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Full Length Research Paper

Bacteriological and environmental characterisation of the water quality in the Danube River Basin in the Galati area of Romania

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In order to contribute with date to the Danube River Basin which is a prime European waterway, this analysis was carried out on the one hand to investigate the possibilities of sanitary risks that are incurred by the riverside population as they are engaged in professional recreational activities that impose a direct contact between man and water, that is intensely developed along the aquatic system and on the other hand to indicate a clear cut picture of the final level of coliforms and *Escherichia coli* that is actually present in the Galati industrial segment of the Danube River. Total coliforms, faecal coliforms and *E. coli* could attain values reaching 1.5×10^{3}, 9.5×10^{2} and 6.4×10^{3} CFU/ml, respectively for the aquatic ecosystems analysed. A variation of these parameters with respect to the ecodynamical characteristic of the Danube water quality such as temperature, pH, total dissolved solids, salinity and hydrogen sulphite reveal the preponderant role that abiotic factors play in the dispersion of bio-contaminants in a broad basin ecosystem. While the persistence of *E. coli* during the sampling period from June to September confirm the fact that there is a continuous faecal pollution of this medium. The high presence of organic pollutants in this medium, combined with the presence of coliforms and *E. coli,* could be related to an accumulation of waste matter all along the ecosystem, also due to the lack of wastewater treatment plants for domestic and industrial discharges, the high impact of human activities across the international river basin and the difficulties encountered in the natural operational processes of self purification.

Key words: Total coliforms, faecal coliforms, *Escherichia coli*, water quality, Danube River, Galati-Romania.

INTRODUCTION

Fecal pollution indicators (coliforms and *E. coli*) exist in humans and animals in most parts of the world. There is a lot of controversy over the survival and transmission of the fecal coliform group into streams and rivers (Hirotani and Yoshiro, 2010). Fecal source tracking is emerging as a reliable discipline to understand the outcome of micro-organisms of sanitary value in most ecosystems. The faecal pollution of water from a health point of view is the contamination of water with disease-causing organisms (pathogens) that may inhabit the gastrointestinal tract of mammals, but with particular attention to human faecal sources as the most relevant source of human illnesses (Ashbolt, 2004). Ingestion of water contaminated with faeces is responsible for a variety of diseases important to humans via what is known as the fecal-oral route of transmission. Food, air, soil, and all types of surfaces can also be important in the transmission of fecal pathogens, and thereby implicated in disease outbreaks. Indeed, some are considered beneficial to the host as they can

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intensive agriculture, fish farming, recreation and tourism. Contamination by potentially toxic elements in the natural out compete pathogens for space and nutrients, complement the biochemical potential of the host's gastrointestinal tract, and help in the development of the host immune system. Nonetheless, animal faeces can also carry a number of opportunistic pathogens, which are capable of inflicting debilitating illnesses, and in some cases, death as stipulated by Schriewer et al. (2010).

Microbial water quality is traditionally monitored using culture-based techniques that selectively promote the growth of bacterial indicators of fecal pollution. In the recent years, the WHO have strongly promoted the use of the fecal bacterium *Escherichia coli* as the principal fecal indicator for waters. Previously, a larger group of bacteria related to *E. coli* but not all specific to faeces (known as the total coliforms) had been promoted, but with the advent of more rapid and selective methods it is more reliable to directly measure *E. coli*. The most commonly used indicators for surface waters are the fecal coliforms and *E. coli*, although another bacterial group, the entercocci are often used to better assess health risks associated with marine recreational waters (Simpson et al., 2002).

Currently used indicators are easy to grow and are often present in higher numbers than pathogens. In contrast, the detection and counting of most pathogens require time-consuming concentration methods to increase the chances of detection, and quantification (Medema et al., 2006). More importantly, pathogens from the three main microbial groups (viruses, bacteria and parasitic protozoa) often require different isolation and identification protocols, and consequently the diversity of pathogens present in a fecally impacted water body cannot be captured using one technique (Al-Megrin, 2010). Fecal pollution of waters has a significant impact on other areas besides the water industry (USEPA, 2002). For example, many food borne outbreaks can be directly or indirectly attributed to the contact of foods with polluted water during a number of food production/ processing steps. Since disinfection treatment demands (that is, total chlorine and energy) increase with fecal pollution could also have a considerable economic impact on areas that depend on tourism. For example, beach closures can be devastating to a local economy. The same thing can be said for areas that depend on fishing and shellfisheries. The high level of nutrients and pathogens that fecal pollution introduces to aquatic environments can have a negative impact on the receiving biota and overall ecosystem health. Water containing total or faecal coliforms above the drinking water guidelines should not be used for drinking or food preparation without disinfection as stipulated by Kirschner et al. (2009).

The Danube which is the second longest river in Europe, flows through several countries from where it receives discharges of agricultural, industrial and urban effluents. It is therefore a theatre for heavy navigation, environment is one of the major problems for human health and environmental quality. There is an accrued persistence, toxicity, and bioaccumulation of pathogenic micro-organisms such as protozoa cysts, helminths eggs and especially bacterial flora.

One of the great advances in the history of public health has been the recognition that a community's fecal matter carries all the diseases in that population (WHO, 2004). If it's not properly treated, even one sick person's wastes can spread the disease epidemically (WHO and UNICEF, 2004). So it is necessary to continuously monitor aquatic ecosystems like the Danube River Basin for the presence of pathogenic microorganisms. Due to the enormous popularity of the river for recreation, domestic and industrial activities, there is a need to inform the public of the potential health risks involved with boating, kayaking, canoeing and other anthropogenic activities on the river. With the advent of regular water quality monitoring and public notification programs at water related recreational areas throughout the country, there has been increased interest in developing models to predict water quality conditions without relying on bacteria data and instead correlating precipitation or other easily measured surrogate explanatory variables to bacteria concentrations.

