Waste water contamination in River Ganges at Farakka and its effect on morpho – biomolecular system of selected edible plant species

Sanat Kumar Das[1], Suchisree Jha[1] , Phalguni Bhattacharyya[2]✉,Tania Chakraborty Ghatak[2] and Amitava Ghosh[3]

[1]*Young Professional, ICAR – CISH Malda chapter, Malda*
[1] *Department of Environmental Studies, Asutosh College , University of Calcutta, Kolkata.*
[2]*Department of Botany, Shibpur Dinabundhoo Institution (College), Howrah*
[3]*Department of Botany, Asutosh College, University of Calcutta, Kolkata*

Email ID: phalgunimaldacollege@gmail.com

Abstract

Waste water contamination due to industrial and developing urbanization with different fresh water sources specially in terms of irrigation is a serious matter of concern. Sampling of water of River Ganges from the area surrounding the NTPC , Farakka , Murshidabad district , West Bengal as well as domestic effluents were taken into consideration. We have analyzed the waste water sample for total hardness , total dissolved solid , concentration of As , Fluoride , pH , concentration of Fe in ppm and heavy metal concentration (mg / L) of Cd , Pb , Cr , Cu and also study their effect on certain morphological and biochemical parameters of two important vegetable *Momordica charantia* L. (Bitter gourd , Cucurbitaceae) and *Abelmoschus esculentus* Moench (Ladies Finger, Malvaceae). These include percentage of germination of seeds , seedling growth , root and shoot length , internodal length and leaf size as morphological and cholorophyll and protein parameters as biochemical changes. Antioxidant or free radical scavenging study is a clear evidence for stress induction. We have studied antioxidant activity using capacity of free radical scavenging effect of stable DPPH free radical with a simultaneous study of anti oxidative enzymes catalase and peroxidase. We have analyzed the synthesis of naturally occurring low molecular weight (LMW) plant peptide (s) , 3.0 kDa to 0.5 kDa in response to waste water treatment in two species of sample plant. The result showed the inhibitory effect of waste water on plant morpho – biochemical parameters as well as on the low molecular weight peptides which have definite role on plant growth and development.

Key words : Waste Water , antioxidant , Low molecular weight Peptide

Introduction :

Environmental pollution problems resulting from waste of factories have been one of the most controversial problems for the public in recent years. The problem of environmental pollution on account of essential industrial growth is, due to the problem of disposal of industrial waste as well, whether solid, liquid or gaseous. Polluted water, in addition to other effects, directly affects soil not only in industrial areas but also in agricultural fields and river beds, thereby creating secondary source of pollution (Kisku et al., 2000). Various industries have been continuously adding lot of waste water containing high level of nutrients, heavy metals and hazardous substances to the cultivable land (

Malaviya and Rathore, 2007). These effluents not only increase the nutrient level, but also excess tolerance limits and cause toxicity (Mishra et al., 1999). Water and soil pollution due to industrialization is a cosmopolitan problem, creating acute insanitation and adverse effects on soil and crops when waste waters are used for irrigation. Heavy metals such as copper, cadmium, chromium, lead, mercury, and selenium get into water from many sources, including industries, automobile exhaust, mines, and even from natural soils (Khan *et al*., 2008). Plants grown in contaminated soils or irrigated with municipal wastewater when consumed by peoples can result in health problems (Wahid *et al*., 2004) like diarrhea, mental retardation, liver and kidney damage. Seed germination and growth are vital for continuation of life of seeds and seedlings are extremely vulnerable to environmental stresses due to presence of polluting agents in the environment especially during seed hydration period which is very important for irrigation and triggering the intricate sequences of metabolism essential for germination and growth of seedlings. The effect of industrial effluents on growth and yield parameters of agricultural crops and soil properties has been extensively studied. But only few studies are made to find out the effect of industrial effluents on germination. Direct use of effluent water to the crops results in poor germination, lesser seedling growth and vigour index. The mixing of heavy elements in water of the Ganges near the effluent discharge points of NTPC (National Thermal Power Corporation) , Farrakka showed detrimental effect on morphological parameters of selected plant species *Momordica charantia* L. (Bitter gourd , Cucurbitaceae) and *Abelmoschus esculentus* Moench (Ladies Finger, Malvaceae). Industrial effluents were analyzed for the presence of heavy metals and recorded. The documentation under this project will reflect a general study of the effect of waste water on daily consumed vegetables. The collection of NTPC effluents and study of its effect need a rigorous research.

