UNIVERSITA' DEGLI STUDI DI CATANIA

Dipartimento di Scienze Biologiche, Geologiche e Ambientali

Corso di Dottorato di Ricerca in

Scienze Geologiche, Biologiche e Ambientali

Presidente: Prof.ssa Agata Di Stefano

Dr PIETRO ZUCCARELLO

"Study of Sicilian Surface Freshwater Catchments for Environmental and Peoples Risk Assessment"

TESI DI DOTTORATO DI RICERCA

Tutor Scientifico
Chiar.ma Prof.ssa M. Ferrante

Co-Tutor Scientifico
Prof.ssa G. Oliveri Conti

XXXI Ciclo di Dottorato di Ricerca

Abstracts

Due to the population growth, urbanization and economic development, demand for freshwater in urban areas is increasing throughout Europe. At the same time, climate change and pollution are also affecting the availability of water supplies. Sicily, a southern island of Italy, suffers from a growing strong water scarcity and drought. The freshwater basins of Sicily are not well characterized and classified. In last decades, in Sicilian freshwater surface reservoirs several *Planktothrix rubescens* and *Microcystis aeruginosa* blooms have reported. A further actual worldwide problem for all surface freshwaters basins is the increasing of heavy metals concentrations in their waters.

Aims of this research doctoral project were: *a)* perform a census of Sicilian natural and artificial freshwater basins and identify which of these are suitable for use for drinking purposes; *b)* carry out a risk assessment related to presence of Microcystins and heavy metals (As, Pb, V, Cd, Al); *c)* evaluate the waters toxicity through ecotoxicological bioassays and verify if they would be sensitive and reliable to investigate on toxicity of mixtures of heavy metals and Microcystins.

It was carried out the monitoring of 15 surface basins, among the 30 existing in Sicilian territory, through seasonal chemical, physical and microbiological analysis of the waters such as required by Italian law, the Legislative Decree 152/2006. Moreover, it was performed speciation of cyanobacteria community, cell counting, and PCR analysis. Finally, it was carried out acute (V. *fischeri*), subacute (T. *platyurus*) and chronic (D. *magna*) ecotoxicological assays.

There is much reassurance about quality chemical status of basins. In fact, there are no heavy metals above the reference values. Only Aluminum and Iron are present often in large concentration. The high levels of total

nitrogen and phosphorus give information about the inflow of partially or completely untreated urban and rural wastewater in all basins.

However, in 50% of examined dams, there were the presence of several cyanobacteria species. In particular, it was detected cyanobacteria bloom in Disueri in period between July and September 2017. *Microcystis sp.* and *Cylindrospermopsis raciborskii* were detected (108 and 107 cell/L, respectively) and by mid-August were replaced by *Anabaenopsis sp.* and *Plankthotrix rubescens*, still growing in mid-September (107 and 106 cell/L, respectively). MCs concentrations in every samples were low, in agreement with the lack of qualitative amplification of the mcyE gene.

The distance biplot of PCA showed that Disueri, Pozzillo, Trinità and Arancio dams are located near together and are strongly influenced by N, Fe, Al, V, Mn, F, Mn, Cl, pH, Ca and Nitrite. In recent past, all these dams were currently affected by harmful algal bloom.

V. *fischeri* and D. *magna* assays seem to respond to the number of cyanobacteria cells rather than toxins concentrations. Instead, T. *platyurus* assay show a significant positive relationship between MCs concentrations measured by ELISA test.

In conclusion, the chemical quality of Sicilian surface freshwaters catchments is good and it could be improved it with a better treatment and management of wastewaters got into the surface waters. Concerning the Microcystins contamination, since in every analyzed sample the concentration was below the WHO reference value for drinking waters (1 µg/L) it seems not to be a high and worrying risk for human and environmental health in the brief time.

The simultaneous execution of *V. fischeri* and *T. platyurus* bioassays could favor the monitoring of waters both economically and technically.

PCA analysis seems to be reliable to be used as predictive models for cyanobacteria growth.

Finally, respecting these describes condition, it would be possible the use of waters of all monitored basins as drinking after an adequate treatment according to Italian Legislative Decree 152/2006.

A causa della crescita della popolazione, dell'urbanizzazione e dello sviluppo economico, la domanda di acqua dolce nelle aree urbane è in aumento in tutta Europa. Allo stesso tempo, i cambiamenti climatici e l'inquinamento influenzano anche la disponibilità di risorse idriche. La Sicilia, un'isola meridionale dell'Italia, soffre di una crescente scarsità d'acqua e siccità. I bacini d'acqua dolce della Sicilia non sono ben caratterizzati e classificati. Nell'ultimo decennio, sono state segnalate diverse fioriture di P. *rubescens* e M. *aeruginosa*. Un ulteriore problema a livello mondiale per tutti i bacini di superficie delle acque dolci è l'aumento delle concentrazioni di metalli pesanti nelle loro acque.
Obiettivi di questo progetto di ricerca sono stati: a) eseguire un censimento dei bacini d'acqua dolce naturali e artificiali siciliani e identificare quali di questi sono adatti per l'uso a scopo potabile; b) effettuare una valutazione del rischio relativa alla presenza di microcistine e metalli pesanti (come, Pb, V, Cd, Al); c) valutare la tossicità delle acque attraverso i saggi ecotossicologici e verificare se questi siano sensibili e affidabili per indagare sulla tossicità delle miscele di metalli pesanti e microcistine.
È stato effettuato il monitoraggio di 15 bacini di superficie, tra i 30 esistenti nel territorio siciliano, attraverso analisi stagionali chimiche, fisiche e microbiologiche delle acque come richiesto dalla legge italiana, il D.Lgs. 152/2006. Inoltre, è stata eseguita la speciazione della comunità dei cianobatteri, il conteggio delle cellule e l'analisi della PCR. Infine, sono stati eseguiti saggi ecotossicologici acuti (V. fischeri), subacuti (T. platyurus) e cronici (D. magna).

Ci sono molte rassicurazioni sulla qualità chimica dei bacini. Infatti, non ci sono metalli pesanti sopra i valori di riferimento. Solo alluminio e ferro sono presenti spesso in grande concentrazione. Gli alti livelli di azoto totale e fosforo forniscono informazioni sull'afflusso di acque reflue urbane e rurali parzialmente o completamente non trattate in tutti i bacini.

Tuttavia, nel 50% delle dighe esaminate vi era la presenza di diverse specie di cianobatteri. In particolare, è stata rilevata una fioritura di cianobatteri nella diga Disueri tra luglio e settembre 2017. *Microcystis sp.* e *C. raciborskii* sono stati rilevati e verso la metà di agosto sono stati sostituiti da *Anabaenopsis sp.* e *P. rubescens*. Le concentrazioni di MC in tutti i campioni erano basse, in accordo con la mancanza di amplificazione qualitativa del gene *mcyE*.

Il biplot di distanza della PCA ha mostrato che le dighe Disueri, Pozzillo, Trinità e Arancio si trovano vicine e sono fortemente influenzate da N, Fe, Al, V, Mn, F, Mn, Cl, pH, Ca e Nitriti. Negli ultimi tempi tutte queste dighe sono state colpite dalla proliferazione di cianobatteri nocivi.

I test ecotossicologici al V. *fischeri* e D. *magna* sembrano rispondere positivamente al numero di cellule di cianobatteri piuttosto che alle concentrazioni di tossine. Invece, il saggio di T. *platyurus* mostra una correlazione positiva significativa tra le concentrazioni di MCs misurate mediante test ELISA.

In conclusione, la qualità chimica dei bacini idrici di superficie delle acque siciliane è buona e potrebbe essere migliorata con trattamenti adeguati e una gestione migliore delle acque reflue immesse nelle acque superficiali. Per quanto riguarda la contaminazione da MCs, poiché in ogni campione analizzato la concentrazione era inferiore al valore di riferimento dell'OMS per le acque potabili (1 μg/L), non sembra esserci un rischio elevato e preoccupante per la salute umana e ambientale nel breve periodo.

L'esecuzione simultanea di prove biologiche di V. *fischeri* e T. *platyurus* potrebbe favorire il monitoraggio delle acque sia dal punto di vista economico che tecnico. L'analisi PCA sembra essere affidabile per essere utilizzata come modello predittivo per la crescita dei cianobatteri.

Keywords: Cyanobacteria, Surface Freshwater Catchments, ecotoxicological assays, heavy metals, Microcystins, Health Risk Assessment.

Introduction

Due to the population growth, urbanization and economic development, demand for freshwater in urban areas is increasing throughout Europe. At the same time, climate change and pollution are also affecting the availability of water supplies. Approximately one fifth of the total freshwater abstracted in European countries supplies public water systems. Among various problems related to water supplies, an important issue is the “quantitative” status or “disposable volume" of the freshwater supplies.

The quantitative status can be affected by phenomena such as droughts, floods and water scarcity and, currently, there is an imbalance in much of Europe's surface waters due to water use often exceeding the same water availability. Climate changes are also a further important driving force for both floods and droughts. Several of the social or socioeconomic impacts of water scarcity and droughts are related to public water supply. A deficiency in water supply negatively affects the quality of life of individuals and communities. Depending on the frequency, duration and extent of the interruptions in water supply, public health can be at risk and safety issues can arise.

Actually, increasing drought risk is a worldwide widespread problem, in particular for Mediterranean countries.

The freshwater basins of Sicily, a southern island of Italy, are not well characterized and classified; therefore, there are considerable knowledge gaps on the environmental condition of Sicilian surface freshwater basins used as supplies for municipal waterworks and for irrigation.

Compared to other Italian regions, Sicily suffers from a growing strong water scarcity and drought. There are numerous watercourses and surface basins in Sicily but only few of these have a significant flow and extension. Climate change along with unsustainable human management of water

resources are the causes of the widespread and frequent periods of drought affecting agricultural activities and the lives of the resident people.

To prevent urban water crisis, we need to manage all water resources effectively at every stage, especially the management of clean freshwater source. However, water management in Sicily is a complex issue.

Due to growing input of anthropic pollutant in surface freshwater bodies, in association with global warming, in all over the world more frequently harmful "algal" bloom (HABs) have occurred, especially toxic cyanobacteria bloom. In last decades, in Sicilian freshwater surface reservoirs several *Planktothrix rubescens* and *Microcystis aeruginosa* blooms have reported. These species can produce harmful toxins, known as Microcystins. Potentially, cyanobacterial toxins can accumulate in drinking water from surface basins also after the treatments. So, they have come to be a significant source of illness for local consumers.

So, it needs to monitor the Sicilian surface basins to know quality of waters for a better management of the proliferation and accumulation of cyanobacteria cells and their harmful biotoxins. This occurrence could lead to increased economic costs due to necessary adjustment of water filtration plants. In addition, HABs is harmful for fish, birds and animals with consequent negative effects in freshwaters ecosystem.

A further actual problem for all surface freshwaters basins is the increasing of heavy metals concentrations in their waters.

Heavy metals can cause health damage to consumers of the drinking water and, in view of an integrated management to maintain a good water quality, this toxicological aspect cannot be overlooked.

Although several adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues and it is even increasing in some parts of the world. Health concerns associated with heavy metals in drinking water arise primarily from their ability to cause

adverse health effects, mostly after prolonged periods of exposure to low doses, with exception to massive accidental contaminations of drinking water supplies. Toxic metals represent the ultimate form of persistent environmental pollutants because they are chemically and biologically indestructible and can give bioaccumulation and bio-magnification in aquatic ecosystem.

As metal contamination of drinking water supplies has been identified to be a common problem in many European countries, we intend to assess the content of Aluminum, Arsenic, Lead, Cadmium and Vanadium in surface freshwaters for their effect on the ecosystem and the health whose ingest it. Aluminum has been associated with several neurodegenerative diseases, such as dialysis encephalopathy, amyotrophic lateral sclerosis and Parkinsonism dementia, and, Alzheimer's disease.

An improvement of environmental monitoring systems would be innovative in this context. In the last years the development of new techniques and sophisticated assays has allowed the environmental monitoring plans to improve by ecotoxicological tests, overcoming the limitations of the study by classic biomonitoring, aimed for the simple assessment of concentration of contaminants in the abiotic field, and which shows inefficacy in the identification of the mechanisms by which contaminants affect biological communities. The use of several ecotoxicological tests overcomes this limit by studying the "toxicity state" of water supply. In fact, the presence of chemical contaminants in the environment is not imperatively a sign of adverse effects, but it is mandatory to establish how the mixture of pollutants can modify survival of bacteria and crustaceans.

Ecotoxicological methods may be used to establish water quality but some scientific problems still need to be solved, for example verify if ecotoxicological test is reliable when carried out on water polluted both by

algal biotoxins and heavy metals. Indeed, we don't still know if there are additive, synergistic, potentiating or antagonistic effects that can affect the evaluation of toxicity through ecotoxicological tests.

Chapter 1

Italian Use of Surface Waters like Drinking Water

Italy is an EU Member States that suffers the worsening of the quality of water destined for human consumption. In recent years, in fact, the need to use of the surface water from natural or artificial basins by local aqueducts to make up for the continuous increase in the demand for water destined for human consumption is emerging. From 2008 to 2012 there was a 3.8% increase in drinking water consumption not due to the increase in demand but rather due to a greater dispersion of water by the distribution water systems, which aren't more efficient.
The available ISTAT data allow describing, for the year 2012, the several use of the waters: civil, industrial, agricultural (irrigation and animal husbandry) and energy production. A total of 26.6 billion m^3 of water was used for all these activities in 2012. The 54.5% of the water demand comes from the agricultural sector, followed by the industrial sector (20.7%), the civil sector (19.5%) and the energy sector (5.3%). The agricultural use of water (equal to 14.5 billion m3) derives for 93.7% from irrigation practices and the remaining 6.3% from animal husbandry. However, the volume withdrawn from the environment to meet the entire water demand is estimated at 34.2 billion m^3. The difference between the volumes taken and used shows the overall level of water dispersion.

In 2015, in Italy, every citizen residing in a provincial capital consumed an average of 89.3 m^3 of drinking water, or 245 litres a day (268 litres in 2012). The municipal water network managers have authorized the total dispensing of 1.63 billion m^3 of drinking water in the 116 municipalities, corresponding to almost 30% of the entire Italian population.

To ensure the current level of consumption, the volume fed into the network is much higher than actually consumed and amounts to 2.64 billion m3 of drinking water. Therefore, for each citizen, an annual volume of 145 m^3 was put into the network, corresponding to 396 liters per day.

Overall, the volume of total water losses in the municipalities of provincial capital amounts to 1.01 billion m^3 in 2015, corresponding to a daily dispersion of 2.8 million m^3 of drinking water.

The level of losses is inevitably also linked to the number of connections and utilities served, certainly higher in large cities.

Below, the first tables shows the volume of water taken for drinking from various sources for entire Italy and for region by region; the second table reports the water fed into the municipal drinking water distribution networks and the water supplied by the municipal drinking water distribution networks, highlighting the total water losses (ISTAT, 2012).

Table 1. Volume of water withdrawn for drinking (m^3*1000) – year 2012

	Water Spring	Water Well	River	Natural Lake	Artificial Basins	Sea or Brackish Waters	Total
Italy	3495751	4527555	446646	71973	908772	7947	9458646
Lombardy	264711	1200996	1577	45428	758	0	1513471
Lazio	858371	300014	3592	24052	74	0	1186103
Campania	470269	457594	58	0	25002	0	952922
Veneto	230330	418943	63142	2385	0	0	714799
Sicily	169735	419456	4631	0	113350	6853	714025
Piedmont	293108	337726	20741	0	2746	0	654321
Emilia Rom.	41461	310655	108318	0	46117	0	506551
Tuscany	89509	236792	130225	0	4219	1094	461840
Calabria	194311	170930	46723	0	10027	0	421992
Sardinia	39655	40818	3521	0	246026	0	330020
Basilicata	40145	0	0	0	286632	0	326777
Abruzzo	232150	59716	11288	0	0	0	303154
Liguria	29760	132764	34155	0	47385	0	244065
Friuli V. G.	59613	163863	9614	0	1010	0	234100
Trentino	166075	32354	2853	108	0	0	201390
Puglia	560	88481	0	0	89827	0	178868
Marche	110698	36930	6208	0	21745	0	175581

Molise	114489	42671	0	0	13854	0	171014
Trento	105931	16101	2853	108	0	0	124993
Umbria	43738	71212	0	0	0	0	114950
Bolzano	60143	16253	0	0	0	0	76396
Valle d'Aosta	47063	5640	0	0	0	0	52703

Table 2. Water introduced into the municipal drinking water distribution networks and water supplied by the municipal drinking water distribution networks (m^3*1000).

	Water introduced into networks m^3	Water supplied by networks m^3
Italy	8356851	5232233
Lazio	942139	516828
Campania	827498	448616
Veneto	633860	407899
Sicily	693425	377372
Piedmont	600418	371995
Emilia-R.	484124	360072
Puglia	448166	292999
Tuscany	425577	261687
Calabria	327662	211612
Liguria	241338	166057
Abruzzo	231355	133546
Sardinia	293175	132414
Marche	164962	117332
Friuli V. G.	204305	112634
Trentino	146914	109250
Umbria	105473	64893
Trento	85439	63471
Bolzano	61474	45779
Basilicata	70591	43437
Molise	54329	28672
Valle d'Aosta	27433	21427

It is necessary to increase the use of every water sources present, especially from the surface basins. Unlike groundwater, which is of better quality and so they don't normally require complex processes of potabilization, surface water must be always treated to eliminate or reduce pollutants. The Italian regions with the highest share of surface water used are: Basilicata (83.6%), Sardinia (75.1), Tuscany (57.8%), Emilia-Romagna (55.6%) %) and Puglia (50.6%) (ISTAT, 2014).

Sicily is the bigger Italian region, it is the fourth for number of population and it is the second for use of water for agriculture activities (ISTAT 2016). Compared to other Italian regions, Sicily suffers from a growing strong water scarcity and drought. There are numerous watercourses and surface basins in Sicily but only few of these have a significant flow and extension. Climate change along with unsustainable human management of water resources are the causes of the widespread and frequent periods of drought affecting agricultural activities and the lives of the resident people.

As is also apparent from EEA report 2012, the freshwater basins of Sicily are not well characterized and classified; therefore, there are considerable knowledge gaps on the environmental condition of Sicilian surface freshwater basins used as supplies for municipal waterworks and for irrigation (Figures 1, 2 and 3).

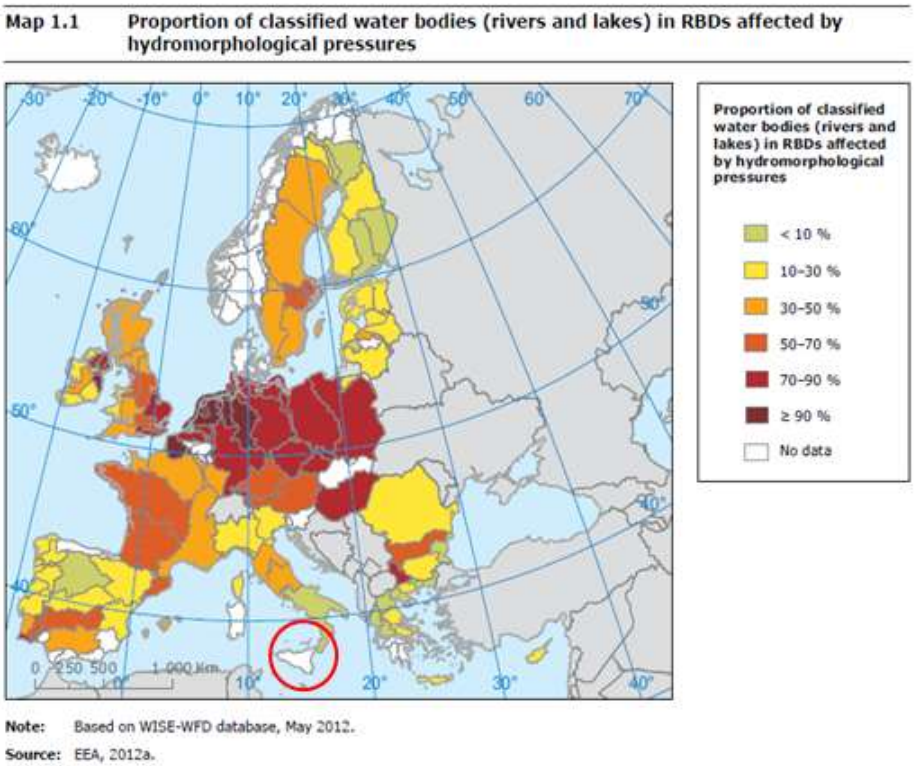


Figure 1. Note: RBDs= River basin districts

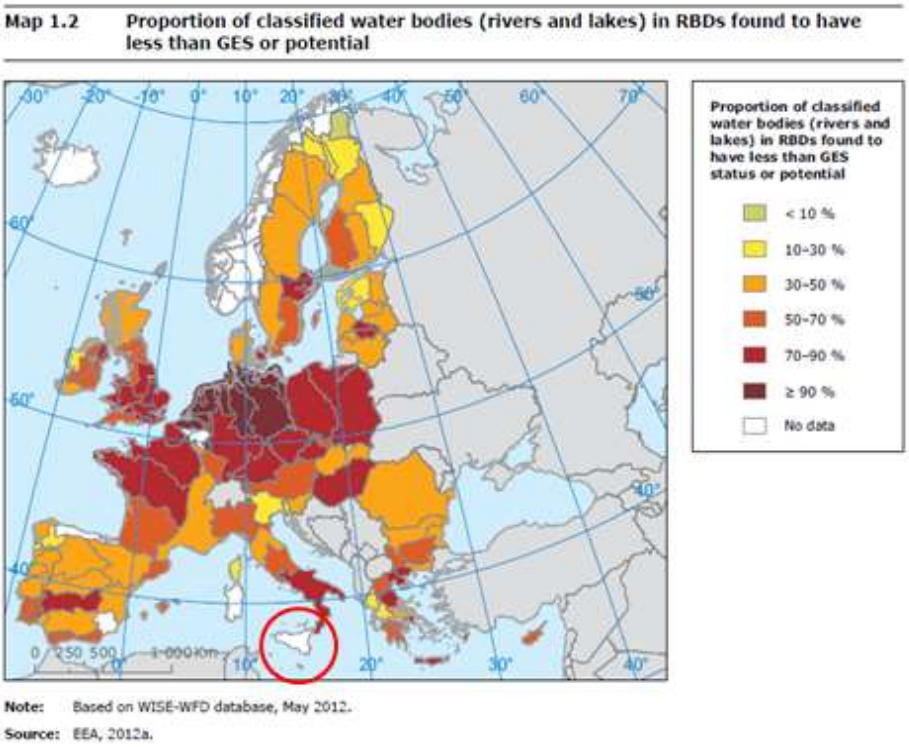


Figure 2. Note: 'good ecological status' (GES), 'good ecological potential' (GEP).

