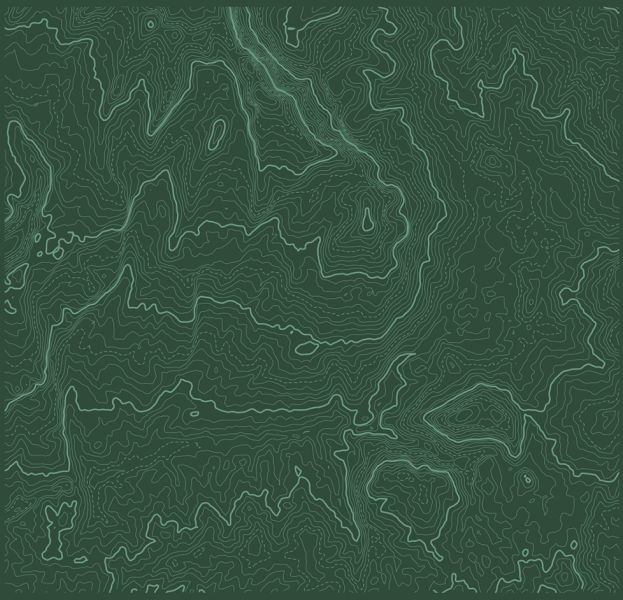
BIOINDICATORS AND BIOMONITORING:

WATER QUALITY CONTROL AND SAMPLE MANAGEMENT FROM LA PURISIMA DAM IN THE STATE OF GUANAJUATO, MEXICO, INVOLVING SAFETY MEASURES AGAINST COVID-19



ARMANDO GUERRERO AGUILAR
ALMA HORTENSIA SERAFÍN MUÑOZ
CHRISTOPH SCHÜTH

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PREFACE

This handbook is meant to serve as a guideline for researchers for the field and laboratory work required for the hydrochemical and biological monitoring survey of superficial freshwater aquatic ecosystems, such as rivers and reservoirs in the Guanajuato River Basin, but it can also prove useful for projects aiming at assessing aquatic ecosystems in rivers and reservoirs with similar characteristics, using an integrated methodology. Nevertheless, the researcher must ensure that the conditions in the study site are favourable for a biomonitoring program.

This work includes an introduction to the concepts of bioindicators and biomonitoring, the applicability of the different existing techniques, a description of the case study areas of the project, as well as the suggested sampling points, the methodology for sample collection, measurement of field and laboratory parameters and data analysis methodologies. The handbook includes a sample record sheet and the instructions on how to carry out a biomonitoring program.

It must also be mentioned that this document was developed during the COVID-19 pandemic, therefore, protective measures for the participants are included in the "Sampling methodology" chapter.

This handbook was developed as part of the collaboration project between the Department of Applied Geosciences of the Technical University of Darmstadt, and the Department of Environmental Engineering of the University of Guanajuato, titled: "Assessment of the status of ecosystems in La Purisima Dam using bioindicators and the impacts of the COVID-19 pandemic".

CHAPTER I

1. Introduction

Superficial water bodies such as rivers and reservoirs provide the foundations for the development of freshwater aquatic ecosystems, as well as for many anthropogenic activities. For the latter reason, they have also become one of the most threatened ecosystems around the world. With the increasing demands from human activities and the stress that they set on the environment, it has become clear that the preservation and restoration of water resources are of paramount importance. A wide variety of new technologies are being implemented in traditional water monitoring methodologies for a more efficient detection and removal of contaminants in a wide variety of water ecology applications, such as nanotechnology for water purification, smart water distribution systems for urban areas, and DNA metabarcoding for aquatic ecosystems biomonitoring (Zulkifli *et al.,* 2017; Prachi *et al.,* 2013; Byeon *et al.,* 2015; & Keck *et al.,* 2018).

Biological monitoring or biomonitoring can be defined as the observed responses that organisms manifest to determine if their environment is favorable for them

to live in. Environmental (natural) or anthropogenic (artificial) factors may disrupt the balance of aquatic ecosystems resulting in a biological response that can be studied to assess the condition of the ecosystem. These biological studies, in parallel with the traditional physical and chemical analysis can lead to a more accurate understanding of the water quality status of a certain water body and, therefore, to a better discernment of the state of an ecosystem as a whole (Bartram *et al.,* 1996; Bytyçi *et al.,* 2018; & Boyanov, 2015).

The novel COVID-19 (SARS-CoV-2) pandemic was declared by the WHO on the 13th of March 2020. It is caused by the Severe Acute Respiratory Syndrome Coronavirus 2, and by May 2021 it has affected nearly 200 countries around the world. Regarding its environmental impacts, the COVID-19 pandemic has had immediate and long-term effects, some of them have been positive and some negative (Zambrano-Monserrate *et al.,* 2020; & Saadat *et al.,* 2020). So far, most of these effects have been qualitative observations, given that quantitative research has not had enough time to be developed (Cheval *et al.,* 2020).

Located within the Guanajuato River Basin is the Guanajuato River, which discharges its waters into the La Purisima Dam, both aquatic ecosystems are heavily impacted by anthropogenic activities. It is widely believed that the Municipality Landfill, located in the vicinity of the Guanajuato River is a major source of pollution of both water bodies. It is also theorized that the increase

in the volumes of urban solid wastes during the COVID-19 pandemic from personal protective equipment, single-use packaging from restaurants and other delivery products have had a negative impact on the sites of study.

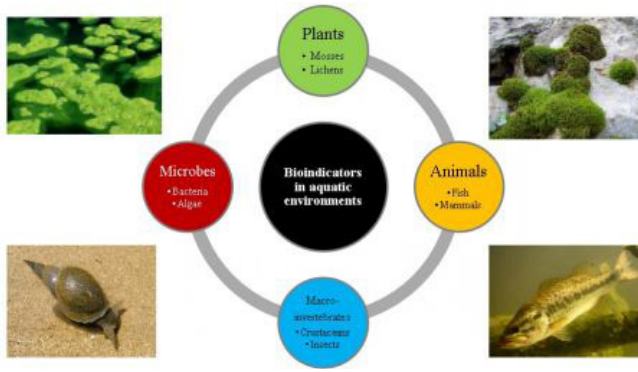

Figure 1. Common bioindicators used in biomonitoring
Author's work

1.1. Degradation processes in rivers and reservoirs

Rivers are natural flowing watercourses, usually of freshwater, that are flowing towards an ocean, sea, lake, or another river. These aquatic environments have provided humans with ecosystem services for millennia. Since the wake of the Industrial Revolution rivers have been progressively affected by anthropogenic stressors such as the increase in demand for water for agricultural, industrial and urban activities, increase in wastewater volumes, and the destruction of riverine habitats.

