 0.07 mM to 0.3 mM.

Polyphenol peroxidase (PPO): K_m and V_{max} for PPO was done according to the method given by **[12]**. The enzyme activity was assayed at five concentrations of catechol substrate ranging from 1.7 $x10^{-3}$ M to 8.3 $x10^{-3}$ M. Estimation of Protein [**13**].

Statistical Analysis: Each treatment was analyzed with a minimum of 3 replicates and the Standard Deviation (SD) was calculated. All the data reported as MEAN ± SD (Minimum of 3 replicates).

III. RESULTS

Lineweaver and Burk (1/V vs 1/[S]) plot was used to determine the kinetic constants Km and Vmax of enzymes (catalase, guaiacol peroxidase and polyphenol oxidase) in crude extracts of roots and shoots of the sampling sites were S1 (Purani Jhusi), S2 (Daragunj), S3 (Nasirpur), S5 (Chheonki), S6 (Maheva) and S7 (Naini Gaon) present in trans-Ganges/Yamuna sites of Prayagraj. The units for Km and Vmax are mM and Umg-1min-1, respectively.

- **Catalase (V_{max} and K_m) (1.11.1.6):**

Table 1: K_m and V_{max} of catalase in crude extracts of *E. crassipes*.

*Kinetic constants**	*Trans-Ganges*		*Trans- Yamuna*	
	SHOOTS	*ROOTS*	*SHOOTS*	*ROOTS*
K_m *(mM)*	*0.213 ± 10*	*0.469 ± 80*	*0.274 ± 22*	*0.659± 72*
V_{max}*(µmol/min)*	*30.22 ± 0.94*	*66.9± 4.5*	*41.2 ± 1.06*	*85.85± 4.37*

**All values given are MEAN ± SD of three replicates*

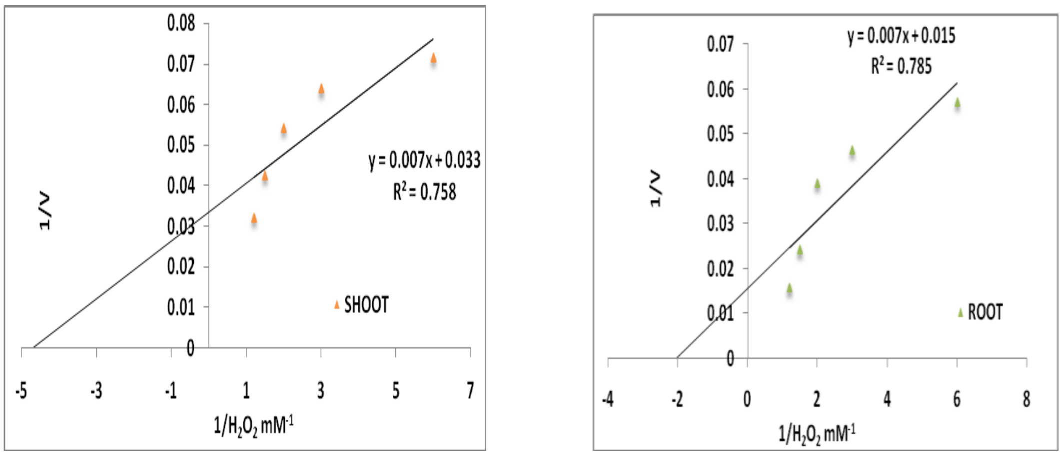

Fig.2: *Lineweaver-Burk plot of catalase in crude extracts of E. crassipes (shoots and roots) from Trans-Ganges sites.*

