TESIS DOCTORAL

MANEJO SOSTENIBLE DEL RIEGO CON AGUAS REGENERADAS

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SUSTAINABLE IRRIGATION MANAGEMENT WITH RECLAIMED WATER

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Table of content

Page

Index of figures

Index of pictures

Index of tables

Abbreviations and symbols

ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
B	Boron
BOD	Biological Oxigen Demand
C	Control treatment
Ca	Calcium
$CaCO_3$	Calcite
CARM	Comunidad Autónoma de la Región de Murcia
Cd	Cadmium
CEA	Cost-effectiveness analysis
CEBAS	Centro de Edafología y Biología Aplicada del Segura
CHS	Confederación Hidrográfica del Segura
CIWEM	Chartered Institution of Water and Environmental Management
Cl	Clorine
cm	Centimeter
cm^2	Square centimeter
CO_2	Carbon dioxide
CO_3	Carbonate
COD	Chemical oxigen demand
Co	Copper
Cr	Chromium
CSIC	Consejo Superior de Investigaciones Científicas
Cu	Copper
cfu	Colony formed unit
CVM	Contingent valuation method
CW	Constructed wetland
DALYs	Disability-adjusted life year
DI	Deficit irrigation
EC	Electrical conductivity
ECw	Electrical conductivity of the irrigation water
ECe	Electrical conductivity of the saturated paste extract
EEB	Enrichment broth
EEUU	United States

EHEC	Enterohemorrhagic E.Coli
EPA	Environmental Protection Agency
ESAMUR	Entidad Regional de Saneamineto y Depuración de Aguas Residuales
ETc	Crop evapotranspiration
ETo	Reference evapotranspiration
ETp	Potential evapotranspiration
EU	European Union
E.coli	*Escherichia coli*
FAO	Food and Agriculture Organization of the United Nations
FC	Fecal coliforms
Fe	Iron
g	Gram
GEC	Global Environment Centre
GIS	Geographic Information System
h	Hour
ha	Hectare
hm^3	Cubic hectometer
HE	Helminths eggs
H_2O	Water
HCO_3	Bicarbonate
HLR	Hidraulic loading rate
HPLC	High-performance liquid chromatography
HRT	Hidraulic retention time
HSSF	Horizontal subsurface flow
IW	Irrigators association
IWMI	International Water Management Institute
K	Potasium
K_2O	Potasium oxide
kg	Kilogram
L	Liter
Mg	Magnesium
MBR	Membrane bioreactors
MF	Microfiltration

meq	Miliequivalent
mg	Miligram
min	Minute
ml	Mililiter
Mm^3	Million cubic meter
mmol	Milimole
Mn	Manganese
MWRI	Ministry of Water Resources and Irrigation
N	Nitrogen
n	Number of samples
Na	Sodium
NaCl	Sodium chloride
ND	No data
NF	Nanofiltration
NO_3- N	Nitrate nitrogen
NO_4- N	Ammonia nitrogen
Ni	Nickel
O_2	Oxigen
OLS	Standard linear regression
OM	Organic matter
P	Phosphorus
Pb	Lead
P_2O_5	Phosphorus pentoxide
PCA	Plate Count Agar
PCR	Polimerase chain reaction
p.e.	Person equivalent
PHCS	Plan Hidrológico de la Cuenca del Segura
pH	Hydrogen ion activity
PIB	Gross National Product
Pn	Net Photosynthesis
pppy	per person per year
RDI	Regulated deficit irrigation
RO	Reverse osmosis
RW	Reclaimed water
s	Second

SAR	Sodium adsorption ratio
SAT	Soil aquifer treatment
SDI	Subsurface drip irrigation
SF	Surface flow
SO_4	Sulfate
SOCs	synthetic organic chemical
SS	Suspension solids
SSC	Soluble solid content
SSF	Subsurface flow
SWC	Soil water content
SWS	Soil water store
t	Ton
TA	Titratable acidity
TC	Total coliforms
TDS	Total disolved solids
TDR	Time-domain-reflectometry
TEV	Total economic value
TOC	Total organic carbon
TP	Total phosphorus
TSB	Tryptic soy broth
TSS	Total suspension solids
TW	Transfer water
UF	Ultrafiltration
UNESCO	United Nations Educational Scientific and Cultural Organization
UNECE	United Nations Economic Commission for Europe
UNEP	United Nations Environment Programme
UNPD	United Nations Development Program
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
VCC	Vancomycih, cefixime and cefsulodin
VPD	Vapour pressure déficit
VSSF	Vertical Subsurface flow
WFD	Water Framework Directive
WHO	World Health Organization

