COMPREHENSIVE WATER QUALITY MONITORING REPORT

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Enhancing Knowledge on Biodiversity and Assessing Ecological Status of the Lower Catchments of Neretva River in Bosnia and Herzegovina

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"The Critical Ecosystem Partnership Fund is a joint initiative of l'Agence Française de Développement, Conservation International, the European Union, the Global Environment Facility, the Government of Japan and the World Bank. A fundamental goal is to ensure civil society is engaged in biodiversity conservation."

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1. INTRODUCTION

Water is one of the most widespread substances in nature and is a basic necessity for survival of all living beings. Today, water has become the most preious natural resource in the world. However, globalisation and rapid growth of worlds' population is reciprocally reflected on the level of environmental pollution. For Bosnia and Herzegovina as a country extremely rich in water resources it is of great importance to preserve qulity of water in rivers, which are increasingly used for sports, recretion and economic purposes.

Mostar is an important touristic destination, hence problems related to water quality are becoming more pronounced. Human and economic well being of the area largley depends on Neretva. It is used for agricultural and industrial production, including electrical energy, maintains a large number of fish farms, and offers excellent conditions for rest and relaxation. Vicinity of the famous Old Bridge, one of the most significant touristic destinations in Bosnia and Herzegovina and important cultural and historical monument under UNESCO protection, offers perfect backgound for development of touristic offers and recreational activities, such as swimming, rowing, sports fishing and and world renowned diving competitions.

The river ecosystem can be imagined as more or less continuous flow reactor in which the biomass of all hydrobionts participates in the cycles of matter and energy circulation, ensuring natural equilibrium. Chemical characteristics and nutrient concentrations in rivers are variable and volatile factors, especially dependent on anthropogenic impact, when rivers are used as recipients of wastewaters. The ecosystem is never static, it is in a constant dynamic state in which complex feedback mechanisms control the activity and abundance of microbes and other hydrobionts. Basic living conditions in surface water ecosystems are determined by complex ecological factors such as hydromorphological paramatres (depth, riverbed shape, vegetation, sediment type, etc.), geological substrate, altitude and physical, chemical and biological parametres.

1.2. General characteristics of aquatic ecosystems

Water is essential for survival of all living orgaanisms, but this precious resource is becoming increasingly endangered by the growth of human population, which requires high quality water for personal use and commercial activities. Overexploitation and pollution of water can lead to degradation of quality and quantity of water, which impacts not only functioning of ecosystems but also availability for human use and activities.

Water resources are not only reservoirs that provide water for use. These ecosystems are complex matrices that require careful management to ensure their future sustainability. Additionally, use of water habitats requires knowledge of important links between ecosystem characteristics and ways in which human activities can change interaction between physical, chemical and biological processes that govern functioning of an ecosystem. In many cases, value of rivers and benefits they offer decrease when river ecosystems are impaired. Surface and underground waters are the most significant water resources (Figure 1).

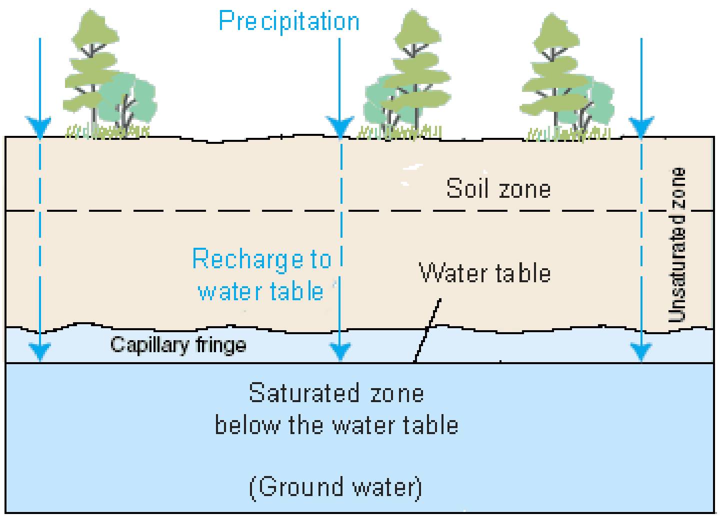


Figure 1. Accumulation of underground water (United States Geological Survey, 2007)

In Europe, surface waters make up approximately 75% of water that is used for general purposes, while 25% is drawn from underground water sources, with only a very small percentage of water being drawn from desalination and recycling. Quality of water resources is threatened by contamination by communal wastewaters, industrial waste, and overusage of pesticides and artificial fertilisers (Dickie, 2005).

1.3. Water quality

Providing clean and safe water to people worldwide and promotion of sustainable use of water resources, were set as fundamental aims by UN in 2000.

Continuous monitoring of quality and quantity of surface and groundwaters is a necessary activity for all levels of government: local, national and international. The *Federal Ministry of Agriculture, Water Management and Forestry* is the competent authority responsible for establishing procedures, legislation and conducting monitoring of the quality of water resources in Federation of Bosnia and Herzegovina.

Water quality and quantity are closely related although they are often not measured simultaneously. Water quantity is usually measured at isolated hydrological stations, that record water level, outflow and flow rate. Water quality is determined by analysing water samples collected at specifically selected sites. Costs related to monitoring only a few parametres that determine water quantity compared to numerous parametres that determine water quality, usually means that monitoring of water quality is not conducted as often as water quantity. However, water qulity results are essential for determining and tracking spatial and temporal oscillations and changes in aquatic ecosystem.

Water quality is a function of natural conditions and/or human activities. Without anthropogenic factor, water quality would depend on decomposition of minerals from soil, atmospheric evapotranspiration processes, dust and salt deposition, natural leaching of organic matter and nutrients from soil, hydrological factors that lead to runoffs and biological processes inside the aquatic environment that can change physical and chemical composition of water. As a result, water in natural environment contains various dissolved substances and insoluble particles. Dissolved salts and minerals are essential costituents of high quality water as they help maintain health and vitality of organisms that depend on that ecosystem (Stark *et al.*, 2000).

Water quality is not a static state of the system, nor can it be defined by measuring only one parameter. It is more correct to describe it as a value that varies in time and space and requires regular monitoring to detect spatial and temporal oscillations and changes. In the process of natural water circulation, many pollutants produced by humans in the course of their various activities end up in natural waters. The quality, type and possibility of human pollution of natural waters are very diverse. The usability of water for certain purposes depends on the composition, properties and concentration of certain substances in the water. Water quality is evaluated according to a series of chemical, physical and biological indicators. Some values give a rough indication of water pollution, while others enable direct monitoring of the source of pollution.

1.4. Basic physico-chemical parameters of freshwaters quality

The physical and chemical properties of water, as a set of abiotic factors of the environment, are of crucial importance for the growth of populations of plant and animal organisms in aquatic ecosystems, and determine the quality of water. Change of parameter values, causes change of living conditions, which has ecological significance for aquatic organisms. All abiotic factors are not equally important for the life of plants and animals. The main quality parameters are water temperature, dissolved oxygen, oxygen saturation, pH, water level, water hardness.

1.4.1 Organoleptic properties of water

Water must be clean, clear and unpolluted for all normal life processes to take place. The color, taste and smell of natural waters, if present, are the result of products of aquatic organisms or the process of decomposition of organic substances and are only physical manifestations of other types of pollution. Artificial odors are created when polluted with waste water. The color of water can be yellowish from organic substances or red-brown if humic and fulvic acids and their soluble salts are present, especially humates and fulvates of iron and mangan. In surface waters, i.e. rivers, this is a frequent phenomenon and almost always follows increases in water levels after heavy rains or sudden melting of snow (Tranter and Jones, 2001). Pure water is tasteless and odorless.

1.4.2. Temperature

Temperature affects the speed of biochemical reactions, the rhythm of photosynthesis in algae and aquatic plants, the metabolism of organisms, as well as the way inorganic compounds, chemical pollutants, parasites, and other pathogens affect organisms. Determining the water temperature at the time of water sampling for analysis is important for determining a number of other parameters. Temperature is important for the process of natural self-purification of water, and it can also affect the availability of dissolved oxygen, nutrients, and other substances in the water column (e.g., ammonia). Temperature directly affects the metabolism of organisms. An increase in temperature accelerates biochemical processes, which results in higher oxygen consumption, and an increase in temperature decreases the solubility of oxygen in water. The final result is the gradual death of organisms.

Aquatic organisms have evolved to live in water of a certain temperature, and often have a very low level of tolerance to temperature changes. Different temperatures are characteristic for each type of organism, and temperature resistance is the hallmark of the species (Maletin *et al.,* 2000). Due to changes in temperature, organisms can become more susceptible to toxic waste, parasites and diseases. Microorganisms do not have the ability to thermoregulate and therefore the temperature of the cell corresponds to the temperature of the external environment (Đukić *et al.,* 2000). Therefore, although water bodies have the ability to adapt to atmospheric temperature extremes, even moderate changes in water temperature can have serious consequences for aquatic life, including bacteria, algae, invertebrates and fish.

The temperature of natural waters is related to the temperature of the environment (atmosphere) and changes cyclically with the change of day and night and climatic seasons. The maximum daily temperature is usually reached a few hours after noon, while it is minimum at dawn. Warm waters are more susceptible to eutrophication—a build-up of nutrients and possible algal blooms—because photosynthesis and bacterial decomposition occur faster at higher temperatures.

Thermal pollution results from direct contacts, such as the discharge of industrial cooling water into rivers, or indirectly through human activities such as the removal of riparian vegetation that shades the river or the construction of structures that obstruct river flow (Arthington *et al.,* 2000). The temperature of the water depends on the climatic conditions, the geological structure of the terrain, the connection of the underground water with the surface water and the mobility of the water. The temperature of water systems increases from the source to the estuary in the summer period (in winter it is the other way around), and fluctuations are the smallest at the source, which is the result of greater stability of groundwater temperatures.

1.4.3. Turbidity and suspended solids

Turbidity refers to the degree of water clarity. Water turbidity is caused by dispersed substances, especially colloids, then microorganisms, gas bubbles, etc. These substances scatter and absorb incoming light and reduce the depth of light penetration into the water.

The greater the amount of suspended particles in the water, the greater the turbidity and the lower the clarity. Turbidity determines how deep light can penetrate the water, but is not related to color: tannin-rich waters are highly colored but usually clear, with very low turbidity. If the penetration of light into the water is limited, the photosynthesis of green plants in the water is also limited. This means that less food and less oxygen is available to aquatic animals. Organisms that can perform photosynthesis in low light or control their position in the water, such as blue-green algae, have an advantage in highly turbid waters.

Turbidity values are not measures of the concentration, type, or size of particles present, although turbidity is often used as an indicator of the total amount of material dissolved in water (total solutes). Turbidity is often expressed as the total amount of suspended particles. The "water turbidity" indicator is used to determine the suitability of water for drinking, as well as water used for bathing and in general for leisure. The biggest sources of turbidity in surface waters are mainly phytoplankton, microorganisms, and metabolite products, but in the vicinity of the coastal zone, particles can also include clay, sand, or silt due to coastal erosion, as well as organic waste from the watercourse itself or from the waters that discharge into the river (US EPA, 1997).

Increased concentrations of suspended substances indicate the impact of discharged wastewater from settlements, construction, forestry, agriculture, industry, and other human activities. Suspended particles absorb heat, and therefore the water temperature rises faster in cloudy than in clear water. Then, since warm water contains less dissolved oxygen than cold water, the concentration of dissolved oxygen decreases. Suspended particles settle within the space between gravel and rock at the bottom of the river bed and alter habitat conditions, and may cause undesirable changes in bottom communities (Ward and Tockner, 2001). During the deposition of biodegradable substances, anaerobic conditions can develop at the bottom of the receiver, with the appearance of gases that are the product of such processes. Suspended particles can clog the gills of fish, and cause illness, slower growth, or even death. Ions and molecules of harmful substances such as excess nutrients and toxic substances, as well as colonies of microorganisms, including pathogenic ones, can be adsorbed on suspended substances. This can be a problem for drinking water, which often requires chlorination to kill harmful bacteria. Turbidity is influenced by: rainfall and water flow, riverbed and bank erosion, waste water, excessive algal growth, coastal vegetation, water flow velocity, watercourse type, soil type and salinity (Barth *et al.*, 2007).

1.4.5. Salinity and electrical conductivity

Salinity is an indication of the concentration of dissolved substances in water. The ions responsible for salinity are cations (calcium, Ca^{2+}; magnesium, Mg^{2+}; sodium, Na^{+}; and potassium, K^{+}) and anions (carbonates, CO_3^{2-} and HCO_3^{2-}; sulfates, SO_4^{2-}; and chlorides, Cl^{-}). The level of salt in aquatic systems is important for aquatic flora and fauna because species can only survive within a certain salinity range (Friedl *et al.*, 2004). Although some species are well adapted to survive in saline habitats, the growth and reproduction of many species can be inhibited by increasing salinity. While an appropriate salt concentration is vital to aquatic plants and animals, salinity outside the normal range can cause stress or death to the organism. Salinity also affects the availability of nutrients to plant roots. Depending on the type of salts present, salinity can increase water clarity. Salinity is measured by comparing the dissolved salts in a water sample with a standardized solution.

The level of total mineralization can be estimated by measuring total dissolved particles or by measuring conductivity. Electrical conductivity, or conductivity, measures the capacity of water to conduct an electric current, a property directly proportional to the concentration of dissolved ions. Electrical conductivity is often used as a proxy for measuring salinity since it is significantly higher in saline systems than in non-saline waters (Dodds, 2002). The specific electrical conductivity, however, also depends on the type of mineral that is dissolved in the water. The specific electrical conductivities of different solutions can only be compared if they are determined at the same temperatures.

Communal, agricultural, and industrial effluents contribute ions to receiving waters, and may also contain substances that are weak conductors (organic compounds) that change the electrical conductivity of receiving waters. Therefore, electrical conductivity can be used to detect sources of pollution (Stoddard *et al.*, 1999). Contaminating effluents can change the electrical conductivity of water in different ways. For example, wastewater will increase conductivity due to chloride, phosphate, and nitrate content, but oil and metal ion spills would lower conductivity.

1.4.6. pH value and alkalinity

Hydrogen ion concentration (pH) is a measure of acidity or alkalinity. In water, a certain number of water molecules (H_2O) dissociate to form hydrogen (H^+) and hydroxyl (OH^-) ions. If the relative proportion of hydrogen ions is greater than the proportion of hydroxyl ions, then the water is defined as acidic. If hydroxyl ions predominate, then water is classified as alkaline. The relative proportion of hydrogen and hydroxyl ions is measured on a negative logarithmic scale from 1 (acidic) to 14 (basic); 7 represents a neutral value (US EPA, 1997; Friedl *et al.,* 2004). Many compounds dissolve more easily in acidic than in neutral or alkaline waters. The pH of the soil around the roots of the plant affects the absorption of nutrients; pH also affects the solubility of heavy metals in water and the concentration of total suspended solids in rivers.

