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EFFECTS OF ORGANIC MANURES AND INORGANIC FERTILIZERS ON GROWTH, YIELD AND SHELF LIFE OF TOMATO (Lycopersicon esculentum Mill.)

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INTRODUCTION

Horticulture is a branch of agriculture concerned with cultivation, production and sale of various crop plants. Horticulture crops include fruits, vegetables, nuts, spices, condiments, mushrooms, and ornamental plants, medicinal and aromatic plants. L.H. Bailey is considered as the Father of American Horticulture and M.H. Marigowda is considered as the Father of Indian Horticulture. Horticulture crops are a source of dietary fibres, vitamins, minerals, nutrients, flavour, aroma etc. This sector enables the population to eat a diverse and balanced diet. Horticulture crops serve as raw materials for various industries viz., confectionery, oils and paints, perfumery and cosmetics, processing, pharmaceutical, etc. Tomato production has been faced by many challenges in most countries. Farmers use heavy doses of pesticides and chemical fertilizers in order to get better yield. The continuous application of these chemical fertilizers and pesticides decreases the soil fertility by increasing the concentration of heavy metals in the soil. Also, many poor farmers cannot buy high yielding varieties as they
are expensive. Meanwhile, the price of fertilizers increases, year by year, due to the higher amount of fertilizers needed in the second and third seasons as compared with that in the first season to maintain the current yield production at economical level.(Selim. M., 2020). Farmers, in the developing countries having low level of education and insufficient training regarding proper agricultural practices is another reason for the low production of crops. Inorganic fertilizers, also known as mineral or chemical fertilizers, have high level of nutrients and these nutrients are released quickly as there is no need of decomposition. As a result, inorganic fertilizers result in increased yield. However, there are many disadvantages in using inorganic fertilizers for a long time. These are destruction of soil texture and structure, which leads to soil erosion and increased acidity as a result of leaching. Besides, inorganic fertilizers are costly and pose negative effects on environment if managed improperly. Organic fertilizers, on the other hand, maintain the quality of soil by improving biological activity, structure and chemistry of soil. They are known to increase soil organic matter content by gradually releasing the nutrients. However, decomposition of organic material is strongly affected by temperature and soil moisture. This means that nutrients may be released continuously even when the plants do not need them. Also, organic fertilizers are low in nutrient content and not all organic materials are available in many regions, these fertilizers alone do not meet the nutrient requirements of the crop. Tomato is a heavy yielder and hence requires sufficient fertilizers for growth and high yield. Because of the high cost of purchasing inorganic fertilizers and inadequate nutrient content in organic fertilizers, some farmers use a mixture of organic and inorganic fertilizers. Various studies have revealed that the combination of organic and inorganic fertilizers proven to be effective in increasing productivity and soil fertility has also been increased in an integrated manner. The present study was conducted to investigate the “Effect of organic manures and inorganic fertilizers on the growth, yield, yield attributes and shelf life of tomato (Lycopersicon esculentum Mill.).” The study was conducted during kharif season 2020.

Materials and methodology
The experiment was conducted with the seeds of the tomato cultivar Pusa Ruby. It is an early maturing variety. Plants are indeterminate. It is suitable for cultivation during autumn, winter and spring-summer seasons. It is a high yielding variety. The fruits are flattish round, small medium and red. The fruits have a good market value. The experiment was laid out in a randomized block design (RBD). There were a total of 8 treatments and each treatment was replicated three times. So, there were a total of 24 plots. The size of the plot taken was 2 m × 1.8 m. All the treatments were randomized separately in each replication. Raised nursery bed of 3 metres length and 1 metre width was prepared. The bed was made with a fine tilth and properly pulverized and solarised. Seeds were sown in the furrows about 2 cm deep and 8 cm apart. Only healthy and disease free seeds are used. After the sowing of seeds, the seed bed was covered with FYM and then watered. Nursery bed was regularly irrigated during evening hours. 3.2.3 Preparation of experimental plots. The experimental field was prepared with deep ploughing to a fine tilth by repeated harrowing and levelling. After ploughing, the field was laid out according to the layout and the plots were prepared. Before the removal of seedlings, the nursery bed was properly irrigated to avoid injury and damage to the roots while pulling the seedlings. Recommended dose of inorganic fertilizer like N (Urea), P2O5 (Single Super Phosphate) and K2O (Muriate of Potash) were added to the respective plots. Half dose of nitrogen and full dose of potassium and phosphorus were applied at the time of transplanting where, the remaining dose of N is given after 30 days and 60 days of transplanting. The organic fertilizers applied were cattle dung and vermicompost. Cattle dung was given at the rate of 5 and 10 t/ha whereas, vermicompost was added at the rate of 5 and 10 t/ha. Each organic fertilizer was given in the allocated plots. The fertilizers were mixed well by ploughing.

The heights of all the tagged plants were measured using a centimetre scale. The height was measured from the base of the main stem to the apex of the plants. Plant height was measured from 30 days of planting up to 90 days to observe the growth rate of plants. The average of five plants was calculated and recorded for each treatment.

The number of branches was counted from 30 days of planting up to 90 days when the plants had ceased to grow. The total number of branches per plant was recorded from the sampled and tagged plants from each plot and the mean was computed.

The number of days taken from the date of transplanting to the opening of first flower in 50% population under each treatment was recorded as days to 50% flowering. Number of flower cluster per plant was taken from five tagged plants during the flowering season and the average value was calculated and
recorded. The number of flowers per cluster was recorded from the five sampled plants under each treatment and the mean value was calculated. Number of flower per cluster were calculated for 5 plants.

RESULTS

The growth characters i.e. plant height and number of branches was significantly influenced by different treatments over control. Plant height and number of branches increased with the advancement of plant growth. Plant height and number of branches per plant was recorded at regular intervals from 30 DAT to 90 DAT. The maximum plant height was 74.30 cm recorded with the treatment T8- 50% NPK + 50% VC. The second best treatment was T7- 75% NPK + 25% VC. Both the treatments were significantly superior to the rest of the treatments. Also, the number of branches per plant was recorded maximum with the treatment T8-50% NPK + 50% VC that showed 9.8 branches. The second best treatment was T7- 75% NPK + 25% VC showed 8.7. The different treatment combinations have significantly influenced on days to 50% flowering and the number of flower clusters per plant. The minimum number of days taken to 50% flowering was obtained by treatment T4- 100% VC showed 39.8 days to 50% flowering followed by treatments T8-50% NPK + 50% VC and T7- 75% NPK + 25% VC showed 40.4 and 41.2 days to 50% flowering respectively. Also, the number of flower clusters per plant was maximum in treatment T4- 100% VC i.e. 14.8 flower cluster per plant. The second best treatment was T8- 50% NPK + 50% VC followed by treatment T7- 75% NPK + 25% VC showed 14 and 13.4 respectively. The maximum number of flowers per cluster was recorded highest in treatment T8- 50% NPK + 50% VC showed 7.8 number of flowers per cluster and T7- 75% NPK + 25% VC produced 7 number of flowers per cluster. Different treatments significantly influenced the number of days taken to first fruiting. Treatment T8- 50% NPK + 50% VC took minimum number of days i.e. 49.6 to first fruit setting.

DISCUSSION AND CONCLUSION

The present study revealed that plant growth characters i.e., plant height and number of branches per plant gradually increased with the age of the plant. Plant height and number of branches is the indicator of growth and vigour of the plant. The height and number of branches per plant was taken at regular intervals. The data revealed that the height and number of branches were significantly influenced by the application of different combinations of organic manures and inorganic fertilizers. The maximum height 74.30 cm and number of branches 9.8 was recorded by treatment T8-50% NPK + 50% VC followed by T7- 75% NPK + 25% VC. Increased growth parameters might be due the organic manures applied in combination with NPK fertilizer. This might be due to the fact that organic manures have supplied additional amount of nutrients both micro and macro nutrients and have improved the soil physical and chemical properties. Also, many of the nutrients in vermicompost are changed to their available forms in order to make them easily utilisable by plants. Similar findings were recorded by Wang et. al. (2017), Eswaran and Mariselvi (2016), Chanda et. al.(2011) and others. It was also observed that the least increase in height and number of branches was recorded by treatment T1 (control with no fertilizer). It showed the importance of fertilizers for growth of the plant.

REFERENCES

MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF STAPHYLOCOCCUS PSEUDINTERMEDIUS FROM CANINE PYODERMA IN SHIVAMOGGA REGION OF KARNATAKA

Prashantha M K; Shambulingappa B E*; Sundareshan S; Kotresh A M; Rudresh B H; Madhavaprasad C B and Arun S J
Veterinary College Shimoga (KVAFSU, Bidar), Karnataka

ABSTRACT

A study on bacteriological investigation of canine pyoderma cases was conducted at the Veterinary College, Shivamogga. Exudate/pus/lesion swabs were collected from clinical cases of canine pyoderma (n=126) and subjected to isolation and identification of bacterial isolates by phenotypic methods. The bacteriological processing of the samples resulted in the recovery of 95 staphylococcal isolates and 18 other bacterial isolates. On culture, staphylococci were the most predominantly (n=95, 75.39%) isolated organisms. The PCR was employed as molecular method in this study for the detection of species of staphylococcal isolates by targeting nuc gene and it was also used for the detection of virulence gene and antibiotic resistance gene in staphylococcal isolates by targeting siet gene and mecA gene, respectively, by using primers published earlier. One of the S. pseudintermedius isolates which confirmed by PCR and sequencing of partial nuc gene was used as positive reference strain for further screening of isolates by PCR.

Based on nuc gene-based PCR, out of 95 staphylococcal isolates obtained, 82 (86.1%) of the isolates were found belonging to S. pseudintermedius. And out of 82 S. pseudintermedius isolates, siet gene was detected in 69 (86.1%) isolates. S. pseudintermedius was found to be predominant bacterial pathogen responsible for pyoderma in dogs.

Keywords: Canine pyoderma, Staphylococcus pseudintermedius, Nucgene, siet gene, mec A gene, virulence gene.

INTRODUCTION

Pyoderma is one of the most frequently seen conditions in small animal practice and most of the pyoderma cases in dogs are associated with Staphylococcus species, which are opportunistic pathogen and infection tends to develop secondarily to an underlying cutaneous, metabolic or immunological abnormality (Craig, 2003). Around 90 per cent of pyoderma cases in dogs are associated with bacteria belongs to Staphylococcus species, especially S. intermedius is one of the causative agents of canine bacterial skin infections, such as otitis externa, pyoderma and abscesses (Kloos and Bannerman, 1994). Staphylococcus intermedius is an opportunistic bacterial pathogen causing various diseases in dogs. Staphylococcal strains designated up to 2005 as S. intermedius species are currently assembled into the so-called S. intermedius group (SIG), consisting of S. intermedius, S. pseudintermedius and S. delphini (Sasaki et al., 2007). It is the S. pseudintermedius, and not S.intermedius, is the species of the S. intermedius group (SIG) that colonizes and causes infections in dogs and cats (Perreten et al., 2010). The novel species S. pseudintermedius is the most significant of the SIG from a clinical point of view. Being an important canine opportunistic pathogen often isolated from dermatitis, otitis and other secondary infections (Sasaki et al., 2007), the SIG particularly S. pseudintermedius, has been implicated as a common cause of pyoderma in dogs (Becker et al., 2005).
It is difficult to differentiate *S. intermedius* from *S. pseudintermedius* during routine diagnostic procedures, but the vast majority of canine isolates are *S. pseudintermedius*. It has therefore been proposed to report all strains belonging to the SIG from dogs as *S. pseudintermedius*; unless genomic investigations prove that the strain belongs to other related species of SIG (Devriese *et al*., 2009). The most common cause of pyoderma in dogs is the coagulase-positive *S. pseudintermedius* (previously misidentified as *Staphylococcus intermedius*) (Jones *et al*., 2007).

*Staphylococcus pseudintermedius* is one of the most common pathogens isolated from skin and post-operative infections in dogs and cats (Stegmann *et al*., 2010). Hence the present study was undertaken with an objective to isolate *Staphylococcus* species from canine pyoderma in Shivamogga region of Karnataka and an attempt was made to identify the *Staphylococcus pseudintermedius* by molecular methods.

**MATERIAL AND METHOD**

The study was conducted in the Department of Veterinary Microbiology, Veterinary College, Shivamogga, a constituent institute under Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka State.

Isolation of bacterial agents from samples collected from canine pyoderma cases:

a. **Collection of clinical samples and the data pertaining to the cases:**

Clinical cases of canine pyoderma presented to teaching veterinary clinical complex Shivamogga were used for collecting samples in this study. The veterinarians who were practicing in the study area (in and around Shivamogga) who volunteered to send from their respective district polyclinic veterinary dispensaries/Veterinary hospitals were also used in the study. All the cases of pyoderma such as papules, pustules, erythema, alopecia, pruritus and epidermal collarettes were selected as subjects for bacterial culture and antimicrobial sensitivity assay. The detailed information of the cases with regard to the breed, age, sex of the dogs including the lesions observed and the underlying causes were recorded for further analysis.

b. **Culturing of samples for Isolation of bacteria**

The clinical material collected using sterile swabs from the lesions was initially inoculated in BHI broth and incubated for 12-24 hrs at 37°C. Primary identification of bacterial agents were carried out based on colony morphology, and Gram's staining. A loopful of the inoculum was then streaked on to pre-prepared mannitol salt agar and MacConkey agar petriplates and incubated for 24hrs at 37°C and examined as per the standard procedure described by Cruickshank *et al*. (1975).

**Identification of bacterial agents by phenotypic methods**

**Grams staining and cultural characters**

Gram's staining kit, which contained Crystal violet, Gram's iodine, Decolorizer and Safranin was procured from M/s Hi-Media, Mumbai. Staining of all the culture isolates was carried out as per the instructions mentioned in the kit and differentiated as gram positive and gram negative and documented.

On the agar plates colony morphology and lactose fermenter/non fermenters were recorded. Grams staining of the pure colonies so obtained on the plates were carried out and the primary identification of bacterial isolates were done based on colony morphology. Then the selective plating of the isolates was carried out for further confirmation. Then relevant biochemical tests were carried out for gram positive and gram-negative isolates so obtained as per the standard procedure (Collee *et al*., 1989) as follows.

**Biochemical tests**

The recovered isolates confirmed by Gram's staining were further subjected to biochemical tests such as coagulase and catalase tests to confirm coagulase positive staphylococci and the aerobic bacterial isolates and other isolates were confirmed by conventional biochemical tests like oxidase and IMVIC tests.

**Coagulase test**

All the staphylococcal isolates confirmed preliminarily by Gram's staining were first subjected to tube coagulase test. For this, about 0.3 ml of 18 hr old *Staphylococcus* culture in BHI broth was mixed with 0.5ml of diluted rabbit plasma (1:4 in PBS) and incubated overnight at 37°C. The result was recorded at 1 hr, 4 hr and after overnight incubation. Formation of clot/ stiff gel which remained in place when tube was tilted through 90° angle or inverted was considered as positive for coagulase production. The tubes were read negative when plasma remained liquid or showed only
b. Determination of purity and yield of the DNA samples

The purity and concentration of the extracted genomic DNA was estimated by UV spectrophotometry. An aliquot of 20µL of DNA sample was dissolved in 0.98 ml of sterile DW. The diluted DNA was transferred into 1 ml microcuvette and the optical density (OD) was read at 260nm and 280nm in a UV spectrophotometer. Sterile DW was used as blank (Boesenberg et al., 2012).

The ratio of 260/280 OD was calculated. A ratio of 1.7 to 1.9 was considered as pure. Further, the purity of the DNA sample was checked by electrophoresis on 0.8 per cent agarose gel.

c. Agarose gel electrophoresis for confirmation of DNA

DNA was confirmed by agarose gel electrophoresis and was carried out as per Lee et al. (2012). The 0.5 µg DNA was used to check the purity by electrophoresis on 0.8 % agarose gel.

d. Preparation of the gel

Agarose (0.8%) was prepared in Erlenmeyer flask by adding 0.8g of agarose to 100ml of running buffer (TAE buffer: 40 mM Tris-acetate, 1 mM EDTA) as per Viljoen et al., 1993.

e. Setting up of gel apparatus and separation of DNA fragments

Loading dye was added to the DNA samples to be separated. DNA size marker was loaded along with samples. Lid was replaced into the gel box. The gel was placed in such a way that cathode (black leads) was closer the wells than the anode (red leads). Gel running was carried out until the dye has migrated to an appropriate distance (Lee et al., 2012).

f. Observing separated DNA fragments

When electrophoresis has completed, power supply was turned off and lid of the gel box was removed. Gel removed from the gel box. Excess buffer from the surface of the gel was drained off. Gel tray was placed on paper towels to absorb any extra running buffer. Gel was removed from the gel tray and exposed to UV light. DNA bands which were shown up as orange fluorescent bands were documented. Simultaneously the gel was observed and the photos were taken in the gel documentation unit and the gel was properly disposed.

Molecular detection of Staphylococcus pseudintermedius (nuc gene) by PCR

Procedure

The PCR was carried out targeting nuc gene as per the
procedure described by Chitra et al. (2015). The PCR was carried out using published primers of Chitra et al. (2015) as shown in table 1.

**Table 1: Oligonucleotide sequences of *S. pseudintermedius* nuc gene primers.**

<table>
<thead>
<tr>
<th>Name of the primer</th>
<th>Primer sequence 5’—3’</th>
<th>Product size (bp)</th>
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<tr>
<td>Staph nuc-F</td>
<td>AAACACCGAGTAATACGCCG</td>
<td></td>
</tr>
<tr>
<td>Staph nuc-R</td>
<td>TTAGCGTTCACAATGGTTCAG</td>
<td>780</td>
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</table>

The reaction mixture of 25 µl each was prepared in 0.2 ml thin-walled PCR tubes placed in mini cooler as shown below (Table 2).

**Table 2: Details of the contents of PCR mixture for nuc gene-based PCR.**

<table>
<thead>
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<th>Reagents (Concentration)</th>
<th>Volume</th>
</tr>
</thead>
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<tr>
<td>Master mix (Ampliqon Taq DNA Polymerase Master Mix RED, 2x)</td>
<td>12.5 µL</td>
</tr>
<tr>
<td>Staph nuc-F (12.5 µL/ml)</td>
<td>1 µL</td>
</tr>
<tr>
<td>Staph nuc-R (12.5 µL/ml)</td>
<td>1 µL</td>
</tr>
<tr>
<td>Template (Staphylococcal DNA)</td>
<td>3 µL</td>
</tr>
<tr>
<td>Nuclease Free Water</td>
<td>7.5 µL</td>
</tr>
<tr>
<td>TOTAL</td>
<td>25 µL</td>
</tr>
</tbody>
</table>

After mixing the contents, tubes were centrifuged to collect the contents in the bottom. The amplifications were performed in a thermal cycler (Biorad T 300) and the thermal conditions were set as detailed as per Chitra et al., 2015.

After completion of PCR reaction, 3 µl of the amplified product was loaded onto a pre prepared 1.5-2 per cent agarose gel (prepared in 1x TAE buffer) containing ethidium bromide at the concentration of 1 µl/10ml. marker (DNA ladder), positive control, negative control and no template control was also loaded onto one well each, and gel was made to run in a gel electrophoresis unit using a 1x TAE buffer. Later gel was read under gel documentation unit and the images were captured using gel documentation system; Gel Doc XR (Bio-Rad., U.S.A).

**Reference strain**

Two of the PCR positive samples which were commercially sequenced (Eurofins Genomics India Pvt Ltd.,) and the sequencing results obtained were edited using Mega version software and then compared with sequences deposited in NCBI. These samples which showed 100% similarity with the corresponding partial gene sequence of *nuc* of *S. pseudintermedius* were used as positive reference strains in the study.

**Detection of virulence gene (siet gene) using PCR**

**Procedure:** The isolates were subjected to PCR for detection of siet gene responsible for its virulence. The PCR was carried out using published primers and the procedure described by Ananda Chitra et al. (2018) as shown in below table 3. The reaction mixture of 25 µl each was prepared in 0.2 ml thin-walled PCR tubes placed in mini cooler as shown below (Table 4).

**Table 3: Oligonucleotide sequences of *S. pseudintermedius* siet gene primers.**

<table>
<thead>
<tr>
<th>Name of the primer</th>
<th>Primer sequence 5’—3’</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph siet -F</td>
<td>TGCAGGTCTCTCA ATCTTTAAC</td>
<td></td>
</tr>
<tr>
<td>Staph siet -R</td>
<td>CTTGCAACTCTGCACGCAATC</td>
<td>465</td>
</tr>
</tbody>
</table>
After mixing the contents, tubes were centrifuged to collect the contents in the bottom. The amplifications were performed in a thermal cycler (Biorad T 300) and the thermal conditions were set as per AnandaChitra et al., 2018.

After completion of PCR reaction, 3µl of the amplified product was loaded onto a pre prepared 1.5-2 per cent agarose gel (prepared in 1x TAE buffer) containing ethidium bromide at the concentration of 1 µl/10ml. marker (DNA ladder), positive control, negative control and no template control was also loaded onto one well each, and gel was made to run in a gel electrophoresis unit using a 1x TAE buffer. Later gel was read under gel documentation unit and the images were captured using gel documentation system; Gel Doc XR (Bio-Rad., U.S.A).

Detection of mecA gene by PCR

Procedure

The PCR was carried out using published primers of Chitra et al. (2015) as shown in below (Table 5). The reaction mixture of 25 µl each was prepared in 0.2 ml thin walled PCR tubes placed in mini cooler as shown below (Table 6).

Table 4: Details of the contents of PCR mixture for siet gene-based PCR.

<table>
<thead>
<tr>
<th>Reagents (Concentration)</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master mix (Ampliqon Taq DNA Polymerase Master Mix RED,2x)</td>
<td>12.5 µL</td>
</tr>
<tr>
<td>Staph siet-F (12.5 µL/ml)</td>
<td>1 µL</td>
</tr>
<tr>
<td>Staph siet-R (12.5 µL/ml)</td>
<td>1 µL</td>
</tr>
<tr>
<td>Template (Staphylococcal DNA)</td>
<td>3 µL</td>
</tr>
<tr>
<td>Nuclease Free Water</td>
<td>7.5 µL</td>
</tr>
<tr>
<td>TOTAL</td>
<td>25 µL</td>
</tr>
</tbody>
</table>

Table 5: Oligonucleotide sequences of S. pseudintermedius mecA gene primers.

<table>
<thead>
<tr>
<th>Name of the primer</th>
<th>Primer sequence 5′— 3’</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph mec A -FC</td>
<td>AAACTACGGTAACATTGATCGC</td>
<td>210</td>
</tr>
<tr>
<td>Staph mec A -R</td>
<td>GCCTATCTCATATGCTGTTCCT</td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Details of the contents of PCR mixture for mecA gene-based PCR.

<table>
<thead>
<tr>
<th>Reagents (Concentration)</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master mix (Ampliqon Taq DNA Polymerase Master Mix RED,2x)</td>
<td>12.5 µL</td>
</tr>
<tr>
<td>Staph mec A -F (12.5 µL/ml)</td>
<td>1 µL</td>
</tr>
<tr>
<td>Staph mec A -R (12.5 µL/ml)</td>
<td>1 µL</td>
</tr>
<tr>
<td>Template (Staphylococcal DNA)</td>
<td>3 µL</td>
</tr>
<tr>
<td>Nuclease Free Water</td>
<td>7.5 µL</td>
</tr>
<tr>
<td>TOTAL</td>
<td>25 µL</td>
</tr>
</tbody>
</table>
After mixing the contents, tubes were centrifuged to collect the contents in the bottom. The amplifications were performed in a thermal cycler (Biorad T 300) and the thermal conditions were set as per Chitra et al., 2015.

After completion of PCR reaction, 3µl of the amplified product was loaded onto a pre prepared 1.5-2 per cent agarose gel (prepared in 1x TAE buffer) containing ethidium bromide at the concentration of 1 µl/10ml. marker (DNA ladder), positive control, negative control and no template control was also loaded onto one well each, and gel was made to run in a gel electrophoresis unit using a 1x TAE buffer. Later gel was read under gel documentation unit and the images were captured using gel documentation system; Gel Doc XR (Bio-Rad., U.S.A).

RESULTS AND DISCUSSION
In the present study, staphylococci were confirmed by biochemical tests and all the staphylococcal isolates were catalase and coagulase positive. In this study the total number of staphylococcal isolates obtained was 95 (75.5%) out of 126 samples collected and processed.

Identification of S. pseudintermedius by molecular method
Ninety-five staphylococcal isolates confirmed phenotypically were further subjected for the PCR targeting nuc gene for the identification of S. pseudintermedius.