WTP	Willingness to pay
WTA	Willingness to accept
WUE	Water use efficiency
WWTP	Wastewater treatment plant
Zn	Zinc
%	Percentage
ºC	Celsius degree
$\mathbf{g_s}$	Stomatal conductance
$\mathbf{\Psi_{stem}}$	Predawn leaf water potential
$\mathbf{\Psi_m}$	Soil matric potential

Resumen

El gran desafío al que se enfrenta la agricultura en el futuro próximo, será, por un lado, el de aumentar la producción de alimentos con menos consumo de agua, especialmente en los países con recursos hídricos limitados, y por otro lado, el uso adecuado de aguas no convencionales tales como las aguas residuales tratadas para riego. En este sentido, la salinidad de casi todos los tipos de efluentes depurados sigue alta, y por lo tanto su uso a medio y largo plazo en el riego podría afectar significativamente a las propiedades físicas y químicas del suelo, especialmente en los sistemas de agricultura moderna e intensiva llevadas a cabo en zonas áridas y/o semiáridas. Por todo ello, es preciso el desarrollo de estrategias adecuadas para la gestión del suelo, el agua y los cultivos cuando estos recursos hídricos no convencionales se utilizan para el riego.

La cuenca del Segura (Murcia), es la única cuenca española cuyos recursos naturales no pueden cubrir la demanda de los distintos usuarios. En esta Región, el sector agrícola consume el 84% de los recursos disponibles (CHS, 2009) y por esta razón, el uso de las aguas regeneradas es especialmente importante. En Murcia existen 92 estaciones depuradoras de aguas residuales "EDAR" produciendo más de 102,1 hm^3 al año (ESAMUR, 2009), lo que supone el 6% de los recursos hídricos renovables anuales (PHCS, 1995). El principal problema asociado al uso de estas aguas es la salinidad ya que el 93% de ellas tiene una conductividad eléctrica (CE) superior a 2 $dS\ m^{-1}$ y el 37% presenta valores de CE superiores 3 $dS\ m^{-1}$ (ESAMUR, 2005).

Los cítricos representan el 25% de los cultivos de regadío, siendo uno de los cultivos más importantes en la Región de Murcia. Esta región produce más del 41% de la producción nacional de limón. Esta importante contribución se debe principalmente al uso intensivo de técnicas de fertirriego localizado, donde el 80% de la superficie total se encuentra bajo riego por goteo (CARM, 2009).

En esta tesis, la primera aproximación a la investigación del uso de aguas regeneradas fué llevada a cabo en el cultivo del limón (Capítulo II). El objetivo de este trabajo fue el estudio de los efectos del agua regenerada sobre el rendimiento del limón, comparando dos fuentes de aguas residuales tratadas, la primera obtenida con un tratamiento secundario (Cartagena) y la segunda obtenida mediante la mezcla de tratamiento terciario con agua de pozo (Campotéjar). Se

estudiaron los efectos del uso del agua regenerada sobre las propiedades químicas del suelo, el estado mineral de la hoja, la producción agrícola y la calidad y seguridad de las cosechas. Los principales resultados obtenidos mostraron que la posibilidad de mezclar agua regenerada con agua de pozo es una buena solución para evitar los problemas asociados con el uso de aguas residuales en la agricultura. La alta salinidad y la elevada concentración de Cl y B fueron las principales restricciones asociadas al uso de estas aguas durante el experimento. La carga microbiana presente en las aguas regeneradas no supuso ningún riesgo de contaminación para los frutos de limón.

Hay muy poca información acerca de la interacción entre el uso de aguas salinas en condiciones de déficit hídrico. Por ello, los principales objetivos del segundo estudio realizado en esta tesis fueron evaluar los efectos combinados de diferentes calidades de agua de riego (trasvase Tajo-Segura, comunidad de regantes y agua regenerada) junto con estrategias de riego deficitario controlado sobre el rendimiento de los cultivos de mandarinos bajo condiciones de clima mediterráneo. También se estudiaron los efectos sobre la fisiología de las plantas y el estado mineral de la hoja, la composición química del suelo y de la solución nutritiva en la zona radicular. Igualmente se evaluó el rendimiento y la calidad de los frutos (capítulo III). Las reducciones en la producción no fueron significativas entre tratamientos, a pesar de que fué detectada una tendencia en la reducción del número de frutos en los tratamientos con agua regenerada. Esta reducción fue más pronunciada en el tratamiento deficitario que recibía agua de baja calidad. Los efectos combinados de las estrategias de RDC y de la utilización de aguas regeneradas puede mejorar algunos parámetros de calidad del fruto tal y como se pudo observar en el aumento del contenido en vitamina C. La situación hídrica de las plantas y el crecimiento vegetativo no fueron significativamente afectados por el uso de las aguas regeneradas. Sin embargo, el estrés hídrico generado en los tratamientos de riego deficitario produjeron reducciones en el potencial hídrico del tallo, cuyos valores fueron inmediatamente recuperados cuando el periodo de déficit hídrico desapareció. Aunque no se advirtieron síntomas de toxicidad a nivel foliar durante el experimento, si que se observó una tendencia a la acumulación de sales en el suelo durante el último año de ensayo en el tratamiento combinado de agua regenerada y riego deficitario controlado. En este sentido, es importante señalar que en zonas áridas y semiáridas, la combinación de estrategias de RDC con el uso de aguas regeneradas, puede verse afectada a largo plazo debido a la acumulación de sales y boro.

