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A Study on Aquatic Macrophytes and Their Role as an Indicator of Water Pollution in Darrang District, Assam, North East India

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Abstract

Pollution is one of the highly discussed subjects globally in the recent past due to its adverse impact on the ecosystem. Many organisations have been managing plants as an indicator of pollution maybe it is soil, water, or air. In view of the above, a study was endeavoured to understand the role of aquatic macrophytes in understanding the water pollution in Darrang district of Assam between 2017-18 years. As many as four plant species namely *Commelina benghalensis* L., *Hygroryza aristata* (Retz.) Nees ex Wight & Arn., *Sagittaria sagittifolia* L. and *Enhydra fluctuans* Lour. were selected based on their availability around the year in the region. For the present study, two sampling sources were selected namely Bega River flows in the middle of Mangaldai town and the other was a channel/stream of Brahmaputra River near Ganderimari and Mowamari Chapari. The physico-chemical properties of the water were analysed to assess the water quality of the water bodies. Morphological and anatomy of the leaf of the plants were done to ascertain the effects of pollution. The result shows the pollutants have influenced greatly in the growth of the plants studied with a varied range.

Keywords: Water Pollution, Physicochemical Properties, Plant Growth, Morphological Changes, Chlorophyll Content, Stomatal Size.

Introduction:

Water is the most crucial element needed for living beings to survive on this planet. The advancement of societies on this planet is being determined by the water bodies or availability of water resources from ancient times. Plants' photosynthesis process is also incomplete without water molecules. On earth, only 3% of the total water of the planate is fresh water and only 1.2% of the water can be used as drinking water. Various aquatic factors such as biological oxygen demand (BOD), electric conductivity (EC), dissolved oxygen (DO), pH (acidic or basic), total coliform, turbidity, total dissolved solids (TDS) etc. are the main indicators of water quality.

With the advancement of societies, industrialisation, urbanisation etc. in modern times water pollution is a growing problem. Water pollution occurs in all types of water bodies; both freshwater bodies like ponds, lakes, and rivers as well as in marine bodies like coastal and deep-water seas. Major causes of water pollution are the deposition of acid, organic sewage, detergents, agricultural chemicals, industrial effluents, silt, oil, and heat into the water bodies. Some of the major water pollutants are cadmium (Cd), arsenic (As), copper (Cu), chromium (Cr), mercury (Hg), lead (Pb), nickel (Ni), molybdenum (Mo), zinc

(Zn) etc. which gets into water sources from different industries, e-waste, fuel spills, medical waste, mining, smelters, thermal power plants. (Verma, 2013)

It has been observed that plants growing in urban areas are affected greatly by varieties of pollutants (oxides of nitrogen and sulphur, hydrocarbons, particulate matter, hydrogen fluoride, phenoxyacid nitrate etc). The rapid addition of toxic substances to the environment is responsible for altering the ecosystem and ultimately affecting the producers i.e., the plants. Deposition of acidic substances causes acidification of water by lowering its pH value below 7. Thus, low pH causes degradation of water quality and further nutrient deficiency and the consequent general reduction in the abundance of aquatic plants in the affected water body. Also, the values of dissolved oxygen, alkalinity, conductivity, total dissolved solute, and other chemical properties of the water are affected by the pollutants (Dwivedi A. K., 2017). Physical properties of the water like colour, taste, odour etc. are also altered due to pollution. Water pollution causes changes in species composition. The number and abundance of acid-tolerant species increase while those of sensitive species decrease. Macrophytes are generally absent in extremely acidic water (Mearns A. et al., 2018). Water bodies are generally polluted by pollutants such as heavy metals, organic matter, sewage from both industrial and domestic sources, environmental conditions, biomedical waste, e-waste etc. The pollutants affect the aquatic plants in many ways resulting in the plants adaptation to the new conditions. As a result of these adaptations, various morphological and anatomical as well as physiochemical changes occur (Nivova D.J. et al., 1983). The roots of aquatic plants are generally affected adversely in highly acidic water and result in poor plant growth and later death of the plant species. Plants with deep water roots and rhizomes are less affected, while plants with short root systems are severely affected. One of the most common impacts of pollution is the gradual disappearance of chlorophyll and concomitant yellowing of leaves, which may be associated with a consequent decrease in the capacity for photosynthesis (Swami A., 2018). Exposure to pollutants causes a reduction in the concentration of plant photosynthetic pigments viz. chlorophyll and carotenoids, which affects the plant productivity, germination of seeds, length of pedicles and the number of flowers or inflorescences (Elisabeth et al., 2006). The addition of organic matter and its rapid decomposition resulted in increased nutrients in the water. The supply of many nutrients causes nutrient enrichment of the water body. In such conditions, planktonic green algae and blue-green algae grow very rapidly causing water blooms. In addition to these many types of hydrophytes like Salvinia, Azolla, Eichhornia etc. also become abundant. All this rapid growth of planktonic and free-floating hydrophytes reduces light penetration into deeper layers of the water body and as a result the submerged flora gradually declines (Gabrielle T. et al., 2006) Various detergents from domestic and industrial use directly released and washed down into the water bodies also cause serious effects on the plants.

