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MADFORWATER

DevelopMent AnD application of integrated technological and management solutions FOR wasteWATER treatment and efficient reuse in agriculture tailored to the needs of Mediterranean African Countries

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Content

1 Introduction

This Deliverable describes the standard operating procedures (SOPs) relative to the MADFORWATER wastewater (WW) treatment and irrigation technologies. The SOPs are aimed at homogenizing analytical procedures, performance data and optimized operational parameters. More precisely, for the 11 WW treatment technologies (WP2) and for the 6 irrigation technologies (WP3) this Deliverable reports:

- the performance indicators that will be utilized to rate the MADFORWATER technologies between each other and in relation to the corresponding benchmark technologies;
- a preliminary evaluation of the environmental impact and of the measures that will be taken to mitigate it;
- the adopted health and safety procedures, aimed at minimizing the risks for researchers in the respect of local, national and EU legislation.

In addition, for the 11 WW treatment technologies a specific section of the Deliverable is dedicated to the harmonization of the analytical procedures, in order to ensure the comparability of the results obtained by the different WP2 partners. Moreover, a final section is dedicated to an overview of the main international standards for treated WW reuse for irrigation, as well as of the national standards relative to the 3 target countries of MADFORWATER (Morocco, Tunisia, Egypt).

This deliverable responds to Ethic Requirement 2 of the Ethics Summary Report: "The applicant must ensure that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project" and Ethic Requirement 3 "The applicant must provide further information about the possible harm to the environment caused by the research and state the measures that will be taken to mitigate the risks". This Deliverable is related to the activities included in Task 2.1 "Standard Operating Procedures (SOPs), including health & safety procedures, for the harmonization of the experimental approach relative to the experimental activities".

2 Wastewater treatment technologies (WP 2)

The MADFORWATER project will develop, at laboratory scale, and/or adapt to the local conditions of the target MAC countries (Tunisia, Morocco and Egypt) a number of technologies for the treatment of different wastewaters (municipal, agro-industrial, industrial) and of drainage canal water. Primary, secondary and/or tertiary treatments have been selected for each type of wastewater, according to its composition and the quality of effluents currently produced by existing treatment plants in selected basins of the target MACs (Table 2.1). In particular, some technology will be specifically developed for the treatment of a single type of wastewater, whereas some will be adapted to different wastewaters.

Table 2.1 – MADFORWATER wastewater treatment technologies

Techn. ID	Technology description	WW[a]	Treatment[b]
1	Canalized lagoon with nitrification/denitrification and disinfection capacity	DCW, MWW	II + III, III
2	Nitrifying trickling filters filled with innovative high specific-surface carriers	MWW	II
3	Constructed wetlands with PGP bacteria for tertiary treatment (N, P, emerging pollutants, heavy metals)	MWW	III
4	Enzymatic degradation of fungicides, dyes and emerging pollutants with immobilized laccases	MWW, FVPWW, TWW	III
5	Catalytic disinfection beds activated by solar UV light	MWW	III
6	Flotation/flocculation integrated process	FVPWW	I
7	Membrane filtration + phenolic compounds adsorption with selective resins + anaerobic digestion in biofilm reactor	OMWW	I + II
8	Aerobic sequenced batch reactor with lime addition	OMWW	II
9	Granulated sludge bioreactor	TWW	II
10	Moving Bed Biological Reactor	TWW, FVPWW	II
11	Dyes adsorption with innovative resins	TWW	III

[a] DCW: drainage canal water; MWW: municipal wastewater; FVPWW: fruit and vegetable packaging wastewater; TWW: textile wastewater; OMWW: olive mill wastewater.
[b] I: primary treatment, II: secondary treatment, III: tertiary treatment.

For the treatment of municipal wastewater (MWW), a secondary treatment based on nitrifying trickling filters filled with innovative high specific-surface carriers (Technology n. 2) will be developed, along with four tertiary treatments as finishing steps specifically tailored to the disinfection of the effluents (Technology n. 5 – Catalytic disinfection beds activated by solar UV light), to the removal of emerging pollutants (Technology n. 4 – Enzymatic degradation with immobilized laccases) or potentially able to provide a combined effect (Technology n. 1 – Facultative canalized lagoons and Technology n. 3 – Constructed wetlands with PGP bacteria). Since the drainage water of the Nile Delta canals (DCW) receives effluents of different origin (mainly municipal and industrial), the possibility to convert drainage canals into facultative canalized lagoons (Technology n. 1) able to act as secondary and tertiary treatment will be investigated.

The treatment of two types of agroindustrial westawaters, namely olive mill wastewaters (OMWW) and fruit and vegetables packaging wastewaters (FVPWW), will be addressed. For OMWW treatment, a primary treatment aiming at removing suspended solids and polyphenols will be optimized, along with secondary treatment via anaerobic digestion in packed bed bioreactors (Technology n. 7), or via aerobic biological treatment in sequenced batch reactors (Technology n. 8). For FVPWW, a Flotation/flocculation integrated process (Technology n. 6) will be developed as primary treatment, followed by secondary treatment via aerobic moving bed biological reactors (Technology n. 10) and a tertiary treatment specifically tailored to the removal of dyes via immobilized laccases (Technology n. 4).

For TWW treatment, two secondary treatment approaches (Technology n. 9 – granulated sludge aerated bioreactor and Technology n. 10 – two-stage (anaerobic/aerobic) Moving Bed Biological Reactor equipped with a micro-bubble aerator) will be developed, along with two tertiary treatment specifically addressed to the removal of dyes, namely Technology n. 4 – Enzymatic degradation with immobilized laccases and Technology n. 11 - adsorption with innovative resins.

All the technologies will be investigated and optimized at the laboratory scale individually during the first phase of the project. Then, technologies addressing the same treatment step for the same wastewater type will be compared, in terms of technical performance, LCA, CBA and social and technical suitability in relation to the local context, and the most promising treatment trains for each wastewater will be selected for pilot-scale experimental testing and demonstration to be performed in the selected sites of the target MACs. This final technology comparison and integration step requires an homogenous approach is followed for all technologies, since the very beginning of the technology development and adaptation process, in terms of i) analytical methods used for each monitored parameter, (ii) performance data used for the technical evaluation of the different technologies, (iii) operational parameters optimized and (iv) type of input provided for the LCA and CBA of each technology.

2.1 Homogenization of the analytical methods

The harmonization of analytical methods used for the monitoring of the process parameters is an essential step in order to obtain easily comparable results for different technologies. Therefore, the main parameters whose monitoring will be mandatory during technology development have been identified for each wastewater type, according to the information available on its composition (Table 2.1.1).

Table 2.1.1 – Mandatory parameters that will be monitored for each wastewater

Mandatory parameter (units)	MWW and DCW	OMWW	FVPWW	TWW
COD (mg/L)	X	X	X	X
Total Suspended solids (mg/L)	X	X	X	X
N-NH_3 (mg/L)	X			
N-NO_3 (mg/L)	X			
Total N (mg/L)	X			
Total P (mg/L)	X			
Total and fecal coliforms (MPN/100mL)	X			
Viruses (genome copies/100 mL)	X			
Polyphenols (mg/L)		X		
Fungicides Thiabendazol and Imazalil (mg/L)			X	
Heavy metals (mg/L)				X
Dyes (mg/L)				X

The analytical methods used by different partner laboratories to monitor the mandatory parameters have been then collected and compared, in order to verify they can provide easily comparable information, and/or to agree between laboratories on common analytical methods to use. Table 2.1.2 shows the analytical methods agreed to use by the different partner laboratories for the monitoring of the mandatory parameters. For simplicity, Table 2.1.2 shows only the reference standard ISO method, if available, or the type of analytical method selected. Details on the analytical methods, in terms of principle, type of sample/wastewater it is suitable for, working range, main potential interferences, etc., are presented and compared, when different, in the following sections.

The survey evidenced that, in all cases ISO standards are available for the analysis of a given parameter (e.g. COD, TSS, N-NH_4^+, N-NO_3, total N, total P, total and fecal coliforms), almost all partner laboratories will use analytical procedures or commercial kits based on the same ISO standard. When ISO or other international standards are not available, analytical procedures under development/optimization in partner laboratories using different analytical approaches will be shared between laboratories, in order to allow the use of a common procedure. For each analytical methodology under optimization, the main parameters for the method validation will be assessed, such as the specificity/selectivity, precision, accuracy, range, robustness against external influences and/or cross-sensitivity against interference from the matrix of the sample.

Table 2.1.2 – Analytical methods used to monitor the selected mandatory parameters by MADFORWATER partners

Mandatory parameter (units)	UNIBO	FHNW	NJU	TUC	UTM
COD (mg/L)	ISO 15705:2002	ISO 15705:2002	ISO 6060:1989	ISO 15705:2002	ISO 15705:2002
Total Suspended solids (mg/L)	ISO 11923:1997	ISO 11923:1997	ISO 11923:1997	ISO 11923:1997	ISO 11923:1997
Ammonium Nitrogen N-NH_4^+ (mg/L)	ISO 7150-1:1984 or Ion-Selective electrode	ISO 7150-1:1984	ISO 11732:2005	ISO 7150-1:1984	ISO 7150-1:1984
Nitrate Nitrogen N-NO_3 (mg/L)	ISO 7890 1-2:1986 or IC-CD	ISO 7890 1-2:1986	ISO 13395:1996	ISO 7890 1-2:1986	ISO 7890 1-2:1986
Total Nitrogen (mg/L)	ISO 11905:1997	ISO 11905:1997	ISO 29441:2010	ISO 11905:1997	ISO 5663:1984*
Total Phosporus (mg/L)	ISO 6878-1:2004	ISO 6878-1:2004	ISO 15681-2-2003	ISO 6878-1:2004	ISO 6878-1:2004
Total coliforms (CFU/100mL)	ISO 9308-2:2012	ISO 9308-2:2012	ISO 9308-2:2012	ISO 9308-2:2012	ISO 9308-2:2012
Fecal coliforms (CFU/100mL)	ISO 9308-2:2012	ISO 9308-2:2012	ISO 9308-2:2012	ISO 9308-2:2012	ISO 9308-2:2012
Viruses (genome copies/100 mL)					q-PCR and q-RT-PCR

Mandatory parameter (units)	UNIBO	FHNW	NJU	TUC	UTM
Total phenolic compounds (mg/L)	HPLC-UV				Folin Ciocalteu colorimetric method
Fungicides: thiabendazol and imazalil (mg/L)		LC-MS			LC-MS
Heavy metals (mg/L)		ISO 17294:2016	ISO 11885:1996		ISO 15586:2003
Dyes (mg/L)		LC-VIS	LC-VIS	LC-VIS	LC-MS

** The method is referred to Total Kjeldahl Nitrogen*

COD analysis

Chemical Oxygen Demand (COD) will be monitored for all wastewaters. Four partner laboratories have selected analytical methods for the measurement of COD based on the International reference ISO 15705:2002, while one has selected a method based on the International reference ISO 6060:1989. Both methods are based on the same principle and known as dichromate method, although the former produces a lower amount of toxic wastes. The test consists of the oxidation by digesting the samples with sulfuric acid and potassium dichromate in the presence of silver sulfate and mercury (II) sulfate. Silver acts as a catalyst to oxidize the more refractory organic matter. Mercury reduces the interference caused by the presence of chloride ions. After the digestion, the amount of dichromate consumed for the oxidation is determined by measuring the Cr(III) formed at a wavelength of 600 nm ± 20 nm. The results are expressed as ST-COD, related to the absorbance measured. In case of atypical colouring or turbidity after the digestion, a titrimetric determination is required. The method is applicable to any aqueous sample, which includes all sewage and waste waters, having ST-COD values up to 1000 mg/l and a chloride concentration not exceeding 1000 mg/l undiluted. Samples with higher ST-COD values require pre-dilution. For samples with a low COD, the precision of the measurement will be reduced. The method oxidizes almost all types of organic compounds and most inorganic reducing agents. It has a detection limit (4.65 times the within-batch standard deviation of a blank or very low standard) of 6 mg/l for photometric detection at 600 nm. For the reduced calibration range up to 150 mg/l, an alternative wavelength 440 nm ± 20 nm may be used. For a further reduced calibration range up to 50 mg/l, an alternative wavelength of 348 nm ±15 nm may be used. At 348 nm and 440 nm, the absorbance of the remaining chromium(VI) is measured. In method 6060, a single range from 30 – 700 mg/l is mentioned.

Detailed testing has shown good comparison between the 15705 method and the 6060 method. However, it should not be assumed that method 15795 is comparable in all cases to that of ISO 6060 without testing, particularly when there is a problem in obtaining a 2 ml representative sample (e.g. samples with high content of suspended solids).

Most of the partners have also indicated the intention to use commercial kits from the same provider.

TSS analysis

The measure of Total Suspended Solids (TSS) in all wastewaters will be executed according to the ISO 11923:1997 by all the partners. The test consists on the filtering of the samples through a glass-fibre filter, using a vacuum or pressure filtration apparatus, then the filter is dried at 105 °C and the solids on it are determined by weighing. The method is suitable for raw waters, wastewaters and effluents. The lower limit of quantification is approximately 2 mg/l and no upper limit has been established. Floating oil and other immiscible organic liquids will interfere. Water samples are not always stable, which means that the content of suspended solids depends on storage time, means of transportation, pH value and other factors. Results obtained with unstable samples need to be interpreted with caution. The result of the determination depends to some extent on the type of filter used. It is therefore necessary that the type of filter be specified. The size distribution of particles in different water samples can vary widely. Therefore, there can be a variable correlation between results obtained with filters of different pore diameter, and no conversion factor can be given for the conversion of results obtained with one type of filter to another. The partners will therefore agree on the type of filter to be used.

N-NH_4^+ analysis

The measurement of ammonium nitrogen (N-NH_4^+) in MWW and DCW will be performed either using spectrometric methods based on the ISO 7150:1984 or ISO 11732:2005 standards, or with Ion-Selective electrodes. The main features of each method are described below.

The method ISO 7150:1984 is a manual spectrometric method. In particular, the blue compound formed by reaction of ammonium with salicylate and hypochlorite ions in the presence of sodium nitrosopenta-cyanoferrate(III) (sodium nitroprusside) is measured at 655 nm. Then, the ammonium nitrogen content, in milligrams per litre, is determined from the absorbance of the test solution compared with the absorbance of the blank test solution and the calibration graph. When using cells of optical path length 40 mm and a 40 ml test portion, the limit of detection lies within the range N-NH_4^+ = 0,003 to 0,008 mg/l. Application to excessively coloured or saline waters shall be preceded by distillation. The only serious interferences that may be encountered are those from aniline and ethanolamine, and represent the interference to be expected from primary amines in general. However, such substances are seldom encountered at appreciable concentrations in water samples.

In method ISO 11732:2005, determination of ammonium nitrogen is made by flow analysis and spectrophotometric detection. Methods using flow analysis are automating wet chemical procedures and are particularly suitable for the processing of large sample series at a high analysis frequency (up to 100 samples per hour). The ISO 11732:2005 describes two types of flow analysis: flow injection analysis (FIA) and continuous flow analysis (CFA). Both methods consist of the automatic dosage of the sample introduced into a flow system (manifold) in which the sample reacts with the reagent solutions on their way through the manifold. The sample preparation may be integrated into the manifold. The reaction product is measured in a flow detector. In the FIA method, the sample containing ammonium is mixed with a continuously streaming flow of an alkaline solution. The ammonia formed is separated in a diffusion cell from the solution over a hydrophobic semipermeable membrane and taken up by a streaming recipient flow containing a pH indicator. Due to the resulting pH shift, the indicator solution will change its colour, which is measured continuously in the flow photometer. In the CFA method, ammonium present in the sample flowing continuously in a gas-segmented carrier stream reacts in alkaline solution with hypochlorite (ClO^-), which has previously been liberated from dichloroisocyanurate. The chloroamine formed reacts under catalysis of nitroprusside with salicylate at a temperature of 37 °C to 50 °C to form a blue-green indophenol dye which is quantitatively measured in a flow photometer at 640 nm to 660 nm.

