Isolation and screening of bacterial enteropathogens for assessing the water quality of samples collected from different areas of Patna in Bihar

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Abstract : *The present study was conducted to isolate and screen bacterial enteropathogens from water collected from different localities of Patna, Bihar. Five different strains of bacterial enteropathogens namely Escherichia coli, Shigella sp., Salmonella sp., Enterobacter sp. and Klebsiella spp. were isolated from 4 different areas of Patna, proving that water is a reservoir for bacterial enteropathogens. Escherichia coli and Enterobacter sp. were the most frequently occurring bacterial enteropathogens while the occurrence of Salmonella sp. and Shigella sp. in the water samples was the rarest among the all. Klebsiella sp. was found to be present in nearly all the water samples of all the areas with the maximum abundance in the Mahendru area.*

Antibiotic susceptibility profiles showed that Nalidixic acid was the most effective antibiotic against the isolated bacterial enteropathogens while, Amoxyclav and Ampicillin were least effective.

It was concluded that water is an important reservoir for enteropathogens in the areas of the study. Therefore, it is incumbent upon local communities, government and even school and college students, to realize the significance of water bodies in their surrounding areas and to see that these precious water resources are neither contaminated nor choked.

Key Words: *Antibiotic susceptibility, bacterial enteropathogens, water quality.*

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Introduction :

Environment plays an important role in the transmission of enteric pathogens. Water is known to be a reservoir for the most common and classical bacterial enteropathogens *(*K. Khalil *et. al.,* 1994*)*. In many developing countries including India, water is scarcely available and is contaminated by faecal matter due to a defective and ill-treated water supply system. There is an

indiscrete disposal of excreta, insufficient toilet facilities and lack of knowledge in personal hygiene *(Ekanem et. al., 1991).* The various bacterial enteropathogens harbouring in such an inferior quality of water mainly includes *Salmonella spp., Shigella spp., Campylobacter spp., Escherichia coli, Vibrio cholerae, Aeromonas spp., Plesiomonas spp., Enterobacter spp., Klebsiella spp.*, etc (Adegunloye 2005).

The bacterial enteropathogens show marked variations with respect to their antibiotic susceptibilities. The resistance of enteropathogenic bacteria to commonly prescribed antibiotics is increasing both in developing as well as in developed countries. Resistance has emerged even to newer, more potent antimicrobial agents. Many enteropathogens cause acute clinical disease but the ability to adhere to or invade the intestinal mucosa can promote long-term colonization, causing chronic disease or carrier status.

Keeping in view all these aspects associated with the water quality, our present study was aimed at the isolation and screening of bacterial enteropathogens from different water samples collected from different areas of Patna, Bihar; moreover, assessing the water quality by a comparative study of the microbial load in each of the water sample with the help of statistical analysis.

Materials and Methods :

Sampling area:

Four different areas of Patna, Bihar namely Raja Bazaar, Boring Road, Kankarbag and Mahendru were selected for the present study. The water samples from these areas were used for the isolation and screening of enteropathogens.

Sample collection:

Water samples were collected in sterile vials from tap water, municipal water, hand pump water, well water, pond water and water of river Ganges from each of the above mentioned areas. Total numbers of water samples collected were 21 where 5 samples were collected from each area and 1 from the river Ganges.

Isolation of bacterial enteropathogens:

Deoxycholate Agar (DCA), Mac Conkey Agar (MCA), Methyl-Violet Lactose Agar (MV-L) and Endo agar media were prepared, autoclaved and poured into sterilized Petri plates and allowed to solidify *(Khalil et. al., 1994).* Serial dilutions of water samples were prepared up to 10^{-7} by preparing 0.85% normal saline solution. 0.1ml of inoculum of 10^{-7} dilution was pipetted out and spread on the respective media with the help of spreader. All the inoculated plates were then incubated at 37ºC for 24 – 48 hrs. Colonies on each of the plates were counted and gram staining was done for light microscopy.

Identification of enteropathogens:

Isolates were identified on the basis of standard biochemical tests such as IMViC, Carbohydrate fermentation, Catalase, Gelatinase, Urease and Triple Sugar Iron Agar (TSIA) tests (Black *et. al.*, 1982; Hansen, 2004). All the media and reagents were prepared and inoculated with the respective isolated strains. The colour change was observed and recorded.