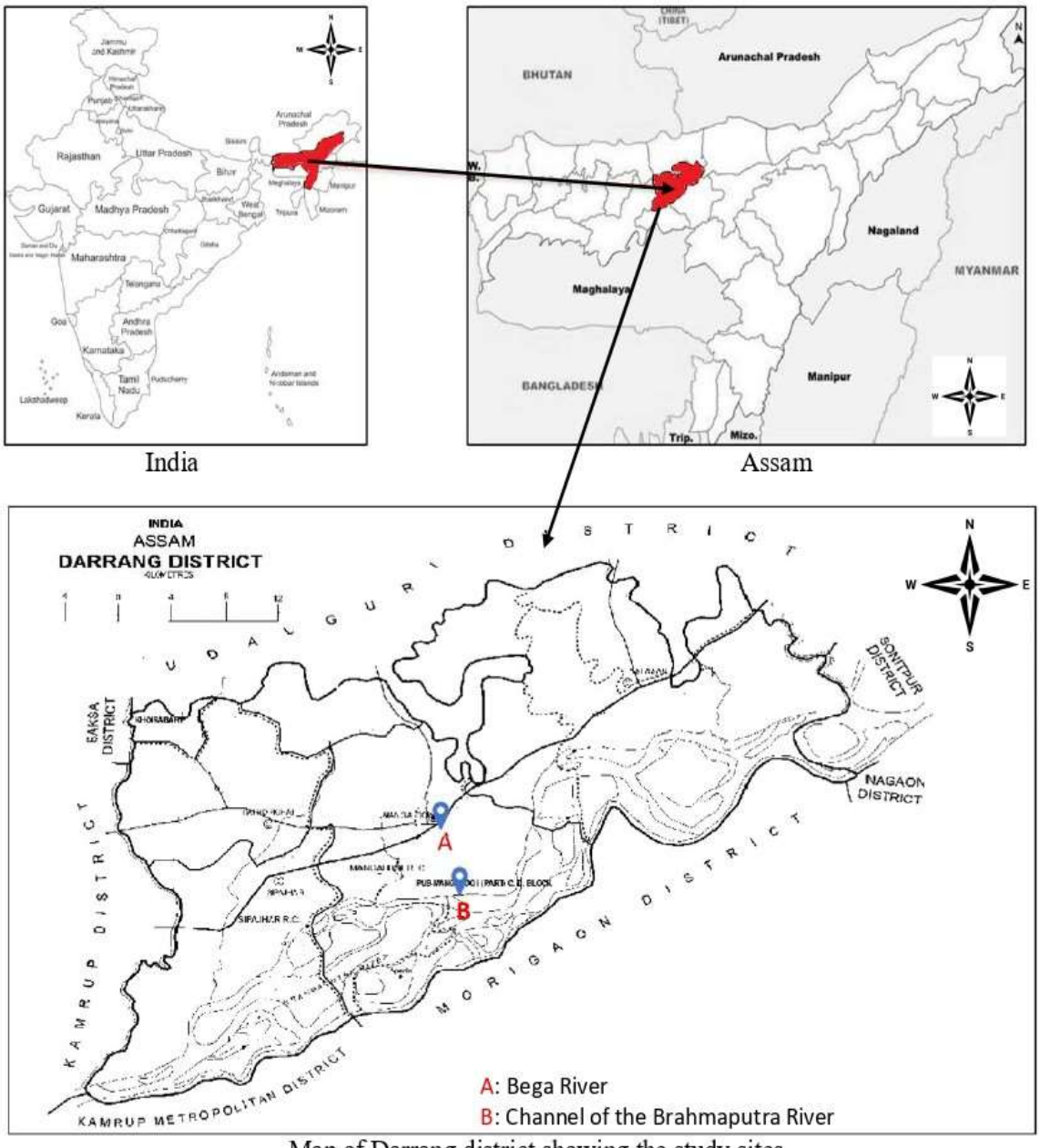
Nowadays, pollution has increased tremendously, affecting the proper growth of plants in its vicinity. The leaf is the sensitive part which is affected by air pollution directly instead of all other plant parts such as the stem and root, pollutants can directly affect the plant by entering the leaf, destroying individual cells, and reducing the ability to produce food. Air pollutants are absorbed by leaves through stomata, while particulates get deposited on their surfaces. Over the years, there has been a continuous increase in human population. Road transportation, vehicular traffic and industries which has resulted in the pollution of air, soil, and water. Hydrophytic plants have a very large surface area and trapping device (Uddin S., 2018), through which the pollutants affect the aquatic plants. It is a well-known fact that the structure of aquatic vegetation in rivers can change because of both nutrient enrichment (Sánchez-Carrillo S., Álvarez-Cobelas, M., 2010) and the presence of pollutants (Bernez I. et al., 2001). In aquatic ecosystem plants get