The objectives of the project were to isolate, identify and characterise total coliforms, faecal coliforms, *E. coli* and some environmental variables in the lower Danube international River Basin in Galati-Romania area which is a contribution to the monitoring efforts to biologically and physico-chemically assess its quality, before it empties its contents into the Black sea.

MATERIALS AND METHODS

Geographical position and description of study site

The Port of Galati is the main Romanian river - maritime port on the Danube. It is situated on the left border of the Danube between km 160 and Mm 76. Its geographical position is Latitude: 45° 25'N Longitude: 028° 05'E. The anchorage zones are established between Mm 76 - 78.5, km 155 - 158 for the seagoing and river vessels and between Mm 80 - 150 and km 158.2 - 159.3 for the empty river vessels. The Port of Galati has four main specialized sectors: Mineral Port - located within km 155.4 and 157.6, specialized for ore, coal, coke and limestone rolled goods; Old Commercial Port located within km 149.34 and 151, specialized for passengers and ballast traffic; Docuri Basin located within Mm 80 and km 149.35, specialized for general cargo, bulk, cereals, ore, coal and coke transportation.

Meteorological information

Hot summers and cold winters characterize the lower Danube Basin. Maximum summer temperatures vary between +35 and +40°C and average summer temperatures are between +25 and +22.4°C. Minimum winter temperatures vary between - 20.2 and - 28.6°C and average winter temperatures vary between - 5.7 and - 1.7°C. Prevailing winds are from the North - Northeast and Southeast during winter and spring months. Average wind velocity

Table 1. Mean climatic characteristics of the study region Galati Romania.

Parameter/Months	June	July	August	September
Temperature (°C)	20.2	22.0	21.4	17.2
Precipitation (mm)	68.0	46.0	46.0	42.0
Snowfall (cm)	-9999.9	-9999.9	-9999.9	-9999.9

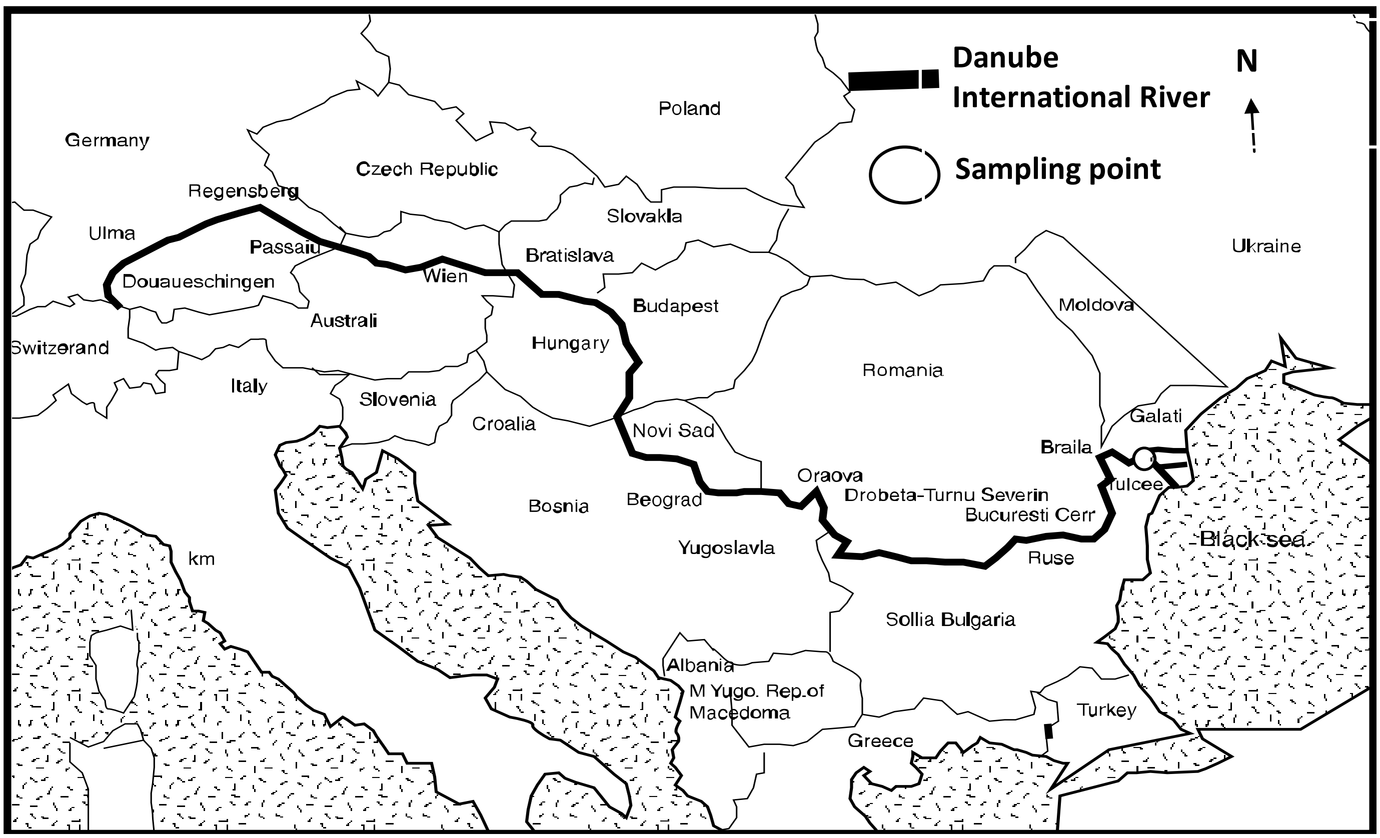

Figure 1. Map of the Danube international water basin, indicating the sampling sites.