Antibiotic Susceptibility Test:

The Kirby-Bauer modified disc diffusion method was used in determining antibiotic sensitivity pattern of bacterial isolates (Obi *et. al.*, 1997). The following antibiotics (Hi-Media Disc in mcg) were tested: Co-Trimoxazole (25mcg), Norfloxacin (10mcg), Oxytetracycline (30mcg), Cefuroxime (30mcg), Amoxyclave (30mcg), Gentamicin (10mcg), Nalidixic acid (30mcg), Nitrofurantoin (300mcg), Cephalothin (30mcg), and Ampicillin (25mcg). Mueller-Hinton agar was prepared, autoclaved, poured in Petri dishes and

then left to solidify (Wasfy *et al.*, 2000). Suspension of each bacterial culture was prepared and 0.1ml of this suspension was spread on the plates with the help of spreader. Then, antibiotic discs were placed on medium. Plates were incubated at 37ºC for 24-48 hrs. Zone diameter was measured in millimetres using the ruler.

Statistical analysis:

Frequency of occurrence of the isolated bacterial enteropathogens in different water samples in Raja Bazaar, Boring Road, Kankarbag and Mahendru areas respectively, were calculated and compared for assessing the water quality in these areas *(*Khalil *et. al.,* 1994*).* Moreover, the antibiotic susceptibilities of the bacterial isolates were also calculated (Wasfy *et. al.*, 2000) to determine the association between different water sources and antimicrobial susceptibilities.

Results :

A total of 5 isolates were obtained from 21 water samples analyzed. The distribution of bacterial enteropathogens according to the source of water in each of the four areas is presented in fig. 1, 2, 3 and 4.
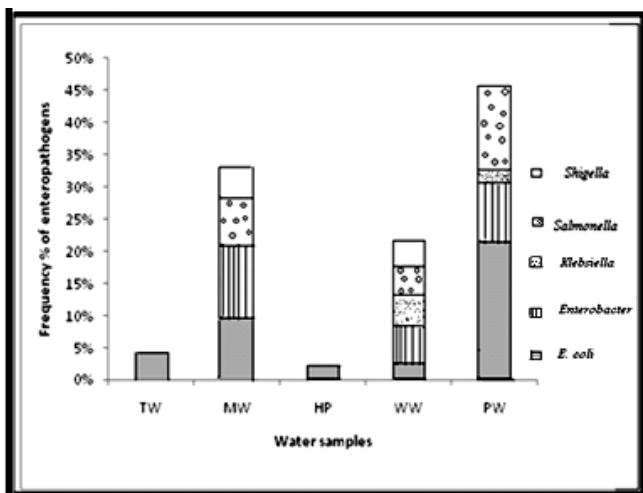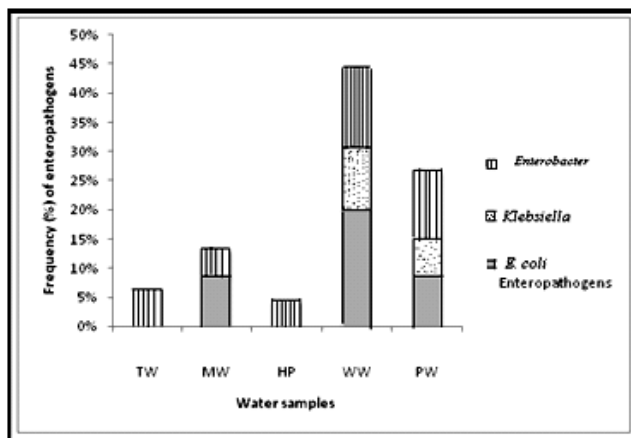

Fig 2: Frequency of bacterial enteropathogens in water from different sources in Boring Road locality.
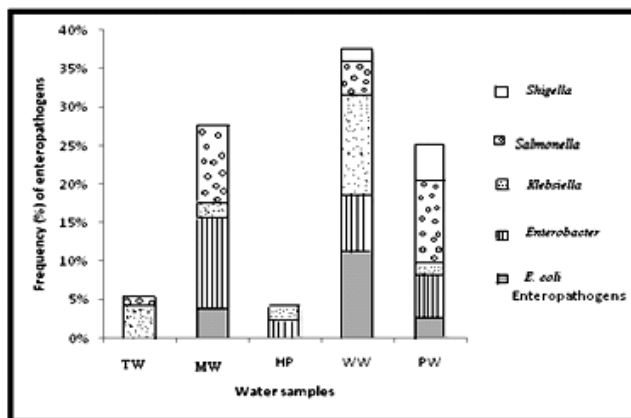