affected by the pollutants present in the water which enters their body through absorption. In some cases where air pollution is low but water pollution level is high, some plants get themselves adapted to accumulate the pollutants, and their productivity level or rate of photosynthesis increases or decreases as those plants can absorb enough nutrients from their sources (Joshi D.M. et al., 2009). The pollutants that reach the water bodies, thus affect the aquatic plants in various aspects. This causes the plants to adapt to the new situation and because of these adaptations, various morphological, anatomical, and physiochemical changes occur. But because of their adaptive nature, some changes take place in their morphology and anatomy. For example, the size of foliage, length of petiole or stem or internodes and nodes, length of root, the diameter of stem, petiole or root, different colouration etc. varies with the changing environmental pollution level. Similarly, changes in anatomical characters such as root or stem anatomy, anatomy of leaf, stomata and their distribution on leaf etc. also takes place. Plants are always dependent on different environmental conditions and their effects are always reflected in the plants in their growth and development. The growth and development of the plants are very much unaffected or unhampered in a normal environment. But, when changes in the environmental factors (biotic and abiotic) occur, the growth of plants gets affected. To adapt themselves, the plants try to survive in such conditions by making changes in their physiology (Stevovic S. et al., 2010). In a aquatic ecosystem algae are the unavoidable participants of the ecosystem. Algae are very sensitive to pollutants and they readily accumulate pollutants through their metabolism process. The algal metabolism is also very sensitive to the variations in environmental and natural disturbances (Omar W. M. W., 2010) (Devikrishna C. S. et al., 2001).

Materials and methods

1. Study Site: In the present investigation, two different water bodies of Darrang district were selected. The first study area was the Bega River flowing through the middle of Mangaldai town and the other study area was a channel of the Brahmaputra River near Ganderimari and Mowamari Chapari. From Bega River, three different sites were selected as study sites for the collection of water samples as well as plant specimens. Also, from the Channel of the Brahmaputra River, three different sites were selected for the same purpose. Water samples from these six sites were collected for analysis to identify the quality of water.

Fig1: Study site location



Map of Darrang district showing the study sites

2. Study of water quality: For the assessment of the water quality of the selected sites, the following physiological and physico-chemical parameters of water were selected and tested using standard/ established protocols.

1) Colour and Odour
2) pH
3) Dissolved Oxygen
4) Total dissolved solid
5) Alkalinity
6) Free CO_2

3. Collection of the plant samples: The plants selected for the study can be found in the study sites whole year. The plants were collected during the same phenolic stage for the comparison study. Mature plants

were selected for the study and leaf samples were collected from the middle of the mature plants. The plants selected for the study are *Commelina benghalensis* L., *Hygroryza aristata* (Retz.) Nees ex Wight & Arn., *Sagittaria sagittifolia* L. and *Enhydra fluctuans* Lour.

4. Study of periphytic algae: Algae are very much sensitive to the quality of the water. To check the microscopic algae species that are growing at the water bodies water samples from both the sites were tested under the microscope and the algal species were documented.

5. Estimation of total chlorophyll content: The chlorophyll content of the plant samples was estimated by using the standard method described by Zilha A. et al. (Zilha A. et al., 2016). The values of chlorophyll content were measured in milligrams per gram of leaf tissue. Fresh mature leaves were taken for the estimation of the chlorophyll.