PCR was carried out for the 95 staphylococcal isolates targeting nuc gene. Of these 82 isolates yielded 780bp amplicon specific for S. pseudintermedius (Plate-6). Two PCR products of representative samples (sample 6 and 10) were sent for the commercial sequencing (Eurofins Genomics India Pvt Ltd). The chromatogram and the nucleotide sequence obtained by sequencing were analyzed and edited using Mega version software. Further the edited nucleotide sequences were compared with deposited sequences of NCBI using BLAST tool and the sequence showed 100% sequence similarity with corresponding nuc gene sequence partial of S. pseudintermedius (Table 7, Fig.1).

The results are supported by the work done by Bannoehr and Guardabassi (2012) who reported that S. pseudintermedius was the most prevalent coagulase-positive staphylococci inhabitant of the skin and mucosa of dogs and cats. It was also the major bacterial pathogen isolated from canine infections. Similar work done by Ruzauskas et al. (2016) reported in a study that 192 samples (76.8%) of the 250 samples collected from dogs tested positive for Staphylococcus species. The percentage was higher in non-treated animals (89.5%).

Detection of thermonuclease (nuc) gene
It is difficult to differentiate S. intermedius from S. pseudintermedius during routine diagnostic procedures, but the vast majority of canine isolates are S. pseudintermedius. It has therefore been proposed to report all strains belonging to the SIG from dogs as S. pseudintermedius; unless genomic investigations prove that the strain belongs to a related species (Devriese et al., 2009). Conventional microbiological diagnostic tests often fail to distinguish between S. pseudintermedius and S. intermedius, such that S. pseudintermedius were frequently misidentified as S. intermedius or S. aureus (Sasaki et al., 2007 and Van Hoovels et al., 2006).

In a study by Sasaki et al. (2007), detection of the nuc gene, encoding thermonuclease, was carried out for SIG strains by PCR and PCR products were sequenced directly.

Chitra et al. (2015) in a study reported that PCR targeting nuc gene was a useful tool in preliminary identification of S. pseudintermedius and was also used to differentiate S. pseudintermedius isolates from other coagulase positive staphylococcal species such as S. aureus and Staphylococcus intermedius organisms.

In this present study, a total of 95 staphylococcal isolates were subjected for species detection by nuc gene-based PCR, of which, 82 (86.3%) isolates gave positive results yielding amplified products of 780bp.

The results are supported by a study of Becker et al., (2005) that except for the limitations with isolates of hoofed animals, the S. intermedius nuc PCR assay has potential for rapid identification of S. intermedius and differentiation from other CoPS including S. aureus and the nuc gene was amplified in 94.9% of staphylococcal isolates.
Bannoehr et al. (2007) reported that the lack of unique phenotypic markers for *S. pseudintermedius* in comparison to the other SIG members has precluded its identification without DNA sequencing. Importantly, due to the presence of common phenotypic markers, *S. pseudintermedius* is occasionally misidentified as *S. aureus* in human clinical diagnostic laboratories (Pottumarthy et al., 2004).

Ananda Chitra et al. (2018) developed species specific PCR and screened 91 samples collected between February 2013 and February 2014 from various skin infections of dogs of different breeds, age and sex. *S. pseudintermedius* was isolated from 53 (59%) animals. A higher rate of isolation of *S. pseudintermedius* have been reported in pyoderma cases from Japan (76%) by Onuma et al. (2012), Germany (76%) by Ruscher et al. (2009) and South Korea (61%) by Yoon et al. (2010). Lesser rate of isolation like 52% from both diseased and healthy dogs was noticed in Poland by Garbacz et al. (2013) and 55 per cent from healthy dogs in Tunisia by Elhani et al. (2015). However, a very lower isolation rate was observed with 16 per cent from healthy and diseased dogs in south China by Feng et al. (2012) and 26.5 per cent from pyoderma cases in North China by Wang et al. (2012).

Table 7: PCR based detection of *nuc, siet,* and *mecA* genes in staphylococcal isolates.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>No. of isolates subjected for PCR</th>
<th>Gene targeted</th>
<th>No. of isolates positive by PCR (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95</td>
<td><em>nuc</em> gene</td>
<td>82 (86.31 %)</td>
</tr>
<tr>
<td>2</td>
<td>82</td>
<td><em>siet</em> gene</td>
<td>69 (84.14 %)</td>
</tr>
<tr>
<td>3</td>
<td>82</td>
<td><em>mecA</em> gene</td>
<td>03 (3.65 %)</td>
</tr>
</tbody>
</table>

![Plate 6: Partial amplification of nuc gene of *S. pseudintermedius* by PCR.](image)
SUMMARY

Nuc gene-based PCR detection can be used a reliable diagnostic tool for laboratory diagnosis of *S. pseudintermedius* infections and in this study, it detected 86.3% of the staphylococcal isolates as *S. pseudintermedius*.

Siet gene encoding exfoliative toxin an important virulence factor associated with skin affections was detected in 72.6 per cent of the *S. pseudintermedius* isolates.

REFERENCE


A STUDY OF HISTOPATHOLOGICAL CHANGES IN THE GILL AND LIVER TISSUES OF FRESHWATER FISH *HETEROPNEUSTES FOSSILIS* EXPOSED TO CYPERMETHRIN TOXICITY

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Department of Zoology
SPW Degree & PG College, Tirupati

**ABSTRACT**

The synthetic pyrethroid, cypermethrin toxicity was studied in freshwater fish *Heteropneustes fossilis* and the tissues like gill and liver were chosen for Histopathological effects by light microscopy, for 4 days and 7 days under sublethal concentrations. No Histopathological effects were observed in control group and 4 days group. Hence 7 days group was selected for the present study. Significant changes in Hepatic cells of liver were observed like cloudy swelling of hepatocytes, vacuolar degeneration, and dilation of sinusoids, Hepatic lesions, and Karyo Lexis. In Gill, oedema, Epithelial lining, curling of lamellae were observed. This study demonstrates the potential of electron microscopy in particular SEM, as a tool for detecting cypermethrin induced damage to liver and surface of gill lamellae.

**Keywords:** Organo phosphate Pesticide, Cypermethrin (CYP), Heteropneustes fossilis, Histopathology, Necrosis, Hypertrophy.

**INTRODUCTION**

Synthetic Pyrethroids considered to be an effective insecticides in Agriculture and Aquaculture due to their high insecticidal toxicity with low mammalian toxicity (Elliot et al., 1974) and easily biodegradable. These insecticides occur by ingestion of contaminated food uptake of waterborne residue and through the integument of observed material (Kerr & vas, 1973). The uptake of insecticide in fish was reported to be usually, mostly through the gills (Holden, 1962; Addison, 1976). Fish–specific organs such as the gills and their late metabolic action against this type of pesticide make fish highly susceptible to the toxicity of pyrethroid pesticides. Oxidative stress plays an important role in the neurological, reproductive and developmental toxicity caused by pyrethroids. Moreover, changes in antioxidant enzyme activity following pyrethroid pesticide exposure make fish more susceptible to oxidative stress caused by environmental pollutants. In this an attempt was made to examine occurrence of pyrethroid pesticides in the aquatic environment and oxidative stress induced toxicity in fish exposed to pyrethroids.

The synthetic pyrethroids were widely used in Agriculture, Animal Husbandry, post harvest technology is hazardous to the aquatic organisms. It also affects Environmental factors such as $P$, turbidity, alkalinity, dissolved Oxygen, temperature and conductivity. The lethal effect on aquatic organisms (Jayantha Rao, K. 1984) is in turn influenced by the rate of pollutants entering the water. Aquatic organisms, including fish are frequently exposed to a wide variety of environmental pollutants leading deleterious effects, especially when these contaminants are slightly
decomposable, exhibit a high biological effectiveness and possess a high potential for accumulation or synergetic effects (Au., 2004). Fishes the most diverse group of vertebrate fauna are important component of the food chain and any effect of toxicant may have adverse influence on the nutritional value of fish and on human beings through their consumption.

Fish are typical experimental models for toxicological investigations (Shiekh and Lee, 2008). They are often used to assess the biological impacts of contaminants and water quality because their response to low concentration of toxic substances (Ayas et al., 2007). In the present study, an attempt was made to examine the sublethal effects of different concentrations of Cypermethrin on the gills liver and muscle for 0 days, 4 days and 7 days.

Histopathological study:

a. Light Microscopy

Methodology

The *Heteropneustes fossilis*, a freshwater fishes were procured from surrounding tanks of Kakinada, Andhra Pradesh and transferred to large 100 L glass aquaria (120X45X80 cm) containing dechlorinated tap water for acclimatization for a period of 20 days. They were fed daily with meat and liver of chicken. Afterwards the fish were exposed to according their biomass ratio (Donaldroff et al., 1951) to different concentrations of Cypermethrin.

The LC₅₀ was determined (65 µg/1/48h) tenfold lower concentration of the LC₅₀ was selected as sublethal concentration (0.52 µg/L) and the fishes were exposed to 4 day and 7 days. Controls were maintained simultaneously without toxicants and analyses were done on zero day, 4 th day and 7 th day of experimentation. Tissues like liver and muscle were selected for Histopathological observations. Fishes were sacrificed and the tissues like Gill and liver were fixed in 10% neutral buffered formation. Gill alone were processed by double embedding technique. The fixed tissues were dehydrated in an increased gradient of alcohol (78, 80, 90 and 100%) for 30 minutes each and were eventually dried in Acetone and cleared in Xylene for 30 minutes. The tissues were then infiltrated by embedding by embedding in molten wax and sectioned at 6µ. The Paraffin sections were then mounted on slide, stained with Haematoxylin and counter stained with Eosin and were mounted in Canada balsom. Histopathological lesions were examined and photographed with the help of Intel Pentium QX3 computer attached microscope under 400X lens.

RESULTS AND DISCUSSION

Histology is useful technique for investigating the toxic effect of various pollutants. Such a study also offers opportunity to locate the effect of pollutants in various organs and systems of animals. This type of study in fish has to a great extent is handicapped because of inadequate histological literature concerning various fish organs (Hinton et al., 1977). Considerable interest has been shown in recent years in histopathological studies while conducting sub lethal test in fish. Tissue changes in test organisms exposed to sub lethal concentration of toxicants are a functional response of organisms which provides information on the nature of toxicants.

In fish, gill is the front organ to which any pollutant comes into contact. Fish gill is very sensitive to changes in the composition of the environment and is an important indicator of waterborne toxicants. Consequently, injury to gill epithelium is a common response observed in fish exposed to a variety of contaminants. The severity of damage to the gills depends on the concentration of the toxicant and the period of exposure.

The present histopathological observations of liver tissues showed degenerated hepato -pancreatic tissue, congested blood vessels, blood cells among hepatocytes BC, appearance of blood streaks among hepatocytes (ABS), vacuolar degeneration (VD), as well Necrosis and damage of hepatocytic cell wall and disposition of hepatic cords, (Aldora et al.1998) reported that after cypermethrin exposure, hepatocytes showed nuclear change and cytoplasmic vacuolation.

In Gill tissue histopathological alterations have showed remarkable changes in the structure. The changes include epithelial lining lifting (EL), bulging of tips of primary filaments (BTPG), degenerated secondary Lamella (DGLS), curling of secondary gill filaments (CSG), and atrophy of secondary lamella (ASL), fusion of secondary gill filaments (FSG). The damage of gills of fish exposed to the higher concentrations showed shortened and clubbing of ends of the secondary gill lamellae and necrosis in the primary lamella was well
marked. Hyperplasia and Hypertrophy of nuclei were also noticed. The changes like Pyknotic Nuclei, Vacuolization and degeneration of epithelial cells and pillar cells and lifting of the epithelial layer from the secondary lamellae were also significant.

Liver is an important organ and plays fundamental role in the bio-transformation, in the uptake and detoxification of foreign composition (Gerrihofer et al., 2001) in the body and thus liver is a target organ of xenobiotics. It is also one of the most affected contaminants in water (Camargo and Martinez, 2007) and as a consequence it undergoes different levels of damage. Cypermethrin exposed fish liver showed degeneration in the hepatocytes, necrosis and aggression of inflammatory cells, dilation and congestion may be attributed to the direct toxic effects of pollutants, since the liver is the principle organ responsible for detoxification in vertebrates generally and in fish particularly. The liver during CYP exposed fish had vacuolated cells showing evidence of fatty degeneration. Vacuolations of hepatocytes is a common response associated with exposure of fish to a variety of toxic chemicals which might be an indication of imbalance the rate of synthesis of substances in the Parenchyma cells and the rate of their release into the circulation (Gngerich, 1982).

cytoplasmic vacuolization observed in the liver tissue.

A-C CONTROL (Gill): PGL-primary gill Lamella, SGL-Secondary gill Lamella, CA-Central Axis, ILR-Inter Lamellar Region, Sublethal- B, DPGL-Degenerated Primary Gill Lamella, DSGL- Degenerated Secondary Gill Lamella Sublethal- C, LD-Lamellar Disorganization LF-Lamellar Fusion,


ACKNOWLEDGEMENT
The author is grateful to University Grants Commission (SERO-HYD) for sanctioning Minor Research Project (MRP-7095-16, Dt: 14-9-2018) to carry out the research project.
REFERENCES


ANALYSIS OF OXIDATIVE STRESS MARKERS IN CROSSBRED COWS WITH SUBCLINICAL MASTITIS AND CONCURRENT METABOLIC AND INFECTIOUS DISEASES


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Veterinary College, Vinobanagara, Shivamogga, INDIA

Research Article

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ABSTRACT

The aim of this study was to evaluate the oxidative status in crossbred cows with subclinical mastitis and concurrent metabolic and infectious diseases. The crossbred cows were grouped as Gp-C (n=6) as controls, Gp-M (n=6) with clinical mastitis, Gp-TS consists cows affected with tenositis of teat, Gp-RA with ruminal acidosis, Gp-ND with diarrhea along with dehydration and loss of appetite, Gp-RTI with coughing, fever, decreased appetite, varying degrees of dyspnea, Gp-RB with a history of failure to conceive after at least two successive inseminations. Oxidative stress parameters like catalase, SOD, GPx, GSH and malondialdehyde were estimated spectrophotometrically. The antioxidant enzymes like catalase, super oxide dismutase (SOD), Glutathione Peroxidase (GPx) levels were significantly declined and the nonenzymatic parameters reduced glutathione (GSH) and malondialdehyde (MDA) are significantly elevated in affected animals than in healthy animals. The results of the oxidative stress parameters indicate imbalance of antioxidant profile and oxidative stress in the animals with various disorders and previous illness along with subclinical mastitis.

INTRODUCTION

Bovine mastitis is an economic burden for dairy farmers and its management is crucial for the sustainability of any dairy business. Mastitis is defined as the inflammation of udder tissue because of infection. Mastitis is manifested as clinical and subclinical form. Subclinical mastitis is characterized by changes in the milk, such as an increased pH value, chloride content, or leucocyte count, in the absence of obvious swelling of the udder or clots in the milk. It was observed that, for every clinical case of mastitis in the herd, there are 15–40 subclinical cases contributing to an elevated somatic cell count (Bailey, 2009).

Many of the research efforts are directed at understanding the nature of pathogenic bacteria that are responsible for the most intra-mammary infections. The susceptibility of the animal to develop mastitis may be affected by factors beyond the mammary gland. Therefore, occurrences of other diseases are important determinants of clinical mastitis. Most metabolic diseases like milk fever, ketosis, retained placenta, and displacement of abomasum in dairy cows occur during lactation. Majority of the infectious diseases including mastitis and metritis also occur at the same time. Therefore, there is a close relationship between metabolic and infectious diseases and both of these types of disease may lead to inflammation and oxidative stress. Investigation of oxidative stress status in cows with subclinical mastitis and co-infections or disorders may pave the way for understanding the disease and aid in decision making in livestock health management.

*Corresponding author: amkotresh@gmail.com
The role of free radical biology and antioxidant systems in health and disease is gaining considerable interest currently in human medicine as well in veterinary medicine. An oxidative stress is the excess of reactive oxygen species and the absence of optimal amounts of antioxidants in the body. Various infectious diseases of farm animals, such as pneumonia, enteritis and mastitis are associated with oxidative stress. Different enzymes can prevent the formation of radicals or scavenge radicals or hydrogen peroxide and other peroxides. Among antioxidant enzymes, superoxide dismutase and catalase have been demonstrated in milk (Swaisgood, 1995). Lipid peroxidation is a process triggered by a number of reactive oxygen species and it results in production of various products like lipid hydroperoxides and malondialdehyde (MDA). Increased plasma MDA concentration is considered as a marker of lipid peroxidation. MDA concentrations were found to be increased in the serum, milk and tissue of rats with mastitis. (HadiEslami, et al., 2015). During peripartum period antioxidantive status of dairy cows is seriously impaired and consequently both the oxidative stress and inflammatory response may present the predisposing factors to their higher susceptibility to intramammary infections (IMI) and mastitis (Turk et al., 2017).

There is therefore a close relationship between oxidative stress that may lead to inflammation of various tissues including mammary gland, which can be responsible for causing mastitis. The present study is undertaken to assess the oxidative stress that could affect the origin of inflammation of the mammary gland. The present study would help to take appropriate preventive measures for avoiding mastitis which is a very costly disease worldwide.

MATERIALS AND METHODS

Animal Selection
The crossbred lactating cows (n=42) from various organized farms in central Karnataka were selected based on the history of previous illness and grouped as detailed below for the study. The cows (Gp-C, n=6) which were considered as controls, had history of no co-infections or previous illness for a period of 3 months. The cows (Gp-M, n=6) with clinical mastitis were considered as positive controls. The experimental groups (n=6 in each group) were considered as following. Gp-TS consists cows affected with stenosis of teat, Gp-RA with ruminal acidosis, Gp-ND with diarrhea along with dehydration and loss of appetite, Gp-RTI with coughing, fever, decreased appetite, varying degrees of dyspnea, Gp-RB with a history of failure to conceive after at least two successive inseminations.

California Mastitis Test (CMT)
The California Mastitis Test (CMT) as per Schalm and Noorlander (1957) was performed by pouring each sample of well-mixed milk to a level of 3 ml in one quarter of a partitioned plastic paddle, then adding 3 ml of the commercial CMT reagent by means of an automatic pipette. The paddle, which held four samples, was rotated quickly by hand 10 times.

Interpretation and scoring of the CMT test: Depending upon the degree of gel formation, the grades were assigned according to Sastry (1978) as follows: Negative - no change in consistency. Trace - no visible change in consistency, but when paddle is tipped a slime is momentarily seen on the bottom. 1+ - a gel or thick slime forms, but when the paddle is swirled the solution does not move into the centre. 2+ - a thick lumpy gel forms, which, when swirled, quickly moves toward the centre. 3+ - a distinct gel forms which tends to adhere to the bottom of the paddle, and during swirling a distinct central peak form.

Estimation of enzymatic antioxidants

Superoxide dismutase (SOD) activity
Total SOD activity of blood plasma is determined according to the method of McCord and Fridovich (1969) as modified by by Holland et al (1982). Briefly, the analysis was carried out as following. The assay reaction mixture was composed of 0.1 mM EDTA, 50 µM acetylated ferricytochrome C (freshly prepared), 200 µM of xanthine, 20 mU/ml of xanthine oxidase and 50 µl of samples in 0.05 M potassium phosphate buffer, pH 7.8 in a total reaction volume of 1 mL. 910 µL of assay pre-mix and 50 µL sample were taken and mixed thoroughly. Subsequently, the enzymatic reaction is initiated by adding 20 mU of xanthine oxidase (in 40 µL of assay buffer) to the reaction mixture and the decrease in absorption at 550 nm is observed for a period of 0 to 5 min. The SOD activity of blood plasma is expressed as units/ml.

Catalase activity:
Catalase (CAT) activity of a sample is determined
according to the method of Aebi (1984). This assay is carried out using sample’s ability to decompose hydrogen peroxide (H$_2$O$_2$) into H$_2$O and O$_2$ and the rate of decomposition of H$_2$O$_2$ can be followed directly by the decrease of its absorbance at 240 nm over a period of time (usually 30 seconds). Blood sample (50 µL) was mixed with 450 µL of RBC lysis buffer and kept for 5 minutes for efficient erythrocyte lysis. Then the resultant blood lysate was used for evaluation of catalase enzymes.

**Total glutathione peroxide (GPx) activity**

Total glutathione peroxidase (GPx) activity is determined according to the method of Pagila and Valentine (1967) as modified by Lawrence and Burk (1976). The assay mixture is consisted of 1 mM EDTA, 0.2 mM NADPH, 1 mM GSH, 1 mM NaN$_3$, 1 U/mL glutathione reductase, 1.5 mM cumene hydroperoxide and the enzyme source or sample in 0.1M potassium phosphate buffer, pH 7.0. In a total volume of 1 mL, on the day of the experiment, an assay pre-mix is prepared taking all the above reagents except cumene hydroperoxide and sample/standard. Sample (0.1mL) is added to 0.8 mL of the above assay mixture and allowed to incubate for 5 min at room temperature before initiation of the enzymatic reaction by the addition of 0.1 mL of pre-warmed cumene hydroperoxide solution. The decrease in absorbance of NADPH at 340 nm is monitored for 5 min.

\[
\text{Total Gpx activity (units/mL of sample)} = \frac{\Delta A_{340}/\text{min}}{\text{DF}} x \frac{V}{6.22} x \frac{1.05}{0.05} x \frac{1.0}{0.5} = \frac{f}{F} \times 21
\]

Where, \(\Delta A_{340}/\text{min} = \text{Change in absorbance at 340 nm/min of sample or blank, } \text{DF} = \text{Dilution factor of the original sample, if any, before adding to the reaction mix, } V = \text{Sample volume in mL, } 6.22 = \text{Millimolar extinction coefficient of B-NADPH at 340 nm}

**Determination of Blood Glutathione (GSH) level:**

Glutathione (GSH) in whole blood was determined by method of Butler et.al. 0.5 ml of the sample was added to 2 ml of phosphate solution, followed by the addition of 0.25 ml of DTNB reagent. The absorbance was measured at 412 nm within 5 minutes of the addition of DTNB reagent against blank (prepared using 0.5ml of 1% metaphosphoric acid. GSH concentration = Absorbance of sample X concentration of standard in 0.5 ml. GSH concentration expressed as µg/ml.

**Malondialdehyde (MDA) Assay**

The peroxidative damage in plasma was evaluated in terms of lipid peroxidation (LPO). Lipid peroxidation in tissue samples was measured as thiobarbituric acid-reactive substance called malondialdehyde (MDA) formed per ml of plasma according to Yagi et al.(1984). Taking the Spectrophotometric measurement of the standard solution, which is obtained by reacting 0.5 nmol of tetramethoxypropane with TBA by steps 4-6, as F and that of the sample as f, the lipid peroxide level (Lp) can be expressed in terms of malondialdehyde.

\[
\text{Plasma } Lp = 0.5 x f/F x 1.05/0.05 x 1.0/0.5 = f/F \times 21 \text{ (nmol/ml of blood)}
\]

**Statistical analysis**

The values obtained from the various experiments were expressed as Mean ± S.E with ‘n’ unequal to number of animals or samples. Data obtained were statistically subjected to one-way analysis of variance (ANOVA) followed by Bonferroni’s post hoc Multiple Comparison Test using Graph Pad Prism software programme (GraphPad® software Inc., Version 5.0; San Diego, CA, USA). difference was considered significant at \(p<0.05\) or lower.

**RESULTS**

**California. Mastitis Test (CMT)**

Out of 170 lactating animals screened by CMT 35.2% of the cows were found positive. The mean CMT scores among the groups were enlisted (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>CMT Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gp-C</td>
<td>0.00</td>
</tr>
<tr>
<td>Gp-M</td>
<td>3.00</td>
</tr>
<tr>
<td>Gp-TS</td>
<td>2.75</td>
</tr>
<tr>
<td>Gp-RA</td>
<td>2.75</td>
</tr>
<tr>
<td>Gp-ND</td>
<td>3.00</td>
</tr>
<tr>
<td>Gp-RTI</td>
<td>2.25</td>
</tr>
<tr>
<td>Gp-RB</td>
<td>2.75</td>
</tr>
</tbody>
</table>

Note: Values are mean, Means with different superscripts differ significantly within the Column (\(p < 0.05\))
**Estimation of Oxidative Stress Parameters**

Superoxide Dismutase (SOD) activity was significantly (p < 0.05) different from group C with all the groups of affected animals and are significantly lower in groups compared to Group C. Catalase activity values are significantly lower (p < 0.05) in groups compared to control. Glutathione peroxidase (GPx) activity showed significant difference in the GSHPx activity observed between Groups C with all the Groups and GSHPx levels are significantly lower in groups M, TS, RA, ND, RTI, RB, compared to C (p < 0.05). There is significant (p<0.05) difference between Group M and Group C. (Table 2).