The pH value of the water system is very important because it is closely related to biological productivity. Although the tolerance levels of environmental oscillations vary from species to species, pH values between 6.5 and 8.5 usually indicate good water quality and this range is usually characteristic of surface waters. The natural acidity of rainwater is caused by the dissolution of atmospheric carbon dioxide (CO_2). Hydrogen ions that reach water through precipitation are neutralized by carbonate and silicate minerals during the passage of water through the soil. This neutralization capacity that the soil possesses determines whether acid precipitation will affect the water quality of the water body into which it flows. The ability of a river bed to establish chemical balance and neutralize the acidity of rainwater depends on the period the water spends in the soil as well as the levels of calcium carbonate, bicarbonate, and silicate minerals (Friedl *et al.*, 2004; Wetzel and Likens, 2000). In waters with beds are chemically unbalanced, usually those containing hard igneous rocks, dissolved organic acids predominate, which can lower the pH value of the watercourse to 4.0. Under these conditions, acidic watercourses are created that have very low values of hardness and mineral content, and with that, a low degree of biological productivity. Conversely, sedimentary rock beds, especially limestone, which are rich in carbonates, have a high silicate content, are well buffered and produce neutral (pH 7) or slightly alkaline hard water fluids (pH of 7.5 to 8.5).

All plants and animals have the ability to grow within a specific pH range, usually between 6.5 and 8.0. If the pH of the water is outside the normal range for the organism, it can cause stress or even death for the organism. Young fish and insects are particularly sensitive to changes in pH value. The specific pH range for bacteria is between 4 and 9, and the optimum is between 6.5 and 7.5.

Many factors can affect the pH of water. These include: water source, rainfall, time of day, water temperature, amount of plants and algae in the water, geology and soil, e.g., acid sulfate soils, inflow of industrial waters, disturbance of acid sulfate soils by agricultural activities, urban development, mining, atmospheric precipitation (acid rain, deposition of dry particles), burning of fossil fuels, factories, photosynthesis, respiration and salinity. The pH of the water varies during the day, as the balance between photosynthesis and respiration changes with light intensity and temperature. Rain is naturally slightly acidic due to dissolved carbon dioxide; the water flowing from the limestone zones has a relatively high pH.

Alkalinity is a related term generally used to indicate the capacity of a system to neutralize the effects of acidity. The alkalinity of the water determines the amount of ions in the water that neutralize the hydrogen ions. The neutralization property is the ability of an aqueous system to resist or moderate changes in pH. Alkaline compounds in water such as bicarbonates, carbonates, hydroxides, alkali and alkaline earth metals remove H^+ ions and lower the acidity of the water (i.e., increase the pH) (Swedish Environmental Protection Agency, 2002; Godfrey *et al.,* 1996). They are formed by the dissolution of mineral substances in the soil and atmosphere. Some of them, such as phosphates, come from municipal wastewater, or rainwater that washes agricultural soils. Natural waters have a pH of 5.5 to 8.6.

1.4.7. Dissolved oxygen

Oxygen dissolved in water is one of the most important components of the aquatic ecosystem, necessary for the respiration of all aquatic plants, animals and microorganisms. It is a limiting factor in the development of life, because it is necessary for the metabolism of aerobic organisms, and it also affects inorganic chemical reactions. Dissolved oxygen is found between water molecules in the form of microscopic oxygen bubbles. Fish "breathe" by absorbing dissolved oxygen through their gills. The concentration of dissolved oxygen is an important indicator of water quality and the overall condition of the aquatic habitat. High oxygen concentrations usually indicate good water quality. Permanently low concentrations of dissolved oxygen will endanger aquatic life, because it will not meet their needs. Oxygen enters the water through the process of diffusion through the surface of the water, dissolution from the atmosphere, assimilation processes of speed and splashing of water such as rapid waterfalls or rapids in rivers that promote the mixing of water and air (aeration), or as a by-product of the photosynthesis of aquatic plants and algae (Huggins and Anderson, 2005). The rate at which oxygen from the air enters the water and dissolves depends on the level of agitation of the water surface and the depth of the water.

The amount of dissolved oxygen depends to a large extent on temperature and to some extent on atmospheric pressure. Salinity also affects dissolved oxygen concentration, so oxygen levels are low in highly saline waters. The amount of any gas, including oxygen, dissolved in water is inversely proportional to the temperature of the water; as the temperature increases, the amount of dissolved oxygen (gaseous) decreases. At lower temperatures, larger amounts of oxygen are dissolved than in waters with higher temperatures. This relationship is illustrated by the annual cycle for the Murray River in Australia, (Figure 2.).

At high altitudes, due to low atmospheric pressure, dissolved oxygen concentrations are lower. At 1850 meters, the amount (mg/l) of dissolved oxygen in the water is only 80% of the amount at sea level, under otherwise identical conditions. Lowland rivers, which contain more organic matter than mountain rivers, tend to have lower dissolved oxygen concentrations because bacteria use the oxygen to break down organic matter. Microbial decomposition of organic matter is an important process in the cycle of carbon and nutrients in the river. As the amount of organic matter increases (due to their dissolution), the consumption of oxygen also increases. If the consumption of oxygen in an aquatic ecosystem is greater than the ability of that ecosystem to absorb oxygen, the creation of a hypoxic, potentially toxic environment and nutrient overload can occur.

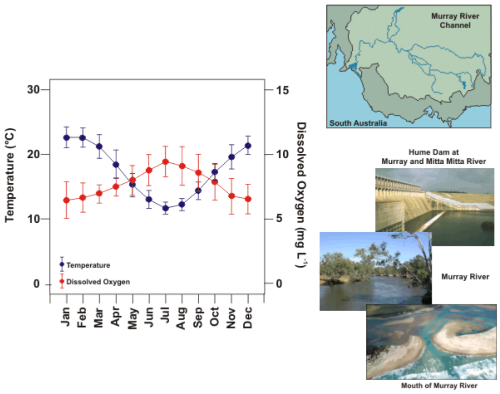


Figure 2. Seasonal oscillations in dissolved oxygen and temperature in the Murray River, Australia. Data are monthly means ± 1 standard deviation. It is noticeable that the highest temperature points coincide with the minimum dissolved oxygen throughout the year. (Murray River, http://www.theage.com.au)

Many productive lakes experience periods of oxygen depletion at great depths, during the warm summer months when a pronounced temperature gradient is established between warm surface water and cold deep water. Oxygen is consumed by the respiration of living organisms, but also by other oxidation processes. A high concentration of algae in surface waters can lead to oxygen depletion at depth as the cells die off and settle to the bottom of the lake, where they are broken down by bacteria. The decomposition process consumes oxygen from the water through bacterial respiration. Oxygen consumption is lower in better quality waters. A decrease in the amount of oxygen in water can also occur due to an increase in the amount of organic substances that are the result of water pollution (Huggins and Anderson, 2005). In connection with this, it is necessary to know the degree of oxygen saturation, which is calculated on the basis of the oxygen content at a certain temperature and indicates the presence of organic substances. In some cases, water can contain too much oxygen and become supersaturated. This represents a potential danger for fish, because in this oversaturated environment the concentration of oxygen in their blood increases, which has negative consequences for circulation. Oversaturation mainly occurs in highly turbulent waters due to aeration, in waters where algae blooms occur due to extremely high temperatures, as well as due to excessive growth of aquatic plants, and thus increased photosynthesis.

1.4.8. Water hardness

Hardness is one of the oldest terms used to describe the qualitative properties of water. It is determined by the concentration of polyvalent metal cations in the solution. The total hardness of water can be carbonate (transient) hardness, caused by the presence of metal ions in the form of carbonates and bicarbonates in water (Ca, Mg) and non-carbonate (permanent) hardness originating from (SO_4^{2-}), chloride (Cl^-), nitrate (NO_3^-) and other calcium and magnesium salts. Since only calcium and magnesium salts are found in significant quantities in natural waters, water hardness is defined as the content of calcium and magnesium salts. Hard water is not dangerous for human and animal health, but it is not suitable for industry due to higher consumption of fuel for heating boilers and the formation of sediment.

The waters of the researched Neretva area are hard waters because it is an area of limestone, dolomite and gypsum. Scientific studies have determined high hardness values, with a range from 8.74 to 16.69°DH (Bajramović and Bobar, 2008). Hardness is most often expressed as the concentration of calcium carbonate ($CaCO_3$) in mg/l. In older literature, the unit "German degree of hardness" is also used.

1.4.12. Organic substances

Organic matter is important for the cycling of nutrients, carbon and energy between producers and consumers in the aquatic ecosystem. Microbiological decomposition of organic matter, inefficient feeding of zooplankton, and excretion of waste products by aquatic animals release stored energy, carbon, and nutrients, and thus make them available again for the metabolic needs of primary producers and bacteria. External sources of organic matter entering aquatic ecosystems via river beds from sources such as waste effluents can enhance microbial respiration and invertebrate activity in aquatic environments. Organic substances affect the biological availability of minerals and elements, and have a protective role in many aquatic habitats, affecting the depth of light penetration. The presence of organic substances in water is not conditioned by municipal or industrial waste water that is discharged into waterways. Sometimes organic substances have so-called humic origin, the result of soil washing by a surface water. Organic substances are also formed by various biological processes in water.

1.5. Hydrological variables

The quantity of water within the system and the rate of recharge affect the chemical and biological properties of all aquatic environments. Water level and flow rate can affect the concentrations of ions and nutrients in the water and determine the physical space along which biological production can take place. Flow rate is not a measure of water quality, but it is an important parameter, due to its direct influence on the chemical composition of the river habitat and receiving waters. Flow rate is directly related to the amount of water transferred from the catchment to the stream channel and can be defined as the volume of water that moves past a given point during a given period of time. Flow is influenced by weather conditions: it increases during snowfall and snowmelt, and decreases during dry periods (Pomeroy and Brun, 2001; Hoham and Duval, 2001). Annual, seasonal and even daily cycles affect suspended and dissolved materials in rivers and the rate at which they reach downstream points. The use of water for irrigation, industry or domestic purposes can seriously reduce the flow in the system. Dams used for electricity generation, especially plants designed to produce electricity during periods of peak demand, often block the flow and release water in large drifts (Smakhtin *et al.*, 2003).

The speed of the water flow, which increases as the amount of water in the river increases, determines the types of organisms and habitats that can survive in that environment. Some organisms prefer fast-flowing rivers, while others prefer slower rivers. The speed of the flow also affects the amount of silt and sediment carried by the river. Sediments in slow-flowing rivers will settle to the bottom, while sediments in faster-moving rivers will remain suspended in the water column, and eventually settle in reservoirs, or near coastal marine areas, where they may be essential for estuarine maintenance and biological productivity within these environments. Rivers with a faster flow, especially if they also have a coarse-grained substrate, will have larger amounts of dissolved oxygen than slower rivers with a smooth bottom. Water flow can also affect the temperature oscillations of river water, as the temperature is more susceptible to the effects of atmospheric conditions when the water flow is slower.

Flow rate affects the river's susceptibility to pollution. Large, fast-flowing rivers can receive a larger influx of pollutants, and through dilution, remain intact or be less threatened. Small, slow rivers have a reduced ability to dilute and break down waste. However, the idea that the solution to pollution is dilution is dangerous, because it does not take into account the effects on receiving waters or sediments where the accumulation of pollutants can have significant negative consequences.

1.6. Biological indicators of water quality - bioindicators

Biological assessments of the state of water systems are based on mutual influences of living communities and habitats, i.e. changes in ecosystems, which occur as a result of altered abiotic factors. Biological assessments are becoming increasingly important, under the complex circumstances of waste discharges into rivers. As the concentration of waste substances in natural waters is constantly changing, especially in watercourses, the state of water quality in certain places depends on the time and method of sampling.

However, the changed conditions of the habitat will affect the organisms of the ecosystem, so that species of organisms that are sensitive to certain changes in abiotic factors will decrease or disappear, while species more resistant to changes will develop. Biological research determines the impact of changes in the water system, which occurred in the research area in the time between two sampling periods. The advantage of biological indicators compared to measuring instruments is that they monitor the synergistic effect of several pollutants, instead of measuring the concentration of each pollutant individually.

Organisms, populations and communities composed of different species make up the biological diversity of an aquatic habitat. From unicellular microbes such as viruses, bacteria, protists, and fungi, to multicellular organisms such as vascular plants, aquatic invertebrates, fish, and aquatic plants, communities of organisms living in or near aquatic ecosystems play a vital role in regulating biogeochemical flows in their environment, and at the same time they are subject to the influences of those same biogeochemical currents. Aquatic organisms, which are often considered the "engineers" of aquatic ecosystems, not only respond to physical and chemical changes in their habitat, but can also promote these changes and play important roles in purifying and detoxifying their habitat (Ostroumov, 2005). The overall biological diversity of an aquatic system ensures that ecosystems can continue to function normally: shifts in species composition through species loss or biological invasion can lead to physical and chemical changes in the environment, which have potentially harmful consequences for the communities of organisms living in the ecosystem, and for the people who rely on the water supply system and other activities. The diversity of aquatic ecosystems can also be affected by physical and chemical changes in the environment.

There is considerable duplication of functions in aquatic food webs, where several species and trophic levels can perform similar self-purification functions of the water mass. For example, both bacteria and fungi play roles in the chemical degradation of pollutants in aquatic ecosystems, and water filtering is performed by invertebrates living in benthic and pelagic habitats (Ostroumov, 2005). Although this duplication of functions offers a kind of support for the maintenance of ecological services, it is no guarantee that ecosystems are protected from further degradation.

The loss of sensitive species can have multiple effects on other resident organisms, leading to catastrophic changes and shifts in the composition of aquatic communities and the functions they perform. As such, the overall diversity of biological communities allows many ecosystem processes to function normally in a steady state. The loss of diversity can lead to a decrease in ecosystem functionality as well as a complete shift of the ecosystem to alternative stable states (Ostroumov, 2005; Scheffer *et al.,* 2001). A simple example of a dramatic change in the state of an ecosystem to an alternative stable state, attributed to water pollution is the change from a clear water state dominated by vascular plants to a turbid water state dominated by phytoplankton, which is very noticeable in waters with a shallower riverbed (Scheffer *et al.,* 2001).

Considering the importance of biological communities to water quality, water pollution is considered an ecological and biological issue, as it impairs the ability of resident and non-resident organisms to use the resources provided by the ecosystem and to maintain the ecological functions they perform. Physical loss of habitat and changes in the chemical composition of water can impair the ability of species to grow, reproduce, and interact with other species in the ecosystem. Excessive loading of piles of sediments in rivers interferes with the life cycle of fish, hindering respiration and covering spawning grounds, and deposited can suffocate benthic organisms. Numerous pollutants have various modes of action, from causing mass mortality to causing chronic disease, in addition to the effects they have on bio-accumulation through the food chain.

Valuation of biological communities present in aquatic habitats reflects ecosystem quality. Biomonitoring is a procedure for assessing the quality of the environment because biological communities integrate the effects of various stressors, and thus give a clear idea of the magnitude of their collective impact. Biota also integrate stressors over time and provide an ecological benchmark of changing environmental conditions. The widespread use of biomonitoring techniques has resulted in part from public interest in the status of individual species and the cost-effectiveness of sampling regimes. Monitoring of biological communities can be done at various trophic levels including microorganisms (bacteria, protists, and viruses), primary producers (algae and vascular plants), primary consumers (invertebrates), and secondary consumers (fish) (Rosenberg, 1998; Reynoldson *et al.* , 1997; Barbour *et al.,* 1999).