6. Macro-morphology study: For the morphological study, the colouration of various parts, length and breadth of plant parts, diameter area of leaves were recorded by externally observing the plants. The length and breadth of various plant parts were observed and noted using a measuring scale.

To calculate the leaf area, the outline of the leaf of the selected plant sample was drawn by keeping it on graph paper.

Leaf area = number of small squares covered by the leaf × area of a small square

7. Micro-morphology study:

7.1. Foliar epidermal study: For the epidermal study, the upper and lower epidermis of the leaves are peeled using the hand peel technique. For ease of peeling the epidermises of leaves were dipped into the peel solution for about half an hour and the epidermis was peeled off.

Imprints method using nail polish: Each leaf was painted with fingernail polish on both the adaxial and abaxial surfaces and allowed to dry. After drying, the nail polish coat is carefully scrapped out from the leaf epidermis. Epidermal strips were taken from the median portion of matured leaves, stained in alcoholic Safranin, and mounted in 50% glycerine jelly for microscopic examination. Epidermal strips from both the adaxial and abaxial surfaces were prepared and mounted separately. Photographs of good preparations were taken.

7.2 Stomatal Index: Stomatal index is calculated by using the following equation.

$$S\,I = \frac{S}{S+E} \times 100$$

(S I = Stomatal index, S = No. of stomata per unit area (Stomatal frequency) E = No. of epidermal cells in the same unit area)

8. Anatomical study:

T.S of leaf: - For micromorphological studies, free-hand sections of leaf and petiole were cut, cleared, and stained with safranine. Numerous temporary and permanent mount of the microscopical section of fresh leaf was made. Then observed under the binocular and trinocular microscope. Photomicrographs of the microscopical section were taken with the help of a trinocular microscope.

Result and discussion:

The experimental findings reveal the following results, which are discussed below. The average data sets from the six plant collection sites were taken.

Water quality analysis:

Table 1: Results of Various Parameters of Water Quality Test (Average Values)

SL No	Sources	Physical-chemical properties						
		Colour	Odour	pH	D.O. Mg/L	Free CO_2 Mg/L	TDS Mg/L	Alkalinity
1	Brahmaputra River channel	Colourless	Stinky	7.23	12.56	42.24	154	179.5
2	Bega River	Brownish white	Odourless	8.59	6.28	64.24	865.6	285.52

The values of these parameters in the water samples show that the water from the Bega River is polluted and unusable and the water samples collected from the Brahmaputra River channel are almost in the range of normal usable water quality.

Study of periphytic algae: The occurrence of algal genus in the plants from both polluted and non-polluted sources are tabulated below-

Table 2: Algae species Found Associated with the Selected Plant Specimen from the Study Sites

Sl. No.	Plant species	Associated algae species	
		Non-polluted site	Polluted site
1	*Commelina benghalensis* L.	*Desmi, Diatom, Lyngbya, Spirogyra*	*Lyngbya*
2	*Hygroryza aristata* (Retz.) Nees ex Wight & Arn.	*Anabaena, Diatom, Nitella, Spirogyra*	*Diatom, Spirogyra*
3	*Sagittaria sagittifolia* L.	*Desmid, Diatom, Spirogyra*	*Spirogyra*
4	*Enhydra fluctuans* Lour.	*Diatom, Spirogyra, Vaucharia*	*Spirogyra, Lyngbya*

In the study, the number of algal species was found higher in the non-polluted sites (channel of the Brahmaputra River) in comparison to the polluted sites (Bega River). Algal species abundance also shows the quality of the water from the two sources. The occurrence and number of periphytic algae also supports that the water quality of the Brahmaputra River Channel provides favourable condition for algal growth in a non-polluted aquatic ecosystem system.