El último capítulo se basó en el uso de Sistemas de Información Geográfica (SIG) como herramienta en la planificación y gestión de proyectos de utilización de las aguas regeneradas (Capítulo IV). En la región de Beira Interior (Portugal), se desarrolló un estudio con el objetivo de identificar sitios potenciales para la reutilización del agua residual tratada mediante un análisis multicriterio basado en el SIG. Para ello, se utilizó un humedal artificial ubicado en la parte noroeste de la región de Beira Interior como fuente de agua regenerada. El trabajo demostró que las instalaciones de humedales artificiales para tratar las aguas residuales pueden contribuir a la recarga de los acuíferos en zonas de escasez de agua, reduciendo los riesgos de contaminación medioambiental y por lo tanto, generando beneficios a las actividades económicas y turísticas en sus áreas de actuación.

Finalmente, se adjuntan dos anexos a esta memoria de investigación. En el Anexo A se describe un estudio de valoración contingente desarrollado en colaboración con el Dpto. de Economía de la Empresa de la Universidad Politécnica de Cartagena (UPCT) en donde se estimó los beneficios de no mercado que la sociedad asigna a la reutilización de las aguas residuales tratadas para uso agrícola. En el anexo B se enumeran las diferentes publicaciones y comunicaciones a congresos generadas, hasta el momento, en el periodo de la tesis doctoral.

Summary

The great challenge in agriculture for the near future, will be, on the one hand, the task of increasing food production with less water, particularly in countries with limited water and land resources, and on the other hand the safe use of non conventional water resources, such as treated wastewater, for irrigation. In this sense, almost all types of treated effluents remain moderately to highly saline and consequently their long term use in irrigation may significantly affect the physical and chemical properties of the soil especially under modern and intensive agricultural system in arid and semi-arid areas, therefore appropriate strategies for managing soil, water and crops may also be needed when these non conventional resources are used for irrigation.

The Segura basin (Murcia) is the only Spanish basin whose natural water resources cannot cover its water demands. The main use with 84% is the agriculture (CHS, 2009), for this reason, the use of reclaimed water in agriculture is especially important. In Murcia-Spain there are at least 92 operating wastewater treatment plants “WWTPs” delivering more than 102.1 hm^3 per year (ESAMUR, 2009), restore up to 6% of the annual renewable water resources (PHCS, 1995). The major problem associated to reclaimed water use in Murcia is salinity. In this Region, 93% of the treated wastewater has an Electrical Conductivity (EC) higher than 2 $dS\ m^{-1}$ and 37% has EC values higher than 3 $dS\ m^{-1}$ (ESAMUR, 2005).

Citrus represent the 25% of the irrigated crops, being one of the most important crops in the Region of Murcia. This Region produces more than 41% of the national lemon production. This important contribution is mainly due to the use of intensive localized fertigation techniques where 80% of the total area is under drip irrigation (CARM, 2009).

In this thesis, the first investigation approximation into reclaimed water was on lemon trees (Chapter II). The aim of this work was to study the effects of reclaimed water on lemon tree performance. In particular, this research was to compare two sources of treated wastewater, one obtained with a secondary treatment (Cartagena) and the other with a tertiary treatment blending with well water (Campotejar), and to study their effects on soil chemical properties, on the leaf mineral status, crop production and fruit quality and safety. The main results obtained shown that the possibility of blending reclaimed water with well water is

a good solution to avoid the problems associated with wastewater use in agriculture. The high salinity, Cl and B concentration were the main restrictions associated with treated wastewater used in the experiment. The use of reclaimed water as irrigation water for lemon trees did not represent a microbial risk for lemon fruit.