is between 1 and 4 m/s. Wind velocity is between 10 to 15 m/s occur between 1 and 5% of the time. Snowfall is not uniformly distributed but varies between 50 and 80 cm for approximately 30 days/year. Fog (particularly during cold periods) an average 55 days/year. Partial freezing of the river can commence in January and usually ceases in February. It is reported that current velocities in the river vary between 2.29 and 2.36 km/h for medium water levels and between 5.95 and 6.04 km/h during flood levels. The mean climatic conditions of the study region are presented in the Table 1.

Water levels

Navigation from the river mouth to Galati is defined as Fluo Maritime and the minimum depth at 94% L.W.L is defined as 7.32 m. At Galati the level "0 " on the gauge scale is 0.86 m above Black Sea Datum and it is reported that during spring floods (snow melting period) the water level in the river can rise to 7.30 m above Black Sea Datum. From the water data it would seem that make maximum use of water depths in the river downstream of Galati the minimum depth of water to be provided at the new berths should be 7 m. The maximum water depth during spring floods would then be in excess of 13.5 m.

Sampling of water from the Danube River Basin

Sampling from Danube River was carried out on monthly basis for a period of four months, from June to September 2010.One point was chosen at the Galati port along the beach, and at the regions of the rivers that are frequently exploited for swimming, fishing, recreational activities and international aquatic transport (Figure 1). The mechanisms of sampling, isolation, identification and counting are illustrated below and are in line with standard procedures for the examination of water and wastewater (APHA, 1992; 1998).

The coliform group of indicator organisms includes some members of this family that are capable of fermenting lactose with the production of acid and gas within 48 h at 35°C. The family of enterobacteriaceaae are Gram negative oxygenic and facultatively

Table 2. Distribution of total coliforms, faecal coliforms and *Escherichia coli* in the Danube River water- Galati.

	June	July	August	September
Total coliforms	$1.5x10^3$	$4.5x10^2$	$20x10^4$	$7.5x10^2$
Faecal coliforms	$9.5x10^2$	$4.5x10^2$	$2.0x10^4$	$6,5x10^3$
Escherichia coli test	Present	Present	Present	Present

onoxigenic nonspore forming, that produce acid glucose and other carbohydrates. The presence of *E. coli* may be attributed to contamination from environmental sources and subsequent multiplication in the medium (Sanja et al., 2009).

Sampling procedures

The cap of the sterile sampling bottle is removed just before sampling in the Danube River. Gloves are used to avoid any contamination of the sample or the researcher. The bottle is plunged downwards below the water surface in the lake, some space is allowed in the sampling bottle to allow the mixing of the sample before pipetting. All samples are preserved at 4°C and tested within one hour of collection so as to avoid any multiplication of the bacteria due to long intervals between the sampling and the actual analysis.

Microbiological analysis and preparation of media

The presumptive test was carried out with a lactose broth test, which is composed of meat extract, NaCl, peptone, Lactose and water. The required pH after sterilization is 6.8 at 25°C. 10 ml of medium is distributed in small tubes containing Durham tube. They are sterilized in autoclave at 121°C. The confirmation test is composed of the Brilliant green lactose broth (BGLB) that is comprised of peptone, lactose, bile, brilliant green, water. The medium is adjusted to a pH of 7.2 at 25°C after sterilization. 10 ml of medium is distributed in small tubes containing Durham tube. They are sterilized in autoclave at 121°C. *E. coli* is cultured by the TBX medium, which is comprised of tryptone, bile, x-glu agar, water. The medium is adjusted to a pH of 7.2 at 25°C after sterilization. 10 ml of medium is distributed in small tubes, they are sterilize in autoclave at 121°C.The positive bleu coloured *E.coli* colonies are directly counted on the petri dishes and are noted as CFU/ml. The Gram coloration test is carried out to identify the morphology and colony forming attributes of *E. coli*

Presumptive test for coliforms

Presumptive test consists in inoculating the sample in lactose broth. The lactose broth tubes are incubated at 35°C. Positive samples are identified after 24±2 h either by the presence of accumulated gas in Durham's tube, either due to lower pH by lactic acid.

Confirmed test for coliforms

The confirmation test consisted in the transfer and inoculation of tubes positive at presumptive test in selective media - brilliant green lactose broth (BGLB). The tubes are incubated at 35°C, and examined for gas production after 48±2 h. The most probable number of coliforms (MPN) will be calculated based on the proportion of positive tubes.

Measurement of physical and chemical characteristics of water

The absorbance spectrum is determined by the UV-Vis spectrophotometer. The pH is measured by the pH meter, temperature by the thermometer, total dissolved solids (TDS), salinity and electronegativity by the conductimetre, hydrogen suphite was determined by the addition of reagents such as cadmium acetate, iodine, HCl, and titrated by sodium thiosulphite solution. The standard curve is evaluated on the spectrophotometer. To determine ammonia, the Nessler reagent is applied, and the reading is done on the spectrophotometer.