Fig 3: Frequency of bacterial enteropathogens in water from different sources in Kankarbag area.
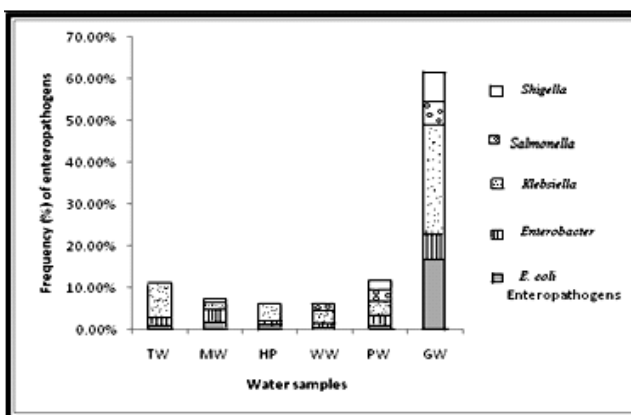

Fig 4: Frequency of bacterial enteropathogens in water from different sources in Mahendru locality.

Fig 1: Frequency of bacterial enteropathogens in water from different sources in Raja Bazaar locality.

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Water was reservoir of enteropathogens like *E. coli, Enterobacter sp., Klebsiella sp., Salmonella sp.* and *Shigella sp.*

Escherichia coli- E. coli was the most predominant enteropathogen in every sample from all localities except hand pump and tap water of boring road and kankarbag (Figs. 2 and 4). Among all the water sources, river Ganges was the main source for isolation of enteropathogens.

Enterobacter sp. – Another dominant genera of enteropathogen was *Enterobacter* as it was isolated from the river Ganges and other water sources but not isolated from the tap water samples of Kankarbag and Raja Bazaar (Figs. 1 and 3).

Klebsiella sp. - Klebsiella was frequently isolated from the river Ganges and to some extent from other sources. However, it was absent in water sample of hand pump of Mahendru, Boring road, Raja Bazaar and also from the tap water and municipal water supply of Boring road and Raja Bazaar (Figs. 1,2 and 4).

Salmonella sp.- Water samples from the slum area were often contaminated with *Salmonella* but was not isolated from the tap water of Raja bazaar and Mahendru. Boring Road area was completely free of *Salmonella* (Fig 2).

Shigella sp. - Shigella was isolated from the sewage which was being dumped into the river Ganges. It was more often isolated from the pond water and well water as compared to other sources. However, municipal water supply of Raja bazaar was contaminated with *Shigella* (Fig 1).

Antibiotic Susceptibility Test :

The antibiotic susceptibility test was carried out for all the isolated bacterial enteropathogens so as to determine their sensitivity against different antibiotics.

Table 1: Antibiotic sensitivity of different enteropathogens

STRAINS	ANTIBIOTICS									
	Ac	Co	Nx	O	Cu	Na	Nf	Ch	A	G
I	0%	0%	0%	0%	0%	26.02%	25.20%	26.02%	0%	22.76%
II	0%	0%	0%	21.74%	18.12%	21.74%	17.39%	9.42%	0%	11.59%
III	0%	19.05%	19.05%	17.01%	0%	12.93%	0%	13.61%	0%	18.37%
IV	0%	12.70%	11.90%	16.67%	0%	22.22%	15.87%	0%	0%	20.63%
V	0%	0%	10.96%	19.18%	0%	21.92%	17.12%	14.38%	0%	16.44%

Escherichia coli was sensitive against Gentamicin, Nalidixic acid, Nitrofurantoin (25.20%) and Cephalothin. Out of these, Nalidixic acid and Cephalothin were most effective (26.02%) and the effectiveness of gentamicin was 22.76%. However, *Escherichia coli* showed resistance against Co-Trimoxazole, Norfloxacin, Oxytetracycline, Cefuroxime, Ampicillin and Amoxyclav (Table 1).