Possible interferences in both methods are given by amines, since volatile amines in the FIA method can diffuse through the membrane and lead to a pH shift, whereas low-molecular amines in the CFA method react similarly to ammonia and consequently lead to erroneously high results. A high concentration of metal ions, which tend to precipitate as hydroxides when pH is raised, can cause poor reproducibility of both methods. Particulate matter in the samples can clog the transport tubes and give rise to a positive interference in the spectrophotometric measurement. In the case of larger particles (> 0.1 mm), it is necessary to filter the sample by membrane filtration, whereas smaller particles can be removed by dialysis. Both methods are suitable for the determination of ammonium nitrogen in various types of waters (such as ground, drinking, surface, and waste waters) in mass concentrations ranging from 0.1 mg/l to 10 mg/l (in the undiluted sample).

Ion-Selective electrodes will be also used to measure the ammonium nitrogen concentration during continuous monitoring campaigns, due to its quick response and high precision. Commercial electrodes, such as the electrode CRISON 9663C, have measuring range from 0.9 mg/l to 9g/l, working conditions from pH=0 to 8 and temperature conditions from 5 to 50°C. Possible interferences are produced by K^+, Na^+ and Ca^+ ions. The concentration of ammonium nitrogen during continuous monitoring campaigns will be also periodically checked using of the spectrophotometric method ISO 7150:1984, in particular when low ammonium nitrogen concentrations are expected, due to the lower concentration limit of the spectrophotometric method compared to that of the electrodes.

N-NO₃ analysis

Nitrate nitrogen ($N-NO_3$) will be measured in MWW and DCW using spectrophotometric methods based on the ISO 7890 1-2:1986 and ISO 13395:1996 standards.

The ISO 7890 1-2:1986 method is based on the reaction of nitrate with 2,6-dimethylphenol in the presence of sulfuric and phosphoric acids to produce 4-nitro-2,6-dimethylphenol, which is measured spectrophotometrically at 324 nm. Potential interference of nitrite-nitrogen up to 5 mg/l is controlled with the use of amidosulfonic acid. Chloride may interfere but can be removed by addition of silver sulfate to the sample and filtration before taking the test portion. The method is applicable to the direct analysis of potable and raw water. A nitrate nitrogen concentration of up to 25 mg/l in the test portion can be determined, with a detection limit of 0.06 mg/L.

The ISO 13395:1996 method allows the determination of nitrite nitrogen and nitrate nitrogen and the sum of both by flow analysis and spectrometric detention. Two types of flow analysis can be used: flow injection analysis (FIA) and continuous flow analysis (CFA). Nitrate in the sample is reduced with metallic cadmium to nitrite. Then, a phosphoric acid reagent solution that is also flowing continuously is admixed. Nitrite that is initially present and nitrite resulting from the reduction of nitrate will diazotize sulfanilamide in acid solution to the diazonium salt which is then coupled with N-(1-naphthyl)ethylenediamine to form a red dye. Nitrite is determined, omitting the cadmium reduction, directly by the above-mentioned diazotization and coupling reaction. The mass concentration of nitrate nitrogen is given by the difference: nitrite/nitrate - nitrite. The method can be applied to various types of waters (such as ground, drinking, surface, and waste waters) in mass concentrations ranging from 0.01 mg/l to 1 mg/l for nitrite nitrogen and from 0.2 mg/l to 20 mg/l for nitrite/nitrate nitrogen, both in the undiluted sample. The range of application can be changed by varying the operating conditions.

In addition to the method described before, nitrate nitrogen concentration will be monitored by UNIBO using an ion-chomatography system quipped with an IonPac AS144U 250 mm column, a conductivity detector combined to an ASRS-Ultraconductivity suppressor system, using a solution of 3.5 mM Na_2CO_3 and 1.0 mM $NaHCO_3$ prepared in ultra-resi-analyzed water as eluent, with a flow rate of 1.2 ml/min. A nitrate nitrogen concentration in the range 0.2 mg/l to 40 mg/l can be determined.

Total Nitrogen analysis

Total nitrogen will be monitored in MWW and DCW according to ISO 11905:1997 or ISO 29441:2010 methods. Both methods allow the determination of nitrogen present in water, in the form of free ammonia, ammonium, nitrite, nitrate and organic nitrogen compounds capable of conversion to nitrate using oxidative digestion with peroxodisulfate.

In method 11905, ammonia, nitrite and organic nitrogen-containing compounds in the test sample are oxidized to nitrate using peroxodisulfate in a buffered alkaline system by boiling at elevated pressure in a closed container. Subsequent reduction of nitrate to nitrite is carried out by passage of the digest through a mixing coil containing copperized cadmium. The resulting nitrite is reacted with 4-aminobenzene sulfonamide and N-(1-naphthy)-1,2-diaminoethane dihydrochloride to produce a pink colour. Photometric measurement is carried out at 540 nm. The method can be applied to the analysis of natural fresh water, sea water, drinking water, surface water and treated sewage effluent. It is also applicable to the analysis of sewage and trade wastes in which the amount of organic matter in the test portion can be kept below 40 mg/l, expressed as carbon (C), when measured by Total Organic Carbon (TOC), or 120 mg/l, expressed as oxygen (O_2), when measured by Chemical Oxygen Demand (COD) according to the respective International Standards. The range of detection is 0.02 mg/L up to 5 mg/L and much higher concentrations can be determined using smaller test portions.

In method 29441, the sample is pre-treated with a buffered peroxodisulfate solution and thermal UV radiation. Nitrate is formed and determined either by flow injection analysis (FIA) or by continuous flow analysis (CFA), according to method ISO 13395:1996 (see section on nitrate nitrogen analysis). The method can be applied for various types of waters such as round, drinking, surface, and waste waters, in mass concentrations ranging from 2 mg/l to 20 mg/l for total nitrogen, all in the undiluted sample. Interferences are possible in samples with extreme pH values and samples with a high buffering capacity. In those cases several dilutions of the sample should be analysed and the consistency of results checked. High concentrations of organic substances can cause problems as the oxidation capacity may not be sufficient. For samples containing more than 100 mg/l of total organic carbon (TOC), reduced yields in the determination of nitrogen may arise. If the presence of more than 100 mg/l of TOC is suspected, the sample should be run with several dilutions in order to check for consistency, or standard addition techniques should be applied.

The Total Kjeldahl Nitrogen (TKN) will be used by a partner laboratory to estimate the total nitrogen, according to method ISO 5663:1984. Using this method only trivalent negative nitrogen is determined. Organic nitrogen in the form of azide, azine, azo, hydrazone, nitrite, nitro, nitroso, oxime or semicarbazone is not determined quantitatively. The method is based on the mineralization nitrogen compounds into ammonium sulfate with sulfuric acid in the presence of selenium as catalyst. Ammonia is liberated from the ammonium sulfate by the addition of alkali and distillation into boric acid/indicator solution. Ammonium ions in the distillate are determined by titration with standard acid. Alternatively, ammonium ion in the mineralizate is directly determined by spectrometry at 655 nm. This method is applicable to the analysis of raw, potable and waste waters. A Kjeldahl nitrogen content can be determined in the range 1 to 10 mg/L. Using a 10 ml test portion, this corresponds to a sample concentration of up to 1000 mg/l. When using this method, the total nitrogen will be estimated by summing the TKN with nitrite and nitrate nitrogen determined with the corresponding methods described in sections above.

Total phosphorus analysis

The analysis of phosphorus in MWW and DCW will be performed according to methods ISO 6878-1:2004 and ISO 15681-1-2:2003.

The ISO 6878-1:2004 method allows the determination of orthophosphates via reaction with an acid solution containing molybdate and antimony ions to form an antimony phosphomolybdate complex. The complex is reduced with ascorbic acid to form a strongly coloured molybdenum blue complex. Measurement of the spectrophotometric absorbance at 880 or 700 nm of this complex is used to determine the concentration of orthophosphate present. Polyphosphate and some organophosphorus compounds are determined by conversion to molybdate reactive orthophosphate by sulfuric acid hydrolysis. Total phosphorous is determined after conversion of organophosphorus compounds to orthophosphate by mineralization with peroxodisulfate. The method is applicable to all kinds of water including seawater and effluents. Phosphorus concentrations within the range of 0.005 mg/l to 0.8 mg/l may be determined in such samples without dilution. A solvent colour extraction procedure with 1-hexanol allows smaller phosphorus concentrations to be determined with a detection limit of about 0.0005 mg/l.

The ISO 15681-1-2:2003 is a variant of method ISO 6878 for orthophosphate and total phosphorus determination by flow analysis (FIA – 15681-1, or CFA – 15681-2). The method includes the digestion of organic phosphorus compounds and the hydrolysis of inorganic polyphosphate compounds, performed either manually as described in ISO 6878 or with an integrated UV digestion and hydrolysis unit. Determination of orthophosphate in the mass concentration ranges from 0.01 mg/l to 1.00 mg/l P, and of total phosphorus in the mass concentration range from 0.10 mg/l to 10.0 mg/l P.

Possible interferences are given by arsenate, which produces a colour similar to that produced by orthophosphate. This interference can be eliminated by reducing arsenate to arsenite with sodium thiosulfate. Silicate may also interfere if present at concentrations greater than 60 times the phosphorus concentration. Fluoride interference is significant above 50 mg/l. Nitrite interference is significant above 5 mg/l. This interference can be eliminated by acidifying the sample after collection.

Enumeration of total and fecal coliforms

The ISO 9308-2:2014 method will be used to monitor the total and fecal coliforms in MWW and DCW. *Escherichia coli* can be also enumerated using this method. The method involves the use of patents concerning Colilert-18 and Quanti-Tray and Quanti-Tray 2000 owned by IDEXX Laboratories. It is based on the growth of target organisms in a liquid medium and calculation of the “Most Probable Number” (MPN) of organisms by reference to MPN tables. The medium contains ortho-nitrophenol galactoside, which is hydrolysed by the enzyme β-D-galactosidase giving rise to the yellow coloured ortho-nitrophenol after growth of coliforms. After incubation, sample that have a yellow colour of equal or greater intensity than that of the comparator sample are considered positive for coliform bacteria. Total and fecal coliforms are independently determined by incubating samples for 18-22 h at different temperatures (35±0.5 °C for total and 44.5±0.2 °C for fecal coliforms). *E. coli* can be also enumerated after incubation at 35 °C and exposure to UV light (365 nm), based upon expression by *E. coli* only of the enzyme β-D-glucuronidase and the presence of 4-methylumbelliferyl glucuronide (MUG) in the medium, which is cleaved into the fluorescent compound methyl umbelliferone. The method can be applied to all types of water, including those containing an appreciable amount of suspended matter and high background counts of heterotrophic bacteria.

Enumeration of viruses

The enumeration of viruses will be carried out only on MWWs and DCWs. Samples will be collected, pre-processed, stored and finally shipped by partners developing MWW and DCW treatment technologies to UTM, that will perform the analysis. Partner UTM will adapt/optimize a q-PCR and a q-RT-PCR method for the detection and quantification of Adenovirus, Rotavirus, Norvovirus and Enterovirus from Chung et al. (2015), J. Appl. Microbiol. 119:876–884 and Magill-Collins et al. (2015), Wilderness Environ Med. 26:312-318. Partner UTM will also optimize and distribute to all partners a procedure for the correct sampling and storage of samples dedicated to the enumeration of viruses, as well as a procedure for viral nucleic acid extraction, storage and shipping. In this respect, all MADFORWATER partners developing MWW and DCW treatment technologies have the general equipment and know-how required for the extraction of nucleic acids.

Total phenolic compounds analysis

Two methods will be used to measure total phenolic compounds in OMWW: the conventional colorimetric test developed by Folin and Ciocalteu (Folin O., Ciocalteu V., J. Biol. Chem. 73: 627-650) and an HPLC method (Frascari et al., 2016, Chem. Eng. J. 283: 293-303). In the Folin–Ciocalteu (FC) method, 25 mL flasks, carefully cleaned with sulfuric acid 25% and washed with de-ionized (DI) water, are filled with 12.5 mL of DI water, 125 µL of sample (diluted as required, to avoid absorbance signal saturation) and 1.25 mL of FC reagent. After 2 min, the reaction is quenched by adding 3.75 mL of sodium carbonate (20% w/v). Finally, the flasks are diluted to the volume mark, and left at 75 °C for 2 h. Then, the absorbance is read at 765 nm. The method is calibrated with acid gallic as external standard. In the HPLC method, the sample is analysed on a C18 Kinetex 2.6 µm 100A Phenomenex column (or an equivalent column) connected to a UV/vis detector set at 264 nm using a mobile phase flow rate of 1.0 mL/min and the following mobile phase gradient: 0–4 min, 100% phase A (HPLC water with 0.1% orthophosphoric acid); 4–6 min, 70% phase A and 30% phase B (acetonitrile); 6–15 min 70% phase A and 30% phase B. The mobile phase gradient is designed to merge all the phenolic peaks into a single broad peak. An internal standard (gallic acid 50 mg/L) is added in each HPLC analysis. Both the traditional FC method and the HPLC method treat the PC mixture as a pseudo component, expressing the total PC concentration as specific PC equivalent (gallic acid). Both methods thus avoid the complexity of taking into account the contribution of each single compound. This approximation is less severe in the case of the FC method, which recognizes the chemical functionality by the selective colorimetric reaction. On the other hand, neither method guarantees that all and only phenolic compounds will be detected: the HPLC method could in principle sum non-phenolic compounds to the actual PCs and/or fail to detect specific PCs, whereas the FC method can be characterized by an interference due to other organic compounds present in OMWW (i.e. proteins and carbohydrates). Cross-checks between the two methods using different OMWWs revealed that the deviation between the two methods ranges between 0.4% and 17%, with an average value (10%) comparable to the relative analytical errors (Frascari et al., 2016, Chem. Eng. J. 283: 293-303).

Analysis of thiabendazol and imazalil fungicides

For the analysis of the fungicides thiabendazol and imazalil in FVPWWs, an LC-MS method will be developed after modification of the analytical procedure described in Sirtori C. et al., 2014, Anal. Bioanal. Chem. 406: 5323, which based on analytes separation on a C18 analytical column using a mobile phase gradient of 0.1 % formic acid and 5 % Milli-Q water in acetonitrile as mobile phase A and 0.1 % formic acid in water (pH 3.5) as mobile phase B.

Heavy metals analysis

The analysis of heavy metals will be carried out in different partner laboratories using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS), Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) or Atomic absorption spectroscopy (AAS).

The international standard ISO 17294 describes the multi-elemental determination of analytes by ICP-MS in aqueous and nitric acid or aqua regia digests. The method measures ions produced by a radiofrequency inductively coupled plasma. Analyte species originating in a liquid are nebulized and the resulting aerosol is transported by argon gas into the plasma torch. The ions produced by high temperatures are entrained in the plasma gas and introduced, by means of an interface, into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge ratios and quantified with a channel electron multiplier. The method allows the determination of 62 elements in water (drinking water, surface water, groundwater, wastewater, and aqua regia and nitric acid digests of water, eluates, industrial and organic wastes, soils, sludges, sediments, and other solid wastes. The 62 elements determined include almost all elements in environmental analysis. Exceptions are halogens, noble gases, hydrogen, nitrogen and oxygen. The technique is based on the measurement specific isotopes of elements. Corrections are made for isobaric interferences and interferences by polyatomics. Correction factors and correction equations are evaluated during the run by analyzing a so-called interference check solution. Trace elements have detection limits less than 1 µg/l, heavier elements down to 0.1 µg/l. Coefficients of variation for (interlaboratory)reproducibility are less than 15% for surface water except for chromium, tin, thallium and vanadium.

ISO 11885 describes the determination of dissolved, particulate and total elements in raw, potable and wastewater by ICP-OES. The 33 elements determined include all important trace elements in environmental analysis. Filtered acidified samples (nitric acid) are analysed to give dissolved elements; the filtrate is digested consecutively with nitric acid and hydrochloric acid to give particulate elements and the whole sample is digested with nitric acid to give total elements. The technique is based on the measurement of the emission at one wavelength, which is highly selective for a specific element. Calibration matrix should match as much as possible the sample matrix. All trace elements have detection limits less than 0.1 mg/l. Systematic deviations are not significant. Coefficients of variation for (interlaboratory)reproducibility are less than 5% except for the alkali metals sodium, potassium, magnesium and calcium and the elements sulfur and vanadium.