1.6.1. Algae and aquatic vascular plants

Algae and aquatic vascular plants (macrophytes) are the primary producers in aquatic ecosystems. Algae are organisms that live suspended in the water column (phytoplankton) or attached to substrates (periphyton). Aquatic vascular plants are usually rooted in the substrate but can also float freely. As primary producers, algae, and to a lesser extent, aquatic vascular plants, form the basis of aquatic food webs. Vascular plants have an additional role in providing habitat and shelter for fish and invertebrates in riparian areas of rivers and lakes. The density of algae and vascular plants in an aquatic ecosystem is primarily controlled by the availability of nitrogen and phosphorus nutrients in the water column and sediments, although light and temperature also play an important role in determining the distribution and density of these organisms.

Algae and aquatic vascular plants generally have rapid reproduction rates and very short life cycles, making them valuable indicators of short-term environmental impacts. Algae and aquatic plants, as primary producers, are the most directly exposed and affected by physical and chemical factors and are sensitive to pollutants that may not visibly endanger other aquatic organisms, or that may endanger other organisms only at high concentrations (Barbour *et al.*, 1999). Sampling such plants is relatively simple, inexpensive, does not disturb the habitat, and standardized methods exist for comparisons within and outside the region. Species diversity and community density (often measured as chlorophyll a in algae) is often used as an indication of algal and aquatic plant growth and production.

1.6.2. Invertebrates: zooplankton and benthic macroinvertebrates

Aquatic invertebrates are consumers that primarily feed on bacteria, algae, and detrital minerals that are either produced within or influenced by surrounding watersheds. Zooplankton is a community of invertebrates suspended in the water column, while benthic macroinvertebrates inhabit the bottom. Invertebrates have the ability to control algae through nutrition, and are an important food source for organisms in higher trophic levels, such as fish and predatory invertebrates.

Invertebrates are good indicators of local conditions because many of them have restricted migration regimes or are immobile, and as such, are useful for examining localized impacts. Individual species of invertebrates react differently to changes in the environment. Changes in the habitat will be reflected in changes in the composition of species spatially and temporally (affected and unaffected localities over time). Therefore, species composition can be used to help assess environmental degradation arising from single or multiple sources (Barbour *et al.*, 1999).

1.6.3. Fish

Fish are the main predators in many aquatic ecosystems. They are dependent on lower species for food, and through nutrition they perform very important roles in controlling the growth and reproduction of lower species. Fish are important not only for the functioning of the ecosystem, but as a fish stock they also represent socioeconomic value. The loss of fish species due to changes in water quality or overfishing can result in dramatic changes in ecosystem dynamics, as the nutritional pressure on invertebrates and algae can weaken, allowing rapid growth and possible algal blooms (Maletin, 2002).

Fish communities can be used as an indication of longer-term and far-reaching consequences of changes in an aquatic ecosystem because fish species have relatively long life spans and are mobile. They tend to integrate the influences of lower trophic levels, and thus provide insight into the overall state of the habitat. Fish are important for assessing contaminants in ecosystems because they are generally at the top of the food chain and are susceptible to bioaccumulation and biomagnification of heavy metals and synthetic organic contaminants. They are relatively simple to collect and identify to species level, and sometimes it is possible to capture and return them intact (non-destructive sampling) when fish tissues and organs are not required for analysis (Barbour *et al.*, 1999).

1.6.4. Microbes

Microbial communities of bacteria, viruses, protists and fungi are numerous in aquatic habitats, but only recently have scientists realized the importance of their contribution to the overall functioning of the aquatic ecosystem. In addition to microorganisms that constantly exist in water, such as decomposers and producers of new organic matter, microorganisms from the digestive system of animals and humans also reach the water, through soil washing and waste water (David, 2005).

Typical characteristics of microbial populations are high abundance, short generation time (i.e. reproduction), and pronounced ability to disperse (Dolan, 2005). Most microbes are heterotrophic organisms, meaning they need organic carbon as fuel for metabolism. Bacteria and fungi are important decomposers of organic matter in aquatic ecosystems, releasing nutrients and minerals into the water column, which become available as fuel for the metabolism of other organisms. Although the study of aquatic viruses is still in its early stages, these viruses appear to infect primarily bacteria and unicellular algae and play important roles in regulating the production and diversity of the microbial food web (Wommack and Colwell, 2000).

Most of the microbes that inhabit aquatic habitats are completely harmless to humans and play an important role in the functioning of the habitat. However, microbial contamination of surface and underground water with pathogenic organisms is certainly the most important issue of water quality, especially in underdeveloped countries, where access to safe, clean water for drinking, bathing and irrigation is often unavailable. The World Health Organization (WHO) points out that the greatest risk for humans arising from microbial contamination of water is the consumption of water contaminated with human or animal feces (WHO, 2004).

When available, water treatment plants have the potential to reduce the presence of coliform bacteria in a sample from very high concentrations to values below analytical detection levels. Although water treatment facilities exist in almost all cities of the developed world, the supply of safe drinking water and sanitation is still lacking in many underdeveloped countries.

1.7. Microbiological indicators of water quality

Microbial monitoring in surface and groundwater is used to detect the presence of pathogenic organisms. There are several broad categories of microbes including bacteria, protozoa, parasitic worms, fungi and viruses. The organisms most often used for microbiological monitoring are fecal indicators: organisms that indicate the presence of fecal contamination of human or animal origin. Tests used to indicate the presence of pathogenic organisms include testing for total coliforms, faecal coliforms, or *Escherichia coli*-specific, among others (Ashbolt *et al.*, 2001). It is important to note that these measures are only indicators, and do not detect all pathogens present in the watercourse (Tallon *et al.*, 2005). It is impossible to carry out all tests to detect all pathogenic microorganisms that can contaminate the water. Therefore, bacteriologists apply practical tests for water quality monitoring, which are performed on the basis of determining the concentration of selected indicator organisms which serve as indicators of the possible presence of certain pathogenic organisms. As effective indicators of the presence of pathogens, indicator microorganisms should be present in the sample in equal or greater numbers and be as or more resistant to environmental factors and purification processes than pathogens. Indicators of fecal contamination should meet the criteria shown in Table 3.

As the majority of microbial pathogens present in natural and wastewater are of fecal origin, the detection of fecal contamination of water is the main objective of water testing. As bacterial indicators of fecal contamination, those species that are found as normal microflora are found almost exclusively or predominantly in human feces, and there are so many of them that they can be easily proven even in large dilutions. These include coliform bacteria, faecal streptococci, sulfite-reducing clostridia, species from the genus Salmonella and species from the genus Proteus. They can survive in the environment for a long time and thus provide a reliable indication of fecal contamination long after they have been expelled from the digestive tract (Morrison, *et. al.*, 2001).

Table 3. Criteria for choosing an ideal indicator organism (Gerba, 2000)

Criteria
The organism should be present in all types of water.
The organism should be present wherever enteric pathogens are present.
The organism should have a longer life span and a better ability to survive than the most resistant pathogen.
The organism does not multiply in the aquatic environment.
Test methods should be simple to apply.
The concentration of the indicator organism should be directly related to the degree of fecal contamination.
The organism should be a member of the intestinal microflora of warm-blooded animals.

7.1. Coliform bacteria

The most common human pathogens, coliform bacteria, are found in the gastrointestinal tract of warm-blooded animals. Historically, coliform organisms, particularly *Escherichia coli*, have been used as indicators of faecal contamination of water and food (APHA 1989). Modern ecological microbiology divides coliform microorganisms into groups of "total coliforms" and "fecal (thermotolerant) coliforms". Total coliforms include all Gram-negative, aerobic and facultatively anaerobic, rod-shaped, non-sporogenic bacteria that ferment lactose with the production of gas and acid at 35^{o}C over 48 hours, such as members of the Enterobacteriacea family. A higher temperature (Eijkman's test) is used to separate total coliforms from fecal coliforms. Fecal coliforms are aerobic and facultatively anaerobic, Gram-negative, non-sporogenic rods that ferment lactose on a selective EC medium at 45.5°C over 48 hours (Bitton, 2005). The fecal coliform group is limited to microorganisms that grow in the gastrointestinal tract of humans and other warm-blooded animals and includes members of at least three genera: Escherichia, Klebsiella, and

Enterobacter. Due to the possibility of cultivation at elevated temperatures, they are called thermotolerant coliforms (TTC) and have become the main indicator of the microbiological state of water.

Another common feature of all coliforms is the presence of constitutive β-galactosidase and a negative cytochrome oxidase reaction. This group is widely distributed in nature. Some members of this family are saprophytes, although they are associated with the digestive tract of warm-blooded animals. *Escherichia coli* is the main thermotolerant coliform (~97%) of the Enterobacteriaceae family. Coliforms were chosen as water quality indicators because they are present and survive longer in septic water than other pathogenic organisms. *E. coli* was chosen as an indicator of fecal contamination of water resources, because it is the only thermotolerant fecal coliform that is exclusively of fecal origin and does not reproduce outside its natural habitat - the intestinal tract of humans and animals, while other coliforms can be present in fresh feces and have the power of survival and growth outside the human and animal digestive tract (Prescott, *et. al.*, 2002).

Table 4. Bacteriological indicators of water quality (Ashbolt *et al.*, 2001)

Definitions of key fecal indicator microorganisms	
Coliforms	Gram-negative, non-sporogenic, oxidase-negative, rod-shaped facultative anaerobic bacteria that ferment lactose (with β-galactosidase) into acid and gas within 24-48h at 36±2°C. Not specific indicators of fecal pollution.
Fecal (thermotolerant) coliforms	Coliforms that produce acid and gas from lactose at 44.5±0.2°C within 24±2h, known as faecal coliforms and fecal indicators.
Escherichia coli	Thermophilic coliforms that produce indole from tryptophan, now also defined as coliforms that can produce β-glucuronidase (although taxonomically up to 10% of environmental *E. coli* cannot). The most suitable group of coliforms to

	indicate fecal pollution from warm-blooded animals.
Fecal streptococci (FS)	Gram-positive, catalase-negative cocci from a selective nutrient medium (e.g. liquid culture of dextrose azide or m Enterococcus agar) that grow on bile aesculin agar at 45°C, belong to the genus Enterococcus and Streptococcus, possess the D antigen of the Lancefield group.
Enterococci	All faecal streptococci that grow at pH 9.6, 10°C and 45°C and at 6.5% NaCl. Almost all are members of the Enterococcus genus, and meet the following criteria: they are resistant to 60°C for up to 30 min and can reduce 0.1% methylene blue. Enterococci are a subspecies of fecal streptococci that grow under the above conditions. Alternatively, enterococci can simply be identified as microorganisms with the ability to grow aerobically at 44±0.5°C and hydrolyze 4-methylumbelliferyl-β-D-glucoside (MUD, β-glucosidase activity detected by blue fluorescence at 366 nm), in the presence of thallium acetate, nalidixic acid and 2,3,5-triphenyltetrazolium chloride (TTC, which is reduced to red formazan) in a specific medium (ISO/FDIS 7899-1 1998).
Sulfite-reducing Clostridium (SRC)	Gram-positive, sporogenous, nonmotile, strictly anaerobic, rod-shaped bacteria, which reduce sulfite to H_2S.
Clostridium perfringens	As for SRC, but also ferments lactose, sucrose and inositol with gas production, ferments milk, reduces nitrates, hydrolyzes gelatin and produces lechitinase and acid phosphatase.

1.8. Water quality guidelines and standards

The quality of water necessary for human needs varies, as do the criteria used to assess water quality. The highest standards of purity are required for water used for drinking, while it is acceptable for water used for some industrial processes to be of lower quality. The water quality required to maintain ecosystem health is largely a product of natural environmental factors and conditions. Some aquatic ecosystems can withstand large changes in water quality without any obvious and recognizable effects on the composition and functioning of the habitat, while other ecosystems are sensitive to small changes in the physical and chemical composition of the water, and degradation of habitat sustainability and loss of biodiversity may occur. Degradation of the physical and chemical quality of water as a consequence of human activity is often gradual, and subtle adaptations of aquatic ecosystems to these changes cannot always be easily observed until dramatic changes in the state of the ecosystem occur. For example, in many shallow European lakes, the gradual enrichment of surface water with plant nutrients has resulted in a shift from systems previously dominated by rooted aquatic plants to systems now dominated by algae suspended in the water column (Scheffer *et al.,* 2001). Regular monitoring of biological, physical, and chemical constituents of aquatic ecosystems can serve to detect extreme situations in which the ability to restore the ecosystem to a normal state extends beyond its capabilities.

As a rule, water quality is determined by comparing the physical, chemical and biological characteristics of a water sample with standard values and water quality guidelines. Guidelines and standard values for drinking water quality are set to enable the supply of clean and safe water for human consumption, thereby protecting human health. Mostly based on scientifically determined acceptable levels of toxicity to humans or aquatic organisms. Guidelines for the protection of aquatic life are more difficult to determine, mainly because aquatic ecosystems vary enormously in composition both spatially and temporally, and because ecosystem boundaries rarely coincide with territorial boundaries. There is an initiative among scientific and regulatory research circles to identify natural environmental conditions for chemicals that are not toxic to humans or animals and to use these as guidelines for aquaculture protection (Robertson *et al.*, 2006; Dodds and Oakes, 2004; Wickham *et al.*, 2005). Other guidelines, such as those created to ensure adequate water quality for recreational, agricultural, or industrial activities, set limits for the physical, chemical, and biological constituents of water needed to safely conduct various activities. According to Tedeschi (1997), surface waters are classified into 4 classes according to the following basic quality indicators (Table 5).

Table 5. Indicators of water quality and classification of waterways

Parametre	Class of Waterway Quality			
	I	II	III	IV
Dissolved oxygen (mg O_2/L)	8	6	4	3
Oxygen sautration (%)	90 – 105	75 - 90	50 – 75	30 – 50
Oxygen supersautration	-	105-115	115-125	125-130
BPK_5 (mg O_2/L)	2	4	7	20
pH	6,8 – 8,5	6,8 – 8,5	5 / 6-9	5 / 6-9
Visible litter	-	-	-	-
Colour	-	-	-	-
Odor	-	-	-	-
Total coliforms in 1L	500	5000	500 000	$> 10^6$
Total fecal coliforms in 1L	200	2000	20 000	> 200000

Other physical-chemical parameters that are indicators of the state of quality for class I and II waters, are shown in Table 6.

Table 6. Other physical-chemical quality parameters and associated classes of watercourses (Tedeschi, 1997)

Tested parameters	Class of watercourses	
	I	II
El. conductivity (µS/cm)	500	600
Total hardness (mg/L $CaCO_3$)	-	-
Total alkalinity (mg/L $CaCO_3$)	200	100
HCO_3^- (mg/L)	-	-
Chlorides (Cl^-) (mg/L)	> 200	< 200
Sulfates (SO_4^{2-}) (mg/L)	> 200	< 200
Calcium (Ca^{2+}) (mg/L)	100 – 250	100 – 250
Magnesium (Mg^{2+}) (mg/L)	30 – 150	30 – 150
Ammonia ($N\text{-}NH_3$) (mg/L)	0,10	0,30
Nitrites ($N\text{-}NO_2$) (mg/L)	0,01	0,05
Nitrates ($N\text{-}NO_3$) (mg/L)	0,5	1,0
Total phosphorus (P mg/l)	0,1	0,3

Class I are waters that, in their natural state or after disinfection, can be used for drinking and in the food industry, and surface waters for breeding noble species of fish (Salmonidae).