Materials and Methods

Water sampling : The water from different sources near NTPC and Farakka industrial town were collected depending on the hour of high discharge in the river. The sources were given in the following:

a. Thermal power plant effluent from NTPC.
b. Normal water of the River Ganges

Collection of waste water and analysis

Sample of waste water was collected in 100 ml capacity plastic containers from the rivers at randomly selected points during day time from 10 A.M. to 10.30 A.M. The waste water was dirty white to little black in colour and had a fecal odor. The analysis was performed after rigorous filtration in collaboration with the Department of Botany, University of North Bengal and District Soil and Water Analysis Unit of Malda , Central Irrigation Department , Malda unit for the study of heavy metals.

Morphological trait study :

a. **Percentage of germination. b. Shoot length c. Root length d. Internodal length e. Leaf size.**

The above morphological parameters were measured by scale and graph paper method. The plants were germinated from the certified seeds (Sutton India Ltd) in plastic pots filled with fertile manured soil. The treatment using sample waste water from different sources were given plants after 4 days of germination and continued for 2, 4 and 6 days. The parameters were measured after 2, 4 and 6 days of treatment against a control set. After measurement the plants were washed (500 gm each in weight) by running fresh water and again by distilled water for several times to wash off the debris. Finally the plants were cut into pieces by knife and put into a mixer with distilled water to obtain extract. The extracts after fine grinding were sieved through a cheese cloth to obtain extracts free from tissue debris. After that volume was measured 100 ml. The extracts was mixed up with 10% methanol and extracted with solvents through a separating funnel. Finally to avoid the lipid and other bio molecules solvent ether was used in the extracts. After evaporation of solvent ether the extracts from two plant species were kept into deep freeze at – 20 degree centigrade for further bio analysis.

Extraction of Plant Exudates through Methanol (10 %) and separate sets are made to study anti oxidative parameters like DPPH based free radical scavenging activity and enzymes related to anti oxidative parameters, Catalase and Peroxidase. The methods of Britton and Mehley, 1955 were used for analyzing Catalase and Peroxidase activity. Britton C, Mehley AC. [1955] Assay of Catalase and Peroxidases. In: Colowick, S.P., Kalpan, N.O. (Eds.), Methods in Enzymology, Assay of Catalase and Peroxidase, Academic Press Inc., New York, Vol. II, pp. 764–775. Cholorophyll was estimated following the method Lichtenthaler and Wellburn Biochem.Society.Trans.603.531.1983 and Estimation of protein concentration was done following **Lowry method:** Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (November 1951). "Protein measurement with the Folin phenol reagent". *J. Biol. Chem.* **193** (1): 265–75.

Chlorophyll estimation :

1 gm of sample was weighed and mixed into a clean mortar. The tissue was grinded into a fine pulp with addition of 20 ml of 80 % Acetone and it was centrifuged and (5000 rpm for 5 min) and the supernatant was transferred to small flask.The tissue residue was grinded with 20 ml of 80 % of Acetone, centrifuge and transfer the supernatant to same flask.The process was repeated until the residue was color less the pestle and the mortar was washed thoroughly with 80 % acetone and the clear washing was collected in the flask. The volume was made up to 100 ml with 80 % Acetone. The absorbance of the solution was read at 480, 510, 645,663 and 652 nm against the solvent (80 % Acetone) as blank.