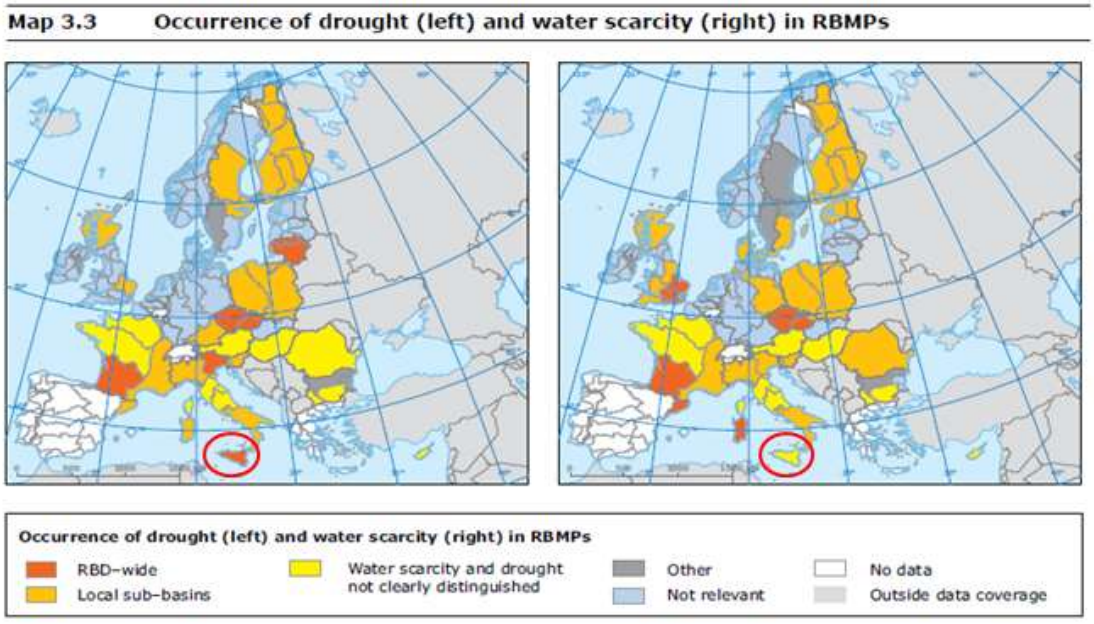


Figure 3. Occurrence of drought (left) and water scarcity (right)

Chapter 2

Current Italian Regulations

Legislative Decree 152/99, and the following Legislative Decree 152/2006, imposed the updating of the patrimony related to the state of the water resources, finalized to their characterization as well as to the identification of the underground water bodies that require specific interventions useful to their protection or to their qualitative recovery.

The cited Decree includes, among the purposes, not only the prevention and reduction of pollution and the restoration of water bodies, but also the protection and improvement of aquatic ecosystems, terrestrial ecosystems and wetlands dependent on aquatic ecosystems in terms of water requirements. The achievement of knowledge of the status of the water bodies through monitoring activities, allows the review and possible updating of the programs of measures to be taken.

The assessment of the quality of each water basin is determined by the value of the Ecological State (High, Good, Sufficient, Poor and Bad) and of the Chemical State (Good, Not Good), which can be evaluated based on the presence of a Biological Quality Element (EQB) such as phytoplankton, and chemical-physical parameters such as the amount of total phosphorus, hypolimnetic oxygen and water transparency.

For phytoplankton, the quality assessment index for Mediterranean artificial basins is the "MedPTI" (ISE-CNR, 2009); for some types of basins, the Ministerial Decree 260/2010 consider the use of the "PTIot index" (Phytoplankton Trophic Index based on optimum-tolerance). These indexes contribute to the composition of the Overall Index for Phytoplankton, ICF (now defined as IPAM/NITMET, New Italian Method of Evaluation of Phytoplankton), which is established by annual sampling and based on the average of the values of two parameters, the index

average biomass and the decomposition index. The calculation of these indices is based on several components, such as the average concentration of chlorophyll A, medium biovolume, PTI (PTIot or PTIspecies), percentage of cyanobacteria present. The ICF is then integrated with these indices to define the Ecological Status of the Surface Water Body.

The chemical status of the water basins is assessed on the analysis of the pollutants included in the priority list (Table 1/A of MD 260/2010 amended by Legislative Decree 172/2015, in accordance with Directive 2013/39/ EU). For the achievement of the Good status, the concentrations of these substances must comply with the Environmental Quality Standards (SQA) in terms of annual average (SQA-MA) or maximum permissible concentration (SQA-CMA). It's enough that only one element exceeds these values to fail the achieve the Good status.

To be used to produce drinking water, surface freshwater must be classified in the categories A1, A2 and A3, according to the physical, chemical and microbiological characteristics, referred to in Table 1/A of Annex 2 to the third part of the legislative decree 152/2006. According to the category of belonging, surface fresh water must be subjected to different treatments: water in the category A1 undergoes physical treatment and disinfection, in the A2 category physical-chemical treatment and disinfection while in the A3 category strong physical-chemical treatment and disinfection.

Table 4: Characteristics of quality concerning heavy metals for surface water intended to produce drinking water

Parameter	Unit of measure	A1 G	A1 I	A2 G	A2 I	A3 G	A3 I
Iron	mg/L	0,1	0,3	1	2	1	-
Manganese	mg/L	0,05	-	0,1	-	1	-
Copper	mg/L	0,02	0,05	0,05	-	1	-
Zinc	mg/L	0,5	2	1	5	1	5
Boron	mg/L	1	-	1	-	1	-
Beryllium	mg/L	-	-	-	-	-	-
Cobalt	mg/L	-	-	-	-	-	-
Aluminium	mg/L	-	-	-	-	-	-
Nickel	mg/L	-	-	-	-	-	-
Vanadium	mg/L	-	-	-	-	-	-
Arsenic	mg/L	0,1	0,05	-	0,05	0,05	0,1
Cadmium	mg/L	0,001	0,005	0,001	0,005	0,001	0,005
Total Chrome	mg/L	-	0,05	-	0,05	-	0,05
Lead	mg/L	-	0,05	-	0,05	-	0,05
Selenium	mg/L	-	0,01	-	0,01	-	0,01
Mercury	mg/L	0,0005	0,01	0,0005	0,01	0,0005	0,01
Barium	mg/L	-	0,1	-	1	-	1

Chapter 3

Cyanobacteria and Cyanotoxins

Cyanobacteria (Cyanophyte) are aquatic organisms that can be found both in fresh and brackish waters, in sea and in thermal waters (Ferrante et al., 2016; Hensea & Beckmanna, 2006; Furnari et al., 2008; Carmichael, 1994; Funari & Testai, 2008). They can be very dangerous since some species could produce very powerful bio-toxins, also known as cyanotoxins.

In favourable conditions, algal cells may reach high concentrations producing known "harmful algal blooms" with strong cyanotoxins release (Ferrante et al., 2010; Ferrante et al., 2013; Sciacca & Oliveri Conti, 2009; Carmichael, 1994).

Temperatures above 20 °C, sunlight and high concentrations of nutrients (mainly phosphorus and nitrogen) favor their proliferation (Hudnell, 2010). These substances are often of anthropogenic origin, deriving therefore from the use of fertilizers, from civil and industrial waste and from outflows from agriculture (Paerl, 2008). Furthermore, the increase in population and the consequent development of urban areas has led to the exhaustion of the wetlands, essential for filtering the waters before reaching the aquifer (Hudnell, 2010).

Therefore, the use of surface freshwaters for several human purposes may not be safe due to the presence of cyanotoxins (Funari & Testai, 2008). These secondary metabolites may represent a risk to human health. They can be classified into four different categories based on the effects observed in human (Carmichael, 1997). They can be classified as:

- ✓ Hepatotoxins (microcystins and nodularins);
- ✓ Cytotoxins (cylindrospermopsins);
- ✓ Neurotoxins (anatoxins, saxitoxins, β-N-methylamine-L-alanine);
- ✓ Dermatotoxins (lingbiatossins and aplisiatossins).

The table below (table 3) summarizes the main cyanotoxins and the related cyanobacteria capable of producing them. Some toxins can be released from multiple kinds of cyanobacteria (Lopez *et al*., 2008). Cyanobacteria belonging to the same genus can also produce different types of toxins. There are also structural variants of cyanotoxins (Davis et al., 2009).

Tabella 3. List of genera of cyanobacteria and associated toxins (Lopez et al., 2008)

Type of toxin	**Cyanobacteria**
Microcystins	*Anabaena*, *Aphanocapsa*, *Hapalosphon*, *Microcystis*, *Nostoc*, *Planktothrix (Oscillatoria)*
Nodularins	*Nodularia spumigena*
Saxitoxins	*Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*
Anatoxins	*Anabaena*, *Aphanizomenon*, *Planktothrix (Oscillatoria)*
Cylindrospermopsins	*Aphanizomenon*, *Cylindrospermopsin*, *Umezakia*
Lipopolysaccharide (LPS)	*Aphanizomenon*, *Planktothrix (Oscillatoria)*
Lingbiatossins	*Lyngbya*
β-N-methylamine-L-alanine (BMAA)	*Anabaena*, *Cylindrospermopsin*, *Microcystis*, *Nostoc*, *Planktothrix (Oscillatoria)*

While the factors that stimulate or promote the blooms are almost known, those that trigger the production of toxin or the dominance of toxic and non-toxic strains are still largely unknown. There is increasing evidence of the role of nutrients in toxin production and in the dominance of toxic strains (Anderson et al., 2002; Davis et al., 2009). In the last decade, some studies have suggested that heating and continuous eutrophication of surface basins favor toxic strains on non-toxic strains (Vézie, et al., 2002).
Among cyanotoxins, *Microcystins* are the most widespread and hazardous (Ferrante et al., 2013; Ferrante et al., 2010). In fact, the LD50 dose for

Microcystins-LR is 50 μg/kg, compared to 500 μg/kg for strychnine and 10.000 μg/kg for sodium cyanide (Samdal et al., 2014; Wood R., 2016). The acute or chronic Microcystins exposure may cause severe and sometimes fatal hepatotoxicity and it may also be associated to increased onset cancer both in humans and animals (Miller et al., 2010; Wood R., 2016). The main action mechanism is the proteic phosphatases inhibition which causes changes in cytoskeleton, oxidative stress, apoptosis (Huang et al., 2015), an increase of certain transcription genes, induction of cell proliferation and liver hypertrophy (Achene, 2011; Funari & Testai, 2008). There are several hypotheses about the eco-physiological role of Microcystins in cyanobacteria: since their release uses active transport processes with a significant energy consumption (Rapala et al., 1997), they can represent their defence and/or attack mechanism against competitors (eg. to improve resistance in the presence of oxidative stress). It has been shown that increased Microcystins release in an environment enhances their survival (Zilliges et al., 2011; Schatz et al., 2007).

In the last decades we are witnessing a progressive increase in the incidence of CHABs (Chorus e Bartram, 1999; Carmichael, 2008; Hudnell, 2010). The numerous studies carried out and the published reports regarding this problem seem to be correlated not only with greater attention to the problem, but above all to the increase in the frequency and severity of these algal blooms, as well as to the appearance in previously unaffected geographical areas (Lopez *et al*., 2008).

Cases of animal poisoning associated with exposure to cyanotoxins in fresh water are reported in the literature: the first case of poisoning due to cyanobacteria blooms was in 1878 in Australia (Francis, 1878). The first case described in humans was a gastrointestinal disorder caused by cyanobacterial toxins in the Ohio River in 1931 (Tisdale, 1931; Veldee, 1931). The most serious case was reported in Brazil, where many dialysis

patients died due to the presence of Microcystins in the water used to produce dialysates (Ferrante et al., 2016). The presence of cyanobacteria blooms has been documented all over the world (Funari *et al*., 2008).

In Italy, the phenomenon of algal blooms in surface freshwater is mainly caused by two Cyanophyceae. The first is the species *Planktothrix rubescens*: this species, thanks to the high demand of nitrogen, which cannot fix from the atmosphere, and the moderate need for phosphorus, is an excellent ecological competitor in most Italian lakes.

P. rubescens blooms mainly during autumn and winter while it be inclined to go in deep in the hypolimnum during the spring and summer (Kurmayer *et al*, 2016). The second most widespread Cianophycea is *M. aeruginosa*: this species generally has a hot stenothermic and prefers waters with high levels of phosphorus. Its blooms are often impressive, both in growth and decline. A cause of its annual cycle and speed of replication can be a serious health risk for bathing, as well as for potability and contamination of fish fauna (Mattei *et al*, 2005).

The following figure 4 shows the distribution of cyanobacteria in the various Italian regions, as reported in the literature (Funari *et al*, 2008).

Figure 4. Distribution of cyanobacteria in the various Italian region.

Chapter 4

Heavy Metals

Heavy metals are chemical elements naturally present in the earth's crust. The wide use of metals in all anthropic activities, from agriculture to industry, has caused the contamination of the atmosphere, waters, food chain with the consequent harmful human exposure. Indeed, they are almost ubiquitous in the environment.

Heavy metals are aluminum, iron, silver, barium, beryllium, cadmium, cobalt, chromium, manganese, mercury, molybdenum, nickel, lead, copper, tin, titanium, thallium, vanadium, zinc, and some metalloids with similar properties such as arsenic, bismuth and selenium. Some of these (e.g. Co, Cr, Cu, Fe, Mo, Zn, V) are essential to normal human physiology but high concentration of these can be cause of toxic effect; instead, others (e.g. As, Pb, Hg, Cd, Al) can lead to high risks of toxicity for human.

It follows a brief report concerned to the five heavy metals considered in this research project (As, Al, Cd, Pb and V).

Arsenic is a metalloid widely present in the rocks, soils, waters and air. Inorganic arsenic is the most abundant. It's largely used to produce the semiconductors, glassware, wood preservatives and pesticides and in many others industrial activities (Garelick et al., 2008).

Inorganic arsenic compounds are also more toxic respect than organo-arsenicals; moreover, trivalent arsenite is more toxic than pentavalent arsenate (Zhang et al., 2015; Mandal et al., 2001).

After oral consumption, the absorption rate of arsenic in the gastrointestinal tract is above to 90%. Arsenic binds to red blood cells, and deposits in the liver, kidneys, muscle, bone, hair, skin, and nails. The major metabolic pathway of arsenic is methylation wherewith it's metabolized into MMA,

and then into DMA. At last, arsenic and its metabolites are mainly excreted through urine (Hong et al., 2014).

Arsenic affects cellular respiration via inhibition of various mitochondrial enzymes and consequentially interfering with oxidative phosphorylation (Goyer, 2001).

Recent studies have shown that the toxicity of arsenic is dependent on more factors as the exposure amount, length, and frequency, beyond those on the age, sex, individual sensitivity, genetics, and nutritional factors (Hong YS et al., 2014).

Moreover, the toxic mechanism behind arsenic seems to be also related to chromosome abnormalities, oxidative stress, altered DNA repair, altered DNA methylation patterns, altered growth factors, enhanced cell proliferation, promotion/progression, suppression of p53, and gene amplification.

A lot of research present in current Literature shows how arsenic affects the onset of skin (Leonardi et al., 2012), lungs (Garcia-Esquinas et al., 2013), bladder (Meliker et al., 2010), liver (Lin et al., 2013), and prostate cancer (Garcia-Esquinas et al., 2013).

At the moment, the previously reported provisional tolerable daily intake (PTWI) for In-As (2.1 μg/kg bw per day) is considered no longer health protective as the BMDL0.5 value was in the same range as the PTWI value. Based on data from an epidemiology study conducted on a highly-exposed population, the In-As lower limit on the benchmark dose for a 0.5% (BMDL0.5), increasing incidence of lung cancer was calculated to be 3 μg/kg bw per day (range: 2–7 μg/kg bw per day), using a range of assumptions to estimate total dietary exposure of the study population to In-As from drinking water and food (Ferrante et al., in press).

Aluminum is the third most abundant element in the earth's crust and it occurs primarily in aluminosilicate minerals. Aluminum is an important

aquatic contaminant due to its ubiquity, toxicity and weak regulatory discharge limits (He & Ziemkiewicz, 2016). It's widely used in the fields of medicine, pharmacy, food technology, and cosmetics. It's also commonly used in food preparation equipment and kitchen utensils. Aluminum exposure occurs mainly through environment, occupational, and diet for humans. Approximately, the 95% of the total of Al, taken by oral way, is excreted through feces (Aronson, 2006).

Public and consumer health considerations, focused on hypotheses about aluminum and neurocognitive impacts, are systematically reviewed and assessed. Indeed, although the mechanisms of how aluminum enters in the brain are not fully known, it is thought that it passes through the blood–brain barrier through transferrin and accumulates in the brain cortex that is rich in transferrin receptors (Bondy, 2016).

It's largely recognized that Aluminum is a neurotoxin, which could cause neurodegeneration. It affects important biological reactions such as axonal transport, neurotransmitter synthesis, synaptic transmission, phosphorylation or de-phosphorylation of proteins, protein degradation, gene expression, peroxidation, and inflammatory responses (Kawahara & Kato-Negishi, 2011).

Although the relation between aluminum and neurodegenerative diseases is still controversial, it seems to be related with brain diseases like Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis (MS) (Ferrante & Conti, 2017; nan-Eroglu & Ayaz, 2018).

New data did not substantially change the LOAEL range of 50–75 mg/kg bw per day, but one of the studies also provided a NOAEL of 30 mg/kg bw per day. This NOAEL was identified from a study in which aluminum citrate was administered in drinking-water. Aluminum citrate is more soluble than many other aluminum compounds and is likely to be more bioavailable from drinking-water than from food. The Committee

concluded that the NOAEL of 30 mg/kg*bw per day was an appropriate basis for establishing a PTWI for aluminum compounds. For adults, the estimates of mean dietary exposure to aluminum-containing food additives from consumption of cereals and cereal-based products are up to the PTWI of 2 mg/kg*bw. Estimates of dietary exposure of children to aluminum-containing food additives, including high-level dietary exposure, can exceed the PTWI by up to 2-fold (JEFCAa).

Vanadium is omnipresent in trace amounts in the environment, in food and, also, in the human body, where it might serve as a regulator for phosphate-dependent proteins. About 80% of the world produced vanadium is being used in steel industry as additive. It can be found in different production area such as those of automobiles, shipyard, fertilizers, catalysts to ceramics, pigments, batteries and several industrial areas. Vanadium pollution is observed in groundwaters and water resources such as rivers, lakes and seas. Drinking waters contamination can cause harmful toxic effects on health and several diseases (Rehder, 2016; Imtiaz et al., 2015).