**DISCUSSION**

Oxidative stress is associated with many diseases including sepsis, enteritis, pneumonia and arthritis in animals of veterinary importance. In dairy cows, it may cause mastitis and reproductive disorders (Turk et al., 2012). The mean activities of SOD (U/mL), CAT(U/mL) and GPx(mU/mL) were 15.04±0.3224, 110.3±4.079, and 68.38±1.413

### Table 2: Assessment of enzymatic antioxidants in subclinical mastitis affected Animals with metabolic disorders and co-infections

<table>
<thead>
<tr>
<th>Group (U/mL)****</th>
<th>SOD Activity (U/mL)****</th>
<th>Catalase Activity (U/mL)****</th>
<th>GPx Activity (µg/mL)***</th>
<th>Reduced GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gp-C</td>
<td>23.07±1.053a</td>
<td>266.1±30.43a</td>
<td>96.37±2.588b</td>
<td>25.3±0.6759a</td>
</tr>
<tr>
<td>Gp-M</td>
<td>15.14±0.6285a</td>
<td>107.8±10.21b</td>
<td>64.35±5.075c</td>
<td>33.18±2.824c</td>
</tr>
<tr>
<td>Gp-TS</td>
<td>16.49±1.219b</td>
<td>123.4±12.98b</td>
<td>73.61±2.963b</td>
<td>36.42±1.41b</td>
</tr>
<tr>
<td>Gp-RA</td>
<td>15.6±0.8349b</td>
<td>108.3±10.44b</td>
<td>69.51±2.975c</td>
<td>32.9±1.108c</td>
</tr>
<tr>
<td>Gp-ND</td>
<td>13.83±0.4097c</td>
<td>85.93±11.4b</td>
<td>63.94±3.62c</td>
<td>30.68±1.674c</td>
</tr>
<tr>
<td>Gp-RTI</td>
<td>14.23±0.9375c</td>
<td>113.7±11.22b</td>
<td>64.82±5.813c</td>
<td>31.43±2.334c</td>
</tr>
<tr>
<td>Gp-RB</td>
<td>13.47±1.031b</td>
<td>97.33±10.76b</td>
<td>65.27±3.602b</td>
<td>31.27±1.94d</td>
</tr>
</tbody>
</table>

**Note:** Values are mean ± SE, Means with different superscripts differ significantly within the Column (p < 0.05).

### Levels of Malondyaldehyde (MDA): There is significant difference between all the Groups with Group C where MDA levels are significantly higher (p < 0.05) in all the groups. Groups M, TS, RA, ND, RTI, RB, as compared to group C. There is significant difference between Group M and C with MDA levels significantly higher in Group M compared to Group C. (Table 3).

### Table 3: Assessment of malondyaldehyde in subclinical mastitis affected animals with metabolic disorders and co-infections.

<table>
<thead>
<tr>
<th>Group</th>
<th>Malondyaldehyde –MDA (nmol/mL)****</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gp-C</td>
<td>14.43±0.383a</td>
</tr>
<tr>
<td>Gp-M</td>
<td>20.41±0.655c</td>
</tr>
<tr>
<td>Gp-TS</td>
<td>19.71±1.209c</td>
</tr>
<tr>
<td>Gp-RA</td>
<td>20.42±0.690c</td>
</tr>
<tr>
<td>Gp-ND</td>
<td>22.02±0.589c</td>
</tr>
<tr>
<td>Gp-RTI</td>
<td>19.97±0.603c</td>
</tr>
<tr>
<td>Gp-RB</td>
<td>20.75±0.600c</td>
</tr>
</tbody>
</table>

**Note:** Values are mean ± SE, Means with different superscripts differ significantly within the Column (p < 0.05).
respectively. These values are significantly lower in animals with subclinical mastitis and compare to healthy animals. These results are in accordance with previous studies on dairy cows with clinical mastitis and subclinical mastitis (Rehman et al., 2017, Sharma et al., 2011). The preventive body antioxidative defense systems can be accomplished by enzymes SOD, GPx and CAT. The decrease in these enzymes in this study can be attributed to excessive production of free radicals and reactive oxygen species. Imbalance in the antioxidant and oxidant system lead to damage of biological macromolecules as evidenced by increased MDA levels (Trevisan et al., 2001). The significant increase in blood MDA levels in the current study are in line with the reported involvement of mammary cellular damage due to oxidative stress in both clinical and subclinical mastitis (Rehman et al., 2017). It was also noted that an increased level of somatic cells can be correlated with an increase in the concentration of MDA thus with increased lipid peroxidation processes. (Andrei et al., 2011). The mean concentration + SE of GSH (µg/ml) is 32.72±0.6882, p<0.05 in affected groups and is significantly increased compared to healthy animals (25.3±0.6759, p < 0.05). These results are in accordance with Kizil et al. (2007) showed significant increases of plasma GSH concentrations in SCMT affected cows (p<0.01).

The oxidative stress among the groups is similar indicating the all the disorders or previous illness had led to the oxidative stress. When reactive oxygen species are not effectively and safely removed oxidative stress may impair the health of dairy cows both metabolic and hormonal disease (Celi, 2011). The disorders and previous illness might have influenced the occurrence of mastitis due to oxidative stress. Many studies have shown that multiple diseases, including mastitis, mammary edema, metritis, and retained fetal membranes most commonly occur during the periparturient period when dairy cows are known to experience oxidative stress (Kankofer, 2002; Miller et al., 1993). All the lactating animals in the study were under oxidative stress aggravated by the concurrent diseases or disorders. Therefore, the aim of the treatment of mastitis should be coupled with antioxidant therapy.

REFERENCES


Water is important for survival of living beings as well as geological and geomorphic processes on the planet Earth. In the present scenario of developmental activities both surface and groundwater are polluted and need attention for checking the pollutant sources. Ghaggar river in northern part of the country is flowing from the states-Himachal Pradesh, Haryana, Punjab, Rajasthan and finally in Pakistan. In the upper part of the Ghaggar river after entering in Haryana in Panchkula district its water quality has been assessed for drinking purpose. Eight river water samples were collected from different locations of Ghaggar River in the month of June 2019. Water samples were analyzed using Field Water Testing Kit prepared by Tamilnadu Water Supply and Drainage Board (TWAD), Chennai for chemical parameters-pH, Hardness, Chloride, Fluoride, Iron, Ammonia, Nitrite, Nitrate, Phosphate and Residual Chlorine. Results of chemical analysis of water samples were categories as per BIS drinking water standards (IS 10500:2012). In the river water samples pH ranges from 6.5 to 7.5; hardness ranges from 100 mg/l to 1170 mg/l; chloride ranges from 50 mg/l to 360 mg/l; fluoride ranges from 0.5 mg/l to 3 mg/l; iron ranges from nil to 10 mg/l; ammonia ranges from 0.5 mg/l to 5 mg/l; nitrite ranges from 0.2 mg/l to 1 mg/l; nitrate ranges from 20 mg/l to 150 mg/l; phosphate ranges from 0.5 mg/l to 1 mg/l and residual chlorine ranges from nil to 2 mg/l. The data interpretation shows that pH in all the eight water samples is desirable for drinking purpose; hardness is desirable at Bitna, Kaushalya Dam, MajriChowk, Peer Muchchalla, Sector-28, Panchkula, Daffarpur and permissible at Jagatpur and non-potable at ChandiMandir; chloride is desirable at Bitna, Jagatpur, Kaushalya Dam, MajriChowk, Peer Muchchalla, Sector-28, Panchkula, Daffarpur and permissible at ChandiMandir; fluoride is desirable at Bitna, Jagatpur, Kaushalya Dam, MajriChowk, Peer Muchchalla, Sector-28, Panchkula, Daffarpur and non-potable at ChandiMandir; iron is desirable at Bitna, Kaushalya Dam, Peer Muchchalla, Sector-28, Panchkula, Daffarpur and permissible at ChandiMandir; ammonia is desirable at Bitna, Sector-28, Panchkula, Daffarpur and non-potable at ChandiMandir; iron is desirable at Bitna, Kaushalya Dam, Peer Muchchalla, Sector-28, Panchkula, Daffarpur; nitrite is desirable in all the eight water samples; nitrate is desirable at ChandiMandir, MajriChowk, Peer Muchchalla, Sector-28, Panchkula and non-potable at Bitna, Jagatpur, Kaushalya Dam, Daffarpur; phosphate is desirable in all the eight water samples and residual chlorine is desirable at Bitna, Jagatpur, Kaushalya Dam, ChandiMandir, Peer Muchchalla, Sector-28, Panchkula, Daffarpur and non-potable at MajriChowk. The study shows that river water is not suitable for drinking purpose in seven water samples except one water sample (Sector-28, Panchkula). The study is highly useful for monitoring the water quality of Ghaggar River.

Keywords: Geospatial technology, Ghaggar River, water, quality, Panchkula, Haryana.

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INTRODUCTION
Water is important for survival of livings on the planet Earth. Rivers are one of the water carriers from one place to other place. In the source areas river water is generally good but it becomes polluted when reaches in consumption areas i.e. populated and industrial areas. Rivers are the nerves of an area and need prime attention to keep them pollution free so that good quality of water may reach to people. But, the situation of water quality of rivers is alarming. Satellite remote sensing data are good source for mapping of rivers and selection of sample sites. Many workers have done good work on river water quality Olajire and Imeokpia (2001), Joshi et al. (2009), Norsaliza and Mohd (2010), Yisa and Jimoh(2010), Aggarwal and Arora (2012), Uddin et al. (2014), Asadi et al. (2017), Alssgeera et al. (2018), Gafri et al. (2018), Raj, Naveen et al. (2018).

STUDY AREA
Ghaggar River originates in Sirmour District of Himachal Pradesh and travels a length of 320 Km through the States of Haryana, Punjab and Rajasthan in India and after that in Pakistan. It enters the territory of Haryana in Panchkula District near Kalka and passes through the Panchkula district to Mohali district in Punjab and again enters in Ambala district of Haryana and then enters into Patiala district in Punjab and it again enters in Kaithal district of Haryana and then crosses to Sangrur district of Punjab and it again enters in Fatehabad district of Haryana and crosses to Mansa district of Punjab and enters in Sirsa district of Haryana and finally enters in Hanumangarh district of Rajasthan and after that in Pakistan. In the present study a small part of the Ghaggar River falling in Panchkula district have been selected for its water quality assessment for drinking purpose (Fig.1).

OBJECTIVE
The main objective of the study was to assess Ghaggar River water quality for drinking purpose in Panchkula district, Haryana.

MATERIALS USED AND METHODOLOGY
Ghaggar River was digitized on Google earth satellite data in ArcGIS software. Eight water samples of Ghaggar River were collected in plastic 250 ml bottles in the month of June 2019 (Fig.2, Table 1). Geo-coordinates of sample sites were noted with the help of mobile GPS. Chemical analysis of eight water samples was done using Field Water Testing kit prepared by Tamilnadu Water Supply and Drainage (TWAD) Board, Chennai for ten chemical parameters viz. pH, hardness, chloride, fluoride, iron, ammonia, nitrite, nitrate, phosphate and residual chlorine (Table 2). Water samples analysis results were categorized into desirable, permissible and non-potable on the basis of BIS (IS:10500:2012) Drinking Water Standards (Table 3).
Table 1: Location of Ghaggar River water samples.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample Locations</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bitna</td>
<td>30.802932°</td>
<td>76.931645°</td>
</tr>
<tr>
<td>2</td>
<td>Jagatpur</td>
<td>30.740461°</td>
<td>76.909989°</td>
</tr>
<tr>
<td>3</td>
<td>Kaushalya Dam</td>
<td>30.785095°</td>
<td>76.914733°</td>
</tr>
<tr>
<td>4</td>
<td>ChandiMandir</td>
<td>30.726251°</td>
<td>76.895649°</td>
</tr>
<tr>
<td>5</td>
<td>MajriChowk</td>
<td>30.699540°</td>
<td>76.877357°</td>
</tr>
<tr>
<td>6</td>
<td>Peer Muchchalla</td>
<td>30.666147°</td>
<td>76.875203°</td>
</tr>
<tr>
<td>7</td>
<td>Sector-28, Panchkula</td>
<td>30.646977°</td>
<td>76.872468°</td>
</tr>
<tr>
<td>8</td>
<td>Daffarpur</td>
<td>30.644242°</td>
<td>76.871799°</td>
</tr>
</tbody>
</table>

Table 2: Results of chemical analysis of Ghaggar River water samples.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample Locations</th>
<th>pH</th>
<th>Hardness (mg/l)</th>
<th>Chloride (mg/l)</th>
<th>Fluoride (mg/l)</th>
<th>Iron (mg/l)</th>
<th>Ammonia (mg/l)</th>
<th>Nitrite (mg/l)</th>
<th>Nitrate (mg/l)</th>
<th>Phosphate (mg/l)</th>
<th>Residual Chlorine (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bitna</td>
<td>7.0</td>
<td>200</td>
<td>50</td>
<td>1</td>
<td>0</td>
<td>0.5</td>
<td>1</td>
<td>150</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Jagatpur</td>
<td>7.5</td>
<td>300</td>
<td>230</td>
<td>0.5</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>150</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Kaushalya Dam</td>
<td>6.5</td>
<td>100</td>
<td>50</td>
<td>0.3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>ChandiMandir</td>
<td>7.5</td>
<td>1770</td>
<td>360</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>0.2</td>
<td>45</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>MajriChowk</td>
<td>7.5</td>
<td>150</td>
<td>150</td>
<td>1</td>
<td>10</td>
<td>3</td>
<td>0.2</td>
<td>20</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Peer Muchchalla</td>
<td>7.0</td>
<td>150</td>
<td>150</td>
<td>1</td>
<td>0.5</td>
<td>2</td>
<td>0.5</td>
<td>45</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>7</td>
<td>Sector-28, Panchkula</td>
<td>7.5</td>
<td>190</td>
<td>170</td>
<td>1</td>
<td>0.3</td>
<td>0.5</td>
<td>0.2</td>
<td>45</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>8</td>
<td>Daffarpur</td>
<td>7.5</td>
<td>200</td>
<td>50</td>
<td>1</td>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
<td>75</td>
<td>1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 3: BIS Drinking Water Standards (IS 10500:2012).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Constituent</th>
<th>Potable</th>
<th>Non-Potable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desirable</td>
<td>Permissible</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>pH</td>
<td>6.5 to 8.5</td>
<td>&lt;6.5 to &gt;8.5</td>
</tr>
<tr>
<td>2</td>
<td>Total Hardness (mg/l)</td>
<td>&lt;200</td>
<td>200-600</td>
</tr>
<tr>
<td>3</td>
<td>Chloride (mg/l)</td>
<td>&lt;250</td>
<td>250-1000</td>
</tr>
<tr>
<td>4</td>
<td>Fluoride (mg/l)</td>
<td>&lt;1.0</td>
<td>1.0-1.5</td>
</tr>
<tr>
<td>5</td>
<td>Iron (mg/l)</td>
<td>&lt;0.3</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Ammonia (mg/l)</td>
<td>&lt;0.5</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Nitrite (mg/l)</td>
<td>&lt;1.0</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Nitrate (mg/l)</td>
<td>&lt;45</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Phosphate (mg/l)</td>
<td>&lt;1.0</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Residual Chlorine (mg/l)</td>
<td>&lt;0.2</td>
<td>0.2-1</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

i. pH
In the Ghaggar River water samples pH ranges from 6.5 to 7.5. pH is desirable in all the eight Ghaggar river water samples (Bitna (7), Jagatpur (7.5), Kaushalya Dam (6.5), ChandiMandir (7), Majri Chowk (7.5), Peer Muchchalla (7), Sector-28, Panchkula (7.5), Daffarpur (7.5)(Fig.3).

ii. Hardness
In the Ghaggar River water samples hardness ranges from 100 mg/l to 1170 mg/l. Hardness is desirable at Bitna (200 mg/l), Kaushalya Dam (100 mg/l), MajriChowk (150 mg/l), Peer Muchchalla (150 mg/l), Sector-28, Panchkula (190 mg/l), Daffarpur (200 mg/l) and permissible at Jagatpur (300 mg/l) and non-potable at ChandiMandir (1170 mg/l)(Fig.4).

iii. Chloride
Chloride ranges from 50 mg/l to 360 mg/l in the Ghaggar river water samples. Chloride is desirable at Bitna (50 mg/l), Jagatpur (230 mg/l), Kaushalya Dam (50 mg/l), MajriChowk (150 mg/l), Peer Muchchalla(150 mg/l), Sector-28, Panchkula (170 mg/l), Daffarpur (50 mg/l) and permissible at ChandiMandir (360 mg/l) (Fig.5).

iv. Fluoride
Fluoride ranges from 0.5 mg/l to 3 mg/l in the Ghaggar river water samples. Fluoride is desirable at Bitna(1mg/l), Jagatpur(0.5mg/l), Kaushalya Dam (0.5 mg/l), Majri Chowk(1mg/l), Peer Muchchalla(1mg/l), Sector-28, Panchkula (1mg/l), Daffarpur (1mg/l) and non-potable at ChandiMandir(3 mg/l) (Fig.6).

v. Iron
Iron ranges from nil to 10 mg/l in the Ghaggar river
ix. Phosphate
Phosphate ranges from 0.5 mg/l to 1 mg/l in the Ghaggar river water samples. Phosphate is desirable in all the eight samples (Bitna (1 mg/l), Jagatpur (0.5 mg/l), Kaushalya Dam (1 mg/l), ChandiMandir (0.5 mg/l), MajriChowk (0.5 mg/l), Peer Muchchalla (0.5 mg/l), Sector-28, Panchkula (0.5 mg/l), Daffarpur (1 mg/l)) (Fig.11).

x. Residual Chlorine
Residual Chlorine ranges from nil to 2mg/l in the Ghaggar river water samples. Residual Chlorine is desirable at Bitna (0 mg/l), Jagatpur (0 mg/l), Kaushalya Dam (0 mg/l), ChandiMandir (0.2 mg/l), Peer Muchchalla (0.2 mg/l), Sector-28, Panchkula (0.2 mg/l), Daffarpur (0.2 mg/l) and non-potable at MajriChowk (2 mg/l) (Fig.12).

GHAGGAR RIVER WATER QUALITY AT SAMPLE SITES

I. Bitna
At Bitna water sample site pH, hardness, chloride, fluoride, iron, ammonia, nitrite, phosphate, residual chlorine is desirable except nitrate (150 mg/l) which is non-potable, hence, the water is non-potable (Fig.13). Nitrate is an anthropogenic pollutant in the Ghaggar river water, therefore, source of the pollutant may be identified and closed.

ii. Jagatpur
At Jagatpur water sample site pH, chloride, fluoride, nitrite, phosphate, residual chlorine is desirable; hardness is permissible and iron, ammonia and nitrate are non-potable, hence, the water is non-potable (Fig.14). Iron, ammonia and nitrate in the Ghaggar

Fig. 8: Ammonia in Ghaggar River water samples.

Fig. 9: Nitrite in Ghaggar River water samples.

Fig. 10: Nitrate in Ghaggar River water samples.

Fig. 11: Phosphate in Ghaggar River water samples.

Fig. 12: Residual Chlorine in Ghaggar River water samples.
permissible and hardness, fluoride, iron, ammonia are non-potable, hence, the water is non-potable (Fig.16). Hardness, fluoride, iron, ammonia in the Ghaggar river water may be due to anthropogenic reasons, therefore, source of the pollutants may be identified and closed.

**v. Majri Chowk**
At Majri Chowk water sample site pH, hardness, chloride, fluoride, nitrite, nitrate, phosphate, residual chlorine is desirable and iron, ammonia are non-potable, hence, the water is non-potable (Fig.17). Iron and ammonia in the Ghaggar river water may be due to nitrate, phosphate, residual chlorine is desirable, hence, the water is potable (Fig.19).

**vi. Peer Muchchalla**
At Peer Muchchalla water sample site pH, hardness, chloride, fluoride, iron, nitrite, nitrate, phosphate, residual chlorine is desirable and ammonia is non-potable, hence, the water is non-potable (Fig.18). Ammonia in the Ghaggar river water may be due to anthropogenic reasons, therefore, source of the pollutants may be identified and closed.

**vii. Sector-28, Panchkula**
At Sector-28, Panchkula water sample site pH, hardness, chloride, fluoride, iron, ammonia, nitrite,
Ghaggar river water quality is non-potable at sample sites- Bitna, Jagatpur, Kaushalya Dam, Chandi Mandir, Majri Chowk, Peer Muchchalla, Daffarpur and potable at Sector-28, Panchkula.

REFERENCES


Nowadays with the rapid development of industries, a large amount of heavy metals contaminated water is released to the environment (Liu et al., 2020). Heavy metals discharged from various industries such as mining, metal finishing, electroplating, glass, textiles, ceramics and storage batteries (Godwin et al., 2019). This leads to inevitable release of heavy metals to the environment. They are non-biodegradable, accumulating in living organisms and also aggravating their hazardous impact on the environment (Poo et al., 2018). The toxicity of heavy metals are the major risk to all the living beings and alters the environment. Most of the essential heavy metals are toxic and carcinogenic in nature (J.M. Patra et al., 2017). Heavy metals react with soil constituents by various mechanisms such as adsorption, fixation and surface precipitation (Campillo et al., 2020). It is therefore important to struggle with remediation of contaminated soils.

Bio remediation is an ecologically-friendly approach that involves the use of living organisms particularly microorganisms, to degrade contaminants that alters toxic
to less toxic compounds (Akpomie Olubunmi O et al., 2016). Several technologies have been used to remove heavy metals, among these, adsorption is a widely recommended method for the removal of heavy metals ions, due to its high efficiency, fast, ease of operation, inexpensive and availability of various adsorbents (Bordoloi et al., 2017). The effect of application of adsorbent is not only a decrease in the bioavailability of pollutants, but also reduced spreading of pollutants in the environment (Koltowski et al., 2017).

In recent days, a cost effective and environmentally friendly remediation approach is the application of Biochar (He et al., 2019). This technological review focuses on the selection of feedstock, production, characterization of Biochar (BC) and its application in promoting the sustainable environment.

**Biochar**

The treatment of biomass by heat under limited oxygen, where the complex chemical compounds are converted into simple, which gives various gaseous compounds termed as “char”. The material that has been obtained from biomass of several feedstocks is named as “Biochar” (K Kalus et al., 2019). The solid biochar can be obtained from biomass by several thermal decomposition processes such as pyrolysis, combustion, gasification and liquefaction. Based on its applications, scientific researchers emerged in the research includes: its mitigation of climatic change, efficient and cost-effective waste management and also acts as amendment to improve soil quality and sustain crop yield (Sohi SP et al., 2010).

**Pyrolysis**

Among various methods, pyrolysis is considered as a feasible method that can quickly convert biomass into solid biochar. Main thermochemical processes such as slow pyrolysis, fast pyrolysis, flash pyrolysis, catalytic pyrolysis and microwave assisted pyrolysis are commonly used for the production of biochar (Manya 2012).

From the literature search it has been understood that in slow pyrolysis, the temperature is gradually increased at a slow rate in the anoxic condition with a heating rate ranging from 5 to 7°C/min, the residence times of vapor persist longer usually for 10–60 s for the production of biochar (Canabarro N et al., 2013, M Sekar et al., 2021) and the particle size for degradation ranging between 5 and 50 mm whereas fast pyrolysis produces bio-oil mainly at the higher temperature increased at a faster rate. Rapid heating rate at 300°C–600°C/min, a short vapor residence time and the particle size less than 1 mm.

**Biochar Terminology**

According to Kanyaporn et al (2012), “Biochar is a stable form of carbon, being more stable than the organic form and capable or remaining in the soil for hundreds and thousands of years”. The pyrolysis technique is relatively simple and low cost and allows significant flexibility in both the type and quality of the biomass feedstock. Liu et al., 2020 described Biochar as “carbon- based porous material prepared by pyrolysis. Senthilkumar et al., 2019 stated that “Biochar can be synthesized from biological material through pyrolysis, in the absence of O2 or limited oxygen conditions. E Sforza et al, (2020) defined “Biochar as a carbon rich material characterized by high porosity with oxygen functional groups and aromatic surfaces. It is produced from the pyrolysis (300°C-700°C) (Wang et al., 2019) along with syngas and bio-oil (Singaravelu 2019).

**Feedstock for the biochar production**

The most important criteria for the production of biochar depends on the stable biomass feedstock selection. The choice of biomass based on the abundance of feedstock, ready availability and low cost (Jang and Kan, 2019).

From the literature it has been used a wide variety of raw materials used such as agricultural waste, municipal solid waste, kitchen waste, crops, sludge, lignocellulosic biomass, non-lignocellulosic material, industrial residues and also living organisms, synthetic polymers/plastics, tyres, coal have been employed (Agrafioti et al., 2014, Liu et al., 2019, J. Wang et al., 2019).

