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Research Article

IMPACT OF AQUATIC POLLUTION ON THE TESTICULAR CELLS OF FRESH WATER CRAB *BARYTELPHUSA CUNICULARIS* (Westwood, 1836)

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ABSTRACT

Aquatic organisms are susceptible to contamination of organic and inorganic discharge. Physico-chemical parameters as Temperature, pH, Dissolved Oxygen, Free Carbon dioxide, Hardness, Chemical Oxygen Demand, Total Solids, Total Dissolved Solids, Suspended Solids, Chloride, Alkalinity, Nitrate, Phosphate and contamination of heavy metals like Fe^{+}, Na^{+} and K^{+} has direct or indirect impact over the metabolic content of targeted cells. Altered physico-chemical parameters influences gametogenic mechanism of crab *Barytelphusa cunicularis.* Testicular cells of *Barytelphusa cunicularis* showed hypertrophy but has reduced content of gametogenic cells leading to infertility. The obtained results were discussed in relation to influence of physico-chemical parameter over reproduction of crab *Barytelphusa cunicularis*.

Keywords: Physico-Chemical Parameter, *Barytelphusa Cunicularis*, Testicular Cells, Histology.

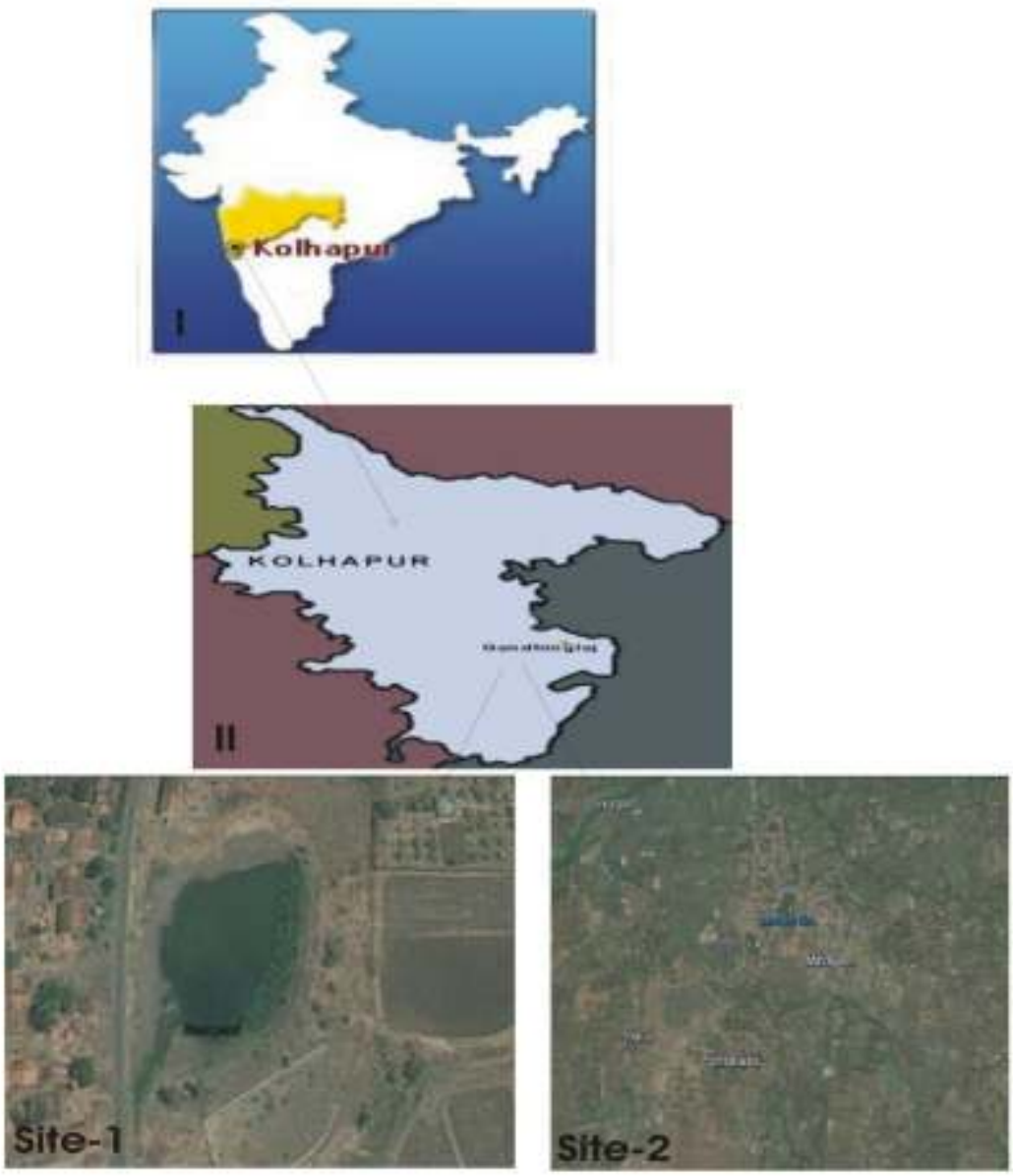
INTRODUCTION

Water is natural resources useful in any development of urban as well as rural areas. Increased population made stress over natural water resources disturbing quality of the water and the hydrological budget. Sewage waste, industrial effluents, agricultural discharge etc. are major sources of water pollution. Industrial waste, fossil fuels and nuclear power plants, carries variety of pollutants which enter into the aquatic body. Aquatic systems extensively contaminated with heavy metals released from domestic, industrial and other man-made activities. Discharge of pollutants from domestic sewages, industrial wastewaters, agricultural runoff and other sources, which can have short term or long term effects over quality of aquatic system[1]. Healthiness of aquatic bodies were depends over its biological diversity and type of physicochemical characteristics [2]. Thus the contamination of freshwater with a wide range of pollutants has became matter of concern over the last few decades [3]. Pollution of aquatic bodies disturb physiology of floral and faunal diversity in it. Majority of invertebrates found affected among which invertebrates including crustaceans were highly sensitive to any change in physico-chemical parameters of aquatic body [4]. Crustaceans are important constituent and has fundamental role in aquatic food chains specifically in nutrient cycle, water quality monitoring and small-scale fisheries with their ecological and economical importantance. Distribution and existence of arthropods were depends over specific environmental parameters as pH and DO [5]. Factors like temperature, salinity etc. held responsible for attending maturity in arthropodan species [6]. Since from last decades natural environment was disturbed by aquatic pollution including pesticides, inorganic chemicals and heavy metals which cause alteration in normal physiology of aquatic animals. Less attention towards the morphology and histology of the reproductive system in freshwater crab *B. cunicularis* in relation with physico-chemical parameters has focused the undertaken work and results have been discussed in relation to reproductive mechanism in selected freshwater crab *Barytelphusa cunicularis.*