Inorganic vanadium compounds can lead to harmful health effects such as carcinogenic, immunotoxic and neurotoxic insults. Concerning to the carcinogenic activity, in vitro studies have evidence that vanadium can induce genotoxic lesions, cell morphological transformation and anti-apoptotic effects in a certain type of cells (Zwolak, 2014).

The neurotoxic effects of V have been mainly attributed to its ability to produce reactive oxygen species (ROS). It seems that the neurotoxicity induced by vanadium exposure generally occurs with co-exposure to other metals (Ngwa et al., 2017). Moreover, other studies have shown a link between vanadium and oxidative stress in the etiology of diabetes (Valko et al., 2005).

Harmful effects of vanadium on human health are still controversial since vanadium compounds, in addition to playing an essential biochemical role,

have shown to be potentially effective against diabetes Type 2, malign tumors including cancer, endemic tropical diseases (such as trypanosomiasis, leishmaniasis and amoebiasis), bacterial infections (tuberculosis and pneumonia) and HIV infections (Rehder, 2016).

Despite extensive investigation of the toxicity of pentavalent V, little is known of the effects of high-level exposure on human health, nor have thresholds been set by national or international bodies or institutions such as the European Food Safety Authority (EFSA) or the World Health Organization (WHO). The Environmental Protection Agency (EPA) alone has suggested a reference dose (RfDo) for oral intake of 0.009 mg/kg/day body weight (bw) based on the observation of decreased hair cysteine levels in rat studies. Research is ongoing to determine whether regulations are needed (Arena et al. 2015).

Cadmium is a widespread toxic pollutant of occupational and environmental concern due to its diverse toxic effects. Major exposure derived from cigarette smoke, food, water and air contamination.

Cadmium has no physiological role in humans and has a long half-life with strong hemoglobin binding and a wide tissue distribution. It can bind with several enzyme, replacing the essential metals such as zinc and calcium, and consequently it blocks their activities.

Cadmium induce several toxic effects including nephrotoxicity, carcinogenicity, teratogenicity and endocrine and reproductive toxicities.

Regarding cancerogenic activity, cadmium affects cell proliferation, differentiation, apoptosis and other cellular processes. Relevant is that cadmium interacts with DNA repair mechanism, with the generation of reactive oxygen species and induction of apoptosis (Rani et al., 2014).

It seems to be Cadmium exposure is related to the disruption of hippocampus region in brain and to consequential neurotoxicity (Karri et al., 2016).

The Codex Committee on Contaminants in Foods, at its Sixth Session, requested that the Committee conduct an assessment of dietary exposure to cadmium from cocoa and cocoa products. The potential dietary exposures to cadmium for high consumers of products containing cocoa and its derivatives in addition to cadmium derived from other foods were estimated to be 30–69% of the PTMI for adults and 96% of the PTMI for children 0.5–12 years of age. The Committee noted that this total cadmium dietary exposure for high consumers of cocoa and cocoa products was likely to be overestimated and did not consider it to be of concern.

Estimated mean population dietary exposure to cadmium from products containing cocoa and its derivatives, from GEMS data: 0.005 to 0.39 μg/kg bw/month (0.02–1.6% of the PTMI); from national data: 0.001 to 0.46 μg/kg bw/ month (0.004–1.8% of the PTMI) (JEFCAb).

Lead is one of the most toxic metals without any biochemical role. Furthermore, among metals, it is one of the most used and it has found a wide range of industrial applications such as paints, water tape, cosmetics, fuel (Shotyk & Le Roux, 2005). Lead environmental or occupational exposure can cause a multitude of toxic effects on human health. It increase production of free radicals (e.g. ROS) and decrease availability of antioxidant reserves (e.g. Glutathione, Vitamin C, Vitamin E, Vitamin B1, Carotenoids) (Patrick , 2006). Moreover, it was observed an alteration of immune system (Basaran & Undeger, 2000), a dyslipidemia with consequent cardiovascular disease (Ademuyiwa et al., 2005), the promotion of brain dysfunction, behavioral changes and cognitive function disorder (Cory-Slechta, 2003; Nehru & Sidhu, 2001).

Chronic lead poisoning is one of the most common disease of environmental and occupational origin especially in developing countries.

It was shown that erythrocyte acetylcholinesterase activity was negatively correlated with blood lead levels and its inhibition would explain one of mechanism of lead-induced neurotoxicity (Ademuyiwa et al., 2007).

The Committee considered the neurodevelopmental effects of lead to be pivotal in its assessment for children. Based on the results of a metaanalysis of epidemiological data, the chronic dietary exposure corresponding to a decrease of 1 IQ point was estimated to be 0.6 μg/kg bw/d (5th to 95th: percentiles 0.2–7.2 μg/kg bw/d). For adults, the Committee concluded that the pivotal data were the increased systolic blood pressures. Based on the averaged median reference slope estimates for blood lead levels versus systolic blood pressure from 4 epidemiology studies, the dietary exposure corresponding to an increase in systolic blood pressure of 1 mmHg (0.1333 kPa) was estimated to be 1.3 (5th to 95th percentiles 0.6–28) μg/kg bw/d. Based on this analysis, the previously established PTWI of 25 mg/kg bw is associated with a decrease of at least 3 IQ points in children and an increase in systolic blood pressure of approximately 3 mmHg (0.4 kPa) in adults. These changes are important when viewed as a shift in the distribution of IQ or blood pressure within a population. The Committee concluded that the PTWI could no longer be considered health protective, and it was withdrawn. Because the analyses do not indicate a threshold for the key effects of lead, the Committee concluded that it was not possible to establish a new PTWI that would be considered health protective. The Committee stressed that these estimates are based on dietary exposure (mainly food) and that other sources of exposure to lead also need to be considered.

Previously established PTWI withdrawn. Not possible to establish a new PTWI that would be considered health protective (JEFCAc).

Chapter 5

Ecotoxicological Bioassay

To monitoring the pollution levels of ecosystems, the simple assessment of contaminants does not allow always-reliable assessments since it is no considered the effects that substances can exercise simultaneously on organisms.

The effect due to substances is related to their binding in the biological receptor, although the specific conditions of the matrix and the environment can influence their interaction. Therefore, it is necessary to integrate the chemical data with that relating to the biological and ecotoxicological investigations (Caciolli et al., 2016).

The effects depend on the type of exposure that can be single (when the organism is exposed only once to the toxic substance); multiple (more exposures later), or chronic (the organism is constantly exposed to the substance). The interaction between different substances to which the organism is simultaneously exposed can also lead to synergistic effects, for example agonist or antagonist.

Ecotoxicological tests are effective tools for the assessment of contamination; their purpose is to verify if a potentially toxic compound would cause a relevant biological response in the organisms exposed.

Toxicology tests can evaluate acute, sub-acute or chronic toxicity. They make it possible to determine a cause-effect relationship, but the results obtained are often valid only for experimental conditions and cannot be extended to other species or complex natural systems as they do not consider the interactions that can occur between biota and environment. In ecotoxicological assays, living organisms under optimal conditions are exposed for a certain time at different concentrations of substance or

sample and the response shown by the organism is evaluated (Maffiotti et al., 1997).

The observed and measured parameter (endpoint) in the different ecotoxicological tests may be mobility, survival, size or growth, number of eggs or children, or any biochemical or physiological variable that can be reliably quantified. The aim of test is to establish a relationship between endpoints and the concentration of substance with the purpose to determine the Lethal Dose 50 (LD50) or that to evaluate the contamination level of a sample.

Vibrio fischeri is a bioluminescent marine bacterium, Gram negative aerobic-anaerobic facultative, which in nature exists both in the free state and as a symbiote of fish and marine cephalopods.

The mechanism by which *V. fischeri* regulates its bioluminescence is related to a "cell-cell" communication system, known as *quorum sensing*, with which the bacteria can communicate with each other. The luxI gene, enclosed in an operon called lux-ICDABE, encodes the synthesis of a specific synthetase which in turn leads to the synthesis of a specific molecule, N-(3-oxohexanoyl)-myoserine lactone, known the autoinducer. Simultaneously, the luxR gene encodes the production of a transcription factor which, after having interacted with the autoinducer, binds to the lux-ICDABE operon, activating the transcription of genes that lead to the synthesis of all the components of the luciferase system as well as an exponential increase in the synthesis of LuxI (Adar et al., 1992).

V. fischeri can emit light of blue-green color at a wavelength of 430 nm. The sequence of reactions that causes the emission of light is associated with the respiratory chain of electron transport; it is catalyzed by the enzyme luciferase which, in the presence of a long chain aliphatic aldehyde and 5-P riboflavin, allows the oxidation of bacterial luciferin with the formation of oxy-Luciferin and the emission of light (Perin, 2004).

For a mechanism that is still not completely clear, when the bacteria come into contact with toxic substances, there is a quantitatively measurable lowering of the luminescence. Probably, toxic substances cause the inhibition of some enzymes or they intervene in the membrane transport processes, leading to a slowing of the energy production reactions and, therefore, a reduction in the emission of bioluminescence.

By *V. fischeri* bioassay is possible to measure quantitatively the **acute toxicity** with a time of exposure among five and thirty minutes. The toxicity of a substance is measured in terms of EC50, ie the concentration for which there is a 50% decrease in the light emitted by the bacteria, while the toxicity of a sample is measured in terms of percentage of light decreasing.

Daphnia magna is a crustacean of freshwater that is easy to breed, of good availability and of small size (5-6 mm individuals, <1 mm newborn), belonging to the order of the Cladoceri, in the subclass of the Branchiopodi. Under normal conditions, Daphnia magna reproduces by parthenogenesis, therefore, a population of daphniids is composed entirely of adult females of various ages with the same genetic makeup, since they are mothers-sisters of each other. Sexual reproduction, instead, happen in response to negative environmental stimuli, so that some newborn organisms, under hormonal influence, become males and mate with adult females, which produce the ephippia, who have only two eggs that do not develop but remain wrapped in a thick layer of carapace, and remain latent until the change in conditions favorable for their development. The bioassay commonly uses this "sleeper" form (ephippia), which can be activated directly before each individual test.

By *D. magna* bioassay is possible to measure quantitatively the **chronic toxicity** with a time of exposure of twenty-one days. The toxicity of a substance is measured in terms of EC50, ie the concentration for which

there is a 50% of mortality, while the toxicity of a sample is measured in terms of percentage of death.

Thamnocephalus platyurus is instead a crustacean of freshwater of small size among 1-3 mm for adults while less of 1 mm for newborns, belonging to the family of Thamnocephalidae.

By *T. platyurus* bioassay is possible to measure quantitatively the **subacute toxicity** with a time of exposure of twenty-four hours. The toxicity of a substance is measured in terms of EC50, i.e. the concentration for which there is a 50% of mortality, while the toxicity of a sample is measured in terms of percentage of death.

Chapter 6

Aim of Doctoral Research Project

In Italy the distribution of surface water is not homogeneous; indeed, among the 400 lakes with surface area greater than 0.2 km^2 with a total water volume of about 150.000.000 of m^3, above125.000.000 of m^3 are located in Northern Italy (Orta, Maggiore, Lugano, Como, Iseo, Idro and Garda); more than 25.000.000 million m^3 are distributed in the remaining part of Italy and most of this volume is located in Central Italy, such as in the lakes of Bolsena, Bracciano, Vico and Trasimeno; only the 3% of the total volume is found in Southern Italy and in the main Italian islands (Sicily and Sardinia), mainly in artificial reservoirs used for drinking and irrigation purposes (Bruno *et al*,. 2016).

Unfortunately, the freshwater basins of Sicily are not well characterized and classified according to the Legislative Decree n. 152 of 3 April 2006 and s.m.i.; therefore, there are significant knowledge gaps on the environmental status of these basins and their potential uses. Moreover, several cases of cyanobacteria blooms phenomena have periodically been reported in many reservoirs across Sicily (Arancio and Castello dams in Agrigento, Trinità dam in Trapani, Pozzillo dam in the province of Enna), the greatest island in south of Italy. In past occurrences, the main species emerged particularly in basins of Sicily where *Microcystins aeruginosa* and *Planktothrix rubescens*, Microcystins producing species can also be found (Naselli-Flores et al., 2007; Naselli-Flores & Barone, 2003). However, the Italian regulation no providing the monitoring for cyanobacteria e cyanotoxins to ensure adequate security of waters.

For this purpose, ecotoxicological methods could be used to objectify the assessment of the quality of surface water quality potentially contaminated by chemical mixtures and cyanotoxins. However, it is not yet reliable that

ecotoxicological bioassays are sensible also to investigate on toxicity of mixtures of every type of contaminants such as heavy metals and Microcystins (De Coninck DI et al., 2013).

Aims of this research doctoral project were: *a)* perform a census of Sicilian natural and artificial freshwater basins and identify which of these are suitable for use for drinking purposes; *b)* carry out a risk assessment related to presence of Microcystins and heavy metals (As, Pb, V, Cd, Al); *c)* evaluate the waters toxicity through ecotoxicological bioassays and verify if they would be sensitive and reliable to investigate on toxicity of mixtures of heavy metals and Microcystins.

Chapter 7

Materials and Methods

7.1 Census of Sicilian freshwater basins

To aim to perform a census of every Sicilian freshwater basins, the **Department of Water and Waste of Sicilian Region**, the **SicilAcque Society** and **ENEL Society** were involved into the research like partner of the Doctoral Project. Through their documentations, it has been possible to contact the manager of each basin who described carefully the management of reservoir, treatment and distribution of water.

After obtaining the necessary authorizations, it was possible to select the basins of interest for our study according to the following characteristics:

- geographical location (at least one per province);
- intended use (giving priority to those already use for drinking);
- historical memory (already affected by cases of "cyanobacteria bloom");
- *significance of the reservoir* according to Annex 1 to Part III of Legislative Decree 152/2006 "*those whose feeding basin is affected by anthropic activities that could compromise the quality and having a liquid mirror surface of at least 1 Km^2".*

7.2 Sampling

For each selected basin, planned sampling was seasonal, but the number of sampling has been performed based on authorizations of reservoirs managers and, mainly, on water quality and risk of cyanobacteria blooms. So, for some basins it was carried out two seasonal sampling, for others three and for other also four. Where it was in act a bloom, it was performed one sampling every week for a full month.

Moreover, based on available of boat, for each basin it was planned sampling in four point (three on surface and one on thermocline). However, where there is no boat it was carried out only one or two sampling in the best points of access on the water.

Preliminarily, to determine the composition of the phytoplankton community and the possible presence of cyanobacteria species, the samplings were initially qualitative and were carried out on the whole water column and the surface of basins through a *type Apstein* net for Plankton with a porosity of 20 μm, complete with collecting cup with filter and tap. A series of netfuls were made up to collect about 200 ml of final sample to be divided into:

- n.1 of 100 ml sterile plastic bottle (polyethylene or polycarbonate) for molecular analysis, not to be fixed with any reagent;
- n.2 of 50 ml plastic bottle (like Falcon, polyethylene or polycarbonate) fixed the first with Lugol solution (final concentration 1%) and the second with formaldehyde (final concentration 4%). These aliquots were used for cyanobacteria species identification and counting.

After, for the determination of the chemical-physical parameters according to Legislative Decree 152/06, in each sampling point about 10 liters of water were sampled by an immersion sampler provided with a 1 L glass bottle with automated closure. Samples were divided in aliquots which stored into different types of containers:

- n.2 x 250 ml Winkler bottles with emery cap for the determination of COD, BOD5 and Dissolved Oxygen;
- n.1 bottle of 1L sterilized glass for microbiological analysis;
- n.1 bottle of 1 L amber glass for the determination of PAHs and pesticides;
- n.1 glass bottle of 1 L for the analysis of anions and other chemical indicators;

- n.1 x 60 ml vials for the determination of Volatile Organic Compounds;
- n.1 PVC bottle of 0.5 L for analysis of metals.

Moreover, to carry out the acute toxicity bioassay with *Vibrio fischeri*, a 10 ml opaque plastic tube was sampled. To perform subacute or chronic toxicity bioassay 1 liter of waters was collected into a sterile plastic bottle. For the determination of Microcystins, a 1 L glass bottle was sampled. For molecular analysis, a 100 ml sterile plastic bottle (polyethylene or polycarbonate), that not to be fixed with any reagent. Finally, for cyanobacteria species identification and counting two 50 ml plastic bottles were sampled and fixed the first with Lugol solution (final concentration 1%) and the second with formaldehyde (final concentration 4%).
In the laboratory, samples were stored in the dark at 4 °C until analysis.

7.3 Analysis

7.3.1 Determination of MCs

Methanol for residual analysis and Trifluoroacetic acid (TFA) were purchased from Sigma Aldrich. Bond Elut SPE C18 cartridges were delivered from Agilent Technology (Santa Clara, United States). Deionized water (> 18 MΩcm-1 resistivity) was produced by a MilliQ water purification system. Glass fiber filter of 1.2 µm and 0.2 µm respectively were obtained from Millipore (Darmstadt, Germany). The Variable Volume Multichannel pipettes were Eppendorf Research plus. An EMD Millipore 47mm Glass Vacuum Filter Holder was used for the filtration of samples.
Extraction and analysis were carried out according to the main available guidelines (WHO, 2003; US EPA, 2014; ISS, 2007). Since it was expected low Microcystins concentrations, it was adjusted the method of guidelines as done by Lawton et al. (1994). For each sample, the first step of process involved water filtration through glass filters of 1.2 µm porosity. The

filtered sample was then treated and analysed to measure free Microcystins while the filter was treated and analysed to measure the intracellular toxins. The filtered waters were purified through an Agilent Bond Elut SPE cartridge C18 activated according to manufacturer's instructions and, finally, samples were eluted with 5 ml of methanol (0.1% of TFA); the extract was dried under nitrogen flow and reconstituted with a 20 ml of ultrapure water (concentration factor was 50). Filters were kept at -20°C for a night and would subsequently be defrosted at room temperature to help the cell lysis. Afterwards they were inserted in a Falcon test tube and extracted twice using a 5 ml of methanol solution in an ultrasonic bath for 15 min at 20°C. Two extracts were then pooled in a single new falcon test tube. Finally, extracts were dried under nitrogen flow and reconstituted with 20 ml of Methanol.

In the same way, blank and quality control sample at concentration of 1 µg/L were extract.

7.3.1.1 ELISA test

Microcystins-ADDA ELISA of the Abraxis LLC (Warminster, PA 18974) and standard solutions of MC-LR for ELISA test were provided by Tecna s.r.l. (Trieste, Italy). A Thermo Scientific Multiskan FC microplate photometer was used to carry out the ELISA tests.

Figure 5. Thermo Scientific Multiskan FC microplate photometer

For the ELISA test, 0.5 ml of extract described in paragraph 7.3.1 were dried under nitrogen flow and reconstituted with 0.5 ml of ultrapure water. ELISA test was performed according to the manufacturer's instructions. This is an indirect competitive immuno-enzymatic test for detection of Microcystins and Nodularins. When toxins were present in a sample they competed with the Microcystins-protein analogue that was immobilized on the plate through the binding sites of the Microcystins/Nodularins antibodies in solution. Then, the plate was washed and the second antibody-HRP label was added. The plate was again washed, and a colour signal would eventually develop. The intensity of colour was inversely proportional to the total concentration of toxins present in the sample. The intensity was detected using a Thermo plate reader with a 450-nm filter. The Microcystins concentration was measured by interpolation using a standard curve performed at every analytical batch. A certified reference standard of MC-LR was also adopted for calibrating at concentrations of 0.15, 0.40, 1.00, 2.00, 5.00 µg/L. According to instructions, there was a logarithmic function between absorbances and concentrations. All samples and standards were analysed in duplicate. In every batch, a negative control sample and a certified spiked check sample of Abraxis would be read. Spiked concentration must be 0.75 ± 0.185 µg/L. The LOD of instrumental method was 0.10 µg/L. Since extracts were concentrated fifty times, real LOD was 2 ng/L.

7.3.1.2 LC-MS/MS Method

Since ELISA test cannot discriminate between Nodularins and Microcystins toxins, it will be necessary to analyse the samples through a specific method in LC-MS; this approach will allow to discriminate only the most toxic Microcystins by other toxins (Hilborn et al., 2007; Yuan et al., 2006; Zeck et al., 2001; U.S.EPA, 2014).

Methanol, Water, Formic Acid of UPLC-MS grade were purchased by Carlo Erba Reagents®.

The extracts obtained by means of the methods described in paragraph 7.3.1, were dried under nitrogen flow and reconstituted with 30 µl of methanol and analyzed by Ultra-High-Pressure Liquid Chromatography with Electro-Spray ionization and by Mass Quadrupole Mass Spectrometry. For this purpose, a Waters UHPLC-ESI-TQD Acquity system was used with the Acquity UPLC® HSS C18 1.8 µm - 2.1x150mm column and mobile phase consisting of water and methanol (both added at 0.1% with formic acid) in percentages of gradient variables during the race. The amount of sample injected into the chromatograph was 1 microliter.