RESULTS

The results obtained in the isolation and identification of faecal coliforms and total coliforms are presented in Figure 9 and Tables 2-3. The environmental variables are analysed in Figures 2-8 and Table 1. pH values in the lower Danube River Basin is slightly basic, and varies from 7.94-8.64. Temperture varies from 22 to 27°C, with the highest values recorded in the month of July and August. TDS range from 256 to 296 mg/L during the study period. Salinity values ranged from 0.2 to 0.3 sal units, while electronegativity oscillated around the value of -88 mEv. The lowest value for hydrogen sulphite was recorded in June (2.91 mg/L), while the highest value was recorded in July (14.14 mg/L). The percentage of saturation of the ecosystem analysed was generally below 50%. The simple correlation coefficient was calculated for Total coliforms and faecal coliforms as reported on Table 3. It revealed a positive value for pH, temperature, TDS, salinity and ammonia and a negative correlation with electronegativity.

Microbiological characteristic

The correlation was negative for H_2S, with the total coliforms, but positive with fecal coliforms. There was a very high and significant correlation that was calculated between the faecal and total coliforms. This was the same for *E. coli* and all the various forms of coliforms put together.

The measured microbiological indicator parameters, which are total coliforms, faecal coliforms and *E. coli*, reveal a persistent dynamics of these pathogens in the Danube International River Basin and are presented in Table 2 and Figure 9. The lowest value of total coliforms is 4.5×10^2 MPN/ml in the month of July, while the highest value is 20×10^4 CFU/ml in the month of August

Table 3. Correlation coefficient between the ecological parameters and the values assessed for the biological parameter (total coliforms and *Eschericha coli*).

Parameter	Coliforms (CFU/ml)	
Microbiological variable	Total	Faecal origin
pH	0.15	0.15
Temperature	0.70	0.68
Total Dissolved Solids	0.56	0.56
Salinity	0.99	0.57
Electronegativity	-0.55	-0.55
Hydrogen sulphite	-0.82	0.069
Ammonia	0.05	0.056
Total coliforms		0.99
Escherichia coli	0.96	

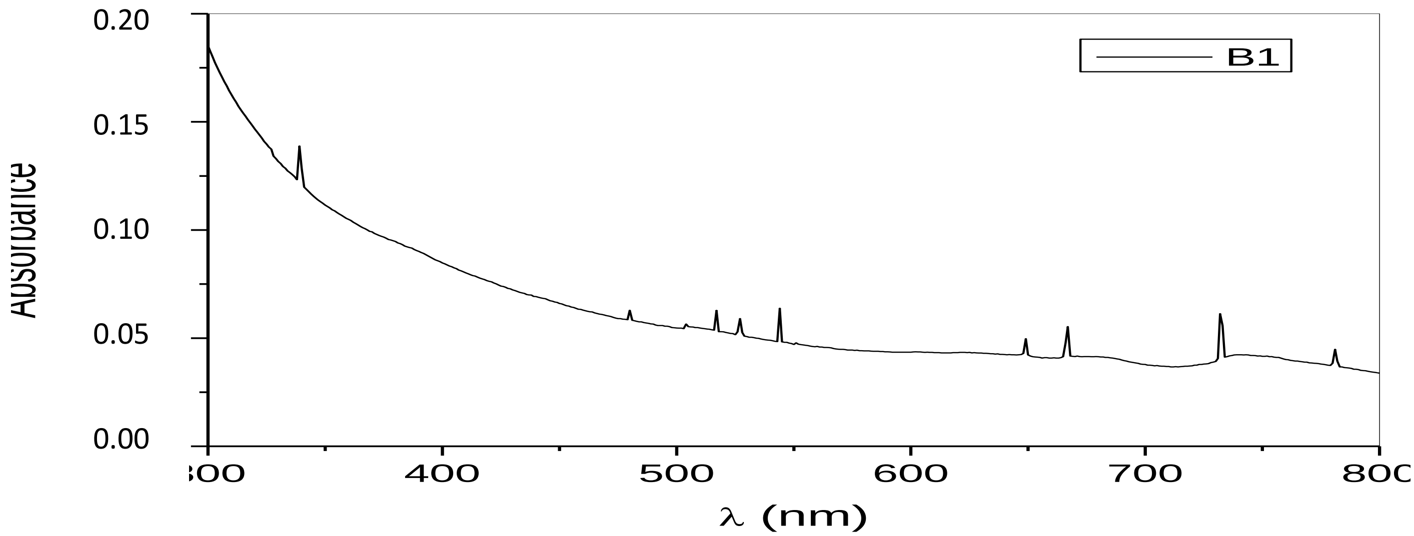

Figure 2. Absorption spectrum of the Danube River Basin.