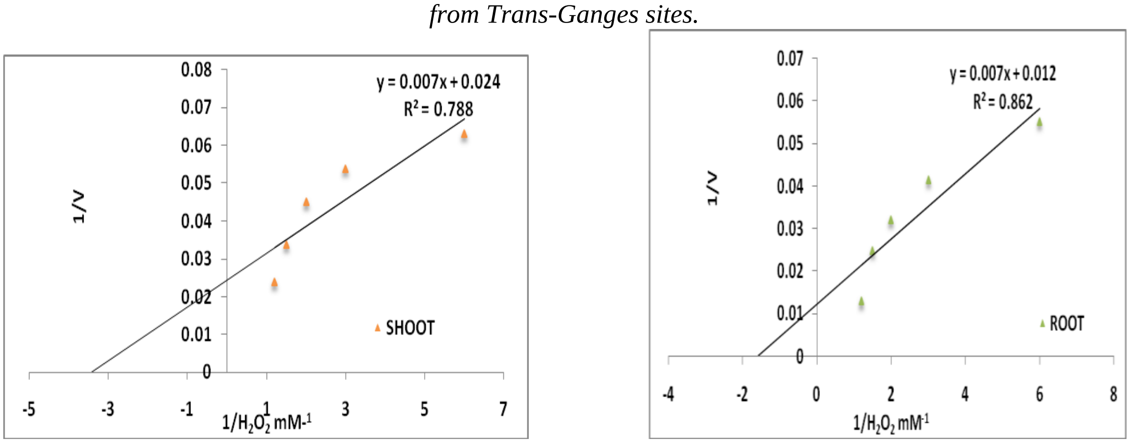

Fig.3: Lineweaver-Burk plot of catalase in crude extracts of E. crassipes (shoots and roots)

From Trans-Yamuna sites.

- **Guaiacol peroxidase (V_{max} and K_m) (1.11.1.7):**

***Table** 2: K_m and V_{max} of guaiacol peroxidase in crude extracts of E. crassipes.*

*Kinetic constants**	*Trans-Ganges*		*Trans- Yamuna*	
	SHOOTS	*ROOTS*	*SHOOTS*	*ROOTS*
K_m *(mM)*	*0.188 ± 0.02*	*0.107 ± 0.014*	*0.086 ± 0.01*	*0.161± 0.027*

V_{max}*(μmol/min)*	*6.6 ± 0.46*	*24.5± 0.50*	*2.76 ± 0.045*	*43.7± 1.8*

*All values given are MEAN ± SD of three replicates

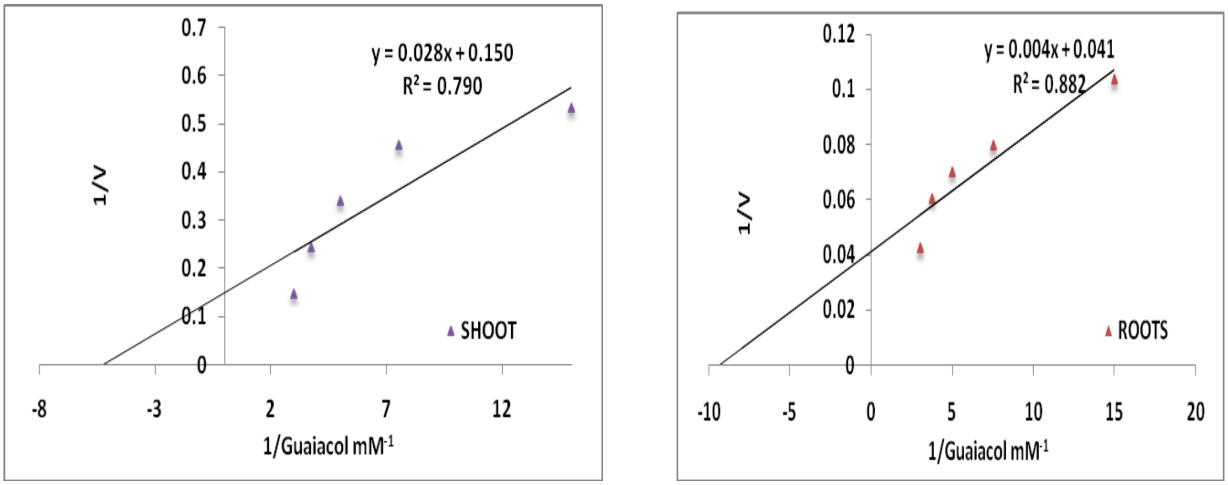

Fig.4: Lineweaver-Burk plot of guaiacol peroxidase in crude extracts of E. crassipes (shoots and roots) from Trans-Ganges sites of Prayagraj.