ISO 11886 describes the determination of trace levels of Ag, Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, and Zn in surface water, ground water, drinking water, wastewater and sediments, using AAS with electrothermal atomization in a graphite furnace. The method is applicable to the determination of low concentrations of elements. Water samples are preserved by acid treatment, filtered and preserved by addition of acid, or digested. Sediment samples are digested. A small sub-sample of sample solution is injected into a graphite furnace of an atomic absorption spectrometer. The furnace is electrically heated. By increasing the temperature stepwise, the sample is dried, pyrolized and atomized. Atomic absorption spectrometry is based on the ability of free atoms to absorb light. A light source emits light specific for a certain element (or elements). When the light beam passes through the atom cloud in the heated graphite furnace, the light is selectively absorbed by atoms of the chosen element(s). The decrease in light intensity is measured with a detector at a specific wavelength. The concentration of an element in a sample is determined by comparing the absorbance of the sample with the absorbance of calibration solutions. Some sample solutions, especially wastewaters and digestions of sediments, may contain large amounts of substances that may affect the results. High concentrations of chloride may cause low results, because the volatility of many elements is increased and analyte loss may occur during the pyrolysis step. Matrix effects may be overcome, partially or completely, by optimization of the temperature programme, the use of pyrolytically-coated tubes and platforms, the use of chemical modifiers, the standard addition technique and the use of background correction. The detection limit of the method for each element depends on the sample matrix as well as of the instrument, the type of atomizer and the use of chemical modifiers. For water samples with a simple matrix (i.e. low concentration of dissolved solids and particles), the method detection limits will be close to instrument detection limits. The minimum acceptable detection limit values for a 20-µl sample volume are in the range 0.1 – 2 µg/l.

Dyes analysis

Dyes will be analysed in TWW only. A method will be developed after modification of the analytical procedure described in Zhao X. and Hardin I.R., 2007, Dyes and Pigments, 73: 322-325, for the analysis of the following dyes in LC-UV or LC-MS: BEZAKTIV BLEU S-2G, BEZAKTIV BLEU S-MATRIX 150, BEZAKTIV ROUGE S-MATRIX 150, TUBANTIN BLEU BRR H.C., TUBANTIN BRUN GGL, TUBANTIN ORANGE GGLN 200.

2.2 Homogenization of the performance indicators

At the end of the 2nd year of research, each WW treatment technology will be evaluated by means of a series of performance indicators, in order to rate the Madforwater WW treatment technologies in comparison with corresponding benchmark or state-of-the-art technologies. The following indicators have been identified, and will be evaluated for each pollutant monitored in the WWs to which each technology is applied (the list of the WWs to which each technology will be applied is reported in Table 2.1).

a) removal efficiency (%);

b) removal rate ($g_{pollutant}$ / ($L_{reactor}$ d));

c) effluent quality, in relation to the main standards for treated WW reuse in irrigation (effluent concentration / threshold concentration); a critical analysis of the main standards for treated WW reuse in irrigation is reported in section 3.4;

d) hydraulic retention time (d);

e) enzyme, catalytic bed or resin half-life (d).

Parameters a) to d) will be evaluated for all the 11 WW treatment technologies, whereas parameter e) will be evaluated only for technologies n. 4, 5, 11.

2.3 Environmental impact of the wastewater treatment technologies

The WP2 technological partners were asked to evaluate the environmental impact of the WW treatment technologies by referring to the following categories:

- Solid waste disposal
- Atmospheric emissions
- Liquid emissions
- Energy consumption
- Chemicals consumption

On the basis of the answers provided by the WP2 partners, the main solid waste consists in biological sludge; only 2 technologies are characterized by atmospheric emissions, consisting in biogas; liquid emissions are basically absent; energy consumption is mainly associated to aeration, whereas several technologies required the addition of chemicals. A short description of the expected environmental impacts of the WW treatment technologies is reported in Table 2.3.1. A more comprehensive evaluation of the environmental impacts and benefits of the Madforwater technologies will be performed in the framework of the Life Cycle Analysis object of Task 2.5. Table 2.3.1 includes only the environmental impacts relative to the actual implementation of the technologies at pilot or full scale, whereas the environmental impacts relative to the laboratory activities required to monitor these technologies (mobile phases for chromatographic analysis, disposable material, etc.) were not considered, as they are basically independent of the considered technology. The treated WW produced by each technology was not considered as a liquid emission with a significant environmental impact, as all the MADFORWATER technologies are expected to produce an irrigation-quality effluent.

Table 2.3.1 – Expected environmental impact of the Madforwater irrigation technologies

Technology ID and description	**Solid wastes**	**Atmospheric emissions**	**Liquid emissions**	**Energy consumption**	**Chemicals consumption**
1 - Canalized lagoon with nitrification/denitrification and disinfection capacity, for the treatment of municipal WW and drainage canal water.	Solid waste consists of the sludge that settles on the bottom of the canals, which may require periodical removal to maintain the correct hydraulic and water purification properties of the lagoon. The produced sludge can be processed through conventional sewage sludge treatments and then either landfilled, incinerated or applied on agricultural land according to its quality.	No atmospheric emissions.	No liquid waste production is expected.	Canalized lagoons do not require energy during their normal operation, since they are designed to operate by gravity flow. Energy is required for pumping of settled sludge during periodical maintenance operations.	No chemical consumption.
2- Nitrifying trickling filters filled with innovative high specific-surface carriers, for the treatment of municipal WW	Solid waste consists of the settled sludge. The produced sludge can be processed through conventional sewage sludge treatments and then either landfilled, incinerated or applied on agricultural land according to its quality.	The process produces an exhaust air stream without any polluting characteristics.	No liquid waste production is expected.	Energy for wastewater pumping and air pumping (if applied)	No chemical consumption.
3 - Constructed wetlands with PGP bacteria for the treatment of municipal WW	Solid waste in CWs consists of dead parts of the plants. Biomass (all live and dead parts together with litter) and dead plant's parts production fluctuates widely throughout the year and among the used vegetation. Maximum aboveground standing crop (the largest value of plant material in a year's period) for submerged plants,	No atmospheric emissions.	No liquid waste production is expected.	Horizontal subsurface flow CWs require very little energy if any; typically less than 0.1 kW m^{-3}.	Fertilizers are rarely applied to CW systems. In case it is recommended, carefully consideration must be taken, because of the potential impacts of the nutrients that

Technology ID and description	Solid wastes	Atmospheric emissions	Liquid emissions	Energy consumption	Chemicals consumption
	commonly used in HSF-CWs, varies from 50 – 500 g m^{-2} dry mass. Dead plant materials can be disposed of in a landfill since heavy metals accumulation in the above ground plant parts, in municipal WW, is negligible, thus no special treatment is required. At the time of CW reconstruction total plant material (roots and shoots) can be properly disposed as a hazardous material as is or after a reduction in volume and/or weight by thermal, microbial, physical, or chemical means and even reclaim the metals with economical value. Gravel, as substrate media, at the end of the system's life can also be disposed after cleaning processing.				might escape in the effluent to the receiving water.
4 - Enzymatic degradation of fungicides, dyes and emerging pollutants with immobilized laccases, for the treatment of municipal WW, textile WW and fruit / vegetable packaging WW	No solid waste production is expected.	The process may produce an exhaust air stream without any polluting characteristics.	No liquid waste production is expected.	Energy for wastewater pumping mixing and/or aeration, if required.	The process requires the following chemicals: nanoparticles, resins, enzymes, titanium dioxde.
5 - Catalytic disinfection beds	No solid waste production is expected.	No atmospheric emissions.	No liquid waste production is	The process does not require energy for	A titanium dioxide-based paint will be

Technology ID and description	Solid wastes	Atmospheric emissions	Liquid emissions	Energy consumption	Chemicals consumption
activated by solar UV light, for the treatment of municipal WW			expected.	pumping, since in large scale it is normally gravity driven.	used.
6 - Flotation/flocculation integrated process, for the treatment of fruit / vegetable packaging WW	Solid waste consists of the settled sludge. The produced sludge can be processed through conventional sewage sludge treatments and then either landfilled, incinerated or applied on agricultural land according to its quality.	The process produces an exhaust air stream without any polluting characteristics.	No liquid waste production is expected.	Energy consumption for wastewater pumping and aeration in the flotation tank.	A non toxic flocculating agent may be used to enhance the flotation process.
7 - Membrane filtration + phenolic compounds adsorption with selective resins + anaerobic digestion in biofilm reactor, for the treatment of olive mill WW	Spent resin. After a given number of adsorption/desorption cycles (to be determined experimentally), the resin must be removed from the adsorption column. On the basis of preliminary data, 4 kg of resin can treat 1 m3 of OMWW. None of the tested resins presents any toxicity. A specific effort will be aimed at the maximization of the number of adsorption/desorption cycles that can be performed with the same resin load. Digestate. It is the material remaining after the anaerobic digestion of dephenolized OMWW. After stabilization through composting, it can be effectively re-utilized as a soil conditioner because it contains nutrients such as ammonium and	Anaerobic digestion of OMWW leads to the production of biogas, composed mainly by methane and carbon dioxide. On the basis of preliminary data, the expected production is about 30 Nm^3/m^3 OMWW. In the real-scale process, the biogas is burned to produce electricity. The process is designed so as to maximize methane production and therefore electricity production.	The condensed ethanol obtained after evaporation of the desorption solvent gets fully re-utilized in subsequent desorption cycles. Therefore, the process does not produced any liquid emissions.	The main energetic consumption of the process is associated to ethanol evaporation. On the basis of preliminary evaluation, the energetic consumption is equal to 50 kWh/m^3 OMWW.	The process requires the following chemicals: - adsorption resin: about 4 kg/m3 OMWW; - ethanol: about 4% of the ethanol used in each cycle gets lost and must therefore be replaced; this corresponds to a consumption of about 10 L/m3 OMWW.

Technology ID and description	Solid wastes	Atmospheric emissions	Liquid emissions	Energy consumption	Chemicals consumption
	phosphate.				
8- Aerobic sequencing batch reactor with lime addition, for the treatment of olive mill WW	Solid waste consists of the settled sludge. The produced sludge can be processed through conventional sewage sludge treatments and then either landfilled, incinerated or applied on agricultural land according to its quality.	The process produces an exhaust air stream without any polluting characteristics.	No liquid waste production is expected.	Energy consumption for wastewater pumping and aeration. A novel high-efficiency air distribution system based on the production of micro-bubbles will be implemented in the SBR in order to reduce energy consumption.	The process requires a periodic addition of lime.
9 - Granulated sludge bioreactor, for the treatment of textile WW	Solid waste consists of the settled sludge. The produced sludge can be processed through conventional sewage sludge treatments and then either landfilled, incinerated or applied on agricultural land according to its quality.	The process produces an exhaust air stream without any polluting characteristics.	No liquid waste production is expected.	Energy consumption for wastewater pumping and aeration.	No chemical consumption.
10 - Moving Bed Biological Reactor, for the treatment of textile WW and fruit / vegetable packaging WW	Solid waste consists of the settled sludge. The produced sludge can be processed through conventional sewage sludge treatments and then either landfilled, incinerated or applied on agricultural land according to its quality.	The anaerobic step will produce biogas, composed mainly by methane and carbon dioxide.	No liquid waste production is expected.	Aeration constitutes the biggest energy-consuming step in the MBBR process, which accounts for 45%–75% of the total energy consumption. A novel high-efficiency air distribution system based on the production of micro-	No chemical consumption.

Technology ID and description	Solid wastes	Atmospheric emissions	Liquid emissions	Energy consumption	Chemicals consumption
				bubbles will be implemented in the MBBR treating textile WW in order to reduce energy consumption.	
11 - Dyes adsorption with innovative resins, for the treatment of textile WW	Spent anion exchange resin. After a long period of operation, the anion exchange resin might be replaced due to its deteriorated performance or fouling. None of the tested resins presents any toxicity.	The anion exchange process doesn't produce any gaseous emissions.	The anion exchange process will produce some concentrated desorption liquid wastes (~ 25 L produced / m3 WW treated), characterized with high salinity and COD. The NaCl can be recovered by evaporation of the liquid waste under sunlight or in furnace.	The main energetic consumptions of the process are associated to (1) stirring the anion exchange resin, (2) pumping the NaCl solution for desorption and (3) recovering NaCl by evaporation of the desorption liquid waste in furnace.	Periodic resin replacement.

2.4 Health and safety procedures relative to the wastewater treatment technologies

In order to evaluate the adequacy of the health and safety procedures that WP2 partners plan to adopt for the WW treatment technologies, each partner was asked to fill in a standardized questionnaire relative to the equipment utilized, the planned protective equipment and storage methods, the planned measures in case of spill or accident, the procedures for the education and training of researchers and the reference legislation to be respected. The questionnaires relative to the 11 WW treatment technologies are reported in Tables 2.4.1 to 2.4.11. A comprehensive examination of these Tables indicates that the WP2 technologies are not characterized by any particularly relevant health & safety issue. The activities characterized by some hazard are mainly those related to the handling of municipal wastewaters and drainage canal water, due to potential exposure to pathogens and hazardous endocrine disrupting micropollutants, if present, during normal operation or as a consequence of accidental spills, and to potential exposure to nanoparticles in case of application of nanoparticle-immobilized enzymes. Some additional hazard may be posed by the use of flammable solvents in technologies including adsorption/desoption processes, or the production of methane-rich biogas via anaerobic processes.

With regard to the reference legislation, all the research activities will be conducted in compliance with EC Regulation No. 1907/2006 (REACH), as amended by EU Regulation No. 453/2010, in addition to the reference laws valid in Italy, Tunisia, Switzerland, China, Greece, the 5 countries where the WP2 technologies will be developed.

Table 2.4.1 – Health & safety procedures relative to technology 1 (Canalized lagoon with nitrification/denitrification and disinfection capacity) (Partner UNIBO, IT).

WW type and characteristics	Secondary effluent of municipal WW treatment (composition varies depending on the origin/selected treatment plant in the three MACs): COD 80-100 mg/l, TSS 5-25 mg/l, N-NH_3 <30 mg/l, N-NO_3 10-30 mg/l, total P ~1 mg/l, total coliforms 200-1000/100 ml. Drainage canal water: COD ~50 mg/L, TSS ~70 mg/l, N-NH_3 ~4 mg/l, N-NO_3 ~17 mg/l, total P ~1.2 mg/l, total coliforms ~2.3 x 10^5/100 ml.
Equipment used	Laboratory scale stirred tank bioreactor (STR) equipped with probes for temperature, pH, dissolved oxygen and redox potential on-line monitoring; automatic control of temperature, aeration, mixing and dissolved oxygen concentration (preliminary kinetic characterization of the process under aerobic/anaerobic, light/dark conditions). Laboratory scale canalized reactor with recirculation line, temperature control and lamps, equipped with probes for temperature, pH, dissolved oxygen and redox potential on-line monitoring; feed and effluent tanks, pumps. The pilot canalized lagoon will be made of cement and properly sealed with synthetic liners (e.g. asphalt, plastic membranes etc.) to avoid possible leaks and hence, contamination of groundwater. The influents to the lagoon will come from the effluents of a secondary treating WWTP or from a Branch canal using either pipes or an open channel that releases water into the lagoon. Supplementary pumps can be used if needed. Equipment for chemical and microbiological analysis.