Calculations –

mg chlorophyll a/g tissue = 12.7 (A663) 2.69(A645) * V/1000*10

mg chlorophyll b/g tissue = 22.9 (A645) -4.68(A663) * V/1000*10

mg chlorophyll /g tissue = 20.2 (A645) +8.02(A663) * V/1000*10

mg chlorophyll /g tissue = 7.6 (A480) -1.49(A510) * V/1000*10

where,

A = Absorbance of specific wavelength

V = Final volume of chlorophyll and carotenoid in 80 % Acetone

W= Fresh weight of the tissue extracted

Extraction of Low molecular weight peptide(s) from waste water irrigated plant species:

The parts of two selected plant were surface sterilized and separately cryo-crushed and extracted with measured amount of chilled distilled water by blender. The extract was cold centrifuged at 10,000 rpm for 30 minutes using protease inhibitor PMSF. The supernatant was subjected to ether wash at acidic pH to remove endogenous hormonal impurities and lipids. It was then passed through separate cation exchange (Dowex-50; 900 meq. in glass column 60 x 2.9 cm) and anion resin (Dowex-2; 700 meq. in glass column 60 cm x 2.9 cm) for trapping amphoteric molecules like proteins, peptides and amino acids. Then concentrated aqueous acidic column eluents were washed 4 times with equal volume of peroxide free ether to remove traces of IAA, ABA, and GA. After discarding of anionic hormones, the extracts were filtered through Millipore ultra filtration system with Amicon filters 10 kDa (YM 10), 3 kDa (YM3) and 0.5 kDa (YC 05) cut off with 1.5 kg/cm^2 N_2 gas pressure. The samples were repetitively filtered and lyophilized. The obtained peptide extract was dissolved in 50 mL distilled water and stored in freeze at -20° C for further analysis. (Ghosh et al. 2015)

Determination of Antioxidant activity of isolated peptide(s)

DPPH -Scavenging activity

Antioxidant activity of LMW peptide was examined by using capacity of free radical scavenging effect of stable DPPH free radical. The radical scavenging activity of the aqueous extracts was measured by DPPH method. In this assay ascorbic acid was used as a standard compounds. The absorbance was measured at 517 nm. Blois. 1958.

Result and Discussion:

Table 1 and 2 demonstrating the analysis of collected effluent waste water from NTPC and normal water from the river Ganges showed the parameters like Total Dissolved solids , Suspended solids, BOD, Total organic carbon, Chemical oxygen demand, Alkalinity (as $CaCO_3$) Grease, Total coliform,

volatile organic compound, Aluminium, Boron, Fluoride, Manganese, Pb, Zn, Cd, Cr, Cu, Ni, As, etc. It was observed that in comparison to Normal Ganges the physico – chemical parameters appeared in higher order indicating pollution level but the concentration of heavy metals like Cr , Cd and Pb showed slight toxic level.

Total dissolved solids ppm	Total hardness ppm	As ppm	Fluoride ppm	Fe ppm	pH
668	750	0.005	6.621	8.2	4.180

Contaminants	Unit	Concentration Medium
Dissolved , total (TDS)	mg/l	500
Suspended solids (SS)	mg/l	220
BOD5 at 20 degree Celsius	mg/l	220
Total organic carbon (TOC)	mg/l	160
Chemical Oxygen Demand (COD)	mg/l	500
Alkalinity (as CaCO3)	mg/l	100
Grease	mg/l	100
Total coliform	CFU 100 m/l	107 -108
Volatile Organic compounds (VOCs)	mg/l	100 – 400
Aluminium (Al)	mg/l	0.1 – 0.2
Boron (B)	mg/l	0.1 – 0.4
Fluoride (F)	mg/l	0.2 – 0.4
Manganese (Mn)	mg/l	0.2 – 0.4

	Pb	Zn	Cd	Cr	Cu	Ni	As
Minimum	99.30	182.00	22.20	118.05	22.00	44.72	0.001
Maximum	168.30	285.00	51.00	190.40	166.50	133.80	2.05
Safe limit	250– 500	300–600	3–6	--------	135–270	75–150	1 – 40