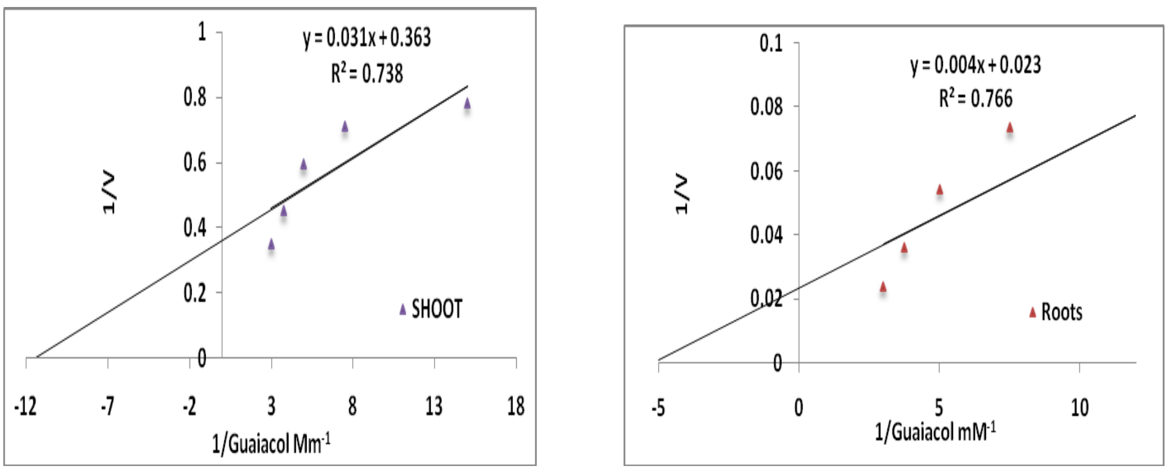

Fig.5: Lineweaver-Burk plot of guaiacol peroxidase in crude extracts of E. crassipes (shoots and roots) from Trans-Yamuna sites of Prayagraj.

- **Polyphenol oxidase (V_{max} and K_m) (1.14.18.1):**

Table 3: *K_m and V_{max} of poly phenol oxidase in crude extracts of E. crassipes.*

Kinetic constants*	***Trans-Ganges***		***Trans- Yamuna***	
	SHOOTS	***ROOTS***	***SHOOTS***	***ROOTS***
K_m ***(mM)***	*2.31 ± 0.9*	*2.4± 0.2*	*5.9 ± 0.2*	*6.73± 0.7*
V_{max}***(μmol/min)***	*0.015 ±0.004*	*0.017± 0.003*	*0.034 ± 0.001*	*0.075± 0.004*