Personal protective equipment (PPE)	Labcoat. Safety eyewear (PINTON CLEAR CE EN166, EN170 (2C-1.2), ANSI (Z87+)). Examination gloves (NR Latex, personal protective equipment of complex design category III, in compliance with 89/686/EEC, type tested to EN 420:20003+A1:2009, EN 374-2:2003, EN 374-3:2003 & EN 388-:2003, CE 0086; Class 1 Medical Device, in compliance with 93/42/EEC, type tested to EN455-1:2000, EN 455-3:2009, EN 455-3:2006, EN 455-4-2009 and ASTM D3578, CE).
Collective protective equipment (CPE)	No special collective protective equipment is required.
Storage methods	The secondary effluent of municipal WW treatment and drainage cal water must be stored in closed jerry-cans at 4°C to reduce bacterial growth.
Procedures in case of spill or accident	Spilled dranage canal water and secondary effluent of municipal wastewater must be covered with adsorbent sand and disposed of properly. The spilled area must then be washed with tap water and disinfected with with chlorinated water. Each waste must be disposed in the right way according to the European Waste Catalogue and the specific procedures of the University of Bologna.
Working environment decontamination	In case of an accident, decontamination with chlorinated water of the equipment and areas spilled by the WW is required.
Designated areas for specific unit operations	No designed areas are required.
Special authorizations and / or required procedures	The Standard Operating Procedure relative to this research activity must be examined and approved by the Prevention and Protection Service of the University of Bologna.
Education and training of research personnel	The research personnel must attend two specific courses on biological and chemical risk, where a qualified personnel explains and illustrates them the correct use of all the PPE and CPE. At the end of the course the research personnel has to fill in a learning verification questionnaire.
Reference legislation	The relevant national legislation in Italy is: the Legislative Decree no. 81 of April 9th 2008 and the Legislative Decree no. 106 of August 3rd 2009, which coordinates and restructures in a single regulation text all relevant legal provisions.

Description of the operational phases and of the related hazards and protection measures		
Operational phases	***Hazards identification***	***Safety instructions***

The canalized facultative lagoon process consists in one operational phase, where the water flows continuously in a canal with the proper geometry that favours the combined activity of photosynthetic oxygenic microalgae, aerobic chemoorganotrophic bacteria and anaerobic/facultative aerobic chemoorganotrophic bacteria. At lab scale, the process will be investigated in two different systems. Firstly, in stirred tank reactors under batch conditions, in order to characterize the removal kinetics under different light intensity and oxygen concentrations typically occurring at different depth in canalized lagoons. Secondly, small scale (0.5 m^3) canalized lagoons will be used under continuous conditions with effluent recycle. At pilot scale, a larger canalized lagoon will be fed with DCW continuously and the effluent used for irrigation.	The main risk is connected to the presence of pathogens in the influent wastewater. No major spill is expected in the lab tests due to low volumes treated. In pilot tests, no hazard is expected.	The operator must avoid any contact with influent wastewater by using the proper PPE.

Table 2.4.2 – Health & safety procedures relative to technology 2 (Nitrifying trickling filters with innovative high specific-surface carriers) (Partner UTM, TN)

WW type and characteristics	Municipal WW: 25-141 mg/L total nitrogen, 8-22 mg/L phosphorus, 80-428 mg/L total organic carbon, Heavy metals (Cu, Hg, Pb, Cr, Ni), presence of coliforms.
Equipment used	Trickling filter system: distribution system, filter media, underdrain system, containment structure, filter feed pump, secondary clarifiers. Molecular analysis of microbial population: Gel electrophoresis unit "D-code electrophoresis system, Biorad", thermocycler. Chemical analysis: BOD analysis system, COD analysis system, spectrophotometer.
Personal protective equipment (PPE)	Lab coat, Safety eyewear, Latex Gloves, paper face mask.
Collective protective equipment (CPE)	Chemical hood, Air extractor active 24/24
Storage methods	The wastewater must be stored in closed jerry-cans at 4°C to reduce bacterial growth and fermentation processes.
Procedures in case of spill or accident	MWW spill: cover with adsorbent sand and dispose properly; the studied WW does not have any specific toxicity.
Working environment decontamination	No special needs for decontamination are required after spills of MWW, except for a disinfection step.
Designated areas for specific unit operations	There is no need to separate specific unit operations
Special authorizations and / or required procedures	No special authorization is required.
Education and training of research personnel	Research personnel is properly educated and trained (they are all graduated from University). Moreover, periodically internal training are organized by Senior researchers.

<table>
<tr><td>Reference legislation</td><td colspan="2">The relevant national legislation in Tunisia is: the Legislative Decree no. 96-62 of July 15th 1996, the Legislative Decree no. 96-41 of June 10th 1996</td></tr>
<tr><td colspan="3">Description of the operational phases and of the related hazards and protection measures</td></tr>
<tr><td>Operational phases</td><td>Hazards identification</td><td>Safety instructions</td></tr>
<tr><td>The treatment process consists in a single operational phase in which municipal WW is pumped on top of the trickling filter to horizontal perforated pipes that distribute it homogenously on the fixed bed the WW flows through downward. Aerobic conditions are maintained either by forced-air flowing through the bed or natural convection of air.</td><td>The main risk is connected to the presence of pathogens in the wastewater, as well as by aerosols generated by forced aeration.</td><td>The operator must avoid any contact with influent wastewater by using the proper PPE. The lab scale reactor is place under chemical hood and the air outlet is disinfected in a chlorine-based sterilizing solution.</td></tr>
</table>

Table 2.4.3 – Health & safety procedures relative to technology 3 (Constructed wetlands with PGP bacteria for tertiary treatment (N, P, emerging pollutants, heavy metals) (Partner TUC, GR)

WW type and characteristics	Effluent of municipal WW secondary treatment: - BOD 2.8 mg/L, COD 19 mg/L, T-N 9.2 mg/L, NH_4-N 0.2 mg/L, T-P 5.3 mg/L - Total Coliforms (TC) 2.13 x 10^5 cfu/100mL - *E. coli* 2.50 x 10^5 cfu/100mL - Enterococci 6.80 x 10^4 cfu/100mL - Ni < 10 μg/L (aver. 7.6 μg/L) - Pb < 20 μg/L (aver. 11 μg/L) - Cr < 4 μg/L (aver. 3 μg/L) - Zn < 300 μg/L (aver. 159 μg/L) - Cd not detected Pilot unit elsewhere may accept secondary treated effluents, which have all organic compounds at a higher concentration.
Equipment used	A Horizontal Subsurface Flow Constructed Wetland (HSF-CW) consists of a cell, made of cement that is properly sealed with synthetic liners (e.g. asphalt, plastic membranes etc.) to avoid possible leaks and hence, contamination of groundwater. The influents to the CW come from the effluents of a secondary treating WWTP using either pipes or an open channel that releases water into the wetland. Supplementary pumps can be used if needed. Substrates may include gravel, soil, sand or other organic materials. Vegetation is comprised of macrophytes similar to those found in a natural marsh.

Personal protective equipment (PPE)	- Cloth lab coat - Protective overalls for heavy duty protection against hazardous liquids, dusts and insects (EN 13034, EN ISO 17491-4) - Safety eyewear (2 - UV Protection, 1.2) (EN 166, EN170). - Latex and nitrile disposable examination gloves (Protection of the user against physical or mechanical injuries, cuts, tears and punctures (EN388), bacteria and germs (EN 455/1-4) and against water penetration EN 420) - EN 345-1:1992 - Safety footwear resistant to water penetration / absorption - Oversleeves from the same material as the protective coverall - Vaccination of the permanent staff of WWTP against infectious diseases
Collective protective equipment (CPE)	No special collective protective equipment is required.
Storage methods	If there is such a need, the wastewater must be stored in borosilicate glass media bottles at 4°C to ensure no further bacterial growth and precipitation processes.
Procedures in case of spill or accident	Municipal WW: - Close of the CW input valve so that the influents bypass the wetland system - Disconnect power in case of pumping system malfunction, to lessen overflows and protect the network - Pumping of wastewater leakage back to the wetland system - In case of long-term failure, care must be taken (e.g., a backup irrigation system) to ensure the maintenance of proper conditions for plants growth. - Wash spilled area with tap and chlorinated water
Working environment decontamination	In case of an accident, decontamination with chlorinated water, of the equipment and areas spilled by the WW is required.
Designated areas for specific unit operations	As long as CW systems are located next to WWTPs, account must be taken for the required surface area and also foresight for possible expansion. No further action is required.
Special authorizations and / or required procedures	No special authorization is required for installation of the CW.
Education and training of research personnel	Personnel must be trained to handle and control properly pumping systems, to repair them in case of a malfunction, to sampling techniques and monitor of vegetation's condition.
Reference legislation	The framework Council Directive 89/391/EEC of 12 June 1989 and the Council Directive 91/383/EEC of 25 June 1991 have been transposed into Greek law in the Presidential Decree 17/1996 "On Measures for the Improvement of Safety and Health of Employees during their Work Activities". The Presidential Decree 95/1999 "On Conditions for the Establishment and Function of Protective and Preventive Services" provide more details on this matter. Further important framework legislation was the Presidential Decree 159/1999 "Modification of PD 17/96" and Law 3144/2003 "On Social Dialogue for the Promotion of Employment, Social Protection and other Provisions".

Description of the operational phases and of the related hazards and protection measures		
Operational phases	***Hazards identification***	***Safety instructions***
Operation is taking place in one phase in which wastewater flows horizontally through the wetland (containing gravel, soil or other media). Duration of the process is controlled by the hydraulic retention time (HRT), i.e., the flow rate of the influent WW. The effluent is discharged to the final receiver or used for reclamation.	As an environmentally friendly technology, no hazardous impacts are revealed, unless in the case of an accident. The only danger is related to the secondary treated wastewater itself, i.e., the system input stream, rather than the proposed technology.	The operator must avoid any contact with influent wastewater by using the proper PPE.

Table 2.4.4 – Health & safety procedures relative to technology 4 (Enzymatic degradation of fungicides, dyes and emerging pollutants with immobilized laccases) (Partner FHNW, CH; Partner UTM, TN)

WW type and characteristics	Different wastewaters will be treated using immobilized laccases: Fruit/vegetable packaging WW containing the fungicides imazalil and thiabendazol; Textile WW containing dyes (BEZAKTIV BLEU S-2G, BEZAKTIV BLEU S-MATRIX 150, BEZAKTIV ROUGE S-MATRIX 150, TUBANTIN BLEU BRR H.C., TUBANTIN BRUN GGL, TUBANTIN ORANGE GGLN 200); Effluent of municipal WW secondary treatment containing the micropollutant bisphenol A and potentially pathogenic bacteria originating from municipal wastewater.
Equipment used	Packed bed reactor (PBR), percolating filter with nanobiocatalysts mixed to porous carriers, or membrane bioreactor (MBR) with freely suspended nanobiocatalysts. Analytical equipment: automated solid phase extraction system, GC-MS, Microplate Reader, LC-MS, BOD analysis system, COD analysis system, spectrophotometer.
Personal protective equipment (PPE)	Lab coat, Latex Gloves, safety eyewear, examination gloves
Collective protective equipment (CPE)	Biological Safety cabinet, Fume hoods
Storage methods	The wastewater must be stored in closed jerry-cans at 4°C.
Procedures in case of spill or accident	In case of WW spill, cover with adsorbent sand and dispose properly. Rinse with tap water and soap. Each waste must be disposed in the right way according to the European Waste Catalogue and local specific procedures for waste disposal.
Working environment decontamination	In case of spills of effluents of municipal WW secondary treatment, disinfection of the contaminated spot should be performed due to possible contamination with pathogenic bacteria. No special needs for decontamination are required after spills of FVPWW and TWW.

<table>
<tr><td>Designated areas for specific unit operations</td><td colspan="2">Handle the wastewater in ventilated rooms or fume hoods. Toxic and carcinogenic chemicals must be stored in a dedicated and ventilated storage wardrobe.</td></tr>
<tr><td>Special authorizations and / or required procedures</td><td colspan="2">At partner FHNW, the Standard Operating Procedure relative to this research activity must be examined and approved by the Prevention and Protection Service of FHNW.</td></tr>
<tr><td>Education and training of research personnel</td><td colspan="2">Both at UTM and FHNW, qualified personnel periodically provide training on the correct use of all the PPE and CPE to the research personnel.</td></tr>
<tr><td>Reference legislation</td><td colspan="2">Reference national legislation in Tunisia (applicable to partner UTM): Legislative Decree no. 96-62 of July 15th 1996 and Legislative Decree no. 96-41 of June 10th 1996.

Reference national legislation in Switzerland (applicable to partner FHNW): 818.123.1 Verordnung über mikrobiologische und serologische Laboratorien, EKAS Richtlinien</td></tr>
<tr><td colspan="3">Description of the operational phases and of the related hazards and protection measures</td></tr>
<tr><td>Operational phases</td><td>Hazards identification</td><td>Safety instructions</td></tr>
<tr><td>Treatment with laccases immobilized on silica nanoparticles or resin particles takes phase in one operational phase, regardless of the type of reactor used (Packed bed, percolating filter, or membrane bioreactor).</td><td>Risks are associated to the use of silica nanoparticles for enzyme immobilization, which may cause nanoparticle inhalation and to the potential exposure to pathogens and hazardous, endocrine disrupting micropollutants (bisphenol A) in the wastewater, when treating effluents of municipal WW secondary treatment.</td><td>The operator must work in fume hood and wear the proper PPE when handling nanoparticles.

The operator must avoid any contact with influent wastewater by using the proper PPE.</td></tr>
</table>

Table 2.4.5 – Health & safety procedures relative to technology 5 (Catalytic disinfection beds activated by solar UV light) (Partner TUC, GR)

WW type and characteristics	Effluent of municipal WW secondary treatment. COD<125 mg/L, microbial counts <10000 per 100 mL, may include very low concentrations of pharmaceuticals and personal care products, endocrine disruptors, heavy metals.
Equipment used	Catalytic disinfection bed: Small scale units: 30X40X5cm with working height of water 1 to 4 cm. TiO2 coated surface (30X40cm) activated by solar UV light. Inclination 1-2%. Sampling ports for measurements of the influent and effluent streams. Measurements: HOBO Temperature Light 3500 DP Logger for continuous measurement of temperature in the liquid phase and illuminance. Liquid samples will be analysed for COD, EDCs, PPCPs, heavy metals as well as microbiological parameters.
Personal protective equipment (PPE)	Labcoat at all times. Safety eyewear (PINTON CLEAR CE EN166, EN170 (2C-1.2), ANSI (Z87+)). Examination gloves (NR Latex, personal protective equipment of complex design category III, in compliance with 89/686/EEC).
Collective protective equipment (CPE)	No need of collective protection equipment
Storage methods	The secondary treated wastewater will be collected and used daily – no storage required.
Procedures in case of spill or accident	Influent & effluent spill: Minimal treatment – collection and disposal in sewer.
Working environment decontamination	Disinfection of floor / labspace with chlorine solution.
Designated areas for specific unit operations	No need to designate a specific area.
Special authorizations and / or required procedures	Operating procedures relative to this research activity must be conforming with the TU-Crete guidelines for Health and Safety.
Education and training of research personnel	All laboratory research personnel must attend a specific course on biological and chemical risks, where qualified personnel (two professors from Environmental Engineering) explain and illustrate the correct use of all PPE and CPE.

Reference legislation	Given the complexity of the legislative framework, in Greece, Law 3850 of 2 June 2010 has collated all OSH matters in a comprehensive "Code of Laws for the Health and Safety of Employees" available at: http://www.elinyae.gr/el/item_details.jsp?cat_id=708&item_id=8438	
Description of the operational phases and of the related hazards and protection measures		
Operational phases	***Hazards identification***	***Safety instructions***
1. Disinfection Step. In this step the water is passing slowly over the TiO2 surface and with exposure to UV light it gets disinfected.	No relevant hazards.	None
2. Storage step. In this step, when no sunlight is available (during the night time) the effluents are stored in a container and pumped back through the disinfection unit during the daylight period and when the flow through the system is low (e.g., 11:00 to 14:00).	- The main risk in this step is amount of effluents collected during the non-daylight hours, if it is higher than the capacity of the storage vessel. - If the bypass system does not work, we may have overflowing of the vessel and a spill on the ground.	- In this case, the additional wastewater is allowed to bypass the system and go directly to final discharge. - In this case, the spilled effluents should be sent to direct discharge and the ground disinfected with a mild solution of chlorine.

Table 2.4.6 – Health & safety procedures relative to technology 6 (Flotation/flocculation integrated process) (Partner FHNW, CH)

WW type and characteristics	Fruit/vegetable packaging WW. Contains dust particles and fungicides (imazalil, thiabendazol)
Equipment used	Wastewater treatment: Dissolved air flotation equipment. Analytical equipment: automated solid phase extraction system, GC-MS, Microplate Reader, LC-MS.
Personal protective equipment (PPE)	Labcoat, safety eyewear, examination gloves
Collective protective equipment (CPE)	Biological Safety cabinet, Fume hoods
Storage methods	The wastewater must be stored in closed jerry-cans at 4°C.
Procedures in case of spill or accident	In case of WW spill, cover with adsorbent sand and dispose properly. Each waste must be disposed in the right way according to the European Waste Catalogue and the specific procedures of FHNW.
Working environment decontamination	In order to decontaminate a surface: wash/rinse with water. For decontamination in case of wastewater spill, an additional disinfection step should be performed.
Designated areas for specific unit operations	Handle the wastewater in ventilated rooms or fume hoods.