Class II are waters that in their natural state can be used for bathing and recreation by citizens, for water sports, for breeding other types of fish (Ciprinidae) or that can be used for drinking after appropriate conditioning (coagulation, filtration, disinfection, etc.) and for the needs of industry that needs clean water.

Class III waters are waters that in their natural state or after appropriate conditioning can be used in agriculture and in industry that does not need clean water.

IV class are all other waters.

Organizational bodies such as the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) have been concerned with the health aspects associated with water resource management for many years. Research is constantly being conducted and as a result documents are regularly published that relate to the safety and condition of the aquatic ecosystem and its importance for human health. According to the WHO, the most common adverse consequences of exposure to faecal-contaminated recreational waters are, among others, enteric diseases, gastroenteritis and diarrhea (WHO, 2001). Causal links between faecal and contaminated bather contamination and acute febrile respiratory illnesses, more serious than gastroenteritis, have also been identified (WHO, 2006).

In order to classify recreational waters as contaminated or unsafe, the level of faecal contamination of the water area should be compared with the amounts of appropriate bacteriological indicators. Sewage discharges, tributary oils, and contamination by infected bathers are considered to be the three most important factors affecting fecal contamination of recreational waters. In assessing microbiological water quality, the sampling program should collect typical representative samples of the tested habitat, and water samples should be routinely collected during environmental and sanitary inspections. It is also essential that a sufficient number of samples are collected to accurately estimate the levels of microbes to which recreational water users are exposed (WHO, 2003b). Monitoring the quality of water resources determines the suitability of water for the intended use.

Table 7. presents an overview of the guidelines set by the Canadian Council of Ministers of the Environment (CCME), the European Economic Community (EEC), the South African Department of Water Affairs and Forestry (Department of Water Affair and Forestry, DWAF) and the United States Environmental Protection Agency (USEPA) for microbiological parameters of recreational water quality.

Table 7. Overview of guidelines for microbiological indicators of recreational water quality

Microorganism	DWAF (1996)	EEC (2000)	CCME (2006)	USEPA (1992)
Escherichia coli	≤ 130/100 ml (recreation); 1/100 ml (irrigation)	0/100 ml	0/100 ml	≤ 400/100 ml (recreation)
Fecal coliforms	≤ 2000/100 ml (r recreation); 10000/100 ml (irrigation); 0/100 ml (household)	0/100 ml; ≤ 10/100 ml (swimming pools)	200/100 ml (recreation)	200-400/100 ml (recreation)
Enterococci	≤30/100 ml (recreation)	100/100 ml (recreation)	≤20/100 ml (recreation)	≤100/100 ml (recreation)
Pseudomonas aeruginosa	No numerical value is indicated	0/100 ml	≤2/100 ml (recreation)	No numerical value is indicated

Microbial contamination of rivers in Bosnia and Herzegovina, as in many other countries, can be greatly affected by precipitation, which leads to relatively short periods of increased fecal pollution. Contamination of recreational waters can lead to health problems due to the presence of infectious microorganisms, originating from human sewage or from animal sources (WHO, 2003a). The number of microorganisms that can cause infection (infectious dose) depends on the specific pathogen, the form in which it appears, the conditions of exposure, and individual susceptibility to infections as well as the immune status. For viral and parasitic protozoan diseases, infectious doses of viable infectious units can be low (Haas *et al.*, 1999; Okhuysen *et al.*, 1999; Teunis and Havelaar, 1999).

1.9. Wastewater

Wastewater is polluted, impure water, which contains impurities or dissolved substances of various origins, which have changed their quality and usability. Wastewater is most often discharged into surface waters as receivers. The total amount of waste water production and producers is increasing daily, which is evident from their increasingly changing qualitative composition (Bitton, 2005; Đukić and Ristanović, 2005; Mara and Horan, 2003). According to their characteristics, they are classified as: fresh, putrefactive, infectious, radioactive, and toxic. The properties of wastewater differ according to its origin, and are divided into municipal and industrial wastewaters. Agricultural wastewater, due to its characteristics and methods of disposal, belongs to a special group of waste water.

1.9.1. Municipal wastewater

Municipal waste water includes sanitary water containing human physiological waste, as well as wastewater from other household needs such as food preparation, cleaning, laundry, personal hygiene, etc. These waters also include atmospheric wastewater, which is formed as surface runoff from urban areas, which carries with it accumulated dirt. A significant characteristic of these waters is the high proportion of organic substances that can be degraded microbiologically into gases and substances with a bad smell and appearance. The amount of household wastewater depends on the average water use, which varies during the day, week and year. The basic characteristics of municipal wastewater are presented in table 8. (Đukić and Ristanović, 2005; Bitton, 2005).

Table 8. Characteristics of municipal wastewater (Đukić and Ristanović, 2005)

Origin	They are created as a result of economic and sanitary activities and physiological human waste.
Quantity	Limited by the limits of water use by the population for physiological and cultural sanitary needs.
External appearance	Similar (uniform)
Release mode	A limited degree of unevenness, which is determined by the sanitary conditions of the population.
Suspended solids	Standard in terms of quality and quantity.
Environmental reaction	Neutral or slightly alkaline.
Chemical composition	Homogeneous, dominated by organic compounds of animal or plant origin.
Toxicity	It is not inherent.
Typical composition properties	There are noticeable changes in concentration that depend on the level of water consumption.
Chemical significance	Predominantly epidemiological significance, and always general sanitation.
Disinfection method	Biologically at typical treatment plants.

1.9.1.1. Properties and composition of municipal wastewater

The composition of wastewater is diverse. Due to the aerobic processes of degradation of organic substances, these waters are poor in oxygen and rich in carbon dioxide, sulfates, nitrates, and many other compounds. The color of sewage water is usually yellow-brown or gray. They are intensely cloudy. Odor is influenced by anaerobic reduction processes that begin after consumption of available oxygen. The temperature varies from 6-12˚C, in winter while summer temperatures can reach up to 20˚C. Sewage water is slightly alkaline, with a pH of around 7.5. Substances present in wastewater are divided into suspended and dissolved substances. Suspended matter contains colloidal matter and particles, as well as living organisms. They are predominantly of organic origin, while dissolved substances are mostly inorganic. Inorganic substances present in sewage are essential ingredients of drinking water, or they can arrive from feces, urine, and general household waste. Chlorides mainly come from urine, and phosphate compounds from urine, feces and synthetic detergents. Nitrogenous compounds in sewage water are ammonia, urea and amino acids which can be free or in the form of proteins. Urine contributes the most nitrogen due to the high amount of urea it contains (Bitton, 2005; Mara and Horan, 2003).

In addition to many organic and inorganic dissolved substances and particles, municipal wastewater also contains insects, arthropods, small fish, solid waste, toxic gases, emulsions, pesticides, herbicides, poisons, drugs, as well as a large number of pathogenic and non-pathogenic microorganisms (> 100,000/ml) (Tyagi *et al.,* 2006). From the health and hygiene point of view, the most important components of wastewater are the causative agents of infectious and parasitic diseases, which, especially if they are present in larger numbers, represent a serious threat to the further use of water from the receiver (Massman *et al.,* 2004).

Escherichia coli

Escherichia coli is a Gram-negative rod found in the gastrointestinal tract of all warm-blooded animals where it forms part of the intestinal flora. Several strains are pathogenic agents of gastrointestinal diseases. The five classes of pathogenic *E. coli* include enterotoxic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroaggregative *E. coli* (Kuntz and Kuntz, 1999; Rusin *et al.,* 2000).

Enterotoxic (ET) *E. coli* causes traveler's diarrhea and has also been identified as a cause of diarrhea in infants and children. The disease is caused by two toxins, a heat-labile toxin and a heat-stable toxin. ET *E. coli* is species specific, meaning that humans carry strains that infect other humans. After ingestion and incubation from 10 to 72 hours, cramps, vomiting, severe diarrhea and dehydration occur, which can last for three to five days. A case of ET *E. coli* poisoning was reported in the USA, which occurred by consuming water contaminated with human sewage (Rusin *et al.,* 2000).

Enteropathogenic (EP) *E. coli* mainly infects children under one year of age. Symptoms of infection are watery diarrhea with mucus, fever and dehydration. Although the disease is under control in North America, Europe and Australia, it is still a major cause of infantile diarrhea in South America, Africa and Asia (Smith and Cheasty, 1998; Rusin *et al.,* 2000).

Enteroinvasive (EI) *E. coli* infections resemble Shigella infections. The disease begins with severe abdominal cramps, watery stools and fever, affects all age groups and is self-limiting, with no known complications. It is unclear whether food transmits enteroinvasive *E. coli,* but food contaminated with human feces can cause illness either directly or through contaminated water. (EI) *E. coli* spreads to neighboring cells through intracellular bacterial multiplication, causes cell death, and then inflammation and ulceration of the colonic mucosa (Menard *et al.,* 1996; Rusin *et al.,* 2000).

An enterohemorrhagic (EH) or verocytotoxic strain of *E. coli* was first described in 1982. *E. coli* produces two toxins, verotoxin I and II, which are very similar to the toxin produced by *Shigella dysenteriae*. Symptoms of the disease include severe cramps and diarrhea (Riley *et al.,* 1983; Rusin *et al.,* 2000). The disease is self-limiting and can last up to eight days. Younger people can develop hemolytic uremic syndrome, which results in kidney damage and hemolytic anemia. The result can be permanent damage or even kidney failure. In elderly patients, additional symptoms include fever and neurological effects, which may lead to thrombotic thrombocytopenic purpura. In the elderly, the disease is fatal in 50% of cases (Smith and Cheasty, 1998).

Enteroaggregative (EA) *E. coli* has been implicated as a cause of diarrhea in children in developing countries, and can also result in serious and even fatal disease. There are indications that this organism is responsible for food poisoning in developed countries. This bacterium adheres to the intestinal mucosa and secretes enterotoxins and cytotoxins resulting in secretory diarrhea and mucosal damage (Nataro *et al.,* 1998).

2. AQUATIC SYSTEM OF THE NERETVA RIVER

The Neretva is the largest and hydrologically richest tributary of the Adriatic Sea in the Balkans. It is the largest karst river in the eastern part of the Dinaric Alps. It springs in the area of Borče, at 1227 m altitude, below the mountain Jabuka (2100 m), in the southeastern part of Bosnia and Herzegovina. Regionally, the Neretva River belongs to the territory of Herzegovina, with a total length of 230 km, of which only the last 22 km belong to Croatia (Figure 9.).

According to the recent reorganization of Bosnia and Herzegovina, Canton 7 is located in this part of the Federation of Bosnia and Herzegovina, which was named Herzegovina-Neretva Canton (HNK) after the Neretva, its largest river. The Neretva system hydrographically drains the largest part of the Adriatic Sea basin. The catchment area is about 10,500 km^2 (Samokovlija Dragičević, 2003). This area is known for its exceptional natural heritage, diversity of landscapes, and natural beauty.

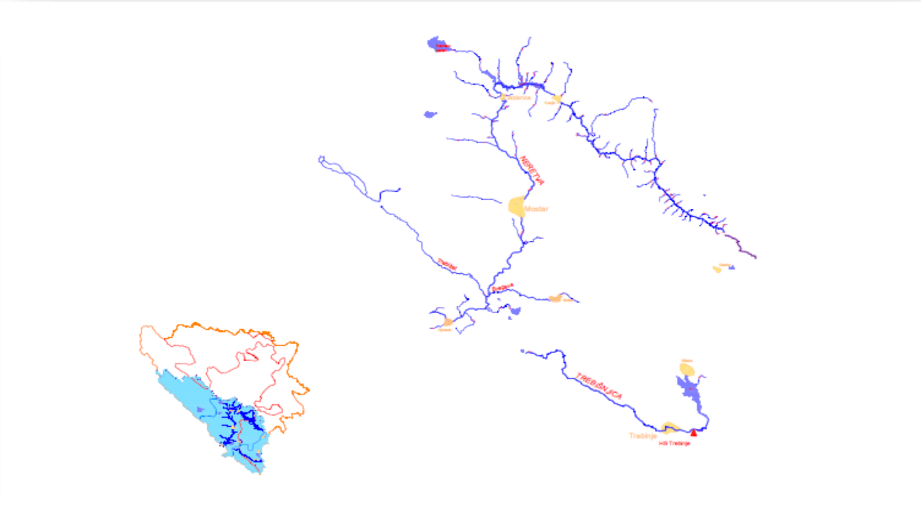


Figure 9. Geographical location of the Neretva river basin. For most of its course, the Neretva flows through Herzegovina. Together with Trebišnjica, it drains the Herzegovinian karst and the hydrologically rich soil, carrying large amounts of water into the Adriatic Sea. (WWF project Living Neretva)

2.1. Geographical and morphological characteristics of the Neretva basin

The area of the Neretva river basin is hydrologically specific, with a limestone-dolomite composition, and is characterized by extremely rich groundwater and sinkholes. Geographically and hydrologically, the Neretva is divided into three catchments, upper, middle and lower. Due to the mountainous relief of the source, the river Neretva is characterized by the specificity of its position: the upper catchments have the character of alpine rivers with a greater drop, many rapids and narrow valleys and strong mechanical strength (Variščić, 2004).

The upper reaches of the Neretva stretch from the source to Konjic. The source and source gorge are located deep in the Dinaric Alps at 1227 m altitude. Through this relatively isolated and craggy limestone terrain, the Neretva flows in untouched rapids and waterfalls through steep gorges up to 600-800 meters deep. In its upper and middle reaches, the Neretva flows through a valley that runs parallel to the Dinaric mountain range, from south to northwest, and covers about 1,390 km^2 with an average fall of 12% (Variščić, 2004). The water of the upper reaches of the Neretva belongs to the first quality class. This part of the Neretva River is hydrologically extremely rich with numerous streams and springs, three large lakes, in addition to numerous others in the wider area of the upper Neretva on the mountains Treskavica (2086 m), and Zelengora (2015 m) with Lelija (2032 m). The basin of the upper course of the Neretva is surrounded by the mountains Visočica (1974 m), Bjelašnica (2067 m), Bitovnja (1700 m) and Vranica (2084 m), and this area is characterized by an exceptional biodiversity of flora and fauna, a high degree of endemism, and represents an invaluable natural resource of Bosnia and Herzegovina and Europe.

The middle course begins with the confluence of the Rama between Konjic and Jablanica, where the Neretva suddenly turns south. From there, the Neretva valley cuts through the mountain ridges at right or sharp angles. This part of the Neretva River is characterized by canyon sides with a height of 800 to 1200 m, with very little vegetation. In the canyon, the Neretva is very fast, with rapids and smaller waterfalls. The gorges are built of limestone, which according to geologists are older than 175 million years (Variščić, 2004). Below Konjic, the Neretva briefly expands into a wide valley with valuable agricultural land. The large artificial lake Jablanica was formed after the construction of a dam near Jablanica. From Jablanica, the Neretva enters the largest canyons of its course, and flows through the steep slopes of the mountains Prenj (2103 m), Čvrsnica (2228 m) and Čabulja (1776 m). Coming out of the canyon near Salakovac, the Neretva flows at the foot of the Velež mountain (1969 m) through the Bijelopolska valley, the Mostar valley and the Mostar field, and then enters its lower course.