Table 1. Normal water from the Ganges

Total dissolved solids ppm	Total hardness ppm	As ppm	Fluoride ppm	Fe ppm	pH
968	850	0.005	9.621	8.2	3.18

Contaminants	Unit	Concentration Medium
Dissolved , total (TDS)	mg/l	800
Suspended solids (SS)	mg/l	520
BOD5 at 20 degree Celsius	mg/l	420
Total organic carbon (TOC)	mg/l	360
Chemical Oxygen Demand (COD)	mg/l	400
Alkalinity (as CaCO3)	mg/l	100
Grease	mg/l	500
Total coliform	CFU 100 m/l	97 -108
Volatile Organic compounds (VOCs)	mg/l	100 – 400
Aluminium (Al)	**mg/l**	0.5 – 0.8
Boron (B)	**mg/l**	0.1 – 0.4
Fluoride (F)	**mg/l**	0.88 – 0.90
Manganese (Mn)	**mg/l**	0.2 – 0.4

	Pb	Zn	Cd	Cr	Cu	Ni	As
Minimum	399.30	182.00	92.20	218.05			0.005
Maximum	468.30	285.00	151.00	290.40			2.05
Safe limit	250– 500	300–600	3–6	--------	135–270	75–150	1 – 40

Table 2. Thermal power plant effluent from NTPC (TPPE)

Considering different source water and their effect on morphological parameters like percentage of germination, root length (RL), shoot length(SL) inter nodal length(INTL) and leaf size (LS) of *Momordica and Abelmouschus* plants for 2 , 4 and 6 days the waste water collected from NTPC power plant Table 3 and 4. The waste water contains too much toxic elements which increase or decrease according to the nature of the effluents and the place of collection. The nature of thermal power effluent of NTPC , proved harmful to the morphological parameters of the two selected species. Bhattacharyya, 2014. In our study we used the waste water collected from different sources from Farraka in a crude way without any dilution and studied its effect.

SOURCE OF WATER	GERMINATION %	TREATMENT 2 DAYS				TREATMENT 4 DAYS				TREATMENT 6 DAYS			
		RL	SL	INTL	LS	RL	SL	INT L	LS	RL	SL	INT L	LS
Normal Ganga water	100	5.9	10.5	8.2	2.5	7.0	10	8.5	2.7	12.5	11.2	6.0	2.9
Normal control	100	10.7	11.3	9.5	3.8	12.2	11	9.2	3.7	12.5	11.9	9.9	3.4
Thermal power plant effluent water NTPC	45	7.5	8.5	4.1	1.3	5.7	5.0	5.2	2.9	10.2	7.0	6.9	2.9

Table 3. Waste water treatment of *Momordica charantia*

SOURCE OF WATER	GERMINATION %	TREATMENT 2 DAYS				TREATMENT 4 DAYS				TREATMENT 6 DAYS			
		RL	SL	INTL	LS	RL	SL	INTL	LS	RL	SL	INTL	LS
Normal control	100	10.2	13.7	14.7	1.2	9.2	17.8	16.3	1.9	10.5	17.5	15.7	2.2
Normal Ganga water	100	7.3	8.5	12.5	1.2	5.4	11.5	9.3	1.0	9.3	6.3	5.4	1.2
Thermal power plant effluent water NTPC	35	8.5	10.0	13.3	1.5	8.1	16.5	15.1	1.7	7.0	14.4	13.5	2.0
DATA OF MEAN OF 10 REPLICATES													

Table 4. Waste water treatment on *Abelmoschus esculentus*

1	2	3	4	5	6
2.13	1.98	1.0	1.45	0.98	1.12

Table 5. Chlorophyll / *Momordica*

1	2	3	4	5	6
1.75	1.70	1.0	1.15	0.78	1.13

Table 6. Protein / Momordica

1	2	3	4	5	6
1.58	1.55	1.12	1.45	0.85	1.12

Table 7. Chlorophyll / Abelmuschus

1	2	3	4	5	6
1.14	1.14	0.85	1.12	0.55	1.0

Table 8. Protein / Abelmuschus

Table 5 – 8 or Figure 1 to 4 showed the concentration of Chlorophyll and Protein in two selected species. Both the species exhibited the reduction in concentrations under NTPC effluent waste water. The data of day 1 showed the effect of normal Ganges water. In *Momordica* the effect of waste water from different place of Farakka region on the cholorophyll and Protein level showed maximum degradation of those two parameters in Farakka sewage and NTPC waste water (Ghosh et al. 2014). In *Abelmouschus* the effect of waste water from different place of Farakka region on the cholorophyll and Protein level showed maximum degradation of those two parameters in Farakka , NTPC waste water.