It is estimated that lakes and reservoirs hold about 90% of the world's available freshwater resources. Lakes are large, lentic, freshwater or saltwater natural water bodies created by glacial, tectonic and volcanic activity. A reservoir is an artificial impoundment created to sustain a variety of human activities, they usually have larger drainage basins than lakes and are usually located in watersheds with extensive agricultural activities. Some of the most common processes that affect lakes and reservoirs are described below (Chapman, 1996; Von Schiller *et al.,* 2017; & Mallin *et al.,* 2006) (Figure 2).

Acidification is the process by which acid inputs surpass the quantity of basic compounds produced in the reservoir, by weathering of minerals or by reduction of acid anions like nitrates (NOx) and sulfates (SOx). Atmospheric pollution, caused by precipitation (dry or wet deposition) of nitrate oxides and sulfur dioxides, is the

main reason of freshwater acidification. Industrial and mining effluents are another source of acidification.

Acidification was one of the most recognized environmental concerns of the late twentieth century. The Industrial Revolution and its rapid increase in the combustion of coal and oil resulted in emissions to the atmosphere of oxides of sulfur and nitrogen, followed by deposition on land and waters. The effects are manifold. Soils get acidified and forest growth is impaired, metals are mobilized as the groundwater gets acidic; this results in increased metal concentrations in drinking water wells, corrosion is accelerated, and our cultural heritage is degraded as statues and other constructions from stones with calcium carbonate dissolve (Almer & Dickson, 2021).

Being the recipients of water that runs off the surrounding landscape, inland waters —streams, rivers, and lakes— are particularly vulnerable. Lakes integrate processes in the surrounding watershed. Hence, many of the natural and anthropogenic conditions of lakes are the function of processes at much larger scales, including, e.g. natural flow with the gravity of organic and inorganic matter, and agricultural fertilizers and chemicals they also respond to altered export of matter from their watersheds due to changing land use, temperature, and precipitation. Accordingly, lakes are not only valuable ecosystems and natural resources *per se,* but also sentinels of environmental change beyond their own boundaries (Williamson *et al.,* 2008). This applies also

to acidification —acids are deposited on land and water, transported downstream along with other chemical species that are mobilized from soils due to increased acidity, with subsequent consequences for aquatic life.

On the other hand, the pH values of most natural, river and lake waters are in the range of 6-9. There are natural and anthropogenic sources that may influence the acidification of surface inland waters. The former depends on geological, geochemical, biological, and climatic factors. Since the 1960s, the problem of lake and river acidification has mostly been related to anthropogenic emissions of chemical compounds that have contributed to acidification either through acid deposition (SO_2, NO_x) or via terrestrial chemical transformations leading to H+ production (NH_3/NH_4 +) (Gałuszka & Migaszewski, 2015).

Eutrophication can be defined as the process by which a reservoir becomes overly enriched by nutrients like nitrogen or phosphorus, which in turn promotes the excessive growth of plants and the blooming of algae. This process results in a reduction of dissolved oxygen, an increase of turbidity, and the production of toxic metabolites. This process is considered one of the most serious causes of reservoir degradation.

Lake eutrophication and water quality deterioration have become a major environmental problem in urban areas and fertilized basins in developing countries across the world (Lin *et al.,* 2020).

In most water bodies including lakes and reservoirs, total phosphorus concentration, chlorophyll-*a* concentration, and Secchi disk visibility, in association with species composition are the common criteria to classify lakes and reservoirs as oligotrophic, mesotrophic, and eutrophic. Nutrient-rich runoff from cultivated land and industrialized and urbanized cities concentrated in phosphorus are the critical factors that drove eutrophication in water bodies. Among others, controlling external loading of nutrient, ecological, and mechanical methods were found to be common mechanisms to prevent and recover lake eutrophication.

Avoiding the factors that are under human control, i.e., a reduction of external loading of nutrients especially targeted on phosphorus reduction into the water basins, relocates sewage, industrial and domestic waste discharges to be lined out of the catchment of the lake. Furthermore, motivating the community to use less phosphorus-containing fertilizers and promoting phosphorus-free detergents are suggested solutions to sustainably prevent and reduce eutrophication in the long run (Ayele & Atlabachew, 2021).

Sedimentation refers to the deposition and accumulation of organic and inorganic matter at the bottom of the reservoir. It is a slow but very common process, and the sediments can have their origin within the lake (autochthonous matter) or from the external watershed (allochthonous matter). An excessive accumulation of sediments represents a serious problem in reservoirs

since these sediments can be transformed into toxic substances, as well as causing structural stress.

Sedimentation is known as the process which fills up natural lakes and man-made reservoirs with sediments to become the end land again. The main reason for this process is the sediment yield transported by the rivers as suspended or bedload into the reservoirs. Bed and suspended sediment load originate from soil and rock erosion in the catchment area of the reservoir. Suspended fine sediments are also the result of surface erosion as well as of crashing and abrasion of coarser sediments transported by rivers. When entering lakes and reservoirs, the coarser sediments such as sand and gravel settle down and form a delta. The finer suspended sediments are deposited over the whole reservoir. During floods, they are periodically transported as turbidity currents like an underwater avalanche directly from the delta along the reservoir to the deepest point in front of the dam.

Today, the worldwide yearly loss of storage capacity due to sedimentation is already higher than the increase of capacity by the construction of new reservoirs for irrigation, drinking water, and hydropower. In Asia, for example, 80% of the useful storage capacity for hydropower production will be lost by 2035. Thus, the sustainable use of the reservoirs is not guaranteed in long term. In the case of deep and long reservoirs, the sedimentation rate is much below the world mean value. Nevertheless, the sedimentation also threatens these

reservoirs since the mentioned turbidity currents are sporadically transporting large volumes of sediments down to the dam. There, the concentrated deposits are hindering the safe operation of the outlet structures as intakes and bottom outlets. Thus, after only 30-40 years of operation, sedimentation has become a serious problem in many reservoirs located even in catchment areas with moderate surface erosion (Schleiss, 2013).

Stratification is a process that significantly influences water quality in reservoirs. It refers to a difference in temperature, which leads to a variation in density (sometimes also caused by a difference in solute concentrations). The different layers present different physico-chemical characteristics; the upper layer is exposed to solar insolation, while the lower layer is detached from the atmosphere, creating anoxic conditions. The sediments will, therefore, form various compounds such as ammonia, nitrates, phosphates, and sulfides.

Stratification is defined as the development of relatively stable light and warm layers above colder deeper layers within a body of water. Thermal stratification is related to water density and is affected by incoming heat, water depth, and the degree of water-column mixing. Lakes receive thermal energy mainly through the lake surface. Part of the shortwave energy from the sun penetrates through the surface and is absorbed in the near-surface layers. In a typical lake, this happens within the uppermost 10 m, but in very clear water lakes this penetration can reach down to tens of meters.