The lower course of the Neretva River with its complex hydrography and the influence of the sea begins downstream from Počitelj. In this part, the Neretva becomes calm and navigable, it is much richer in water and has the characteristics of a lowland river. The last 30 km of the Neretva's course form a specific meandering delta. This extremely rich alluvial delta is listed under the Ramsar Convention as internationally significant.

2.2. Tributaries

In the upper and middle course, the Neretva River receives its tributaries Ljuta, Jasenica, Rakitnica, Trešanica, Neretvica, Rama, Doljanka, Drežanka, and Radobolja from the right side, while the Lađanica, Krupac, Bijela, Idbarčica, Glogošnica, Šištica, Bištica, and Prenjska rivers , flow in from the left. The right tributaries have a longer course with sources at higher altitudes, while the left tributaries are short, generous with water, and with sources only a few tens of meters above the river level. The most important tributaries of the Neretva river downstream from Mostar are: the right tributary Jasenica (drains the waters of the karst fields Rakitno, Kočerinsko and Mostarsko blato through tunnels and channels), Lištica with Ugrovac, and the left tributaries Buna with Bunica and Bregava. In the lower reaches of the

Neretva, it accepts the waters of the large sinkholes of Trebižat and Krupa. Trebižat brings water from the Ričina basin, the Suvaja and Vrljika basins, and the Tihaljina and Mlada basins. Krupa emerges from Hutovo Blato, and is connected to the Trebišnjica River in Popovo polje. In addition to these tributaries, the Neretva is fed by permanent and occasional karst springs and hot springs.

2.3. Hydrogeological and hydrographic characteristics of the Neretva basin

The main characteristic of the Neretva basin is the developed karst watercourses. The fundamental characteristics of the karst watersheds are extensive zones of water collection in mountainous areas and very complex springing conditions at the contacts of weathered permeable carbonate deposits and impermeable rocks. The flow of water is linked to fracture systems, and is characterized by high speeds of underground flows and the occurrence of strong karst springs with large discharge amplitudes. Due to the small retention capacity of the aquifer, the summer period is characterized by a significant decrease in discharge at the springs, and sometimes by complete drying up. Groundwater quality is generally very good, and the only problems are occasional turbidity and bacteriological pollution of the source as a result of heavy precipitation, especially after a long dry period (Fritz and Ramljak, 1992; Herak, 1986).

Watercourses within the Neretva basin have very complex water regimes, characterized by the following phenomena: extremely uneven flow caused by seasonal unevenness of precipitation, which is intense during autumn and winter, and moderate during the growing season; drainage is mainly carried out through the karst subsoil; the orographic and hydrogeological boundaries of the river basins differ significantly due to the complex network of underground karst paths that often have bifurcations, which allows flow in different directions (Herak *et al.*, 1982).

The boundaries of the Neretva watershed can be determined with relative certainty only upstream of Jablanica, because downstream the river flows through extremely karstic terrain where it is difficult to determine the zones of influence, and therefore it is very difficult to determine the boundaries of the watershed. Above-ground and underground watersheds do not coincide, so the stated total basin area of 10,500 km^2 should be taken with caution, because the actual area of the basin could only be determined after extremely extensive, long-term and expensive geological research (Samokovlija Dragičević, 2003). The Neretva brings more than 8,830,000,000 m^3 into the sea annually. The average speed of water flow at the measuring station Žitomislići, south of Mostar, is 378 m^3/s (Goluža, 2006). Water levels are the highest in April and the lowest in August.

Hydropower plants with large artificial hydroaccumulations were built on the Neretva River and its main tributaries. The middle and lower courses of Neretva are largely controlled by four large dams (over 15 m) and their hydroaccumulations, which control water levels downstream. The Jablanica lake with an area of 14 km^2 was created after the construction of the HPP Jablanica. HPP Rama with a large reservoir was built on the river of the same name, and two HPPs with one large hydroaccumulation lake on the river Trebišnjica, which is considered part of the Neretva basin. Between Jablanica and Mostar, the Neretva was dammed three more times by hydroelectric power plants with the Grabovica, Salakovac and Mostar reservoirs, resulting in loss of its former natural appearance and function.

Figure 10. Hydroelectric power plants with hydroaccumulations on the Neretva basin (Samokovlija Dragičević, 2003)

2.4. Climatic characteristics of the area

The climate represent a natural framework that conditions the development of natural processes and life activities. The most important climatic parameters for surface water systems are temperature, amount and intensity of precipitation, evaporation and evapotranspiration. The climatic characteristics of the Neretva river area vary with the distance from the sea. The lower stream has a Mediterranean climate, the middle part has a continental climate, and the upper stream has a mountain climate. The temperature ranges from -29 to +43°C, and the annual evaporation is 500 - 900 mm. The average annual flow of the river is 269 m^3/s, the lowest is 44 m^3/s, and the highest is 2179 m^3/s (Margeta and Fistanic, 2000).

In the researched area of the Neretva river basin, the sub-Mediterranean climate prevails, with mild winters, warm summers and abundant precipitation during the colder part of the year. The lowest average temperatures in January are between 3.4 and 4.8˚C, while the average temperatures in July are over 24˚C, and the maximum temperature is over 40˚C. The annual amount of precipitation is from

1000 mm to over 1800 mm. During the summer months, the amount of precipitation is below 30 mm, and during the late autumn and winter the maximum values reach up to 150-230 mm/month (the maximum values are in December). The mildest climate is in the Neretva valley in the lower and middle reaches. The strongest winds are bura and yugo. In the winter period in the area of Herzegovina, the bura is dominant and brings dry and cold weather. Yugo blows from the direction of the Adriatic Sea and is pronounced in autumn and spring. It is saturated with moisture and brings rain in autumn.

A very important physical characteristic of water is its temperature, because water temperature is a limiting factor in the development of aquatic biotope organisms. The activity of plants and animals in the water depends on daily and seasonal variations, and large deviations have a disastrous effect on the living world. Average minimum water temperatures of the Neretva were measured in February (2.6˚C), and maximum in July (12.5˚C). The Neretva is one of the coldest rivers in the world, with upper water temperatures of only 7–8˚C during the summer months.

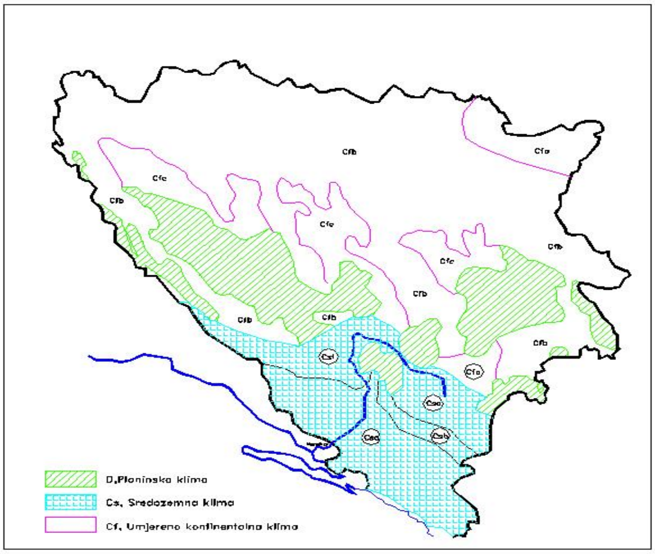


Figure 11. Main climate types in Bosnia and Herzegovina (Jukić, 2006)

Bearing in mind the multiple importance of the Neretva River for the population of Herzegovina and the wider region, as well as the scarce monitoring programs and scientific-research works related to water quality from a physical-chemical, and especially microbiological aspect, the goal of research into this river system was set.

The main goal of this work was to implement an extensive monitoring program of the, in order to assess the current state of water quality, and to examine the impact of municipal waste water on the physico-chemical and microbiological characteristics of the Neretva, Radobolja, Buna, Bunica, Bregava and Trebizat rivers, and Salakovac and Mostar lakes. In addition, the goal was to monitor and analyze changes in selected parameters and water quality oscillations throughout the seasons, and to analyze spatial longitudinal variations in the quality of the Neretva River's water flow through Mostar and to identify localities that show the greatest pressure of anthropogenic influence.

The work process was approached by defining the following tasks: selection of locations for water sampling in the investigated area; selection of appropriate physical, chemical and microbiological quality indicators; determining the current state of water quality by analyzing the measured values of certain parameters from selected measuring points, monitored during 20 month cycles; assessment of the degree of damage to the Neretva River; statistical processing of the obtained results of physico-chemical and microbiological analyzes and interpretation of the results in accordance with their accuracy and reliability; calculation of water quality index and accompanying data analysis.

3. *Materials and Methods*

3.1. Selection of water quality parametres

Water quality is a basic and most significant characteristic of any aquatic ecosystem. It is a predetermining factor for development of aquatic biota. As such, water quality assessment is a fundamental aspect for research and conservation of aquatic resources. It is a clear indication of ecosystem health which allows direct identification of threats to homeostasis of an aquatic ecosystem. Selection of parametres used in water quality assessment is a crucial step in order to obtain precise and accurate data on condition of water and possible sources of impairment. Regular monitoring of water quality detects changes (good and bad) and suggests remediation measures. Monitoring is necessary to identify problems and focus attention to where it is needed the most. Water can thus be classified and its potential usage determined. Parametres chosen are the most common ecological indicators of water quality and represent several different categories of waterway impairment: water temperature, dissolved oxygen, pH, electrical conductivity, total coliforms and *Escherichia coli*.

The temperature of water influences and regulates many chemical, physical and biological processes, including metabolic rates of aquatic organisms. Importantly, it regulates oxygen solubility in water and influences autopurification rate. Water temperature is essential to determine a range of other parametres. Stability of water temperatures over time is a main indicator of an aquatic ecosystem stability.

Important indicator of the condition of an aquatic ecosystem is the concentration of oxygen dissolved in water. Oxygen is essential for respiration and survival of aquatic organisms. In a fast flowing, large and turbulent stream such as Neretva, oxygen uptake is considerably higher compared to slow moving streams and lakes. Concentrations of oxygen in water will vary depending on the physical, chemical and biochemical activities in the river. Under normal circumstances, dissolved oxygen measurements in streams are usually 9 to 12 mg/l.

Oxygen concentrations will vary over a 24-hour (diurnal) cycle, even in pristine waterbodies, due to rates of photosynthesis, (Ridanovic, *et al.*, 2010).

pH value of an aquatic ecosystem is an essential paramtere as it is closely related to biological productivity. pH value between 6.5-8.5 indicates good water quality and it is usually characteristic for surface waters. Neretva's basin is mildly alkaline due to its karstic substrate.

Electrical conductivity is the capacity of water to conduct electrical current and represents degree of mineralization of water. It is directly proportional to concentration of dissolved ions and can be used to detect sources of pollution. Conductivity is increased e.g., due to presence of phosphates, nitrates and chlorides.

It is crucial that selection of parametres reflects the most significant categories of waterway impairment. Sewage outflows into Neretva and its tributaries have negative impacts on water quality, which is obvious from hygienic and aesthetic aspects. Wastewater can affect receiver waters in a variety of ways, causing degradation of water quality by physical, chemical and biological pollution, to the extent that water becomes harmful to human health (Ridanovic, *et al.,* 2017). Coliforms and fecal coliforms are direct indicators of water quality and communal wastewater overload (Dukic and Ristanovic, 2005; Bitton, 2005). Presence of indicator bacteria in water highly correlates with presence of pathogenic organisms that may cause serious water-borne infections (Boufafa, *et al.,* 2020).

This study aims to investigate degree of correlation of total coliforms as the general bacteriological water quality indicator with electrical conductivity as a measure of water mineralization and determine whether there is any predictive value in testing these parametres. Electrical conductivity is often overlooked in WQM protocols and should be given more emphasis due to its ease of measurement and extensive set of data provided by EC value alone. It was also aimed to obtain data on bacteriological water quality and examine bacteriological load of aquatic ecosystems at selected sites.

3.2. Selection of sampling sites

Sampling was conducted at river Neretva, its five tributaries and two large artificial lakes on Neretva. Two sites were selected at each river in order to avoid experimental bias and to collect representative samples across the research area. The sites were selected based on proximity to major pollution sources. Aquatic ecosystems tested were: Lake Salakovac, Lake Mostar, and five rivers: Neretva, Radobolja, Buna, Bunica, Bregava and Trebizat.

Water parametres were measured by: a digital thermometre with automatic calibration (Hanna), pH-metre with electrode (Hanna), conductometre with automatic temperature compensation (Trans Instruments), digital oxymetre (Greisinger electronic). Microbiological sampling was conducted during two seasons spring (May) and autumn (November) to reflect any differences in microbial numbers after prolonged draught during summer and after spring floods, i.e., high and low water levels. Samples were analysed at Institute for Public Health FBIH, by BAS EN ISO 9308-1/2015 method.

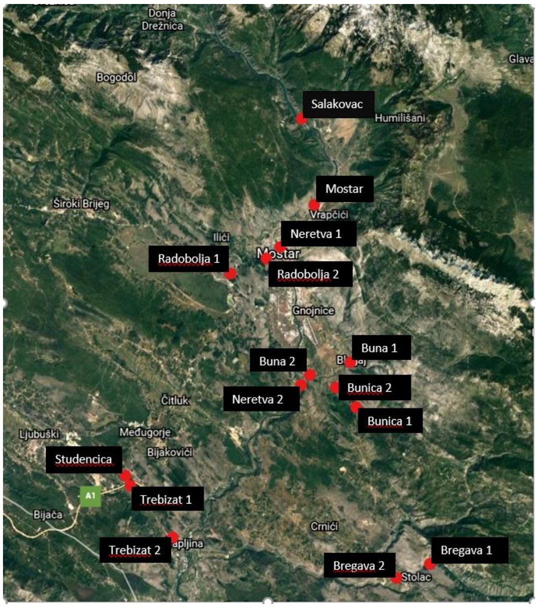


Figure 12. Microbiological sampling sites

During the selection of sampling sites, safety and the ease of access to water with all sampling equipment were taken into account. As part of the fieldwork, a detailed visual inspection of watercourses in the research area was carried out. The selected sampling locations are river areas with the most significant anthropogenic impacts.

Neretva

A total of seven sites on the Neretva were selected, with a distance between the first and the last of about 55 km. The first locality is located on Lake Salakovačko, 20 km north of Mostar. The second locality is Mostar lake in the northern suburb of Mostar. In the narrower city area, two localities, Carina and Mahala, were selected, upstream and downstream from significant sources of pollution. The fifth locality is located on the Neretva river downstream of the Buna canals and the mouth of the Buna. The sixth locality is 20 km south of Mostar in Žitomislići, while the seventh is in Čapljina.

Radobolja

The only river that flows through the City of Mostar. It is exposed to strong anthropogenic influence. Radobolja was assessed at 8 sites.

Buna and Bunica

Buna and Bunica were assessed at five sites each, starting at the source of the rivers, followed by three sites along their middle courses, with final sites being in proximity to the estuaries.

Bregava

Bregava was assessed at four sites. The first one was on the locality Do, in vicinity of mHHP. The second and third site were upstream from Stolac, while the fourth site was within the urban centre of Stolac. The area downstream from the town was not regularly assessed due to inaccesibility of the terrain, the river sinks underground, delta is usually dry, for at least 6 months annually.

Trebizat

Trebizat was assessed at 10 sites. The sheer lenght of the river from its sourcce at Pec Mlini to its estuary in Neretva demanded a large number of sites for sampling to account for specificities along its watercourse.