Figure 6. Waters UHPLC-ESI-TQD Acquity System

The reading was performed using the MRM acquisition method, selecting the ionic transitions from the values of m/z (ratio between the mass of the single ion fragment and its electric charge) obtained from the analysis of suitable reference materials and shown in the following table:

Table 4. Cyanotoxins and their ionic transitions (m/z) (Turner AD et al., 2018)

Analytes	ESI	Fragment 1	Fragment 2
Microcystin-LR	+	994.6>134.8	994.6>126.8
Microcystin-YR	+	1045.3>135	1045.3>127
Microcystin-LW	+	1025.5>135	1025.5>127
Microcystin-LY	+	1001.5>107	1001.5>134.8
Microcystin-LF	+	985.7>212.7	985.7>134.9
Microcystin-LA	+	910.6>134.9	910.6>106.9
Microcystin-RR	+	520>134.8	520>126.9
NODULARINA	+	824.8>134.8	824.8>102.9

7.3.2 Determination of Heavy Metals

From each sample, aliquots of 10 ml were removed, and metals of interest were extracted and quantified. The samples were mineralized in an Ethos Touch Control (TC) microwave system (Milestone S. r.l., Italy) equipped with pressurized vessels, using a heated mixture of strong acids. A digestion solution was prepared with 6 ml of 65% nitric acid (HNO_3) (Carlo Erba) and 2 ml of 30% peroxide hydrogen (H_2O_2-Carlo Erba) over a 50 min operation cycle at 200 °C. After mineralization, the vessels were opened if a temperature <25 °C was reached, then the content was decanted in falcon tubes and ultra-pure water (Merck) was added to the samples up to 30 ml; for quantification of metals an ICP-MS Elan-DRC-e (Perkin–Elmer, USA) was used. Analytical blanks were processed in the same way as the samples, and concentrations were determined using standard solutions prepared in the same acid matrix. Standards for the instrument calibration were prepared with a multi-elements certified reference solution ICP Standard (Merck). The method detection limits (MDL) estimated were (mg/L): As 0.013, Cd 0.002, Pb 0.001, V 0.025, Al 0.2. For each batch of mineralization, a laboratory-fortified matrix (LFM) was processed for the quality control and we obtained recovery rates between 90 and 110%.

Figure 7. ICP-MS Elan-DRC-e (Perkin–Elmer, USA)

7.3.3 Cyanobacteria Analysis

Speciation of cyanobacteria community, cell counting, and PCR analysis were carried out by **Department of Environment and Health of Istituto Superiore di Sanità (ISS) in Rome** and by **Department of Technological Innovation (DIT) of INAIL in Rome.**

7.3.3.1 Speciation of Cyanobacteria Community

The analysis of the cyanobacteria community is carried out by using sedimentation chamber. These cylindrical plexiglass settling chambers have a variable volume range - 1, 5, 10, 25, 50, 100 ml - and the choice of one chamber rather than another depends on the abundance of individuals present in the sample. Preparation of the sample analysis chamber must be carried out with great care: first it is necessary to homogenize the sample collected in 50 ml plastic bottles and spiked with Lugol solution or formahldeyde by inverting it rhythmically, but slowly, for a certain number of times (about 50); afterwards the chamber must be filled with the contents of the bottle up to the edge, finally the same must be closed with a glass plate making a vacuum, being careful to avoid the formation of air

bubbles. The sample so prepared should be left to sediment for 24h in the dark, at room temperature.

The specific determination in the different samples was made using an inverted microscope (Olympus BX50). For the taxonomic recognition of the various species, specific manuals have been used (Komárek & Anagnostidis, 1998, 2005; Komárek, 2013).

7.3.3.2 Cell Counting

Aliquots (ml) of sample, fixed in formaldehyde as described in paragraph 7.3.1, are filtered using a filtration apparatus consisting of a pump (MILLIPORE) connected to a ramp on which the filtration devices are mounted. The samples are filtered on 0.2µm (25 mm diameter) black polycarbonate filters (Whatman). Each filter is mounted on a glovebox, previously cleaned with 70% ethyl alcohol on which a drop of fluorescence immersion oil (Olympus) is placed. Another drop is interposed between the filter and the coverslip in such a way as to create two zones with a homogeneous refractive index. The slide thus prepared can be stored in the dark at -20 °C for about two months and may be refrozen after reading.

The cyanobacteria count was performed using an epifluorescence microscope (Olympus BX51). The filaments/colonies were counted in autofluorescence (530-550ex/590em broadband) with a 10x objective, using an image analysis software (ImageJ). For each sample, the number of cells per filament/colony was determined as the average number of cells counted on at least 50 strands colonies.

7.3.3.3 PCR Analysis

For each sample, 0.5 L of were filtered within 24 h of sampling, under sterile conditions on Supor filters, 0.2µm porosity, 47 mm diameter (Pall

Supor-200 P / N 60301) (Boström et al., 2004). Thereafter, the filters, dried on absorbent paper, were stored in Petri dishes at -20 °C until analysis.

To verify the presence and the quality of cyanobacterial DNA in the samples, a preliminary PCR was performed using primers designed inside the PC operon, which generates a product of about 685 bp in various cyanobacterial genera (Vichi et al., 2012). This first analysis is to be considered preliminary, because it had the sole purpose of checking the presence and the quality of the cyanobacterial DNA present in the samples. Qualitative PCRs were then performed to search for markers capable of indicating potential toxicity in the cyanobacterial species observed (*Cylindrospermopsis, Microcystis, Anabaenopsis, Pseudoanabaena* and *P. rubescens*). PCR products were visualized by 2% agarose gel electrophoresis.

Afterwards, for the cylindrospermipsin-synthase gene, amplification of the cyrJ marker was followed which in some *C. raciborskii* strains generates a fragment of about 550 bp (Maire et al., 2010).

Instead, for the saxitoxin-synthase gene, amplification of the stxB marker was followed, which, in some *C. raciborskii* strains, generates fragments of variable size between 270 and 957 bp (Hoff-Risseti et al., 2013).

Finally, for the MC gene, the amplification of the mcyE marker was followed by using discriminating primers for the genera *Microcystis* and *Planktothrix* (Rantala et al., 2006) which generates 370 bp fragments.

7.3.4 Eco-toxicological Assay

7.3.4.1 Acute Toxicity by Vibrio fischeri Assay

For the determination of acute toxicity by Vibrio fischeri, the analyzes were performed according to the APAT CNR IRSA 8030 - 2003 method using a luminometer Microtox® model 500 for the measurement of the

bioluminescence emitted by the bacteria. Inhibition of bacterial viability was expressed as percentage values.

Luminescent bacteria were purchased in the freeze-dried and frozen state of the NRRL-B-11177 strain and stored at controlled temperature (-20 °C). The strain must be reactivated before the analysis according to the indications provided by the manufacturer.

The bacterial suspension, divided into aliquots in the cuvettes, was incubated for about 15 minutes at 15 °C. Therefore, the luminous intensity emitted by the bacteria was measured with the luminometer Microtox®. This measure is referred to as I_0. Then, 2.5 ml of each sample or control solution were added to the respective cuvette with the bacterial suspension. For each sample, the light intensity emitted by the bacteria was measured after 5, 15 and 30 minutes. The values obtained correspond respectively to I_5, I_{15} and I_{30} for each cuvette. The ratio between intensities at time *t* and time *0* gives the percentage of inhibition, the index of acute toxicity.

It was also analysed a control sample with potassium dichromate which inhibition must be 60%.

Figure 8. Microtox® model 500 - Modern Water, United Kingdom.

7.3.4.2 Subacute Toxicity by Thamnocephalus platyurus Assay

Subacute toxicity was carried out by Thamnotoxkit F purchased from Microtox®. The bioassay is performed in a multiwell plate using instar II-III larvae of the fairy shrimp *Thamnocephalus platyurus*, which were

hatched from cysts as first step of analysis process. To do this, cysts were solubilized in 10 ml of solution B (produced according to manufacturer instruction) and incubated for 24 h at 25 °C under light of 3500 lux.

Then, each well was filled with 10 ml of sample and 10 larvae of *T. platyurus* were transferred on each one. Also, it was analysed a blank sample. Every sample was analysed in triplicate. The multiwell plate were again incubated for 24 h at 25 °C in darkness. Finally, it was counted the alive individuals through a dissection microscope.

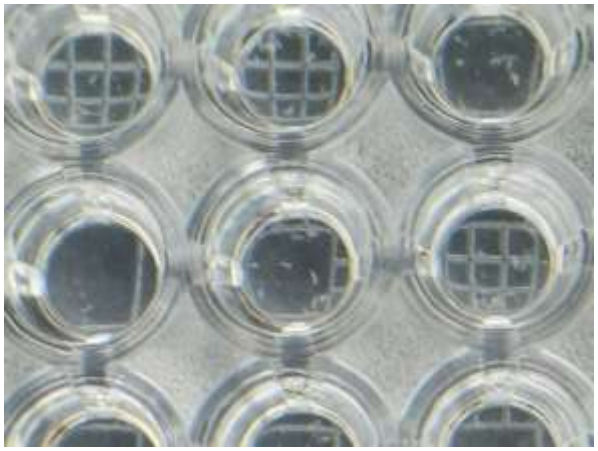


Figure 9. Multiwell plate with *T. platyurus* under dissection microscope

7.3.4.3 Chronic Toxicity by Daphnia magna Assay

Daphnia magna bioassay was performed in **GhEnToxLab of Department of Animal Science and Aquatic Ecology of Ghent University (Belgium)**. The test was carried out only for waters of dams affected of cyanobacteria blooms potentially contaminated from produced cyanotoxins.

Cultures of twenty *D. magna* clonal lineages were established by hatching twenty different resting eggs collected from the sediment layer of the habitat of the nine natural populations. Exposures were performed according to OECD guideline 211 (OECD, 2008). For each clone and treatment ten individual replicate animals less than 24 h old were exposed in polyethylene cups containing 50 mL of the diluted sample (dilution factors: 50, 25, 12.5, 6.25, 0). Media were renewed three times a week.

Animals in the treatments were fed daily with an alga mix consisting of *Pseudokirchneriella* and *Chlamydomonas*. Animals were fed 0.125 mg C per animal in the first week, 0.25 mg C in the second week and 0.375 mg C in the last week of the exposures. Exposures lasted for 21 days under controlled laboratory conditions (25 ± 1 °C, 16 h:8 h light–dark cycle with a light intensity of 14 μmol m−2 s−1). Total reproduction was recorded in all replicates as total number of juveniles produced per female over the entire test duration of 21 days.

7.3.5 Statistical Analysis

IBM SPSS Statistics 20.0 and R software were used for the statistical analysis. Pearson's correlation was applied to explore any linear relationship between analysed parameters. Tukey test was performed to show significant difference for each parameter between dams and for each parameter between season.

The Principal Components Analysis (PCA) on the distance matrix was used to show spatial disposition of dam and parameters that influence the water quality, especially where there was an algal bloom. For each parameter of each dam, it was considered the average value obtained by all sampling. Data were standardized before analysis and the results were displayed in a biplot distance (Legendre P. & Legendre L., 2012; Saleem M et al., 2019).

Chapter 8

Results

8.1 Census of Sicilian freshwater basins

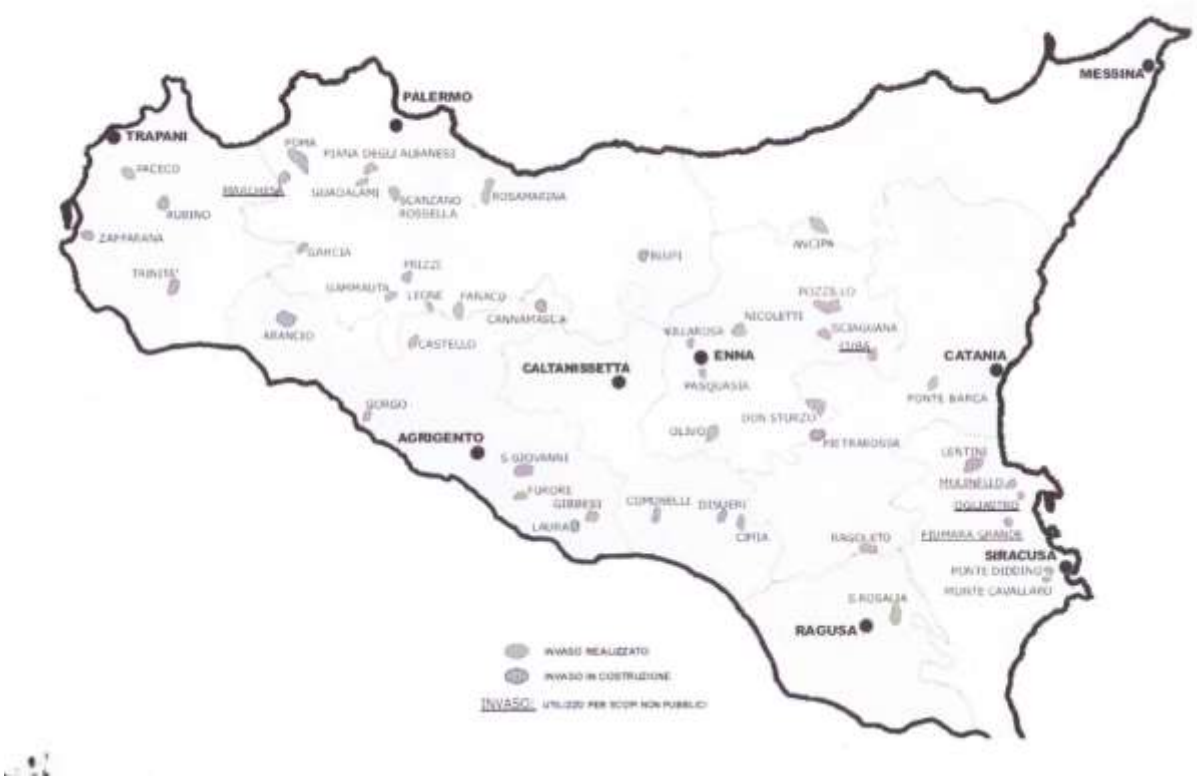


Figure 10. Sicilian surface freshwater catchments.

For each surface water basin analysed, the parameters in which the exceeding of the limits of Legislative Decree 152/2006 for the use of surface water for drinking purposes are highlighted in red.

In the territory of **Trapani** there are 4 artificial reservoirs of surface water currently used for irrigation purposes:

• the Trinità lake was built in 1959 by blocking the course of the Delia river in the territory of the municipality of Castelvetrano (TP). The total surface of the catchment area is 200 km^2. The lake occupies 69 m above sea level,

with a liquid surface of 2.13 km^2 for a volume of 20.3 Mm^3; its maximum depth is 22 m with an average annual depth of 9.5 m. The annual outflow is around 7.6 Mm^3. The basin was affected by blooms of *Planktothrix rubescens* between 2010 and 2012.

Table 5. Physico-chemical and microbiological analyzes related to Lago Trinity

	UNIT OF MEASURE	SUMMER	WINTER	SPRING
Chrome	µg/L	5,4	0,9	1,5
Aluminium	µg/L	937,8	191,7	128,6
Vanadium	µg/L	22,3	1,0	1,0
Manganese	µg/L	209,6	26,5	27,6
Nickel	µg/L	6,7	10,9	5,9
Zinc	µg/L	31,5	<	48,9
Cupper	µg/L	1,3	3,1	2,5
Arsenic	µg/L	<0,3	0,9	0,8
Selenium	µg/L	<0,2	0,4	0,3
Cadmium	µg/L	<0,1	<	<0,1
Lead	µg/L	<0,2	<	0,2
Barium	mg/L	0,1	0,2	0,1
Boron	mg/L	0,4	0,6	0,2
Iron	µg/L	807,1	314,1	114,2
Potassium	mg/L	9,8	7,2	7,6
Calcium	mg/L	258,8	186,1	177,5
Magnesium	mg/L	45,1	28,9	28,4
Sodium	mg/L	131,3	92,6	80,4
Mercury	µg/L	<0,13	<	<0,13
TEMPERATURE	°C	27,8	12,6	16,2
VISIBILITY	mt	0,8	0,9	1,0
SALINITY	ppm	2,6	2,7	3,0
TOTAL NITROGEN	mg/L	0,8	8,1	4,8
BOD5	mg/L	4,0	4,0	5,0
COD	mg/L	39,8	9,0	11,3
DISORDED OXYGEN	% di saturation	135,0	114,7	117,0
SURFACTANS	mg/L	<0,4	<0,4	<0,4
AMMONIUM	mg/L	<0,03	<0,03	<0,03
CONDUCIBILITY	uS/cm	1752,3	1227,3	1352,7
pH	Unit pH	9,0	7,8	7,6
TURBIDITY	NTU	21,4	10,4	6,9
HARDNESS	°F	83,2	58,4	56,0
FIXED RESIDUE 180°	mg/L	1261,3	920,5	1014,5
VOC	ug/L	<	<	<
PESTICIDES	ug/L	<	<	<
PAH	ug/L	<	<	<

PHOSPHORUS	mg/L	0,1	0,0	0,1
PHOSPHATE	mg/L	<0,1	<0,1	<0,1
CLORIDE	mg/L	120,0	113,0	131,3
SULFATE	mg/L	550,0	497,7	606,3
FLUORIDE	mg/L	0,8	1,0	1,4
NITRATE	mg/L	<0,9	6,3	<0,90
NITRITE	mg/L	<0,04	0,1	<0,04
CYANIDE	mg/L	<1	<1	<1
COLONIES COUNTING 37°C	UFC/100ml	>300	>300	190,0
COLONIES COUNTING 22°C	UFC/ml	>300	>300	200,0
COLIFORMS BACTERIA 37°C	UFC/100ml	282,5	1010,0	16166,7
E. COLI	UFC/100ml	0,0	11,7	3,3
INTESTINAL ENTEROCOCKS	UFC/100ml	10,5	5,3	0

In the territory of Palermo there are 11 surface basins of which 10 are intended for drinking and 1 irrigation:

- **the Fanaco lake** was built in 1956 by blocking the high course of the river Platani in the territory of Castronovo di Sicilia (PA), collects the waters of a catchment area whose total area is 84.5 km^2, constituted for 46 km2 by a direct basin and for the remaining 38.5 km^2 from connected basins. The lake occupies a maximum of 679 m above sea level with a liquid surface of 1.5 km^2 for a volume of 22.9 Mm3; has a maximum depth of 47.5 m and an average depth of 15.3 m. The annual outflow is about 15.8 Mm^3. The waters are used for drinking purposes.

Table 6. Physico-chemical and microbiological analyzes related to Lago Fanaco

	UNIT OF MEASURE	SUMMER	AUTUMN	WINTER	SPRING
Chrome	µg/L	12,6	ND	2,0	<
Aluminium	µg/L	1514,1	ND	415,4	140,0
Vanadium	µg/L	1,4	ND	1,5	0,7
Manganese	µg/L	34,3	ND	8,9	6,6
Nickel	µg/L	5,5	ND	5,2	4,0
Zinc	µg/L	192,3	ND	24,4	<
Cupper	µg/L	1,6	ND	14,7	1,8
Arsenic	µg/L	<0,3	ND	0,3	0,3
Selenium	µg/L	<0,2	ND	0,5	0,3

Cadmium	µg/L	<0,1	ND	0,1	<
Lead	µg/L	<0,2	ND	0,9	0,9
Barium	mg/L	0,2	ND	0,1	0,1
Boron	mg/L	0,1	ND	0,0	0,1
Iron	µg/L	1588,9	ND	394,3	103,1
Potassium	mg/L	4,2	ND	3,0	3,8
Calcium	mg/L	77,2	ND	73,2	40,1
Magnesium	mg/L	10,3	ND	6,1	5,7
Sodium	mg/L	19,3	ND	15,8	14,7
Mercury	µg/L	0,1	ND	<	0,5
TEMPERATURE	°C	25,0	17	14,4	13,0
VISIBILITY	mt	2,0	0,5	1,5	1,0
SALINITY	ppm	0,4	0,7	0,9	0,7
TOTAL NITROGEN	mg/L	6,0	7	6,7	7,9
BOD5	mg/L	2,0	0,4	7,5	4,5
COD	mg/L	29,5	13	19,0	11,4
DISORDED OXYGEN	% di saturation	103,0	92	117,0	122,0
SURFACTANS	mg/L	<0,4	<0,4	<0,4	<0,4
AMMONIUM	mg/L	<0,03	<0,03	0,2	<0,03
CONDUCIBILITY	uS/cm	333,0	340	429,5	328,0
pH	Unit pH	7,9	7,9	8,0	7,8
TURBIDITY	NTU	2,9	14	5,4	17,5
HARDNESS	°F	23,5	ND	20,8	12,4
FIXED RESIDUE 180°	mg/L	256,5	255	323,0	246,0
VOC	ug/L	<	<	<	<
PESTICIDES	ug/L	<	<	<	<
PAH	ug/L	<	<	<	<
PHOSPHORUS	mg/L	<0,02	<	0,0	0,1
PHOSPHATE	mg/L	<	<	<	<
CLORIDE	mg/L	15,5	ND	15,0	16,0
SULFATE	mg/L	47,5	ND	40,5	42,0
FLUORIDE	mg/L	0,3	<0,05	0,1	0,2
NITRATE	mg/L	1,0	ND	3,5	4,0
NITRITE	mg/L	<0,04	ND	<0,04	0,2
CYANIDE	mg/L	<1	<1	<1,0	<1
COLONIES COUNTING 37°C	UFC/100ml	>300	>300	>300	>300
COLONIES COUNTING 22°C	UFC/ml	>300	>300	>300	>300
COLIFORMS BACTERIA 37°C	UFC/100ml	ND	1000	350,0	170,0
E. COLI	UFC/100ml	ND	1000	0	170,0
INTESTINAL ENTEROCOCKS	UFC/100ml	ND	20	25,0	6,0

- **the Piano del Leone lake** was built in 1933 by blocking the course of the river Sosio in the territory of the municipality of Castronovo di Sicilia (PA) and collects the waters of a direct water catchment area

whose total area is 24.5 km^2. The water body occupies the maximum flooded elevation of 829.28 m above sea level with a liquid surface of 0.6 km^2 for a volume of 4.83 Mm^3; has a maximum depth of 29.2 m and an average depth of 8.1 m. The annual outflow is around 10.2 Mm^3. The use of water is for drinking and irrigation purposes.