Little is known about the interaction between deficit irrigation and saline water. For this reason, the main objectives of the second study were to evaluate the combined effects of different irrigation water qualities (Tajo Segura water transfer canal, irrigators association and reclaimed water) and the regulated deficit irrigation strategies on mandarin tree crop performance under Mediterranean climate conditions and their effects on plant physiology and leaf mineral status, soil chemical properties and water content, yield and fruit quality (Chapter III). Yield reductions were not significant between treatments, although a tendency to reduced number of fruit was detected in the reclaimed water treatments. This reduction was more pronounced under combined conditions of reduced irrigation quality water and regulated deficit irrigation. The combined effects of RDI strategies and of using reclaimed water can increase some fruit quality parameters such as vitamine C on mandarin trees. Plant water relations and vegetative growth were not significantly affected by the use of reclaimed water. However, water stress in the RDI treatments induced some reductions in the stem water potential that inmediatly were recovered when deficit period disappeared.

Although no leaf toxic visual symptoms were seen during the experiment,a tendency was identified in terms of salts accumulation in the soil during last season in RW-RDI treatment. In this sense, it is important to remark that in arid and semi-arid areas, the combination of RDI strategies and reclaimed water-use can be affected in the long-term because of the salts and boron accumulation.

The last work was based in the use of Geographical Information Systems (GIS) as a tool in the planning and management of water reuse projects (Chapter IV). A work in the Beira Interior region (Portugal) was developed with the aims at identifying potential sites for reclaimed water use, using a GIS-based multi-criteria analysis. A constructed wetland (CW) system located in the northwest part of the Beira Interior region was used as the source of reclaimed water. The work also intended to show that small wastewater treatment facilities may be used for aquifer recharge in areas of water scarcity, reducing the discharge of residuals loads to the environment and benefiting economic and tourist activities.

Finally, two annexes attached to this research report. Annex A describes a contingent valuation study developed in collaboration with the Department of Business Economics at the Polytechnic University of Cartagena (UPCT) where it was estimated the non-market benefits that society gives to the reuse of treated wastewater for agricultural use. In Annex B lists the various publications and conference papers generated, so far, during the period of the doctoral thesis.

I INTRODUCTION TO AGRICULTURAL USE OF RECLAIMED WATER

Wastewater has been produced ever since humans have existed on earth. It is any water that has been degraded in quality after being directly or indirectly subjected to anthropogenic influences such as domestic and commercial water uses, agricultural practices and/or industrial processing. Thus, the amount of wastewater has continuously increased over years as the human population has enlarged and progressed.

The reuse of wastewater has been practiced for centuries whenever the access to natural water resources was limited. Their reuse was either directly for agricultural purposes, or indirectly for human consumption when the mixture wastewater/riverwater was used for drinking. In the 19th century human populations had become so concentrated that outbreaks of life-threatening diseases in London were traced to a common public drinking water pump that was contaminated with sewage from a nearby house (Snow, 1854). Since that time, the practice of wastewater collection and treatment has been progressively developed and perfected, using some of the most technically sound biological, physical, chemical, and mechanical techniques available.

In the 21st century, water shortage, water quality degradation, underground water depletion, population growth and demographic unbalances between rural and urban areas as well as crop water requirements and prolonged periods of drought have became evident challenges in the arid and semiarid areas. Consequently, concerns about water conservation and saving are becoming a priority as health concerns. Thus, the human community has begun to understand the urgent need, on the one hand, to enhance the productivity of water use and on the other hand, to reduce the amount of pollutants in the used water they are discharging to the environment.

Irrigated agriculture provides more than 40% of the current food production; nonetheless, it is the primary user of diverted water reaching a proportion that exceeds 70-80% of the globally available resources (Fereres and Connor, 2004). Moreover, inefficient use of water is a notorious phenomenon in the agricultural sector, especially when expressed in terms of currency returns per cubic meter of water applied and compared with other sectors such as the industrial or tourism ones. The latter could have higher potential economic-productivity. In this sense, considerable amounts of water could be saved from irrigated agriculture in favor of more productive sectors. Nevertheless, continuous population growth and its consequent increase in food and water demand restrict the options at hands to a few possibilities such as: (i) implementing precise agriculture that produces more crops per available drop of water and, (ii) finding new resources such as the use of brackish and treated wastewater resources. The latter is constantly available whenever humanity exists and progresses and therefore has the potential to augment water supplies and narrow the gap between fresh-water availability and demand in water-scarce countries and regions (Qadir et al., 2007).

Since the eighties, about 80 countries and regions, representing 40% of the world's population, have experienced water stress, and about 30 of these countries are suffering water scarcity during a large part of the year (Gleick, 1993). Recently, several countries have encountered a water shortage (Allan, 2001) and, datasets and maps published in recent years show that more and more countries will become water-stressed because of increased water scarcity (Seckler et al., 1998; FAO, 2003). By the year 2025 about 60% of the world's population will have to cope with a lack of water (Cosgrove and Rijsberman, 2000). Therefore, the inevitable prospects of water scarcity are driving a new approach in water resources management, incorporating the principles of sustainability, environmental ethics, and public participation as is clearly imposed by the European Wastewater Directive (91/271/EEC) which prohibits the delivery of wastewater effluents to the environment before reducing possible hazards to their minimum. Thus, the volume of treated wastewater is in continuous increase due to environmental concerns and the progressive implementation of the European Wastewater Directive "EWD" (91/271/EEC).