3.3. ***Monthly monitoring program***

In order to assess the ecological situation in detail and to carry out a detailed analysis of water quality, the tests were carried out every month from May 2020 to December 2021. They covered the complete cycles of seasonal variations. Many physical, biological and chemical characteristics of river systems vary temporally as a result of seasonal changes in climate and environmental conditions. Sampling periods are precisely determined to account for temporal variation and maximize data consistency and reproducibility. The late summer period is characterized by high temperatures and minimal water levels. The river ecosystem is most susceptible to anthropogenic stress during the low water levels in late summer.

Precipitation that reaches the area can directly and/or indirectly cause short-term changes in primary production and the values of some parameters. Intense long-term precipitation increases the water level and the speed of water flow. Increased inflow of wastewater flowing from city streets can result in increased turbidity, increase or decrease water temperature, and accelerate oxygen consumption and nutrient breakdown. The increase in cloud cover associated with precipitation can also significantly reduce solar heating of water and air. In a similar way, larger temporal (eg, shorter days, colder temperatures) and spatial (eg, reduced coastal vegetation and shade, land cultivation) changes affect the rate of primary production and respiration. In order to take into account the potential effects of short-term climatic phenomena (eg, rain, air temperature and solar heating) on the values of the measured parameters and to assess river processes during sampling, fieldwork was not conducted for at least five days after a major precipitation event.

4. Results and Discussion

4.1. Temporal and spatial variations of physico-chemical parameters of water in the Neretva basin

Each water system has unique physical and chemical properties, which largely depend on the climatic, geomorphological and geochemical conditions in the basin and the underlying aquifer. In absence of human activitues, up to 90-99% of global fresh waters would have natural chemical concentrations suitable for aquatic life and for human consumption. Research of the water system of the Neretva river included determination of certain physico-chemical parameters, with the aim of obtaining as realistic and complete a picture of the current state of the Neretva as possible, considering the pronounced anthropogenic influence, and the discharge of untreated municipal wastewater.

The main goal of this work is to determine the impact of anthropogenic pollution on the physico-chemical and microbiological characteristics of water in the lower Neretva river basin, and to determine the level of pollution of the Neretva and its tributaries, through a detailed analysis of microbiological and physico-chemical indicators of water quality. The research is based on incomplete bacteriological data on the population of indicator microorganisms and physical-chemical indicators of water quality.

All parameters were analyzed in monthly cycles, in the period from May 2020 to December 2021, on seven rivers and two lakes. A total of 40 sites was assessed. The water quality in the Neretva river as evaluated based on the Regulation on the categorization of watercourses "Official Gazette FBiH", number 2/92 and 13/94.

WQM Lake Salakovac				
	Water Temperature °C	pH	Electrical Conductivity (μS/cm)	Dissolved Oxygen (mg/ml)
May-20	13.2	8	200	10.2
Jun-20	15.2	8.3	200	10.1
Jul-20	21.9	8.4	200	9.2
Aug-20	22.9	8.3	300	8.7
Sep-20	21.7	8.5	300	9
Oct-20	15.9	8.5	200	9.1
Nov-20	11.2	8.4	300	10.5
Dec-20	8	8.4	300	12
Jan-21	7.2	8.7	200	10.2
Feb-21	10.3	8.6	200	9.6
Mar-21	9.2	8.3	200	10.7
Apr-21	8.7	8.4	200	10.4
May-21	12.2	8	200	9.9
Jun-21	22.3	8.2	200	9
Jul-21	22.9	8.2	200	10.1
Aug-21	23.6	8.1	300	8.5
Sep-21	21.9	8.4	200	9.3
Oct-21	15.1	8.4	200	9.4
Nov-21	10.8	8.2	300	9.9
Dec-21	8.4	8.2	300	11.3

WQM Lake Mostar				
	Water Temperature °C	pH	Electrical Conductivity (µS/cm)	Dissolved Oxygen (mg/ml)
May-20	11.7	8	200	10.4
Jun-20	15.1	8.2	200	10.2
Jul-20	20.7	8	200	10.4
Aug-20	22.4	8.1	300	10.1
Sep-20	21	8.1	200	10.2
Oct-20	13.7	8.3	200	9.6
Nov-20	12.4	8.2	300	10.2
Dec-20	8.7	8.3	300	11.6
Jan-21	8.4	8.6	200	11.1
Feb-21	8.3	8.4	200	9.9
Mar-21	9.1	8.1	200	10.7
Apr-21	9.3	8.5	200	10.5
May-21	12.2	8	200	9.9
Jun-21	21.1	8.1	200	9.6
Jul-21	21.6	8.2	200	8.6
Aug-21	23.1	8.2	300	10.2
Sep-21	21.3	8.2	200	10.8
Oct-21	13.3	8	200	10.2
Nov-21	11.4	8.3	300	10.6
Dec-21	8.9	8.2	300	11.5

WQM Neretva (Average values)				
	Water Temperature °C	pH	Electrical Conductivity (µS/cm)	Dissolved Oxygen (mg/ml)
May-20	12.56	8.06	200	10.5
Jun-20	13.12	8.22	220	10.66
Jul-20	15.1	8.6	220	10.52
Aug-20	15.36	8.1	300	10.02
Sep-20	15.44	8.1	220	10.18
Oct-20	13.58	8.06	200	10.6
Nov-20	11.36	8.08	300	10.92
Dec-20	9.1	8.2	233.3	11.33
Jan-21	7.7	8.65	225	11.92
Feb-21	8.5	8.5	250	10.4
Mar-21	9.4	8.42	200	11.3
Apr-21	9.92	8.54	200	10.82
May-21	11.54	8.4	200	10.98
Jun-21	13.62	8.12	200	9.66
Jul-21	15.98	8.1	240	9.98
Aug-21	16.02	8.1	300	10.46
Sep-21	15.5	8.12	240	10.38
Oct-21	12.82	8.28	240	10.4
Nov-21	11.32	8.2	300	10.94
Dec-21	9	8.23	266.7	11.7

WQM Radobolja (Average values)				
	Water Temperature °C	pH	Electrical Conductivity (µS/cm)	Dissolved Oxygen (mg/ml)
May-20	12.8	8.38	300	11.54
Jun-20	14.18	8.21	300	9.74
Jul-20	16.15	8.31	266.66	11.1
Aug-20	15.3	8.13	300	10.3
Sep-20	13.1	8.06	300	10.1
Oct-20	11.32	8.32	200	10.67
Nov-20	11.31	8.12	200	10.67
Dec-20	10.87	8.12	271.43	11.7
Jan-21	10.64	8.12	257.14	11.54
Feb-21	11.07	8.21	257.14	11
Mar-21	11.28	8.31	257.14	10.78
Apr-21	10.97	8.5	257.14	10.78
May-21	12.67	8.55	237.5	10.88
Jun-21	15.44	8.3	242.85	10.17
Jul-21	15.96	8.1	280	10.26
Aug-21	13.25	8.17	300	10
Sep-21	13.75	8.07	325	10.22
Oct-21	11.41	8.21	214.28	10.65
Nov-21	11.28	8.21	200	10.85
Dec-21	10.78	8.16	275	11.68

WQM Buna (Average values)				
	Water Temperature °C	pH	Electrical Conductivity (μS/cm)	Dissolved Oxygen (mg/ml)
May-20	11	7.84	240	10.74
Jun-20	11.7	7.98	200	11.56
Jul-20	12.7	8	240	10.26
Aug-20	11.9	7.96	200	10.6
Sep-20	12.74	8.16	240	9.84
Oct-20	10.6	7.88	300	11.12
Nov-20	10.6	8.14	300	11.9
Dec-20	10.72	7.95	325	11.62
Jan-21	9.77	8.2	275	11.22
Feb-21	9.32	8.32	200	10.7
Mar-21	10.28	8.26	200	10.8
Apr-21	10.28	8.44	200	9.74
May-21	10.32	8.26	200	10.78
Jun-21	10.82	8.42	220	10.42
Jul-21	13.46	8.14	240	10.4
Aug-21	13.1	8.12	240	10.76
Sep-21	14.12	8.22	260	10.38
Oct-21	11.3	8.08	300	9.96
Nov-21	10.13	8.1	300	10.53
Dec-21	10.12	8.15	300	11.3

WQM Bunica (Average values)				
	Water Temperature °C	pH	Electrical Conductivity (μS/cm)	Dissolved Oxygen (mg/ml)
May-20	12.74	7.72	300	10.68
Jun-20	13.44	7.7	300	11.08
Jul-20	15.54	7.78	300	10.96
Aug-20	13.93	7.73	300	10.66
Sep-20	14.46	7.76	300	10
Oct-20	11.68	7.92	300	11.44
Nov-20	11.06	7.86	340	11.62
Dec-20	10.25	7.85	350	11.8
Jan-21	10.16	8.16	266.67	11.33
Feb-21	6.76	8.3	200	11.04
Mar-21	10.2	8.14	200	11.32
Apr-21	12.08	8.28	260	10.24
May-21	12.66	8.02	260	11.12
Jun-21	15.06	8.44	320	11.38
Jul-21	17.02	8	280	11.08
Aug-21	17.72	8.06	300	10.38
Sep-21	16.96	8.08	300	9.74
Oct-21	12.74	8.22	300	11.42
Nov-21	11.38	8.1	320	10.98
Dec-21	10.65	8.15	300	10.8

WQM Bregava (Average values)				
	Water Temperature °C	pH	Electrical Conductivity (µS/cm)	Dissolved Oxygen (mg/ml)
May-20	14.92	8.32	300	11.1
Jun-20	13.67	8.3	225	11.2
Jul-20	14.05	8.25	225	11.4
Aug-20	16.27	8.22	250	10.6
Sep-20	16.82	8.22	300	9.85
Oct-20	11.27	8.17	400	11.52
Nov-20	10.25	8.27	300	11.65
Dec-20	10.72	8.22	300	11.2
Jan-21	10.47	8.25	300	11.25
Feb-21	10.8	8.3	300	10.65
Mar-21	11.15	8.27	325	10.85
Apr-21	10.92	8.32	350	10.42
May-21	11.47	8.17	300	10.12
Jun-21	13.67	8.4	250	9.82
Jul-21	14.5	8.1	275	11.52
Aug-21	15.8	8.12	275	10.65
Sep-21	16.2	8.15	325	10.05
Oct-21	11.57	8.12	325	11.22
Nov-21	10.15	8.22	300	11.22
Dec-21	10.35	8.15	325	11.12

WQM Trebizat (Average values)				
	Water Temperature °C	pH	Electrical Conductivity (µS/cm)	Dissolved Oxygen (mg/ml)
May-20	14.67	7.95	800	9.71
Jun-20	16.12	7.92	844.44	10.1
Jul-20	16.85	7.88	822.22	9.72
Aug-20	15.86	7.91	850	9.47
Sep-20	15.81	8.01	733.33	9.31
Oct-20	12.44	8.06	833.33	11.17
Nov-20	11.36	8.16	425	10.6
Dec-20	10.7	8.1	574	10.35
Jan-21	10.45	8.22	475	11.85
Feb-21	11.47	8.22	477.77	10.7
Mar-21	12.14	8.4	544.44	10.42
Apr-21	13.24	8.42	588.88	10.1
May-21	13.98	8.43	533.33	10.4
Jun-21	15-76	8.57	766.66	9.95
Jul-21	16.61	7.95	875	9.95
Aug-21	16.74	8.11	800	9.27
Sep-21	16.36	8.08	900	9.66
Oct-21	12.66	8.21	777.77	10.48
Nov-21	11.26	8.17	788.88	10.94
Dec-21	10.76	8.12	460	10.12

4.2. Water temperature

Water temperature affects the concentrations of many parameters, as well as physical, chemical and biological processes in water, and is a necessary parameter in every sampling regime. Water temperature values are uniform in all localities and show a high degree of agreement without significant deviations. There are normal seasonal variations that follow the natural annual cycle, related to the geographical location and climatic conditions of the researched area.

Lake Salakovac: minimum temperature of 7.2ºC was measued in winter (January), while the maximum of 23.6ºC was recorded suring summer (August). At the Lake Mostar: minimum temperature of 8.3ºC was measured in February, while the maximum of 23.1ºC was reached in August. In Neretva: minimun value of 7.2ºC was recorded in Junary, while the maximum of 16.9ºC was reached in August. At Radobolja: the lowest temperature of 9.9ºC was measured in December, while the highest at 19.1ºC was recorded in July. In Buna: minimum of 8.1ºC was in February, while the maximum of 19.3ºC was recorded in September. At Bunica: the lowest temperature of 6.4ºC was recorded in February, while maximun of 21.6ºC was measured in August. In Bregava: the lowest temperature was 9.7ºC in Nevember, and the highest 19.9ºC was recorded in September. In Trebizat: the lowest value was 10ºC in February, and the highest of 23.3ºC was in September.

Relatively low summer water temperatures are favorable for numerous chemical characteristics and biological processes, such as the concentration of nutrients, faster decomposition of organic pollutants, etc. The results also show that the temperature of the water does not change significantly with the flow, which also means that there are no sudden fluctuations in the increased discharge of wastewater. During the summer months, in addition to the high ambient temperature, the low water level and the decrease in water flow contribute to the increase in water temperature.

At the Salakovac and Mostar reservoirs, the influence of hydropower plants on the natural flow of the Neretva is pronounced. The watercourse is relatively calm and even with a depth of about 12 m. The shores are rocky with scanty vegetation. In the narrower city area, the course of the Neretva is fast and timid with beech trees, waves, streams and springs. In some places, it calms down, creating inlets in the coves of the coastal belt. The water depth is variable and in the summer it reaches up to 9 meters. It is characterized by coasts with steep rocks, underwater and above-water caves, and numerous sources of drinking water. Coastal vegetation is lush with characteristic Neretva vegetation: wild roses, ash trees, lianas, brambles, beyturans, hemp, ivy, sycamores, russets, mulberries, holm oaks, prilipi, janarik, lovage, wild figs, rosehips, blackberries and willows (Mahić *et al.*, 2007). At the selected site on Buna, the Neretva is characterized by a fairly calm flow without pronounced springs and springs, and banks with low rocks rich in vegetation.

4.3. Concentration of H^+ ions

pH values showed an interesting trend during the monitoring cycle. A significant decrease in the pH value is noticeable in the winter period, as a result of the increased decomposition of organic substances (increased turbidity) and the production of carbon dioxide, which lowers the pH value. Low winter pH values are followed by a significant increase in the spring period as a result of increased primary production.

Lake Salakovac: the lowest pH value of 8ºC was measured in the spring period (May), while the highest value of 8.7ºC was recorded in the winter months (January). Lake Mostar: the lowest pH value of 8ºC was measured in the spring and autumn periods (May and October), while the highest value of 8.6ºC was recorded in the winter months (January). Neretva: the lowest value of 7.9ºC was measured during the spring and summer months, while the highest temperature value of 8.8ºC was recorded in the summer months (April) in Čapljina. Radobolja: the lowest value of 7.9ºC was measured in the winter period (November, December, January, February) at Vrelo, while the highest value of 9.2ºC was recorded in the spring months (May). Buna: the lowest pH value of 7.6ºC was measured in the spring period (May), while the highest value of 8.8ºC was recorded in the summer period (June). Bunica: the lowest pH value of 7.5ºC was measured in the summer period (June), while the highest value of 8.5ºC was recorded in the winter period (January). Bregava: the lowest pH value of 8ºC was measured in the summer period (July), while the highest value of 8.4ºC was recorded during most of the year. Trebizat: the lowest pH value of 7.4ºC was measured in the summer period (July), while the highest value of 8.7ºC was recorded in the spring period (March).