Long-wave energy from clouds and the atmosphere is absorbed within the first centimeters in the lake water body. Thermal energy is also exchanged between the lake bottom and the lake water body, but normally it has only a secondary role in lake thermodynamics (Huttula, 2012). Reservoirs are more complex and vulnerable ecosystems in comparison to rivers, mainly because they lack the self-depurating capacity of rivers, therefore easily accumulating pollutants. Also, it is considered that most of these water bodies are currently under some kind of environmental stress. Lakes and reservoirs are recognized as important sentinels of climate change, integrating catchment and atmospheric climate change drivers. Climate change conceptual models generally consider lakes and reservoirs together despite the possibility that these systems respond differently to climate-related drivers (Hayes *et al.*, 2017).

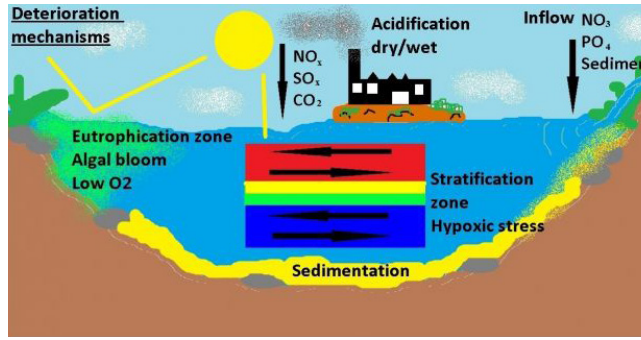

Figure 2. Degradation processes in reservoirs
Author's work

1.2. Environmental effects of the COVID-19 pandemic

The application of biomonitoring methods in times of COVID-19 is a fundamental tool to analyze the correlation of pollution in water bodies before this Era. The COVID-19 global pandemic, caused by the Novel Coronavirus, is one of the most virulent diseases to have afflicted humankind. The first SARS-CoV-2 virus cases were detected in December 2019, in China's Hubei province, being subsequently declared as a Public Health Emergency of International Concern by the World Health Organization (Berekaa, 2021). Most of the environmental impacts have been a direct result of the limitations on the economic, industrial, social and transport sectors. These have physically manifested especially in air, water, and soil. Regarding the focus of this study, special attention is given to the impacts that solid urban wastes, such as single-use items (i.e., medical and hygiene products, food service industry disposables, packing products, etc.) have had on the environment (Parashar *et al.,* 2021; Niñoval, 2021). In India, for example, some works have shown that in this situation, the Water Quality Index estimates indicate an improvement of 37% during the lock-down period. The Biological Oxygen Demand and Chemical Oxygen Demand values reduced by 42.83% and 39.25%, respectively, compared to the pre-lock-down phase, while Faecal Coliform declined by over 40% (Patel *et al.,* 2020).

CHAPTER II

2. Case study areas

2.1. La Purisima Dam

La Purisima Dam and its area of influence are located to the southwest of the municipality of Guanajuato, between the meridians 20°51'54"N and 20°54.25"N, and the parallels 101°15'18"W and 101°17'49"W. It borders to the North with the communities of El Limon and Cienega del Pedregal, to the South with the communities of El Zangarro and El Coyote, to the East with the communities of San Jose Garcia and La Trinidad, and to the West with the communities of Capulin and Cañada de Bustos. The dam receives the tributaries of the Guanajuato, Chapin, and Trinidad rivers (Arredondo *et al.,* 2015).

La Purisima Dam ranks 74 out of 180 biggest reservoirs in Mexico. With an area of 2,728.81 ha, it represents 2.7% of the municipality's territory. According to Bonilla *et al.,* (2015), it has a storage capacity of 195.7 million m^3, a maximum length of 3.9 km during the rainy season and a minimum length of 1.9 km during the dry season. It has a maximum width of 2.5 km during the rainy season and a minimum width of 1.2 km during the dry season.

Its maximum depth during the rainy season is 22 m and it has a minimum depth of 4.3 m during the dry season. The height of the crest is 43 m, and the average temperature of the water is of 19.4 °C (Periódico Oficial del Gobierno del Estado de Guanajuato, 2005).

The dam was inaugurated in 1979 for irrigation and flood control, being one of the four main depositories for the 011-Irrigation District. In 2005, La Purisima Dam and its area of influence were declared a natural protected area. It presents three types of vegetation: deciduous tropical forest, aquatic and underwater vegetation and xerophilous scrub. It houses 215 species of fauna, 43 of which are considered at risk and/or endemic, as well as 137 species of flora with 2 endangered species according to the NOM-059-SEMARNAT-2010 (Diario Oficial de la Federación (DOF), 2010).

La Purisima Dam is situated within the Silao-Romita aquifer recharge area, which has a good permeability due to the quantity and thickness of granular materials it's comprised of, thus acquiring great importance in the recharge of underground water. As a natural protected area, the dam develops an economic function providing a variety of ecosystem services such as provisioning services (water for irrigation, ecosystem for species), regulating services (dilution and assimilation of organic matter) and cultural services (recreation activities).

Regarding the water quality of the dam, it has been stated in previous works that the levels of total phosphorous exceed 20 times the maximum permissible levels

(Cano-Rodríguez *et al.*, 2000). This leads to the growth of algae and the development of eutrophication zones which in turn decreases the amount of dissolved oxygen and this is associated with the documented death of fish and other aquatic life. As for other potentially toxic elements, reported concentrations may exceed 25, 30, 80, and 180 times the maximum permissible limits (MPL) for arsenic, lead, mercury, and selenium respectively (Cano-Rodríguez *et al.*, 2000). The municipal landfill is located in the nearby area and one of the objectives of this study is to assess the influence that it has on the dam. It is believed that some of the nutrients that cause pollution might be derived from the landfill and other sources.

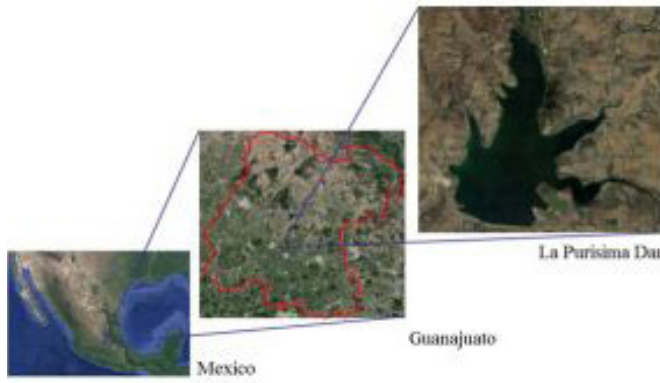

Figure 3. Location of La Purisima Dam in Guanajuato, Gto., Mexico
Author's work

2.2. Guanajuato River

The Guanajuato River originates from runoff in the vicinity of the town of Santa Rosa, located approximately 8 km North of the city of Guanajuato, having as tributaries with steep slopes the La Cata, Duran, San Antonio, Pastita, San Javier, Marfil and Noria Alta streams. Later on, it flows in the urban area and down to the La Purisima Dam, they converge at the delta through the Santa Ana River on the right bank and the La Yerbabuena, El Cubo and El Chapin rivers on the left bank (Miranda-Avilés *et al.*, 2009).