**Preparation of biochar**

From the literature, different methods of biochar production were carried out from various feedstocks using different materials.

The biochar was prepared using porcelain crucible in a closed furnace, during pyrolysis N2 was mixed at 2500ml/min to prevent explosion in the furnace (K.M. Poo et al., 2018). Bird et al., 2012 pyrolyzed the algal biomass separately using batch pyrolysis with gas flame support using a Bigchar™ 1000 pyrolysis unit.
Y.Y. Wang et al 2016 carried out the biochar preparation in a programmable tube furnace by slow pyrolysis at 300°C for 3 hours under anaerobic conditions. And the heating rate was about 25°C min⁻¹ and cooled to room temperature. Pyrolysis was carried out in a muffle furnace and heating rate at 17°C/min (E. Agrafoioti et al., 2014). Few researchers carried out biochar preparation in a bench top, high temperature (upto 1200°C) electric quartz tube furnace. The samples heated at High Heating Temperatures (HHT) such as 350°C, 450°C, 550°C and 650°C with a heating rate of 5°C /min and with the holding time of 60 mins. Slow pyrolysis at 450°C -500°C is a viable choice to obtain high yield and good quality biochar (Yu et al., 2017). Fixed bed vertical, tubular reactor made of quartz glass was used for the preparation of BC, with 40°C/min heating rate reached a higher level upto 500°C, under the flow of N₂ at 100ml/min (Bordoloi et al., 2017). Nitrogen gas used in the pyrolysis process is mainly to avoid oxygen presence inside the reactor (M Sekar et al., 2021)

Characterization of biochar

The characteristics of biochar vary by feedstock and by processing condition (Li et al., 2018). The elemental composition, pH, specific surface area, surface potential and spectral property of the biochar were analyzed by various analyzer such as elemental analyzer, and inorganic elemental constituents by inductively coupled plasma optical emission spectrometer (ICP-OES), pH meter, surface analyzer, zeta potential analyzer and FTIR spectroscopy (Ni et al., 2018). Taghavi et al., 2018 characterized the protein content of brown macroalgae was measured by Kjeldahl method based on national standard of GB/T 6432-1994 and in accordance with GB/T 6433-2006 standard, the amount of lipid was obtained by the solvent extraction method. Volatile and moisture content were measured at a temperature ranging from 30°C to 800°C at 10°C/min with a nitrogen gas flow of 20 L min⁻¹ through thermogravimetric analysis (TGA) using thermogravimetric analyzer (TGA/SDTA851 and METTLER-TOLEDO compact). Based on the standard test for determination of ash content of biomass ASTM E1755, the ash content was obtained.

The surface area is the main physical property which affects the metal sorption (Karthik et al.). The specific surface area was quantified using N₂ multilayer surface area and porosity analyzer and the measured data arranged according to the BET method (Son et al., 2018). The carbon and nitrogen contents of biochar were studied using Stable Isotope Analyzer (Mahdi et al., 2016). And various common analytical techniques of biochar are X-Ray Diffraction (XRD), X-Ray Photoelectron Spectroscopy (XPS), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), X-Ray Spectroscopy (EDS), Scanning Transmission Electron Microscopy (STEM), X-Ray adsorption Spectroscopy (EXAFS) and solid state Nuclear Magnetic Resonance (NMR) were briefly presented (Wang et al., 2019). The pH and electrical conductivity were measured by pH-EC meter, ash content, volatile matter and yield of biochar was determined by American Society for Testing Material (ASTM) method D1762.

Biochar as a biosorbent

Recent researches have shown that biochar is an effective biosorbent which is ecofriendly and easily available in sufficient quantities and it has also been proven to be an effective adsorbent of inorganic and organic pollutants. (Xue et al., 2012). Biochar has been shown to be very effective in immobilizing, adsorbing and sequestering a number of heavy metals from soil and water. The adsorption capacity of biochar depends on its physicochemical properties such as surface area, distribution of pore size, functional groups and cation exchange capacity, while physicochemical properties differ with preparation condition (Wang et al., 2019). These properties play a major role in the interplay between biochar surfaces and heavy metals.

Biochar promotes sustainable environment

For the sustainable environment, biochars help based on its three sustainability factors such as use of sustainable biomass, sustainable production processes, and sustainable end use (Elad et al., 2011). Biochar creates a sustainable agricultural land and environment mainly by neutralizing the soil from acidic state (Jeffery S et al., 2011) by which the soil nutrients and microbial activity is increased. Due to its high-water retention capacity, biochar reduces the leaching of soil and improves the water regime of the soil (Novak JM et al., 2012). The capability of biochar is to adsorb and neutralize the phytotoxic organic compounds by increasing the surface area during pyrolysis (Thies J et al., 2009). Many studies exclaimed that the application of biochar on soil as a greater sorption affinity, it binds with various organic pollutants in the environment.
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36. **Zainab Mahdi, Ali El Hanandeh, Qiming Yu.**
TRACKING ENVIRONMENTAL ISSUES AND ACTIONS

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Review Article

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ABSTRACT

Life support system or environment of Earth is undergoing rapid & unprecedented changes in recent decades. How to follow up and understand emerging environmental issues and concerns from local to global level employing new and emerging tools and technologies is presented in this communication. An experiential perspective for the benefit of students, professionals, researchers and citizens is presented here in to further the debates regarding actions and programmes to be taken up.

Keywords: Anthropocene, global warming, climate change, biodiversity, ground water depletion, pollution, GM organisms, crops, fruits and vegetables, depletion of ozone layer, ozone build up near surface of Earth, wastes, E-wastes, pollution in outer space, interlinking of rivers, sustainable agriculture, forestry, energy, plastics, human-animal conflicts, nuclear weapons, bioweapons, alien species /invasive species, rivers: Ganga, Yamuna, disconnect with nature, value of ecological services, industrial & nuclear accidents, population pressure, pandemics and syndemics, space debris orbiting Earth, and declining number of pollinators, largely bees.

INTRODUCTION

Environment and contemporary issues are concerns of people of all walks of life. How to keep oneself aware about the happenings and actions towards environment protection and addressing environmental issues at local, regional and global levels is to be learnt and devised by people as per their experiences, interests and resources. An experiential account using mass communication media like television, radio, newspapers, journals, magazines, internet, mobile phone, laptop or desktop is being presented in this paper.

Contemporary Environmental Issues

In the present age, Anthropocene, much-discussed environmental concerns, issues and developments are as follows:

Global warming, climate change— Global warming is rise in average global temperature because of increasing concentration of greenhouse gases in the atmosphere. Long term changes in climate parameters such as precipitation, temperature and wind patterns have direct bearing on global warming and climate change.

Biodiversity – the variety and variability at species, genetic and ecosystem levels of all forms of life on Earth constitutes biodiversity.

Ground water depletion– refers to drop in groundwater levels due to sustained withdrawal by pumps and other means for various uses. This situation occurs when withdrawals exceed the replenishment of underground aquifers.

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Pollution – denotes contaminants in natural environment that result in harmful changes in the life support system – air, soil, water (freshwater and marine water). Pollution is due to various chemical substances and forms such as mercury and heavy metals, energy, noise, thermal, light, municipal and industrial wastes, plastics, radioactive or nuclear remains and different kinds of data in present times of digital age.

GM organisms, crops, foods– A genetically modified organism (GMO) is one whose genetic material has been altered using genetic engineering techniques to produce better quality products and fulfil the needs of mankind all over the world.

Depletion of ozone layer – Gradual thinning of Earth's ozone layer in the upper atmosphere, caused by chemical compounds - gaseous chlorine or bromine released from industry and other human activities. Thinning of ozone layer is most marked in the polar regions, especially over Antarctica. Ozone depletion is of concern because it leads to increased amount of ultraviolet (UV) radiation that reaches Earth's surface, due to which health problems like skin cancer, cataracts, genetic and immune system damages reportedly occur.

Ozone build up near surface of Earth – Since 1900, ozone concentration near the Earth's surface has been increasing mainly due to vehicular exhausts and emissions from factories, power plants, and refineries. This is an issue of immense concern in present times.

Wastes (solid, semisolid, liquid, gaseous, municipal, industrial, construction, biomedical, commercial, mining, radioactive and agricultural) refers to garbage, detritus, sludge, and other discarded materials.

E-wastesare electrical and electronic equipment, whole or in part discarded as waste by the consumer or bulk consumer as well as rejects from manufacturing, refurbishment and repair processes.[Published in The Gazette of India, Extraordinary Part-II, Section 3, Sub-Section (I)].

Government of India, Ministry of Forest and Climate Change 23rd March, 2016.

Pollution in outer space of Earth –The increasing number of debris, junk, wastes, trash, garbage, chemicals due to defunct manmade objects in space, or in Earth's orbit, because of accidents of space crafts or their destruction as the space crafts have become defunct – all these have polluted the outer space and are also posing grave threat to the weather forecasting, communication, and military satellites.

Interlinking of rivers – it is a civil engineering mega project in our country that envisages to interlink various rivers in the country by forming a series of reservoirs and canals with an aim that water is equitably available to all and to mitigate the problems of drought and flooding in several parts of the country.

Sustainable agriculture, forestry– is farming in sustainable ways, whereby society’s present food, feed and textile needs, without compromising ability of current or future generations to meet their needs. Whereas sustainable forestry is the management of forests according to the principles of sustainable development. It has to keep a balance between ecological, economic and socio-cultural paradigms.

Energy(renewable, alternative energy sources) – Energy moves the wheel of life. All kinds of processes require energy. Energy can be obtained from various sources which can be divided into non-renewable and renewable resources. Fossil fuels – oil, natural gas and coal are non-renewable resources of limited availability. Alternate sources of energy – renewable resources, such solar energy, wind energy, hydropower, energy from biomass, geothermal energy have been widely tapped in recent times.

Plastics – The term plastic is derived from Greek word 'plastikos' meaning fit for molding. These are synthetic materials made from organic polymers like polyethylene, PVC, and nylon. Being easily moldable they are used to make various objects such as toys, medical devices, automobiles, packaging materials, buildings and various fixtures in them, bottles, household articles and several products used in modern life.

Human wildlife conflicts – Are abbreviated as HWC. It refers to varied interactions between people and wild animals, with consequences for people, their resources, wildlife and their habitats (IUCN 2020). HWC are caused by competition for shared natural resources between people and wildlife. These influence food security and the well-being of both people and animals. In many regions these conflicts have intensified over recent decades as a result of human population growth and the transformation of land use. HWC are serious global threats, hampering
sustainable development, food security and conservation in urban and rural landscapes.

Nuclear weapons – Nuclear weapons (= atom bomb, nuke, atomic bomb, nuclear warhead, A-bomb, or nuclear bomb) are explosive devices that derive their destructive force from nuclear reactions – either fission (fission bomb) or from a combination of fission and fusion reactions (thermonuclear bomb). Both types of bombs release huge amounts of energy from relatively small amounts of matter.

Bioweapons – Also known as Biological Weapons, Biological Warfare, Bioterrorism attacks and Germ Warfare are biological organisms, and substances (toxins or infectious agents such as bacteria, viruses, insects, fungi and replicating entities) derived directly from living organisms, that can be used to cause death or injury to humans, animals, or plants.

Biological agents, like anthrax, botulinum toxin and plague can pose a difficult public health challenge causing large numbers of deaths in a short amount of time while being difficult to contain. Bioterrorism attacks also result in epidemics, for example, Ebola or Lassa viruses have been used as biological agents.

Bioweapons constitute a subset of a larger class of weapons referred to as weapons of mass destruction, which also includes chemical, nuclear and radiological weapons. The use of biological agents is a serious problem, and the risk of using these agents in a bioterrorist attack is increasing in recent years.

Alien species / invasive species – Invasive alien species are ones that are/get introduced, accidentally or intentionally, outside their natural habitats and in long run they invariably threaten biological diversity. Alien species / invasive species are from all taxonomic groups, including animals, plants, fungi and microorganisms. Many of them lead to extinction of native species and eventual ecological disturbance. These impacts have marked socio-economic value. Invasive species adversely affect the invaded habitats and bioregions, causing ecological, environmental, and/or economic damage. On the other hand, many a times, their spread has beneficial effects too.

Rivers: Ganga, Yamuna – Yamuna is the second-largest tributary river of the Ganga and the longest tributary in India. It travels a total length of 1,376 kilometres. Ganga, a trans-boundary river of Asia, flows through India and Bangladesh. It flows to a length of 2,525 kms and empties into the Bay of Bengal. These rivers are lifelines of the places through which these passes. On the other hand, they are also conduits of various by products of societies.

Disconnect with nature, value of ecological services – With growing urbanization, and globalization, humans are increasingly being disconnected with nature. People are realizing and are reconnecting with nature. Ecological services such as supporting (nutrient cycling, soil formation), provisioning (food, fresh water, medicines, oils, fats, essential oils, gums & resins, wood, fibre, fuel), regulating (climate, flood, drought, disease, water purification), cultural (aesthetic, spiritual, educational, recreational), security (personal safety, ensured resources access, security from disasters), basics for a fulfilling life (adequate livelihood, nutrition, shelter), health (strength, vitality, wellness, pure air, water and food), social relations (cohesion, respect, helping attitude), freedom (individual’s values, being self).

Industrial & nuclear accidents – Three Mile Island accident (1979), SL-1 accident (1961), The Bhopal disaster (night of 2–3 December 1984), Chernobyl disaster (1986), and Fukushima Daiichi nuclear disaster (2011) are notable industrial and nuclear accidents. Such accidents involved loss of precious life and large monetary costs for remediation of the affected sites.

Population pressure – The world population is growing by more than 90 million per year, of which 93% is in developing countries. This could hamper their economic development. Population increase in past decades has been a gradual phenomenon and Earth’s natural resources were being replenished by nature for this increase. Recent studies indicate that besides population increase natural resources are being consumed in greater amounts than their rate of replenishment. The increase in human population has become a major cause of environmental concern of late.

Pandemics and Syndemics – Pandemic is global epidemic or infectious disease that spreads to more than one continent. Syndemic, a synergistic epidemic, is aggregation of two or more concurrent or sequential epidemics or disease clusters in a population with biological interactions, which lead to burden of disease. This term was developed by Merrill Singer in mid 1990s.
Space debris orbiting Earth—Millions of pieces of debris (defunct satellites, pieces of rockets and spacecrafts, equipments, chemicals and tools that astronauts accidently dropped in space) are swirling around at high speeds in space near Earth. These are matters of concern as they have caused accidental collisions in space damaging communications, weather and other satellites and rockets causing environmental and economic damage. There are objects greater than 10cm 34,000 in number; objects 1-10cm in size 900,000 in number; and objects 1mm to 1cm in size 128 million in number (Source: European Space Agency, 8 January 2021), (LiveMint, 16.01.2021).

Declining number of pollinators, largely bees—because of loss of habitat, use of agrochemicals, pesticides and climate change has bearing on production of crops, fruits and vegetables.

The list as above may not be complete as of now. It could be expanded and updated every now and then.

One needs to be aware that following actions are being taken and realised for the safeguard of both living beings and Earth's environment.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Actions taken</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>Local, national, regional, global</td>
</tr>
<tr>
<td>2.</td>
<td>Institutions &amp; organizations – UN (United Nations), UNEP (United Nations Environment Programme), IPCC (Intergovernmental Panel on Climate Change)</td>
</tr>
<tr>
<td>3.</td>
<td>Bills, legislations, policies, Protocols</td>
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<td>4.</td>
<td>Governance/ monitoring – Ministry, NGT (National Green Tribunal), EPCA [Environmental Pollution (Prevention and Control)] Authority, CPCB (Central Pollution Control Board)</td>
</tr>
<tr>
<td>5.</td>
<td>NGO (Non-governmental organization)</td>
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<tr>
<td>6.</td>
<td>Environment days – House sparrow, forestry, water, meteorological, migratory birds, ozone, oceans &amp; no tobacco day.</td>
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<tr>
<td>7.</td>
<td>Environment years – April 2019 to March 2020 Construction Technology Year</td>
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<td>8.</td>
<td>Green buildings</td>
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<td>9.</td>
<td>Forests Rights Act – Forest Act (Schedule Tribes and other traditional forest dwellers (recognition of forest rights Bill, 2006)</td>
</tr>
<tr>
<td>10.</td>
<td>Civil nuclear liability bill</td>
</tr>
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<td>11.</td>
<td>Rejuvenation &amp; restoration of defunct water bodies</td>
</tr>
<tr>
<td>12.</td>
<td>Environment education</td>
</tr>
<tr>
<td>13.</td>
<td>E-waste (management) Amendment Rules, 2018</td>
</tr>
<tr>
<td>15.</td>
<td>Mass awareness campaigns</td>
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<td>16.</td>
<td>Green budgets</td>
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<tr>
<td>17.</td>
<td>Non-conventional &amp; renewable energy resources</td>
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<tr>
<td>18.</td>
<td>Green political agendas</td>
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<td>19.</td>
<td>Smart cities</td>
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</tbody>
</table>
To be an informed citizen and how to track environmental issues, and be an environmental protection soldier, the following resources can easily be tapped:

**How to track? – modes of mass communication**
- News papers
- World Wide Web
- Mobile phone
- Laptop/desktop
- Internet

The above-mentioned resources have following attributes:

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Nuances</th>
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<tbody>
<tr>
<td>1.</td>
<td>Latest issues, decisions developments</td>
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<tr>
<td>2.</td>
<td>Written medium</td>
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<tr>
<td>3.</td>
<td>Powerful medium</td>
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<tr>
<td>4.</td>
<td>Responsible</td>
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<tr>
<td>5.</td>
<td>Specific thrust</td>
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<tr>
<td>6.</td>
<td>Powerful media for molding opinions</td>
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<tr>
<td>7.</td>
<td>Affordable</td>
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<tr>
<td>8.</td>
<td>Fast evolution taking place, paper to digital sources are increasing day by day</td>
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<tr>
<td>9.</td>
<td>Daily tracking is essential</td>
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<tr>
<td>10.</td>
<td>Record keeping is a must</td>
</tr>
<tr>
<td>11.</td>
<td>Information and facts are retrievable</td>
</tr>
<tr>
<td>12.</td>
<td>Interpretations need to be holistic and sound</td>
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<tr>
<td>13.</td>
<td>Different perspectives need to be considered</td>
</tr>
<tr>
<td>14.</td>
<td>Tracking with newspaper – with www / original source is of immense help</td>
</tr>
<tr>
<td>15.</td>
<td>Article can be retrieved with the help of google any time</td>
</tr>
<tr>
<td>16.</td>
<td>Editorials add mature, objective analyses to ones insight</td>
</tr>
<tr>
<td>17.</td>
<td>+ &amp;– aspects of one and all may be taken into account too</td>
</tr>
</tbody>
</table>

Following figures exemplify latest updates about environment.
One gets to know of the current announcements and schemes by the Ministry of Environment, Forest and climate change.

The following screen shot displays how making quality water available in market turns out to be a profitable enterprise.

Information regarding mass vaccination for protection against Covid 19 has been reported variedly by different newspapers.
Companies

ISW Steel’s net profit jumps nearly 14 times to ₹2,669 cr.

RII’s consolidated profit rises 12.5% to ₹13,101 cr.
Editorial on E-waste, given below, expands ones understanding of this issue.
The power of technology’s deployment in getting quick and reliable data like count of elephants from space is demonstrated by the following screen shot of Live Mint, 22.01.2021.

Various dimensional data on climate change as of now can be noted from the following newspaper report.
This account has demonstrated how a regular tracking using the simplistic sources of data and information on environment and its different issues can be recorded by an environmentally-conscious person.

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ASSESSMENT OF GROUNDWATER QUALITY FOR DRINKING PURPOSE IN CHARKHI DADRI BLOCK IN CHARKHI DADRI DISTRICT, HARYANA

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Research Article

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ABSTRACT

Water is important for living organisms on the Earth. Present development activities have put pressure on water resources. Groundwater is under more stress because of high exploitation for drinking, irrigation and industrial purposes. In the arid to semi-arid climatic zones groundwater is very important for sustaining day to day activities and agriculture and industrial purposes. The present study area Charkhi Dadri block in Charkhi Dadri district, Haryana was selected to study groundwater quality for drinking purpose. The geo-coordinates of the study area are latitudes 28.49° N to 28.70° N and longitudes 76.05° E to 76.47° E and covers an area of 396.57 sq. km. In the study area ten groundwater samples were collected in double capped 250 ml plastic bottles. Geo-coordinates of the sample locations were noted using mobile GPS. Chemical analysis of all the ten groundwater samples were done using Tamilnadu Water Supply and Drainage Board (TWAD), Chennai prepared Field Water Testing kit for twelve chemical parameters viz. pH, alkalinity, hardness, chloride, total dissolved solids (TDS), fluoride, iron, nitrite, nitrate, ammonia, phosphate and residual chlorine. Chemical analysis results were entered in excel software and prepared bar graphs. Result of groundwater samples analysis were compared with BIS (IS 10500:2012) drinking water standard to know the suitability of groundwater for drinking purpose. The study shows that pH ranges 7 to 8, alkalinity 200 mg/l to 450 mg/l, hardness 130 mg/l to 800 mg/l, chloride 40 mg/l to 1200 mg/l, TDS 552 mg/l to 2820 mg/l, fluoride 0.5 mg/l to 5 mg/l, iron nil to 0.3 mg/l, ammonia nil to 1 mg/l, nitrite 0.2 mg/l to 1 mg/l, nitrate 45 mg/l to 100 mg/l, phosphate nil in all the ten groundwater samples and residual chlorine nil to 0.2 mg/l. The study is highly useful for planning and monitoring of groundwater for drinking purpose in the study area.

Keywords: Groundwater, quality, drinking, Charkhi Dadri, Haryana.

INTRODUCTION

Water is important for survival of living beings on the Earth. Though plenty of water is available on the planet Earth but the useable amount for living beings is very less. In the present developmental activities demand of water is increased many folds. For drinking purpose good quality water either surface or groundwater is required but the availability of good quality water is again very less. Groundwater is easily available, hence, most exploited for drinking, irrigation and industrial uses. In arid to semi-arid regions of the world groundwater is the main source for drinking, irrigation and industrial uses. The need of the hour is to assess good quality groundwater resources at least for drinking purpose. Nas and Berktay (2010), Machiwal et al. (2011), Sarala and Ravi Babu (2012), Singh and Kumar (2014), Spanos et al. (2014), Annapoorna and Janardhana (2015), Punia et al. (2015), Nelly and Mutua (2016), Vijaya Lalitha et al. (2016), Selvakumar et al.

*Corresponding author: anup0106@yahoo.com
(2017), Chaudhary and Satheeshkumar (2018), Madhav et al. (2018), Bunkar and Kumar (2019), Jha et al. (2020), had done work on groundwater quality study for drinking purpose.

STUDY AREA
The study area Charkhi Dadri block in Charkhi Dadri district of Haryana is falling between the latitudes 28.49° N to 28.70° N and longitudes 76.05° E to 76.47° E and covers an area of 396.57 sq. km (Fig. 1). Climate of the area is semi-arid type. Geologically the alluvium and blown sand present and geomorphologically alluvial plain and aeolian plain are present in the study area.

OBJECTIVE
The main objective was to assess groundwater quality for drinking purpose in the study area.

MATERIALS AND METHODOLOGY
In the study area ten groundwater samples were collected in double capped 250 ml plastic bottle from hand pumps (HP) and tube wells (TW). Geocoordinates of the sample locations were taken using mobile GPS. Chemical analysis of ten groundwater samples were done using Tamilnadu Water Supply and Drainage (TWAD) Board prepared Field Water Testing kit for twelve chemical parameters viz. pH, alkalinity, hardness, chloride, total dissolved solids (TDS), fluoride, iron, nitrite, nitrate, ammonia, phosphate and residual chlorine (Table 1). Result of chemical analysis of groundwater samples were entered in excel software and prepared bar graphs. Result of groundwater samples analyses were compared with BIS (IS 10500:2012) drinking water standards (Table 2) to know the suitability of groundwater for drinking purpose.

Figure 1: Location map of the study area.

Table 1: Result of groundwater samples analysis in the study area.