Special authorizations and / or required procedures	The Standard Operating Procedure relative to this research activity must be examined and approved by the Prevention and Protection Service of FHNW.	
Education and training of research personnel	Qualified personnel will periodically educate and explain the correct use of all the PPE and CPE.	
Reference legislation	Relevant national legislation in Switzerland: 818.123.1 Verordnung über mikrobiologische und serologische Laboratorien, EKAS Richtlinien	
Description of the operational phases and of the related hazards and protection measures		
Operational phases	***Hazards identification***	***Safety instructions***
The Flotation / flocculation integrated process takes place in a single phase.	No relevant hazards	None.

Table 2.4.7 – Health & safety procedures relative to technology 7 (Membrane filtration + phenolic compounds adsorption with selective resins + anaerobic digestion in biofilm reactor) (Partner UNIBO, IT)

WW type and characteristics	Olive mill WW with 2 g/L total phenolic compounds; 10 g/L total carbohydrates; <0.1 g/L total lipids, 3 g/L total proteins.
Equipment used	Microfiltration and adsorption/desorption process: paper filter (25 μm) and filter holder (diameter 12.5 cm); 4 PVC columns (lenght 0.5 m, inner diameter 0.024 m); quartz sand; resin XAD16; peristaltic pump (1:200 rpm); Teflon pipes; steel fittings; autosampling system composed by 15 electrovalves with timers; low pressure rotatory evaporator; vacuum pump; liquid chromatography (HPLC) for the analysis of polyphenols and volatile fatty acids. Anaerobic digestion process: CSTR inox AISI 316 L steel reactor of 30 L volume with automatic pH correction system (temperature resistent pH-probe, membrane pump, automatic control unit); thermostatic bath with a thermocouple for temperature control; two inox impellers; N_2 bag (connected to the headspace of the reactor) to avoid depression; volumetric gas counter; hydraulic guard at the end of the methane pipeline to avoid overpressure; silicone and Teflon tubes; gascromatograph for the analysis for biogas composition; HPLC for the analysis of volatile fatty acids.
Personal protective equipment (PPE)	Labcoat. Safety eyewear (PINTON CLEAR CE EN166, EN170 (2C-1.2), ANSI (Z87+)). Examination gloves (NR Latex, personal protective equipment of complex design category III, in compliance with 89/686/EEC, type tested to EN 420:20003+A1:2009, EN 374-2:2003, EN 374-3:2003 & EN 388-:2003, CE 0086; Class 1 Medical Device, in compliance with 93/42/EEC, type tested to EN455-1:2000, EN 455-3:2009, EN 455-3:2006, EN 455-4-2009 and ASTM D3578, CE).

Collective protective equipment (CPE)	Chemical hood for the dispersion of the produced biogas into the atmosphere. Air extractor active 24/24 for the dispersion of the produced biogas into the atmosphere, in case of breakdown of the chemical hood. The anaerobic digester is equipped with a pressure control system to avoid any overpressure or depression event.
Storage methods	The wastewater must be stored in closed jerry-cans at 4°C to reduce bacterial growth and fermentation processes.
Procedures in case of spill or accident	Olive mill WW spill: cover with adsorbent sand and dispose properly; the studied WW does not have any specific toxicity. Ethanol spill: cover with adsorbent sand and dispose properly; open all windows and doors to reduce ethanol concentration in the atmosphere. Digestate spill: cover with adsorbent sand and dispose properly; the digestate must be sterilized, as it could contain pathogens.
Working environment decontamination	No special needs for decontamination are required after spills of OMWW or ethanol: after collecting the spilled liquid as described above, wash/rinse the surface with water. For decontamination in case of digestate spill, an additional disinfection step should be performed. Each waste must be disposed in the right way according to the European Waste Catalogue and the specific procedures of the University of Bologna.
Designated areas for specific unit operations	Ethanol must be stored in a dedicated and ventilated storage wardrobe. Anaerobic digestion must be conducted in a large room (> 50 m^3) to eliminate any risk of reaching explosive methane concentrations in the atmosphere in case of failure of both methane dispersion equipments.
Special authorizations and / or required procedures	The Standard Operating Procedure relative to this research activity must be examined and approved by the Prevention and Protection Service of the University of Bologna.
Education and training of research personnel	The research personnel must attend two specific courses on biological and chemical risk, where a qualified personnel explains and illustrates them the correct use of all the PPE and CPE. At the end of the course the research personnel has to fill in a questionnaire in order to verify their learning.
Reference legislation	The relevant national legislation in Italy is: the Legislative Decree no. 81 of April 9th 2008 and the Legislative Decree no. 106 of August 3rd 2009, which coordinates and restructures in a single regulation text all relevant legal provisions.

Description of the operational phases and of the related hazards and protection measures

Operational phases	***Hazards identification***	***Safety instructions***

1. Adsorption process. OMWW is pumped from the OMWW container to the filtration apparatus by means of a peristaltic pump at a flow rate of 10-30 mL/min. From the outlet of the filtration apparatus, OMWW enters the adsorption column, from where it exits after a 20-60 minutes retention time. The exiting WW is collected in a plastic container closed with plastic film. The operator has the following tasks: - take WW samples at different sections of the equipment, by means of a 2 mL plastic syringe, for the analysis of polyphenols, VFAs and COD; - perform periodic measurements of flow-rate and pressure drop.	The only risk in this phase is the attainment of an excessively high pressure in the column inlet (> 3 bar), which could lead to failure of the column. However, the choice to use PVC columns instead of glass ones minimizes the possible consequences of column failure.	Pressure must be supervised during the entire process, either automatically or by the operator; in case of attainment of a total pressure of 2 bar, the inlet flow rate must be decreased. The operator must wear lab coat, gloves and safety eyewear.
2. Desorption process. Acidified ethanol (0.5% HCl 1 N) is pumped to the column inlet by means of a peristaltic pump at a flow rate of 10-50 mL/min. The exiting solvent is collected in a plastic container closed with plastic film. The operator has the following tasks: - take WW samples at different sections of the equipment, by means of a 2 mL plastic syringe, for the analysis of polyphenols, VFAs and COD; - perform periodic measurements of flow-rate and pressure.	The main risk in this phase is the attainment of an excessively high pressure in the column inlet (> 3 bar), which could lead to failure of the column. However, the choice to use PVC columns instead of glass ones minimizes the possible consequences of column failure. A further risk is connected to ethanol flammability.	Pressure must be supervised during the entire process, either automatically or by the operator; in case of attainment of a total pressure of 2 bar, the inlet flow rate must be decreased. No flames must be used in the vicinity of the equipment. The operator must wear lab coat, gloves and safety eyewear.
3. Solvent regeneration and phenolic mixture recovery. The polyphenol-rich solvent collected at the exit of the column is introduced into a low-pressure rotatory evaporator connected to a vacuum pump. Ethanol slowly evaporates, leaving the desorbed material in the reboiler of the evaporator. Ethanol is then re-condensed and collected in order to be re-utilized in subsequent cycles.	The only risk in this phase is connected to ethanol flammability.	No flames must be used in the vicinity of the equipment. The operator must wear lab coat, gloves and safety eyewear.

4. Anaerobic digestion process. The dephenolized OMWW is fed to the 30 L anaerobic digester in a semi-continuous way. Solid organic residues could be added as co-digestion matrices. The digester is operated at 38-40 °C, at atmospheric pressure, and agitated at 500-600 rpm. An amount of digestate equal to the amount of substrates added daily is removed from the bottom of the equipment in order to maintain a constant reaction volume. The digestate is centrifuged, so as to separate the liquid fraction (to be tested for its potential re-utilization for irrigation) and the solid fraction (to be tested for its potential re-utilization as a fertilizer). The produced biogas is sent to a chemical hood by means of a Teflon pipeline. The operator has the following tasks: - take liquid digestate samples by means of a 2 mL plastic syringe, for the analysis of VFAs and COD; - take biogas samples by means of a 1 mL glass syringe, for the analysis of biogas composition.	This phase is characterized by the following hazards: - biological risk, as the digestate can contain pathogens; - explosions due to methane flammability; - H_2S inhaling.	The operator must: - avoid any contact with biological digestate by PPE use (particular attention during the daily feeding practise); - check that the line connected to the N_2 bag works correctly, to avoid oxygen entrance in case of reactor depression and avoid any possible primer near the reactor; - make sure that the Teflon pipeline that connects the digester to the chemical hood is not damaged, and that the extractor fan works properly.

Table 2.4.8 – Health & safety procedures relative to technology 8 (Aerobic sequenced batch reactor with lime addition) (Partner UTM, TN)

WW type and characteristics	Olive mill WW with total phenolic compounds > 2 g/L; total carbohydrates 10 g/L; total lipids <0.1 g/L, Tannin-like compounds, total proteins 3 g/L, limited growth of bacteria and yeasts.
Equipment used	Bioreactor components: Timer, air pump, humidifier, reactor, magnetic stirrer. Molecular analysis of microbial population: Gel electrophoresis unit "D-code electrophoresis system, Biorad", thermocyclor. Chemical analysis: BOD analysis system, COD analysis system, speedvac, spectrophotometer.
Personal protective equipment (PPE)	Lab coat, Safety eyewear, Latex Gloves, paper face mask
Collective protective equipment (CPE)	Chemical hood, Air extractor active 24/24
Storage methods	The wastewater must be stored in closed jerry-cans at 4°C to reduce bacterial growth and fermentation processes.

Procedures in case of spill or accident	OWW spill: cover with adsorbent sand and dispose properly; the studied WW does not have any specific toxicity.
Working environment decontamination	No special needs for decontamination are required after spills of OMWW, except for rinsing with water and soap.
Special authorizations and / or required procedures	There is no need to separate specific unit operations
Education and training of research personnel	Periodic internal training is organized by Senior researchers
Reference legislation	Relevant national legislation in Tunisia: Legislative Decree no. 96-62 of July 15th 1996; Legislative Decree no. 96-41 of June 10th 1996.

Description of the operational phases and of the related hazards and protection measures		
Operational phases	***Hazards identification***	***Safety instructions***
The treatment process consists in sequential stages consisting in filling with WW (no aeration, mixing provided by mechanical means), reaction (aeration), settling of sludge (no aeration and no mixing) and decanting of treated wastewater (no aeration and no mixing).	Aerosols can be generated during the aerated stage.	The lab scale reactor is placed under chemical hood and the air outlet is disinfected in a chlorine-based sterilizing solution.

Table 2.4.9 – Health & safety procedures relative to technology 9 (Granulated sludge bioreactor) (Partner UTM, TN)

WW type and characteristics	Textile WW with grease and oils <10 mg/L, phosphorus 0.5 mg/L, anionic detergents 1.92 mg/L, Chlorides 5380 mg/L, Sulfate 1180 mg/L, sodium 3280 mg/L, Heavy metals (Al, B, Cd, Co, Cr, Cu, Hg, Ni, Fe, Zn)
Equipment used	Bioreactor Molecular analysis of microbial population: Gel electrophoresis unit "D-code electrophoresis system, Biorad", thermocyclor. Chemical analysis: BOD analysis system, COD analysis system, spectrophotometer.
Personal protective equipment (PPE)	Lab coat, Safety eyewear, Latex Gloves, paper face mask
Collective protective equipment (CPE)	Chemical hood, Air extractor active 24/24
Storage methods	The wastewater must be stored in closed jerry-cans at 4°C.
Procedures in case of spill or accident	TWW spill: cover with adsorbent sand and dispose properly; the studied WW does not have any specific toxicity.
Working environment decontamination	No special needs for decontamination are required after spills of TWW, except for rinsing with water and soap.
Designated areas for specific unit operations	There is no need to separate specific unit operations
Special authorizations and / or required procedures	No special authorization is required.

Education and training of research personnel	Periodically internal trainings are organized by Senior researchers.		
Reference legislation	Relevant national legislation in Tunisia: Legislative Decree no. 96-62 of July 15th 1996; Legislative Decree no. 96-41 of June 10th 1996		
Description of the operational phases and of the related hazards and protection measures			
Operational phases		***Hazards identification***	***Safety instructions***
The treatment process consists in a single phase.		Aerosols can be generated by aeration.	The lab scale reactor is placed under chemical hood and the air outlet is disinfected in a chlorine-based sterilizing solution.

Table 2.4.10 – Health & safety procedures relative to technology 10 (Moving Bed Biological Reactor) (Partner TUC, GR; Partner FHNW, CH)

WW type and characteristics	Fruit / vegetable packaging WW containing dust particles and fungicides (imazalil, thiabendazol). Textile WW. Initially, synthetic textile WW will be used containing glucose (1000 mg/L COD) as an organic carbon source, the required inorganic nutrients for microbial growth and the model dye compounds that are used by the GITEX company in Tunisia. Following the runs with synthetic WW, real effluents will be employed that have the following characteristics: COD around 300 mg/L, Total Suspended Solids around 200 mg/L and BOD from 100 to 500 mg/L.
Equipment used	A Moving Bed Biofilm Reactor (MBBR) made of stainless steel used for anaerobic-aerobic treatment; biofilm carriers made of polyethylene with a density of 0.98 g/cm3, a height of 10 mm and a diameter of 10 mm with cross inside, which are kept within the reactor by an outlet sieve; a diffuser for providing compressed air to the wastewater of the aerobic MBBR; mixers for the transfer of carriers inside the anaerobic MBBR; a calibrated rotameter for the monitoring of the air flow-rate; a water heater; level sensors for the controlling of the feeding
Personal protective equipment (PPE)	Labcoat. Safety eyewear (PINTON CLEAR CE EN166, EN170 (2C-1.2), ANSI (Z87+)). Examination gloves (NR Latex, personal protective equipment of complex design category III, in compliance with 89/686/EEC, type tested to EN 420:20003+A1:2009, EN 374-2:2003, EN 374-3:2003 & EN 388-:2003, CE 0086; Class 1 Medical Device, in compliance with 93/42/EEC, type tested to EN455-1:2000, EN 455-3:2009, EN 455-3:2006, EN 455-4-2009 and ASTM D3578, CE).

Collective protective equipment (CPE)	Chemical hood for the dispersion of the produced biogas into the atmosphere. Air extractor active 24/24 for the dispersion of the produced biogas into the atmosphere, in case of breakdown of the chemical hood.
Storage methods	If needed, the wastewater must be stored in closed jerry-cans at 4°C to eliminate bacterial growth and fermentation processes.
Procedures in case of spill or accident	Fruit / Vegetable packaging WW and Textile WW spill: cover with adsorbent sand and dispose properly; the studied WWs do not have any specific toxicity. Digestate spill: cover with adsorbent sand and dispose properly; the digestate must be sterilized, as it could contain pathogens.
Working environment decontamination	No special needs for decontamination are required after spills of the studied WWs: after collecting the spilled liquid as described above, wash/rinse the surface with water. For decontamination in case of digestate spill, an additional disinfection step should be performed. Each waste must be disposed in the right way according to the European Waste Catalogue and the specific procedures of TUC and FHNW.
Designated areas for specific unit operations	Anaerobic digestion must be conducted in a large room (> 50 m^3) to eliminate any risk of reaching explosive methane concentrations in the atmosphere in case of failure of methane dispersion equipment.
Special authorizations and / or required procedures	The Standard Operating Procedure relative to this research activity must be examined and approved by the Prevention and Protection Service of TUC and FHNW.
Education and training of research personnel	At TUC, the research personnel have attended specific courses on techniques of chemical and biochemical processes. At FHNW, qualified personnel will periodically educate and explain the correct use of all the PPE and CPE.
Reference legislation	Relevant national legislation in Greece: Law 3850 of 2 June 2010, the Draft: Community strategy 2007-2012 on health and safety at work. Relevant national legislation in Switzerland: 818.123.1 Verordnung über mikrobiologische und serologische Laboratorien, EKAS Richtlinien.