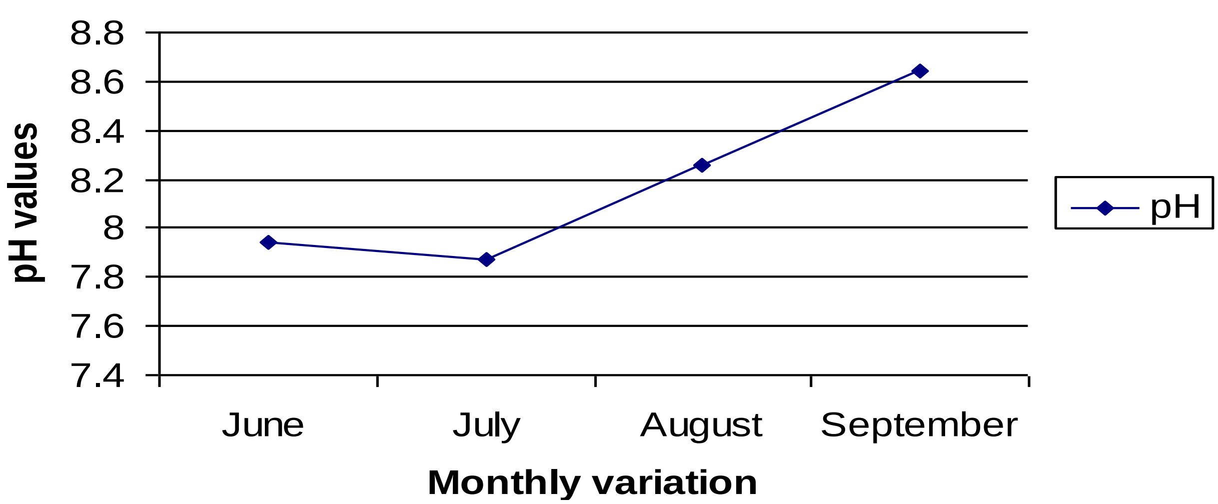

Figure 3. Variation of pH in the Danube River.

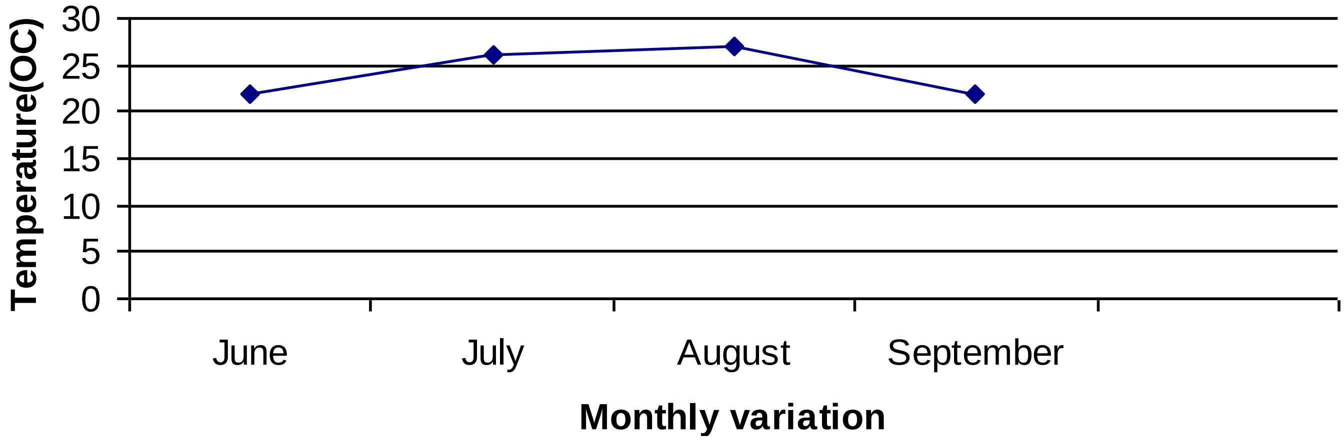

Figure 4. Variation of temperature in the sampling point.

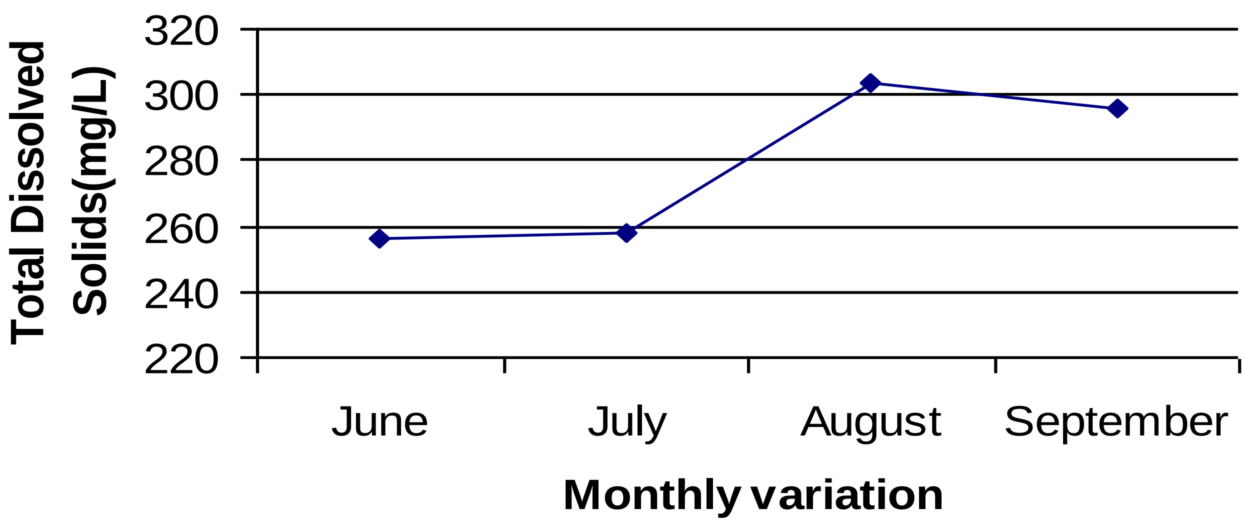

Figure 5. Dynamics of dissolved solids in the study area.

as indicated in Table 1. For the case of faecal coliforms, the lowest value of 4.5 $\times 10^2$ CFU/ml is recorded in the month of July, while the highest value of 20 $\times 10^3$ CFU/ml is enumerated in the month of August. At the first experimentation phase of isolation and identification of *E.* coli from the direct samples of the Danube, it was observed in the months of July and September which presented values of 6.1×10^2 and 6.4 $\times 10^3$ CFU/ml, respectively. After inoculation of the positive tubes, it was observed that *E. coli* was cultured in all the samples assessed.