The pH values affect aquatic life and the use of water for recreational purposes. Bacteria survive in the pH range of 5-10, while the optimal values of this factor are in the range of 6.5-8.5. All measured pH values belong to the first class of watercourses.

4.4. Electrical conductivity

Electrical conductivity is a measure of water's ability to conduct electricity and directly depends on the concentration of ionized substances in the water. Electrical conductivity was relatively uniform at the measuring sites during the test period, while seasonal unevenness was observed, with somewhat lower values recorded in spring, as a consequence of the increase in primary production and consumption of dissolved salts.

At all sites with exception of Trebizat the electrical conductivity values were uniform throughout the monitoring period, and ranged between 200 and 400 (μS/cm), regardless of the season. Trebizat: the lowest value wsa 300 (μS/cm) at Tihaljina and maximum was 1300 (μS/cm) at Ribnjak Mlade. According to the obtained electrical conductivity results, there is no significant contamination with inorganic substances. This confirms the acceptable limit of electrical conductivity and does not give cause for concern. All measured values of electrical conductivity belong to the I class of watercourses.

4.5. Dissolved oxygen

Oxygen is necessary for the maintenance of life in the river, but also for the decomposition of biological loads created by various natural and anthropogenic sources. Oxygen concentrations in all samples are quite high, and show no significant spatial or seasonal deviations. The values were in the range from XX (O2 mg/l), measured in summer near Bristol, to XX (O2 mg/l) in Mahala. The annual mean value was XX (O2 mg/l) (SD XX), with a median of XX (O2 mg/l). All measured oxygen concentrations were in the first class of water quality.

Lake Salakovac: the lowest value of 8.5ºC was measured in the summer period (August), while the highest value of 12ºC was recorded in the winter months (December). Lake Mostar: the lowest value of

8.6ºC was measured in the summer period (September), while the highest value of 13.2ºC was recorded in the winter months (January). Neretva: the lowest value of 9.2ºC was measured in the summer period (August), while the highest value of 11.6ºC was recorded in the winter months (July). Radobolja: the lowest value of 8.5ºC was measured in the summer period (June), while the highest value of 12.4ºC was recorded in the winter period (December). Buna: the lowest value of 8.9ºC was measured in the summer period (September), while the highest value of 12.7ºC was recorded in the winter period (November). Bunica: the lowest value of 9ºC was measured in the summer period (September) at the spring, while the highest temperature value of 12.5ºC was recorded in the summer months (June) at the mouth. Bregava: the lowest value of 9ºC was measured in the summer period (September), while the highest temperature value of 12ºC was recorded in the winter months (November). Trebizat: the lowest value of 7.9ºC was measured in the spring period (May), while the highest temperature value of 12.3ºC was recorded in the winter months (January).

The amount of dissolved oxygen is inversely proportional to the temperature of the water, and the low average temperature is partly responsible for the high oxygen concentration. In large and deep rivers such as the Neretva with enough sunlight, a lot of drops and turbulence, there is greater aeration (ventilation) of the flows, which in turn increases the amount of oxygen in the water, enables oxidative decomposition processes, and reduces the effect of pollution by organic substances. It is believed that a river with a high oxygen content has a great power of self-purification precisely because of the possibility of regulating oxidation processes. Mijatović et al., (2004) state that the movement of the water mass thus improves water quality and helps in the decomposition of contaminants. In waters that receive waste water in addition to cooling water, the ability to self-purify is significantly reduced (Tedeschi, 1997).

In addition to temperature, the ability of water to dissolve a certain amount of oxygen also depends on atmospheric pressure. Oxygen in water obeys Henry's law, so solubility is roughly proportional to the partial pressure of oxygen in air. The higher the air pressure, the higher the partial pressure of oxygen, so water at sea level may contain slightly more oxygen than mountain streams at the same temperature. Another factor that greatly affects the oxygen regime is the operation of hydroelectric power plants upstream of Mostar. Increased water aeration caused by the operation of the turbines increases the oxygen level in the water.

At a certain barometric pressure and temperature, water can dissolve a certain amount of oxygen and this solution is saturated. Since the temperature of the water affects the level of dissolved oxygen in the water, the oxygen saturation values were examined. By using the saturation value, it is possible to remove the influence of temperature, which enables spatial and temporal comparisons to be made.

Consistently high values of dissolved oxygen are extremely important. In general, variations in oxygen levels do not appear to be due to discharges from point sources. However, there are other important mechanisms controlling oxygen levels in the littoral zone, which may be very different from those in the pelagic zone. For example, a well-developed layer of aquatic macrophytes and associated periphytes will significantly raise the oxygen level during photosynthesis, and consume oxygen through respiration at night. More extensive studies are needed to assess diurnal variation in oxygen levels as well as seasonal variation between sampling sites. In addition, the impact of dams and hydropower plants on the river ecology of this area should be further clarified.

4.6. Microbiological characteristics of the Neretva

The mixing of water favors the process of self-purification of water, during which some microorganisms die off, especially those that are not permanent inhabitants of the water (Đukić *et al.,* 2000). This is particularly the case with indicators of fecal pollution. Such a condition is confirmed by bacteriological examination and shows the ability of natural self-cleaning, which is characteristic of the course of this river without signs of ecological pressure.

The lowest values of total coliforms 70 (cfu/100 ml) were recorded at source of Buna in autumn, and the highest of 6200 (cfu/100 ml), during spring at Neretva. *Escherichia coli* values were highest at Buna's estuary in Autumn 1300 (cfu/100ml), while at Salakovac, Bunica 2 and Trebizat 1 none were recorded.

During microbiological reserach, water temperature values were uniform across sites and show a high degree of concordance without significant deviations. Normal seasonal variations that follow the natural annual cycle, related to the geographical position and climatic conditions of the researched area, are noticeable, Table 1 and Table 2. The lowest temperature of 9.9 ºC was measured in spring at Buna 1 (the source), while the highest temperature of 15.3 ºC was recorded in autumn at Trebizat 2, close to delta. Relatively low water temperatures are favorable for a number of chemical characteristics and biological processes, such as nutrient concentration, faster decomposition of organic pollutants. During the research, the daily temperature at all sites did not show significant variation from the first to the last measuring station. The water temperature in streams does not change significantly with the flow, suggesting that there are no sudden fluctuations in temperature associated with discharges of municipal wastewater or other sources of thermal pollution.

pH values show a flat trend with no substantial outliers during the monitoring cycle. There is a slight decrease in pH values in autumn at certain sites, as a result of increased decomposition of organic matter (increased turbidity) and the production of carbon dioxide, which lowers the pH. Marginal increase in pH values in spring indicates an increase in the rate of primary production. pH values affect life in water and the use of water for recreational purposes. Bacteria survive in the pH range of 5-10, while the optimal values of this factor are in the range of 6.5-8.5. All measured pH values belong to the I class of quality. Tendency towards alkaline values is due to mineral composition of karstic substrate.

Electrical conductivity is a measure of the ability of water to conduct electricity and directly depends on the concentration of ionized substances in water. Electrical conductivity was relatively uniform at sampling sites during testing period, while seasonal inconsistency was observed, with slightly lower values recorded in the spring as a result of increased primary production and consumption of dissolved salts. It can be noted, however that conductivity values at river Trebizat are significantly higher compared to other waterbodies. The highest values of 1000 (µS / cm) and 800 (µS / cm) were measured in autumn, while during spring values were 600 (µS / cm), at both sites. According to obtained results, there is a significant load of dissolved inorganic substances. It is known that Trebizat is very rich in minerals, Ca and Mg carbonates, responsible for rich tuff formations and travertine waterfalls, which are unique natural attributes of this river.

Table14. Analytical values of water quality parametres in autumn

	Water Temperature °C	pH	Electrical conductivity (µs/cm)	Dissolved oxygen (mg/ml)	Total coliforms (cfu/100 ml)	*Escherichia coli* (cfu/100 ml)
Lake Salakovac	11.2	8.4	300	10.5	3200	0
Lake Mostar	12.4	8.2	300	10.2	400	10
Neretva 1	11	8	300	10.5	3800	760
Neretva 2	11.4	8.1	300	11.1	1800	270
Radobolja 1	10.9	7.8	200	10.1	2000	120
Radobolja 2	11.5	8.2	200	10.9	2500	440
Bunica 1	11.4	7.8	400	11.2	340	17
Bunica 2	10.2	7.9	300	11.6	890	91
Buna 1	10.4	7.9	300	10.7	70	21
Buna 2	11	8.2	300	12.4	2800	1300
Bregava 1	10.3	8.3	300	11.8	2600	40
Bregava 2	10.5	8.2	300	12	1800	790
Trebizat 1	11.5	8.4	1000	10.9	5800	20
Trebizat 2	11.2	8.1	800	10.8	5500	30

Table 15. Analytical values of water quality parametres in spring

	Water Temperature °C	pH	Electrical conductivity (µs/cm)	Dissolved oxygen (mg/ml)	Total coliforms (cfu/100 ml)	*Escherichia coli* (cfu/100 ml)
Lake Salakovac	12.2	8	200	9.9	2940	3
Lake Mostar	10.8	8.2	200	10.6	240	11
Neretva 1	10.3	8.2	200	10	6200	360
Neretva 2	11.9	8.7	200	11.7	730	25
Radobolja 1	11.1	7.9	200	10.7	480	18
Radobolja 2	13.1	9.2	200	11	5900	310
Bunica 1	12.5	7.9	300	10.8	2200	10
Bunica 2	12.7	8	300	11	1300	0
Buna 1	9.9	8.2	200	10.4	900	70
Buna 2	10.7	8.2	200	11	2600	30
Bregava 1	11.6	8.1	300	10.3	286	7
Bregava 2	12.1	8.2	300	11.2	511	30
Trebizat 1	14.9	8.7	600	10.7	2700	0
Trebizat 2	15.3	8.6	600	10.4	3500	30

Oxygen is necessary for the maintenance of life in water, but also for decomposition of biological loads caused by various natural and anthropogenic sources. Oxygen concentrations in all samples are high, and do not show significant spatial or seasonal deviations. Values ranged from 9.9 (O_2 mg / l), measured in spring at the Lake Salakovac, to 12.4 (O_2 mg / l) at Buna 2 (delta) in Autumn. All measured oxygen concentrations were in the first class of quality. The amount of dissolved oxygen is inversely proportional to water temperature, and low average temperature is partly responsible for high oxygen concentrations. In large and deep rivers such as the Neretva with enough sunlight, a lot of falls and turbulence, there is more aeration (aeration) of streams, which in turn increases the amount of oxygen in the water, allows oxidative decomposition processes and reduces effects of organic pollution. It is believed that a river with a high oxygen content has a great power of self-purification precisely because of its capability of regulating oxidation processes. The movement of water mass thus improves water quality and helps to decompose contaminants. In waters that receive wastewater, the ability to self-purify is, however, significantly reduced (Tedeschi, 1997).

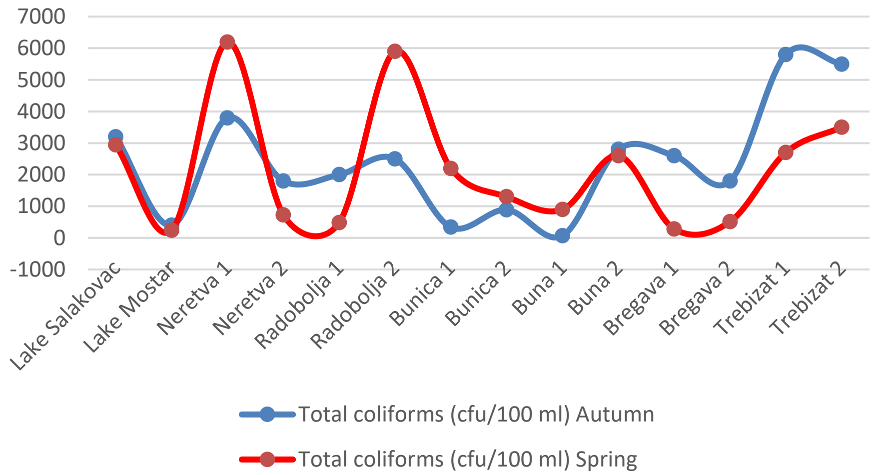


Figure 16. Comparison of total coliform values during two sampling seasons

As microbiological indicators of water quality, the number of total coliforms in 100 ml and the number of *Escherichia coli* in 100 ml of water were used. Tables 1 and 2, respectively, show a significant bacteriological load across all sites, with all values exceeding prescribed normatives for water safety. Wastewater from urban households is discharged untreated directly into surface waters. The overall results of bacteriological parameters are alarming and show a very high level of contamination of tested waterbodies. A characteristic seasonal profile of microbiological parameters was not marked at examined localities, with total coliform values being slightly lower during autumn, Graph 1, while *E.coli* values, overall were lower in spring. Spatial longitudinal variations were pronounced. All absolute values of bacteriological parameters exceed, many times, the allowed values, hence all tested waterbodies are under high bacteriological load. The risk to human health, from water, arises from the presence of pathogenic microorganisms. Many of these microorganisms originate from water contaminated with human and animal feces, which may contain a variety of intestinal pathogens that cause diseases ranging from mild gastroenteritis to serious, and sometimes fatal, dysentery, cholera, and typhoid. Depending on the frequency of some other diseases in the community, other viruses and parasites may also be present.