Description of the operational phases and of the related hazards and protection measures		
Operational phases	***Hazards identification***	***Safety instructions***

1. Anaerobic process. The sludge samples are collected from the wastewater treatment plant of a textile dyeing factory. After inoculation, raw TWW is pumped by a peristaltic pump into the anaerobic MBBR, where the temperature is kept at 26°C by a water heater. The anaerobic MBBR, acting as the anaerobic reactor, is sealed with a cap when operated. Anaerobic unit plays a main role in COD removal. The biomass is kept in movement by small fluidizing carriers with high surface area which help the circulation of water inside the reactor. Trace elements are simultaneously added to the influent to facilitate the growth of microorganisms. Sparging of nitrogen gas in bioreactor allows for operation under anoxic conditions. The N2 gas is monitored using a mass spectrometer; COD, nitrite, sulfate, pH, conductivity, and colour are regularly measured in the influent. The digestate is centrifuged, so as to separate the liquid fraction and the solid fraction. The produced biogas is sent to a chemical hood by means of a Teflon pipeline. The operator has the following tasks: - take liquid digestate samples by means of a 2 mL plastic syringe, for the analysis of VFAs and COD; - take biogas samples by means of a 1 mL glass syringe, for the analysis of biogas composition.	This phase is characterized by the following hazards: - biological risk, as the digestate can contain pathogens; - explosions due to methane flammability; - H_2S inhaling.	The operator must: - avoid any contact with biological digestate by PPE use (particular attention during the daily feeding practise); - check that the line connected to the N_2 bag works correctly, to avoid oxygen entrance in case of reactor depression and avoid any possible primer near the reactor; - make sure that the Teflon pipeline that connects the digester to the chemical hood is not damaged, and that the extractor fan works properly.

2. Aerobic process. The supernatant flows into the aerobic MBBR for further treatment, and then the effluent is overflowed out of the reactor to the receiving storage tank. The biomass inside the reactor is growing on the biofilm carriers that move freely in the water volume of the reactor, kept within the reactor volume by a sieve arrangement at the reactor outlet. To provide oxygen to the water and fluidize the biofilm carriers, an aerator is fixed at the centre bottom of the reactor. The air flow-rate is monitored by a calibrated rotameter. The dissolved oxygen concentration (DO) is measured using an oxygen electrode that is often calibrated. During continuous operation of the MBBR, effluent samples are immediately filtered through 0.45 μm filter paper and then analyzed. Soluble COD and $NH4^{+}$-N are also measured according to the Standard Method for Water and Wastewater Examination. BOD_5 is measured via the oxygen consumption of bacteria breaking down organics in the sample over a 5-day period under standardized conditions.	None	None

Table 2.4.11 – Health & safety procedures relative to technology 11 (Dyes adsorption with innovative resins)

WW type and characteristics	Textile WW: COD 300-700 mg/L, BOD 69-125 mg/L, TSS 104-357 mg/L, Chlorides 5380 mg/L, Sulphate 1180 mg/L, Sodium 3280 mg/L, Al<9.0 mg/L, Ca<0.01 mg/L, Co<0.01 mg/L, Cr<0.01 mg/L, Cu<0.2 mg/L, Fe<0.5 mg/L, Mn<1.0 mg/L, Hg<0.005 mg/L, Ni<0.01 mg/L, Pb<0.01 mg/L, Zn<0.1 mg/L, As, Se: not detectable. Synthetic Textile WW: pH 7-8, COD 300-700 mg/L, BOD 60-130 mg/L, color 200, residual dye (different kind of dyes will be used separately such as direct dyes, reactive dyes and so on in which the concentration will be depend on COD and color content), Al<9.0 mg/L, Mn<1 mg/L, Fe<0.5 mg/L, Cu, Zn, Cr, As, Cd, Co, Ni, Pb, <0.2 mg/L

Equipment used	anion exchange process: anion exchange resin -Designed anion exchange reactor -muffle furnace, constant temperature incubator -TOC analyser, UV-vis spectrophotometer, Ion chromatography, ICP-OES/MS, HPLC for wastewater characteristics analysis
Personal protective equipment (PPE)	PPE includes protective glasses, gloves and gas mask.
Collective protective equipment (CPE)	Chemical Fume Hoods that can capture and remove contaminants to prevent overexposure to personnel within the lab.
Storage methods	The wastewater must be stored in closed jerry-cans at 4°C.
Procedures in case of spill or accident	TWW spill: cover with adsorbent sand/paper and dispose properly Desorption liquid waste spill: cover with adsorbent sand/paper and dispose properly
Working environment decontamination	No special needs for decontamination are required after spills of TWW: after collecting the spilled liquid as described above, wash/rinse the surface with water. Each waste must be disposed in the right way according to National Hazardous Waste List of China and the specific procedures of Nanjing University.
Designated areas for specific unit operations	No specific area needed.
Special authorizations and / or required procedures	The Standard Operating Procedure relative to this research activity must be examined and approved by the Prevention and Protection Service of the Nanjing University.
Education and training of research personnel	The research personnel must attend two specific courses on biological and chemical risk, where a qualified personnel explains and illustrates them the correct lab work. At the end of the course the research personnel has to fill in a questionnaire in order to verify their learning.

Reference legislation	Safety management regulations for dangerous chemicals, Http://www.gov.cn/gongbao/content/2011/content_1825120.htm; Precursor chemicals management regulations, Http://www.gov.cn/gongbao/content/2005/content_91170.htm; Regulations of the people's Republic of China on the administration of chemicals, Http://www.gov.cn/gongbao/content/2011/content_1860782.htm; Identification and classification management approach for the physical danger of chemicals, Http://www.gov.cn/gongbao/content/2013/content_2473885.htm; Registration management approach for dangerous chemicals, Http://www.gov.cn/flfg/2012-07/11/content_2180852.htm; Interim provisions on the supervision and administration of hazardous chemicals, Http://www.gov.cn/flfg/2011-09/13/content_1945888.htm; Environmental management measures for new chemical substances, Http://www.gov.cn/gongbao/content/2010/content_1671246.htm; Classification of radioactive sources, Http://www.gov.cn/gzdt/2005-12/28/content_140242.htm; Occupational health management measures for radiation workers, Http://www.gov.cn/gzdt/2007-06/21/content_655923.htm; Biological safety management regulations in pathogenic microorganisms laboratory, Http://www.gov.cn/gongbao/content/2005/content_63265.htm; Biological safety environment management approach in pathogenic microorganisms laboratory, Http://www.gov.cn/gongbao/content/2007/content_588180.htm; Safety supervision regulations of gas cylinder, Http://www.gov.cn/gongbao/content/2003/content_62428.htm

Description of the operational phases and of the related hazards and protection measures

Operational phases	***Hazards identification***	***Safety instructions***
1. Adsorption process. TWW is pumped into the anion exchange reactor, which has a stirring bar to mix anion exchange resin with wastewater. The retention time is in the range of 20~30 min. - take TWW samples from inlet and outlet for analysis.	The only risk in this phase is leakage of the TWW from the reactor and the pump lines.	The reactor will be checked at regular intervals. The pump lines will be replaced new ones at regular intervals.

2. Desorption process. -Pump a determined portion of mixture of anion exchange resin and textile wastewater into the regeneration tank. Then textile wastewater is pumped back to the treatment container and anion exchange resin is retained in the regeneration tank by a filter. -Pump 10% NaCl solution into the renegeration tank and start stirring for a determined period. -Draw the liquid waste to the storage tank. -Pump some textile wastewater into the regeneration tank -Stir and pump the mixture of resin and wastewater back to the main treatment container.	The main risk in this phase is leakage of the TWW and regeneration solution. In addition, the pump might be clogged with anion exchange resin.	The container will be checked at regular intervals. In order to avoid the clog of the pump, diaphragm pump is preferred.

3 Irrigation technologies (WP3)

The MADFORWATER project includes in WP3 the development and adaptation to the local conditions of the target MAC countries of the following 6 irrigation technologies:

A. Increased crop resistance to water scarcity and salinity through the addition of plant growth promotion bacteria (Task 3.1.1)
B. Large spectrum soil moisture sensor calibrated for saline water (Task 3.1.2)
C. Low-pressure micro-sprinkler adapted to dry climates and to treated WW (Task 3.2.1)
D. Low-pressure calibrated nozzle adapted to dry climates and to treated WW (Task 3.2.2)
E. Re-engineered surface irrigation systems based on calibrated gated pipes (Task 3.2.3)
F. Open source software tool to determine the optimal irrigation amount and schedule (Task 3.1.3).

The 6th technology (F) will not be covered in this Deliverable. Indeed, as it is a modelling technology, it does not have a significant environmental impact, it does not require specific health & safety measures and it cannot be evaluated by means of performance indicators.

3.1 Homogenization of the performance indicators

At the end of the 2nd year of research, each technology will be evaluated by means of a series of performance indicators, in order to rate the Madforwater irrigation technologies in comparison with corresponding benchmark or state-of-the-art technologies. As the above-listed 5 irrigation technologies are intrinsically characterized by significant differences between each other, it is not possible to identify a single set of performance indicators common to all the 5 technologies. The selected indicators are therefore listed in Table 3.1, for each of the 5 technologies. The indicators common to at least 2 technologies are reported in blue.

Table 3.1 – Performance indicators of the irrigation technologies. The indicators common to at least 2 technologies are reported in blue.

Technology ID and description	Performance indicators
A - Increased crop resistance to water scarcity and salinity through the addition of plant growth promotion bacteria (Task 3.1.1)	• Plant growth: plant height after a given time • Plant growth: plant weight (dry matter) after a given time • Plant growth: root weight (dry matter) after a given time • Crop productivity: $kg_{product}$ / year / $m^3_{water\ supplied}$ • Proline content • Leaf chlorophyll content
B - Large spectrum soil moisture sensor calibrated for saline water (Task 3.1.2)	• Range of water salinity accepted by the tensiometer • Stability of measurement, evaluated for different levels of treated WW salinity • Effectiveness of the signal correction algorithm • Resistance to aging, when used with treated WW
C - Low-pressure micro-sprinkler adapted to dry climates and to treated WW (Task 3.2.1)	• Energy consumption referred to a standardized condition in terms of water flow rate, type and length of pipe, land topography and plot surface area (kWh/m^3) • Water saving, in relation to the benchmark technology mainly applied in the target MACs for the context and type of crop targeted by this irrigation technology (%) • Resistance to clogging, both in the lab and under field conditions, when tested with various types of treated WW • Range of operating pressure required to respect the intended range of droplet diameter • Fraction of the discharged water volume constituted by small droplets (< 500 µm), that could generate aerosols • Uniformity of droplet diameter distribution, when tested with various types of treated WW
D - Low-pressure calibrated nozzle adapted to dry climates and to treated WW (Task 3.2.2)	• Energy consumption referred to a standardized condition in terms of water flow rate, type and length of pipe, land topography and plot surface area (kWh/m^3) • Water saving, in relation to the benchmark technology mainly applied in the target MACs for the context and type of crop targeted by this irrigation technology (%) • Resistance to clogging, both in the lab and under field conditions, when tested with various types of treated WW • Anti-leakage property of the emitter at low suction pressure (2 m) • Range of discharge rate, with specific focus on the possibility to work in a low range (60-80 L/h) • Stability of the above-listed parameters during a long-term operation in field conditions with minimum maintenance (once a month) compared to standard systems (once a week)
E - Re-engineered surface irrigation systems based on calibrated gated pipes (Task 3.2.3)	• Energy consumption referred to a standardized condition in terms of water flow rate, type and length of pipe, land topography and plot surface area (kWh/m^3) • Water saving, in relation to the benchmark technology mainly applied in the target MACs for the context and type of crop targeted by this irrigation technology (%)

	• Conveyance efficiency, defined as the water flow rate applied divided by the volume of water pumped into the system (%) • Coefficient of uniformity, expressing the degree of variation of the flow rate between the different calibrated nozzles connected to the same gated pipe (%)

3.2 Environmental impact of the irrigation technologies

The WP3 technological partners were asked to evaluate the environmental impact of the irrigation technologies by referring to the following categories:

- Energy consumption
- Chemicals consumption
- Solid waste disposal
- Atmospheric emissions
- Liquid emissions

On the basis of the answers provided by the WP3 partners, the environmental impact of the Madforwater irrigation technologies is expected to be limited to the first two categories, whereas the production of solid, liquid or gaseous wastes was rated not relevant by all partners. On the other hand, the small environmental impact of the irrigation technologies is expected to be largely offset by the benefits related (i) to the large reduction in water consumption in comparison to the benchmark technologies traditionally used in the target MAC countries, and (ii) to the possibility to effectively use the Madforwater technologies with treated WW, thus reducing groundwater catchment. A short description of the expected environmental impacts of the irrigation technologies is reported in Table 3.2 (limitedly to the two categories for which a not negligible impact is expected). A more comprehensive evaluation of the environmental impacts and benefits of the Madforwater irrigation technologies will be performed in the framework of the Life Cycle Analysis object of Task 3.4.

Table 3.2 – Expected environmental impact of the Madforwater irrigation technologies

Technology ID and description	Expected environmental impact
A - Increased crop resistance to water scarcity and salinity through the addition of plant growth promotion bacteria (Task 3.1.1)	• **Energy consumption** is foreseen for the production of the bacterial inoculum to be supplied through the irrigation system. The actual energy consumption depends on the bacterium/bacteria that will be selected on the basis of the in vitro and greenhouse tests. To minimize energy consumption, fast-growing bacteria will be preferentially chosen. • **Chemicals** will be required to produce the growth medium utilized to grow the bacterial cells. To minimize the cost of the technology and the consumption of chemicals, bacteria able to grow on cheap substrates, including organic wastes, will be preferentially chosen.
B - Large spectrum soil moisture sensor calibrated for saline water (Task 3.1.2)	This technology will be characterized by a negligible environmental impact for all the 5 examined categories.
C - Low-pressure micro-sprinkler adapted to dry climates and to treated WW (Task 3.2.1) D - Low-pressure calibrated nozzle adapted to dry climates and to treated WW (Task 3.2.2)	• **Energy consumption**: on the basis of previous experience, the operation of both the emitter and the calibrated nozzle will be characterized by a very low energy consumption (<0.2 kWh/m^3 of WW distributed). • **Chemicals**: the only chemical that will be used is chlorine, required to remove the biofilm formed in pipes and emitters between consecutive tests. About 10 L of chlorine will be used over the whole experimental period, for each

	technology.
E - Re-engineered surface irrigation systems based on calibrated gated pipes (Task 3.2.3)	• **Energy consumption:** by optimizing pipe size diameters, a balance between pipe costs and energy consumption will be obtained without compromising the performance of the gated pipes.

3.3 Health and safety procedures relative to the irrigation technologies

In order to evaluate the adequacy of the health and safety procedures that WP3 partners plan to adopt for the irrigation technologies, each partner was asked to fill in a standardized questionnaire relative to the equipment utilized, the planned protective equipment and storage methods, the planned measures in case of spill or accident, the procedures for the education and training of researchers and the reference legislation to be respected. The questionnaires relative to the 5 irrigation technologies are reported in Tables 3.3.A to 3.3.E. A comprehensive examination of these Tables indicates that the WP3 technologies are not characterized by any particularly relevant health & safety issue. The only activities characterized by some biological hazard are those characterized by the use of real treated WW (technologies B, C and D). However, as illustrated in Tables 3.3.B, 3.3.C and 3.3.D, the use of treated WW will be limited to the tests operated in the field, and will be associated to the application of strict safety measures aimed at avoiding any contact of researchers with treated WW.

With regard to the reference legislation, all the research activities will be conducted in compliance with EC Regulation No. 1907/2006 (REACH), as amended by EU Regulation No. 453/2010, in addition to the reference laws valid in Italy, Tunisia and France, the 3 countries where the WP3 technologies will be developed (see detailed list in Tables 3.3.A to 3.3.E).