DISCUSSION

Microbiological water quality investigations of lotic and lentic ecosystems are very rare, despite their importance in accompanying the role of large water bodies for cases of recreation, tourism and aquaculture. An attempt to adequately monitor scientific data in large scale river bodies is a priority to some organisations in Europe as stipulated by Kirschner et al. (2009). This microbiological data in the Danube gives a strong signal to the environmental community in Galati-Romania to embark on the creation of water treatment facilities in order to prevent the transmission of communicable diseases by population that explore its water, as total and faecal pollution is a crucial problem affecting most urban water systems (Eleria, 2002). These values are indicated in Table 2 and Figure 9. The Danube is an open ecosystem, with rather unstable ecodynamical values, thus the presence of *E. coli* which does not form spores and the exponential presence of total and faecal coliforms as presented in Table 2 reveal the continuous input of these

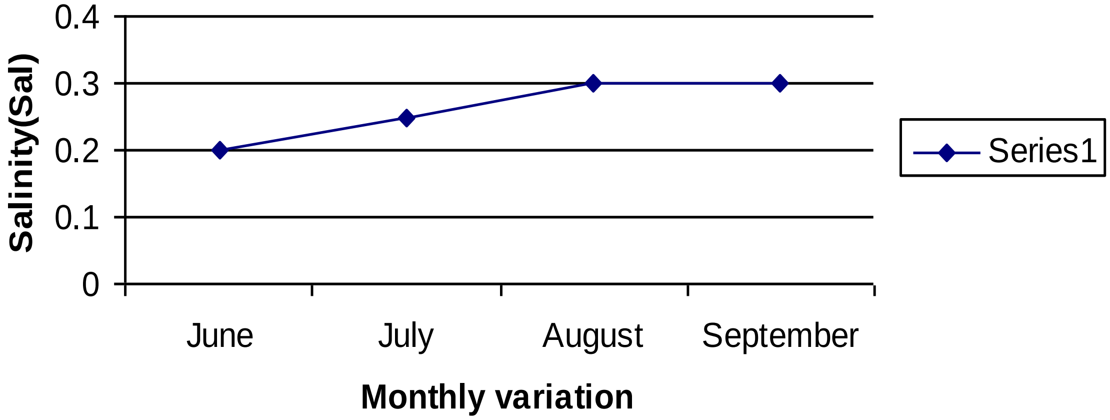

Figure 6. Variation of salinity in the sampling region.

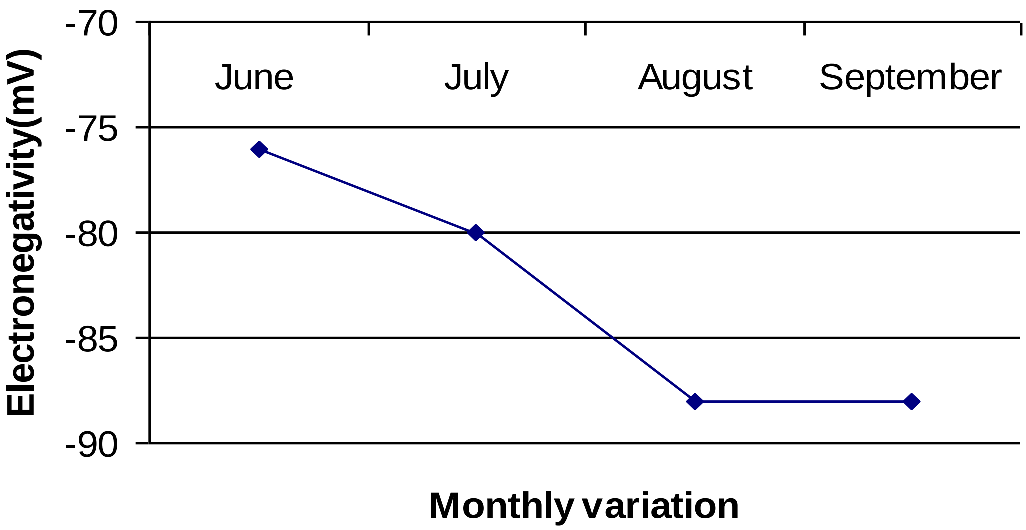

Figure 7. Monthly distribution of electronegativity in the Danube River.