MATERIALS AND METHODS

For the present study water samples were collected from two selected animal collection sites. Samples were brought to the laboratory by using plastic containers for analysis of different physico-chemical parameters by standard methods [6, 7]. Heavy metal analysis was carried out by using Atomic Absorption Spectrophotometer (AAS) (Kemito company- 201). During study parameters like temperature, pH, dissolved oxygen and free CO_2 etc. were determined at the sampling sites respectively.

For histological study, testes were dissected, fixed in Bouin's fixative for 24 hr and washed. Testicular organ was dehydrated and processed for routine microtechnique procedure. Sections at 6-7 µm thickness were taken and stained with hematoxylene and eosin.



Map-1: Sampling sites: I-Nool pond, II-Mahagoan river.

RESULTS

During the study various physico-chemical parameters were estimated related to pollution point of view the data obtained were as follows:

Temperature:

We found that, surface water temperature was ranged between 19°C to 31°C. It was recorded minimum during winter and maximum in summer. The monthly variations showed that, the temperature of water followed the seasonal pattern and were fluctuated according to the prevailing atmospheric temperature. Thus on an average the maximum range of temperature was seen at Mahagoan river 27.75ºC (Table No. I, Fig.1-A) while minimum at Nool pond 22.2 ºC throughout the year (Table No. II, Fig. 2-A).

pH:

The pH is an important factor for determining productivity of an ecosystem. During the assessment year we found that, the pH concentration was ranged between 7.42 and 8.38 (Table No. I, II and fig.1, 2, 3-B).

Dissolved oxygen:

Oxygen content of water has direct relation with productivity of water body and health and survival of organism living in it. The average range of DO was found 3.25mg/l (Table No. II, Fig.1-C). at Mahagoan river while 33.5 mg/l at Nool pond (Table No.I,Fig.1-C). High values of dissolved oxygen might be due to increased photosynthetic activity while lower values may be because of its utilization during decomposition of organic matter and respiration by micro and macro organisms.

Free CO_2:
Free CO_2 concentration in water indicates the presence of decomposable organic matter, bacterial action and physiological activities of biotic components. The maximum free CO_2 Value was 55.77 mg/l at Nool pond whereas, minimum 9.02mg/l at Mahagoan river (Table No. I, II, Fig.1-C).

Alkalinity:
Alkalinity of natural water is the result of bicarbonate content and is usually expressed in terms of presence of calcium carbonate. The total alkalinity of water is caused by the cations of Ca^+, Mg^+, Na^+, NH_4^+ and Fe^+ combined either as carbonates or bicarbonates or occasionally as hydroxides. The maximum value recorded for alkalinity was 128.75mg/l at Nool pond, while minimum 18mg/l at Mahagoan river (Table No. I, II, Fig.1-C).

Chloride:
The most important source of chloride in natural water is discharge of sewage. The minimum value of chloride found was 19.1775mg/l at Mahagoan river, whereas maximum 242.07mg/l at Nool pond (Table No. I, II, Fig.1-C). Water analysis from Nool pond indicated that it was contaminated due to organic content of sewage, agricultural wastes inducing fertilizers etc. with discharge by surrounding areas.