Chlorophyll content analysis:

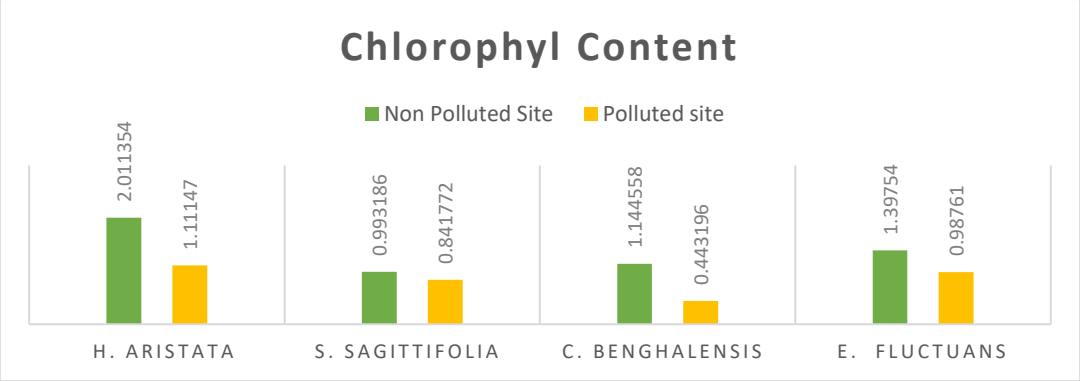
Table 3: Chlorophyll Content of The Selected Plants from the two Study Sites.

Sl No.	Plant species	Total Chlorophyll content (mg/gram of tissue)	
		Plant from Non-polluted source (Brahmaputra River channel)	Plant from Polluted source (Bega River)
1	*Commelina benghalensis* L.	1.144558	0.443196

	Hygroryza aristata (Retz.) Nees ex Wight & Arn	2.011354	1.11147
3	*Sagittaria sagittifolia* L.	0.993186	0.841772
4	*Enhydra fluctuans* Lour	1.39754	0.98761

The findings are graphically represented below

Fig2: Graphical representation of the chlorophyll content



The quantitative estimation of total chlorophyll content in the leaf of the selected plant specimen from the non-polluted and polluted source differentiation. The total chlorophyll content in the plant samples from the non-polluted source is found to be more than that of polluted source samples.

Name of the plants	Source of Collection	Leaf				Stem (internode)		Root	
		Length (cm)	Breadth (cm)	Area (cm^2)	Colour	Length (cm)	Colour	Length (cm)	Colour
Commelina benghalensis L.	Brahmaputra River	5.1	1.84	62.58	Green	4.72	Light Green	11.4	Brown
	Bega river	7.48	2.08	111.24	Dark Green	4.98	Dark Green	5.62	Brown
Hygroryza aristata (Retz.) Nees ex Wight & Arn	Brahmaputra River	5.06	1.46	54.24	Green	4.5	Brown	5.7	Brownish Green
	Bega River	3.7	1.32	43.16	Yellowish green	5.8	Brown	3.68	Brownish Green
Sagittaria sagittifolia L.	Brahmaputra River	11.48	3.36	241.86	Green	29.06	Green	7.98	White