In the area of the Guanajuato River at the height of the Noria Alta road distributor, the river is seriously obstructed by stones, rocks and garbage, causing a decrease in the natural flow of the river. In the communities of Santa Teresa and Noche Buena there are banks for the extraction of material from the river and this has caused damage to the banks of the river and the change of its channel continuously, which is why it currently registers an approximate width of up to 250 m.

This situation makes the lower part of the Santa Teresa community vulnerable to flooding. The Guanajuato River in its path from the Noche Buena community to the front of the town of Cuevas registers serious problems of silt and damaged riverbanks. Another problematic is that the river serves as a depository of solid domestic wastes by the population of the communities along with

its flow and also domestic wastewater is discharged into its waters.

This situation has severely polluted the river where, reportedly, along the riverbed blackish, foamy and oily water can be observed (García-Ledesma, 2015). The rivers finally discharge their waters into the La Purisima Dam, greatly contributing to its pollution.

Figure 4. Guanajuato River
Author's work

2.3. Municipal landfill

The Municipal Landfill is located to the southwest of the City of Guanajuato, on top of one of the hills that adjoin the west bank of the Guanajuato River. Its geographical coordinates are 20°59'58.81"N, 101°19'9.72"W. It is approximately 800 m long and has a width of approximately 150 m. There is very little information recorded about the landfill and most of it comes from media agencies, which frequently report news regarding the poor conditions present at the site.

It is known that the landfill has been operating out of the established Mexican normativity NOM-083-SEMARNAT-2003 (DOF, 2015) for at least a decade and that it has gotten so out of control that there have been official reports indicating that the site must be shut down and relocated.

Local reports have exposed the current situation at the landfill, where the lack of fencing allows livestock to "graze" inside the landfill, making it a potential infection point. It is known that several fires occur within the landfill each year, making it also a source of air pollution. Also, the unregulated disposition of solid urban wastes generates leachates that have the potential to pollute groundwater as well as superficial water bodies like the adjoining Guanajuato River and La Purisima Dam (Segura, 2020).

It is theorized that these leachates are indeed a source of pollution in the Guanajuato River and ultimately in the La Purisima Dam and that this situation has been aggravated by the COVID-19 pandemic due to the volume increase of solid urban wastes.

Figure 5. Municipal Landfill
Author's work

CHAPTER III

3. Bioindicators and Biomonitoring

3.1. Bioindicators

The term "bioindicator" refers to an organism that reveals the presence of an environmental stressor (e.g., pollutants, excess nutrient) by a physical, chemical or behavioral response. Bioindicators provide a more qualitative assessment of the effects of different pollutants present in the ecosystem and also a sense of how long they have been present. Different types of bioindicators are useful and suitable for a wide range of applications.

In this context, bioindicators are used to evaluate the health of an ecosystem considering its relationship with human activities. Animals (fish, birds, macroinvertebrates, etc.) (Chovanec *et al.,* 2003), plants and fungi (mosses, lichens, tree rings, etc.), and microorganisms (algae, diatoms, etc.) (Shankar, 2013), are all examples of commonly used bioindicators in environmental assessment studies. These species composition, abundance and distribution vary depending on the disturbance gradient of their sustaining ecosystem. Therefore, observing trends over time or across the disturbance gradients might

allow land and ecosystem managers to predict the probable changes in the ecosystem and to initiate measures to mitigate such impacts on time (Berhane *et al.,* 2014).

Some authors categorize bioindicators as early warning, diagnostic, and compliance species. Early warning indicators can reveal the first signs of disturbance in the environment before most other species are affected. Diagnostic indicators are those used to investigate observed environmental disturbances. Compliance indicators are those species, which are used to verify maintenance or restoration goals have been achieved (Berhane *et al.,* 2014; Hamza-Chaffai, 2014).

Bioindicators can be grouped into accumulation indicators; those that can store pollutants without any visible changes in their metabolism, and response indicators; those that present symptoms of environmental stress when taking up small amounts of harmful substances. According to Holt and Miller (2010), regardless of the environment, geographic region, organism, or type of disturbance, a good bioindicator always presents certain characteristics: a) abundant and common, b) economical/commercial importance, c) good indicator ability and d) well-studied.

Therefore, given that an ideal bioindicator cannot live outside the supporting ecosystem being assessed (e.g., desert, forest, freshwater, grassland, marine, terrestrial, tundra, etc.), by observing their behavior it is possible to assess areas of contamination.

3.2. Biomonitoring

"Biomonitoring" is defined as the observation of biological communities or individual organisms and their responses to physical or chemical changes in their environment over time. Biomonitoring can provide qualitative assessments, by observing and recording these changes, or quantitative evaluations by chemical analysis of substances present in the tissues of organisms. Bioindicators are sampled to evaluate risks to human health and the environment for communication to the public or government policy makers through a range of ecological census techniques and taxonomic identification (e.g., biotic and diversity indices, multimetric and multivariate approaches, DNA-metabarcoding, etc.) (Derocles *et al.,* 2018).

Biological monitoring can be divided into active biomonitoring; includes all the methods that insert organisms under controlled conditions into the site to be monitored, and passive biomonitoring; uses organisms and communities of organisms that are a natural component of the ecosystem and appear there spontaneously (Zimmermann *et al.,* 1994). In order for a biomonitoring study to be statistically reliable, it should be carried out in different weather conditions or seasons.

Biomonitoring lies at the core of ecosystem conservation, management and restoration. As biomonitoring is an obligation today, biomonitoring programs are framed by government organizations (e.g., Australian River

Assessment System, Canadian Aquatic Biomonitoring Network, Joint Nature Conservation Committee in the United Kingdom, Water Framework Directive, etc.) (Derocles *et al.,* 2018). Cost-effectiveness, simple application, and reproducibility of the methodologies are some of the characteristics which make biological monitoring such a valuable ecological assessment tool. Nevertheless, the researcher must consider the importance of having a good taxonomic knowledge of the indicator species, as well as of their physiological processes of uptake and retention of environmental contaminants.