In Murcia-Spain there are at least 92 operating wastewater treatment plants "WWTPs" delivering more than 102 hm^3 per year (ESAMUR, 2009), which restore up to 6% of the annual renewable water resources (PHCS, 1995). The free availability of this considerable volume of water against the negative impact of water shortage in the region has renewed and enhanced the use of urban treated wastewater in modern agriculture (mainly drip irrigation).

I.1 TYPES AND CHARACTERISTICS OF WASTEWATER

When public water supplies are used for domestic, commercial, and industrial purposes, a wide variety of known and unknown constituents are added that end up as wastewater. The most common wastewater used for recycling is the liquid waste collected through the municipal sewerage network. Other wastewaters include industrial water, storm water run-off and drainage water.

Municipal wastewater or urban wastewater consist of a combination of some or all of the domestic effluent produced, water produced by commercial establishments and institutions (including hospitals), industrial effluent and storm water which has not infiltrated the soil, as well as other forms of urban runoff (Van der Hoek, 2004). Therefore wastewater contains one or more impurities at levels higher than in freshwater, including salts, metals, metalloids, residual drugs, organic compounds, endocrine-disrupting compounds, and the active residues of personal care products and/or pathogens.

Industrial water is wastewater produced from processes at industrial or commercial premises. It includes all waterborne waste from these facilities except sewage. Industry effluents are very varied, so industrial wastewater contains varying amounts of a large number of different synthetic organic chemicals (SOCs), with structures ranging from simple to extremely complex. Among industrial wastewater it is predominantly wastewater from food processing industries that has high potential to be reused in agriculture since the main constituents are organic substances. Table 1 specifies some food processing industries which could be employed for reclaimed water use.

Storm water refers to surface water runoff that originates from precipitation. As storm water runs over surfaces it can accumulate disease-causing microorganisms and other contaminants such as oils, grease, tars, and metals from roadway runoff, pesticides and herbicides, fertilizers, animal feces and decayed humic materials. Therefore, care must be taken to ensure that untreated storm water is used in a safe and controlled manner, or that storm water is treated to an appropriate level before use.

The last source is the drainage water generated by irrigated agriculture and surface runoff that has passed through the soil profile and entered the drainage system. This water contains salts and the residues of agrochemicals such as pesticides, fertilizers, and soil and water amendments. Typical drainage water is saline and sodic, contains salts that can impair plant growth but rarely contains metals or pathogens. However, it can lead to soil salinization and waterlogging, which impair productivity on millions of hectares of agricultural land. Contingent upon the levels and types of salts present, and the use of appropriate irrigation and soil management practices, agricultural drainage water can be used for different crop production systems (Rhoades, 1999; Oster and Grattan, 2002). Two types of drainage systems (subsurface tile drainage and tubewell drainage) are practiced on large areas suffering salt and shallow water table problems (picture 1).

Table 1: Selected food industries and composition of industrial wastewater (Source: Kretzschmar, 1990 cited in DONTA).

Industry	**Contaminants**	**N** mg l^{-1}	**P** mg l^{-1}	**K** mg l^{-1}
Distilleries	alkali, acids, soda, chlorine-compounds	25	1	20
Brewery/Malting	yeast, carbohydrates, settleable solids	40	5	50
Fish processing	scale, fats, oils, org. Acids, Salts, H_2O_2	500	-	-
Potato flour	none	500	140	96
Canning	salts, organic acids, detergents, corrosive substances	60	10	35
Dairy	disinfectants	35	10	20
Starch	salts, acids	300	45	415
Cider	detergents	870	160	-
Sugar	strontium, tar, prussic (cyanic) acid	50	10	-

Picture 1: Drainage system installed in large agricultural areas suffering soil salinity problems in Salinas Valley, California.

I.2 HEALTH AND ENVIRONMENT RISKS AND PUBLIC CONCERNS

Health and environmental aspects are particularly sensitive issues and important prerequisites, since wastewater effluent will not be used and/or be accepted to replace conventional or possibly other non-conventional water sources for irrigation, unless it is adequately treated and safely applied (Salgot et al., 2003; Gerba and Rose, 2003).

The total solid waste present in wastewaters does not exceed 1 percent of its composition; even though, several contaminants may present serious threats to human and animal health as well as to the water bodies, the soil and the plant production system.