Table 3.3.A – Health & safety procedures relative to technology A (addition of plant growth promotion bacteria)

WW type and characteristics	Neither real nor synthetic WW will be used for this technology.
Equipment used	Bacterial cultures provided either as liquid cultures or lyophilized cells. Bacteria will be grown in glass flasks and/or in Petri dishes during the initial enrichment, isolation and characterization *in vitro*, performing operation under laminar hoods. Fermenters will be possibly used to produce higher amount of culture for selected bacteria. During greenhouse test, liquid culture of the bacteria at suitable concentration will be provided to the plants using plastic tubes and pipettes.
Personal protective equipment (PPE)	Wear protective gloves/protective clothing. Safety glasses, to prevent eye exposure, are recommended to be used when handling the product. Avoid prolonged or frequent repeated skin contact,

<table>
<tr><td></td><td colspan="2">especially with broken skin. Chemical protective gloves to a standard EN374 should be used. Avoid formation of dust, splashing and formation of aerosols.</td></tr>
<tr><td>Collective protective equipment (CPE)</td><td colspan="2">No special ventilation is necessary. However if operating conditions create high airborne concentrations of this material, based upon available information and in the absence of occupational exposure limits the use of a vapour mask to a minimum standard of EN405 with filters compliant with NF EN143 norm (type FFP1) is recommended.</td></tr>
<tr><td>Storage methods</td><td colspan="2">Temperatures consistently exceeding 30°C and long-term freezing conditions must be avoided. The substance should be handled under conditions of good industrial hygiene and in conformity with any local regulations in order to avoid unnecessary exposure. The product is formulated using a range of microorganisms specially selected from the natural environment and that are known to be non-pathogenic to humans, animals or plants. It is advised to cover open wounds when in use.</td></tr>
<tr><td>Procedures in case of spill or accident</td><td colspan="2">The preparation is expected to biodegrade rapidly. Therefore, it is not anticipated to bioaccumulate. The preparation is not anticipated to pose any environmental hazard. There is no ozone depletion, photochemical ozone creation or global warming potential. No data on toxicity specifically to soil organisms, plants and terrestrial animals are available. Electric interrupter and fire extinguishers will be used if necessary.</td></tr>
<tr><td>Working environment decontamination</td><td colspan="2">Not relevant.</td></tr>
<tr><td>Special authorizations and / or required procedures</td><td colspan="2">The bacteria that will be selected are neither class 3-4 biological agents nor carcinogens. Only bacteria Generally Regarded As Safe (GRAS) will be selected.</td></tr>
<tr><td>Education and training of research personnel</td><td colspan="2">All new workers, including students, are trained by the Health and Safety delegate of the Institute. A protocol for the product use will be provided according to the final bacterial formulate. Bacterial cultures will be provided to the plant during irrigation procedure.</td></tr>
<tr><td>Reference legislation</td><td colspan="2">The relevant national legislation on safety in workplaces in Italy and Tunisia will apply. For Italy: Legislative Decree no. 626 of September 19th 1994, Legislative Decree no. 81 of April 9th 2008 and Legislative Decree no. 106 of August 3rd 2009, which coordinates and restructures in a single regulation text all relevant legal provisions. For Tunisia: Law N. 91-63 of 29 July 1991; Decree N. 2009-644 of 2 March 2009.</td></tr>
<tr><td colspan="3">Description of the operational phases and of the related hazards and protection measures</td></tr>
<tr><td>Operational phases</td><td>Hazards identification</td><td>Safety instructions</td></tr>
<tr><td>1) Dilution/ resuspension of the bacterial cells</td><td>Contact with bacteria</td><td>Precautionary statements: wear protective gloves/protective clothing. Safety glasses, to prevent eye exposure, are recommended to be used when handling the product. Avoid prolonged or frequent repeated skin contact, especially with broken skin. Chemical protective gloves to a standard EN374 should be provided. Avoid formation of dust, splashing and formation of aerosols.</td></tr>
<tr><td>2) Dispensing to the plants</td><td>Contact with bacteria</td><td>Protective gloves, protective clothing.</td></tr>
</table>

Table 3.3.B – Health & safety procedures relative to technology B (Large spectrum soil moisture sensor)

WW type and characteristics	All the lab-scale tests will be performed with either synthetic WW (salt spiked water) or disinfected WW. Real treated WW will be used only in the field tests. Any direct contact of researchers with treated WW will be avoided.
Equipment used	Soil samples in which the sensor will be buried and dripping system to apply the reproduced WW, supplied by a tank and pump. In the agricultural field, tests will be conducted in soil containers to control all WW inputs.
Personal protective equipment (PPE)	Experiments will be conducted initially in an isolated room, then in agricultural plots with domestic WW. Any contact with the WW will be prevented according to Irstea's health & safety rules.
Collective protective equipment (CPE)	Information panels.
Storage methods	WW will be manufactured onsite or taken from the outlet of the local WW treatment plant.
Procedures in case of spill or accident	Gates and security valves. Electric interrupter and fire extinguishers.
Working environment decontamination	All the lab-scale tests will be performed with either synthetic WW or disinfected WW, therefore no specific lab decontamination measures are required.
Special authorizations and / or required procedures	Under Irstea's health and safety protections policy, no additional procedures are required, considering the nature of the effluent used.
Education and training of research personnel	All new workers, including students, are trained by the Health and Safety delegate of the Institute.
Reference legislation	The French health and safety regulation at work will apply: Decree No. 2011-774 of 28 June 2011, amending Decree No. 82-453 of 28 May 1982 on hygiene, industrial safety and medical prevention in public service; NF X35-102 for the office; NF EN 294 for safety distances.

Description of the operational phases and of the related hazards and protection measures		
Operational phases	***Hazards identification***	***Safety instructions***
1) WW preparation	Contact with bacteria, projections	Irstea's health and safety plan, including the extensive use of personal protection equipment (glasses and gloves).
2) Experiments with soil	Contact with bacteria	Irstea's health and safety plan, the WW will be used for watering a soil container that will welcome the tensiometer. Protection against contact, projections and leakage will be the object of particular attention.

Table 3.3.C – Health & safety procedures relative to technology C (Low-pressure micro-sprinkler)

WW type and characteristics	All the lab-scale tests will be performed with either synthetic WW (added with salt, mineral and organic particles) or disinfected WW. Real treated WW will be used only in the field tests. Any direct contact of researchers with treated WW will be avoided.	
Equipment used	Pipes and fittings, valves, tank and pump, prototype of mini-sprinkler.	
Personal protective equipment (PPE)	Experiments will be conducted initially in an isolated room, then in agricultural plots with domestic WW. Any contact with the WW will be prevented according to Irstea's health & safety rules.	
Collective protective equipment (CPE)	Information panels.	
Storage methods	WW will be manufactured onsite or taken from the outlet of the local WW treatment plant.	
Procedures in case of spill or accident	Gates and security valves. Electric interrupter and fire extinguishers.	
Working environment decontamination	All the lab-scale tests will be performed with either synthetic WW or disinfected WW, therefore no specific lab decontamination measures are required.	
Special authorizations and / or required procedures	Under Irstea's health and safety protections policy, no additional procedures are required, considering the nature of the effluent used.	
Education and training of research personnel	All new workers, including students, are trained by the Health and Safety delegate of the Institute.	
Reference legislation	The French health and safety regulation at work will apply: Decree No. 2011-774 of 28 June 2011, amending Decree No. 82-453 of 28 May 1982 on hygiene, industrial safety and medical prevention in public service; NF X35-102 for the office; NF EN 294 for safety distances.	
Description of the operational phases and of the related hazards and protection measures		
Operational phases	***Hazards identification***	***Safety instructions***
1) WW preparation of the WW	Contact with bacteria, projections	Irstea's health and safety plan, including the extensive use of personal protection equipment (glasses and gloves). The WW will be prepared in a separate tank that will be connected to a pump and to the pipe supporting the emitters.
2) Experiments in the lab	Contact with bacteria, projections	Irstea's health and safety plan, protection against contact, projections and leakage will be the object of particular attention. All the WW used will stay in a close loop circuit and will be recycled at the end of the experiment.
3) Experiments in the field	Contact with bacteria, projections	Irstea's health and safety plan, protection against contact, projections, runoff and leakage will be the object of particular attention. The WW used in field studies will be that which is normally disposed in the environment after treatment.

Table 3.3.D – Health & safety procedures relative to technology D (Low-pressure calibrated nozzle)

WW type and characteristics	All the lab-scale tests will be performed with either synthetic WW (added with salt, mineral and organic particles) or disinfected WW. Real treated WW will be used only in the field tests. Any direct contact of researchers with treated WW will be avoided.	
Equipment used	Pipes and fittings, valves, tank and pump, prototype of low pressure, anti-leakage emitter.	
Personal protective equipment (PPE)	Experiments will be conducted initially in an isolated room, then in agricultural plots with domestic WW. Any contact with the WW will be prevented according to Irstea's health & safety rules.	
Collective protective equipment (CPE)	Information panels.	
Storage methods	WW will be manufactured onsite or taken from the outlet of the local WW treatment plant.	
Procedures in case of spill or accident	Gates and security valves. Electric interrupter and fire extinguishers.	
Working environment decontamination	All the lab-scale tests will be performed with either synthetic WW or disinfected WW, therefore no specific lab decontamination measures are required.	
Special authorizations and / or required procedures	Under Irstea's health and safety protections policy, no additional procedures are required, considering the nature of the effluent used.	
Education and training of research personnel	All new workers, including students, are trained by the Health and Safety delegate of the Institute.	
Reference legislation	The French health and safety regulation at work will apply: Decree No. 2011-774 of 28 June 2011, amending Decree No. 82-453 of 28 May 1982 on hygiene, industrial safety and medical prevention in public service; NF X35-102 for the office; NF EN 294 for safety distances.	
Description of the operational phases and of the related hazards and protection measures		
Operational phases	***Hazards identification***	***Safety instructions***
1) WW preparation	Contact with bacteria, projections	Irstea's health and safety plan, including the extensive use of personal protection equipment (glasses and gloves). The WW will be prepared in a separate tank that will be connected to a pump and to the pipe supporting the emitters.
2) Experiments in the lab	Contact with bacteria, projections	Irstea's health and safety plan, protection against contact, projections and leakage will be the object of particular attention. All the WW used will stay in a close loop circuit and will be recycled at the end of the experiment.
3) Experiments in the field	Contact with bacteria, projections	Irstea's health and safety plan, protection against contact, projections, runoff and leakage will be the object of particular attention. The WW used in field studies will be that which is normally disposed in the environment after treatment.

Table 3.3.E – Health & safety procedures relative to technology E (Re-engineered surface irrigation systems based on calibrated gated pipes)

WW type and characteristics	The gated pipe technology is supposed to be used with water characterized by high salinity level. However during design and testing, the quality of water is not important. therefore clean fresh water will be used.	
Equipment used	Pipes and fittings, tank and pump, low pressure & anti-leakage emitter.	
Personal protective equipment (PPE)	High visibility jackets, protective clothes from wind and water, boots. Electricity socks and pumps will be placed away from the experiment to avoid any electric hazard. Fork lift will be used for heavy pipe movement	
Collective protective equipment (CPE)	Not relevant.	
Storage methods	Large water tanks already available at the IAMB site.	
Procedures in case of spill or accident	Gates and security valves. Electric interrupter and fire extinguishers.	
Working environment decontamination	Only clean water will be used.	
Special authorizations and / or required procedures	As the experimental site is property of IAMB, no authorisation is needed.	
Education and training of research personnel	All new workers, including students, are trained by the Health and Safety delegate of the Institute.	
Reference legislation	The relevant national legislation on safety in workplaces in Italy will apply: Legislative Decree no. 626 of September 19th 1994, Legislative Decree no. 81 of April 9th 2008 and Legislative Decree no. 106 of August 3rd 2009, which coordinates and restructures in a single regulation text all relevant legal provisions.	
Description of the operational phases and of the related hazards and protection measures		
Operational phases	***Hazards identification***	***Safety instructions***
1) Gated pipe installation	Tripping and heavy good lift	Fork lift will be provided and PPE will be ensured
2) Testing the gated pipes under different operating conditions	Broken pipes, electric / pumping fault	Gates and security valves. Electric interrupter and fire extinguishers

3.4 Overview of the main standards for the reuse of treated wastewater in agriculture

This section analyses the main international standards for treated WW reuse for irrigation (ISO 16075, WHO 2006 guidelines, FAO 2006 guidelines), as well as the national standards of the 3 target countries of Madforwater (Morocco, Tunisia, Egypt). In addition, also the US EPA standards are examined (EPA 600/R-12/618 of 2012). The main purpose of international guidelines is to ensure public health and environmental protection, by combining different types of measures: wastewater treatment, crop restriction, control of wastewater application and human exposure control. The above-mentioned standards are summarised in Tables 3.4.1 (organic content, suspended solids, pathogens), 3.4.2 (salinity, specific ions, total nitrogen, infiltration) and 3.4.3 (trace elements).

In several standards, irrigation water is grouped into quality classes in order to guide the user to the potential advantages as well as problems associated with its use. Each quality class generally corresponds to a type/level of WW treatment process, but also to possible restrictions in terms of the types of crops that can be irrigated, as illustrated in detail in Table 3.4.1. It should be noted that the international standards provide general guidelines, but their application has to be adjusted to the specific local / national conditions through a proper national legislation. As a matter of fact the conditions of water use in irrigation are very complex and difficult to predict, the suitability of water for irrigation will greatly depend on climatic conditions, physical and chemical properties of the soil, the salt tolerance of the crop grown and the management practices.

Table 3.4.1 reports the international and national limits relatively to organic content (BOD, COD), suspended solids (TSS, turbidity) and pathogens.

- **Organic matter**: as shown in Table 3.4.1 , while some standards report limits for both BOD and COD, others regulate only BOD, while there are no standards for organic matter at all in WHO. US EPA and ISO 16075 have a very strict water quality standard for unrestricted irrigation (10 mg/L). But if we consider irrigation for non-food crops/processed crops and pastures the standards range of the US EPA and ISO 16075 is 20-35 mg/L. in comparison with international thresholds, Egypt and Tunisia have quite strict standards (respectively 20 mg/L and 30 mg/L, for BOD), whereas Morocco has a less strict limit (100 mg/L).

- **Turbidity or Total Suspended Solids (TSS):** US EPA and ISO 16075 recommend a level < 2 NTU only for directly consumed crops or unrestricted irrigation, but no recommendation is given by WHO or by the considered North African countries. Most countries and international organizations that do not have a standard for turbidity have a standard for suspended solids. A high level of turbidity can affect the performance of the irrigation facility, and can lower the hydraulic conductivity of the soil; this can in turn pollute the soil surface through surface flow (*Vinten et al., 1983; Ragusa et al., 1994*). In addition, since various viruses and bacteria can be attached to and migrate along with the solid particles, the elimination of suspended solids is related to the elimination of germs (*Guidelines for Water Reuse 600/R-12/618; Environmental Protection Agency: Washington, DC, USA, 2012*). The ISO 16075 TSS threshold for unrestricted irrigation is 10 mg/L whereas the range of values for restricted irrigation is quite large (20 - 140 mg/L). EPA regulates the TSS content (< 30 mg/L) only for treated

WW used for processed food crops/non-food crops. The US EPA TSS limit is very close to that of Tunisia's and Egypt's standards (30 mg/L and 20 mg/L, respectively). No standard is given by Morocco for turbidity or TSS.

- **Pathogens:** Based on the total number of *E. Coli*, the WHO guidelines recommend 1000 CFU/100 mL for the strictest standard, relative to unrestricted wastewater, whereas EPA (which regulates fecal coliforms) applies a stricter standard (200 CFU/ 100 mL for processed food crops and non-food crops). It should be noted that *E.Coli* represents just a fraction of the fecal coliforms. Compliance with these standards, aimed at the prevention of infectious diseases, requires a relevant cost for WW treatment. According to *Shuval* (1997), the US EPA guidelines, compared to those of WHO, will cost an additional 30 million $ per prevented enteric disease. Indeed, there have been criticisms towards the US EPA guidelines since it might be impossible for developing countries to adopt them, due to the required cost and technology. ISO 16075 suggests a quite strict maximum of 100 thermo-tolerant coliform / 100 mL for unrestricted irrigation, and a maximum of 10000/100 mL for restricted irrigation. The ISO guidelines provide a low microbe standard for unrestricted irrigation, since they are based on epidemiological studies of developing countries where much of the population have now acquired immunity towards enteric infection (*Angelakis et al., 2008; Blumenthal et al., 2000).* WHO has recommended new guidelines for wastewater irrigation which consider the human health risk through epidemiological studies and quantitative microbial risk assessment (QMRA), a process for estimating the risk of exposure to microorganisms. Morocco has a severe threshold for fecal coliforms (1000 CFU/ 100 mL). Egypt's standard for pathogens is close to Morocco's, whereas only egg parasites (intestinal nematodes <1/1000 mL) are regulated in the Tunisian standards for pathogens.