Table 7. Physico-chemical and microbiological analyzes related to Lago Pian del Leone

	UNIT OF MEASURE	SUMMER	AUTUMN	WINTER	SPRING
Chrome	µg/L	3	ND	1,3	0,7
Aluminium	µg/L	338	ND	131,9	170,3
Vanadium	µg/L	<4,4	ND	1,3	1,0
Manganese	µg/L	417	ND	9,2	9,5
Nickel	µg/L	3	ND	4,5	4,7
Zinc	µg/L	5	ND	11,9	<
Cupper	µg/L	4	ND	14,0	2,2
Arsenic	µg/L	<0,3	ND	0,3	0,5
Selenium	µg/L	<0,2	ND	0,7	0,6
Cadmium	µg/L	<0,1	ND	<	<
Lead	µg/L	<0,2	ND	0,6	0,9
Barium	mg/L	0,1	ND	0,0	0,1
Boron	mg/L	0,0	ND	0,0	0,0
Iron	µg/L	353,8	ND	157,0	161,3
Potassium	mg/L	3,0	ND	1,9	1,8
Calcium	mg/L	61,4	ND	69,9	58,3
Magnesium	mg/L	6,5	ND	3,7	4,0
Sodium	mg/L	18,5	ND	11,0	13,8
Mercury	µg/L	0,1	ND	<	0,2
TEMPERATURE	°C	24,5	14		15,5
VISIBILITY	mt	1,0	0,2	0,5	1
SALINITY	ppm	0,4	0,78		0,68
TOTAL NITROGEN	mg/L	14,5	<0,5	6,8	7,1
BOD5	mg/L	3,5	<	10	<3,8
COD	mg/L	14,0	19	22	8
DISORDED OXYGEN	% di saturazione	78,5	107	107	112
SURFACTANS	mg/L	<0,4	<0,4	<0,4	<0,4
AMMONIUM	mg/L	0,2	ND	0,164	<0,03
CONDUCIBILITY	uS/cm	365,0	377	390	329
pH	Unità di pH	7,7	7,2	8,1	8
TURBIDITY	NTU	5,5	29	2,6	11
HARDNESS	°F	18,0	ND	18,96	16,19

FIXED RESIDUE 180°	mg/L	277,5	282,75	300	246,75
VOC	ug/L	ASSENTI	ASSENTI	ASSENTI	0,1
PESTICIDES	ug/L	ASSENTI	ASSENTI	ASSENTI	ASSENTI
PAH	ug/L	ND	ASSENTI	ASSENTI	ASSENTI
PHOSPHORUS	mg/L	<0,02	ND	0,04	0,05
PHOSPHATE	mg/L	<0,48	ND	ND	
CLORIDE	mg/L	12,5	ND	11	13
SULFATE	mg/L	36,5	ND	28	34
FLUORIDE	mg/L	0,3	<0,05	0,1	0,3
NITRATE	mg/L	<0,9	ND	5	3
NITRITE	mg/L	<0,04	ND	0,1	0,2
CYANIDE	mg/L	<1	<1	<1,0	<1
COLONIES COUNTING 37°C	UFC/100ml	>300	>300	>300	>300
COLONIES COUNTING 22°C	UFC/ml	>300	>300	>300	>300
COLIFORMS BACTERIA 37°C	UFC/100ml	ND	700	100	60
E. COLI	UFC/100ml	ND	100	0	0
INTESTINAL ENTEROCOCKS	UFC/100ml	ND	0	0	4

• **the Scanzano-Rossella lake** was built in 1965 by barring the Scanzano and Rossella torrents on the border between the territories of the municipalities of Piana degli Albanesi and Monreale (PA). The water is used for the full water availability of the city of Palermo. The total surface of the catchment area is 86 km^2 and consists of 59.4 km^2 of connected basins. The lake occupies a maximum area of 527 m above sea level with a liquid surface of 1.64 km^2 for a volume of 20.38 Mm^3; has a maximum depth of 33 m and an average depth of 12.4 m. The annual outflow of about 21.8 Mm^3.

Table 8. Physico-chemical and microbiological analyzes related to Lago Scanzano-Rossella

	UNIT OF MEASURE	SUMMER – AUTUMN	WINTER - SPRING
Chrome	µg/L	1,8	2,2
Aluminium	µg/L	192,3	1402,3
Vanadium	µg/L	<4,4	2,1
Manganese	µg/L	10,3	16,0
Nickel	µg/L	6,5	3,5
Zinc	µg/L	17,5	6,2
Cupper	µg/L	1,9	1,7
Arsenic	µg/L	1,0	0,4

Selenium	µg/L	0,3	<
Cadmium	µg/L	<0,1	<
Lead	µg/L	<0,2	0,3
Barium	mg/L	0,1	<
Boron	mg/L	0,1	0,1
Iron	µg/L	255,1	<
Potassium	mg/L	3,6	3,0
Calcium	mg/L	53,8	48,6
Magnesium	mg/L	8,3	6,5
Sodium	mg/L	24,7	20,6
Mercury	µg/L	<0,13	<
TEMPERATURE	°C	24,3	11,8
VISIBILITY	mt	1,0	1,1
SALINITY	ppm	0,4	0,8
TOTAL NITROGEN	mg/L	0,6	5,9
BOD5	mg/L	3,5	8,0
COD	mg/L	21,8	18,5
DISORDED OXYGEN	% di saturation	109,3	119,8
SURFACTANS	mg/L	<0,4	<0,4
AMMONIUM	mg/L	0,3	0,1
CONDUCIBILITY	uS/cm	372,5	367,0
pH	Unit pH	7,5	7,9
TURBIDITY	NTU	8,7	23,7
HARDNESS	°F	16,8	14,8
FIXED RESIDUE 180°	mg/L	279,3	275,3
VOC	ug/L	<	0,2
PESTICIDES	ug/L	<	<
PAH	ug/L	<	<
PHOSPHORUS	mg/L	0,1	0,1
PHOSPHATE	mg/L	<	<
CLORIDE	mg/L	18,0	24,5
SULFATE	mg/L	32,3	39,5
FLUORIDE	mg/L	0,3	0,3
NITRATE	mg/L	<0,9	4,0
NITRITE	mg/L	<0,04	0,1
CYANIDE	mg/L	<1	<1
COLONIES COUNTING 37°C	UFC/100ml	>300	>300
COLONIES COUNTING 22°C	UFC/ml	>300	>300
COLIFORMS BACTERIA 37°C	UFC/100ml	21250,0	2625,0
E. COLI	UFC/100ml	30,0	25,0
INTESTINAL ENTEROCOCKS	UFC/100ml	3,5	43,3

- **Rosamarina Lake** is an artificial basin created in 1992 blocking the course of the San Leonardo river. The waters are used for irrigation, drinking and industrial purposes. The total surface of the catchment area is

500.5 km^2, extending within the territory of the province of Palermo. The lake occupies a maximum area of 175 m above sea level with a liquid surface of 5.41 km^2 for a volume of 130 Mm^3; has a maximum depth of 69.5 m and an average depth of 24 m. The annual outflow is around 80 Mm^3.

Table 9. Physico-chemical and microbiological analyzes related to Lago Rosamarina

	UNIT OF MEASURE	SUMMER	WINTER	SPRING
Chrome	µg/L	1,9	0,8	1,3
Aluminium	µg/L	175,1	277,3	125,5
Vanadium	µg/L	0,8	0,8	0,7
Manganese	µg/L	32,0	4,8	26,6
Nickel	µg/L	7,0	6,8	3,4
Zinc	µg/L	17,6	<	17,5
Cupper	µg/L	2,7	3,2	5,3
Arsenic	µg/L	<0,3	0,5	<0,3
Selenium	µg/L	0,3	0,4	<0,2
Cadmium	µg/L	<0,1	<	<0,1
Lead	µg/L	1,3	<	1,6
Barium	mg/L	0,1	0,0	0,1
Boron	mg/L	0,3	0,1	0,1
Iron	µg/L	163,0	283,0	150,8
Potassium	mg/L	5,9	5,8	3,2
Calcium	mg/L	370,4	102,8	39,8
Magnesium	mg/L	23,2	20,5	9,1
Sodium	mg/L	77,8	74,8	17,6
Mercury	µg/L	<0,13	<0,13	<0,13
TEMPERATURE	°C	23,6	14	24,7
VISIBILITY	mt	1,2	0	1
SALINITY	ppm	1,1	1,83	2,13
TOTAL NITROGEN	mg/L	1,4	13,1	13,6
BOD5	mg/L	4,5	<3,8	13
COD	mg/L	26,0	11	30
DISORDED OXYGEN	% di saturation	98,3	104	117
SURFACTANS	mg/L	<0,4	<0,4	<0,4
AMMONIUM	mg/L	<0,03	<0,03	<0,4
CONDUCIBILITY	uS/cm	945,5	847	976
pH	Unit pH	7,7	7,9	7,9
TURBIDITY	NTU	4,5	11,8	1,54
HARDNESS	°F	102,0	34,08	13,67
FIXED RESIDUE 180°	mg/L	709,3	635,25	731,25
VOC	ug/L	<	<	<

PESTICIDES	ug/L	<	<	<
PAH	ug/L	<	<	<
PHOSPHORUS	mg/L	0,2	0,07	<0,02
PHOSPHATE	mg/L	<	<	<
CLORIDE	mg/L	61,8	61	64
SULFATE	mg/L	337,8	305	350
FLUORIDE	mg/L	0,5	0,42	0,82
NITRATE	mg/L	5,0	8	12
NITRITE	mg/L	0,1	0,2	0,15
CYANIDE	mg/L	<1	<1	<1
COLONIES COUNTING 37°C	UFC/100ml	180,0	>300	160
COLONIES COUNTING 22°C	UFC/ml	>300	>300	>300
COLIFORMS BACTERIA 37°C	UFC/100ml	600,0	230	180
E. COLI	UFC/100ml	0,0	10	0
INTESTINAL ENTEROCOCKS	UFC/100ml	3,0	3	0

- **Poma Lake** was built in 1970 by blocking the course of the river Jato in the territory of the town of Partinico (PA), it is used for drinking and irrigation purposes. The total surface of the catchment area is 299.45 km^2 and consists of approximately half of the connected basins. The lake occupies 196.75 m above sea level with a liquid surface of 5.37 km^2 for a volume of 78.3 Mm^3; has a maximum depth of 46.8 m and an average depth of 14.6 m. The annual outflow is about 39.1 Mm^3.

Table 10. Physico-chemical and microbiological analyzes related to Lago Poma

	UNIT OF MEASURE	WINTER	SUMMER
Chrome	µg/L	6,6	1,6
Aluminium	µg/L	3528,2	531,4
Vanadium	µg/L	7,7	1,5
Manganese	µg/L	52,6	12,6
Nickel	µg/L	7,1	3,1
Zinc	µg/L	<	10,0
Cupper	µg/L	5,0	3,2
Arsenic	µg/L	1,3	0,6
Selenium	µg/L	0,5	0,3
Cadmium	µg/L	<	<0,1
Lead	µg/L	1,5	<0,2
Barium	mg/L	0,1	0,1
Boron	mg/L	0,1	0,1
Iron	µg/L	3586,5	478,8

Potassium	mg/L	5,3	4,9
Calcium	mg/L	67,9	64,1
Magnesium	mg/L	20,8	22,2
Sodium	mg/L	55,8	62,4
Mercury	µg/L	<	<0,13
TEMPERATURE	°C	13,3	17,5
VISIBILITY	mt	0	0,5
SALINITY	ppm	1,26	1,29
TOTAL NITROGEN	mg/L	12	6
BOD5	mg/L	<	5
COD	mg/L	7,7	15
DISORDED OXYGEN	% di saturation	120	105
SURFACTANS	mg/L	<0,4	<0,4
AMMONIUM	mg/L	<0,03	<0,03
CONDUCIBILITY	uS/cm	593	603
pH	Unit pH	7,9	8
TURBIDITY	NTU	81,9	11
HARDNESS	°F	25,49	25,16
FIXED RESIDUE 180°	mg/L	444,75	452,25
VOC	ug/L	<	<
PESTICIDES	ug/L	<	<
PAH	ug/L	<	<
PHOSPHORUS	mg/L	0,14	0,04
PHOSPHATE	mg/L	<	<
CLORIDE	mg/L	64	60
SULFATE	mg/L	100	113
FLUORIDE	mg/L	0,62	1,13
NITRATE	mg/L	6	<0,90
NITRITE	mg/L	0,2	<0,04
CYANIDE	mg/L	<1	<1
COLONIES COUNTING 37°C	UFC/100ml	>300	>300
COLONIES COUNTING 22°C	UFC/ml	>300	>300
COLIFORMS BACTERIA 37°C	UFC/100ml	1300	2500
E. COLI	UFC/100ml	150	30
INTESTINAL ENTEROCOCKS	UFC/100ml	100	2

- **Piana degli Albanesi Lake** was built in 1923 by blocking the course of the river Belice right in the territory of the municipality of Piana degli Albanesi (PA) and is one of the oldest Italian artificial basins. The use is mainly hydroelectric and in a secondary way the waters are used for irrigation and for the water supply of the city of Palermo. The catchment area occupies a total area of 41.35 km^2, consisting of 3.75 km^2 of connected basins. The lake occupies a maximum area of 612 m above sea level with a

liquid surface of 3.78 km^2 for a capacity of 39.9 m^3; has a maximum depth of 35.8 m and an average depth of 10.6 m. The annual outflow is around 15,5 Mm^3.

Table 11. Physico-chemical and microbiological analyzes related to Lago Piana degli Albanesi

	UNIT OF MEASURE	WINTER	SUMMER
Chrome	µg/L	1,2	1,0
Aluminium	µg/L	296,0	19,6
Vanadium	µg/L	0,8	3,1
Manganese	µg/L	12,0	1,0
Nickel	µg/L	3,5	4,6
Zinc	µg/L	<	<0,5
Cupper	µg/L	2,6	2,7
Arsenic	µg/L	<	0,7
Selenium	µg/L	<	0,4
Cadmium	µg/L	<	<0,1
Lead	µg/L	<	<0,2
Barium	mg/L	0,0	0,0
Boron	mg/L	0,1	0,1
Iron	µg/L	291,8	23,8
Potassium	mg/L	2,9	2,5
Calcium	mg/L	45,5	46,9
Magnesium	mg/L	8,7	10,2
Sodium	mg/L	18,6	17,1
Mercury	µg/L	<0,13	<0,13
TEMPERATURE	°C	13	24,1
VISIBILITY	mt	1	1,5
SALINITY	ppm	0,69	0,74
TOTAL NITROGEN	mg/L	6	4,9
BOD5	mg/L	4	4
COD	mg/L	8,7	18
DISORDED OXYGEN	% di saturation	108	103
SURFACTANS	mg/L	<0,4	<0,4
AMMONIUM	mg/L	0,063	<0,4
CONDUCIBILITY	uS/cm	336	361
pH	Unit pH	7,8	7,9
TURBIDITY	NTU	13,1	3
HARDNESS	°F	14,94	15,89
FIXED RESIDUE 180°	mg/L	252	270,75
VOC	ug/L	<	<
PESTICIDES	ug/L	<	<
PAH	ug/L	<	<
PHOSPHORUS	mg/L	0,08	<0,02

PHOSPHATE	mg/L	<	<
CLORIDE	mg/L	22	24
SULFATE	mg/L	41	42
FLUORIDE	mg/L	0,16	0,32
NITRATE	mg/L	3	1
NITRITE	mg/L	0,1	<0,04
CYANIDE	mg/L	<1	<1
COLONIES COUNTING 37°C	UFC/100ml	200	>300
COLONIES COUNTING 22°C	UFC/ml	>300	>300
COLIFORMS BACTERIA 37°C	UFC/100ml	83	4000
E. COLI	UFC/100ml	3	0
INTESTINAL ENTEROCOCKS	UFC/100ml	8	14

In the territory of **Caltanissetta** there are 3 artificial reservoirs of surface water currently used for irrigation purposes:

• **Disueri Lake**, originally, was obtained through a gravity dam built between 1939 and 1948 that barred the Gela river in the territory of the municipality of Gela, but as a result of subsequent problems it was decided to build a new barrier placed a little further downstream and which was completed in 1997. The total surface of the catchment basin, with no connected basins, is 239 km^2. The maximum flooded quota is 163.91 m above sea level with a liquid surface of 1.85 km^2 for a volume of 28.2 Mm^3; with a maximum depth of 31 m and an average depth of 15,2 m. But due to the interruption, the capacity has been significantly reduced to small volumes. The annual outflow is around 1.9 Mm^3.

Table 12. Physico-chemical and microbiological analyzes related to Diga Disueri

	UNIT OF MEASURE	SUMMER	WINTER	SPRING
Chrome	µg/L	8,4	<	6,8
Aluminium	µg/L	3474,2	455,4	4550,3
Vanadium	µg/L	15,5	1,6	10,4
Manganese	µg/L	204,8	38,6	419,2
Nickel	µg/L	7,6	7,2	9,7
Zinc	µg/L	<0,5	3,5	2,5

Cupper	µg/L	1,8	2,1	6,7
Arsenic	µg/L	<0,3	1,4	4,4
Selenium	µg/L	0,4	0,4	0,7
Cadmium	µg/L	<0,1	<	<0,1
Lead	µg/L	<0,2	<	1,4
Barium	mg/L	0,1	0,1	0,1
Boron	mg/L	0,5	0,3	0,4
Iron	µg/L	2900,0	<	2322,5
Potassium	mg/L	17,2	13,2	14,9
Calcium	mg/L	55,5	99,4	73,7
Magnesium	mg/L	44,0	32,7	38,6
Sodium	mg/L	180,4	127,8	138,8
Mercury	µg/L	0,1	0,7	<0,13
TEMPERATURE	°C	23,7	15	25,5
VISIBILITY	mt	0,2	1	0,2
SALINITY	ppm	1,67	2,58	2,64
TOTAL NITROGEN	mg/L	5	9	5
BOD5	mg/L	59	10	5
COD	mg/L	116	35	21
DISORDED OXYGEN	% di saturation	89	89	124
SURFACTANS	mg/L	<0,4	<0,4	<0,4
AMMONIUM	mg/L	<0,03	0,171	<0,4
CONDUCIBILITY	uS/cm	1354	1169	1192
pH	Unit pH	8	7,8	8
TURBIDITY	NTU	227	9,3	39,1
HARDNESS	°F	31,98	38,28	34,30
FIXED RESIDUE 180°	mg/L	285	876,75	894
VOC	ug/L	<	0,1	<
PESTICIDES	ug/L	<	<	<
PAH	ug/L	<	<	<
PHOSPHORUS	mg/L	0,3	0,16	0,01
PHOSPHATE	mg/L	<	<	
CLORIDE	mg/L	ND	130	161
SULFATE	mg/L	ND	309	305
FLUORIDE	mg/L	0,58	0,88	0,82
NITRATE	mg/L	<0,9	4	<0,90
NITRITE	mg/L	<0,04	1	<0,04
CYANIDE	mg/L	<1	<1	<1
COLONIES COUNTING 37°C	UFC/100ml	>300	>300	>300
COLONIES COUNTING 22°C	UFC/ml	>300	>300	>300
COLIFORMS BACTERIA 37°C	UFC/100ml	>100	1300	15000
E. COLI	UFC/100ml	10	13	10
INTESTINAL ENTEROCOCKS	UFC/100ml	ND	8	7

In the territory of Enna there are 9 artificial reservoirs of surface water, of which 1 is currently used for drinking purposes and 8 irrigation:

- **Pozzillo lake** was built in 1959 by blocking the Salso River course in the municipality of Regalbuto (EN), its waters are used for hydroelectric purposes and in a secondary way for irrigation purposes. The total surface of the catchment area is 577 km^2. The lake at the maximum flooded altitude 366.5 m above sea level with a liquid surface of 7.9 km^2 for a volume of 154.42 Mm^3; has a maximum depth of 51.5 m and an average depth of 19.5 m. The annual outflow is about 82.9 Mm^3.