In case of *Enterobacter,* Nalidixic acid and Oxy-tetracycline were highly effective showing about 21.74% effectiveness. *Enterobacter* was also sensitive against Nitrofurantoin (17.39%), Cephalothin (9.42%), Gentamicin (11.59%) and Cefuroxime (18.12%). It was resistant against Co-Trimoxazole, Norfloxacin, Cefuroxime, Ampicillin and Amoxyclav (Table 1).

Klebsiella was highly sensitive against Co-Trimoxazole and Norfloxacin (19.05%). Other effective antibiotics were Nalidixic acid (12.93%), Cephalothin (13.61%), Oxy-tetracycline (17.01%) and Gentamicin (18.37%). However, *Klebsiella* was resistant against Cefuroxime, Ampicillin, Nitrofurantoin and Amoxyclav (Table 1).
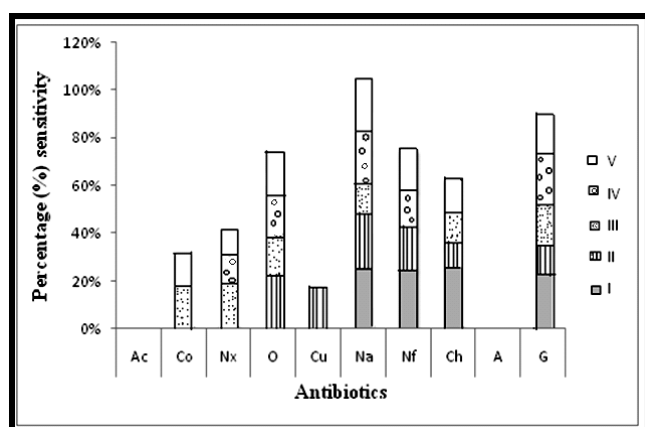

Fig 5: Antibiotic sensitivity of enteropathogens.

where, I - *E. coli,* II – *Enterobacter,* III – *Klebsiella,* IV – *Salmonella,* V – *Shigella*

Salmonella was sensitive against many antibiotics, out of which Nalidixic acid was the most effective (22.22%). It was also sensitive against Norfloxacin (11.90%), Co-Trimoxazole (12.70%), Nitrofurantoin (15.87%), Oxy-tetracycline (16.67%) and Gentamicin (20.63%). *Salmonella* was resistant against Cefuroxime, Ampicillin, Amoxyclav and Cephalothin (Table 1).

Shigella was highly sensitive against Nalidixic acid (21.92%). It was also sensitive against other antibiotics- Norfloxacin (10.96%), Cephalothin (14.38%), Gentamicin (16.44%), Nitrofurantoin (17.12%) and Oxy-tetracycline (19.18%). However, Cefuroxime, Amoxyclav, Co-trimoxazole and Ampicillin were not at all effective. *Shigella* showed resistance against these antibiotics (Table 1).

Hence, among all these antibiotics, Amoxyclav and Ampicillin were least effective. No inhibition zone was formed when enteropathogens were treated with these two antibiotics. However, Nalidixic acid was the most effective antibiotic for enteropathogens. Thus, the relative effectiveness of different antibiotics provides the basis for a sensitivity spectrum of the organism.

Discussion :

This study was conducted to elucidate that the water is a reservoir for common and classical bacterial enteropathogens in Patna.

Acute diarrhoea due to bacterial infection is an important cause of morbidity and mortality in infants and young children in most developing countries including India (Adegunloye, 2005). Clarification of the enteropathogens involved in diarrhoeal disease in the country is an essential step towards the implementation of effective primary health care activities against the disease (Olowe *et. al.*, 2003).

In most developing countries the local conditions vary in different localities and so is the quality of water. The tap water, municipal water, hand-pump water, well water, pond water and the Ganges water were considered as samples for the isolation of bacterial enteropathogens in the present study. The physical, chemical and biological characteristics of each of these water samples were quite different in different sampling areas of Patna.

The results showed that bacterial species (*Escherichia coli, Salmonella sp., Shigella sp., Klebsiella sp., Enterobacter sp.*, *Pseudomonas species, and Alcaligenes species*) were isolated from the water samples collected from different sampling areas of Patna.

Of all the bacterial isolates, *Escherichia coli, Salmonella sp., Shigella sp., Klebsiella sp., Enterobacter sp.*, were selected for the study.