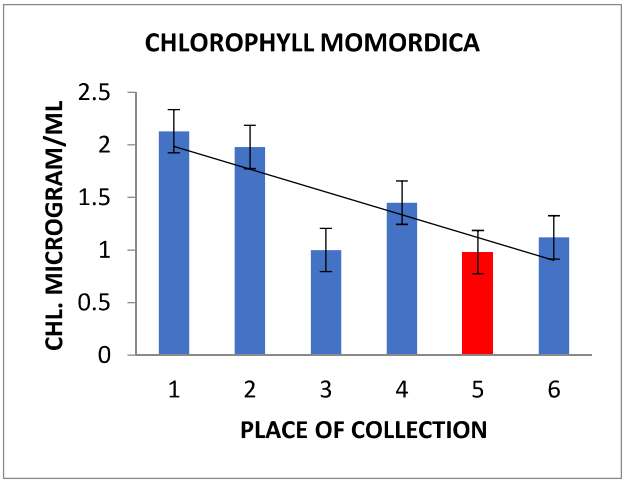

Figure 1

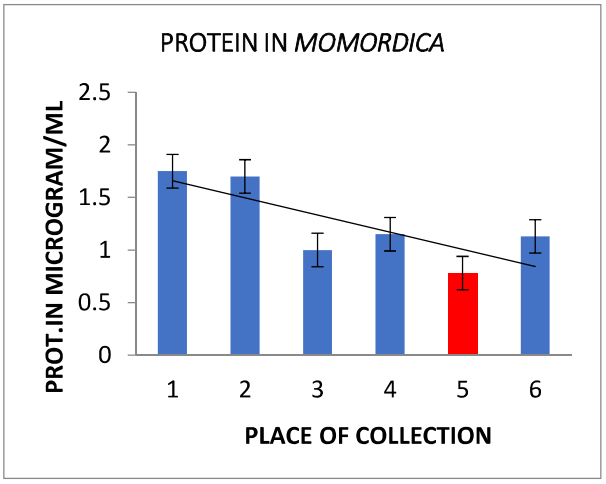

Figure 2

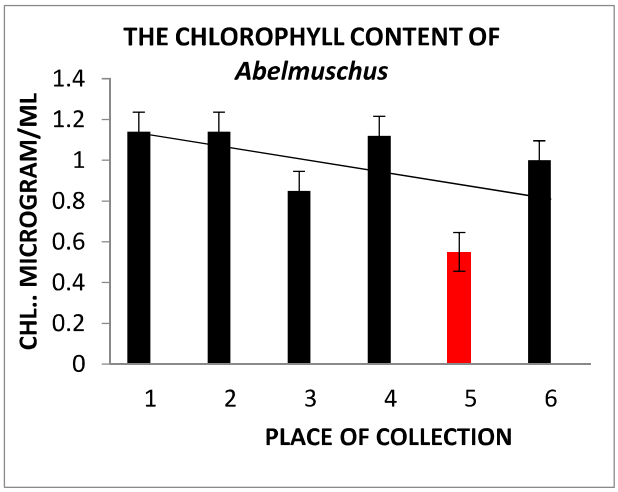

Figure 3