The utility assessment of any wastewater source depends on the water quality parameters that affect the receiving body. For instance, if the receiving stream for a wastewater is a lake, the nutrients like nitrogen and phosphorus in the wastewater may be the primary concern. Nutrients discharge to a lake or river can cause eutrophication (Thomas et al., 2010), a condition that degrades water quality by increasing algae growth, depleting dissolved oxygen concentrations, and increasing sedimentation. If the receiving stream is a high quality river, the primary concern may be the oxygen consuming organics in the water. Oxygen depletion in the river could lead to deterioration in the quality and diversity of fish species, and severe oxygen depletion will cause fish kills.

If the wastewater is to be treated and incorporated directly or indirectly into the potable water distribution network, the primary concerns are the presence of pathogen (Carincross and Mara and, 1989), heavy metals and/or carcinogenic substances (Ono et al, 1996) and any substances that could potentially produce health disorders. In this sense, the presence of high nitrate levels in drinking water holds the risk to produce what is called “blue baby” symptoms or Methemoglobinemia (Greer et al., 2005). Until infants reach about six months of age, their digestive system secretes lower amounts of gastric acid and the pH level in their digestive system is higher than most adults. Adults with a diminished capability to secrete gastric acid also can experience a rise in pH in their digestive system. In both situations, bacteria can proliferate, increasing the transformation of nitrate to nitrite. Once in the blood, nitrite oxidizes iron in the hemoglobin of red blood cells to form methemoglobin, which lacks hemoglobin's oxygen-carrying ability.

The failure to properly treat and manage wastewater for irrigation purposes may generate adverse health effects. Leafy vegetables, eaten raw, can transmit contamination from farm fields to consumers. Hookworm infections are transmitted by direct exposure to contaminated water and soils. Most studies that have investigated the risk from consumption of irrigated vegetables have linked a high prevalence of infection in a population with the widespread use of wastewater in agriculture (picture 2).

Picture 2: African farmers exposed to health risks in Ghana.

Other studies proved reclaimed water safety because of the treatment reliability. In the 1970s and 1980s, two studies were conducted in California to develop a reliable wastewater treatment system to produce irrigation water and ensure production of agricultural crops in consort with protection of public health: The Pomona Virus Study (Sanitation Districts of Los Angeles County, 1977) and the Monterrey Wastewater Reclamation Study for Agriculture (Engineering-Science, 1987) (picture 3). A major result of these studies was the demonstration that even food crops that are consumed uncooked could be successfully irrigated with reclaimed water without adverse environmental or health effects (Sheikh et al., 1990). Recent studies demonstrate the safety and suitability of reclaimed water in agricultural irrigation (York et al., 2008).

Almost all types of treated effluents remain moderately to highly saline and consequently their long term use in irrigation may significantly affect the physical and chemical properties of the soil especially under modern and intensive agricultural system in arid and semi-arid areas (Ayars et al., 1993; Hillel, 2000; Pérez-Sirvent et al., 2003; Angin et al., 2005). Inappropriate scheduling of water and fertilizers application has led to salinity buildup under long term use of drip irrigation (Darwish et al., 2005), and the prolonged use of wastewater for irrigation has increased the compaction of the receiving soil and reduced its capacity of holding nutrients (Wang et al., 2003).

Picture 3: Aerial view of the experimental farms and the wastewater treatment plant "WWTP", where the Monterrey Wastewater Reclamation Study for Agriculture was developed.

Wallach et al. (2005) reported the development of soil water repellency under irrigation with secondary treated sewage effluent in field soils. Soil water repellency affected the uniformity of moisture content distribution in the soil profile following irrigation events in the summer, and rainfall events in the winter. Such non-uniform wetting can adversely affect agricultural production and lead to contamination of underlying ground water resources. That is why the use of poor quality water requires some changes from standard irrigation practices, such as selection of appropriately salt tolerant crops, improvements in water management and, in some cases, the adoption of advanced irrigation technology as reported by Oster (1994).

Historically, the quality of irrigation water has been determined by the quantity and kind of salt present in these water supplies. Although crops vary considerably in their ability to tolerate saline conditions (Mass and Grattan, 1999), in general, as salinity increases in the treated wastewater used for irrigation, the probability of certain soil, water, and cropping problems increases. Establishing a net downward flux of water and salt through the root zone is the only practical way to manage a salinity problem (Westcot and Ayers, 1985). Under such conditions, good drainage is essential in order to allow a continuous movement of water and salt below the root zone. Long-term use of reclaimed water for irrigation is not generally possible without adequate drainage. Where drainage water salinity exceeds crop threshold levels the water can be blended with freshwater. Blending, which can be done before or during irrigation, enables farmers to extend the volume of water available (Rhoades, 1999; Oster and Grattan, 2002).