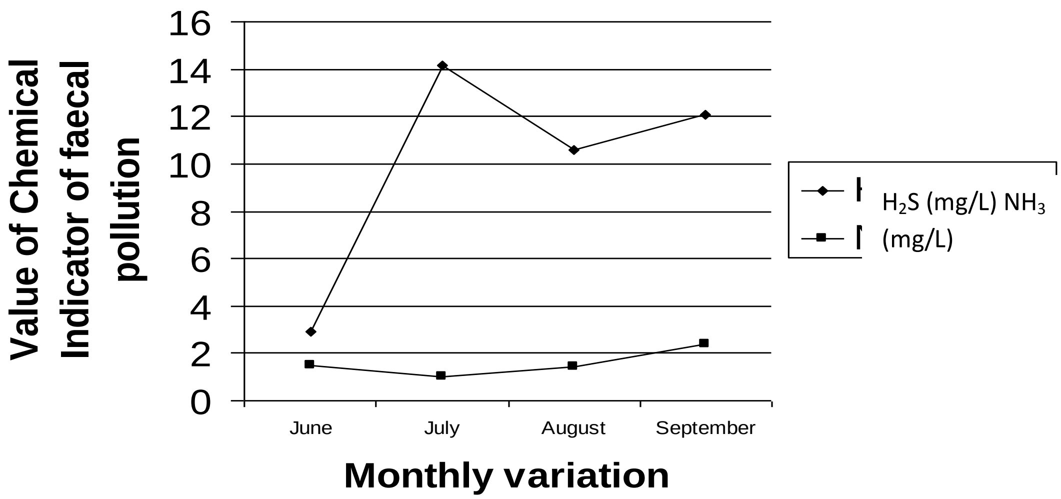

Figure 8. Distribution of some chemical variables (hydrogen sulphite and ammonia) in the water.

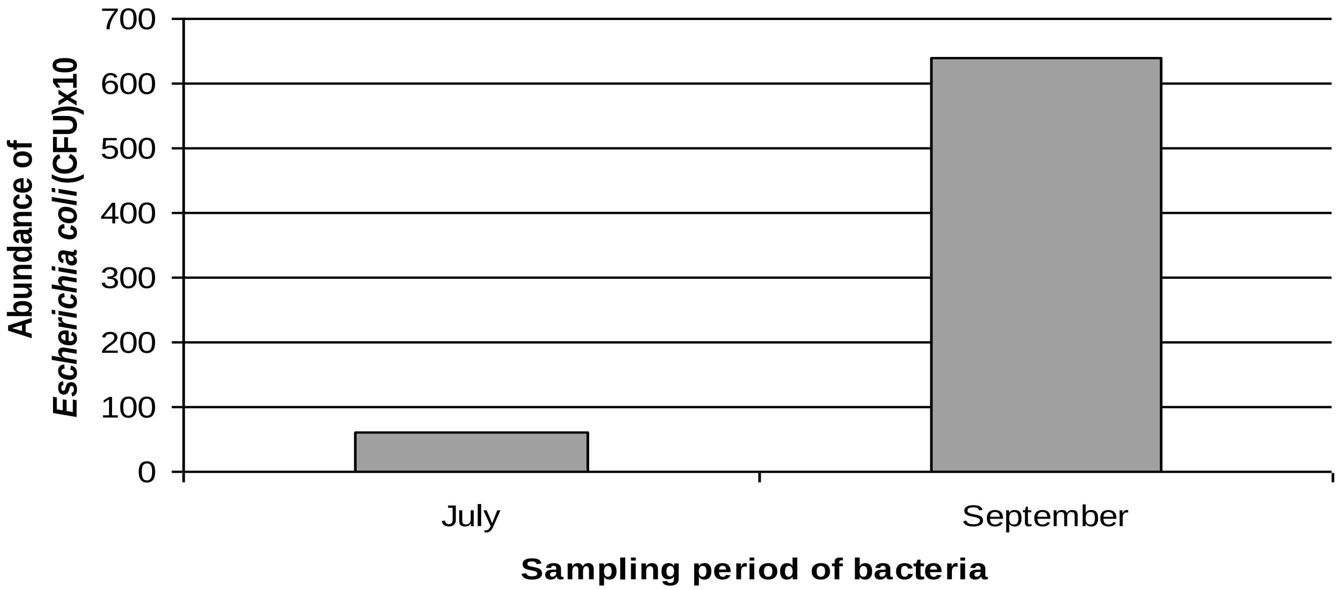

Figure 9. Comparative analysis of *E. coli* presence in July and September in the Danube-Galati port, Romania.

infectious agents in the medium as stipulated by Ju-Yong et al. (2008).

The monthly values are very important with values reaching 20 × 10^4 CFU/ml for total coliforms. These pathogens will keep on accumulating in the open system (Bell et al., 1994). The values obtained could be spatio-temporarily linked to the number of visitors in this ecosystem and the also the role played by point and non point sources in the bio-contamination of the aquatic ecosystem (Cieslak et al., 1993; Kelsey et al., 2004). These pathogens could be free living, particle associated or in an intermediary state, depending on the organic and inorganic conditions of the medium as stipulated in the findings of Basemer et al. (2005). Thus the positive relationship between the biological parameters and the ecological values analysed, which are pH, temperature, salinity, and inorganic substances proof the necessary role played by abiotic parameters in the survival and transmission of microorganisms in streams and rivers. Bacteria levels in a river are a function of initial loading and the disappearance rate which, in turn, is a function of the time or the distance of travel from the source and of other factors including: temperature, salinity, and light intensity as presented in our findings. According to Frankel and Bazylinski (2006) and Uroz et al. (2009), the presence of some minerals like iron are indispensable in the architectural set up of magnetotactic bacteria, this could be the case with hydrogen sulphite which presents a high abundance in the Danube River.

The results as presented in Table 2 and Figure 9 gives us a clear picture of the pattern of transmission of faecal coliforms and total coliforms in this study region as presented by Kirschner et al. (2009), in their microbiological findings in the Danube River Basin. Some of the biocontaminants can enter the river waters from municipal, industrial and agro-industrial sources and also from numerous diffuse sources as cooking, playing of various games, restauration and lodging by the visitors (Evenson and Strevett, 2006; Micic and Hofmann,2009). However, source tracking procedures are very necessary in order to be precise on the origin of the microbiological flora that flows into this medium (Rasmussen and Ziegler, 2003; Blanch et al., 2004). A planned implementation of the major guidelines applicable to water and wastewater use is imperative in the hydro-ecological exploitability of this ecosystem (Covaci et al., 2006; Korajkic et al., 2010; Santo Domingo et al., 2007).