**All values given are MEAN ± SD of three replicates.*

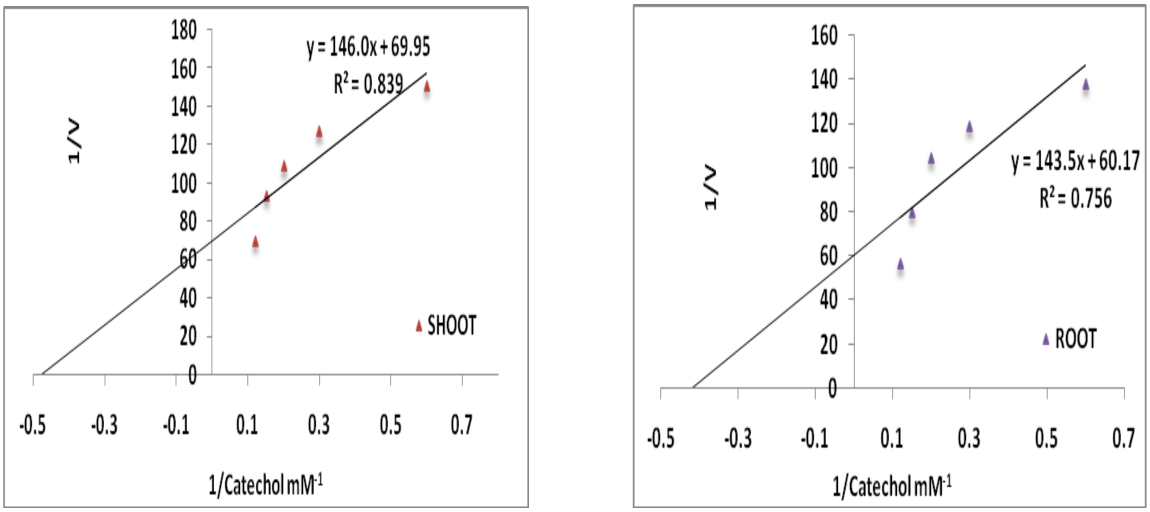

Fig.6: Lineweaver-Burk plot of polyphenol oxidase in crude extracts of E. crassipes from Trans-Ganges sites of Prayagraj.

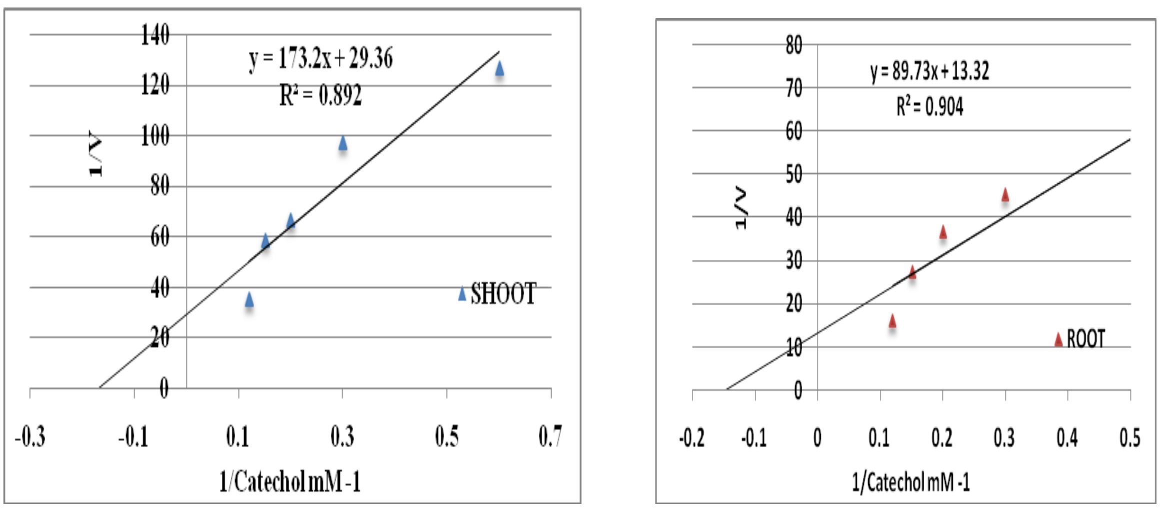

Fig.7: Lineweaver-Burk plot of polyphenol oxidase in crude extracts of E. crassipes from Trans-Ganges sites of Prayagraj.

An increase in the antioxidants activity is often believed to indirectly refle on increase in reactive oxygen species formation due to the environmental stresses appears to be directly or indirectly. From the study in Prayagraj, K_m (mM) of crude extract of shoots of *E. crassipes* from *trans*-Ganges sites were in order of PPO (2.31 ± 0.9)> CAT (0.213 ± 0.01)> GPX (0.188 ± 0.02). Similarly, the pattern of increase was observed in K_m (mM) of roots of E.*crassipes* from *trans*-Ganges sites *i.e.*, PPO (2.4± 0.2) > CAT (0.469 ± 0.08) > GPX (0.107 ± 0.014. The V_{max} ($Umg^{-1}min^{-1}$) of crude extract of shoots of E. *crassipes* were in order of CAT (30.22 ± 0.94)> GPX (6.6 ± 0.46)>PPO (0.015 ±0.004), while the pattern of increase was observed in V_{max} ($Umg^{-1}min^{-1}$) in crude extract roots of *E. crassipes i.e.*, CAT (66.9± 4.5> GPX (24.5± 0.50)>PPO (0.017± 0.003) from *trans*-Ganges sites.