Indiscriminate disposal of human and animal excreta in open spaces was observed in all the four areas of our study. Toilets were not being used by the people in the slum areas. Open defecation in the field and inside the houses were common, particularly in very young children. These children seldom use toilet as a habit, which promotes the spread of enteric organisms shed in the faeces of infected persons and domestic animals.

In water samples from the Raja Bazaar area, *E. coli* and *Enterobacter* were found to be dominating while *Klebsiella* and *Shigella* were less frequently isolated indicating that the tap water and hand-pump water was safe for consumption whereas the presence of *Salmonella* and *Shigella* in the municipal water reflects the ill-drainage system of the area.

In water samples from Boring Road area, we were unable to isolate *Shigella* and *salmonella* illustrating that the water samples were potable. But still *E. coli, Enterobacter and Klebsiella* continue to persist in the water sample which is a matter of concern.

In water samples from Kankarbag area, the tap water contained a lesser load of bacterial enteropathogens. However, the pond water was the most contaminated with the enteropathogens depicting the unsatisfactory sanitary conditions of the area.

In water samples from Mahendru, the Ganges water was the most potent carrier of enteropathogens particularly *Shigella sp.* and *Salmonella sp. Escherichia coli, Enterobacter* and *Klebsiella* were found to be dominating in all the water samples indicating heavily contaminated water systems. The sewage being dumped in the River Ganges was the major source of *Shigella* and *Salmonella* occurrence resulting in the water pollution.

Therefore, on comparing the different water samples collected from different localities of Patna it was found that *Escherichia coli* and *Enterobacter* spp. were the most frequently occurring bacterial enteropathogens in the water samples while the occurrence of *Salmonella sp.* and *Shigella sp.* in the water samples was the rarest among the all. *Klebsiella spp.* was found to be present in nearly all the water samples of all the areas with the maximum abundance in the Mahendru area.

For the evaluation of bacteriological quality of water, *Escherichia coli* is generally accepted as an indicator. A water sample is considered contaminated if any *Escherichia coli* or faecal coliform is isolated from it. A high inoculum dose required for *Salmonella* and *Shigella* infections indicates that a highly contaminated source or sufficient time is needed to achieve such large doses in vehicles of transmission. Consequently, water rather than person to person spread is the cause for coliform pathogen infections. *Escherichia coli*, *Enterobacter* and *Klebsiella* infections require a grossly unsanitary environment for the transmission and diarrhoeal outbreak, which explains that enteritis by them, is rare in countries and cities with generally high water hygiene (Black *et. al.,* 1982).

In the present study, water supplied in the slum areas administered by hand pumps was found to be highly contaminated with bacterial enteropathogens probably due to seepage of faecal matter from ground level.

In a study from South Africa, it was shown that the incidence of diarrhoea decreased when the quality of water was improved by building deep and enclosed wells and the use of potable water from surface wells was discontinued. A morbidity survey revealed a significant relationship between the sanitary quality of the water supply system of a

community and the incidence of gastrointestinal illness (Esrey *et. al.*, 1985).

Many bacteriological studies have suggested that level of toilet hygiene might influence the incidence of enteric illnesses. Heavy surface contamination of water by enteropathogens has been found in areas close to the disposal of excreta, especially in outbreaks of shigellosis.

Municipal water from the houses of the boring road area was the least contaminated while that of Raja Bazaar area was contaminated the most with the bacterial enteropathogens. One possible explanation could be that there may be some kind of leakage in the plumbing, drainage and septic tanks lying down under the ground, where any leakage from rusting and damaged pipes could possibly contaminate the water with faecal matter from the sewerage. Moreover, the water supply of most of the houses investigated was almost a century old and was probably damaged.

Well water was found to be frequently contaminated with the enteropathogens may be because the water was collected and stored in open wells which were uncovered and easily accessible to birds, dust and insects.

The tap water of all the four areas was relatively less contaminated and potable as compared to other water samples. This was probably because the water was being pumped from underground and stored in the closed tanks which were being cleaned routinely and sanitation was properly maintained. But still contamination may result from leaking pipes carrying the water from the tanks to the tap.

The Ganges water was found to be unsafe for human consumption. It was heavily contaminated with *Klebsiella* (26.68%), *Escherichia coli* (16.67%), *Shigella* (7.22%), *Enterobacter* and *Salmonella* (5.55%).