Warmer months resulted in the higher *E. coli* concentrations in the lower Danube due probably to an increase in the metabolic and reproductive potentialities of the bacteria analysed. The combined assessment of biological parameters and the ecohydrological indicators produces near real-time visual information on which to base water quality management decisions. Auer and Niehaus (1993) found that the fecal coliform bacteria death rate is impacted by both solar radiance and water temperature. Myers et al. (1998) found that the bacteria decay rate was a measure of the die-off of bacteria resulting from ultraviolet light and temperature stress, cell starvation, predation by other bacteria and protozoans, and removal by filter feeders. They also determined that transport, dilution, dispersion, and concentrations of fecal coliform are strongly influenced by the timing, spatial distribution, and amount of rainfall, runoff, and stream flow and that light penetration, which is reduced by turbidity, is the most important factor in determining decay rates. These phenomenons can be attributed to the lower Danube River Basin, which is classified as one of the major European Inland lotic medium. Young and Thackston (1999) found that fecal bacteria counts in urban tributaries were much higher in sewered basins than in non sewered basins and in general were related to housing density, population, development, percent impervious area, and domestic animal density. Mallin et al. (2000) found that fecal coliform densities were strongly correlated with turbidity (positively) and salinity (negatively). These are in line with our results, which show an ecosystem that is subjected to a very high human pressure.

Rainfall - Storm water runoff is a significant source of pollutants to the river, which can include bacteria, viruses, and sediment, to which the substrate pollutants attach as indicated by Mallin et al. (2000). Storm rainfall characteristics and conditions prior to the storm are significant factors in the transport and concentration of pollutants in the river. Stream flow-river flow is the primary transport medium of fecal coliform bacteria (Christensen et al., 2000). Bacteria concentrations in the river tend to increase during the hydrograph rise and decrease during the hydrograph recession due to watershed wash off processes, this phenomenon is known as hysteresis and is not only particular to the Danube River Basin, but to major ecological setups that are constantly being exposed to mitigating meteorological constraints. The spatial–temporal variability of bacteria concentrations in this river, reveal a consequential diarrheic incidence in the community exploiting the resource. Samples were collected at the same location, at the same time of the day, representing a clear cut picture of the river's health (Clark and Norris, 2000).

Temporal conditions may vary substantially between daily bacteria samples due to the natural die-off of bacteria, additional inputs of bacteria to the river, and/or the transport of pollutants within a 24 h period (Balzer et al., 2010). High frequency data over time need to be collected throughout the whole so as to determine the response of the river to various storm volumes, durations, and intensities. This project was an effort to predict bacteria concentrations in the lower Danube River Basin using the necessary variables applicable in hydro-biological and environmental evaluations. Correlation coefficients were estimated between fecal coliform concentrations and a variety of hydrological, environ-mental, and meteorologic variables further explain the necessity of combining scientific data in order to predict a model for the dispersal of bacteria in aquatic systems as presented by Abdelzaher et al. (2010). Exposure to fecally contaminated water does not always translate into infection. However, the higher the fecal bacterial levels in water such as the case of the Danube River Basin can easily be translated to illnesses in the community. This might explain why the correlation between the total coliforms, faecal coliforms was very high in our samples.

Poor hygienic conditions also accelerate the faecal-oral route of pathogen transmission. Pathogen levels in water and predisposition of the person exposed play important roles in the manifestation of the disease. Impact of expo-sure to faecal pathogens tends to be a greater problem for immuno-compromised and immuno-suppressed people, like infants, the aging, and those experiencing debilitating illnesses such as pneumonia and the acquired immune diseases.

Among the diseases associated with poor microbial water quality, those causing dehydrating diarrhea are of critical importance as they could lead to death within 48 h after the initial symptoms as analysed in the findings of Manja et al. (1982). These extreme cases are more predominant in countries where overcrowding and poor sanitary conditions are the norm (Francy et al., 2002). The presence of faecal coliforms indicates the contami-nation of water with faecal waste that may contain other harmful or disease causing microorganisms, including bacteria, viruses, protozoa or other infectious agents (Brewster et al., 1994). Drinking water contaminated with these organisms can cause stomach and intestinal illness including diarrhoea and nausea.

Conclusion

These results proof the presence of total coliforms, faecal coliforms and *E. coli* in the lower Danube River Basin in Galati-Romania port area in quantities that impose the application of environmental securing measures to avoid the propagation of communicable diseases at proportions that can cause water borne outbreaks to the community exploiting the water for domestic and industrial purposes. This research therefore acts as an impending tool in erasing the public misperception regarding water availa-bility and its management. Due to issues like climate change, poorly managed water usage, agricultural irrigation practices, and population growth, the sustain-ability of water resources has now become an important issue globally.

The expected increase in the total production of human and animals faeces (due to population growth and meat consumption), is bound to have a detrimental effect on microbial water quality in both coastal and inland waters of developed countries if safeguarding measures are not being applied. In developing countries the future is grimmer, because of lack of economical resources and appropriate sanitary conditions. Better management practices, remediation strategies, and overall awareness of the global issues associated with fecal pollution are needed in order to improve the chances of sustainable water resources for future generations in all countries. These will re-inforce strategies for the potabilisation of water resources that occupies two-thirds of the earth, but remains a very limiting, expensive and precious commodity.

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