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Sample Location</th>
<th>Latitude (°N)</th>
<th>Longitude (°E)</th>
<th>Source</th>
<th>pH</th>
<th>Alkalinity (mg/l)</th>
<th>Hardness (mg/l)</th>
<th>Chloride (mg/l)</th>
<th>TDS (mg/l)</th>
<th>Fluoride (mg/l)</th>
<th>Iron (mg/l)</th>
<th>Ammonia (mg/l)</th>
<th>Nitrite (mg/l)</th>
<th>Nitrate (mg/l)</th>
<th>Phosphate (mg/l)</th>
<th>Residual Chlorine (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dadri</td>
<td>28.60</td>
<td>76.28</td>
<td>HP</td>
<td>7</td>
<td>450</td>
<td>800</td>
<td>450</td>
<td>2040</td>
<td>1.5</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>Samaspur</td>
<td>28.65</td>
<td>76.37</td>
<td>HP</td>
<td>8</td>
<td>380</td>
<td>550</td>
<td>70</td>
<td>1200</td>
<td>1.5</td>
<td>0</td>
<td>1</td>
<td>0.5</td>
<td>75</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>Morwala</td>
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<td>76.39</td>
<td>HP</td>
<td>7</td>
<td>230</td>
<td>280</td>
<td>40</td>
<td>660</td>
<td>5</td>
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<td>0</td>
<td>0.3</td>
<td>45</td>
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<td>0</td>
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<td>4.</td>
<td>Imlota</td>
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<td>76.44</td>
<td>HP</td>
<td>8</td>
<td>350</td>
<td>800</td>
<td>850</td>
<td>2376</td>
<td>3</td>
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<td>1</td>
<td>0.5</td>
<td>100</td>
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<tr>
<td>5.</td>
<td>Ghosola</td>
<td>28.56</td>
<td>76.26</td>
<td>HP</td>
<td>7</td>
<td>380</td>
<td>420</td>
<td>170</td>
<td>1164</td>
<td>5</td>
<td>0</td>
<td>0.5</td>
<td>0.2</td>
<td>45</td>
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<td>0.2</td>
</tr>
<tr>
<td>6.</td>
<td>Makrani</td>
<td>28.49</td>
<td>76.27</td>
<td>TW</td>
<td>7</td>
<td>400</td>
<td>700</td>
<td>1070</td>
<td>2604</td>
<td>5</td>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
<td>75</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>7.</td>
<td>Atelakhur</td>
<td>28.59</td>
<td>76.10</td>
<td>HP</td>
<td>7</td>
<td>280</td>
<td>130</td>
<td>50</td>
<td>552</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>45</td>
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<td>8.</td>
<td>Charkhi</td>
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<td>76.22</td>
<td>HP</td>
<td>7</td>
<td>200</td>
<td>400</td>
<td>100</td>
<td>840</td>
<td>5</td>
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<td>0.5</td>
<td>0.5</td>
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<td>9.</td>
<td>Dohki</td>
<td>28.66</td>
<td>76.16</td>
<td>HP</td>
<td>8</td>
<td>550</td>
<td>800</td>
<td>1200</td>
<td>2820</td>
<td>2</td>
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<td>0.5</td>
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<td>75</td>
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<td>0</td>
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<td>10.</td>
<td>Chhapar</td>
<td>28.63</td>
<td>76.11</td>
<td>TW</td>
<td>8</td>
<td>420</td>
<td>600</td>
<td>1100</td>
<td>2544</td>
<td>1.5</td>
<td>0</td>
<td>0.5</td>
<td>1</td>
<td>75</td>
<td>0</td>
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</tbody>
</table>
RESULTS AND DISCUSSION

i. pH
In the study area pH ranges 7 to 8 (Table 1, Fig.2). As per BIS (10500:2012) drinking water standards pH is desirable 6.5-8.5 and non-potable <6.5 and >8.5 (Table 2). pH is desirable in all the ten groundwater samples (Dadri, Samaspur, Morwala, Imlota, Ghasola, Makrani, Atela Khurd, Charkhi, Dohki, Chhapar) in the study area.

ii. Alkalinity
In the study area alkalinity ranges 200 mg/l to 450 mg/l (Table 1, Fig.3). As per BIS (10500:2012) drinking water standards alkalinity is desirable <200 mg/l, permissible between the range 200 mg/l-600 mg/l and non-potable >600 mg/l (Table 2). Alkalinity is permissible in all the ten groundwater samples (Dadri, Samaspur, Morwala, Imlota, Ghasola, Makrani, Atela Khurd, Charkhi, Dohki, Chhapar) in the study area.

Table 2: Drinking water standards (BIS: 10500:2012).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Constituent</th>
<th>Potable</th>
<th>Non-Potable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Desirable</td>
<td>Permissible</td>
</tr>
<tr>
<td>1.</td>
<td>pH</td>
<td>6.5 to 8.5</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Alkalinity (mg/l)</td>
<td>&lt;200</td>
<td>200-600</td>
</tr>
<tr>
<td>3.</td>
<td>Total Hardness (mg/l)</td>
<td>&lt;200</td>
<td>200-600</td>
</tr>
<tr>
<td>4.</td>
<td>Chloride (mg/l)</td>
<td>&lt;250</td>
<td>250-1000</td>
</tr>
<tr>
<td>5.</td>
<td>Total Dissolved Solids (TDS) (mg/l)</td>
<td>&lt;500</td>
<td>500-2000</td>
</tr>
<tr>
<td>6.</td>
<td>Fluoride (mg/l)</td>
<td>&lt;1.0</td>
<td>1.0-1.5</td>
</tr>
<tr>
<td>7.</td>
<td>Iron (mg/l)</td>
<td>&lt;0.3</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Ammonia (mg/l)</td>
<td>&lt;0.5</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Nitrite (mg/l)</td>
<td>&lt;1.0</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>Nitrate (mg/l)</td>
<td>&lt;45</td>
<td>-</td>
</tr>
<tr>
<td>11.</td>
<td>Phosphate (mg/l)</td>
<td>&lt;1.0</td>
<td>-</td>
</tr>
<tr>
<td>12.</td>
<td>Residual Chlorine (mg/l)</td>
<td>&lt;0.2</td>
<td>0.2-1.0</td>
</tr>
</tbody>
</table>

Fig. 2: pH in groundwater samples.

iii. Hardness
Hardness ranges 130 mg/l to 800 mg/l (Table 1, Fig.4) in the study area. As per BIS (10500:2012) drinking water standards hardness is desirable if less than 200 mg/l, permissible between the range 200 mg/l-600 mg/l and non-potable if more than 600 mg/l (Table 2). Hardness is desirable in one groundwater sample (Atela Khurd), permissible in five groundwater samples (Samaspur, Morwala, Ghasola, Charkhi, Chhapar) and non-potable in four groundwater samples (Dadri, Imlota, Makrani, Dohki) in the study area.

Fig. 3: Alkalinity in groundwater samples.
iv. Chloride

In the study area chloride ranges 40 mg/l to 1200 mg/l (Table 1, Fig.5). As per BIS (10500:2012) drinking water standards chloride is desirable if less than 250 mg/l, permissible between the range 250 mg/l-1000mg/l and non-potable if more than 1000mg/l (Table 2). Chloride is desirable in five groundwater samples (Samaspur, Morwala, Ghasola, Atela Khurd, Charkhi), permissible in two groundwater samples (Dadri, Imlota) and non-potable in three groundwater samples (Makrani, Dohki, Chhapar) in the study area.

v. Total Dissolved Solids (TDS)

In the study area TDS ranges 552 mg/l to 2820 mg/l (Table 1, Fig.6). As per BIS (10500:2012) drinking water standards TDS is desirable if less than 500 mg/l, permissible between the range 500 mg/l - 2000 mg/l and non-potable if more than 2000 mg/l (Table 2). In the study area TDS is permissible in five groundwater samples (Samaspur, Morwala, Ghasola, Atela Khurd, Charkhi) and non-potable in five groundwater samples (Dadri, Imlota, Makrani, Dohki, Chhapar).

vi. Fluoride

In the study area fluoride ranges 0.5 mg/l to 5 mg/l (Table 1, Fig.7). As per BIS (10500:2012) drinking water standards fluoride is desirable if less than 1.0 mg/l, permissible between the range 1.0 mg/l - 1.5 mg/l and non-potable if more than 1.5 mg/l (Table 2). Fluoride is desirable in one groundwater sample (Atela Khurd), permissible in three groundwater samples (Dadri, Samaspur, Chhapar) and non-potable in six groundwater samples (Morwala, Imlota, Ghasola, Makrani, Charkhi, Dohki) in the study area.

vii. Iron

In the study area iron ranges nil to 0.3 mg/l (Table 1, Fig.8). As per BIS (10500:2012) drinking water standards iron is desirable if less than 0.3 mg/l and non-potable if more than 0.3 mg/l (Table 2). In the study area iron is desirable in all the ten groundwater samples (Dadri, Samaspur, Morwala, Imlota, Ghasola, Makrani, Atela Khurd, Charkhi, Dohki, Chhapar).
Ammonia

Ammonia ranges nil to 1 mg/l (Table 1, Fig.9) in the study area. As per BIS (10500:2012) drinking water standards ammonia is desirable if less than 0.5 mg/l and non-potable if more than 0.5 mg/l (Table 2). Ammonia is desirable in seven groundwater samples (Morwala, Ghasola, Makrani, Atela Khurd, Charkhi, Dohki, Chhapar) and non-potable in three groundwater samples (Dadri, Samaspur, Imloita) in the study area.

Nitrite

In the study area nitrite ranges 0.2 mg/l to 1 mg/l (Table 1, Fig.10). As per BIS (10500:2012) drinking water standards nitrite is desirable if less than 1 mg/l and non-potable if more than 1 mg/l (Table 2). In the study area nitrite is desirable in all the ten groundwater samples (Dadri, Samaspur, Morwala, Imloita, Ghasola, Makrani, Atela Khurd, Charkhi, Dohki, Chhapar).

Nitrate

In the study area nitrate ranges 45 mg/l to 100 mg/l (Table 1, Fig.11). As per BIS (10500:2012) drinking water standards nitrate is desirable if less than 45 mg/l and non-potable if more than 45 mg/l (Table 2). Nitrate is desirable in two groundwater samples (Ghasola, Atela Khurd) and non-potable in eight groundwater samples (Dadri, Samaspur, Morwala, Imloita, Makrani, Charkhi, Dohki, Chhapar) in the study area.

Phosphate

In the study area phosphate is nil in all the ten groundwater samples (Table 1, Fig.12). As per BIS (10500:2012) drinking water standards phosphate is desirable if less than 1.0 mg/l and non-potable if more than 1.0 mg/l (Table 2). In the study area phosphate is desirable in all the ten groundwater samples (Dadri, Samaspur, Morwala, Imloita, Ghasola, Makrani, Atela Khurd, Charkhi, Dohki, Chhapar).

Residual Chlorine

In the study area residual chlorine ranges nil to 0.2 mg/l (Table 1, Fig.13). As per BIS (10500:2012) drinking water standards residual chlorine is desirable if less than 0.2 mg/l, permissible between the range 0.2 mg/l-1.0 mg/l and non-potable if more than 1.0 mg/l (Table 2). In the study area residual chlorine is desirable in eight groundwater samples (Dadri, Samaspur, Morwala, Imloita, Ghasola, Makrani, Atela Khurd, Charkhi, Dohki, Chhapar) and permissible in two groundwater samples (Ghasola, Makrani).
CONCLUSIONS
In the study area pH, iron, nitrite and phosphate is desirable in all the ten groundwater samples while alkalinity is permissible in all the ten groundwater samples. Hardness is desirable in one groundwater sample, permissible in five groundwater samples and non-potable in four groundwater samples. Chloride is desirable in five groundwater samples, permissible in two groundwater samples and non-potable in three groundwater samples. TDS is permissible in five groundwater samples as well as non-potable in five groundwater samples. Fluoride is desirable in one groundwater sample, permissible in five groundwater samples and non-potable in six groundwater samples. Ammonia is desirable in seven groundwater samples and non-potable in three groundwater samples. Nitrate is desirable in two groundwater samples and non-potable in eight groundwater samples. Residual Chlorine is desirable in eight groundwater samples and permissible in two groundwater samples. This study is highly useful for planning and monitoring of groundwater quality for drinking purpose in the study area.

REFERENCES


ABSTRACT

Groundwater is an important source for drinking, Agriculture, domestic and industrial purposes and makes about two third of the freshwater resource of the world. The quality and quantity of the groundwater is a serious concern for the India as eighteen percentage of world population live in India and just 4% of world fresh water availability in India. Quality of groundwater is a serious concern for mankind as it is directly linked to human health. Due to the rapid increase in the population in last four decades makes a big challenge to provide quality of water. Industrialization and other man-made activities are also contributing in decline trained of groundwater. The groundwater is being polluted with highly toxic contaminants such as arsenic, fluoride, nitrate, chloride, chloride, fluoride, lead and heavy metals. Fluoride and chloride were found as most common contaminants in Agra region. Fluoride and chloride are coming into the groundwater from deep underground rocks bearing fluorine and chlorine. Geological structure of rocks is also responsible for Fluoride and chloride pollution in the groundwater of the region. Major groundwater problems in Agra region are a significant decline in water level, an occurrence of fluoride, saline groundwater in a deeper zone, less groundwater recharge and more surface runoff of monsoon rainfall. The comparison of analyzed ground water samples with the WHO, APHA standards are presented. The study was concluded by over exploitation of groundwater for the drinking, domestic, irrigation purposes and the leaching of industrial wastes and municipal solid waste (MSW) is one of the leading emerging sources of contamination of groundwater in Agra region.

Keywords: Groundwater, Agra Region & Contaminants.
concentration, the range of fluoride concentration was found from 0.11 mg/l to 12.8 mg/l. It is observed that about 73% of fluoride samples, 80% of pH samples, 82% of total hardness samples, 88% of total alkalinity samples, 48% of chloride samples, 64% of calcium samples and 99% of magnesium samples were not meeting the requirement of IS 10500 (1990) [2]. Physico chemical characteristics of groundwater of Agra city were studies by analysing samples from 12 different sites of the Agra city and found that water is not meeting the requirement of Bureau of Indian standard and WHO standard, Precautionary measures were recommended before drinking to prevent adverse health impacts to citizen of the region [3]. A study of the shallow aquifers of Marks Nagar, Unnao district of Uttar Pradesh revealed that high concentration of fluoride and consequent health hazard (Fluorosis) and infestations detected in human population and in cattle. Samples were collected from shallow hand pumps and dug wells, the only available source for drinking and domestic purposes, around 87.5% of collected water samples were exceeding permissible limit of 1.5 mg/l as prescribed by Bureau of Indian Standard (BIS) [4]. The ground water quality studies shows that conductivity is an important physicochemical water quality parameter having correlation with conductivity are temperature, pH, alkalinity, total hardness, calcium, total solids, Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), chloride ion and iron in water [5]. The investigations on the iron contamination in groundwater at Tangail municipality of Bangladesh shows a link between pH and iron concentration. Lower value of pH in collected samples are have higher value of iron concentration, which is evident that pH is indirectly proportionate to iron concentration. All these calculated measurements were exceeded the prescribed limit for iron given by WHO, Indian standard and Bangladesh standard [6]. Research studies on pollution by leachate from a waste management site in southwestern Bangalore city which causes toxic surface water and groundwater contamination. Groundwater quality samples were analyzed from 20 stations from the vicinity of the Haralukunte lake for their physicochemical quality of water, revealed that there is a significant evidence for high degree of pollution and is not fit for drinking purposes [7]. In another research results found major groundwater problems in Agra are a significant decline in water level, an occurrence of Fluoride, Saline ground water in a deeper zone, less groundwater recharge and more surface runoff of monsoon rainfall. Majority of the district is having alluvial plains. About 70% of the area is under agriculture use and surface water such as canal water and groundwater are being used for irrigation purposes [8]. Research Studies on heavy metal concentration in drinking water with respect to Zinc, copper, cadmium, manganese, lead and arsenic in Kamrup district of Assam, shows that excess concentration of Cd, Mn, and Pb present at some locations and it making water not fit for drinking purposes [9]. Fluoride, boron and nitrate concentration in groundwater of different villages in Indira Gandhi, Bhakra and Gang canal catchment area of northwest Rajasthan were analyzed by adopting established methodologies. Fluoride is analyzed by SPADNS method, boron was tested by colorimetric method using carbine as the color developing agent and nitrate was estimated by phenol di sulphonic acid method. 81.67 % of fluoride samples were exceeding the prescribed limit of fluoride, 34 % of boron samples were found with the high concentration of boron and 300 water samples were tested for nitrate in which 97.33% samples were observed within the prescribed range and 2.33 % were observed out of prescribed range i.e. 45 mg/l [10].

Gupta Et al Studied groundwater quality with special reference to the Fluoride concentration from 261 villages of Kheragarh Tehsil of Agra district. the range of fluoride concentration was found from 0.11 mg/l to 12.8 mg/l. It is observed that about 73% of fluoride samples, 80% of pH samples, 82% of total hardness samples, 88% of total alkalinity samples, 48% of chloride samples, 64% of calcium samples and 99% of magnesium samples were not meeting the requirement of Bureau of Indian Standard i.e. IS 10500 (1990). Piped water supply is proposed to the residents for drinking and other cooking purposes [11]. Water quality index is established with the help of various physico-chemical parameters in different seasons. Water quality index for rainy season, winter season and summer season are 96, 101.7 and 106.3 respectively, it indicates poor quality of water (Chatterji and Raziuddin 2002). The water quality rating study shows that it is not suitable for human uses. It is also observed that water body is suffering with relatively high load of pollution in summer season compared to winter and rainy season [12]. Water quality index with reference to chloride, the electrical conductivity indicating poor ground water quality of the study area. Sodium
Absorption Ratio (SAR) shows significant high percentage of sodium in most of the study locations, indicating ground water quality is not suitable for irrigation purposes. [13] Physico chemical parameters of tap water of Chandigarh, was measured by the testing against parameters like color, odor, temperature, pH, Turbidity, Electrical conductivity, Total dissolved solids, Dissolved oxygen and salinity. Results of the parameters were found well within the limit prescribed by Bureau of Indian standard and World Health Organization (BIS/WHO).[14] Heavy metals such as Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn in 39 water supplying wells 5 water storage tank in which iron was reported higher than standard in 03 wells, high concentration of iron in ground water in the city of Bafgh and Ardakan and it was found that due to industrial activities of iron extraction and its processing such as pelletizing and rolling would be the root cause of contamination. The sludge and effluent flowing from sewage treatment plant is also being released in to environment.[15] The physico-chemical analysis of groundwater was conducted for the villages of Kadi tehsil at Mehsana district of Gujarat where ground water is the only source of drinking water in most of the villages. There was a positive correlation observed between pH and fluoride in ground water which indicates high alkaline nature of the water promotes leaching of fluoride ion and affect the concentration of fluoride in ground water. A negative relationship was observed between the pH and bicarbonate which is generally found in deeper zone of groundwater and positive relationship was observed in shallow groundwater. [16] In another research study conducted to estimate the current status of Pus dam of Pusad Tehsil of Yavatmal district, Maharashtra, reveals that there is no harmful contaminant for pisciculture, irrigation and drinking water. There was positive correlation found with temperature and pH, TDS, total hardness, chloride, phosphate, BOD. The transparency has been found with positive correlation with chloride, dissolved oxygen and biological oxygen demand while nitrate negatively correlated with transparency.[17] Ground water from 53 open wells in Sangamner area of Ahmednagar was analyzed, the results showed higher values which are close to river channel and downstream of the river of Pravara. Dominant cations are calcium and magnesium followed by sodium, predominant anions are chloride followed by bicarbonate, sulphate and nitrate ions. Study also reveals that intensive irrigation has a serious impact on the quality of water of the area, which is suggested that excessive use of fertilizers and irrigation caused deterioration of groundwater quality. The remedial measures like controlled use of fertilizers and mixed culture of cropping pattern will help for sustainable agriculture and water use planning.[18] Various research studies of the assessment of water quality were reviewed for physico-chemical parameters and iron. It was found that water pollution is not only devastating to people, but also destroying animals, plants and flora and fauna of aquatic life and reduces its reproductive ability. The sustainable practice suggest that individual and community can help to minimize the water pollution by adopting good management practices and housekeeping to ensure lesser amount of waste water generated. The present study focused on the assessment of ground water quality of some places in Tehsil Bah, Uttar Pradesh, India.

Present Study
The Agra district is located in the western part of Uttar Pradesh and universally renowned place because of Taj Mahal. The quality of water is serious concern for mankind as it is directly linked with human health and common man. The groundwater makes about two third of the freshwater resource of the world. Majority of rural population (85%) depend on groundwater for drinking purposes and domestic uses. Due to rapid increase in the population, industrialization and other man-made activities, the groundwater is being polluted with highly toxic contaminants. Groundwater of Agra region is contaminated with salinity, Fluoride, Chloride, Nitrate and Arsenic. Baroli Ahir area of Agra district and Kheragarh area of Agra district were found high concentration of fluoride i.e., more than 1.5 mg/l (prescribed by IS: 10500:2012) and fluoride is coming to the groundwater from fluoride bearing rocks.

Leaching of industrial waste and municipal solid waste (MSW), Use of chemical fertilizer and pesticides are one of the emerging sources of contamination for ground water quality. Groundwater may have many substances in the form of solution or suspension. Calcium, magnesium, sodium, potassium, iron, cobalt, zinc, copper, chloride, nitrate, fluoride, sulfate, arsenic, pesticides and a variety of other substances may find in different concentration in the groundwater. About 70% groundwater used for irrigation purposes is having serious impacts on quantity and quality of groundwater, uses of water for irrigation is required to minimized by adopting scientific approach during irrigation.
Scarcity and declining trends of groundwater is imposing high level of risk to the gross domestic products (GDP) specially energy sector, thermal energy is the main source of the country, thermal power plants in the country are facing shortage of water during summer and other seasons another sector that is micro, small and medium enterprises (MSME) is also hampered to its production and supply chain of goods and services. Scarcity or declining trends of groundwater may dilute our goal of self-reliant Bharat. Quality of water is utmost required to minimize the health risk, that should meet the standard limit prescribed by national and international bodies such as BIS, WHO, EN etc.

Providing good quality of water for drinking purpose to rising population of the world is extra burden on the water resources and a challenge to entire world to meet the quality of water for domestic, industrial, agricultural and other purposes resulted in unsustainable practices of water conservation from natural resources.

In India, Groundwater quality is depleting with reference to the presence of contaminants above the acceptable limits. Some major reasons of groundwater contamination are as following:

(I) The difference between pumping of groundwater and recharge or replenish of groundwater.
(ii) Sewage and Domestic Wastes.
(iii) Industrial discharges.
(iv) Improper handling of MSW and medical wastes.
(v) Agricultural discharges (Pesticides and chemical fertilizers).
(vi) Thermal pollution.

Physicochemical Parameters of Ground Water
IS: 10500 (2012) is Indian standard for drinking water is formulated by bureau of Indian standard, in these standard limits are given as Acceptable Limit and Permissible Limit in the Absence of Alternative Source.[19] World health organization (WHO) is a global standard providing limits for drinking water internationally. The Requirement (Acceptable Limit) and Permissible Limit in the Absence of Alternative Source in accordance with IS 10500, 2012 is given in Table 1 to 3.

Drinking Water Standards limits in accordance with IS: 10500, 2012

<table>
<thead>
<tr>
<th>Sr.</th>
<th>Organoleptic and Physical Parameters</th>
<th>Requirement (Acceptable Limit)</th>
<th>Permissible Limit in the Absence of Alternative Source</th>
<th>Unit</th>
</tr>
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<tbody>
<tr>
<td>1.</td>
<td>Colour</td>
<td>5</td>
<td>15</td>
<td>Hazen</td>
</tr>
<tr>
<td>2.</td>
<td>Odour</td>
<td>Agreeable</td>
<td>Agreeable</td>
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<tr>
<td>3.</td>
<td>pH value</td>
<td>6.5 - 8.5</td>
<td>No Relaxation</td>
<td>NA</td>
</tr>
<tr>
<td>4.</td>
<td>Taste</td>
<td>Agreeable</td>
<td>Agreeable</td>
<td>NA</td>
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<tr>
<td>5.</td>
<td>Turbidity</td>
<td>1</td>
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<td>NTU</td>
</tr>
<tr>
<td>6.</td>
<td>Total dissolved solids</td>
<td>500</td>
<td>2000</td>
<td>mg/l</td>
</tr>
</tbody>
</table>

Table 2:

<table>
<thead>
<tr>
<th>Sr.</th>
<th>General Parameters Concerning Substances Undesirable in Excessive Amounts</th>
<th>Requirement (Acceptable Limit)</th>
<th>Permissible Limit in the Absence of Alternative Source</th>
<th>Unit</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>Aluminium (as Al)</td>
<td>0.03</td>
<td>0.2</td>
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</tr>
<tr>
<td>2.</td>
<td>Barium (as Ba)</td>
<td>0.7</td>
<td>No Relaxation</td>
<td>mg/l</td>
</tr>
<tr>
<td>3.</td>
<td>Boron (as B)</td>
<td>0.5</td>
<td>1</td>
<td>mg/l</td>
</tr>
</tbody>
</table>
2.0 Materials and Methods:
Methods used to analyze physicochemical parameters in ground water are prescribed in American public health association (APHA)[20] and Bureau of Indian standard (BIS). Physico-chemical Parameters included for the assessment of ground water quality are pH, Total dissolve solids, Electrical conductivity, Temperature, Total hardness, Calcium, Magnesium, Sulphate, Fluoride, Alkalinity, Chloride, Nitrate, Nitrite, Potassium and Sodium.
Grab Sampling is the most used technique for groundwater samples in which single samples collected from a specific site in a short period of time thus a single sample represent the whole quality of water.
Test methods to analyze above mentioned parameters are followed from available Indian standard (IS 3025) and American public health association (APHA 23rd Edition), 2017 by using equipment/glassware and reference materials/reagents given in table-4.[21]

**CONCLUSION**
The quality of groundwater is being deteriorate day by day due to over exploitation of groundwater. the rate of the of underground water for domestic, Industrial, Agricultural and other purposes is more against the recharge or replenish of groundwater. Increasing desertification and loss of green cover are reducing the land’s capacity to recharge groundwater aquifer and regional water tables. Extensive groundwater extraction contributes to the decline the groundwater quality and loss of vegetation cover also.