The widespread development and application of bioindicators has occurred primarily since the 1960s. Over the decades, the range of bioindicators used to assist in studying all types of environments (i.e., aquatic and terrestrial) has expanded, including all major taxonomic groups (Holt & Miller, 2010). An early example of the application of biological indicators can be traced back to the early years of the industrial revolution. Canaries were kept in underground coal mines to perform as early-warning signals for the miners in the United Kingdom (Cairns *et al.,* 1993). Given the hypersensitivity of these birds to small concentrations of carbon monoxide and methane gas, these birds served as a biological indicator of unsafe conditions for the workers.

CHAPTER IV

4. Selection of a biomonitoring method

When selecting a biological monitoring method, one must consider certain factors in order to meet the objectives of the assessment. Selecting an adequate biomonitoring technique from the existing methods will depend majorly on the scope of the study and the availability of resources. The following are the principal methods used to conduct a biomonitoring study (Bartram, 1996):

- Biological tissue analysis: to determine the concentration of certain substances in living organisms.
- Morphological studies: observations of cellular and structural changes in living organisms.
- Controlled environments: measurements of beneficial or toxic effects on living organisms under controlled conditions in situ or in the laboratory.
- Ecological methods: based on community structure and diversity.
- Physiological and biochemical methods: based on community metabolism or biochemical effects in individuals or communities.

4.1. Ecological methods

The Saprobic System is the oldest biological water quality classification. It is based on the use of bioindicators such as algae, macroinvertebrates and fish to assess the concentrations of dissolved oxygen in river systems, which varies depending on the levels of organic pollutants. However, the use of the Saprobic System alone is considered limited by other factors that can influence species depletion and has fallen out of use in the last decades. Nevertheless, it laid the foundations for other, more integrated biomonitoring methodologies.

Ecological methods involve indices that are expressed as numbers or scores that have been derived or transformed from quantitative data (Ortiz-Burgos, 2016). Over the years several indices have been developed and adapted to be applicable to a specific region.

Any biomonitoring method must be complemented with information on the chemical and physical characteristics of the habitats where they are to be applied. These approaches produce numerical indices in which the measure of the index value is related to a qualitative assessment of the studied ecosystem or the water quality (e.g., from polluted to clean). A survey campaign at the sites of study can help evaluate the most suitable organisms, sampling technique and test efficiency. Some of the most widely used ecological indices are listed below (Cairns, 1993; Li *et al.,* 2010; & Kohlmann *et al.,* 2018):

· Biotic indices: combines the relative abundance based on certain taxonomic groups with their sensitivities or tolerances into a single index or score (Tolkamp, 1983). The Biological Monitoring Working Party score (BMWP) and the Average Score per Taxon (ASPT) are a good example of the adaptability and standardization of these methods.

· Diversity indices: these indices assume that the abundance (number of individuals), evenness (uniformity in distribution) and richness (number of species) of taxa in a system decreases with environmental degradation. Examples of these indices are the Shannon-Wiener Index, 1949 (Ortiz-Burgos, 2016), and the Simpson Index, 1949 (Tolkamp, 1983).

· Multi-metric approach: combines variables or metrics (such as composition, functional, richness and sensitivity metrics), which represent various structural and functional attributes of an ecosystem, into a non-dimensional index, which can be used to assess a site's overall condition. By combining different categories of metrics, the multi-metric assessment is regarded as a more reliable tool than assessment methods based on single metrics. Currently, multi-metric approaches often include local adaptations of the BMWP as a core metric.

· Multivariate approach: multivariate approaches, also called model-based procedures are predictive statistical analyses that assess the deviation between the observed aquatic community and reference conditions predicted from environmental physicochemical parameters.

4.2. The Biological Monitoring Working Party (BMWP) score and Average Score per Taxon (ASPT)

This method has its basis on the principle that different species of aquatic macroinvertebrates have different tolerances to pollution. It was developed as a result of the need for standardization and to reduce the effort and taxonomic expertise necessary for routine biological monitoring based on bioindicators. It is a scoring system that relies on the identification to the family level and it is not specific to any single river, catchment or geographical area. It can be used to reflect the impact of organic pollution such as urban waste waters, which is most suitable for the conditions present at La Purisima Dam and the Guanajuato River.

Macroinvertebrates are collected using an adequate sampling technique such as the "kick sampling technique" from different substrates (rocks, gravel and other sediments) in the lake or riverbed. The macroinvertebrates are then separated and identified to the family

taxonomic level. Each family is then given a score between 1 and 10, representing their sensitivity to environmental pollution; 1 being the most resilient organisms and 10 being the most sensitive. The score of each family is added to obtain the BMWP score (see Table 1) (Gutiérrez-Fonseca *et al.*, 2014).

After the BMWP score is obtained, the Average Score per Taxon (ASPT) is also calculated (see Table 3). The ASPT represents the average tolerance scores of the macroinvertebrate's families identified, ranging from 0 to 10. A BMWP score bigger than 100, and an ASTP value bigger than 4, is an indicator of good water quality (Bartram, 1996).

GROUP	FAMILIES	SCORE
Mayflies	*Siphlonuridae, Heptageniidae, Leptophlebiidae, Ephemerellidae, Potamanthidae, Ephemeridae*	10
Stoneflies	*Taeniopterygidae, Leuctridae, Caprniidae, Perlodidae, Perlidae, Chloroperlidae*	10
River bug	*Aphelocheridae*	10
Caddis or Sedge flies	*Phryganeidae, Molannidae, Beraeidae, Odontoceridae, Leptoceridae, Goeridae, Lepidostomatidae, Brachycentridae, Sericostomatidae*	10
Crayfish	*Astacidae*	8
Dragonflies	*Lestidae, Agriidae, Gomphidae, Cordulegasteridae, Aeshnidae, Corduliidae, Libelluiidae*	8
Mayflies	*Caenidae*	7
Stoneflies	*Nemouridae*	7
Caddis or Sedge flies	*Rhyacophilidae, Polycentropidae, Limnephilidae*	7

Snails	*Neritidae, Viviparidae, Ancylidae*	6
Caddis or Sedge flies	*Hydroptilidae*	6
Mussels	*Unionidae*	6
Shrimps	*Corophiidae, Gammaridae*	6
Dragonflies	*Platycnemididae, Coenagriidae*	6
Bugs	*Mesoveliidae, Hydrometridae, Gerridae, Nepidae, Naucoridae,* Notonectidae, *Pleidae, Corixidae*	5
Beetles	*Haliplidae, Hygrobiidae, Dytiscidae, Gyrinidae,* Hydrophilidae, *Clambidae, Helodidae, Dryopidae, Elmidae, Chrysomelidae, Curculionidae*	5
Caddis or Sedge flies	*Hydropsychidae*	5
Craneflies/Blackflies	*Tipulidae, Simuliidae*	5
Flatworms	*Planariidae, Dendrocoelidae*	5
Mayflies	*Baetidae*	4
Alderflies	*Sialidae*	4
Leeches	*Piscicolidae*	4
Snails	*Valvatidae, Hydrobiidae, Lymnaeidae, Physidae, Planorbidae*	3
Cockles	*Sphaeriidae*	3
Leeches	*Glossiphoniidae, Hirudidae, Erpobdellidae*	3
Hog Louse	*Asellidae*	3
Midges	*Chironomidae*	2
Worms	*Oligochaeta* (whole class)	1