Toxicity due to a specific ion occurs when that ion is taken up by the roots and accumulates in the plant in amounts that result in damage or reduced yield. The ions of most concern in treated wastewater are sodium, chloride, and boron (picture 4). The source of boron is usually household detergents or discharges from industrial plants. Chloride and sodium also increase during domestic usage, especially where water softeners are used. For sensitive crops, toxicity is difficult to correct without changing the water supply. The problem is usually accentuated by severe (hot) climatic conditions (Westcot and Ayers, 1985).

Picture 4: Boron toxicity in pistachio tree irrigated with drainage water, Fresno, California.

In addition to their effects on the plant, sodium in irrigation water may affect soil structure and reduce the rate at which water moves into the soil as well as reduce soil aeration (picture 5). If the infiltration rate is greatly reduced, it may be impossible to supply the crop or landscape plant with enough water for good growth. A permeability problem usually occurs in the first few centimeters of the soil and is mainly related to a relatively high sodium or very low calcium content in this zone (Westcot and Ayers, 1985). At a given Sodium Adsorption Ratio (SAR), the infiltration rate increases as salinity increases or decreases as salinity decreases. Therefore, SAR and ECw should be used in combination to evaluate the potential permeability problem. Sometimes, treated wastewaters are relatively high in sodium and the resulting high SAR is a major concern in planning wastewater reuse projects. Chemical or biological amendments are needed over time to prevent soil structural degradation when irrigating exclusively with sodic water. On calcareous soils that contain appreciable amounts of precipitated or native calcite ($CaCO_3$), the dissolution of calcite in the root zone is enhanced by adding acid formers and by the actions of plant roots that increase the levels of carbon dioxide, thereby providing soluble calcium to offset sodium effects (Qadir et al., 2005).

Picture 5: Soil permeability loss because of the irrigation water sodicity, Fresno, California.

Clogging problems with sprinkler and drip irrigation systems have been reported when treated municipal wastewater is used. The most frequent clogging problems occur with drip irrigation systems. In drip irrigation, vortex emitters were more sensitive to clogging than labyrinth emitters and no significant difference was observed between the same kind of emitter placed on soil or sub-soil; in filters, gravel media and disk filters assured better performance than screen filters (Capra and Scicolone, 2007).

Another possible problem of wastewater reuse is the excessive residual chlorine in treated effluent. Possible damages on plant foliage could occur when sprinklers are used and the concentration of residual chlorine is higher than 1 mg l^{-1}.

The development of sustainable water reclaiming schemes needs to include an understanding of the social and cultural aspects of water reuse. Even for non-potable reuse purposes, the public attitude plays an important role, including the perception of water quality, willingness to pay or to accept any wastewater reuse project (Lazarova, 2000). Studies of public attitudes to water reuse have been carried out since the late 1950s (originally in the United States, but more recently in Europe, Central America, and Africa). Some work has demonstrated that acceptance of water-recycling schemes in general is influenced by the degree of human contact associated with the reuse application. Uses for garden irrigation and toilet flushing are consistently preferred over uses for food preparation and cooking (Bruvold, 1985) (Kantanoleon et al., 2007), even in places where they have been practiced on a large scale for decades without any adverse effects to the public health.

Estimates of non-market costs and benefits, such as health and environmental effects, can inform policies on regulatory targets and intervention programs (WHO, 2005). The most common is contingent valuation, which uses measures of willingness to pay or willingness to accept to quantify non priced goods and services, including non beneficial ones, in cases where health and environmental impacts interact in order to evaluate policy choices more comprehensively. A pioneering study in Spain and among the first in Europe, was carried out in Murcia, a semiarid region where reclaimed water plays a growing role because of the conventional water scarcity. The objective was to estimate the non-market benefits that society attaches to the use of reclaimed water for agricultural purposes. The study proved that the local population obtains significant social non-market benefits from this form of water reuse. This complete study can be seen in Appendix A.

I.3 INTERNATIONAL QUALITY STANDARDS / REGULATIONS AND GUIDELINES

The requirements in regulations about the use of reclaimed water all over the world are based primarily on defining the extent of needed treatment of wastewater together with numerical limits on bacteriological quality, turbidity and suspended solids. The State of California pioneered efforts to promote water reclamation and reuse, being the first reuse regulations promulgated in 1918 (Asano and Levine, 1996).