Hardness:
Hardness results from the presence of divalent cations of which $Ca^{+\ +}$ and $Mg^{+\ +}$ which are most abundant in groundwater. Total hardness values were maximum 195.5 mg/l at Nool pond while minimum 56.25 mg/l at Mahagoan river (Table No. I, II, Fig.1-C).

Total solids:
The average range of total solid content was maximum at site I (710.5 mg/l), while minimum at site VI (66 mg/l) (Table No. I, II, Fig.1-D).

Total dissolved solids:
Study showed that, concentration of total dissolved solid was found high at Nool pond 673 mg/l which has decreased potability and reduced utility of water for drinking, irrigation and industrial purposes. Minimum range of TDS was seen at Mahagoan river 60 mg/l (Table No. I, II, Fig.1-D).

Suspended solids:
The average range of suspended solids was maximum at Nool pond 37.5 mg/l while minimum at Mahagoan river 6 mg/l (Table No. I, II, Fig.1-D). Obtained results Indicated enrichment of suspended solids at Nool pond which was unfit for drinking, irrigation and also for industrial purpose.

COD:
COD found reliable parameter for analysis of water pollution. In the present study maximum range of COD found at polluted site Nool pond 115.25 mg/l while minimum at control site Mahagoan river 5.75mg/l (Table No. I, II, Fig.2-D).

Sodium:
The maximum range of sodium content was at Nool pond 91.5mg/l while minimum at Mahagoan river 6.25mg/l (Table No. I, II, Fig.2-D). Maximum sewage discharge was found at site I (Nool pond) leading to its pollution as compared to others.

Potassium:
The high concentration of potassium content was noted at site I (72.5 mg/l) while minimum at site II (1mg/l) (Table No. I, II, Fig.2-D).

Iron
Iron content of water was higher at Mahagoan river 1.33 mg/l (Table No. II, Fig.2-E) while minimum at Nool pond 0.25 mg/l (Table No. I, Fig.1-E).

Nitrate:
Maximum range of nitrate was 0.233mg/l (Table No. I, Fig1-E) found at Nool pond while 0.015mg/l at Mahagoan river (Table No. I, II, Fig.2-E).

Phosphate:
Less concentration of phosphate was observed at Mahagoan river 0.354 mg/l (Table No. II, Fig.3-E) due to the decreased land drainage, sewage and fertilizer disposal from the agricultural lands. Higher concentration was found at Nool pond 2.94 mg/l (Table No. I, Fig.1-E) due to continuous discharge of drainage, sewage and fertilizer.

Anatomy of reproductive system of crab *B. cunicularis:*
The reproductive system of male freshwater crabs *B. cunicularis* consists of a paired testes, vasa deferentia, penises (papillae), gonopods-1 and gonopods-2. The testes and vasa deferentia joined together to have H-shaped appearance (Fig. A and B). The testes lie in the cephalothorax on top of the hepatopancreas just below carapace. Testes were creamy white colored with soft texture. Each testis was elongated and lobulated and extended anterodorsally on the cephalothorax and continued laterally up to stomach. The width and diameter of each testis was not uniform with length. Width of each testis along its length were measured 2 to 6 mm.Total length of the testis ranges between 20 to 30 mm. Comparatively right testis was slightly bigger that of the left testis. Distal ends of testes and anterior ends of vasa deferentia were joined together to form a commissure or cross bridge so as to give 'H' shape structure (Fig. B). Testes produce spermatozoa and carried away in the vasa deferentia which opened ventrally.

Histology of testis:
Microscopically testis of *B. cunicularis* consists of several testicular lobules or seminiferous tubules or testicular acini with a central collecting duct. Entire testes get enveloped by a fibrous layer made up of collagen fibers. Internally, each testis contains large number of oval lobules or follicles. For histomorphological differentiation Heamatoxylene-Eosine was used. Histologically testicular follicles were lined by a single layer of germinal epithelium which gives spermatogonial cells which was stained with blue colour (PLATE-I Fig. C). The lumen of testicular cell stained with pink colour. Non-germinal cells as Sertoli cells acting as accessory cells, sustentacular cells, interstitial cells and nurse cells or nutritive cells were found. These cells were nutritive in function helping the process of spermatogenesis and spermiogenesis.

During the experimentation Mahagoan river was found suitable for growth and development of crab having major reproductive capacity, where as animals collected from Nool pond showed reduced spermatogenesis indicating less reproductive capacity due to contamination of water by organic or inorganic contents.