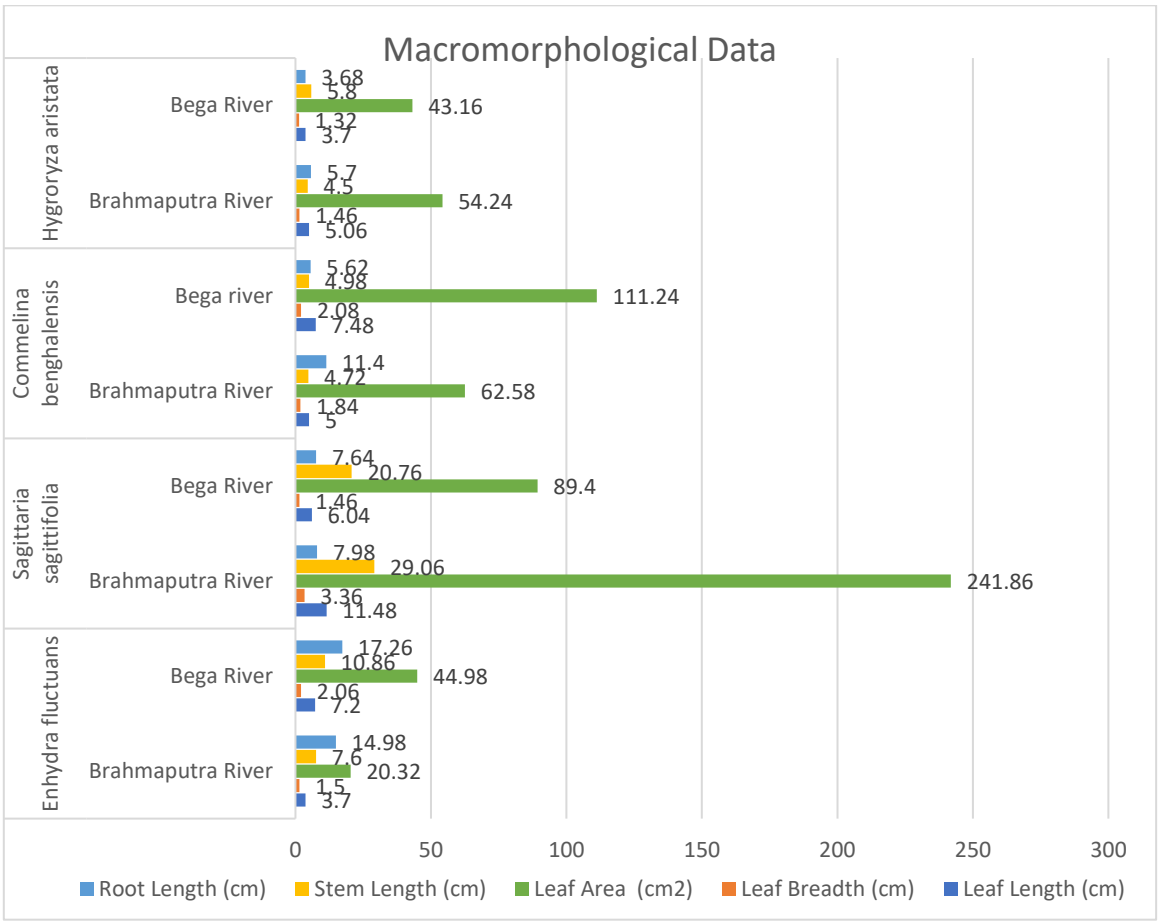
	Bega River	6.04	1.46	189.4	Light Green	20.76	Light Green	7.64	White
Enhydra fluctuans Lour	Brahmaputra River	3.7	1.5	20.32	Green	7.6	Reddish Brown	14.98	White
	Bega River	7.2	2.06	44.98	Light Green/ Yellowish Green	10.86	Light Green	17.26	White

Macro morphology study: Findings of the macromorphological observation are tabulated below –

Table 4: Macro-Morphological Features of The Selected Plants from the Study Sites

The findings of the macromorphological observation are graphically represented below-

Fig3: Graphical representation of the macromorphological findings



Micromorphological Data: Findings of the micromorphological observation are tabulated below –

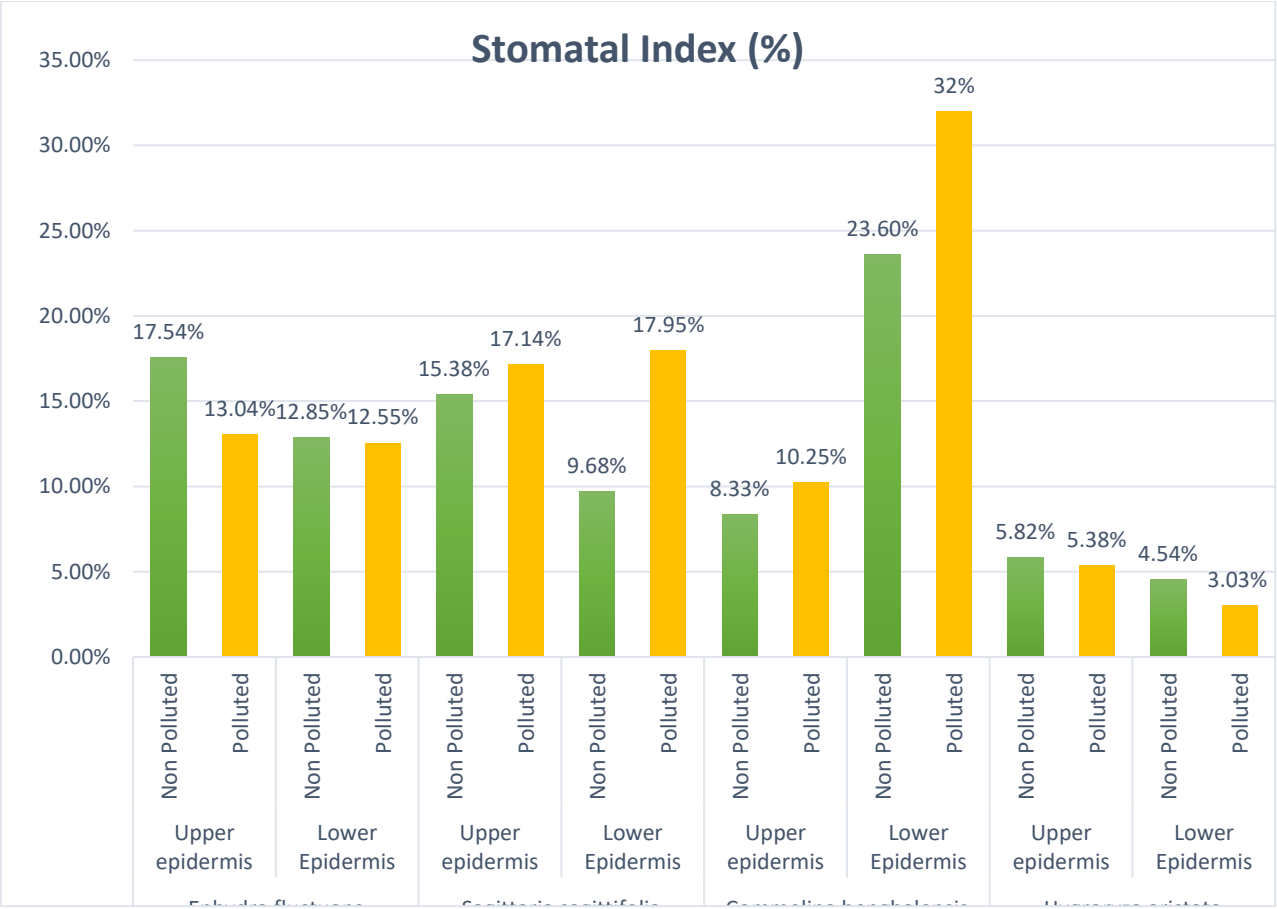
Table 5: Micro-Morphological Features of The Selected Plants from the Study Sites