The pattern of the order in *trans*-Yamuna sampling sites of K_m (mM) was observed as PPO (5.9 ± 0.2> CAT (0.274 ± 0.022)> GPX (0.086 ± 0.01) in shoots of E. *crassipes* while, in roots K_m (mM) order was PPO (6.73 ± 0.7)> CAT (0.659± 0.072) > GPX (0.161± 0.027) from *trans*-Yamuna sites. Similarly, the pattern of the order of V_{max} ($Umg^{-1}min^{-1}$) observed was as CAT (41.2 ± 1.06)> GPX (2.76 ± 0.045)> PPO

(0.034 ± 0.001) in shoots of E. *crassipes* and in crude extracts of roots of E. *crassipes* the V_{max} ($Umg^{-1}min^{-1}$) order was CAT (85.85± 4.37)> GPX (43.7±1.8)> PPO (0.75 ± 0.004) from *trans*-Yamuna sites.

From the overall results, it was concluded that the pattern of V_{max} shows the increasing order as **CAT> GPX> PPO**; the order for K_m was as **PPO> CAT> GPX** observed in both the shoots and roots of E. *crassipes* present in *trans*-Ganges/Yamuna sites of Prayagraj. The kinetic constants (V_{max} and K_m) of enzymes in roots from *trans*-Yamuna sites were higher than in shoots of *E. crassipes* in comparison to the *trans*-Ganges sampling sites. K_m is an approximate measure of the affinity of the substrate for the enzyme. Large K_m value represents low affinity of substrate for enzyme and high K_m value is indicative of high affinity for enzymes. The results observed indicates that PPO has maximum K_m showing low affinity of substrate followed by CAT and GPX in both shoots and roots of *E. crassipes* from *trans*-Ganges/Yamuna sites. V_{max} is the maximum velocity at which the enzyme catalysed a reaction and all its active sites is saturated with substrate. V_{max} for CAT was reported to be maximum followed by GPX and PPO in *E. crassipes* present in *trans*-Ganges/Yamuna sites of Prayagraj.
Further work with partially/purified enzymes are required to substantively contribute towards understanding the detailed mechanism of induced oxidative stress in the E. *crassipes*.

However, a few reports on kinetic data of plants growing in aquatic eco-systems are available The present study is in agreement with the study done by **[14]** who stated that for CAT, the K_m and V_{max} treated samples were 0.72 mM, 0.33mM/ min. mg protein, respectively over the control sample. **[15]** study reported that the CAT V_{max} and K_m of cabbage leaf was 31.12 $\mu Mmin^{-1}$ and 25 mM, respectively. In the study of **[16]** on the effect of crude oil on the lentil shoots the kinetic parameters of the CAT, the K_m and V_{max} for the control and treated samples were 1.7 mM, $0.64mMmin^{-1}$ mg^{-1} protein, and 0.72 mM, 0.33mM $min^{-1}.mg^{-1}$ protein, respectively were investigated. **[17]** reported in his study that the K_m and V_{max} values of PPO for mulberry leaf and mushroom were 35 mM and 3 Uml^{-1}, and, 20 mM and 5 Uml^{-1}, respectively. Polyphenol oxidase activity (PPO) was extensively studied in jackfruit for its role in enzymatic browning. PPO and the phenolic compound play a vital role in defensive mechanism against pest and diseases. For the studies in jack fruit waste, PPO was purified and characterized and its kinetic constant for PPO was found to be 15.82 mM (K_m) and 2182 Uml^{-1} min^{-1} (V_{max}) using catechol as substrate [**18].**