The biological scores allocated by groups of organisms by the Biological Monitoring Working Party (BMWP) score

ASPT SCORE CATEGORY INTERPRETATION	
> 5	Excellent
4 - 4.5	Good
3 - 3.5	Moderate
2 - 2.5	Poor
1 - 1.5	Very poor

1. BMWP score system

Author's work adapted from BMWP

BMWP SCORE	CATEGORY INTERPRETATION
> 100	Very good, Unpolluted, Unimpacted
71 - 100	Good, Clean but slightly impacted
41 - 70	Moderate, Moderately impacted
11 - 40	Poor, Polluted, Impacted
0 - 10	Very poor, Heavily polluted

Table 2. BMWP value interpretation

ASPT SCORE	CATEGORY INTERPRETATION
> 5	Excellent
4 - 4.5	Good
3 - 3.5	Moderate
2 - 2.5	Poor
1 - 1.5	Very poor

Table 3. ASPT value interpretation

CHAPTER V

5. Fieldwork campaigns and site selection

A total of 19 samples in 3 different sites is suggested to carry out the study. Four fieldwork campaigns are proposed to collect all the necessary in situ data for the continuation of the project:

1. "Survey campaign": this campaign is meant as a first approach to the study area; locate the main sites of interest, possible sampling points and best access routes. It'll also provide the opportunity to collect quantitative data from preliminary field measures of water samples as well as qualitative data such as photos, testimonies from the local population, site observation, etc.

2. "La Purisima campaign": *in situ* parameters will be measured from the dam's main tributaries, water and sediment samples will be collected for lab analysis and biomonitoring protocols will be implemented. Qualitative data will also be collected.

Figure 6. "La Purisima campaign" suggested sampling points
Author's work

Outer samples:

- Guanajuato River: 20°54'38.60"N,101°16'44.14"W.
- Trinidad River: 20°53'31.15"N,101°15'36.19"W.
- El Cubo River: 20°52'31.54"N,101°15'37.85"W.
- El Chapin River: 20°52'11.74"N,101°16'22.27"W.
- Gate: 20°52'1.83"N,101°17'15.72"W.
- El Capulin Stream: 20°53'30.22"N,101°17'28.06"W.

Inner samples:

- La Purisima North: 20°53'20.29"N,101°16'21.88"W.
- La Purisima East: 20°52'58.53"N,101°16'21.88"W.
- La Purisima South: 20°52'21.04"N,101°17'2.98"W.
- La Purisima West: 20°52'49.82"N,101°17'21.88"W.
- La Purisima Center: 20°52'41.97"N,101°16'56.20"W

3. "Guanajuato River campaign": the same procedures will be applied here in the most

representative sites along the Guanajuato River: downstream, midstream and upstream from the municipal landfill. The idea is to investigate the influence that the landfills' leachates could have on the water quality of the river.

Figure 7. "Guanajuato River campaign" suggested sampling points
Author's work

- Guanajuato River-Landfill: 20°58'53.55"N, 101°18'32.80"W.
- Guanajuato River after landfill: 20°58'14.01"N, 101°18'26.11"W.
- Guanajuato River-Santa Teresa: 20°57'40.99"N, 101°18'44.89"W.
- Guanajuato River delta: 20°54'38.64"N, 101°16'45.22"W.

4. "Municipal Landfill campaign": sampling of pollution sources like leachates and qualitative data collection like testimonies and observations about the landfill's situation during the COVID-19 pandemic are the main aim of this campaign.

Figure 8. "Municipal Landfill campaign" suggested sampling points
Author's work

- Landfill North: 20°59'59.55"N, 101°19'9.59"W.
- Landfill East: 20°59'53.42"N, 101°19'0.75"W & 20°59'47.08"N, 101°18'54.12"W
- Landfill West: 20°59'49.34"N, 101°19'7.05"W.
- Landfill South: 20°59'41.39"N, 101°18'57.22"W.

It is recommended that each of the campaigns be carefully planned before their execution in order to maximize the efficiency of the resources, including time, personnel

and materials. Each campaign would involve two working days; one to carry out the field work and one to conduct the laboratory analysis. Also, in order to statistically validate the obtained data, a seasonal monitoring (dry and rainy seasons) should be considered (Bartram 1996 & Jerves-Cobo *et al.*, 2020).

CHAPTER VI

6. Sampling methodology

There will be three different types of samples that will be analyzed in the laboratory: water samples, sediment samples and leachate samples. Photographs and observations of the sites where the samples have been taken are to be registered to maintain a more complete record of the fieldwork.

In consideration of the current COVID-19 pandemic, all field and laboratory activities were carried out only while wearing all of the recommended personal protection equipment to prevent exposure (WHO, 2020):

- Respiratory protection: disposable medical masks.
- Hand protection: disposable surgical hand gloves.
- Preventive measures: contact tracing sheet, frequent hand sanitization, 1.5 meter distance.

Furthermore, the contact details of each of the research participants and the people that they interacted with were kept in a record sheet to be able to trace compromised contacts.

Figure 9. Adequate use of personal protective equipment for researchers
Author's work

6.1. Water samples

1. Locate the representative sampling points; the representative sampling points are suggested in *Figure 4*. The researcher should identify areas of special interest such as eutrophication zones, stratification zones, clear zones, zones with anthropogenic activity, and water entry and exit points.

2. Opaque 100 ml polyethylene sampling bottles with an internal sealer to prevent leaks are to be labelled with the following information: site of collection, code of the sample and date of sampling.

3. The sample bottle and cap must be rinsed at least three times with the water from the site of interest to ensure that it only contains the desired water sample.

4. Fill the 100 ml bottle to the top and close it tightly. Refrigerate the samples to < 6 °C until the

water analysis is carried out (for laboratory analysis 48 hours are recommended) (Ramsey, 2015).

6.2. Sediment samples

1. Sediments are to be collected at the same locations where water samples were collected.

2. Opaque 100 ml polyethylene sample plastic bottles can be also used to collect the sediments. The bottles should be labelled with the following information: site of collection, code of the sample and date of sampling.