It is required to recharge the groundwater resources and to maintain the sustainable approach for groundwater to maintain the difference of recharge or replenish of groundwater and the pumping of groundwater. It is also advised to monitor the groundwater quality on regular intervals to know about the current status of groundwater quality. It is individuals and community’s responsibility to minimize the water pollution by adopting sustainable approaches for groundwater management.

**REFERENCES**

**Table 4: Test Parameters with Equipment, Glassware, RM and Reagents.**

<table>
<thead>
<tr>
<th>Test Parameter</th>
<th>Equipment/ Glass wares</th>
<th>Reference Material/Reagents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (as Ca)</td>
<td>Burette/pipette</td>
<td>Calcium</td>
</tr>
<tr>
<td>Chloride (as Cl)</td>
<td>Burette/pipette</td>
<td>Sodium Chloride</td>
</tr>
<tr>
<td>Colour</td>
<td>Nessler Tubes</td>
<td>Potassium chloroplatinate, Cobaltous chloride</td>
</tr>
<tr>
<td>Magnesium (as Mg)</td>
<td>Burette/pipette</td>
<td>Magnesium</td>
</tr>
<tr>
<td>Odour</td>
<td>Thermometer/ Erlenmeyer flask</td>
<td>Odour free water</td>
</tr>
<tr>
<td>pH</td>
<td>pH Meter</td>
<td>pH Buffer</td>
</tr>
<tr>
<td>Taste</td>
<td>Erlenmeyer flask</td>
<td>Sodium Chloride</td>
</tr>
<tr>
<td>Total Alkalinity (CaCO₃)</td>
<td>Burette/pipette</td>
<td>Sodium Carbonate</td>
</tr>
<tr>
<td>Total Dissolved Solids</td>
<td>Balance/Oven</td>
<td>NA</td>
</tr>
<tr>
<td>Total Hardness (CaCO₃)</td>
<td>Burette/pipette</td>
<td>Calcium carbonate</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Turbidity Meter</td>
<td>Hydrazine sulfate, hexamethylenetetraamine</td>
</tr>
<tr>
<td>Fluoride (as F)</td>
<td>Spectrophotometer</td>
<td>Fluoride</td>
</tr>
<tr>
<td>Iron (as Fe)</td>
<td>ICP/AAS/Spectrophotometer</td>
<td>Iron</td>
</tr>
<tr>
<td>Sulphate (as SO₄)</td>
<td>Spectrophotometer</td>
<td>Sulphate</td>
</tr>
<tr>
<td>Arsenic (as As)</td>
<td>ICP/AAS</td>
<td>Arsenic</td>
</tr>
<tr>
<td>Nitrate (as NO₃)</td>
<td>Spectrophotometer</td>
<td>Nitrate</td>
</tr>
<tr>
<td>Total Coliform</td>
<td>Incubator/Autoclave</td>
<td>Reference culture</td>
</tr>
<tr>
<td>E-coli</td>
<td>Incubator/Autoclave</td>
<td>Reference culture</td>
</tr>
</tbody>
</table>


21. State profile, Ground water scenario of Uttar Pradesh UP, Central Ground water Board (CGWB), Ministry of Water Resources, Govt. of India.
Waste generation in India has been increasing, and there are no adequate ground implemented policies for the safe disposal of waste. It increases land and water pollution. Some industries execute zero waste management at their level, but that is not enough to reduce the load on the city's main landfill and disposal site. It is the need of the hour to implement the micromanagement of waste. There are large numbers of schools and colleges in India which generate the same type of waste. These campuses can demonstrate and influence the students, faculties, and other staff and visitors to adopt and successfully implement sustainable practices. Schools and colleges can play an essential role in changing society by teaching students about the new waste management technologies. There are numerous opportunities to introduce new technologies into waste management. The challenge is to encourage the development of technologies that are most conservative of natural resources and that are cost-effective. This paper shows the zero waste management policy in Campus of Techno India NJR Institute of Technology, Udaipur. Separation of waste at source is a vital and essential part of zero waste management. Biodegradable waste is used for composting, and plastic is used for brick manufacturing, a college start-up project. E-waste and hazardous waste are handed over separately to authorized recycling firms.
2016, all gated societies and campuses have been advised to develop the treatment and segregation of waste within their premise.

The Campus has adopted the principles of the 'best practicable environmental option' to deliver its waste management services. The Campus applies a ‘waste hierarchical approach’ to reduce, reuse, recycle, and recover waste products in preference to waste disposal to landfill.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Block Name</th>
<th>Ground Coverage (Sq. M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Academic Block</td>
<td>3377.01</td>
</tr>
<tr>
<td>2</td>
<td>WorkShop</td>
<td>606.3</td>
</tr>
<tr>
<td>3</td>
<td>Hostel Building</td>
<td>597.9064</td>
</tr>
<tr>
<td>4</td>
<td>I 3 lab</td>
<td>275.394</td>
</tr>
<tr>
<td>5</td>
<td>Road</td>
<td>2668.166</td>
</tr>
<tr>
<td>6</td>
<td>Area of Covered by Tiles</td>
<td>1613.306</td>
</tr>
<tr>
<td></td>
<td><strong>Total Area</strong></td>
<td><strong>9138.824 Sq. M</strong></td>
</tr>
</tbody>
</table>

This Institute has to ensure that all the campus wastes are disposed of responsibly by using proper waste segregation mechanism at the source and, if possible, converting it into a value-added environmentally friendly product. As per the guidelines provided by the Indian Ministry of Urban Development ((MoUD) in the form policies of SWM rules 2016, all gated societies and campuses have been advised to develop the treatment and segregation of waste within their premise (2).

The Campus has adopted the principles of the 'best practicable environmental option' to deliver its waste management services (3) (4). The Campus applies a ‘waste hierarchical approach’ to reduce, reuse, recycle, and recover waste products in preference to waste disposal to landfill (5) (6). Figure 1 shows a schematic diagram of solid waste management of the Campus.

![Figure 1: Interrelationships between the functional elements in Solid Waste Management in Campus.](image-url)

...
- Waste Segregation: Source segregation via separate bins as per the waste. Following color code is used for different types of wastage.

- Dimensions and 3D views of the collection and processing center on the college campus are shown in Figures 3 and 4.

**Figure 2:** Color code of bins for different types of waste in Campus.

**Figure 3:** Area and Dimensions of Processing Center (Outer Dimensions in meter).

**Figure 4:** Processing Center for Waste at the Campus.
Management Staff

- **Supervisor** for the supervision of supporting staff.
- **Supporting staffs** will be responsible for:
  1. Cleaning and separate the waste from each facility
  2. Collection of separated waste from different colored bins
  3. Transport all waste to the collection center of Campus
  4. Support all recycling processing activities

Resources for Waste management

There are three sets of four colored bins of 120 liter capacity for each location on Campus. Locations of bins are mentioned in figure 6. One collection center and one processing center are also located on Campus as mentioned in figure 6. The location of black colored dustbin is inside the Campus in a particular room.

Three sets (60 Liter) of blue and green bins are kept in each wing of each floor. Red bins of 60-liter capacity are kept in each washroom of the Campus. There are two trolleys on Campus for the collection of waste from dustbins of 60-liter capacity.

Liquid Waste Management

Wastewater management options and technologies can be functionally divided into two segments. Firstly, septic tanks are used for sewage waste water. Secondly, wastewater from bathrooms is treated through coagulants for the separation of soap and other suspended particles, and this treated water is used for gardening (7) (8).

Rain Water harvesting system

A rainwater harvesting system has been made for Techno India NJR campus through visual inspection, total station, theodolite, and GIS survey. Visual inspection has been done. Rainwater can be harvested through roofs of the academic block, workshop, hostel building, and I3 lab of the college (9) (10). Some more area which is occupied by tiles and road was also considered for rainwater harvesting. The total built-up area for collecting rainwater is 9138.824 m². Average rainfall in Udaipur is 689 mm/year, and 6296.138 m³ volume of water can be saved through recharge in bore well. Two points have been selected based on the survey of the Campus (11).
Solar Energy
To minimize the cost of energy and settle for a sustainable alternative, Institute is choosing to go green (12). It has installed solar panels to generate electricity outside the building blocks at the sides of roads on Campus and rooftop of the hostel building.

CONCLUSION
Sustainable solutions for waste management for institutes are the demand of the situation where generations of waste are increasing. It is required to implement new technologies and systems to improve waste management. This initiative will enhance the knowledge of students about benefits of these sustainable solution and energy saving. This paper is a case study of college campus who adopting the systems of Solid waste management, Waste water reuse, Rainwater harvesting and use of solar energy.

REFERENCES
EVALUATION OF MICROBIAL LOAD AND SELECTED HEAVY METALS CONTAMINATION IN THE RIVER BEAS (PUNJAB) INDIA

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Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, India

ABSTRACT

Aim: To analyse the heavy metal contamination and microbial load in upstream (Talwara), midstream (Urmar Tanda) and downstream (Harike Pattan) stretches of the river Beas in Punjab.

Methodology: Month-wise samples were collected in triplicate during the period of May to December, 2019. Heavy metal parameters such as Arsenic, Cadmium, Nickel, Lead and Zinc were analysed by Atomic Inductively Coupled Plasma Mass Spectrometry following standard protocol. Microbial load in water samples was calculated in terms of Total Plate Count, Total Coliform and Faecal Coliform as per following standard protocol. Statistical analysis involved calculating Analysis of Variance at 5% significant level (p<0.05).

Results: The study revealed that heavy metal parameters like Arsenic, Cadmium, Nickel, Lead and Zinc ranged in mgl⁻¹ from 0.0 to 0.009, 0.0 to 0.02, 0.0 to 0.004, 0.0 to 0.001, and 0.0 to 0.036 respectively. Total Plate Count ranged from 0.32 x10⁴ to 3.2 x10⁵ cfu ml⁻¹ whereas, Total Coliforms and Faecal Coliforms ranged from 4.0 to 1100 MPN 100 ml⁻¹ and 0.0 to 460 MPN 100 ml⁻¹ respectively. The mean concentrations of analysed metals were recorded in the order of Zinc > Cadmium > Arsenic >Nickel >Lead; and most of them were under the permissible limits; however, the presence of faecal coliforms in the water is alarmingly high at Harike Pattan.

Interpretation: This investigation revealed that the river Beas water is suitable for supporting aquatic life, bathing, irrigation and other purposes. The microbial load and the heavy metal parameters need to be monitored regularly, so that their adverse effects on living beings or aquatic organisms may be minimized. River stretch near Harike Pattan and Urmar Tanda being an important habitat of endangered freshwater Dolphin (Platanista gangetica minor), Smooth-Coated Otter (Lutrogale perspicilliata) and Gharial (Gavialis gangeticus) is more vulnerable to the toxic levels of heavy metals and contamination of faecal coliform.

Keywords: Beas river, Coliforms, Heavy metals, Microbial load.

INTRODUCTION

Freshwater is essential for the survival of life on earth. It is not only fundamental for human beings, but also for plants and animals. Moreover, the rivers provide irrigation, potable water, cheap transportation, hydroelectricity, and livelihood to a large population on the earth (Smith and Gleick, 2012). With the continuous growth of population, rapid developments in agriculture, mining, urbanization, industrialization, hydro-electrical generation activities, and motor vehicle pollution, river water contamination with hazardous waste is becoming a common phenomenon (Shivayoginath et al., 2012; Sharma and Walia, 2016).

Human interference, inadequate freshwater supply, and inappropriate management are the major causes that lead to an increase in the pollutant load of a water body (Shankhwar et al., 2015; Ingole et al., 2015). In the state of...
The river Beas is 470 km long and it is one of the major tributaries of the Indus River system. It flows for about 256 km in Himachal Pradesh and for about 214 km in Punjab. It originates in the Himalayas in central Himachal Pradesh about 2050 m above mean sea level on the southern slope of Rohtang pass from two sources Beas Kund and Beas Rishi at a latitude of 32°2’ N and a longitude of 77°05’ E in central Himachal Pradesh.
adversely affected the pristine purity of water, ecology and biodiversity of river along with a massive killing of different types of fish species. Restoration programme of the river Beas was undertaken by the Punjab state government and different NGO’s along with a ban on fish harvesting and the regular biomonitoring of the river. In the year 2019, a 185 km long stretch of the river Beas was included in the list of International Ramsar Site as “Beas Conservation Reserve”. Presently, river Beas is passing through a critical phase of ecological transition due to climate change, anthropogenic pollution, and waste discharge from the industries.

Among the natural trace components of an aquatic environment, heavy metals constitute an integral part. Nowadays, the levels of heavy metals have increased manifold due to agricultural and industrial activities. Micro, small and medium scale industries are daily releasing their effluents into the water bodies and are deteriorating the quality of water. Like-wise, lead a toxic heavy metal which is absorbed through food, water and inhalation (Ferner 2001) while arsenic is organized in the environment through natural processes and a range of anthropogenic activities (Kinniburg and Smedley 2001; Kapaj et al 2006, and Walter and Carter 1995). Sufficient quantities of heavy metals get accumulated in river water and soil, and these are ultimately absorbed by the aquatic organisms present in the water, which can easily be biomagnified through the food chain. This can lethally impact the aquatic organisms and can also inflict mortality in the resident fish stocks (Almeida et al 2002; Megeer et al 2000; Xu et al., 2004). Rivers also play a major role in transporting municipal and industrial wastewater and runoff from agricultural and mining land (Hussain et al., 2017). The bacteriological examination of water has a special significance in pollution studies as it is a direct measurement of hazardous effect of contamination and pollution on human health. Atlas and Bartha (1993) considered that bacteria are fated determinants of pollution released into the environment whereas Clark and Pagel (1977) pointed bacteria as reliable indicators of contamination. The coliforms are the major microbial indicator of monitoring water quality (Brenner et al., 1993; Craun, 1978; Grant, 1997). The Total coliform and faecal coliform counts are the mainly used bacteriological procedures for the assessment of water quality (Geldreich and Clarice, 1966; Gleeson and Gray, 1997. Mcdaniels et al., 1985, Sood et al.,2008) and the detection of *Escherichia coli* provides definite evidence of faecal pollution (Kataria et al.1997; Pathak and Gopal 2001, Kistemann *et al.* 2002). However, more MPN of coliforms are indicating heavy bacterial contamination (Matta and Bisht 2018, Spancer & Ramsay 1978.).

In view of the above works and to fill the research literature gap, the present study was conducted with the objectives of analysing the heavy metal contamination and microbial load in upstream, midstream and downstream stretches of the River Beas in Punjab. Furthermore, this research was vital for evaluating the ecological status of the river and its database generation. The study also helped to check the suitability of the usage of river water for anthropogenic activities like irrigation, bathing, and fisheries purposes, and the ability of the river water to support aquatic fauna. Additionally, the assessment of heavy metals and microbial load in the river Beas water is also poorly documented.

**MATERIALS AND METHODS**

**Study area:** Samples were collected from three sampling sites in River Beas; one each from upstream, midstream and downstream stretches in Punjab. Talwara (31°57′09″N, 75°53′43″E) stretch of the river was selected for upstream (Site-1), whereas Urmar Talwara (31°41′36″N, 75°31′29″E) for midstream (Site-2) and Harike Pattan (31°9′2″N, 74°57′5″E) site for downstream (Site-3) during the present study.

**Collection of water samples:** Sampling was done during 2019-20 at 3 sampling sites seasonally during pre-monsoon (May-June), monsoon (July-September) and post-monsoon seasons (October-December). The samples were collected at monthly interval basis in triplicate in 1 litre sterilized polyvinyl plastic bottles for the analysis of heavy metals parameters and in 100 ml capacity sterilized plastic vials for the analysis of microbial load. All the bottles and vials were properly labelled and were carried in white trays. Samples were brought in insulated corrugated boxes to the College of Fisheries, GADVASU, Ludhiana. Water was stored at 4°C till further analysis.

**Assessment of heavy metals and microbial load:** Heavy metal parameters such as Arsenic (As), Cadmium (Cd), Nickel (Ni), Lead (Pb) and Zinc (Zn) were estimated by Atomic Inductively Coupled Plasma Mass Spectrometry (The Agilent 7700 series ICP-MS) following standard protocol (APHA, 2017). Microbial load in water samples was estimated in terms of Total Plate Count (TPC), Total Coliform and Faecal Coliform as per following standard protocol.
Data analysis: Significant statistical variations between different heavy metals and microbial load parameters through Analysis of Variance in SPSS v25 at 5% significant level ($p<0.05$) were calculated.

**RESULTS AND DISCUSSION**

**Heavy metals in water**

The aim of the present study was to visualize and determine the water quality status of river Beas concerned with the concentrations of heavy metal (Arsenic, Cadmium, Lead, Nickel and Zinc). The research work was performed during May, 2019 to December, 2019. Month-wise variations in average values of heavy metals in the river Beas water (Mean±S.E.) are given in Table 1. The site-wise variations in average values of heavy metals in river Beas water (Mean±S.E.) are depicted in Table 2. The maximum and minimum values of heavy metals at different sampling sites in the river Beas are mentioned in Table 3.

**Arsenic (As)**

In present study, the arsenic concentration ranged from 0-0.008 mg l$^{-1}$ (Figure 3). The maximum mean value of arsenic was found in the month of June at sampling site Harike Pattan (0.008 mg l$^{-1}$), Urmar Tanda (0.0073 mg l$^{-1}$) and Talwara (0.007 mg l$^{-1}$). The average minimum values of arsenic was recorded to be nil in December at all three sampling sites (0.0 mg l$^{-1}$) followed by 0.0 mg l$^{-1}$ at Talwara & Harike Pattan and 0.0013 mg l$^{-1}$ at Urmar Tanda during the month of November (Figure 3). Arsenic values for all the three sampling sites varied significantly as per site-wise as well as month-wise observation. The arsenic concentration recorded throughout the study period were within the permissible limit of arsenic (0.2 mg l$^{-1}$) as recommended by Water Quality Standards in India (Source IS 2296:1992) whereas 2 mg l$^{-1}$ prescribed by WHO (2004) and 0.01 mg l$^{-1}$ by BIS 10500 (2012) in drinking water.

**Cadmium (Cd)**

In the present study, the cadmium traces in the river Beas water ranged from 0.00-0.016 mg l$^{-1}$. The maximum mean values of cadmium were recorded at Urmar Tanda (0.016 mg l$^{-1}$) in the month of July followed by 0.013 mg l$^{-1}$ at Talwara during June &
September and Harike Pattan (during June, July, September and October) (Table-3). The minimum traces of cadmium were noticed to be nil in the month of May, November and December at all the three stretches studied. As per site-wise sampling observations, Urmar Tanda site was reported with maximum traces of Cd followed by Harike Pattan and Talwara site. As per the permissible limit of cadmium 0.01 mg l\(^{-1}\) (WHO 2008), the cadmium concentration at all three sampling sites was reported as slightly beyond limit (0.013 to 0.016 mg l\(^{-1}\)) (Figure 4). The higher concentration of cadmium in river Beas water was recorded during monsoon and it was not detected in May, November and December at all three sampling sites. In the present study, high concentration of cadmium in the month of July might be due to increased run-off from catchment area as cadmium generally enters in water with agricultural run-off which consists of fertilizers, pesticides and other agro-chemicals. Pollution status of cadmium level in River Beas was found below the permissible limit as reviewed by Kumar et al. (2017).

Cadmium values recorded at Talwara site were almost equal to permissible limit in the month of July. Braich and Jangu (2015) reported average concentration of cadmium in Harike wetland as 0.01 mg L\(^{-1}\) which is in corroboration with the present investigation. The higher concentration of cadmium was also reported from surface water of river Sutlej by Setia et al. (2020). The variations in cadmium concentrations were significantly different among the months with in the
Table 3: Maximum and minimum values of heavy metals and microbial load at different sampling sites in the river Beas during the study period (May 2019 –December 2019).

<table>
<thead>
<tr>
<th>Values/Sites</th>
<th>Talwara</th>
<th>Urmar Tanda</th>
<th>Harike Pattan</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arsenic (mg/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>0.008</td>
<td>0.008</td>
<td>0.009</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Average</td>
<td>0.0026</td>
<td>0.0028</td>
<td>0.0037</td>
</tr>
<tr>
<td>(±)SE</td>
<td>0.00028</td>
<td>0.00032</td>
<td>0.00027</td>
</tr>
<tr>
<td><strong>Cadmium (mg/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Average</td>
<td>0.0073</td>
<td>0.0080</td>
<td>0.0078</td>
</tr>
<tr>
<td>(±)SE</td>
<td>0.00056</td>
<td>0.00057</td>
<td>0.00069</td>
</tr>
<tr>
<td><strong>Lead (mg/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>0.000</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Average</td>
<td>0.000</td>
<td>0.000</td>
<td>0.00013</td>
</tr>
<tr>
<td>(±)SE</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Nickel (mg/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>0.004</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.001</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Average</td>
<td>0.0017</td>
<td>0.0012</td>
<td>0.0013</td>
</tr>
<tr>
<td>(±)SE</td>
<td>0.00012</td>
<td>0.00009</td>
<td>0.00008</td>
</tr>
<tr>
<td><strong>Zinc (mg/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>0.018</td>
<td>0.014</td>
<td>0.036</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Average</td>
<td>0.0071</td>
<td>0.0074</td>
<td>0.011</td>
</tr>
<tr>
<td>(±)SE</td>
<td>0.0004</td>
<td>0.0007</td>
<td>0.0006</td>
</tr>
<tr>
<td><strong>Total Plate Count (cfu/100ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>7.4×10^4</td>
<td>5.9×10^4</td>
<td>3.3×10^5</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.32×10^4</td>
<td>2.5×10^4</td>
<td>3.1×10^4</td>
</tr>
<tr>
<td>Average</td>
<td>3.56×10^4</td>
<td>4.32×10^4</td>
<td>1.44×10^5</td>
</tr>
<tr>
<td>(±)SE</td>
<td>0.209</td>
<td>0.158</td>
<td>0.165</td>
</tr>
<tr>
<td><strong>Total Coliforms (MPN/100ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>460</td>
<td>460</td>
<td>1100</td>
</tr>
<tr>
<td>Minimum</td>
<td>5</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Average</td>
<td>54.25</td>
<td>141</td>
<td>314.7917</td>
</tr>
<tr>
<td>(±)SE</td>
<td>6.44</td>
<td>17.75</td>
<td>25.55</td>
</tr>
<tr>
<td><strong>Faecal Coliforms (MPN/100ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>11</td>
<td>48</td>
<td>460</td>
</tr>
<tr>
<td>Minimum</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Average</td>
<td>15.29</td>
<td>20.91</td>
<td>72.75</td>
</tr>
<tr>
<td>(±)SE</td>
<td>1.18</td>
<td>3.16</td>
<td>1.86</td>
</tr>
</tbody>
</table>
sites while among the sites it was noticed significant variation (p<0.05).

**Lead (Pb)**

In the present investigation, the average value of lead concentration was recorded to be 0.001 mg L\(^{-1}\) in the month of September only at Harike Pattan site (Figure 5), whereas it was nil in all the remaining months. Lead was also absent in the other two sampling sites throughout the study. Thus, absence of lead content in most of the water sampling sites is good indicator that the river Beas water still maintaining a good quality. The results obtained in present study regarding presence of lead were not in agreement with the previous investigation reported by Kaur et al. (2019) in river Sutlej, Kumar et al. (2020) in river Sutlej, Beas and Harike wetland and Brraich and Jangu (2015).