Figure A: Male crab

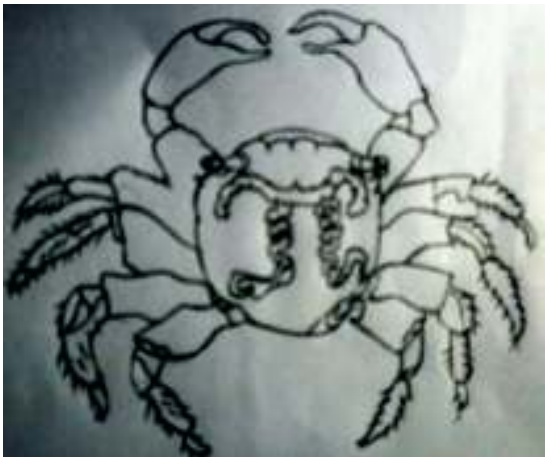
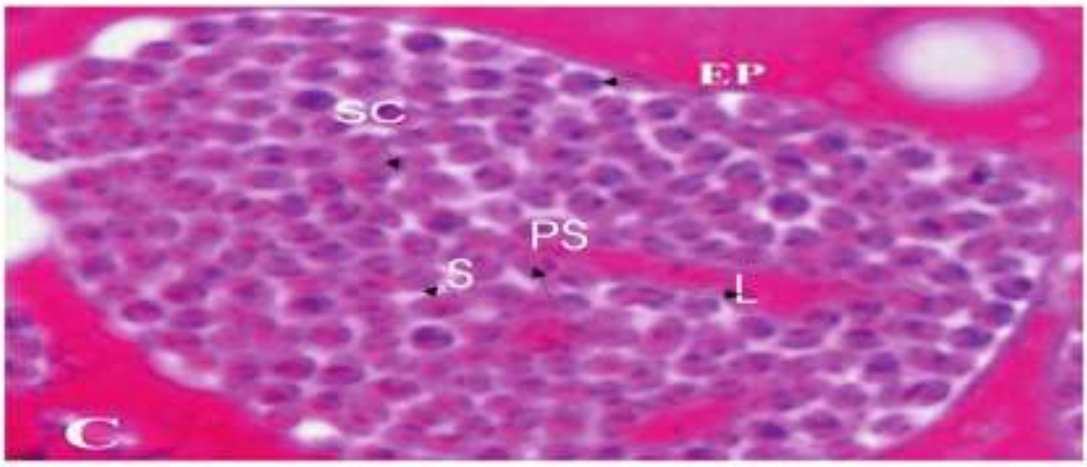


Figure B: Anatomy of testis

PLATE - I



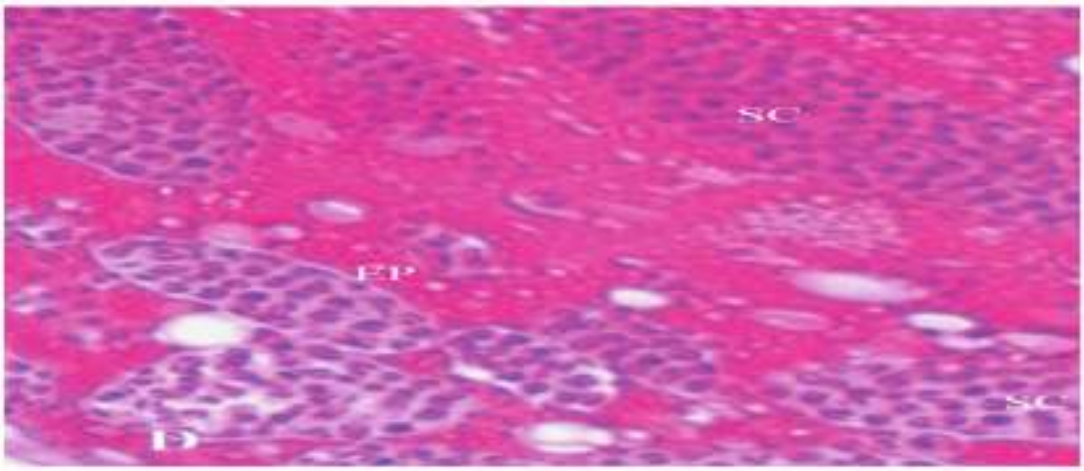


Fig. C-Testicular cell of control site
Fig. D- Testicular cell of polluted site
EP-Germinal epithelium, PS-Primary spermatocytes, SC-Spermatozoa, L-Lumen S-Spermatogonia

DISCUSSION

Physico-chemical study:

Pollution in aquatic ecosystems, especially river systems, found major concern. Contamination of water has subsequently affected all living organisms in the system [10]. Temperature essential variable environmental factors, since it influence the growth and distribution of flora and fauna. The excessive amount of nutrients in water bodies along with higher temperature favors the growth of algae and aquatic