3. Using a scoop or small shovel the top sediment layer (2 cm) should be collected from the river or dam bed.

4. Slowly retrieve the scoop or shovel out of the water and tilt as to allow the surface water to drain off while maintaining the pouring water in the sediment.

5. Empty the sediment into the sample bottle avoiding objects such as branches and leaves. Repeat the process until the sample bottle is full and refrigerate until analysis (Awal *et al.,* 2019).

6.3. Leachate samples

1. Sample sites in the landfill have been suggested in Figure 6, paying special attention to the areas

where leachate pools might be located. It is suggested that the researcher identify the low points of the landfill, where points of possible leachate flows might be found.

2. Leachates can be sampled and preserved in opaque 100 ml polyethylene plastic bottles with an internal sealer. The bottles should be labelled with the following information: site of collection, code of the sample and date of sampling.

3. Leachate samples can be collected with a syringe and then emptied into the bottles. If the leachates present hardened material a spatula can be used to collect the material.

4. Fill the bottle to the top and close the lid tightly. Refrigerate the sample to < 6 °C until analysis (leachate samples may be stored up to 48 hours) (American Public Health Association *et al.,* 1998).

6.4 Biological samples

Algae (Holm-Hansen & Riemann, 1978):

1. Carefully mix the sample by inverting the bottle (avoiding bright light) and measure a suitable volume into a measuring cylinder (between 100 ml and 1 L depending on the turbidity of the sample).

2. Using a gentle vacuum (i.e., a hand-operated vacuum pump), pass as much of the measured sample through a fresh GF/C filter paper as possible.

3. Add 0.2 ml of $MgCO_3$ suspension (1.0 g $MgCO_3$ in 100 ml H_2O) to the last of the sample, as it is being filtered, to preserve it on the filter.

4. Do not allow the filter to dry whilst adding the sample to the filter cup.

5. Once the sample has passed through the filter rinse the sides of the filter cup with about 50 ml of distilled water.

6. Allow the filter to dry for a few seconds and then fold it in half, with the sample folded inside.

7. Place the filter in a Petri dish or small polythene bag labelled with the sample identifier and volume filtered and, if storage is necessary, place the filter in its container in the dark, in a freezer at -20 °C. If the sample cannot be frozen until several hours later keep it cool and in the dark (note the length and conditions of storage).

8. Do not store frozen samples for more than a few months.

Macroinvertebrates (Stark *et al.,* 2001):

1. "Kick sampling" is a technique that involves agitating the stones or sediment of a river or stream by foot and collect the sample in a hand net that is held downstream.

2. Use a pond net to collect macroinvertebrates during a fixed period of time from each of the major habitat types such as gravel and silt. Note:

each habitat type represents a part of the complete sample for the site.

3. Remove large objects from the sample and empty the content into the labelled sampling bottle.

4. If sorting and identification cannot be carried out in the field, the sample should be preserved with alcohol (90% ethanol or greater); macroinvertebrate samples should be carefully inverted a few times before transportation to ensure the sample has adequate contact with the preservative. The samples can be preserved for up to 3 months for analysis.

Figure 10. Sampling approaches in rivers and reservoirs for a) water, b) sediments, c) algae, and d) macroinvertebrates

Author's work based on: b) (https://www.knkx.org), c) (https://www.usgs.gov) y d) (https://www.canterbury.ac.nz)

CHAPTER VII

7. Field and laboratory analysis

In situ parameters are to be measured using portable instruments such as meters and spectrophotometers. All field information must be recorded in the fieldwork data registration sheets. This includes the following categories (see Table 4):

- Sample information: number of samples, site of the sample, code of the sample, date and time.
- Location: East (X) UTM and North (Y) UTM coordinates, altitude (Z).
- Physical parameters: temperature (°C), electrical conductivity (µS/cm) and total dissolved solids (mg/l).
- Chemical parameters: pH, alkalinity (mg/l) and dissolved oxygen (% & mg/l).
- Metalloids: Total As (mg/l).
- Also, field observations regarding bioindicators

Laboratory analysis results should also be recorded in the data registration sheets. According to the NOM-127-SSA1-1994, the following parameters are

to be measured using the corresponding analytical technique (see Table 4) (DOF, 1995):

· Physical parameters: turbidity (NTU).

· Chemical parameters: biological oxygen demand (mg/l), total nitrogen (mg/l) and total phosphorous (mg/l).

· Biological parameters: total coliforms (cfu/100 ml) and faecal coliforms (cfu/100 ml).

· Heavy metals: Hg (mg/l), Cd (mg/l) and Pb (mg/l).

· Metalloids: As V (mg/l).

The analytical techniques for the different types of samples are listed below:

· Water samples: Anion analysis: turbidimetry (determination of sulfates), volumetry (determination of chlorides). Cation analysis: atomic absorption spectroscopy (Cd & Pb) and hydride generation (As & Hg) (Tuzen *et al.*, 2009; & Benković *et al.*, 2012).

· Sediment analysis: loss-on-ignition (organic matter content determination) (Davies, 1978), inductively coupled plasma atomic emission spectroscopy (As, Cd, Hg & Pb).

· Cationic leachate analysis: atomic absorption spectroscopy (Cd & Pb) and hydride generation (As & Hg).

· Biological monitoring analysis: most probable number (total coliforms and faecal coliforms determination), BMWP score and ASPT method, and measurement of Chlorophyll-a.

7.1. Wet mount for microscopy

A wet (or fresh) mount is a simple way to prepare a specimen for microscopic examination. Fresh preparations are used to observe live microorganisms. There are two main techniques to prepare a wet mount; "between slide and covers" and "sloping drop". A between slide and covers preparation consists of placing a drop of liquid with the microorganisms on a slide and then covering it with a coverslip. A sloping drop preparation is performed by placing a drop of the material on a coverslip and covering it with a slide (inverted) with a central excavation (excavated slide). The preparation must be sealed with petroleum jelly around the excavation.

The advantage of this last technique is that the preparation does not dry out and can be observed for a longer time. However, the disadvantage of fresh observation is that it does not allow to increase the contrast of the preparation. Therefore, its use, with a bright field light microscope, is quite limited. Normally, to observe living microorganisms, optical or electronic microscopes are used. A recommended optical microscopy technique is described below (Johnson *et al.,* 1996):

1. Suspend samples in distilled water on standard microscope slides with cover slips.
2. Observe sample through a transmission microscope with a monochromatic filter.