Table 13. Physico-chemical and microbiological analyzes related to Lago Pozzillo

	UNIT OF MEASURE	SUMMER	AUTUMN	WINTER	SPRING
Chrome	µg/L	2,1	1,5	1,5	<0,7
Aluminium	µg/L	508,6	221,1	92,4	46,4
Vanadium	µg/L	<4,4	0,8	0,6	<0,4
Manganese	µg/L	46,7	432,3	83,4	3,4
Nickel	µg/L	9,4	7,4	7,7	8,7
Zinc	µg/L	11,0	2,8	1,6	12,5
Cupper	µg/L	3,7	11,9	15,0	3,8
Arsenic	µg/L	1,3	1,0	0,7	0,5
Selenium	µg/L	0,3	0,3	0,4	<0,2
Cadmium	µg/L	<0,1	<	<	<0,1
Lead	µg/L	<0,2	0,6	0,5	<0,2
Barium	mg/L	0,1	0,1	0,1	0,1
Boron	mg/L	0,4	0,4	0,2	0,3
Iron	µg/L	511,8	245,8	103,8	52,2
Potassium	mg/L	11,4	11,3	9,4	9,3
Calcium	mg/L	90,4	99,1	93,3	100,2
Magnesium	mg/L	34,2	36,3	28,3	30,3
Sodium	mg/L	182,2	206,8	112,1	149,6
Mercury	µg/L	<0,13	<0,13	<0,13	<0,13
TEMPERATURE	°C	22,5	ND	9,0	16,0
VISIBILITY	mt	0,5	1,0	1,0	1,0
SALINITY	ppm	1,7	ND	2,8	2,6
TOTAL NITROGEN	mg/L	0,9	6,7	11,5	ND
BOD5	mg/L	11,8	24	15,5	4,0
COD	mg/L	51,3	58	37,3	10,8
DISORDED OXYGEN	% di saturation	106,8	95	89,7	112,0
SURFACTANS	mg/L	<0,4	<0,4	<0,4	<0,4
AMMONIUM	mg/L	<0,03	<0,03	0,3	0,1

CONDUCIBILITY	uS/cm	1361,0	1501	1237,3	1133,8
pH	Unit pH	8,0	7,8	8,0	8,2
TURBIDITY	NTU	13,7	80	2,2	5,9
HARDNESS	°F	36,6	39,67	34,9	37,5
FIXED RESIDUE 180°	mg/L	1024,3	1125,75	927,9	866,5
VOC	ug/L	<	0,2	0,11	<
PESTICIDES	ug/L	<	<	<	<
PAH	ug/L	<	<	<	<
PHOSPHORUS	mg/L	0,2	0,06	0,1	0,1
PHOSPHATE	mg/L	<	<	<	<
CLORIDE	mg/L	197,0	11	191,0	ND
SULFATE	mg/L	276,8	28	256,5	ND
FLUORIDE	mg/L	5,9	1,93	0,2	ND
NITRATE	mg/L	26,0	6	5,0	ND
NITRITE	mg/L	3,6	0,06	0,1	ND
CYANIDE	mg/L	<1	<1,0	<1	ND
COLONIES COUNTING 37°C	UFC/100ml	>300	>300	96,0	126,3
COLONIES COUNTING 22°C	UFC/ml	>300	>300	>300	>300
COLIFORMS BACTERIA 37°C	UFC/100ml	3975,0	1200	96,8	198,8
E. COLI	UFC/100ml	0	200	19,3	10,0
INTESTINAL ENTEROCOCKS	UFC/100ml	8,8	25	5,0	2,3

- **the Ancipa Dam** was built in 1953 by blocking the Troina River in the territory of the municipality of Troina in the province of Enna. It has been in operation since 1954 and collects the waters of a catchment area whose total area is 103.13 km^2. made up of about half of the connected basins. The lake occupies the maximum flooded part 950 m above sea level with a liquid surface of 1.41 km^2 for a volume of 31.05 Mm^3; has a maximum depth of 70.5 m and an average depth of 22 m. The annual outflow is around 58 Mm^3. The use is mainly hydroelectric; a part of the invaded waters is used for drinking and irrigation purposes.

Table 14. Physico-chemical and microbiological analyzes related to Diga Ancipa

	UNIT OF MEASURE	SUMMER	WINTER
Chrome	µg/L	1,5	4,0
Aluminium	µg/L	245,8	1698,6
Vanadium	µg/L	1,0	3,7
Manganese	µg/L	23,6	56,9
Nickel	µg/L	1,3	5,6
Zinc	µg/L	0,5	5,5
Cupper	µg/L	1,4	13,0
Arsenic	µg/L	<0,3	0,6
Selenium	µg/L	<0,2	0,3
Cadmium	µg/L	<0,1	<
Lead	µg/L	<0,2	1,1
Barium	mg/L	0,0	0,1
Boron	mg/L	0,0	0,1
Iron	µg/L	317,0	1187,5
Potassium	mg/L	1,2	2,1
Calcium	mg/L	30,4	36,1
Magnesium	mg/L	5,0	6,4
Sodium	mg/L	10,0	11,2
Mercury	µg/L	<0,13	<0,13
TEMPERATURE	°C	21,0	
VISIBILITY	mt	2,0	0,5
SALINITY	ppm	0,2	ND
TOTAL NITROGEN	mg/L	0,3	5,2
BOD5	mg/L	3,8	4
COD	mg/L	8,8	10
DISORDED OXYGEN	% di saturazione	95,8	63
SURFACTANS	mg/L	<0,4	<0,4
AMMONIUM	mg/L	0,0	<0,03
CONDUCIBILITY	uS/cm	219,8	250
pH	Unità di pH	7,8	7,7
TURBIDITY	NTU	81,3	79,3
HARDNESS	°F	9,7	11,66
FIXED RESIDUE 180°	mg/L	167,5	187
VOC	ug/L	<	0,1
PESTICIDES	ug/L	<	<
PAH	ug/L	<	<
PHOSPHORUS	mg/L	0,1	0,08
PHOSPHATE	mg/L	<	<
CLORIDE	mg/L	8,8	61
SULFATE	mg/L	14,3	46
FLUORIDE	mg/L	0,1	1,4
NITRATE	mg/L	<0,9	2
NITRITE	mg/L	<0,04	<0,04
CYANIDE	mg/L	<1	<1,0

COLONIES COUNTING 37°C	UFC/100ml	>300	>300
COLONIES COUNTING 22°C	UFC/ml	>300	>300
COLIFORMS BACTERIA 37°C	UFC/100ml	150,0	2200
E. COLI	UFC/100ml	0	200
INTESTINAL ENTEROCOCKS	UFC/100ml	9,5	60

• **Nicoletti Lake** was built in 1971 in the territory of the municipalities of Enna and Leonforte (EN), blocking the course of the Dittaino river. The reservoir collects the waters of a catchment basin whose total area is 101.27 km2 and consists of 51.77 km^2 of connected basins. The lake occupies 387.1 m above sea level with a liquid surface of 1.77 km^2 for a volume of 24.1 Mm^3; has a maximum depth of 38.8 m and an average depth of 13.6 m. The annual outflow is around 6 Mm^3. The use is predominantly irrigated, but a part of the potted waters is used for industrial purposes.

Table 15. Physico-chemical and microbiological analyzes related to Diga Nicoletti

	UNIT OF MEASURE	WINTER	SUMMER
Chrome	µg/L	<	1,0
Aluminium	µg/L	87,4	281,2
Vanadium	µg/L	<	0,8
Manganese	µg/L	3,4	6,3
Nickel	µg/L	5,3	4,4
Zinc	µg/L	<	1245,0
Cupper	µg/L	2,4	4,1
Arsenic	µg/L	0,4	0,7
Selenium	µg/L	<	<0,2
Cadmium	µg/L	<	<0,1
Lead	µg/L	<	0,7
Barium	mg/L	0,1	1,2
Boron	mg/L	0,3	1,2
Iron	µg/L	<	1208,3
Potassium	mg/L	11,3	12,2
Calcium	mg/L	87,3	91,5
Magnesium	mg/L	29,4	34,3
Sodium	mg/L	157,3	167,5
Mercury	µg/L	<	<0,13
TEMPERATURE	°C	13	24,8
VISIBILITY	mt	1	1
SALINITY	ppm	5,49	2,72

TOTAL NITROGEN	mg/L	9	3
BOD5	mg/L	<	9
COD	mg/L	8	24
DISORDED OXYGEN	% di saturation	177	101
SURFACTANS	mg/L	<0,4	<0,4
AMMONIUM	mg/L	0,0165	<0,4
CONDUCIBILITY	uS/cm	2366	1230
pH	Unit pH	8,1	7,9
TURBIDITY	NTU	5,59	7,06
HARDNESS	°F	33,89	36,95
FIXED RESIDUE 180°	mg/L	1774,5	922,5
VOC	ug/L	<	<
PESTICIDES	ug/L	<	<
PAH	ug/L	<	<
PHOSPHORUS	mg/L	0,05	2,58
PHOSPHATE	mg/L	<	<
CLORIDE	mg/L	66	16
SULFATE	mg/L	431	130
FLUORIDE	mg/L	0,6	0,15
NITRATE	mg/L	2	<0,90
NITRITE	mg/L	<0,04	<0,04
CYANIDE	mg/L	<1	<1
COLONIES COUNTING 37°C	UFC/100ml		>300
COLONIES COUNTING 22°C	UFC/ml		>300
COLIFORMS BACTERIA 37°C	UFC/100ml		20000
E. COLI	UFC/100ml		300
INTESTINAL ENTEROCOCKS	UFC/100ml		>100

• **Olivo Lake** is an artificial basin built in 1982 blocking the course of the Braemi stream in the municipality of Piazza Armerina (EN). The purpose is of irrigation type. The total surface of the catchment area is 102 km^2, of which 42 km^2 are made up of connected basins. The lake occupies 451.2 m above sea level with a liquid surface of 1.2 km^2 for a volume of 18 Mm^3; has a maximum depth of 45.4 m and an average depth of 15 m. The annual outflow is around 10.2 Mm^3.

Table 16. Physico-chemical and microbiological analyzes related to Diga Olivo

	UNIT OF MEASURE	SUMMER	WINTER	SPRING
Chrome	µg/L	4,1	<	2,0
Aluminium	µg/L	224,7	170,1	424,2
Vanadium	µg/L	13,5	1,0	1,9
Manganese	µg/L	27,3	34,1	83,5
Nickel	µg/L	3,3	4,0	3,2
Zinc	µg/L	<0,5	<	5,0
Cupper	µg/L	<1	1,5	3,7
Arsenic	µg/L	<0,3	1,9	2,9
Selenium	µg/L	<0,2	<	<0,2
Cadmium	µg/L	<0,1	<	<0,1
Lead	µg/L	<0,2	<	1,0
Barium	mg/L	0,1	0,1	0,1
Boron	mg/L	0,4	0,3	0,3
Iron	µg/L	257,0	<	278,0
Potassium	mg/L	8,8	8,2	8,4
Calcium	mg/L	64,8	79,5	63,6
Magnesium	mg/L	38,7	43,7	47,7
Sodium	mg/L	115,1	121,6	121,2
Mercury	µg/L	<0,13	<0,13	<0,13
TEMPERATURE	°C	27	13	25,3
VISIBILITY	mt	1	1,5	1
SALINITY	ppm	1,31	2,36	2,51
TOTAL NITROGEN	mg/L	3,4	7,4	3
BOD5	mg/L	4	12	4
COD	mg/L	23	32	12
DISORDED OXYGEN	% di saturazione	125	146	122
SURFACTANS	mg/L	<0,4	<0,4	<0,4
AMMONIUM	mg/L	<0,03	0,074	<0,4
CONDUCIBILITY	uS/cm	1080	1073	1137
CHLOROPHYLL	mg/L	<0,01		
pH	Unità di pH	7,8	8	7,8
TURBIDITY	NTU	ND	5,5	3,25
HARDNESS	°F	32,12	37,83	35,51
FIXED RESIDUE 180°	mg/L	810	804,75	852,75
VOC	ug/L	<	<	<
PESTICIDES	ug/L	<	<	<
PAH	ug/L	<	<	<
PHOSPHORUS	mg/L	0,1	0,06	0,03
PHOSPHATE	mg/L	<	<	<
CLORIDE	mg/L	118	148	149
SULFATE	mg/L	213	243	260
FLUORIDE	mg/L	0,57	0,94	0,73
NITRATE	mg/L	<0,9	0,4	<0,90
NITRITE	mg/L	<0,04	<0,04	<0,04

CYANIDE	mg/L	<1	<1	<1
COLONIES COUNTING 37°C	UFC/100ml	>300		>300
COLONIES COUNTING 22°C	UFC/ml	>300		>300
COLIFORMS BACTERIA 37°C	UFC/100ml	2000		10
E. COLI	UFC/100ml	0		10
INTESTINAL ENTEROCOCKS	UFC/100ml	6		58

• **Sciaguana Lake** was built in 1992 in the territory of the municipalities of Regalbuto and Agira (EN), blocking the course of the Sciaguana stream. The waters are used for irrigation purposes. The total area is 64.89 km^2. It has a total capacity of 11.9 Mm^3, a useful capacity of 9.9 Mm^3 and a usable capacity of 5 Mm^3.

Table 17. Physico-chemical and microbiological analyzes related to Diga Sciaguana

	UNIT OF MEASURE	WINTER	SUMMER
Chrome	µg/L	<	1,3
Aluminium	µg/L	142,6	188,0
Vanadium	µg/L	1,6	1,2
Manganese	µg/L	38,5	7,8
Nickel	µg/L	11,1	8,8
Zinc	µg/L	5,1	15,0
Cupper	µg/L	3,2	5,1
Arsenic	µg/L	1,7	1,8
Selenium	µg/L	0,4	0,4
Cadmium	µg/L	<	<0,1
Lead	µg/L	<	0,7
Barium	mg/L	0,1	0,1
Boron	mg/L	0,9	0,4
Iron	µg/L	<	193,0
Potassium	mg/L	21,8	22,1
Calcium	mg/L	190,9	195,3
Magnesium	mg/L	103,6	112,3
Sodium	mg/L	292,5	287,8
Mercury	µg/L	0,1	<0,13
TEMPERATURE	°C	12,5	25,2
VISIBILITY	mt	1	1,5
SALINITY	ppm	2,49	6,09
TOTAL NITROGEN	mg/L	11,6	6,4
BOD5	mg/L	<	10
COD	mg/L	13	25
DISORDED OXYGEN	% di saturazione	149	132

SURFACTANS	mg/L	<0,4	<0,4
AMMONIUM	mg/L	<0,03	<0,4
CONDUCIBILITY	uS/cm	1131	2606
pH	Unità di pH	8,3	7,9
TURBIDITY	NTU	4,93	14
HARDNESS	°F	90,31	95,01
FIXED RESIDUE 180°	mg/L	848,25	1954,5
VOC	ug/L	<	<
PESTICIDES	ug/L	<	<
PAH	ug/L	<	<
PHOSPHORUS	mg/L	0,10	0,13
PHOSPHATE	mg/L	<	<
CLORIDE	mg/L	17	59
SULFATE	mg/L	1020	187
FLUORIDE	mg/L	0,43	0,37
NITRATE	mg/L	11	<0,90
NITRITE	mg/L	0,2	0,1
CYANIDE	mg/L	<1	<1
COLONIES COUNTING 37°C	UFC/100ml		>300
COLONIES COUNTING 22°C	UFC/ml		>300
COLIFORMS BACTERIA 37°C	UFC/100ml		4000
E. COLI	UFC/100ml		10
INTESTINAL ENTEROCOCKS	UFC/100ml		2

In the territory of **Ragusa** there is 1 artificial reservoir of surface water currently used for irrigation purposes:

• **Santa Rosalia Lake** was built in 1981 by blocking the course of the Irminio river in the territory of the municipality of Ragusa. The waters are used for irrigation and to meet the drinking water needs of the city of Ragusa. The total surface of the catchment area is 97.65 km^2. The lake occupies a maximum area of over 382 m above sea level with a liquid surface of 1.45 km^2 for a volume of 24.7 Mm^3; has a maximum depth of 39.3 m and an average depth of 17 m. The annual outflow is around 20.2 Mm^3.

Table 18. Physico-chemical and microbiological analyzes related to Diga S. Rosalia

	UNIT OF MEASURE	SUMMER	WINTER	SPRING
Chrome	µg/L	1,9	<	2,2
Aluminium	µg/L	319,0	43,5	172,6
Vanadium	µg/L	8,4	2,7	0,6
Manganese	µg/L	4,3	5,5	13,7
Nickel	µg/L	3,4	6,0	5,2
Zinc	µg/L	<0,5	<	20,0
Cupper	µg/L	<1	3,3	2,6
Arsenic	µg/L	<0,3	0,8	0,4
Selenium	µg/L	0,2	0,6	0,4
Cadmium	µg/L	<0,1	<	<0,1
Lead	µg/L	<0,2	0,3	<0,2
Barium	mg/L	0,0	0,1	0,1
Boron	mg/L	0,1	0,1	0,2
Iron	µg/L	270,0	120,3	95,0
Potassium	mg/L	3,3	3,4	6,1
Calcium	mg/L	56,0	68,9	110,5
Magnesium	mg/L	13,1	9,5	23,1
Sodium	mg/L	21,0	18,1	70,0
Mercury	µg/L	<0,13	<0,13	<0,13
TEMPERATURE	°C	27,0	14	24,6
VISIBILITY	mt	3,0	2	1,5
SALINITY	ppm	0,4	2,49	0,78
TOTAL NITROGEN	mg/L	0,6	9,3	3,8
BOD5	mg/L	5,5		2
COD	mg/L	21,0	15	9
DISORDED OXYGEN	% di saturazione	113,5	ND	103
SURFACTANS	mg/L	<0,4	<0,4	<0,4
AMMONIUM	mg/L	<0,6	0,0387	<0,4
CONDUCIBILITY	uS/cm	371,5	1140	386
pH	Unità di pH	7,7	8	8
TURBIDITY	NTU	16,0	24,5	1,8
HARDNESS	°F	19,4	21,10	37,12
FIXED RESIDUE 180°	mg/L	282,5	ND	284,25
VOC	ug/L	<	<	<
PESTICIDES	ug/L	<	<	<
PAH	ug/L	<	<	<
PHOSPHORUS	mg/L	<0,02	<0,02	<0,02
PHOSPHATE	mg/L	<	<	<
CLORIDE	mg/L	29,0	26	31
SULFATE	mg/L	39,0	34	40
FLUORIDE	mg/L	0,4	0,43	1,1
NITRATE	mg/L	<0,9	4	<0,90
NITRITE	mg/L	<0,04	0,1	<0,04
CYANIDE	mg/L	<1	<1	<1

COLONIES COUNTING 37°C	UFC/100ml	>300	>300	>300
COLONIES COUNTING 22°C	UFC/ml	>300	>300	>300
COLIFORMS BACTERIA 37°C	UFC/100ml	5000,0	450	19000
E. COLI	UFC/100ml	150,0	6	32
INTESTINAL ENTEROCOCKS	UFC/100ml	69,0	3	20

In the territory of Agrigento there are 6 reservoirs currently used for irrigation and 2 for drinking:

• **Arancio Lake** was built in 1952 by blocking the course of the river Caboj on the border between the territory of Sambuca di Sicilia and that of Sciacca (AG), and it is used for irrigation purposes. The total surface of the 209 km2 catchment basin consists of connected basins. The lake occupies a maximum area of 180 m above sea level with a liquid surface of 3.7 km^2 for a total volume of 38.4 Mm^3; has a maximum depth of 30.3 m and an average depth of 10.4 m. The annual outflow is around 18 Mm^3. As well as for irrigation purposes it can be used for recreational purposes (rowing, water skiing).