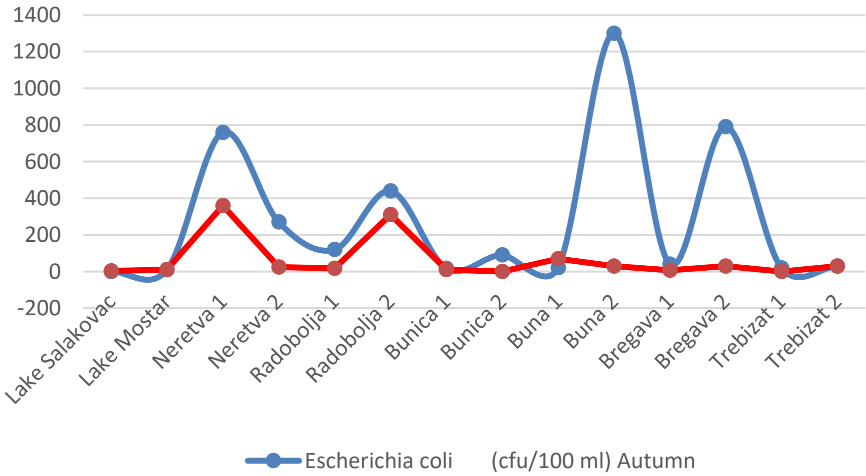
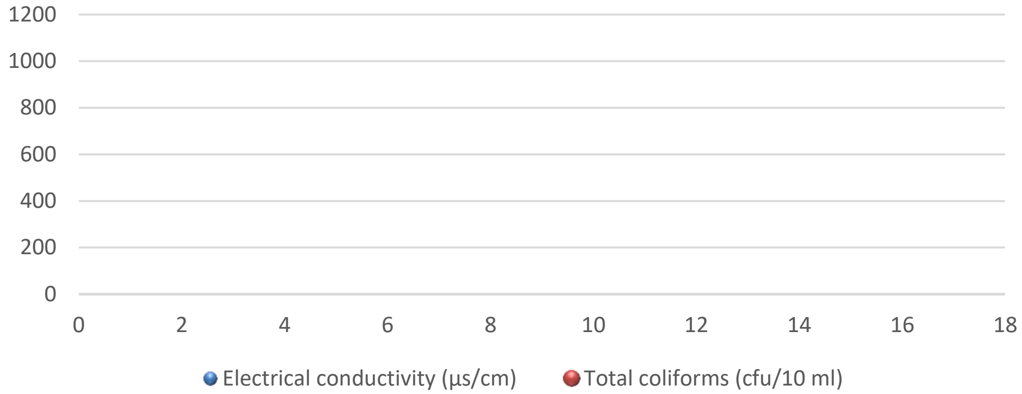
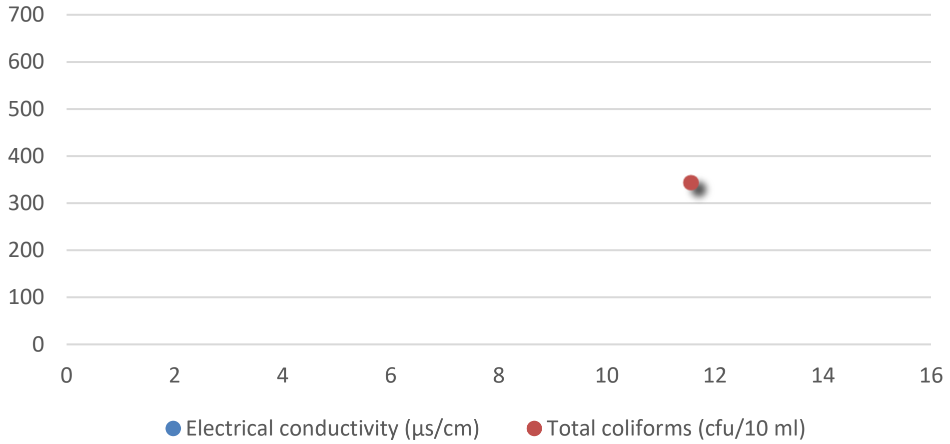


Figure 17. Comparison of E. coli values during two sampling seasons



Correlation = 0.712

Figure 18. Correlation of electrical conductivity and total coliforms in November



Correlation = 0.206

Figure 19. Correlation of electrical conductivity and total coliforms in May

The initial hypothesis was that there would be a positive correlation between electrical conductivity and the value of total coliforms. However the results show that conductivity is not a reliable indicator of the presence of total coliforms. Figure 18 shows that degree of correlation in autumn was 0.712, while it was 0.206 in spring, Figure 19. Correlation values obtained do not indicate a high degree of dependence of the two variables. Whether another abiotic factor affects correlation should be examined in future studies.

The electrical conductivity pattern shows that increased numbers of total coliform bacteria do not cause an increase in electrical conductivity, but elevated electrical conductivity indicates a higher degree of mineralization hence suggesting an increased number of bacteria. Presence of tuff in water facilitates prolonged survival rates of bacteria, as shown in Tables 14 and 15, for bacterial counts in Trebizat. The bacteria have an abundance of suitable substrate for growth, resulting in larger counts in travertine rivers. Electrical conductivity is increased due to solutes in water. Calcium can exhibit marked seasonal and spatial dynamics as a result of biological activity. Similarly, chloride concentrations are not heavily influenced by biological activity, whereas sulphate and inorganic carbon (carbonate and bicarbonate) concentrations can be driven by production and respiration cycles of the aquatic biota (Wetzel, 2001). The presence of tuff in Trebizat indicates that water is saturated with $CaCO_3$, hence the higher conductivity values. The level of total mineralization can be estimated by measuring total dissolved particles or by measuring the electrical conductivity, or the capacity of water to conduct electricity, a property directly proportional to the concentration of dissolved ions. Electrical conductivity is often used as a substitute for measuring salinity as it is significantly higher in saline systems than in freshwaters (Dodds, 2002). The specific electrical conductivity, however, also depends on the type of minerals dissolved in the water.

Trebizat is a slow-moving river, with water temperatures exceeding 20°C during summer. This is turn increases rates of primary production, fostering growth of specific vegetation in this submediterranean belt of the Adriatic province. Communal, agricultural, and industrial effluents contribute ions to receiving waters, and may also contain substances that are weak conductors (organic compounds) that alter the electrical conductivity of receiving waters. Therefore, electrical conductivity can be used to detect sources of pollution (Stoddard *et al*., 1999). Contamination effluents can change the electrical conductivity of water in a variety of ways. For example, wastewater will increase conductivity due to chloride, phosphate, and nitrate content, which will in turn increase rate of primary production and formation of tuff.

Monitoring the presence of pathogenic bacteria is a necessary component of evaluating the quality of water, which people use directly or indirectly. Uses include drinking, personal hygiene, recreation, irrigation of fruits and vegetables, washing food and dishes. Monitoring to detect pathogenic organisms can be carried out without accompanying measurements of physico-chemical parameters, and does not require large costs. If the water is used for personal hygiene or recreation, there is a risk of accidental ingestion of intestinal pathogens as well as the risk of other infections, especially eye, ear and nose. Less than 103 coliforms per 100 ml represents a low risk of intestinal infections, although the risk of viral infections is always present (Kloot *et al.,* 2006). The maximum acceptable concentration of faecal coliforms in natural waters, such as rivers, is 2000 microorganisms/100 ml and numbers above these limits can cause infections. On average, the concentrations of bacteria during the test significantly exceeded the permitted limits for river water. The action of waves or the flow of water in rivers increases the level of dissolved oxygen in the water and supports the growth of the aerobic population of bacteria. During the spring period, a decrease in the concentrations of all tested parameters was observed. This is caused by dilution due to the increased water level during the natural April maximum. Soil moisture in the spring provides favorable conditions for the soil to be a reservoir of coliform bacteria, and by washing contaminated soil, there is a seasonal variation in the number of bacteria. In the research of Ivenson et al., (1986) on seasonal variations and the increase in the number of bacterial population in the river, the inflow and amount of waste water and river sediment certainly have an influence.

Relatively low water temperature values throughout the year favor the longer survival of bacteria originating from the gastrointestinal tract of warm-blooded organisms. In addition to this water temperature, the sudden inflow of water due to the sudden large amount of precipitation affects a fairly high number of bacteria detected during the research of the winter period. Cyclical variations in the number of faecal coliforms in water have been attributed to the influx of water due to rainfall (Potter, 1960; Cody et al., 1961; Solo-Gabriele *et al.,* 2000). However, according to the research results, it is clearly seen that lower temperatures do not significantly affect the reduction of the number of coliform bacteria. This condition can be attributed to the preservation or survival of the number of bacterial populations at low temperatures of the Neretva River. When the temperature drops, the growth and reproduction of microorganisms stops and the metabolism suddenly decreases, but the microorganisms retain their ability to live, that is, the survival time of microorganisms increases at low temperatures (Đukić *et al.,* 2000; Popović and Bevanda, 1986).

Environmental factors strongly influence the fluctuation of the number of bacteria. This is not the result of just one factor, but a group of factors acting as a whole. Temperature, light, turbidity, amount of precipitation, flow rate, nutrients and environmental pollutants are key factors that influence the growth of bacteria and their abundance in water. The detection of other pathogens besides fecal bacteria is less frequent, mainly because there is no suitable, routinely available methodology. When faecal coliform concentrations are high, viruses can also be detected, but only in samples from 20 to 100 liters of water. Enteroviruses are found in sewage in a much lower concentration than bacterial pathogens; they are measured as plaque forming units (PFU) and rarely present more than 1,000 units per liter.

Most of the microorganisms are retained in surface waters and transported over greater distances, while in underground waters, due to filtration through porous rocks, they are retained only relatively close to the source of pollution. Under normal conditions, water can break down biological material, since heterotrophic microorganisms break down organic matter into CO2, water and usable ions (phosphates, nitrates and sulfates). With fast water flow, aeration is constant, and waste materials are quickly diluted and removed. However, when water is stagnant or passes over waste materials, it cannot break down biological material, so it soon turns into contaminated water (Duraković *et al.,* 2000; Mayer, 2004;). Waste materials, plastic, wooden and rubber products, foam and paper were observed in all investigated localities in the water course, which disrupt the water flow and promote the growth of microbes and the formation of biofilms. The degree of contamination is influenced by the number of microorganisms in the soil, as well as the types and amounts of nutrients that the water dissolves in the soil. In a study by Paulse *et al.,* (2007), increased numbers of microbes were detected in materials on which biofilm communities grew. Research has also shown that pathogens survive longer in water and soil where organic matter is readily available as a suitable nutrient and substrate for the development of microorganisms (Perri and Fallon, 1998; Fischer *et al.,* 2003).

River water used for irrigation should not contain more than 1000 microorganisms/100ml. Contamination can not only cause serious diseases in people, but also have serious consequences for the prosperity of the Neretva valley, where agricultural activities are the main form of economy. If water is harvested for irrigation, monitoring of faecal bacteria is recommended because there is a risk of food contamination. This risk is reduced when irrigation stops some time before picking, because many bacteria cannot survive for a long period if they are not under optimal conditions of temperature and nutrients (Coles et al., 2004). The use of contaminated water at any stage of food preparation represents a significant risk to human health, as food is an ideal medium for the growth of bacteria. All water that may come into contact with food must be checked for fecal contamination, which indicates the presence of organic pollution of human origin. Other naturally occurring microorganisms, such as algae and protozoa, can in some situations be useful for assessing the degree of pollution.

A comparison of microbial concentrations shows that all city localities are contaminated. If an adequate wastewater drainage system is not established, the continuous contamination of the river will not only represent a health problem in our community, but will also have negative consequences for the environment of the wider region. The efficiency of the current river monitoring and purification strategies by the relevant municipal, cantonal and federal authorities should be completely reviewed in order to implement the most urgent and radical rehabilitation and protection measures, with the aim of preventing further degradation of the Neretva River.

Certainly, the main source of bacteriological pollution of the river is wastewater of fecal origin, which reaches the river from various sources (households, catering facilities, industry). It has long been known that human feces contain at least 4 times more faecal coliforms than faecal streptococci, but that animal feces contain at least 1.4 times more faecal streptococci than faecal coliforms (Quershi and Dutka, 1979). Geldreich (1970) hypothesized that surface waters with a ratio of faecal coliforms to streptococci greater than 4 were most likely to have suffered mainly from human pollution, while those with a ratio of less than 0.7 were mainly contaminated by the feces of wild and domestic animals. However, given the fact that faecal streptococci survive in water longer than faecal coliforms, the interpretation of the results of their relationships must be approached with caution. Metcalf and Eddy (1991), and Almeida *et al.*, (2005) state the existence of many different sources of fecal contamination, and domestic sewage as the most important source of wastewater produced by humans, which contains between 107 and 1010 total coliforms in 100 ml. While the representation of certain types of fecal pollution in

5. CONCLUSIONS

Results show that the water temperature in tested streams does not change significantly with the flow, suggesting that there are no sudden in temperature fluctuations associated with sources of thermal pollution. All measured pH values belong to the I class of quality for watercourses. Tendency towards alkaline values is due to mineral composition of karstic substrate. There is a significant load of dissolved inorganic substances in Trebizat, a river very rich in minerals responsible for tuff formation, which causes elevated electrical conductivity values compared to other sites. Oxygen concentrations in all samples are high, and do not show significant spatial or seasonal deviations. Bacteriological impact greatly changes the character of water quality. All absolute values of bacteriological parameters exceed, many times, the allowed values. The researched aquatic ecosystems are under high bacteriological load. Correlation values obtained do not indicate a high degree of dependence of the two variables. However, natural properties of waterbody can significantly influence bacterial load. It was found that in the stream with high degree of mineralization, bacterial numbers were higher compared to streams with lower mineralization. Tuff particles in water form an excellent substrate for bacteria, promoting higher survival rates. Electrical conductivity, although an excellent indicator of degree of mineralization, does not have a predictive value in determining numbers of bacteria present in a sample. More extensive studies are needed to further assess issues addressed in this study. The obtained results are good basis for further research in order to directly determine causes of pollution and suggest measures for reduction or complete elimination of them.

Analytical values of general, physical and chemical indicators of surface water quality naturally fluctuated depending on the sampling season and hydrometeorological conditions in the basin. During the test, based on the measured parameters, the water quality was in class I/II.

Analyzes of physical and chemical indicators do not indicate significant changes in the quality of water in Neretva, as a result of receiving untreated wastewater. The research established that from the aspect of physico-chemical quality, the water of the Neretva River is in good condition and meets the requirements of the EU Water Framework Directive.

The quantitative-qualitative composition of the bacterial population indicates that the Neretva River upstream of Mostar is not seriously polluted and that there are no major signs of ecological pressure. However, after receiving untreated city wastewater, water quality according to indicators of fecal contamination falls to the level of III and IV class watercourses.

The maximum allowed value of coliform bacteria in water used for recreation is 2000 in 100 ml. These values were recorded in the summer period, when the water level of the Neretva, which is maintained at the biological minimum all summer, is the lowest, and water quality deteriorates due to the reduced flow rate and the accumulation of contaminants from fecal wastewater. The results indicate a high degree of contamination and a significant burden of fecal waste. A long-term tendency of deterioration of water quality was also observed, especially from the bacteriological aspect, where the results of autumn monitoring in 2009 showed a higher rate of pollution than the analyzes performed in the same period of the previous year.

Due to the natural self-purification of the Neretva watercourse and with a favorable dilution ratio, an improvement in bacteriological quality was noticed after only ten kilometers downstream from the city, although the state of water quality from a bacteriological aspect, even in that area, cannot be considered ecologically favorable.

It would certainly be necessary to strongly support research on the effects of known water pollutants in a longer period and during more permanent exposure, where bacteriological populations with pathogenic indications must have a great advantage, especially during the summer season, at least in certain locations where the risk to water quality is the greatest. Studies should also be conducted using comparative analyzes of water quality conditions in different rivers, as well as analyzes of temporal and spatial variations.

Uncharacteristic precipitation profiles, periods of drought, and various sources of pollution have increased in Bosnia and Herzegovina in the last few years. The availability and quality of water are threatened to such an extent that there is already a need for alternative sources of drinking water. At the national level, river waters have become highly polluted due to increased urbanization and the accompanying increase in water pollution. The lack of adequate sanitary facilities are largely responsible for the pollution of surface waters and their banks with fecal and solid waste, which causes the accumulation and reproduction of potential pathogens in these water resources. If an adequate wastewater drainage system is not established, the continuous contamination of the river will not only represent a health problem in our community, but will also have negative consequences for the environment of the wider region, and even for aquaculture activities in the Maloston Bay. The efficiency of the current river monitoring and purification strategies by the relevant municipal, cantonal and federal authorities should be completely reviewed in order to implement the most urgent and radical rehabilitation and protection measures, with the aim of preventing further degradation of the Neretva River.

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