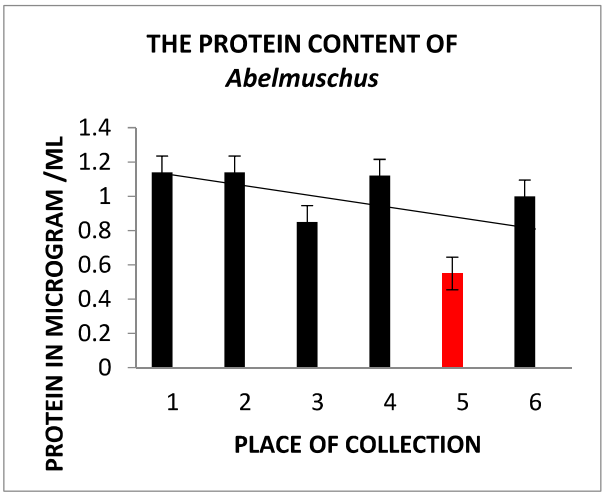

Figure 4

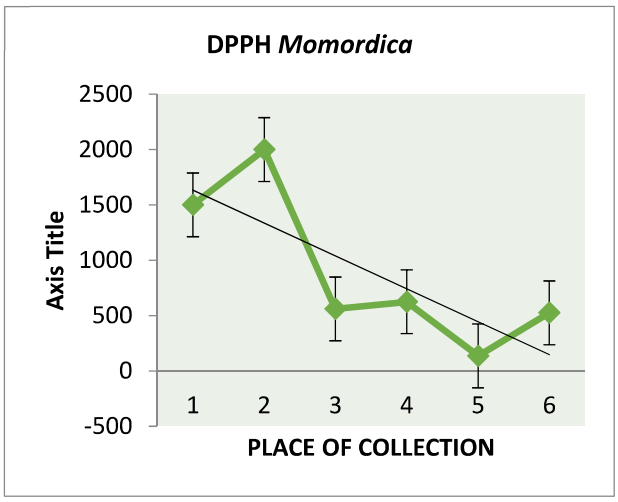

Figure 5.

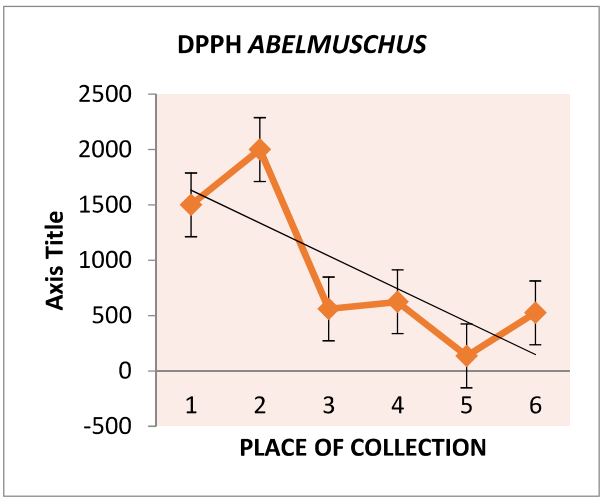

Figure 6.