In study of **[19],** which had reported that K_m value of 1.48 mM for *M. indica* kernel peroxidase using pyrogallol as substrate and a V_{max} of 0.29 enzyme units/g kernel. Similar research study by various authors on kinetic parameters for horseradish peroxidase calculated from Lineweaver-Burk plots gave a K_m of 0.8 mM using pyrogallol as substrate **[20];** a K_m of $8x10^{-5}$ and V_{max} of 1.53 when hydroquinone was used as substrate **[21]. [22]** Reported a K_m of 58 µM and V_{max} of 3.36 units/nmol for french bean peroxidase using pyrogallol as substrate. [**23**] also reported a K_m of 10.50 mM and a V_{max} of 28.50 nmol min^{-1} mg^{-1} as kinetic parameters detected for guaiacol-dependent peroxidase activity in *Crocus sativus*. Hence, the affinity of peroxidase for its substrate could be directly related to the available hydroxyl groups in the substrate.

The study of **[24],** reported that based on their linear regression analysis (Lineweaver-Burk plot) over the substrate concentration range of 0.05 x 10^{-3}M to 0.5 x 10^{-3}M the K_m and V_{max} of the three plant peroxidases (for H_2O_2 in presence of O-dianisidine) were: 0.250 mM and 9.09 uM min^{-1} (tobacco peroxidase), 0.370 mM and 11.11 uM min^{-1} (cabbage peroxidase) and 0.277 mM and 10.02 uM min^{-1} (radish peroxidase) respectively. Kinetic studies revealed that Tobacco peroxidase (source- *N. tabaccum*) had lower apparent K_m values and it has more peroxidases than the other two plant peroxidases examined. **[25]; [26]; [27]** study determined the kinetics factors of the enzyme revealed that K_m of the enzyme in the treated sample was much lesser than that of the control, which suggested that affinity of CAT became much higher for its substrate. This result proposed that the plant moved to deal with oxidative stress due to the presence of crude oil.-polluted soil. It has been shown that some stressful environmental factors such as heavy metals and organic pollutants could affect the CAT activity. Catalase dismutates H_2O_2 into H_2O and O_2, which is found in peroxisomes, cytosol and mitochondria **[28]**. POX decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and antioxidants.

Polyphenol oxidases catalyses both the hydroxylation of monophenols and oxidation of o-diphenols to o-quinones. The V_{max} value for PPO had the highest affinity for catechol and catechol was the most efficient phenolic substrate for the plants [29]**.** PPO is located in the thylakoid membrane of chloroplasts and their phenolic substrates are located in the vacuoles in higher plants In stress condition, the free radical species (forms of active oxygen) are increased, which enhances the activities of these detoxifying enzymes [30]**.** In the systems involving enzyme, substrate, and metal ion, the metal may react with the substrate, the protein-active groups of enzymes, as well as the enzyme-substrate complex. Information is available on enzyme kinetics (V_{max} and K_m values) in the presence of heavy metals. The V_{max} and K_m values represent the maximum reaction velocity and the affinity of the enzyme for the substrate, respectively.

IV. CONCLUSION AND FUTURE SCOPE

Summarizing the overall finding of the study, it has been concluded that *E. crassipes* has the ability of improving the physico-chemical factors of water along with the accumulating and removal capacity of the toxic metal substances from sampling sites. *E. crassipes* reduced the properties of toxic metal stress by increasing the antioxidant enzyme activities and shows the effective changes in biochemical parameters and kinetic constants of enzymes in shoots/roots of *E. crassipes* present in stressed environment for its better survival in contaminated water bodies at both the *trans*-Ganges/Yamuna sites of Prayagraj. This study determines that *E. crassipes* retains all the characteristics of an aquatic plant for use in phytoremediation of wastewater bodies by removing heavy metal contents and improving physicochemical characteristics of the

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