Name of the plants	Source of Collection	Micromorphology of Leaf (Average Values)					
			Epidermal cell size (µm)	Size of stomata (µm)	Thickness of the epidermal	Thickness of photosynthetic layer (µm)	Stomatal Index (%)

					layers (µm)		
Commelina benghalensis L.	Brahmaputra River	Upper epidermis	31.54×19.54	24.42×6.49	35.312	48.643	8.33%
		Lower epidermis	17.43×30.21	24.04×5.17	30.208		23.60%
	Bega River	Upper epidermis	32.13×15.76	21.4 ×6.14	33.572	63.27	10.25%
		Lower epidermis	31.4×17.76	25.10×6.7	31.443		32.00%
Hygroryza aristata (Retz.) Nees ex Wight & Arn	Brahmaputra River	Upper epidermis	32.13×19.87	13.77×7.30	5.556	94.535	5.82%
		Lower epidermis	28.3×18.76	12.02×7.12	10.854		4.54%
	Bega River	Upper epidermis	29.73×19.54	14.28×7.35	8.95	67.64	5.38%
		Lower epidermis	17.43×30.21	13.627×7.85	8.84		3.03%
Sagittaria sagittifolia L.	Brahmaputra River	Upper epidermis	50.1×75.87	33.59×4.12	17.285	127.43	15.38%
		Lower epidermis	76.32×48.12	33.65×4.19	25.804		9.68%
	Bega River	Upper epidermis	51.32×74.34	29.50×11.32	14.89	140.89	17.14%
		Lower epidermis	49.65×75.5	25.64×11.22	15.21		17.95%
Enhydra fluctuans Lour.	Brahmaputra River	Upper epidermis	30.54×10.54	15.64×4.65	16.64	140.11	17.54%
		Lower epidermis	28.43×10.26	14.37×4.12	9.811		12.85%
	Bega River	Upper epidermis	32.63×17.65	16.45×4.84	13.23	129.57	13.04%
		Lower epidermis	30.4×14.84	14.16×4.71	13.87		12.50%

The findings of the stomatal index of the observed plant samples from the two different water bodies are graphically represented below-

Fig4: Graphical representation of the stomatal index



A comparative analysis was conducted for the macro-morphology and micro-morphology of the selected plant species from the non-polluted and polluted sites.

In the study of *Commelina benghalensis* L. differences in colouration of leaves, petiole, and roots in the species from the two different rivers was observed. The specimen from the polluted source shows dark green leaves as well as dark green internodes. In the observation of the structure and size of the petiole and stem differences among them is also found. The size of the petiole of the specimen from polluted source is larger than that of non-polluted site. The size of the internodes in sample from polluted sites is longer in comparison to that of the non-polluted specimen (Table 4). The roots of the plant sample from the non-polluted site were found to be longer than that of the plants from the polluted site. (Table 4).