weeds [11]. High pH value normally associated with increased photosynthetic activity in water [12]. pH of water has important indication in its quality and provides information about geochemical equilibrium or solubility calculations [13]. Variation in pH of water represents indicator of a major productivity of natural water [14]. [15,] documented that, the pH range between 6.7 to 8.4 considered to be safe for aquatic life to maintain productivity. However, pH below 4.0 and above 9.6 found hazardous to aquatic life. The pH intensity influences aquatic productivity in rural areas. Oxygen is an index of the physical, chemical and biological processes. Transfer of oxygen across the air, water interface depends upon temperature, partial pressure of gases in atmosphere, dissolved salt concentration, wave action, relative solubility, photosynthetic activity of plants and respiration by bacteria, plants and animals in the water [15]. [16] documented that, high values of dissolved oxygen during summer might be due to increased photosynthetic activity while less values may be because of its utilization during decomposition of organic matter and respiration by micro and macro organisms. The pH has direct relationship with total alkalinity, reported by [17]. Alkalinity has important one in aquatic life because it protects against increased pH range. [14] documented that, Chloride concentration between 4-10 ppm indicates purity of water. The limit of chloride concentration for drinking water is specified as 600 mg/l [18]. The excess sodium and chloride in drinking water may induce heart failure and hypertension [19]. The increased hardness in summer season was mainly attributed to rising temperature, thereby increasing the solubility of Ca^{++} and Mg^{++} salts [20]. Hardness in water is also derived from CO_2 released in bacterial action from soil through in percolating water. Hardness of water increases due to the result of interaction between water and geological fragmentation. [21] has noticed that, hardness found essential for normal growth of aquatic ecosystem. Water as a universal solvent, dissolved different type of materials as compare to other solvents [11]. The total concentration of dissolved solids in a water body found useful parameter in describing the chemical density and productivity of the water [22]. High content of dissolved solids has elevated density of water, influencing osmoregulation and has reduced gas solubility of water for drinking, irrigation and industries [23]. The domestic pollution and human interference has also contributed enrichment of dissolved solids in aquatic bodies [15]. The higher concentration of total suspended solids was found due to insoluble organic matter in sewage. According to [24] the disposal of sewage and industrial effluent contributed suspended matter to the rivers. [18] recorded that, 30-80 % human diseases were occurred due to impurities of water. The industrial discharge and sewage disposal has increased the content of sodium [25]. Potassium has vital role in the metabolism of freshwater organisms [15]. Cell membrane continually pumps the potassium and sodium, which required in the expenditure of large amount of energy [26]. Iron proved the vital element of life. It is a natural component of soil and its concentration can be influenced by hudge industrialization. Iron concentration in surface water varied greatly, from 61 ppm to 2680 ppm [27]. The direct and indirect effect of iron contamination has decreased species diversity and abundance of plankton and benthic invertebrates [28]. Fe found to be induced free radicals that, causes breaks in nucleic acid and oncogene activation.

Histopathology of testis:

According to [29,] histopathological studies were useful in evaluating the pollution of aquatic bodies in addition, analysis of histological changes in targeted organs. Heavy metal has caused adverse effects on the health of organisms. They act at cellular and molecular level which ultimately lead into physiological and biochemical disorders leading to death [30]. [31] observed histological changes in the gametic tissues of freshwater female crab, *Barytelphusa cunicularis* exposed to heavy metal pesticides. [32] observed the effects of pesticide on different stages in the spermatogenesis in the freshwater prawn, *Macrobrachium kistensis*. Histopathological changes in the gonads of freshwater crab, *Barytelphusa cunicularis* observed by exposure to lethal and sublethal concentration of pesticides [31]. [33] studied the effect of heavy metal pollutant cadmium chloride on histopathology of the freshwater crab, *Barytelphusa guerini*.The crabs from Mahagoan river showed normal testicular activity (PLATE-I Fig.C). The testis consists number of seminiferous tubules with a lumen inside of varying size and shape. The germinal zone consists of spherical spermatogonial, primary spermatocytes and spermatozoa with crescent shaped head while the crabs from Nool pond (PLATE-I Fig. D) showed variations in testicular activities. Lower numbers of testicular cells were noted with disorganization of tubules and germinal epithelium.

From the present study, it can be concluded that the diversity and distribution of crustaceans get controlled by increased (temperature, depth, pH and alkalinity) with biotic factors including food availability, predation, alternation of parthenogenetic and gamogenetic reproduction modes. The present histopathological study on testis showed progressive damage and degeneration and it is clearly evident with the progressive contamination of water i.e. extent of tissue damage increases with the increasing pollution.

CONCLUSION

Damage of gonad in contaminated water lead to decline in reproductive activity. Injury of testis resulted in the disturbance of overall metabolism and several physiological processes of crab. Thus aquatic pollution found to inhibit gonadal maturity.The diminishing testicular activity was indicated by the disorganization of seminiferous tubules and lower number of spermatocytes and the degeneration of germinal epithelium.