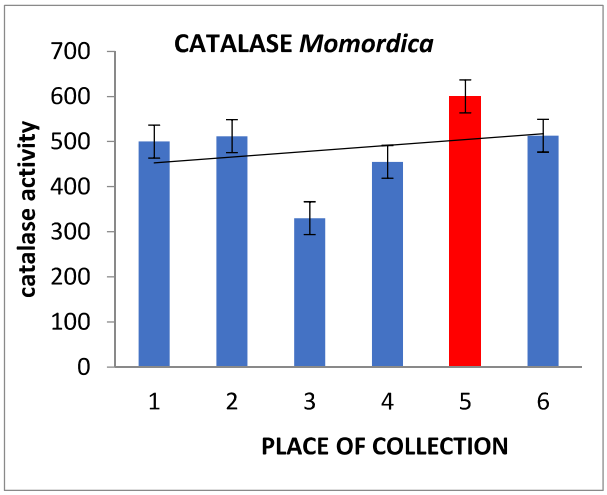

Figure 7

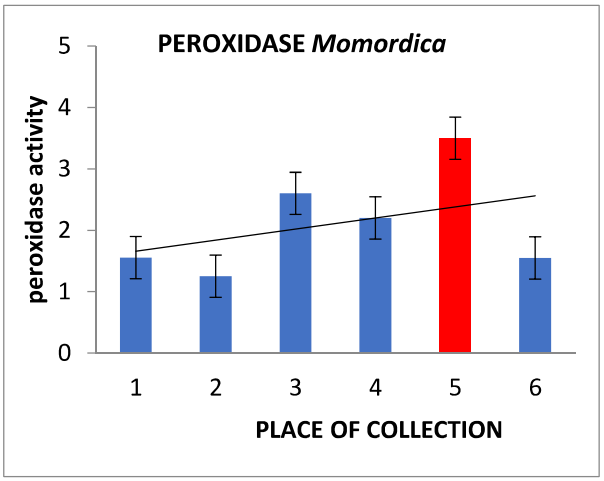

Figure 8

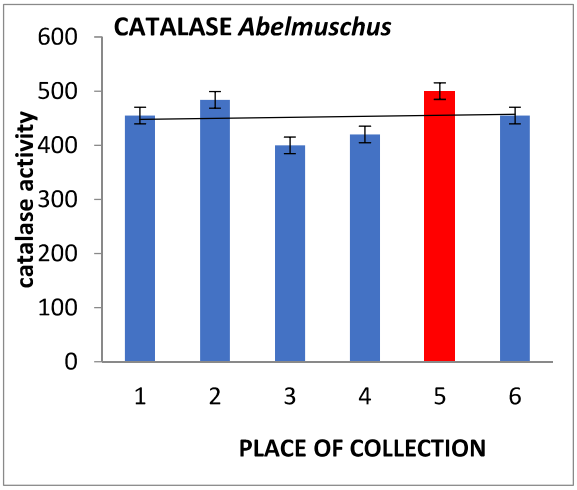

Figure 9

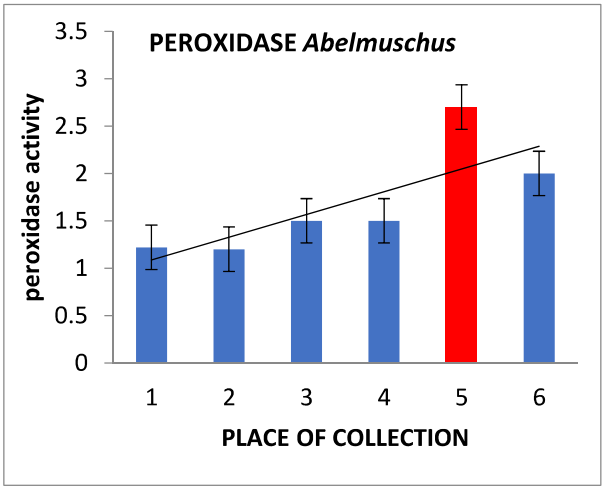

Figure 10

In Figure 5 and 6 it was observed from the curve of DPPH that highest inhibition took place in the NTPC waste water treated *Momordica* plant extract. The same observation was made using *Abelmouschus* plant extract which support the waste water related pollution in those plant metabolic

system. Accumulation of toxic heavy metals leads to stress conditions in the plant system by interfering with the metabolic activities and physiological functioning of the plants. Heavy metals are known to cause membrane damage, structural disorganization of organelles, impairment in the physiological functioning of the plants and ultimately growth retardation. Heavy metals stimulate the formation of reactive oxygen species (ROS) such as superoxide radicals (O_2^o), hydroxyl radicals (OH^o) and hydrogen peroxide (H_2O_2) either by transferring electron involving metal cations or by inhibiting the metabolic reactions controlled by metals. In order to survive under the stress condition, plants have enzymatic and non enzymatic antioxidants to scavenge free radicals.