The water samples from pond contained all the four types of enteropathogens with a relatively higher frequency of *Escherichia coli* and *Salmonella* than *Klebsiella*, *Enterobacter* and *Shigella*.

Management of diarrhoea and other enteropathogenic diseases may require the administration of antibiotics. However, several bacteria are known to be resistant to a wide array of antibiotics. (Obi *et. al.,* 1998; Wasfy *et. al.,* 2000).

Results presented showed multiple antibiotic resistance of all bacteria isolates to ampicillin, erythromycin, tetracycline, chloramphenicol and co-trimoxazole. Multiple antibiotic resistance refer to resistance to two or more classes of antibiotics. The multiple antibiotic resistance of *Salmonella, Shigella, Campylobacter, Aeromonas* and *Plesiomonas* demonstrated in this study accords with other findings of Obi *et. al.,* 1997, 1998, 2004). Erythromycin used to be the drug of choice for campylobacteriosis but increasing resistance of *Campylobacter* to erythromycin is well known. In this study, 35% of *Campylobacter* isolates were resistant to erythromycin. Strains of *Salmonella*, particularly *Salmonella typhi*, accounted for several outbreaks in the United States and world-wide, partly due to resistance to chloramphenicol, ampicillin and trimethoprim. This resistance pattern simulates the 20% and 47% resistance rates of *Salmonella* obtained in this study to chloramphenicol and ampicillin, respectively. Antibiotic susceptibility profiles showed that all enteric bacterial isolates were markedly sensitive to nalidixic acid, gentamicin, ciprofloxacin, nitrofurantoin, oxy-tetracycline and cephalothin. These drugs may, therefore, be of value for enteric infections requiring empiric antibiotic therapy. These reported susceptibilities are in harmony with reports of other investigators (Berkman *et. al.,* 1997; Wasfy *et. al.,* 2000). It should be noted that susceptibility of bacteria to antibiotics is not static and resistance

may be due to antibiotic abuse, antibiotic overuse or may be chromosomally or plasmid mediated (Obi *et. al.*, 1998).

Escherichia coli was sensitive against Gentamycin (22.76%), Nalidixic acid (26.02%), Nitrofurantoin (25.20%) and Cephalothin (26.02%). In case of *Enterobacter,* Nalidixic acid and Oxy-tetracycline were highly effective showing about 21.74% effectiveness. *Klebsiella* was highly sensitive against Co-Trimoxazole and Norfloxacin (19.05%). *Salmonella* was sensitive against many antibiotics, out of which Nalidixic acid was most effective (22.22%). *Shigella* was highly sensitive against Nalidixic acid (21.92%).

Hence, Nalidixic acid was the most effective antibiotic for enteropathogens. Among all these antibiotics, Amoxyclav and Ampicillin were least effective. Antibiotic usage must therefore be carefully regulated and monitored.

In many developing countries like India and in India, cities specifically like Patna, water is frequently contaminated by human and animal excreta due to a defective and ill-planned water supply system, substandards of sanitation and insufficient facilities for excreta disposal and toilets. Faecally contaminated water can take part in the transmission of enteric agents directly by ingestion, or indirectly due to scarcity of water and insufficient knowledge in personal hygiene (Ekanem *et. al.,* 1991) which may contribute to an increase in the incidence of diarrhoeal disease.

Conclusion :

From the results it may be concluded that water is an important reservoir for enteropathogens in the areas of the study. The original source of water may not be that unsafe but it becomes contaminated after distribution and storage by faecal matter in unhygienic and inadequate sanitary conditions. These conditions are suitable for the growth of enteropathogens.

Bacterial enteropathogens showed marked variations with respect to their antibiotic susceptibility. Therefore, periodic monitoring of antibiograms is necessary to detect any changing patterns. The multiple resistance of isolates to some antibiotic classes are of great public health concern and calls for caution in the indiscriminate use of antibiotics on humans and animals.

Since the bacterial enteropathogens tend to cause a number of gastrointestinal diseases so their study is very crucial with respect to developing some critical measures against their spread.

While the government needs to put into place mechanisms to sustain water reservoirs, it is equally incumbent upon local communities, and even school and college students, to realize the significance of water bodies in their surrounding areas and take it upon themselves to see that these precious water resources are neither contaminated nor choked.

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