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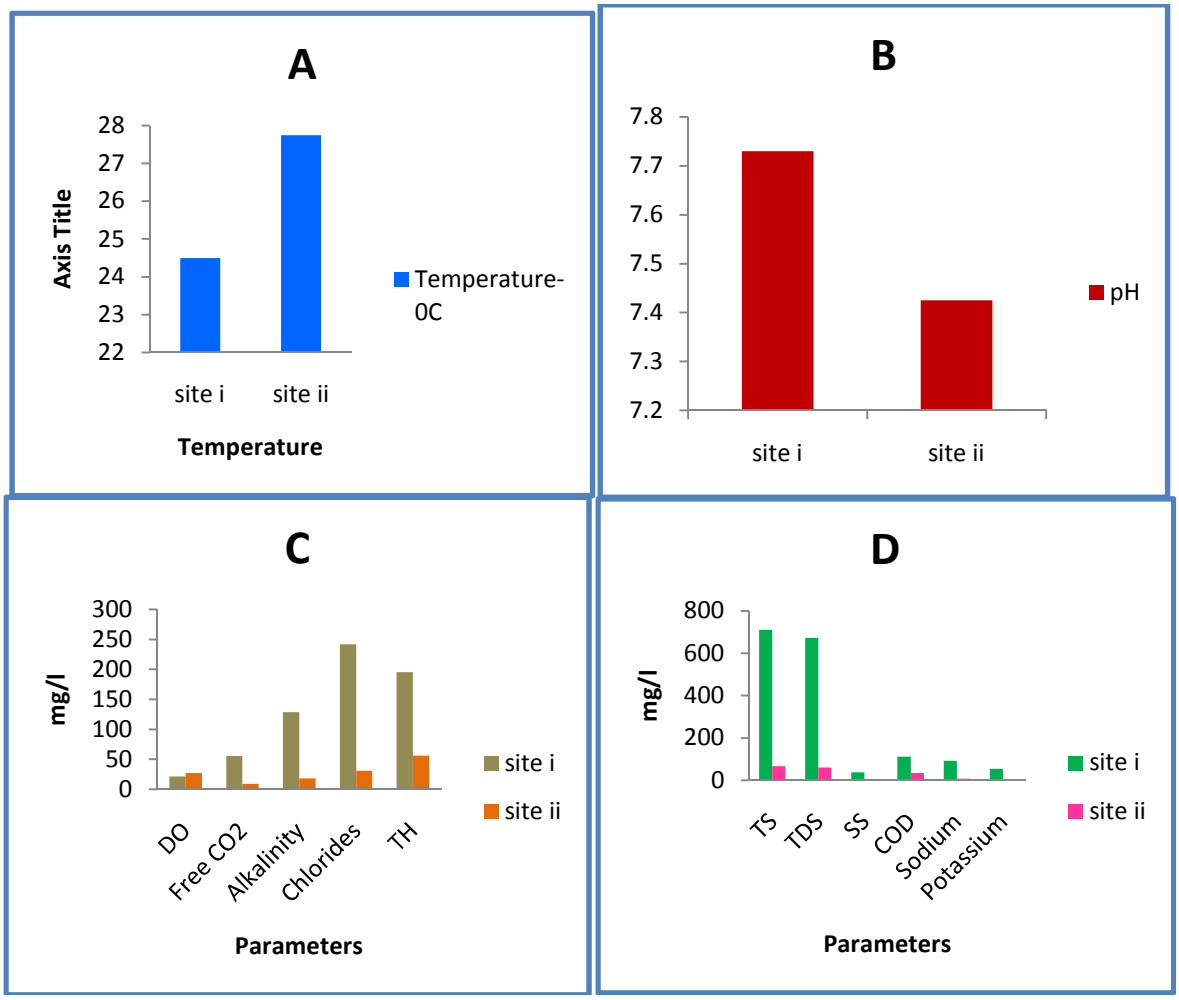

Figure 1: Graphical presentation of physico-chemical parameters in summer season.

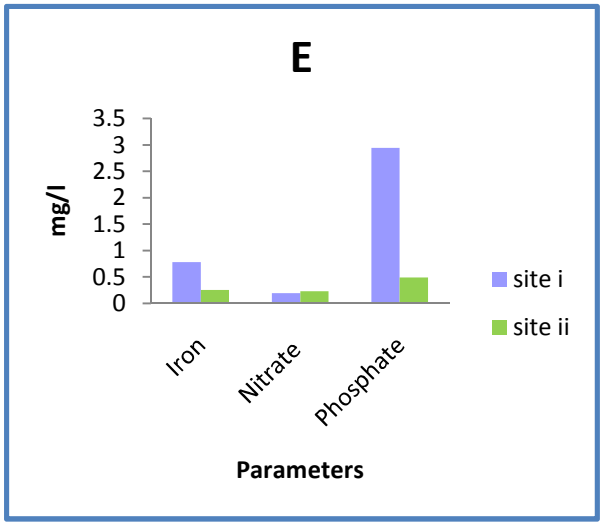

Figure 2: Graphical presentation of physico-chemical parameters in rainy season.