**Nickel (Ni)**

In present study, the traces of nickel in water ranged from 0.0 to 0.003 mg L\(^{-1}\). The maximum value of nickel was observed in the month of June at Talwara site (0.003 mg L\(^{-1}\)) followed by Harike Pattan (0.002 mg L\(^{-1}\)) during the month of August and September. The maximum value of nickel concentration at Urmar Tanda was recorded to be 0.001 mg L\(^{-1}\) in most of the months except in May and December months, where it was nil (Figure 6). The minimum values showed inverse trend as minimum in November and December at Harike Pattan site. The variations in values of nickel were significantly varied among the months as well as among the sites in post monsoon months of November and December (p<0.05). In spite of the fact, the acceptable limit of nickel is 0.02 mg/L recommended by BIS (Bureau of Indian Standard) (2012) & WHO (1993) and the values of nickel in Beas water recorded during present investigation were within the prescribed limit.

**Zinc (Zn)**

In present study, the trace of zinc ranged from 0.0 to
The maximum mean value of zinc (0.033 mg l⁻¹) was recorded at Harike Pattan site in the month of September followed by 0.017 mg l⁻¹ at Talwara in May and 0.013 mg l⁻¹ at Urmar Tanda during July and September. Minimum values of Zn (0.001 mg l⁻¹) were recorded in post-monsoon months at Talwara and Urmar Tanda site in November and December, whereas at Harike Pattan it was minimum (0.005 mg l⁻¹) in the month of October (Figure 7). The concentration of zinc was reported nil in the month of November at Talwara site. The value of zinc varied insignificantly among the sites in all the months except post monsoon months when significant variation was reported. The minimum value of zinc during post-monsoon months might be ascribed due to rainfall and high water velocity which caused river water dilution. Kaur et al. (2019) recorded zinc contamination in the range of 0.47 – 0.96 mg l⁻¹ in river Sutlej, which was higher as compared to the present investigation. The permissible limit of zinc as per Water Quality Standards in India (Source IS 2296:1992) & BIS (Bureau of Indian Standard) 10500 (2012) is 15 mg l⁻¹, whereas WHO (1993) recommended health based guidelines is 3.0 mg l⁻¹. Zinc concentration observed during the present investigation was under the permissible limit.

The mean concentrations of all the metals analysed during the present study were found to be within the permissible limit at Talwara, Urmar Tanda and Harike Pattan sites of the river Beas except one metal i.e., cadmium (Cd) which showed the slightly higher values as compared to its permissible limit. The values of nickel (Ni) and copper (Cu) were also observed within the permissible limit. The concentration of lead (Pb) was slightly higher in the month of August at Talwara site. The concentration of iron (Fe) was observed slightly higher in the month of October at Talwara site. The concentration of manganese (Mn) was observed higher in the month of September at Urmar Tanda site. The concentration of chromium (Cr) was observed higher in the month of August at Talwara site. The concentration of cobalt (Co) was observed higher in the month of September at Harike Pattan site. The concentration of thallium (Tl) was observed higher in the month of August at Talwara site. The concentration of arsenic (As) was observed higher in the month of August at Talwara site. The concentration of selenium (Se) was observed higher in the month of August at Talwara site. The concentration of cobalt (Co) was observed higher in the month of September at Harike Pattan site. The concentration of molybdenum (Mo) was observed higher in the month of August at Talwara site. The concentration of nickel (Ni) was observed higher in the month of September at Harike Pattan site. The concentration of copper (Cu) was observed higher in the month of September at Harike Pattan site.
different metal concentration during the present study were recorded in decreasing order as Zn>Cd>As>Ni>Pb with negligible traces of Lead from all the sites except in the month of September at Harike Pattan.

**Microbiological assays**

Month-wise variations in the average values of microbial load in river Beas water (Mean±S.E.) are depicted in Table 1. Site-wise variations in average values of microbial load in the river Beas water are mentioned in Table 2. Maximum and minimum values of microbial load at different sampling sites in the river Beas are presented in Table 3.

**Total plate count (TPC)**

Total plate count (TPC) ranged from 0.32 x10⁶ to 3.2 x10⁸ cfu ml⁻¹ at different sampling points of the river Beas water. The maximum mean value (3.1 x10⁷ cfu ml⁻¹) of TPC was detected in monsoon months at Harike Pattan followed by Talwara (6.9 x10⁷ cfu ml⁻¹) and Urmar Tanda (5.5 x10⁷ cfu ml⁻¹). The average minimum value (0.33 x10⁶) was observed in post monsoon month at Talwara site followed by 2.7 x10⁶cfu ml⁻¹ in August at Urmar Tanda and 3.3 x10⁶cfu ml⁻¹ in June at Harike Pattan. Site-wise mean maximum value of TPC was recorded form Harike Pattan (14.37 x10⁶ cfu ml⁻¹) followed by Urmar Tanda (4.32 x10⁶ cfu ml⁻¹) and Talwara (3.56 x10⁶ cfu ml⁻¹) (Figure8). The mean values differed significantly during different months within the sites and also among the sites (p<0.05). The number of total plate count (TPC) varied greatly depending on water origin and across the tested growth conditions. TPC is a bacteriological substrate used for the determination of aerobic, mesophilic organisms that grow in aerobic conditions under moderate temperature.

Seasonal fluctuations revealed that TPC was highest during monsoon month at Harike Pattan and lowest during post-monsoon month at Talwara due to contamination problem emerged from various sources like ill managed waste water treatment plant, sanitary sewer pipes leakage etc. Sood et al (2008) also collected samples from 32 sites in the river Ganga for physico-chemical and microbiological analysis during different season. It was found that the total viable counts (TVC) were in order of magnitude of 10⁶ ml⁻¹, which is substantially higher than those prescribed by Bureau of Indian Standards (BIS 2012). The present results obtained for Total plate count and Most probable number were similar to the results obtained by Okonoko et al. (2008). According to ICMSF (1986), TPC level of about 10⁷ is the maximum limit under which a product is considered to be good. However, Lakhmanan et al. (1984) reported TPC above 10⁷ is considered as poor quality. The counts increased gradually from upper stretch to lower stretch, and the Harike Pattan spot (lower stretch) were found to be more contaminated.

**Total Coliform**

The numbers of total coliform bacteria were ranged from 4.0 to 1100 MPN 100 ml⁻¹ at different sampling sites of the river Beas throughout the study period. The month of October (post-monsoon) were noticed with maximum numbers (1100 MPN 100 ml⁻¹) of total coliform bacteria at Harike Pattan site (Table-3, Figure-9). The lower values (7.0 MPN 100 ml⁻¹) of coliforms were reported in monsoon month at Talwara and Harike Pattan followed by 12.33 MPN 100 ml⁻¹ at...
Umar Tanda. Overall monthly average coliform abundance (453.55 MPN 100 ml\(^{-1}\)) was reported in the month of October (post-monsoon). Site-wise average maximum coliforms (314.79 MPN 100 ml\(^{-1}\)) was recorded at Harike Pattan followed by 141.0 MPN 100 ml\(^{-1}\) and 54.25 MPN 100 ml\(^{-1}\) at Umar Tanda and Talwara respectively (Figure 9). The mean values differed significantly during different months within the sites and also between the sites (p< 0.05).

The maximum recorded value of coliforms at Harike Pattan (lower stretch) during the study period may be attributed to discharge of effluent from thermal power plant located in the vicinity of river Beas as total coliforms also represent the thermo-tolerant bacteria. Presence of thermotolerant coliforms always indicates faecal contamination. Usually, more than 95 per cent of thermotolerant coliforms isolated from water are the gut organism *Escherichia coli*, the presence of which is definitive proof of faecal contamination (Bartram and Balance 1996). Sharma and Walia (2016) reported presence of coliform and *E. coli* during winter in all sampling spots in river Beas in Himachal Pradesh. The permissible limit of total coliform is selected as 5000 MPN ml\(^{-1}\) for class C category of water designated for drinking after conventional treatment and disinfection as recommended by CPCB (2012). However, there is no prescribed limit for category D water preferred for propagation of fisheries and wildlife. During the present investigation, total coliform was reported within the recommended limit of CPCB for class C water. Kumar et al. (2017) investigated pollution status in river Beas water, their findings also corroborate with the present study. According to EPA (2003), the permissible level for coliforms should be 0 MPN 100ml\(^{-1}\). The BIS (2012) and WHO (2011) has prescribed the nil presence of coliform and *E. coli* in drinking water. Chandran et al. (2009) found that the presence of coliform was higher in sediment than in overlying water of Vembanadu Lake at Kumarakom region.

**Faecal Coliform**

During the study period, the numbers of coliform bacteria ranged from 0.0 - 460 MPN 100 ml\(^{-1}\), at different sampling sites of the river Beas. The month of October (post-monsoon) was noticed with maximum numbers (460 MPN 100 ml\(^{-1}\)) of coliform bacteria at Harike Pattan site followed by 48 MPN 100 ml\(^{-1}\) at Umar Tanda (Table-1, Figure-10). Singh and Singh (2014) investigated the bacteriological profile of river Gomti in Jaunpur at different spots and reported higher count of TC and FC with the conclusion of higher discharge of faecal organic matter in the river.

The minimum mean values (1.33 MPN 100 ml\(^{-1}\)) of coliforms were reported in October at Talwara followed by 2.33 MPN 100 ml\(^{-1}\) in September at Umar Tanda, whereas minimum value of 3.33 MPN 100 ml\(^{-1}\) was recorded at Harike Pattan. Overall monthly average faecal coliform abundance (169.77 MPN 100 ml\(^{-1}\)) was reported in the month of October (post-monsoon). Site-wise average maximum faecal coliforms (72.74 MPN 100 ml\(^{-1}\)) was recorded at Harike Pattan followed by 20.91 MPN 100 ml\(^{-1}\) and 15.28 MPN 100 ml\(^{-1}\) at Umar Tanda and Talwara respectively (Figure 10). Harike Pattan site is the meeting point of river Beas and Sutlej and is also an inhabited area which is receiving comparatively higher faecal matter resulting into faecal pollution. High level of faecal coliform in Harike Pattan site is due to the discharge of untreated sewage in the river which is a
matter of grave concern. Faecal coliform levels in Harike Pattan sampling site, which is a major tourist attraction due to it being a hotspot of migratory birds, Gharial, Smooth-Coated Otter, and freshwater Dolphin, needs to be examined diligently. The faecal coliform abundance was recorded in the order as post-monsoon > monsoon > pre-monsoon, which was not in agreement with the results of Chandra et al. (2006) who studied bacteriological contamination in river Gola water. The values of faecal coliform at different sites during the study period are presented in (Table-2 & 3, Figure-10). The mean values differed significantly during different months within the sampling sites and also between the sites (p< 0.05). The higher faecal coliform has indicated the tolerance of high temperature. The result coincides with observation reported by Ravichandran and Ramanibai (1988). Increased numbers of faecal coliforms designate high level of faecal contamination and upgrade risk of water-borne diseases viz; cholera, typhoid, dysentery etc. (Hodegkiss, 1988 and Vaidya et al., 2001).

CONCLUSION

The ecological evaluation of the river Beas was diligently measured for different selected heavy metals and microbial load parameters. Overall study revealed that although, water quality at selected sampling sites is suitable to support aquatic life, yet it is recommended that the levels of different heavy metals parameters as well as the microbial load, needs to be monitored regularly. River stretch near Harike Pattan and Urmar Tanda being an important habitat of freshwater Dolphin (Platanista gangetica minor) and Gharial (Gavialis gangeticus) and Smooth-Coated Otter (Lutrogale perspicilliata) is more vulnerable to the detrimental levels of heavy metals and the high number of faecal coliform. Talwara had a better water quality as compared to Urmar Tanda and Harike Pattan, which may be attributed to the fact that it is an upstream site of the Kandi area of Punjab state. The pollution load increases in the river as it traverses through the plains of Punjab, up to its emergence into the River Sutlej at Harike Pattan. There is a significant need to create a mass awareness programme among the people in riparian zone of the river Beas regarding water purity, cleanliness, biodiversity conservation and proper management of solid/liquid waste discharge of pollution causing materials including point and non-point sources of pollution.

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Conflict of Interest: The authors declare that there is no conflict of interest.

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TREES DIVERSITY OF KANWAR TAAL BIRD SANCTUARY, BEGUSARAI, BIHAR

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ABSTRACT

The present work has been carried out to enumerate the different tree species of Kanwar Taal, a Bird Sanctuary, which was added in Ramsar wetlands sites (no. 2436) 'Wetlands of international importance' (Ramsar 2020) and considered as one of the largest natural oxbow lakes in the Indian subcontinent. It is one of the most important wetlands for waterfowl in the Gangetic plain supporting a huge number of various species of migratory and resident avifauna. A total of 61 species of trees include two bamboo species belonging to 51 genera and 29 families have recorded from the Kanwar Taal of Begusarai, Bihar. The recorded trees species with their Vernacular, English names uses have been provided with their respective families and botanical name alphabetically.

Keywords: Kanwar Taal, Bihar, Tree Diversity, Ramsar wetland site.

INTRODUCTION

The Kanwar Taal is situated 30 km away from the main township of the Begusarai district of Bihar. Kanwar Taal has been developed to combat the widespread poaching of these birds. The Bihar state government designated the area as a protected zone in 1986, and the government of India designated it as a bird sanctuary in 1989. It was formed naturally by the meandering of the Budhi Gandak River, which is a tributary of the Ganga. It covers an area of 2600 ha, and during the monsoon, it gets connected to nearby water bodies to form an area of 7,400 ha (Ambastha et al., 2007). Kanwar Taal, a Bird Sanctuary, is one of the largest natural oxbow lakes in the Indian subcontinent (Singh and Jayakumar, 2016). Oxbow lake is a river cut-off due to the high degree of bend and nearness of the meander's limb in very close to each other (Oxford Dictionary, Webster Dictionary). The process of a cutoff may be chute cut or neck cut but ultimately it produces oxbow lake (Strahler and Strahler, 1996). It is one of the most important wetlands for waterfowl in the Gangetic plain hosting a large number of different species of migratory and resident avifauna. It is approximately three times more in than the size of the Bharatpur Sanctuary. It was reported by ornithologist Salim Ali that about 60 migratory birds that come from Central Asia in winter and recorded around 106 species of resident birds (Chauhan, 2015). The soil is sandy, loam, and rich in humus. Water depth varies from 1-5 meters during the monsoon. Tropical monsoon climate typical of the middle Ganges plain, with a verge annual rainfall of 1100 mm concentrated in the period July- September. Temperatures range from 5°C to 45°C. During the monsoon months, the lake is flooded to its capacity; the area is used by the landless Sahani (Mallah) caste, who hold fishing rights. When the water recedes, the exposed lake bed is again used by the landowners for cultivation. Since date, any systematic work has not been carried out on the flora of Kabar lake and number of plant species exist near by the wetland site as tree provides the

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shelter to the local and migratory birds. Keeping in view the importance of kabar lake and tree flora for the shelter of birds, the present study had been planned to enumerate the number of tree species.

MATERIALS AND METHODS
Geographically, Kanwar Taal wetland located between 25°35'00" - 25°40'00" N to 86°05'00" - 86°10'00" E has an elevation of 37–39 m above mean sea level (MSL). Study areas situated at 25° 35’ 32.3” N, 86° 09’ 39.4” E and its ~ 2 KM radius, is a permanent island (Jaimanglagarh Temple) of about 130 ha in the southern corner of the lake. The study was conducted from September to November 2015 several times and tree flora was noted. Various information and data were collected during interaction with local inhabitants. A standard collection method was used following (Jain and Rao 1977). Plants specimens collected in their good condition to identify properly with performed by a plant taxonomist aided by manuals and Floras (Hawthorne, 1990; Arbonnier, 2004; Poorter et al., 2004; Hawthorne and Jongkind, 2006; Sahni, 1998; Randhawa, 1957).

Fig. 1: Map of Kanwar Taal.

Fig. 2: Show study site point by Google earth.
RESULTS AND DISCUSSION

A total of 61 trees species of trees including two species of Bamboo* were observed. The trees belong to 51 genera and 29 families from ~ 2 KM radius of Jaimanglagarh island of Kanwar Taal. The scientific name, family, local name (wherever available), and uses for each species are provided in Table 1.

Table 1: List of tree species reported in Kanwar Taal and adjacent areas.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Family</th>
<th>Scientific Name</th>
<th>Vernacular Name</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anacardiaceae</td>
<td>Mangifera indica L.</td>
<td>Aam</td>
<td>Fruits edible</td>
</tr>
<tr>
<td>2</td>
<td>Annonaceae</td>
<td>Annona squamosa L.</td>
<td>Sharifa</td>
<td>Fruits edible</td>
</tr>
<tr>
<td></td>
<td>Polyalthia longifolia (Sonn.) Thwaites</td>
<td>Ashok</td>
<td>Ornamental and fuelwood</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Apocynaceae</td>
<td>Alstonia scholaris (L.) R.Br.</td>
<td>Chhatpan</td>
<td>Ornamental and fuelwood</td>
</tr>
<tr>
<td>4</td>
<td>Arecaeeae</td>
<td>Borassus flabellifer L.</td>
<td>Taad</td>
<td>Fruits edible</td>
</tr>
<tr>
<td></td>
<td>Cocos nucifera L.</td>
<td>Nariyal</td>
<td>Fruits edible</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phoenix sylvestris (L.) Roxb.</td>
<td>kajjur</td>
<td>Fruits edible</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Bignoniaceae</td>
<td>Haplophragma adenophyllum (Wall.) P. Dop</td>
<td>Marodphali</td>
<td>Fuelwood</td>
</tr>
<tr>
<td>6</td>
<td>Bombacaceae</td>
<td>Bombax ceiba L.</td>
<td>Semal</td>
<td>Fuelwood and furniture</td>
</tr>
<tr>
<td></td>
<td>Ceiba pentandra (L.) Gaertn.</td>
<td>Safed semal</td>
<td>Fuelwood and furniture</td>
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<tr>
<td>7</td>
<td>Boraginaceae</td>
<td>Cordia dichotoma G.Forst.</td>
<td>Lasora</td>
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<tr>
<td></td>
<td>Ebreia laevis Roxb.</td>
<td>Chamrro</td>
<td>Fuel wood</td>
<td></td>
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<tr>
<td>8</td>
<td>Caesalpiniaceae</td>
<td>Baubinia purpurea L.</td>
<td>Kachnar</td>
<td>Ornamental and fuel wood</td>
</tr>
<tr>
<td></td>
<td>Baubinia variegata L.</td>
<td>Kachnar</td>
<td>Ornamental and fuel wood</td>
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<td></td>
<td>Cassia fistula L.</td>
<td>Amaltas</td>
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<td></td>
<td>Delonix regia (Hook.) Raf.</td>
<td>Gulmohar</td>
<td>Ornamental and fuel wood</td>
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<td></td>
<td>Peltophorum pterocarpon (DC.) K.Heyne</td>
<td>Peela Gulmohar</td>
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<td></td>
<td>Tamarindus indica L.</td>
<td>Inli</td>
<td>Fruits edible</td>
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<td></td>
<td>Saraca asoca (Roxb.) de Wilde</td>
<td>Sita Ashok</td>
<td>Ornamental</td>
<td></td>
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<tr>
<td>9</td>
<td>Cannabaceae</td>
<td>Trena orientalis (L.) Blume</td>
<td>Gio</td>
<td>Fuel wood</td>
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<tr>
<td>10</td>
<td>Combretaceae</td>
<td>Terminalia arjuna (Roxb. ex DC.) Wight &amp; Am.</td>
<td>Arjun</td>
<td>Fuel wood</td>
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<tr>
<td>11</td>
<td>Ebenaceae</td>
<td>Diospyros montana Roxb.</td>
<td>Kaladhaa, Kendu</td>
<td>Fuelwood</td>
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<tr>
<td>12</td>
<td>Euphorbiaceae</td>
<td>Trewia nudiflora L.</td>
<td>Ajan</td>
<td>Fuelwood and furniture</td>
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<tr>
<td>13</td>
<td>Fabaceae</td>
<td>Dalbergia sissoo DC.</td>
<td>Shisham</td>
<td>Fuelwood and furniture</td>
</tr>
<tr>
<td></td>
<td>Pongamia pinnata (L.)Pierre</td>
<td>Karanj</td>
<td>Fuelwood and furniture</td>
<td></td>
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<tr>
<td>14</td>
<td>Meliaceae</td>
<td>Azadirachta indica A.Juss.</td>
<td>Neem</td>
<td>Hole parts of the plant used for medicinal purpose</td>
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<td></td>
<td>Melia azedarach L.</td>
<td>Bakain</td>
<td>Fuelwood and furniture</td>
<td></td>
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<tr>
<td></td>
<td>Swietenia macrophylla King</td>
<td>Mahagony</td>
<td>Fuelwood and furniture</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Mimosaceae</td>
<td>Acacia auriculiformis A Cunn. ex Benth.</td>
<td>Sonjhuri</td>
<td>Fuelwood and furniture</td>
</tr>
<tr>
<td>Tree Family</td>
<td>Common Name</td>
<td>Scientific Name</td>
<td>Use</td>
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<tr>
<td><strong>Acacia cagechu (L.f.) Willd.</strong></td>
<td>Khair</td>
<td>Making kattha</td>
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<td></td>
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<td><strong>Acacia nilotica (L.) Delile</strong></td>
<td>Babul</td>
<td>Leaves used for animal feeding, fuelwood, furniture</td>
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<tr>
<td><strong>Albizia lebbeck (L.) Benth.</strong></td>
<td>Siris</td>
<td>Leaves used for animal feeding, fuelwood</td>
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<td><strong>Albizia procera (Roxb.) Benth.</strong></td>
<td>Safed Siris</td>
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<td><strong>Leucaena leucocephala (Lam.) de Wit</strong></td>
<td>Subabul</td>
<td>Leaves used for animal feeding, fuelwood</td>
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<td><strong>Pithecellobium dulce (Roxb.) Benth.</strong></td>
<td>Jangali Jilebi</td>
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<td><strong>Artocarpus lacucha Buch.-Ham.</strong></td>
<td>Barhal</td>
<td>Fruits edible</td>
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<td><strong>Ficus bipinid L.f.</strong></td>
<td>Katgularia</td>
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<td><strong>Ficus racemosa L.</strong></td>
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<td><strong>Moras alba L.</strong></td>
<td>Shahtoot</td>
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<td><strong>Streblus asper Lour.</strong></td>
<td>Sabora, Sihora</td>
<td>Fuel wood</td>
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<td></td>
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<tr>
<td><strong>Moringaceae</strong></td>
<td><strong>Moringa oleifera Lam.</strong></td>
<td>Sahjan, Senjana</td>
<td>Fruits edible</td>
<td></td>
</tr>
<tr>
<td><strong>Myrtaceae</strong></td>
<td><strong>Eucalyptus tereticornis Sm.</strong></td>
<td>Safeda</td>
<td>Fuelwood and construction purpose</td>
<td></td>
</tr>
<tr>
<td><strong>Psidium guajava L.</strong></td>
<td>Amrood</td>
<td>Fruits edible</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Syzygium cumini (L.) Skeels</strong></td>
<td>Jamun</td>
<td>Fruits edible</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Oleaceae</strong></td>
<td><strong>Nyctanthes arbor-tristis L.</strong></td>
<td>Harsingrar</td>
<td>Flowers used for worship</td>
<td></td>
</tr>
<tr>
<td><strong>Phyllanthaceae</strong></td>
<td><strong>Phyllanthus emblica L.</strong></td>
<td>Anola</td>
<td>Fruits edible</td>
<td></td>
</tr>
<tr>
<td><strong>Poaceae</strong></td>
<td><strong>Bambusa bambos (L.) Voss</strong></td>
<td>Kanta Bans</td>
<td>Construction home and furniture</td>
<td></td>
</tr>
<tr>
<td><strong>Dendrocalamus strictus (Roxb.) Nees</strong></td>
<td>Bans</td>
<td>Construction home and furniture</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Proteaceae</strong></td>
<td><strong>Grevillea robusta A. Cunn.ex.R.Br.</strong></td>
<td>Morpatri</td>
<td>Used for fuel wood</td>
<td></td>
</tr>
<tr>
<td><strong>Rhamnaceae</strong></td>
<td><strong>Ziziphus jujuba Mill.</strong></td>
<td>Ber</td>
<td>Fruits edible</td>
<td></td>
</tr>
<tr>
<td><strong>Rubiaceae</strong></td>
<td><strong>Neolamarckia cadamba (Roxb.) Bosser</strong></td>
<td>Kadamb</td>
<td>Fruits edible</td>
<td></td>
</tr>
<tr>
<td><strong>Rutaceae</strong></td>
<td><strong>Aegle marmelos (L.) Corrêa</strong></td>
<td>Bel</td>
<td>Fruits edible</td>
<td></td>
</tr>
<tr>
<td><strong>Citrus maxima (Burm.) Merr.</strong></td>
<td>Tabh</td>
<td>Fruits edible</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Salisaceae</strong></td>
<td><strong>Populus deltoids Marsh.</strong></td>
<td>Poplar</td>
<td>Plywood and box are made of wood.</td>
<td></td>
</tr>
<tr>
<td><strong>Sapotaceae</strong></td>
<td><strong>Madhuca indica J.F.Gmel.</strong></td>
<td>Mahua</td>
<td>Flowers edible</td>
<td></td>
</tr>
</tbody>
</table>
### Table 2: Dominant plant families.