Table 19. Physico-chemical and microbiological analyzes related to Diga Arancio

	UNIT OF MEASURE	WINTER	SUMMER
Chrome	µg/L	3,2	1,2
Aluminium	µg/L	322,1	146,0
Vanadium	µg/L	1,5	0,7
Manganese	µg/L	6,9	34,3
Nickel	µg/L	4,2	8,4
Zinc	µg/L	ND	<
Cupper	µg/L	2,4	2,9
Arsenic	µg/L	1,2	0,9
Selenium	µg/L	0,2	0,4
Cadmium	µg/L	ND	<0,1
Lead	µg/L	0,4	<0,2
Barium	mg/L	0,1	0,1
Boron	mg/L	0,3	0,3
Iron	µg/L	517,8	76,5
Potassium	mg/L	7,1	7,2
Calcium	mg/L	59,5	222,1
Magnesium	mg/L	12,4	34,8

Sodium	mg/L	44,1	94,1
Mercury	µg/L	ND	<0,13
TEMPERATURE	°C	12,9	19
VISIBILITY	mt	0,5	0,5
SALINITY	ppm	1,1	1,14
TOTAL NITROGEN	mg/L	8,1	5,8
BOD5	mg/L	4,5	5
COD	mg/L	9,5	13
DISORDED OXYGEN	% di saturazione	112,0	105
SURFACTANS	mg/L	<0,4	<0,4
AMMONIUM	mg/L	<0,03	<0,03
CONDUCIBILITY	uS/cm	532,0	539
pH	Unità di pH	8,0	7,9
TURBIDITY	NTU	11,0	7,73
HARDNESS	°F	20,0	69,81
FIXED RESIDUE 180°	mg/L	399,0	404,25
VOC	ug/L	<	<
PESTICIDES	ug/L	<	<
PAH	ug/L	<	<
PHOSPHORUS	mg/L	ND	0,07
PHOSPHATE	mg/L	<	<
CLORIDE	mg/L	53,5	127
SULFATE	mg/L	98,0	590
FLUORIDE	mg/L	0,6	1,31
NITRATE	mg/L	5,0	<0,90
NITRITE	mg/L	0,1	<0,04
CYANIDE	mg/L	<1	<1
COLONIES COUNTING 37°C	UFC/100ml	>300	160
COLONIES COUNTING 22°C	UFC/ml	>300	170
COLIFORMS BACTERIA 37°C	UFC/100ml	62,5	110
E. COLI	UFC/100ml	62,5	14
INTESTINAL ENTEROCOCKS	UFC/100ml	7,0	0

In territory of **Siracusa** there is one basin actually used as irrigation, the **Lentini Dam**. Since it was no possible contact the manager of the basin, it is no possible to carry out sampling in this catchment.

In territories of **Catania** and **Messina** there is no significant reservoirs.

8.2 Speciation of Cyanobacteria Community, Cell Counting and PCR Analysis

In following table are summarized for each basin the cyanobacteria community and the concentration of each specie when this reached a significant numerosity.

Table 20. Cyanobacteria community and specie concentration.

	Summer '16	Autumn '16	Winter '17	Spring '17
Fanaco	*Limnothrix redekei* *Borzia sp.* *Merismopedia sp* *Pseudoanabaena sp* *Planktothrix rubescens*	*Borzia sp. (3*10^2)* *Pesudoanabaena sp. (2,0*10^3)* *Limnothrix redekei (6,0*10^2)*	*no ciano*	*no ciano*
Pian del Leone Trinità	*Pseudoanabaena sp* *Planktothrix sp*	*no ciano*	*no ciano*	*no ciano*
Disueri	*Anabaenopsis sp*	*no ciano*	*Microcystis aeruginosa*	*Microcystis aeruginosa (10^8)*
	Pseudoanabaena		*Cylindrospermopsis raciborskii*	*Cylindrospermopsis raciborskii (10^7)*
			Pseudoanabena sp.	*Pseudoanabaena sp.*
Scanzano-Rossella	*Oscillatoriales* *Dolichospermum sp.*	*no ciano*	*no ciano*	*no ciano*
Pozzillo	*P. rubescens* *P. agardhii* *Oscillatoriales* *Planktolyngbya*	*no ciano*	*Planktolyngbya sp. (4,2*10^3)*	*no ciano*
Nicoletti	*no ciano*	*no ciano*	*P. rubescens (6,6*10^4)*	*Planktolyngbya sp. (1,2*10^3)* *Limnothrix redekei (6,0*10^2)*

				*Oscillatoria sp. (1,0*10^3)*
Poma	*no ciano*	*no ciano*	*Pseudoanabaena sp. (5,0*10^2)*	*no ciano*
Piana degli Albanesi	*no ciano*	*no ciano*	*no ciano*	*no ciano*
Olivo	*no ciano*	*no ciano*	*no ciano*	*no ciano*
Sciaguana	*no ciano*	*no ciano*	*no ciano*	*no ciano*
Arancio	*no ciano*	*no ciano*	*no ciano*	*no ciano*
S. Rosalia	*no ciano*	*no ciano*	*no ciano*	*no ciano*
Rosamarina	*no ciano*	*no ciano*	*no ciano*	*no ciano*
Ancipa	*no ciano*	*no ciano*	*no ciano*	*no ciano*

In red it is shown the presence of a harmful bloom of *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii* (sample 86).

According with ISS protocol, in presence of a bloom, it was performed a sampling about every week until to disappearance of blooms. In table below are summarized the cyanobacteria communities detected during one month of sampling in Disueri dam (samples among 91 and 94).

Table 21. Cyanobacteria blooms in Disueri dam

DISUERI			
7-08-2017 (91)	21-08-2017(92)	29-08-2017(93)	12-09-2017(94)
M. aeruginosa *C. raciborskii* *Anabaenopsis sp*	*Anabaenopsis sp.* (10^7) *P. rubescens* (10^6)	*Anabaenopsis sp.* *P. rubescens*	*Anabaenopsis sp.* *P. rubescens*

The results of the *PCR analysis* on sample 86, 91, 92, 93 and 94 showed the presence of cyanobacterial DNA in all the samples analyzed (in the

presence of two positive controls, the *Microcystis* PCC7806 and *P. rubescens* 1460/3 strains).

The amplification of the cyrJ and stxB markers occurred in samples Disueri Dam during the bloom (samples 86, 92, 93 and 94).

Therefore, a potential ability to produce CYN and STX can be assumed for these samples. For the cyrJ gene it is observed the presence of a band of about 550 bp in samples 92 and 93 and 94, and of a band of size between 550 and 600 in the sample 86.

For the stxB gene it was observed the presence of a band of about 270 bp for all positive samples (samples 86, 92, 93 and 94).

The PCR of the mcyE marker did not produce amplified in any of the samples examined. In the positive controls Microcystis PCC7806 and P. rubescens 1460/3, as expected, the amplification of the fragment of 370 bp occurred.

8.3 Cyanotoxins

In table below are schematized the results of ELISA test and UHPLC-MS/MS analysis about detection of Microcystins. In following graph (figure 11) are reported results of ELISA test.

Table 22. Microcystins results by ELISA test and UHPLC-MS/MS analysis

	Season	ID sample	ELISA (ng/L)	UPLC-MS/MS (ng/L)
FANACO	Summer	P27 - RP1	719,5	<5
		P27 - RP2	618,5	<5
LEONE	Summer	P27 - RP3	2,5	<5
		P27 - RP4	<1,5	<5
DISUERI	Summer	P27 - RP7	47,8	<5
S. ROSALIA	Summer	P27 - RP8	9,5	<5
		P27 - RP9	21,9	<5
TRINITA'	Summer	P27 - RP11	41,4	238,9
		P27 - RP12	16,7	<5
		P27 - RP13	52,7	58,7
		P27 - RP14	38,1	18,6
OLIVO	Summer	P27 - RP17	18,5	42
SCANZANO-ROSSELLA	Summer	P27 - RP19	4,3	<5

		P27 - RP20	3,0	<5
		P27 - RP21	3,0	<5
		P27 - RP22	5,4	<5
POZZILLO	Summer	P27 - RP25	219	20
		P27 - RP26	193	6,9
		P27 - RP27	400	410
		P27 - RP28	263	89,1
ROSAMARINA	Summer	P27 - RP31	216	17,1
		P27 - RP32	181	<5
		P27 - RP33	158	<5
		P27 - RP34	110	<5
ANCIPA	Summer	P27 - RP37	4,8	<5
		P27 - RP38	3,9	<5
		P27 - RP39	/	<5
		P27 - RP40	/	<5
		P27 - RP41	/	<5
		P27 - RP42	/	<5
FANACO	Autumn	P27 - RP43	188	<5
LEONE	Autumn	P27 - RP44	4,7	<5
ANCIPA	Autumn	P27 - RP45	21,6	<5
POZZILLO	Autumn	P27 - RP46	3,7	<5
		P27 - RP47	119	23,1
LEONE	Winter	P27 - RP48	1,7	<5
FANACO	Winter	P27 - RP49	37,1	<5
		P27 - RP50	32,8	<5
POZZILLO	Winter	P27 - RP51	36,1	<5
		P27 - RP52	45,1	<5
		P27 - RP53	23,3	<5
		P27 - RP54	8,2	<5
DISUERI	Winter	P27 - RP55	18,7	<5
SCANZANO-ROSSELLA	Winter	P27 - RP56	7,2	<5
		P27 - RP57	7,9	<5
		P27 - RP58	9,4	<5
		P27 - RP59	4,1	<5
OLIVO	Winter	P27 - RP60	25,9	<5
NICOLETTI	Winter	P27 - RP61	728	<5
SCIAGUANA	Winter	P27 - RP62	<1,5	<5
S. ROSALIA	Winter	P27 - RP63	28	<5
TRINITÀ	Winter	P27 - RP64	8,5	<5
		P27 - RP65	9	<5
		P27 - RP66	10,4	<5
ARANCIO	Winter	P27 - RP67	45	<5
		P27 - RP68	36	<5
ROSAMARINA	Winter	P27 - RP69	18	<5
POMA	Winter	P27 - RP70	6	<5
PIAN DEGLI ALBANESI	Winter	P27 - RP71	10,2	<5
FANACO	Spring	P27 - RP72	10,4	<5

		P27 - RP73	17	<5
LEONE	Spring	P27 - RP74	3,4	<5
POZZILLO	Spring	P27 - RP75	30,4	<5
		P27- RP76	25	<5
		P27 - RP77	18,7	<5
		P27 - RP78	27,3	<5
TRINITÀ	Spring	P27 - RP79	54,7	<5
		P27 - RP80	46,6	<5
		P27 - RP81	54,7	<5
ARANCIO	Spring	P27 - RP82	155	<5
POMA	Spring	P27 - RP83	61,5	<5
NICOLETTI	Spring	P27 - RP84	23,6	<5
SCIAGUANA	Spring	P27 - RP85	11,5	<5
DISUERI	Spring	P27 - RP86	360	<5
OLIVO	Spring	P27 - RP87	30,4	<5
S. ROSALIA	Spring	P27 - RP88	21,5	<5
ROSAMARINA	Spring	P27 - RP89	554	<5
PIAN DEGLI ALBANESI	Spring	P27 - RP90	29,3	<5
DISUERI	Bloom	P27 - RP91	393	<5
		P27 - RP92	184	<5
		P27 - RP93	135	<5
		P27 - RP94	65	<5

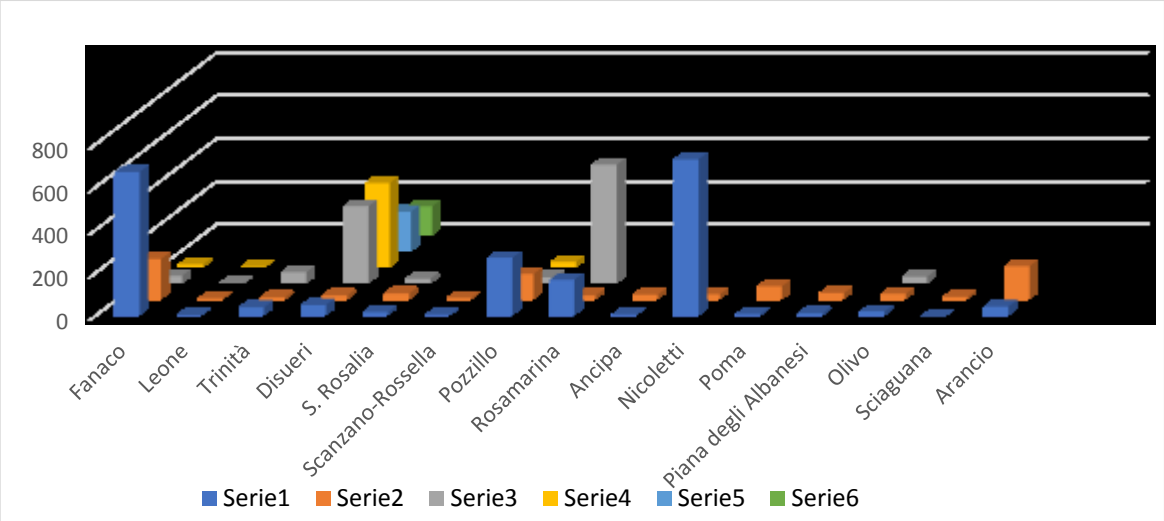


Figure 11. Microcystins results by ELISA test: series (1 to 6) are in chronological order of sampling in each basin.

8.4 Ecotoxicological assay

In following table 23 are schematized the results of *Vibrio fisheri* assay to evaluation of acute toxicity. Toxicity, expressed as percentage of inhibition of bioluminescence, was measured at 5, 15, and 30 minutes.

Table 23. Percentage of inhibition of bioluminescence of *Vibrio fischeri* at 5, 15 and 30 minutes

	Season	ID sample	5 min %	15 min %	30 min %
Fanaco	Summer	RP1/P27	-3,4	-11,8	-11,8
		RP2/P27	24,1	16,3	16,3
Leone	Summer	RP3/P27	-7	-17,1	-17,1
		RP4/P27	-18,1	-22,9	-26,9
Disueri	Summer	RP7/P27	-3,9	-6,3	-6,3
S. Rosalia	Summer	RP8/P27	-7,4	-5,3	-5,3
		RP9/P27	-14,1	-27,3	-27,3
Trinità	Summer	RP11/P27	-44,4	-44,7	-44,7
		RP12/P27	-48,9	-51,3	-51,3
		RP13/P27	-54,3	-53,6	-53,6
Olivo	Summer	RP14/P27	-39	-46,9	-46,9
		RP17/P27	-8	-12,2	-16,4
Scanzano-Rossella	Summer	RP19/P27	-9,8	-12,1	-13,3
		RP20/P27	-8,4	-12,6	-12,8
		RP21/P27	9,1	6,9	2,7
		RP22/P27	4,8	6,5	9,4
Pozzillo	Summer	RP25/P27	-14,4	-12,7	-12,7
		RP26/P27	-9,6	-7,6	-7,6
		RP27/P27	-0,9	-1,2	-1,2
		RP28/P27	-17,8	-19,4	-19,4
		RP29/P27	11,1	21,8	25,4
Rosamarina	Summer	RP31/P27	-20,9	-34	-34
		RP32/P27	-12,9	-15,2	-15,2
		RP34/P27	-5,5	-7,6	-7,6
Ancipa	Summer	RP37/P27	7,8	9,2	7,1
		RP38/P27	-16	-29,2	-43,9
		RP39/P27	3,1	-5,7	-2,1
		RP40/P27	16,6	4	-3,3
Fanaco	Autumn	RP43/P27	0,5	-0,2	-0,4
Leone	Autumn	RP44/P27	3,9	1,9	4,9
Ancipa	Autumn	RP45/P27	-7,5	-11,5	-19,8
Pozzillo	Autumn	RP46/P27	-10,3	-15,2	-21,6

		RP47/P27	2,3	-3,7	-12,3
Leone	Winter	RP48/P27	-5,4	-6,9	-13,6
Fanaco	Winter	RP49/P27	1,9	0,4	-3,7
		RP50/P27	8,5	10,3	10,8
Pozzillo	Winter	RP51/P27	8,1	5,4	7,9
		RP52/P27	-0,4	-3,8	-5,5
		RP53/P27	16,3	15,6	13,5
Disueri	Winter	RP54/P27	4,3	3,8	3,3
		RP55/P27	16,4	11	15,5
Scanzano-Rossella	Winter	RP56/P27	-3,5	-0,7	1,4
		RP57/P27	14,7	11,9	10,5
		RP58/P27	-11,6	-17,5	-14,8
Olivo	Winter	RP59/P27	-6	-13,7	-12,3
Nicoletti	Winter	RP60/P27	-1,4	-6,2	-8
Sciaguana	Winter	RP61/P27	10,1	4,5	-2
S. Rosalia	Winter	RP62/P27	-1,6	-10,2	-15,8
Trinità	Winter	RP63/P27	10,6	13,1	16,4
		RP64/P27	-3,1	-1,5	8,4
		RP65/P27	-11,8	-12,4	-4,5
Arancio		RP66/P27	-20,8	-21,6	-19,3
		RP67/P27	-12,6	-8,7	-3,7
		RP68/P27	-0,9	-0,9	-0,1
Rosamarina	Winter	RP69/P27	-11,9	-9,4	4,4
Poma	Winter	RP70/P27	-19,2	-17,3	-1,2
Piana degli albanesi		RP71/P27	-22,3	-17,9	-1,8
Fanaco	Spring	RP72/P27	-6,8	-8,4	-4,6
		RP73/P27	-3,6	-4,8	-3,5
Leone	Spring	RP74/P27	-8,8	-11,1	-8,3
Pozzillo	Spring	RP75/P27	-7,4	-10,7	-8,6
		RP76/P27	-1,9	-4,2	-1,5
		RP77/P27	-3,1	-3,4	-0,1
Trinità	Spring	RP78/P27	-12,4	-16,4	-15,1
		RP79/P27	-21,4	-27,2	-25,7
		RP80/P27	-6,4	-9,5	-6,3
Arancio	Spring	RP81/P27	-13,1	-12,8	-8
		RP82/P27	-19,7	-23,3	-25,5
Poma	Spring	RP83/P27	-18,5	-21,7	-20,4
Nicoletti	Spring	RP84/P27	-21,6	-24,5	-22,5
Sciaguana	Spring	RP85/P27	-19,9	-24,9	-21,6
Disueri	Spring	RP86/P27	1,8	0,3	4,5
Olivo	Spring	RP87/P27	-9,2	-10,9	-6,7

S. Rosalia	Spring	RP88/P27	-16,8	-17,9	-13,7
Rosamarina	Spring	RP89/P27	-14,5	-16,7	-15,8
Piana degli Albanesi	Spring	RP90/P27	-6	-4,7	-1,9
		RP91/P27	4,9	-1,9	-0,4
Disueri	Bloom	RP92/P27	24,9	20,9	21,9
		RP93/P27	55	52,6	51,3
		RP94/P27	41,6	43,2	43,7

In following graph (figure 12) it is shown the trend of percentage of inhibition in the Disueri Dam. It is possible to observe the growth of toxicity during the cyanobacteria bloom.

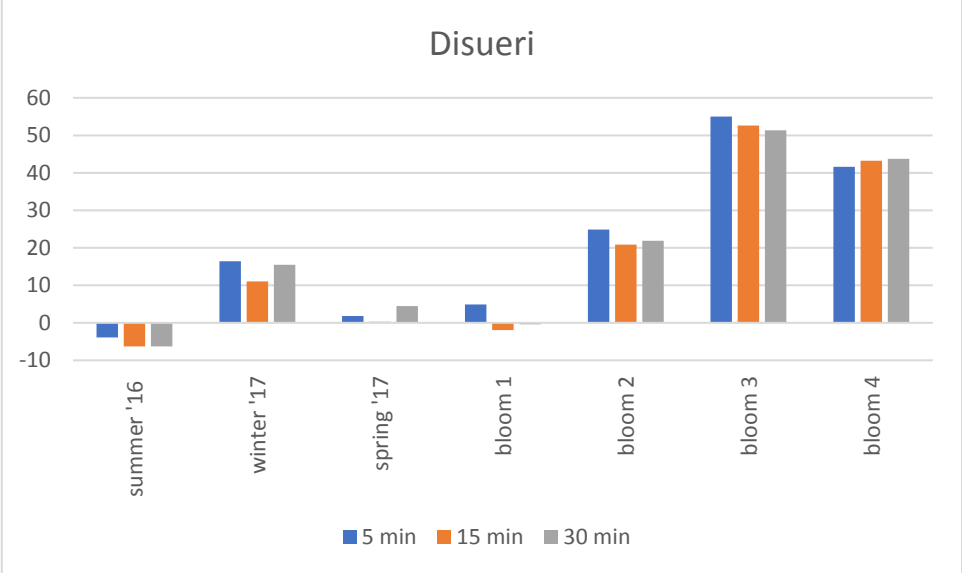


Figure 12. Annual acute toxicity of Disueri Dam through Vibrio fischeri assay

In following table 24 are summarized the results of *Thamnocephalus platyurus* assay to evaluation of sub-acute toxicity. Toxicity, expressed as percentage of mortality, was measured at 24 hours.