Tables 3.4.2 and 3.4.3 report the guidelines initially published by FAO (Ayers and Westcot, 1985) and later adopted by EPA and WHO, as well as some additional threshold values valid in Morocco and Tunisia, relatively to salinity, specific ions, total nitrogen, infiltration and trace elements. Ayers and Westcot (1985) first classified irrigation water into three groups, characterized by different degrees of restriction on use:

- no restriction: any crop and public area can be irrigated (for vegetables and salad crops eaten uncooked, sport fields, public parks where the exposed groups are workers, consumers or public);
- slight to moderate restriction: irrigation is restricted to cereal crops, industrial crops, fodder crops, pasture and trees; some restrictions apply also to the categories of people that could be potentially exposed to this type of irrigation water;
- severe restriction: irrigation is restricted to the same crops of the 'slight to moderate' category, but no exposure at all of workers or the public can occur.

National standards valid in Tunisia, Egypt and Morocco

In **Tunisia** WW reuse in agriculture is regulated by Decree 93–2447 of 13/12/1993, by the Tunisian standard for the use of treated wastewater in agriculture (NT 106-003 of 18 May 1989), by the list of crops that can be irrigated with treated WW (Decision of the Minister of Agriculture of 21 June 1994) and by the list of requirements for agricultural WW reuse projects

(Decision of 28 September, 1995). These decrees prohibit the irrigation with treated WW of vegetables that might be consumed raw. Therefore, most of the recycled wastewater is used to irrigate vineyards, citrus and other trees (olives, peaches, pears, apples, pomegranates, etc.), fodder crops (alfalfa, sorghum, etc), industrial crops (cotton, tobacco, sugar-beet, etc), cereals, and golf courses. Some hotel gardens in Jerba and Zarzis are also irrigated with recycled wastewater. The Tunisian standards also regulate several trace elements with analysis frequency, with threshold values equal to or slightly higher than those set by the WHO 2006, FAO and EPA 2012 standards (Table 3.4.3). A specific authorization is required in Tunisia in each case of irrigation with treated WW.

In **Egypt**, according to the *Egyptian Code for the Reuse of treated Wastewater in Agriculture (501/2005)* the reuse of treated WW is prohibited for the irrigation of vegetables, export-oriented crops (cotton, rice, onions, potatoes, medicinal and aromatic plants), citrus fruit trees and school gardens. The 501/2005 Code classifies wastewater into three grades (A, B, and C) and specifies for each grade the maximum contaminant levels and the crops that can be irrigated, as specified in Table 3.4.1. The 501/2005 Code further stipulates conditions for irrigation methods and health protection measures for farm workers, consumers and inhabitants of neighbouring farms. On the other hand, the 501/2005 Code does not regulate trace elements in irrigation water.

In **Morocco** the application Decree No. 2-97-875 of Feb. 4, 1998 relative to the reuse of treated wastewater stipulated that untreated WW use is prohibited and banished. The Norms and Standards Committee (NSC) is setting objectives for the quality of the receptor medium. Among the suggested norms, there is a project related to quality standards of wastewater designed for irrigation (El-Zanfaly, 2015). Morocco has adapted its limit values to the international guidelines: the limits relative to specific ions, trace elements, total nitrogen and bicarbonate are equal to those set by the WHO 2006, FAO and EPA 2012 standards for unrestricted irrigation. Conversely, in the Moroccan standard the total salt content (which can be expressed in terms of electrical conductivity or total dissolved solids) is about four times the value set by the above-mentioned international guidelines for unrestricted irrigation. Due to the high temperature of the land, a high salt content associated with an intense water evaporation could cause a fast accumulation of salts in the root zone and consequently harmful effects on soil and plants. Indeed, despite the salinity effect on crop growth depends on the crop type, according to Ayers and Westcot (1985) an electrical conductivity of the irrigation water below 700 µS/cm generally does not affect crop growth, whereas values above 3000 µS/cm can cause severe damage.

Table 3.4.1 – International and North African standards for treated WW reuse in agriculture: organic content, suspended solids and pathogens. The notes are explained after Table 3.4.3.

	ISO 16075 [a)]					**WHO**		**EPA 2012**	
	Category of treated wastewater quality [b)]					**Degree of restruction on use [c)]**		**Use**	
	Cat. A (very high quality TWW)	Cat. B (high quality TWW)	Cat. C (good quality TWW)	Cat. D (medium quality TWW)	Cat. E (Exstensively TWW)	unrestricted	restricted	for food crops	for processed food crops or non-food crops
BOD_5 (mg/L)	<10	<20	<35	<100	<35	n.r.	n.r.	<=10	<=30
COD (mg/L)	n.r. [d)]	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Turbidity (NTU)	5	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	<= 2	n.r.
TSS (mg/L)	<10	<25	<50	<140	n.r.	<50	50-100	n.r.	<=30
Coliform (no./100mL)	<100 *thermo-tolerant coliforms*	<1000 *thermo-tolerant coliforms*	<10000 *thermo-tolerant coliforms*	n.r.	n.r.	*E. coli* < 1000	*E. coli* < 10000	*Fecal Coliform* absence	*Fecal Coliform* ≤ 200 (median)
Intestinal nematode (Egg/L)	n.r.	n.r.	n.r.	<5	<5	<1	<1	n.r.	n.r.
Potential irrigation uses	Irrigation of gardens with unrestricted or restricted public access; irrigation of vegetables (consumed raw or processed) and pastures, irrigation of crops other than vegetables, irrigation of fodder and seed crops, irrigation of industrial and energy crops	Irrigation of gardens with unrestricted* [d)] or restricted public access; irrigation of vegetables (consumed raw* or processed) and pastures, irrigation of crops other than vegetables, irrigation of fodder and seed crops, irrigation of industrial and energy crops	Irrigation of gardens with restricted public access*; irrigation of vegetables (consumed raw* or processed*) and pastures*, irrigation of crops other than vegetables*, irrigation of fodder and seed crops, irrigation of industrial and energy crops	Irrigation of gardens with restricted public access*; irrigation of crops other than vegetables*, irrigation of fodder* and seed crops*, irrigation of industrial and energy crops	Irrigation of gardens with restricted public access*; irrigation of vegetables (consumed processed*) and pastures*, irrigation of crops other than vegetables*, irrigation of fodder and seed crops, irrigation of industrial and energy crops	Unrestricted irrigation of vegetables and salad crops eaten uncooked, sport fields, public parks where the exposed groups are workers, consumers or public	Restricted irrigation of cereal crops, industrial crops, fodder crops, pasture and trees	Surface or spray irrigation of food crops which are intended for human consumptio n, consumed raw.	Surface irrigation of food crops which are intended for human consumption (commercially processed) and of crops which are not consumed by humans, including fodder, fiber, and seed crops, or to irrigate pasture land and commercial nurseries.

Table 3.4.1 *(continued)* - International and North African standards for treated WW reuse in agriculture: organic content, suspended solids and pathogens. The notes are explained after Table 3.4.3.

	Morocco		Tunisia	Egypt		
				Grade of treated wastewater[e)]		
	Untreated WW	Treated WW		A	B	C
BOD_5 (mg/L)	<100	<500	<30	<20	<60	<400
COD (mg/L)	<500	<1000	<90	n.r.	<80	n.r.
Turbidity (NTU)	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
TSS (mg/L)	n.r.	n.r.	<30	<20	<50	<250
Coliform (no./100mL)	*Fecal Coliform* <1000	*Fecal Coliform* <1000	n.r.	<1000	<5000	unspecified
Intestinal nematode (Egg/L)	absence	n.r.	<1	<1	<1	unspecified
Potential irrigation uses			Irrigation of crops consumed after industrial processing (vineyards, citrus, olives, peaches, pears, apples..), fodder crops (alfalfa, sorghum, etc), industrial crops (cotton, tobacco, sugar-beet, etc), cereals, and golf courses	Irrigation of mulberry for the production of silk, industrial oil crops and wood trees	Irrigation of plants and trees grown for greenery at touristic village and hotels or inside residential area at new cities; irrigation of fodder/feed crops, trees producing fruits with epicarp (on condition that they are produced for processing purposes such as lemon, mango..), trees used for green belts around cities and a forestation of high ways or roads, wood trees, ornamental plants and fruit trees.	Irrigation of industrial oil crops and wood trees

Table 3.4.2 – International and North African standards for treated WW reuse in agriculture: salinity, infiltration, specific ions, total nitrogen. The notes are explained after Table 3.4.3.

Potential irrigation problem	Parameters	WHO 2006, FAO and EPA 2012					Morocco	Tunisia
			Units	Degree of restriction on use[f)]			Limit values	Limit values
				None	Slight to moderate	Severe		
Salinity	ECw		dS/m	<0,7	0,7-3	>3	12 dS/m	0,7 dS/m
	TDS		mg/L	<450	450-2000	>2000	7680 mg/L	n.r.
Infiltration	SAR	0 - 3	meq/L	> 0,7 ECw	0,7 - 0,2 ECw	< 0,2 ECw	n.r.	-
		3 - 6	meq/L	> 1,2 ECw	1,2 - 0,3 ECw	< 0,3 ECw	n.r.	n.r.
		6 - 12	meq/L	> 1,9 ECw	1,9 - 0,5 ECw	< 0,5 ECw	n.r.	n.r.
		12 - 20	meq/L	> 2,9 ECw	2,9 - 1,3 ECw	< 1,3 ECw	n.r.	n.r.
		20 - 40	meq/L	> 5 ECw	5 - 2,9 ECw	< 2,9 ECw	n.r.	n.r.
Specific Ion Toxicity	Sodium (Na+)	Sprinkler irrigation	meq/L	< 3	> 3		3 meq/L	n.r.
		Surface irrigation	meq/L	< 3	3 - 9	> 9	9 meq/L	n.r.
	Chloride (Cl-)	Sprinkler irrigation	meq/L	< 3	> 3		3 meq/L	n.r.
		Surface irrigation	meq/L	< 4	4 - 10	> 10	10 meq/L	n.r.
	Trace Elements	--> see Table 3.4.3						
Miscellaneous Effects	Total Nitrogen (NO3-N)		mg/L	< 5	5 - 30	> 30	30 mg/L	n.r.
	Bicarbonate (HCO3-)		mg/L	< 90	90 - 500	> 500	518 mg/L	n.r.
pH				normal range 6,5 - 8,5			6,5-8,5	6,5-8,5

Table 3.4.3 – International and North African standards for treated WW reuse in agriculture: trace elements.

Element	Element Symbol	Unit of measurement	Maximum concentration for irrigation				
			WHO 2006	FAO	EPA 2012	Tunisia	Morocco
Aluminum	Al	mg/L	5	5	5	10	5
Arsenic	As	mg/L	0,1	0,1	0,1	0,1	0,1
Beryllium	Be	mg/L	0,1	0,1	0,1	0,05	0,1
Boron	B	mg/L	0,7	3	0,75	2	n.r.
Cadmium	Cd	mg/L	0,01	0,01	0,01	0,01	0,01
Chromium	Cr	mg/L	0,1	0,1	0,1	0,1	0,1
Cobalt	Co	mg/L	0,05	0,05	0,05	0,1	0,05
Copper	Cu	mg/L	0,2	0,2	0,2	0,5	0,2
Cyanide	Cn	mg/L	n.r.	n.r.	n.r.	0,5	1
Fluoride	F	mg/L	1	1	1	3	1
Iron	Fe	mg/L	5	5	5	5	5
Lead	Pb	mg/L	5	5	5	1	5
Lithium	Li	mg/L	2,5	2,5	2,5	n.r.	2,5
Manganese	Mn	mg/L	0,2	0,2	0,2	0,5	0,2
Mercury	Hg	mg/L	n.r.	n.r.	n.r.	0,001	0,001
Molybdenum	Mo	mg/L	0,01	0,01	0,01	n.r.	0,01
Nickel	Ni	mg/L	0,2	0,2	0,2	n.r.	0,2
cloSelenium	Se	mg/L	0,02	0,02	0,02	0,05	0,02
Tin	Sn	mg/L	n.r.	n.r.	n.r.	n.r.	n.r.
Titanium	Ti	mg/L	n.r.	n.r.	n.r.	0,01	n.r.
Tungsten	W	mg/L	n.r.	n.r.	n.r.	n.r.	n.r.
Vanadium	V	mg/L	0,1	0,1	0,1	n.r.	0,1
Zinc	Zn	mg/L	2	2	2	5	2

Notes to Tables 3.4.1, 3.4.2 and 3.4.3.

a) ISO 16075 gives also average values as standards, here is reported only the maximum one.

b) According to ISO-16075 five different water quality levels (A, B, C, D, E) are defined to classify the treated wastewater quality:

- Cat. A: very high quality treated wastewater (TWW) it's the only that should be used without additional barriers* for unrestricted irrigation** and agricultural irrigation of food crops consumed raw. Essentially it's raw water which has undergone physical and biological treatment, filtration and disinfection and its quality must according to the description in this table.
- Cat. B: high quality treated wastewater. Like cat. A it's raw wastewater which has undergone physical and biological treatment, filtration and disinfection and its quality must according to the description in this table. It's potential uses without additional barriers are restricted urban irrigation and agricultural irrigation of processed food crops.
- Cat. C: good quality treated wastewater. It's raw wastewater which has undergone physical and biological treatment and its quality it's according to the description in this table. It's potential use without additional barriers is agricultural irrigation of non-food crops.
- Cat. D: medium quality treated wastewater. It's raw wastewater which has udergone physical and biological treatment and its quality it's according to the description in this table. It's potential uses without additional barriers is restricted irrigation*** of industrial and seeded crops.
- Cat. E: extensively treated wastewater. It's raw wastewater which has undergone natural biological treatment process with long (minimum 10d to 15d) retention time and its quality it's according to the description in this table. It's potential uses without additional barriers is restricted irrigation of industrial and seeded crops.

  *barrier: any means including physical or process steps that reduces or prevents the risk of human infection by preventing contact between the TWW and the ingested produce or other means that, for example, reduces the concentration of microorganisms in the TWW or prevents their survival on the ingested produce
  **unrestricted irrigation: use of TWW for non potable applications in settings where public access is not restricted
  ***restricted irrigation: use of TWW for non potable applications in settings where public access is controlled or restricted by physical or institutional barriers

c) Unrestricted means use of TWW to grow crops that are normally eaten uncooked; restricted means use of TWW to grow crops that not eaten raw by humans.

d) Not recommended

e) The Egyptian Code classifies wastewater into three grades (designated A, B, and C) as follows, depending on the level of treatment it has received, and specifies the maximum concentrations of specific contaminants consistent with each grade.

- Grade A is advanced, or tertiary, treatment that can be attained through upgrading the secondary treatment plants (i.e. Grade B plants) to include sand filtration, disinfection and other processes.
- Grade B represents secondary treatment performed at most facilities serving Egyptian cities, townships and villages. It is undertaken by any of the following techniques: activated sludge, oxidation ditches, trickling filters, and stabilization ponds.
- Grade C is primary treatment that is limited to sand and oil removal basins and use of sedimentation basins.

Despite reuse of treated wastewater is prohibited by law for food and fiber crops, exists a classification of plants and crops irrigable with treated wastewater

f) WHO considers three classes of utilization:

- None: means it can be used for unrestricted irrigation (for vegetables and salad crops eaten uncooked, sport fields, public parks where the exposed groups are workers, consumers or public).
- Slight to moderate: means restricted irrigation (cereal crops, industrial crops, fodder crops, pasture and trees). This class contains more subgroups depending on the possible exposed group (generally workers but also children under 15 years are considered).
- Severe: means that the treated wastewater can be used for localized irrigations of crops (same crops of cat. 'Slight to moderate') if exposure of workers and the public does not occur.

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