The level of Anti-oxidant can be measured by means of DPPH [1,1- diphenyl-2- picrylhydrazyl] assay. This assay was calculated on the basis of $IC_{50.}$ In our experiment the normal control and treated selected plant species by the different waste water were observed at the level of Anti oxidant synthesis. DPPH is used as a substrate to evaluate anti oxidative activity of antioxidant. The method is based on the reduction of methanol DPPH solution in the presence of a hydrogen donating antioxidant due to formation of the non radical form DPPH-H by the reaction. The extract was able to reduce the stable radical DPPH to the yellow coloured diphenyl picrylhydrazine. it has been found that cysteine , glutathione, reduce and decolorize DPPH by their hydrogen donating capability. Blois, 1958.

Waste water treated plant peptide extracts , low molecular weight (3.0 to 0.5 kDa) were analyzed for their effect on seed germination study of ***Vigna catjung*** L. The effect remained inhibitory on seed germination (100% inhibition) but in normal water of the Ganges it showed 90% of the effective germination. However , the peptide extract have high anti - oxidative capacity. This capacity is induced due to stress effect of the waste water (effluent of NTPC) which in support of the observation of Jha et al. (2018).

When we studied effect of waste water on Catalase and Peroxidase activities , free radical scavenging enzymes it was observed that the level of their synthesis increased in NTPC sewage water , Farakka sweage and Belgharia waste water. That has close resemblance with the analysis of these water samples where the presence of heavy metals increased the synthesis of antioxidative enzymes. Heavy metals stimulate the formation of reactive oxygen species (ROS) such as superoxide radicals (O_2^o), hydroxyl radicals (OH^o) and hydrogen peroxide (H_2O_2) either by transferring electron involving metal cations or by inhibiting the metabolic reactions controlled by metals. In order to survive under the stress condition, plants have enzymatic and non enzymatic antioxidants to scavenge free radicals. (Figure 7 – 10)

Acknowledgement :

We do acknowledge the kind cooperation and support of Dr. Manideep Chandra, Honourable Principal of Shibpur Dinobundhoo Institution (College) and also the energetic support and help of the Professors of PG Department of Environmental Science, Asutosh College and UGC, GOI for the smooth execution of the project work.

References :

Blois , M. 1958, Antioxidant Determinations by the Use of a Stable Free Radical Nature : 181, 1199–1200.

Britton C, Mehley AC. [1955] Assay of catalase and peroxidases. In: Colowick, S.P., Kalpan, N.O. (Eds.), Methods in Enzymology, assay of catalase and peroxidase, Academic Press Inc., New York, Vol. II, pp. 764–775.

Ghosh , A; 2014, Int J Pharm Pharm Sci, Vol 8, Issue 1, 428-432

Jha , S. 2018, In vitro antioxidant and antidiabetic activity of oligopeptides derived from different mulberry (Morus alba L.) cultivars . 10 : 4 - 361-367

Kazi , H , Bhattacharyya , P and Ghosh , A . 2014 . Effect of city drain water on low molecular weight peptide profile (0.5 – 3.0 kDa) in relation to germination and antioxidant parameters of common edible seeds. Ind. J. Mul. Acad. Res. 1: 9-16.

Khan, M. A. ; Weber, D. J., 2008. Ecophysiology of high salinity tolerant plants. In: Khan, M. A.; Weber, D. J., Tasks for Vegetation Science vol 40, Springer

Kisku et al., 2000; Uptake and accumulation of potentially toxic metals (Zn, Cu and Pb) in soils and plants of Durgapur industrial belt. Journal of Environmental Biology 32(6):831-8

Lichtenthaler and Wellburn Biochem.Society.Trans.603.531.1983

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (November 1951). "Protein measurement with the Folin phenol reagent". J. Biol. Chem. 193 (1): 265–75.

Malaviya P.S. and VJ Rathod ;2007, Seasonal variations in different physico-chemical parameters of the effluents of Century Pulp and Paper Mill, Lal Kuan, Uttarakhand. Journal of Environmental Biology 28(2):219-24

Wahid , A; 2004, Toxic and osmotic effects of NaCl on sugarcane Bot. Bull. Acad. Sin.) 45: 133-141.