Table 24. Mortality percentage of *T. platyurus* measured at 24 hours

	Season	ID sample	Mortality % at 24 hour
Fanaco	Summer	P27 - RP1	73
Fanaco		P27 - RP2	77
Leone	Summer	P27 - RP3	40
Leone		P27 - RP4	25
Disueri	Summer	P27 - RP7	68
S. Rosalia	Summer	P27 - RP8	47
S. Rosalia		P27 - RP9	55
Trinita'	Summer	P27 - RP11	71
Trinita'		P27 - RP12	50
Trinita'		P27 - RP13	78
Trinita'		P27 - RP14	55
Olivo	Summer	P27 - RP17	25
Scanzano-rossella	Summer	P27 - RP19	15
Scanzano-rossella		P27 - RP20	17,0
Scanzano-rossella		P27 - RP21	25,0
Scanzano-rossella		P27 - RP22	20
Pozzillo	Summer	P27 - RP25	87
Pozzillo		P27 - RP26	97
Pozzillo		P27 - RP27	90
Pozzillo		P27 - RP28	83
Rosamarina	Summer	P27 - RP31	65
Rosamarina		P27 - RP32	71
Rosamarina		P27 - RP33	73
Rosamarina		P27 - RP34	60
Ancipa	Summer	P27 - RP37	15
Ancipa		P27 - RP38	5
Ancipa		P27 - RP39	13
Ancipa		P27 - RP40	18
Fanaco	Autumn	P27 - RP43	65
Leone	Autumn	P27 - RP44	10
Ancipa	Autumn	P27 - RP45	17
Pozzillo	Autumn	P27 - RP46	67
Pozzillo		P27 - RP47	48
Leone	Winter	P27 - RP48	19
Fanaco	Winter	P27 - RP49	38
Fanaco		P27 - RP50	35
Pozzillo	Winter	P27 - RP51	51
Pozzillo		P27 - RP52	46
Pozzillo		P27 - RP53	55
Pozzillo		P27 - RP54	50
Disueri	Winter	P27 - RP55	58
Scanzano-rossella	Winter	P27 - RP56	15
Scanzano-rossella		P27 - RP57	15
Scanzano-rossella		P27 - RP58	20
Scanzano-rossella		P27 - RP59	10

Olivo	Winter	P27 - RP60	20
Nicoletti	Winter	P27 - RP61	85
Sciaguana	Winter	P27 - RP62	20
S. Rosalia	Winter	P27 - RP63	23
Trinità	Winter	P27 - RP64	30
Trinità		P27 - RP65	25
Trinità		P27 - RP66	33
Arancio	Winter	P27 - RP67	30
Arancio		P27 - RP68	33
Rosamarina	Winter	P27 - RP69	10
Poma	Winter	P27 - RP70	15
Pian degli albanesi	Winter	P27 - RP71	13
Fanaco	Spring	P27 - RP72	23
Fanaco		P27 - RP73	20
Leone	Spring	P27 - RP74	7
Pozzillo	Spring	P27 - RP75	43
Pozzillo		P27- RP76	38
Pozzillo		P27 - RP77	35
Pozzillo		P27 - RP78	40
Trinità	Spring	P27 - RP79	35
Trinità		P27 - RP80	33
Trinità		P27 - RP81	35
Arancio	Spring	P27 - RP82	58
Poma	Spring	P27 - RP83	34
Nicoletti	Spring	P27 - RP84	43
Sciaguana	Spring	P27 - RP85	28
Disueri	Spring	P27 - RP86	60
Olivo	Spring	P27 - RP87	20
S. Rosalia	Spring	P27 - RP88	15
Rosamarina	Spring	P27 - RP89	25
Pian degli albanesi	Spring	P27 - RP90	20
Disueri	Bloom	P27 - RP91	67
Disueri		P27 - RP92	55
Disueri		P27 - RP93	57
Disueri		P27 - RP94	43

Daphnia magna assay was performed only on waters of Disueri Dam during the bloom (sample 91, 92, 93 and 94). For each sample the average number of juveniles at 21 days are shown in the table below:

Table 25. Average number of juveniles of *D. magna* born following the exposure in waters of Disueri Dam during the bloom

Dilution	Sample 91	Sample 92	Sample 93	Sample 94
4x	644	1084	962	1223
8x	608	683	810	914
16x	567	597	660	786
32x	422	565	522	717
64x	406	517	607	615
blank	359	345	391	357

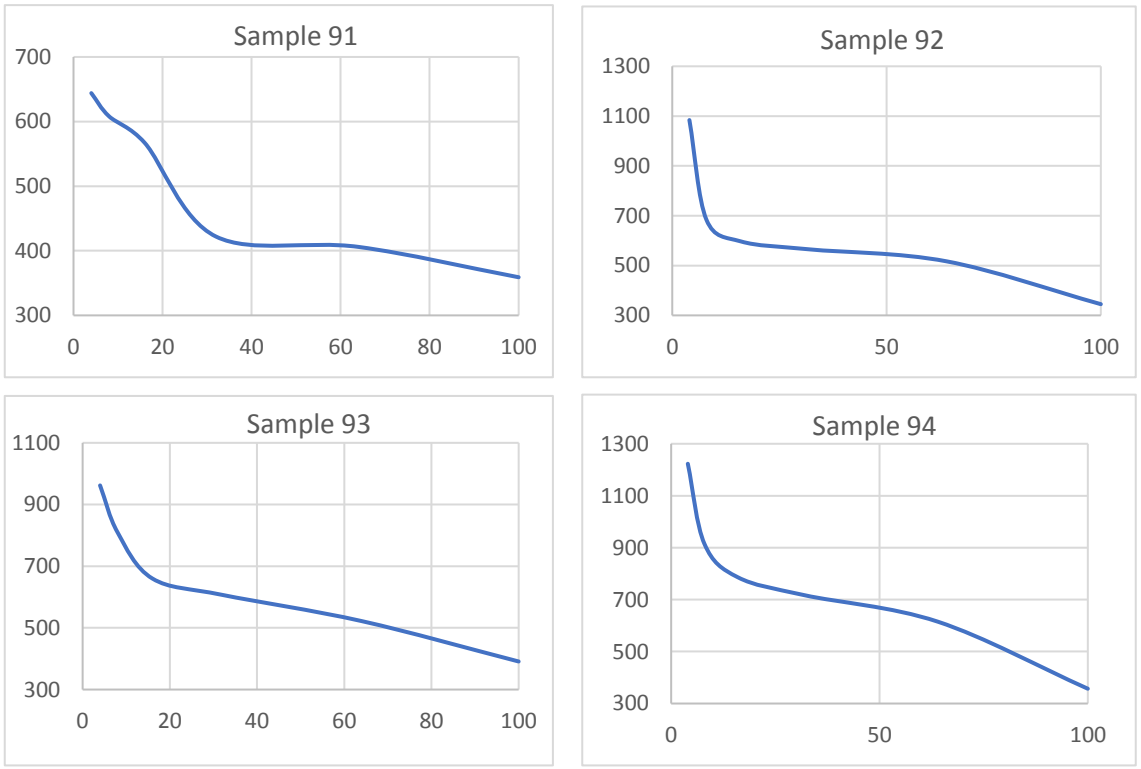


Figure 13. Graphs show reproduction of *D. magna* related to dilution after exposure to the samples (blanks are indicated as dilution 100)

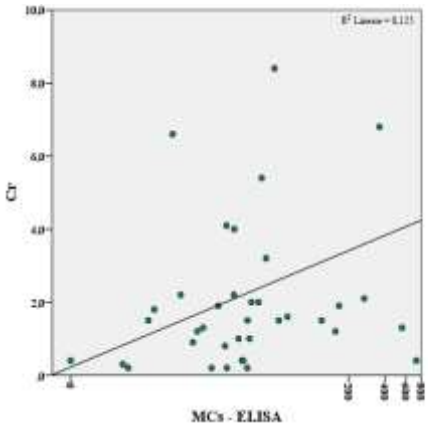
In every samples, no mortality was registered in any replicates of each dilution.

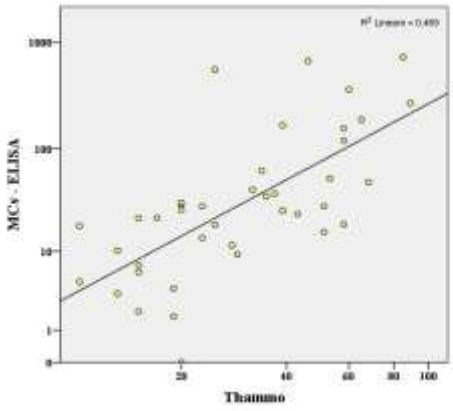
8.5 Statistical Analysis

Pearson's correlation shows the negative linear relationship among *Vibrio fischeri* assay both vanadium (ρ=0.032) and calcium (ρ=0.004). Moreover, there is a positive linear relationship between ELISA test and total chrome (ρ=0.025). Also, *Thamno* test shows positive relationship between ELISA test (ρ=0.001), manganese (ρ=0.022), nickel (ρ=0.010) and sodium (ρ=0.003).

Table 26. Summary of results of Pearson correlation test

	Vanadium	Calcium	Total Chrome	ELISA test	Manganese	Nickel	Sodium
V. fischeri	ρ=0.032 (-)	ρ=0.004 (-)					
ELISA test			ρ=0.025 (+)				
Thamno test				ρ=0.001 (+)	ρ=0.022 (+)	ρ=0.010 (+)	ρ=0.003 (+)





Tukey test shows significant difference between Pozzillo and Disueri dams for aluminum (ρ=0.033), between Leone and every other lake for lead (ρ=0.001), between Leone both Pozzillo and Disueri dams for Thamno test

(respectively ρ=0.031 and ρ=0.029) and, finally, between summer and winter for *Vibrio fischeri* assay (ρ=0.020).

Table 27. Summary of results of Tuckey test

Aluminium	Pozzillo - Disueri ρ=0.033
Lead	Leone – Every lakes ρ=0.001
Thamno test	Leone - Pozzillo ρ=0.031 Leone - Disueri ρ=0.029
Vibrio fischeri	Summer - Winter ρ=0.020

The distance biplot of PCA (PC1 and PC2 were 30% and 21%, respectively) showed that Disueri, Pozzillo, Trinità and Arancio dams are located near together and are strongly influenced by N, Fe, Al, V, Mn, F, Mn, Cl, pH, Ca and Nitrite.

Otherwise, Rosamarina, Olivo, Nicoletti and Sciaguana dams are located near togheter and are strongly influenced by Nitrate, Total Nitrogen, Sulphate, Mg, Ca, K, Na, OD, Fixed residue, Conducibility, hardness, Ba, Zn, As, P.

Instead, Leone, S. Rosalia, Scanzano-Rossella, Ancipa, Poma and Pian degli Albanesi dams are located near together and any parameters do not influence their water quality.

Finally, Fanaco dam is located lonely and Hg, Se and B influence it.

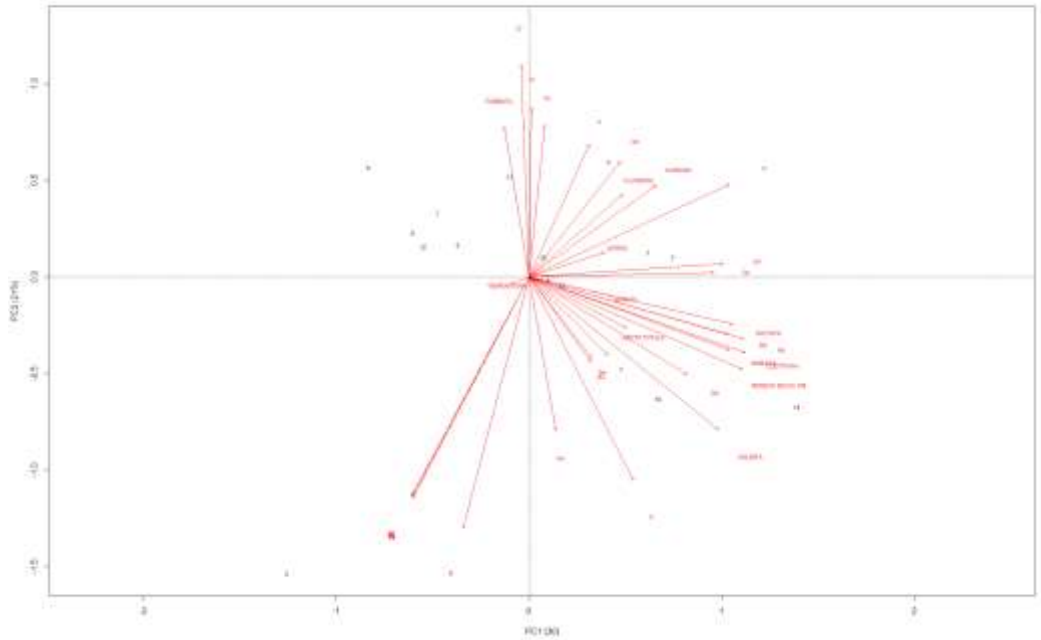


Figure 14. Distance biplot (PC1 and PC2 were 30% and 21%, respectively) shows spatial disposition of dam and parameters that influence the water quality (1=Leone, 2=Fanaco, 3=Trinità, 4=Disueri, 5=S.Rosalia, 6=Scanzano-Rossella, 7=Pozzillo, 8=Rosamarina, 9=Ancipa, 10=Nicoletti, 11=Poma, 12=Pian degli Albanesi, 13=Olivo, 14=Sciaguana, 15=Arancio)

Chapter 9

Discussion and Conclusion

The freshwater basins of Sicily, a southern island of Italy, are not well characterized and classified, yet; therefore, till now there are considerable knowledge gaps on the environmental condition of Sicilian surface freshwater basins used as water's supplies for municipal waterworks and for irrigation.

However, growing strong water scarcity and drought that afflict the biggest island of Italy make necessary to improve the management of every water resource, including the surface freshwater catchments.

Therefore, as first step of this Doctoral research project, it was carried out the monitoring of 15 surface basins, among the 30 existing in Sicilian territory, through seasonal chemical, physical and microbiological analysis of the waters such as required by Italian law, the Legislative Decree 152/2006.

The quality of the waters was established by comparison of values of each studied parameter and reference value.

As shown in paragraph 8.1 there is much reassurance about quality chemical status of basins. Instead, there are no heavy metals above the reference values. Only Aluminum and Iron are present often in large concentration. It could be explained since to eliminate turbidity, the water is left to rest in large tanks, thus undergoing the decanting process and the particles settle on the bottom; clariflocculation is carried out to facilitate the removal of lighter particles: coagulants are added which allow the suspended colloidal substances to be removed. Trivalent ions are the trivalent ions like Al^{3+} deriving from $AlCl_3$ and $Al_2(SO_4)_3$ salts, and as Fe^{3+} deriving from $Fe_2(SO_4)_3$ and $FeCl_3$ salts. These salts alter the zeta potential of colloidal solutions: when the potential is low, the attractive forces

between the particles prevail over repulsions favoring processes such as coagulation and flocculation, forming voluminous and heavier agglomerates that subsequently precipitate together with the metal hydroxides (insoluble) formed during the reaction.

Probably the large use of these salts can overtime lead to an accumulation of soluble fraction of metals. It needs further studies to establish if these high concentrations of Aluminum and Iron derive from anthropic or natural contaminations.

The high levels of total nitrogen and phosphorus give information about the inflow of partially or completely untreated urban and rural wastewater in all basins. The large presence of nutrients promotes microbiological growth. In fact, this is confirmed by the high level of environmental and fecal contaminations. Moreover, the proliferation of cyanobacteria is also favored: in 50% of examined dams, there are the presence of several species. In particular, it was detected cyanobacteria bloom in Disueri in period between July and September 2017. *Microcystis sp.* and *Cylindrospermopsis raciborskii* were detected (108 and 107 cell/L, respectively) and by mid-August were replaced by *Anabaenopsis sp.* and *Plankthotrix rubescens*, still growing in mid-September (107 and 106 cell/L, respectively).

The distance biplot of PCA showed that Disueri, Pozzillo, Trinità and Arancio dams are located near together and are strongly influenced by N, Fe, Al, V, Mn, F, Mn, Cl, pH, Ca and Nitrite. In recent past, all these dams were currently affected by harmful algal bloom (Naselli-Flores, L. & Barone, 2003; Naselli-Flores et al., 2007). The interesting result of PCA could gave important indication about the parameters more influencing the proliferation of M. *aeruginosa* and P. *rubescens*. If this predictive model are reliable, since Rosamarina and Olivo dams are located in proximity of Arancio in the biplot, they could become also favorable habitats for the

growth of these cyanobacteria specie. Some studies in Literature reports the role of different elements in the promotion of the growth (Heath M et al., 2016; Garcia NS et al., 2015; Hou T et al., 2018; Kaushik MS et al., 2015) but no one predictive model was never built considering every parameters, yet.

According to PCA results and the presence of harmful cyanobacteria species, a map of Sicily was created showing the dangerous areas (red), the attention areas (yellow) and the secure areas (blue) (figure 15).

Figure 15. Map of Sicily showing the dangerous areas (red), the attention areas (yellow) and the secure areas (blue).

ELISA MCs concentrations were low, in agreement with the lack of qualitative amplification of the mcyE gene (demonstrated by a 370 bp product in the two positive controls), indicating that the MC-producing fraction within CB population was low. The amplification of both cyrJ (~ between 550 and 600 bp) and stxB (~ 270-300 bp + a high mw variable band) occurred in all samples, concurrently with high density of *C. raciborskii* and *Anabaenopsis sp*. Therefore, although in this moment no positive control was available, we could assume for these samples that there is a potential ability to produce CYN and STX. The detection of MCs levels allowed assessing a very low risk for the population and livestock using the lake's water, while it would been estimated much higher considering the number of cells. The use of a simple qualitative PCR is a

good tool to investigate the presence of potentially toxic CB, and to check for possible new toxin production population. The presence of cyrJ and stxB, once confirmed, could indicate the presence of a *Cylindrospermopsis* strain with potential production of CYN and STX, for the first time in Italy and Europe, and of an *Anabaenopsis* strain producing CYN and STX.

Regarding the detected Microcystins concentrations in entire samples pool, it is important to show the difference of ELISA test and UPLC-MS/MS analysis. In fact, contrarily what report in Literature (Bruno M et al., 2006) the spectroscopy analysis shows a lower concentration of analysed toxins or often their absence respect the ELISA test. It could be explained this difference by chrome interference since there is a significant positive relationship between these ELISA test and chrome concentration. In addition, this could explain the significant positive relationship between higher mortality of *T. platyurus* and MCs concentration measured by ELISA test although it was no highlighted direct correlations between Thamno test and chrome concentrations. However, it needs to analyse also other Microcystins congeners as demethylated analogues.

V. fischeri and *D. magna* assays seem to respond to the number of cyanobacteria cells rather than toxins concentrations. In fact, inhibition of luminosity emitted by *V. fischeri* is higher in Disueri Dam during the bloom reaching also 50% of acute toxicity while D. magna reproduction growing with the increase of cells number in the less diluted samples. Other studies show the positive response of V. *fischeri* and D. *magna* to presence of Microcystis species although they consider also a positive response to toxins for the first assay (D'ors A et al., 2012; J. Asselman et al., 2014).

Instead, *T. platyurus* assay show a significant positive relationship between MCs concentrations measured by ELISA test, leaving the possible interference of chrome that could increase the mortality.

In conclusion, the chemical quality of Sicilian surface freshwaters catchments is good and it could be improved it with a better treatment and management of wastewaters got into the surface waters. This would allow to reduce the supply of nutrients for a continuous improvement of microbiological quality. Concerning the Microcystins contamination, since in every analyzed sample the concentration was below the WHO reference value for drinking waters (1 μg/L) it seems not to be a high and worrying risk for human and environmental health in the brief time.

The simultaneous execution of *V. fischeri* and *T. platyurus* bioassays could favor the monitoring of waters both economically and technically.

Finally, respecting these describes condition, it would be possible the use of waters of all monitored basins as drinking after an adequate treatment according to Legislative Decree 152/2006.

Acknowledge

The present study was funded by PhD scholarship of the University of Catania (Department of Biological, Geological and Environmental Sciences) and the Laboratory of Environmental Hygiene and Food (LIAA) belonging to the Department "GF Ingrassia" of the same University.

Partners of the Project were:

1. Harvard University (Boston) - TH Chan School of Public Health (Prof. A. Zanobetti);
2. Ghent University (Ghent) - GhEnToxLab (Prof. K. De Schampeleare);
3. Istituto Superiore di Sanità (Rome) - Department of Environment and Health;
4. INAIL (Rome) - Department of technological innovation;
5. Sicilian Region - Department of Water and Waste;
6. SicilAcque S.p.A .;
7. Enel S.p.A.

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