7.2. Measurement of Chlorophyll-a for algae:

Phytoplankton chlorophyll is one of the most commonly used biological measurements in water quality assessments and monitoring for freshwater bodies, especially for the determination of the effects of increasing nutrients. For the measurement of Chl-a in the water samples, the standardized UNESCO spectrophotometry method was used. It measures the absorbance of light by Chl-a, at wavelengths of 663 nm, 645 nm and 630 nm; the following steps describe the procedure (Bartram *et al.*, 1996):

Figure 11. Algae and periphyton found at La Purisima Dam

1. Extract the Chl-*a* into 90% acetone solution. Place the top on the tube, label, and store in darkness at 4°C for 10 to 12 hours. Samples can be transported in this form.

2. Extract the microalgae using centrifugation for 10 min at 4000 rpm in centrifuge machine, decant the clear supernatant into a clean centrifuge tube and record volume.

3. Measure the absorbance of the microalgae with a spectrophotometer with wavelengths at 750 nm, 663 nm 645 nm and 630 nm. The blank being a 90% acetone solution.

4. Subtract the absorbance at 750 nm from the other three wavelengths to obtain the turbidity corrected value.

5. Repeat the process for all samples. Some preliminary samples may need to be taken to assess the best sample volume.

6. Use the following equations to calculate the concentration of Chl-a:

$$\text{Chl-a } (\mu g/l) = 11.64\ E_{663} - 2.16\ E_{645} + 0.10\ E_{630}$$

$$\text{Chl-a } (mg/l) = [\text{Chl-a} \cdot v]/V \cdot L$$

Where:

· v = Volume of acetone 90%, L
· V = Volume of water sample, L
· L = Light path of cuvette, cm
· E663 = Value of absorbance at wavelength 663 nm
· E645 = Value of absorbance at wavelength 645 nm
· E630 = Value of absorbance at wavelength 630 nm

The Chl-a determination method for the measure of algal biomass, however, is not considered to be a highly accurate approach (Ramaraj *et al.,* 2013). This is because the concentration of chlorophyll depends on several factors such as the wide species-specific difference in the cellular concentrations of chlorophyll, individual sizes of cells, and the stage in their reproductive cycles, as well as by the precision of the methods used for the analysis. Nevertheless, the level of accuracy of the UNESCO standardized method is considered to be satisfactory for the objectives of the present work.

7.3. Calculation of the BMWP score and ASPT for macroinvertebrates:

1. Separate the macroinvertebrates from other materials using a mesh and sort them into family groups (Bartram, 1996).

2. Identify the family groups and assign them their respective score according to the score system table. Note: even if more than one species occurs for a particular group that group is only recorded once.

3. Add the scores for all groups ticked on the record sheet to give the BMWP score (e.g., if *Oligochaeta, Chironomidae* and *Astacidae* were present the score should be 11).

4. Add up the total number of groups occurring in the sample (for example given in step 6 above the total number is 3).

5. Divide the BMWP score by the total number of groups present to give the ASPT (for the example above 11/3 = 3.66).

6. Record the result as BMWP and ASPT (for the example above BMWP = 11 and the ASPT = 3.66, which would suggest poor, polluted or impacted water quality).

SAMPLE INFORMATION	NUMBER	1	2	3	4	5	6	7	8	9	10
	SITE										
	CODE										
	DATE										
	TIME										
	PERSON										
LOCATION	East (X) UTM										
	North (Y) UTM										
	Z (m.a.s.l.)										
PHYSICAL PARAMETERS	T (°C)										
	E.C. (µS/cm)										
	TDS (mg/l)										
	TURB. (NTU)										
CHEMICAL PARAMETERS	pH										
	HCO3 (mg/l)										
	D.O. %										
	D.O. (mg/l)										
	BOD (mg/l)										
	Total-N (mg/l)										
	Total-P (mg/l)										
BIOINDICATORS	Total Coliforms (cfu/100ml)										
	Fecal Coliforms (cfu/100ml)										
HEAVY METALS	Hg (mg/l)										
	Cd (mg/l)										
	Pb (mg/l)										
METALOIDS	As (mg/l)										
OBSERVATIONS/ REMARKS											

Table 4. Algae and periphyton found at La Purisima Dam

8. Discussion

For the freshwater aquatic ecosystems, such as the ones found at La Purisima Dam and the Guanajuato River, a biomonitoring program, in parallel with the regular physico-chemical analysis, is an efficient and integrated methodology to assess the state of the ecosystems.

The best approach for the riverine ecosystem of the Guanajuato River is the ecological method, such as the Biological Monitoring Working Party (BMWP) in combination with the Average Score per Taxon (ASPT), given that they do not require major expertise in macroinvertebrates taxon identification. This method is also easy to reproduce in the field in terms of sampling, macroinvertebrate counting and indicator species identification.

In the case of La Purisima Dam, the measurement of chlorophyll-a from algae is the best biomonitoring approach, given the substantial presence of this bioindicator at the site, predominantly found at the eutrophication zones. This corroborates previous studies assessments on the high concentrations of nutrients such as nitrogen and phosphorous being discharged into the dam from its principal tributaries. Also, previous survey campaigns have indicated that the presence of bioindicators such as macroinvertebrates is very low. Interviews with local fishermen revealed that the quantity of captured fish has drastically decreased in the past years. This is evidence of the overall deterioration of the ecosystem.

The Municipal Landfill of the City of Guanajuato is currently operating at full capacity and out of the

normativity. The high volume of accumulated solid municipal waste and the high presence of waste leachate ponds are a source of pollution to the neighbouring areas, including water bodies such as the Guanajuato River and La Purisima Dam. According to previous studies, the total impact area is 149,700 m2 (Serafín-Muñoz *et al.*, 2020). Efforts must be made by the authorities to close down, relocate, and rehabilitate the landfill to avoid further ecosystem deterioration.

In order to allow the comparison of data from previous and future studies at the site of study, a well-planned monitoring program must be developed. The program must identify the most relevant sampling points, define the parameters to be measured, incorporate a standardized field and laboratory data blog, and consider at least one monitoring campaign during the dry and rainy seasons in order for the study to be statistically sound.

9. Conclusion

This book provides a reference guide for similar integrated assessments using physico-chemical analysis and biomonitoring techniques in other similar freshwater aquatic ecosystems with the aim to contribute to the evaluation and water quality control according to the established normativity. Additionally, it also contributes to good sanitary sampling practices as a health and safety perspective for the COVID-19 and any further pandemic.

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Bioindicators and Biomonitoring: Water quality control and sample management from La Purisima Dam in the state of Guanajuato, Mexico, involving safety measures against COVID-19 de Armando Guerrero Aguilar, Alma Hortensia Serafín Muñoz y Christoph Schüth terminó su tratamiento editorial en febrero de 2022 en el Programa Editorial Universitario de la Universidad de Guanajuato.

En su composición se utilizó la fuente tipográfica Avenir de 12 puntos, y el cuidado de la edición estuvo a cargo de Martín Eduardo Martínez Granados.