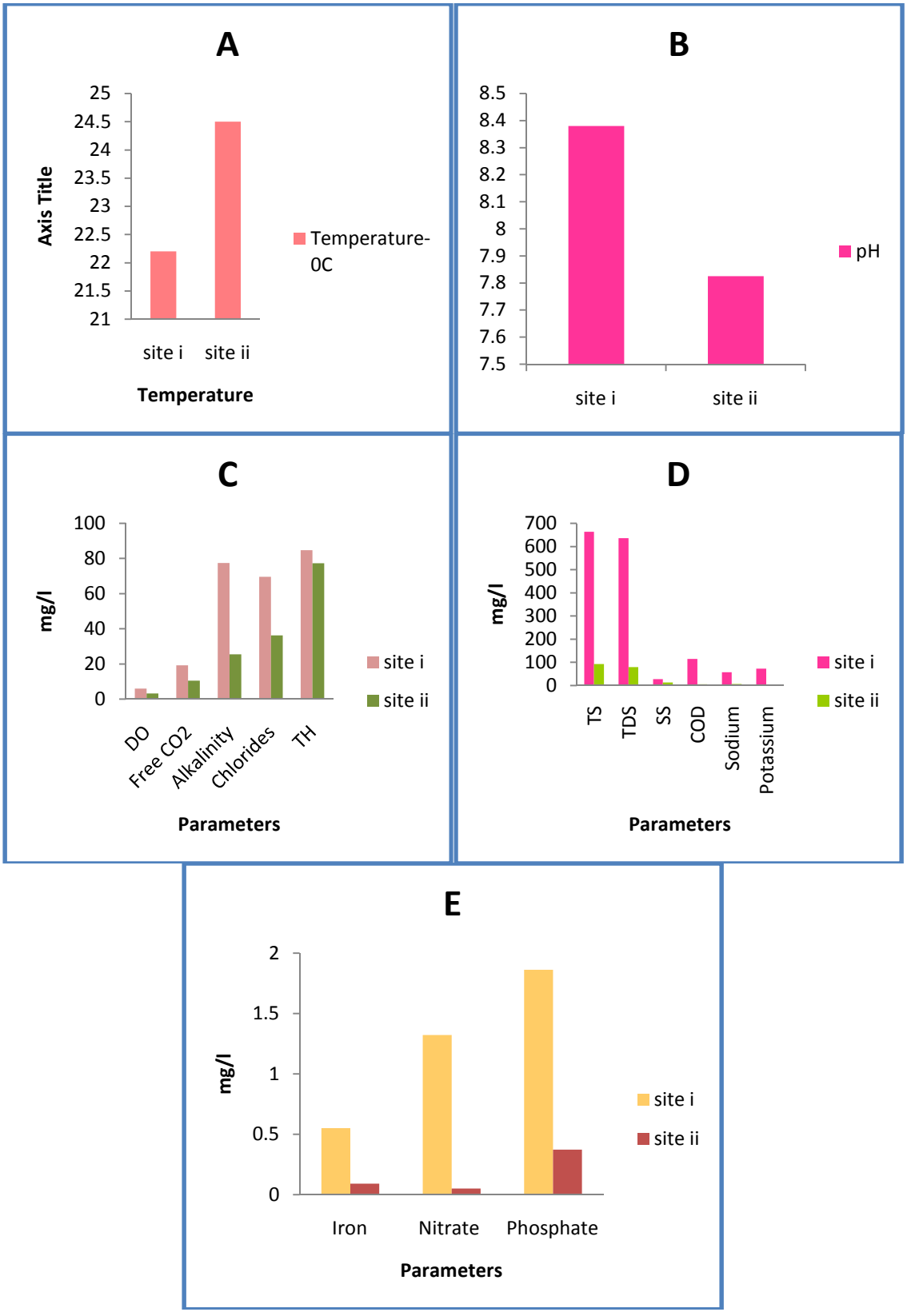

Figure 3: Graphical presentation of physico-chemical parameters in winter season.