The study of the micro morphological characters of the leaves shows that structure and size of the epidermal cells in both the specimen is almost same. The plant species growing in the non-polluted environment have got less stomatal index than the plants from polluted site. (Table 5).

In the plant samples of *Hygroryza aristata* (Retz.) Nees ex Wight & Arn, the colouration of leaves, petioles and roots are different in the plants from the two rivers (Table 4). In the non-polluted site coloure of the specimens leaves is green or light green. But the specimen from the polluted source shows yellowing leaves. In the structure and size of the petiole and stem differences among them is also found. The size of the petiole of the specimen from non-polluted source is larger than that of the polluted source. The size of the internodes in sample from the polluted source is longer in comparison to non-polluted specimen (Table 4). The plant from the polluted source contains longer roots than the non-polluted specimen (Table 4).

Micro morphological observation of the leaves shows that the plant species growing in the non-polluted environment have higher stomatal index than the plants from polluted site. Though the difference between

them is not very much. Structure and size of the epidermal cells in both the specimen is almost same. Stomatal cells are a little bit bigger in case of the specimen from polluted site in comparison to that of the non-polluted site (Table 5).

In the observation of the macro morphological features of the samples of *Sagittaria sagittifolia* L. from the two sites, differences were observed in them especially in their colouration of leaf and petiole. Plants from the non-polluted water body shows clean green colouration of leaf and petiole while the plants samples from the polluted source shows light of pale green colouration. Also, the average size of the leaves from the non-polluted waterbody shows bigger leaf area (Table 4). The Micromorphology of the leaves shows that the plant species growing in the polluted environment have bigger stomatal index than the plants from non-polluted site (Table 5). Photosynthetic layer in the plant samples from the polluted site is thicker in comparison to that of the nonpolluted site plant samples (Table 5).

In *Enhydra fluctuans* Lour. plants from the non-polluted site, the colouration of leaf is green. But in the specimen from the polluted source shows light green or yellowish leaves. The size of the petiole also shows differences. The average leaf area of the plant samples from the polluted site is found to be bigger than that of the nonpolluted site (Table 4).

Micro morphological study of the leaves shows that the photosynthetic region of the plant samples of the nonpolluted site is thicker than that of the non-polluted site plant samples. The structure and size of the epidermal cells in polluted site's specimen is found to be larger than those of the non-polluted site. Stomatal cells are a little bit larger in case of the specimen from polluted source (Table 5).

The yellow colouration of the leaves in the plant from polluted sites may be because of pollutants present in that environment. Svetlana et. al. (Stevovic S. et al. 2014) has commented the same in his research work. Also, the differentiation in the size and structure of petiole, stem and root may be due to the adaptive responds of the plants to overcome the environmental stress due to changes regarding pollution which is similar with the findings of Mahajan S. (Mahajan S., 2015) regarding plants' adaptation to environmental factors. Haworth and his companions (Haworth et. al., 2010) has commented that stomata get affected by the pollutants. Observation on differentiation in the stomatal index because of pollutants on the leaf in the present investigation in all the plants is also similar. In case of *Commelina benghalensis* L. the perfect adaptation and accumulation of organic pollutants can be the reason of perfect growth and development of the species in the polluted area. Smith et. al. (Smith et. al., 1999) has stated when the level of organic pollution is high in the water, the plants show eutrophication. This is confirmatory to luxuriant growth of *Commelina benghalensis* L. in the polluted water body.

Conclusion:

The environmental conditions affect the physiological and metabolic processes of the hydrophytic plants. In the present study it was tried to understand the changes that occurs in the plants due to the pollution and to know the adaptive capability of the selected plants in a polluted environment. As well as by observing the characteristics of the plants we can get a perspective regarding the environmental status of the waterbody where the plant is growing. This will provide data for an indication regarding the quality of water body. The characteristics possessed by the plants in polluted and non-polluted study area might be used for the biomonitoring of the environmental changes of water body. The more detailed study on the physiochemical changes of plant parts and accumulation of pollutants by the adaptively compatible

plants might serve the purpose of identification of indicator species for water pollution. There is a prospect to use such plants for bioremediation and restoration of degraded aquatic ecosystems.

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