<table>
<thead>
<tr>
<th>Family</th>
<th>Number of species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moraceae</td>
<td>9</td>
</tr>
<tr>
<td>Caesalpiniaceae</td>
<td>7</td>
</tr>
<tr>
<td>Mimosaceae</td>
<td>7</td>
</tr>
<tr>
<td>Arecaceae</td>
<td>3</td>
</tr>
<tr>
<td>Meliaceae</td>
<td>3</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td>3</td>
</tr>
</tbody>
</table>

Apart from the Kawar lake which was quite common species of trees *Acacia nilotica*, *Azadirachta indica*, *Bauhinia purpurea*, *Bauhinia variegata*, *Leucaena leucocephala*, *Ficus religiosa*, *Moras alba*, *Mangifera indica*, *Terminalia arjuna*, *Madhuca indica*, *Dalbergia sissoo*, *Ficus beghalensis*, *Ficus hispida*, *Syzygium cumini* etc. This study would be further beneficial if analysed more and experimented with deeper. Conclusively this study could throw some insight into the flora and fauna of the Kawar lake which could be better documented through the application of more novel techniques in the days to come. A preliminary attempt was made to find the Checklist of Tree Diversity of Kanwar Taal. The lake is known to support a rich and diverse aquatic flora. It shows the detritus of habitat, illegal poaching and predominantly bird trapping activity. The present study helps to understand the direct or indirect close relation between indigenous people and wetland. Therefore, has important to explore the flora of the area and the dependence of local communities on it. Human disturbances have influenced the floral and faunal diversity of Kanwar Taal.

**REFERENCE**

EFFECT OF INDUSTRY EFFLUENT ON HISTO-PHARMACOGNOSY OF BOERHAAVIA DIFFUSA LINN. USED AS BLOOD PURIFIER

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Govt. of India, Red Cross Society, New Delhi, India

ABSTRACT

Aims of the Study:
To carried out the effect of Atlas Cycles Industry effluent on pharmacognosy of Boerhaavia diffusa Linn. used as Blood Purifier

Method:
The effluent of Atlas Cycles Industry was analyzed by Trivedi & Goel, 1986 method. Metacalf and Chalk, 1950 was consulted for anatomical studies of selected plant; for chemical analysis Johanson, 1940, Cromwell, 1955 & Trease and Evans, 1983 were followed. TLC was investigated by WHO, 1998.

Results:
The physico-chemical parameters of Atlas cycle industry effluent were found greater values as compared to standard values. The morphological & anatomical parameters were showed decreasing trend in those plants which were collected near the vicinity of Atlas cycle industry. The colour reaction tests resulted only degrees of changes. The number of spots in observation of TLC, stomatal index, palisade ratio, water extractive and alcohol extractive values were reduced in those plants which were collected near the vicinity of Atlas cycle industry where as vein Islet & vein termination number, ash values were comparatively higher in same samples.

Conclusion:
The conclusion of this study is that the plants should not be collected form polluted areas(near the vicinity of any industry) for the preparation of medicines, since majority of parameters reflect decreasing data in those plants which were taken from polluted area.

Keywords: Pharmacognosy, Boerhaavia diffusa, Effluent analysis.

INTRODUCTION
After making a survey of Ghaziabad, it is found that most of the industries are situated near the agricultural land and populated areas. The industrial wastes are being discharged in the earthen and semi earthen areas (fields). In these areas many medicinally important plants are growing. Boerhaavia diffusa Linn. an important medicinal plant is selected in this study which is found in the vicinity of Atlas Cycles Industry, Ghaziabad. The heavy metals and other pollutants enter into the roots of medicinally important plants through industrial effluent and agricultural wastes. Bio-monitors are of the view that the polluted water which is an intricate system of living and nonliving substances like acids, alkalis, chlorides, heavy
metals, dissolved solids, nitrates, sulfates, aquatic life bacteria, fungal forms etc. They are harmful to environment and plant species thus caused a numbers of changes in the morphology, anatomy, chemical constituents, behaviour and reproductive cycle, the flora and fauna of the region. The pollution in the region has affected the growth of various plant species having substantial medicinal value.

Pharmaco-botanical analysis plays a vital role in identification of plants and determination of their purity and quality of raw medicinal material plant. Therefore, an attempt has been made for a comparative study under the impact of industrial pollution on *B. diffusa*. It is a very important medicinal plants having anti-tumor property and commonly used as a blood purifier.

**MATERIALS AND METHODS**

The samples of *Boerhaavia diffusa* Linn. were collected from the area near to Atlas Cycle Industry, Sahibabad, Ghaziabad, UP, India to investigate the effect of industrial pollution. The effluent was analyzed by using the standard methods of Trivedi & Goel (1986). Fresh and matured samples of both the plants are collected from polluted (Atlas Cycle Industry) and non-polluted areas (ALTT Centre, Ghaziabad) to their macro morphological characters. For anatomical studies Metacalf (1980) were consulted. For chemical analysis Johanson, 1940, Cromwell, 1955 & Trease and Evans, 1983 were followed. TLC was investigated by WHO, 1998.

**RESULTS**

**Analysis of Effluent**

The effluent contains data regarding Colour, Odour, BOD, COD, DO, pH, temperature, TS, TSS, TDS, oil and grease, heavy metals etc. The result is given in table -1.

### Table 1: Physico-chemical Characteristics of industrial effluent of Atlas Cycle Industry.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Characteristic of Effluents</th>
<th>Maximum Recommended Concentration</th>
<th>Authority/Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Colour</td>
<td>Yellowish</td>
<td>Should be absent</td>
<td>I.S.I. : 2490</td>
</tr>
<tr>
<td>2.</td>
<td>Odour</td>
<td>--</td>
<td>Odourless</td>
<td>I.S.I. : 2490</td>
</tr>
<tr>
<td>3.</td>
<td>pH</td>
<td>4-6</td>
<td>5.5-9.0</td>
<td>I.S.I. : 2296</td>
</tr>
<tr>
<td>4.</td>
<td>Suspended Solids</td>
<td>200 mg/l</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>5.</td>
<td>Total Dissolved Solids (mg/l)</td>
<td>810 mg/l</td>
<td>2100.0</td>
<td>I.S.I. : 3307</td>
</tr>
<tr>
<td>6.</td>
<td>Total Suspended Solids (mg/l)</td>
<td>1010 mg/l</td>
<td>600.0</td>
<td>I.S.I. : 3306</td>
</tr>
<tr>
<td>7.</td>
<td>Dissolved Solids</td>
<td>720 mg/l</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>8.</td>
<td>Total Solids (mg/l)</td>
<td>840 mg/l</td>
<td>2700.0</td>
<td>-----</td>
</tr>
<tr>
<td>9.</td>
<td>BOD (mg/l)</td>
<td>16.0 mg/l</td>
<td>30.0</td>
<td>I.S.I. : 2490</td>
</tr>
<tr>
<td>10.</td>
<td>COD (mg/l)</td>
<td>200 mg/l</td>
<td>250.0</td>
<td>I.S.I. : 2490,1982</td>
</tr>
<tr>
<td>11.</td>
<td>Oil and Grease (mg/l)</td>
<td>Nil</td>
<td>10.0</td>
<td>I.S.I. : 2490</td>
</tr>
<tr>
<td>12.</td>
<td>Chloride (mg/l)</td>
<td>Nil</td>
<td>600</td>
<td>I.S.I. : 2490</td>
</tr>
<tr>
<td>14.</td>
<td>Chromium (Cr)</td>
<td>5 mg/l</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>15.</td>
<td>Nickel (Ni)</td>
<td>12 mg/l</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>16.</td>
<td>Zinc (Zn)</td>
<td>15 mg/l</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>17.</td>
<td>Cadmium (Cd)</td>
<td>4 mg/l</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>18.</td>
<td>Copper (Cu)</td>
<td>4 mg/l</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>19.</td>
<td>Temperature</td>
<td>500C</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>
Pharmacognostic Studies:

Organoleptic Studies: Macroscopical: Stem is prostrate, ascending, reaching 0.6 – 0.9 m long, often purplish, swollen at the nodes, divaricately branched, slender, cylindrical, minutely pubescent or nearly glabrous. Leaves are arranged in unequal pairs at each node, 1.0-5.0 cm long and 2.5-3.0 cm broad, dark green in colour, ovate, oblong, rounded at apex, glabrous, usually containing white minute scales beneath, base rounded, margin entire, often pink; petiole nearly as long as the blade i.e. 2.0-2.6 cm long and solid in non polluted (fig. a) sites whereas in polluted sites leaves are light green in colour with some white brown patches; petiole solid 1.5-2.0 cm long (fig. b).

**Fig. 1: Morphological differences of Boerhaavia diffusa** Linn. growing in non polluted (a) and polluted (b) areas. Flowers red, pink or white, in small umbels arranged in axillary and terminal panicles; fruits ovate oblong, pubescent, five-ribbed, viscid glandular anthocarps in both the cases. The main differences are in Plate-1(a&b).

Microscopical: Anatomy / Histology: Stem: Single layer circular epidermis covered by thin cuticle with glandular and non-glandular trichomes; hypodermis 3-4 layered collenchymatous followed by 3-5 layers of chlorenchymatous cortex; endodermis well distinct, single layered made up of barrel shaped cells; pericycle represented by few patches of fibers. Vascular bundles arranged in three rings. There are 15-20 vascular bundles in outer ring, 6-14 in middle ring and two large vascular bundles present in inner ring. Pith is large and parenchymatous cell containing micro, rosette and prismatic crystals of calcium oxalate in case of non polluted plants. In case of polluted plants thick cuticle, 5-6 layers of parenchyma, cambium discontinuous and absence of chlorenchyma were observed. The main differences are in Plate 2 (a & b).

**Plate-2: Anatomical differences in** stem of *Boerhaavia diffusa* Linn.growing in non polluted (a) and polluted (b) areas.

**Leaf**: Single layer of epidermis with glandular and non-glandular trichomes; stomata anisocytic and anomocytic found on both the surfaces. Mesophyll differentiated into two regions; 2 layers of palisade and 4-5 layers of spongy parenchyma. Midrib having 4-5 vascular bundles, arranged in arc, raphide (acicular crystal) and microcrystal present in parenchyma of ground tissue in leaf collected from non polluted areas (a&b). The leaf collected from polluted sites shows 2 layered epidermis, 2 layers of collenchyma below the upper epidermis, mesophyll with single layer of palisade and 3-4 layers of spongy parenchyma; one vascular bundle in midrib (c&d). Rosette, micro and idioblasts containing raphides are present in parenchymatous cells. The stomatal index is 16.12 – 20.00 on upper epidermis and 13.93 – 17.64 on lower surfaces in non-polluted leaf. But in case of polluted leaf the stomatal index is 12.70 – 16.27 on upper epidermis and 9.09– 14.81 in lower epidermis. The Palisade ratio is 10.75 – 11.50 in non polluted leaf and 6.25 – 6.95 in polluted leaf. The vein islet number are 12 – 16 in non-polluted and 8 – 10 in polluted samples. The vein termination number values are 14 - 24 in non polluted and 18- 36 in polluted samples (table 2). The main differences are in Plate-3.
Powder Analysis: The colour of powder is green in non polluted plant samples and brown green in

Plate 3: Anatomical differences in the leaves of Boerhaavia diffusa Linn. growing in non polluted (a through midrib) & (b through lamina) and polluted areas (c through midrib) & (through lamina d).

Table 2: Powder analysis of Boerhaavia diffusa Linn. growing in non-polluted and polluted areas.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Characters</th>
<th>Non-polluted</th>
<th>Polluted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Xylem Vessel (mm)</td>
<td>L = 0.62 + 0.08; CV=12.90</td>
<td>L=0.28 + 0.03; CV=14.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W=0.07 + 0.009; CV=12.90</td>
<td>W=0.06 + 0.01; CV=28.78</td>
</tr>
<tr>
<td>2.</td>
<td>Diameter of Xylem Pore (mm)</td>
<td>D = 0.13 + 0.02; CV=10.89</td>
<td>D=0.08 + 0.009; CV=10.69</td>
</tr>
<tr>
<td>3.</td>
<td>Xylem Tracheid (mm)</td>
<td>L=1.37 +0.05; CV=3.65</td>
<td>L=0.14 + 0.007; CV=5.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W=0.15 +0.05; CV=38.16</td>
<td>W=0.07 + 0.01; CV=23.07</td>
</tr>
<tr>
<td>4.</td>
<td>Xylem Fibre (mm)</td>
<td>L = 1.07 + 0.12; CV=11.81</td>
<td>L=0.80+0.15; CV=19.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W=0.06 + 0.09; CV=14.39</td>
<td>W=0.06 + 0.06; CV=10.20</td>
</tr>
<tr>
<td>5.</td>
<td>Palisade Cell (mm)</td>
<td>L=0.087+0.009; CV=10.34</td>
<td>L=0.15+0.012; CV=7.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W=0.04 + 0.003; CV=7.14</td>
<td>W=0.05 + 0.006; CV=11.53</td>
</tr>
<tr>
<td>6.</td>
<td>Palisade Ratio</td>
<td>R = 10.75 – 11.50</td>
<td>R = 6.25–6.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD=11.06+0.32; CV=2.89</td>
<td>SD=6.675+0.26; CV=3.86</td>
</tr>
<tr>
<td>7.</td>
<td>Spongy Parenchyma (mm)</td>
<td>L=0.06+0.003; CV=4.83</td>
<td>L=0.140 + 0.007; CV=5.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W=0.042 + 0.009; CV=21.42</td>
<td>W=0.07 + 0.004; CV=5.63</td>
</tr>
<tr>
<td>8.</td>
<td>Guard Cell (mm)</td>
<td>L=0.15+0.003; CV=2.04</td>
<td>L=0.095 + 0.002; CV=2.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W=0.04 + 0.003; CV=14.60</td>
<td>W=0.03 + 0.004; CV=12.50</td>
</tr>
<tr>
<td>9.</td>
<td>Stomatal Pore (mm)</td>
<td>L = 0.127 + 0.006; CV=4.72</td>
<td>L=0.076+0.008; CV=10.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W=0.042 + 0.006; CV=10.90</td>
<td>W=0.03+0.003; CV=9.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD=17.68+1.33; CV=7.52</td>
<td>SD=14.11+1.36; CV=15.52</td>
</tr>
</tbody>
</table>
Chemical Analysis:

Preliminary Colour Reaction Tests:- The result shows the presence of alkaloids, lignin, tannins, carbohydrates, proteins, sugar, suberin, glucosides, saponin, flavin, steroids and oils in both the cases. Degree of change in colour reaction tests are tabulated in table-3.

Table 3: Colour reaction tests of Boerhaavia diffusa Linn. growing in non-polluted and polluted areas.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Reagents</th>
<th>Test for</th>
<th>Nature of Colour</th>
<th>Degree of Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dragenorff’s Reagent (Cromwell (1955))</td>
<td>Alkaloid</td>
<td>Orange ppt</td>
<td>+++</td>
</tr>
<tr>
<td>2.</td>
<td>Mayer’s Reagent</td>
<td>Alkaloid</td>
<td>Brown</td>
<td>+++</td>
</tr>
<tr>
<td>3.</td>
<td>Wagner’s Reagent (Trease and Evans (1983))</td>
<td>Alkaloid</td>
<td>Brown</td>
<td>+++</td>
</tr>
<tr>
<td>4.</td>
<td>Tannic Acid</td>
<td>Alkaloid</td>
<td>Turbidity</td>
<td>+++</td>
</tr>
<tr>
<td>5.</td>
<td>Hager’s Reagent</td>
<td>Alkaloid</td>
<td>Yellow</td>
<td>++</td>
</tr>
<tr>
<td>6.</td>
<td>Phloroglucinol + HCl</td>
<td>Lignin</td>
<td>Dark Red</td>
<td>+++</td>
</tr>
<tr>
<td>7.</td>
<td>FeCl3</td>
<td>Tannin</td>
<td>Black</td>
<td>+++</td>
</tr>
<tr>
<td>8.</td>
<td>Molisch Test</td>
<td>Carbohydrates</td>
<td>Red</td>
<td>++++</td>
</tr>
<tr>
<td>9.</td>
<td>Millon’s Reagent</td>
<td>Protein</td>
<td>Red ppt</td>
<td>++++</td>
</tr>
<tr>
<td>10.</td>
<td>Xanthoproteic Test</td>
<td>Protein</td>
<td>Yellow</td>
<td>++++</td>
</tr>
<tr>
<td>11.</td>
<td>Bendict’s Reagent after Heating</td>
<td>Sugars</td>
<td>Red Violet</td>
<td>+++</td>
</tr>
<tr>
<td>12.</td>
<td>Sample + Heating with Strong KOH + H2SO4</td>
<td>Suberin</td>
<td>Red Black</td>
<td>++++</td>
</tr>
<tr>
<td>13.</td>
<td>Molisch Test after Hydrolysis</td>
<td>Glucoside</td>
<td>Yellow</td>
<td>++++</td>
</tr>
<tr>
<td>15.</td>
<td>Mg Powder + Conc. HCl</td>
<td>Flavin</td>
<td>Red</td>
<td>++</td>
</tr>
<tr>
<td>16.</td>
<td>Libermann’s Buchard Reagent</td>
<td>Steroids</td>
<td>Violet</td>
<td>++</td>
</tr>
<tr>
<td>17.</td>
<td>Sudan IV</td>
<td>Oils</td>
<td>Violet</td>
<td>++++</td>
</tr>
</tbody>
</table>
**TLC:** The number of spots are 4-8 in non-polluted and 2 in polluted samples (Plate-4). There RF values are tabulated in table-4.

**Table 4: The RF values of Boerhaavia diffusa Linn. growing in non-polluted and polluted areas.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Wavelengths</th>
<th>Non – Polluted</th>
<th>Polluted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RF – values</td>
<td>RF – values</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Sunlight (visible)</td>
<td>0.30,0.34,0.80,0.88</td>
<td>0.80, 0.88</td>
</tr>
<tr>
<td>2.</td>
<td>UV Light (354 nm)</td>
<td>0.26,0.30,0.34,0.36,0.40,0.80,0.88,0.93</td>
<td>0.80, 0.88</td>
</tr>
<tr>
<td>3.</td>
<td>UV Light (365nm)</td>
<td>0.30,0.34,0.36,0.40,0.80,0.88,0.93</td>
<td>0.80, 0.88</td>
</tr>
</tbody>
</table>

**PHYSICAL EVALUATION**

**Fluorescence Behaviour of Plants:** There were no significant results observed with fluorescence behaviour of plant powder and its extracts except some difference in colour only.

**Extractive Values and Ash Values:** The percentage of water and alcoholic soluble extractives are lower in those plants collected from polluted sites, but LOD is higher in polluted samples. Total ash, acid insoluble and sulphated ash are higher in those samples which are collected from the polluted areas. The mean values are tabulated in table-5.

**Table 5: Extractive and Ash values of Boerhaavia diffusa Linn. growing in non-polluted and polluted areas.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Non-polluted</th>
<th>Polluted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Water Soluble</td>
<td>30.250 + 1.400; CV = 4.620</td>
<td>27.090 + 0.960***; CV = 3.543</td>
</tr>
<tr>
<td>2.</td>
<td>Alcohol Soluble</td>
<td>49.730 + 0.820; CV = 1.650</td>
<td>31.880 + 0.210* ; CV = 0.660</td>
</tr>
<tr>
<td>3.</td>
<td>LOD</td>
<td>15.060 + 0.910; CV = 6.042</td>
<td>25.600 + 1.960***; CV = 7.650</td>
</tr>
<tr>
<td>4.</td>
<td>Tota Ash Value</td>
<td>9.150 + 1.070 ; CV = 11.780</td>
<td>14.010 + 0.800** ; CV = 5.600</td>
</tr>
<tr>
<td>5.</td>
<td>Acid Insoluble</td>
<td>1.39 + 0.140 ; CV = 10.066</td>
<td>6.560 + 0.390* ; CV = 5.950</td>
</tr>
<tr>
<td>6.</td>
<td>Sulphated Ash</td>
<td>18.650 + 1.870 ; CV = 10.030</td>
<td>25.830 + 1.290*** ; CV = 5.00</td>
</tr>
</tbody>
</table>
DISCUSSION

The effluent samples collected from the selected industry was analysed for different physico-chemical parameters and has higher values than the recommended values by I.S.I. Similar results were also obtained by Kumar, et al. (1991). The critical observations on the data clearly indicate that the plants growing in polluted sites were badly affected and there was a significant reduction in number of parameters studied as compared to the control plants. Morphological characters were found to be decreased in the selected plant collected from polluted area. Similar observations are reported by Palaniswamy et al. (1995). Angadi and Mathad (1998) have studied the effect of copper, cadmium and mercury on the morphological, physiological and biochemical characteristics of \textit{Scenedesmus quadricauda} (Turp) de Breb. and found the maximum inhibition in the growth, chlorophylls, total DNA, total RNA and protein contents of cells at the higher metal concentrations. Therefore it is observed from various studies that the same species respond differently under different conditions.

Thick cuticle observed in the transverse sections of the stem collected from the polluted area also matched with the findings of Percy et al. (1992). Cuticle is the first point of attack of pollutants; our results indicated an increase in the thickness in cuticle at the polluted sites which indicates that the plants have an effective barrier for the pollutants entry. Trivedi & Singh (1989 & 1990) studied the epidermal features (stomatal density and index) of different plants (\textit{Boerhaavia diffusa} Linn. and \textit{Amaranthus viridis} Linn.) under the impact of air pollution. Significant reduction in cell size of the pollution effected plants was also reported by Ansari and Iqbal (1992). The reduced length of vessel elements coupled with their augmented frequency appears to be the significant adaptations to the stress of pollution. In contrast to the above workers more number of parameters (xylem vessels) observed in the plant samples collected from polluted area over to control population in \textit{Datura inoxia} by Iqbalet \textit{et al.}(1986). Chaudhari and Patil (2001) also observed the inhibition and stimulation in xylem and phloem in pith region under the stress conditions of polluted water. In present findings there is less secondary growth observed in most of the selected plants collected from polluted area. Jabeen and Abraham (1998) also showed less secondary tissue in \textit{Largerstroemia reginae} and \textit{Alstonia scholaris} trees exposed to air pollutants.

Our result indicates that less trichomes frequency, more stomata, presences of collenchyma layers, less layered spongy parenchyma with smaller cell size, less layered ground tissue, decreased ratio of stomatal index and palisade; more numbers of crystals with bigger size in leaves of polluted plant samples. Similar observations were noted by Faroqui & Singh (1990). Low stomatal frequency were observed in the plants grown in polluted areas, which may be an adaptation of ecotypic significance in regulating the limited and controlled entry of harmful gaseous pollutants into the plants tissues, especially when the plant grown in polluted area. Physical evaluation included fluorescence behaviour, extractive and total ash values. The plant samples collected from polluted areas showed quick differentiations to fluorescence behaviour. Water and alcohol extractive values were lowered collected from polluted areas. Ash values were higher comparatively in polluted plant samples. Same observations were made by Sharma and Habib (1995). The percentage of ash content was higher in the plant samples collected from polluted areas as compared to control because ash content of the plants is the direct manifestation of bioaccumulation of minerals observed as macronutrient and micronutrients which take up different functions.

From the observation of TLC, it was seen that the number of spots were decreased in the samples of
plant collected from the polluted areas. Similar observations were studied by Mashaly (1988).

In order to determine the quality of medicinal plants with regard to authenticity pharmacognostical characters viz. macroscopical, anatomical, powder analysis, chemical analysis, TLC, fluorescence behaviour, extractive values and ash values are very important. Anatomy often proves very useful for individual identification of plants so microscopical methods are of great value towards their identification and differentiation of the authenticity of the plant drugs. They provide evidences concerning relationship of groups such as families or help to establish the affinities of genera of uncertain taxonomic status. The number of stomata and epidermal cells, vein-islets and vein termination number per unit area, palisade ratio, stomatal index etc. give constant structure of different species of plants. Moreover, different types of stomata, crystals, fibers, trichomes etc. present in powdered drug help in the identification of plants or differentiation in comparison of same plant, which are collected from the industrial area.

CONCLUSION
It is concluded that the plant under the pollution stress must have suffered in its drug quality. These changes might be due to the presence of heavy metals in effluent.

REFERENCE


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