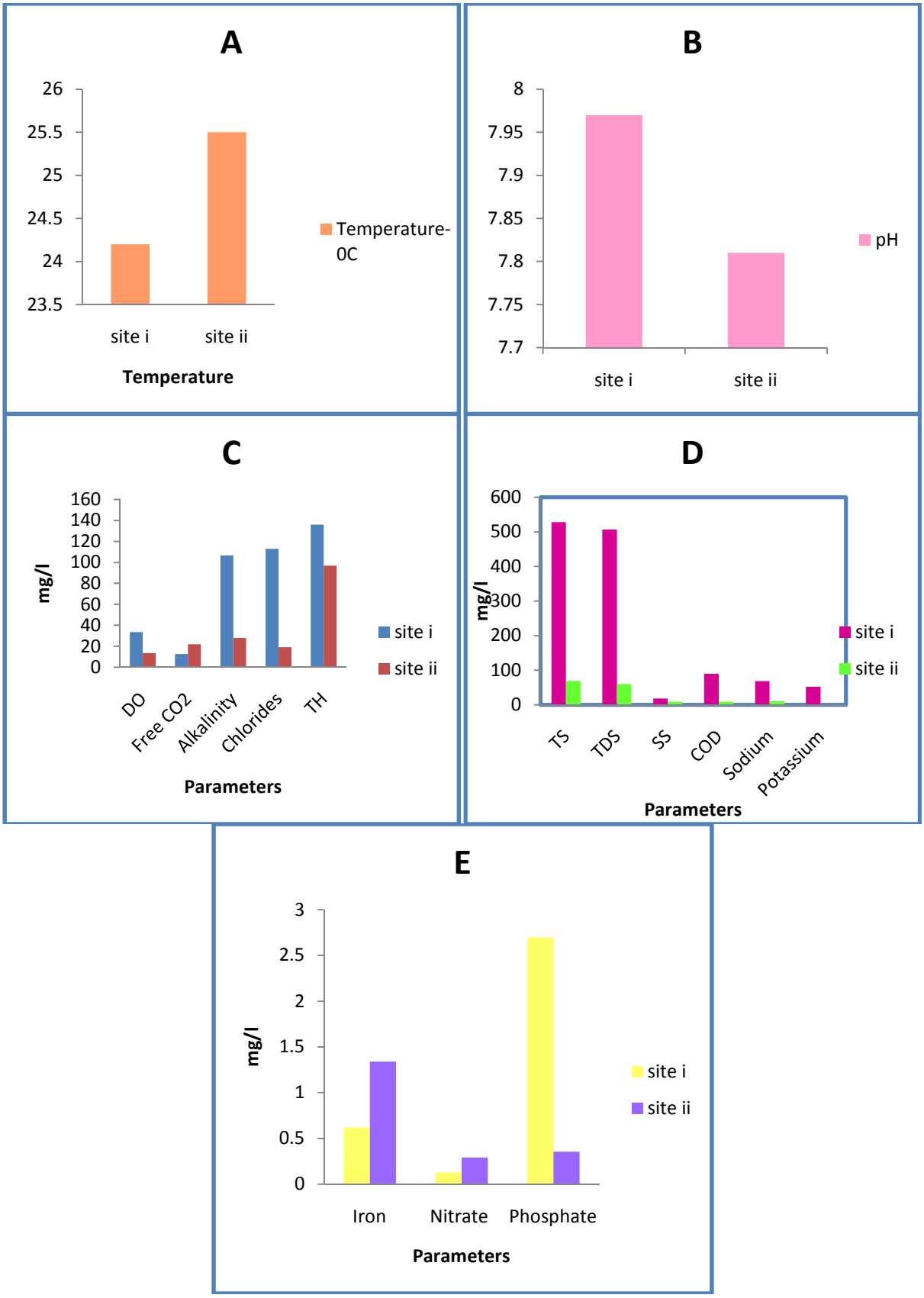

Figure 1, 2, 3: A-Graph of temperature, B- Graph of pH, C- Graph of DO, Free co_2, Alkalinity, Chlorides and TH, D- Graph of TS, TDS, SS, COD, Sodium and Potassium. E- Graph of Iron, Nitrate and Phosphate.

Table I: Seasonal variation in physico-chemical parameters from site- I

Parameter unit	Summer	Rainy	Winter
Temperature-^{0}C	24.5±1.92	22.2±0.5	24.2±2.06
pH	7.73±1.05	8.38±0.63	7.97±1.23
DO(mg/l)	21.35±23.03	6±2.70	33.5±40.60
Free CO_2(mg/l)	55.77±45.57	19.25±8.30	12.65±5.19
Alkalinity(mg/l)	128.75±18.42	77.5±74.50	106.75±59.68
Chlorides(mg/l)	242.07±121.22	69.55±39.54	112.85±58.26
TH(mg/l)	195.5±37.54	84.75±7.08	136±56.66
TS(mg/l)	710.5±119.0784	664±26.280	528±56.850
TDS(mg/l)	673±96.408	636±26.73	507±61.7414
SS(mg/l)	37.5±27.77	28±14.329	18.5±8.2259
COD(mg/l)	112±12.62	115.25±7.973	90±35.251
Sodium(mg/l)	91.5±27.44	57.64±38.19	68.5±11.81
Potassium(mg/l)	53.75±35.71	72.5±0.58	52.5±33.36
Iron(mg/l)	0.783±0.4682	0.55025±0.208	0.62025±0.3760
Nitrate (mg/l)	0.233±0.2340	0.09025±0.0038	0.2905±0.3531
Phosphate(mg/l)	2.945±0.130	1.8625±1.6171	2.6975±0.1466

Table II: Seasonal variation in physico-chemical parameters from site- II

Parameter unit	Summer	Rainy	Winter
Temperature-^{0}C	27.75±2.061	24.5±2.38	25.5±1.9148
pH	7.425±0.527	7.825±0.5377	7.81±1.4354
DO(mg/l)	27±18.092	3.25±0.5	13.5±13.1782
Free CO_2(mg/l)	9.02±2.314	10.45±4.5354	22±14.2576
Alkalinity(mg/l)	18±7.118	25.5±1994	28±21.9696
Chlorides(mg/l)	30.73±1199	36.21±16.78165	19.1775±7.825
TH(mg/l)	56.25±18.83	77.25±17.036	97±66.82315
TS(mg/l)	66±10.708	93±85.2525	69.5±15.2643
TDS(mg/l)	60±10.32	79.5±72.560	60±13.8564
SS(mg/l)	6±2.828	13.5±13.2035	9.5±5.9721
COD(mg/l)	34±35.33	5.75±3.5	9±5.03322
Sodium(mg/l)	7.25±3.20	6.25±3.8622	10.25±6.55
Potassium(mg/l)	1.33±0.577	1±0.8164	1.5±0.707
Iron(mg/l)	0.2572±0.179	1.322±0.670	1.3393±1.871
Nitrate (mg/l)	0.1915±0.0643	0.051±0.037184	0.127±0.03718
Phosphate(mg/l)	0.49±0.2340	0.3725±0.2794	0.354±0.2643

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