The World Health Organization, established in 1989 some microbial water quality guideline for irrigation water, recommending a maximum value of 1000 fecal coliforms per 100 milliliters. In addition, a quality guideline for intestinal nematodes was recommended as less than 1 intestinal nematode egg per liter (WHO, 1989). The latest guidelines (WHO, 2006) for the safe use of wastewater in agriculture have been revised considerably, and the fecal coliform concentration has been replaced by a focus on attributable risks and disability-adjusted life years (DALYs) (table 2). These guidelines require to achive a tolerable additional desease burden of $\leq 10^{-6}$ DALY loss per person per year (pppy), although it is recognized that this index may be too stringent in many developing country settings and that a DALY loss of ≤10^{-5} or even ≤10^{-4} pppy may be sufficiently protective of human health (WHO, 2007).

Table 2: Health-based targets for wastewater use in agriculture (WHO, 2006). The health-based targets are based on quantitative microbial risk assessment (QMRA), indicating $\log_{10}$ pathogen reduction required to achieve a disability-adjusted life year (DALY) index of 10^{-6}.

Exposure scenario	Health-based target (DALY per person per year)	Log_{10} pathogen reduction needed	Number of helminth eggs per litre
Unrestricted irrigation	≤ 10^{-6}		
Lettuce		6	≤ 1
Onion		7	≤ 1
Restricted irrigation	≤ 10^{-6}		
Highly mechanized		3	≤ 1
Labour intensive		4	≤ 1
Localized (drip)irrigation	≤ 10^{-6}		
High-growing crops		2	-
Low-growing crops		4	≤ 1

In contrast to the WHO guidelines that focus mainly on the protection of human and public health, in 1985, the FAO has developed a field guide for evaluating the suitability of water for irrigation (Westcot and Ayers, 1985). This guideline, revised few years later (Ayers and Wescot, 1989), recommend some restrictions on water irrigation use related to 1) salinity, 2) rate of water infiltration into the soil, 3) specific ion toxicity, and 4) to some other miscellaneous effects (table 3). The guide is intended to provide guidance to farm and project managers, consultants and engineers in evaluating and identifying potential problems related to water quality. This guide is still the basic reference applicable to irrigation with treated municipal wastewater (Asano et al., 2006). However it is important to remark the "potential restrictions on use" shown in table 3 are somewhat arbitrary since water quality change occurs gradually and there is no clear cut breaking point. It is also necessary to consider that is not possible to cover all local situations when preparing water quality guidelines.

Additional common guidelines on wastewater reuse in all Mediterranean countries have been proposed by Bahri and Brissaud (2003). These documents are based on minimum requirements which should constitute the basis of water reuse regulations in every country of the region. Due to late development of wastewater treatment in several countries, all of them cannot be expected to comply with the guidelines within the same deadline. However, every country could commit itself to reach the guidelines within a deadline depending on its current equipment and financial capacities.

In Spain the Royal Decree-Law 11/95 of National Sewerage and Water Treatment Plan (1995), elaborated within the context of the implementation of the European Directive 91/271/CEE, has been the main planning tool for the development of the different infrastructures concerning sewerage/municipal wastewater and water treatment. In 1996 the Ministry of Environment drew up its first draft of the regulations in reclaimed water use. After years of debate in a variety of commissions and forums, this draft has turned into the Royal Decree-Law 1620/2007, by which the legal regime of treated wastewater reuses is established (Iglesias and Ortega, 2008). Twelve uses are established and grouped into 5 major sections: 1) Urban, 2) Agricultural, 3) Industrial, 4) Recreational and 5) Environmental. The minimum acceptable quality levels are established for each type of use on the basis of the following parameters: a) Biological: *Nematode* intestinal eggs and *Escherichia coli*; and b) Physicochemical: suspended solids and turbidity. Furthermore, the following have been included as additional parameters: a) *Legionella* spp. in the use of industrial cooling or in those cases where an aerosol risk is expected, in compliance with Royal Decree 865/2003; b) *Taenia saginata* and *Taenia solium*, in cases where pastureland for consumption by milk- or meat-producing animals is irrigated; c) Total phosphorus in environmental and recreational uses (ponds, bodies of water and flowing discharges); d) Total nitrogen in the case of aquifer recharge. It has not been considered advisable at present to include in the regulation project other biological parameters that might serve as virus and protozoon indicators, in view of the fact that there is very little local experience and also because such parameters are not included in the regulations in force in other countries that have greater experience in water reuse (United States, Israel, Japan, etc.) (Iglesias and Ortega, 2008).

Table 3: Guidelines for interpretation of water quality for irrigation (Westcot and Ayers,1985; Ayers and Westcot, 1989).

Potential irrigation problem	Units		Degree of restriction on use		
			None	Moderate	Severe
Salinity					
ECw	dS m^{-1}		≤ 0.7	0.7-3.0	≥3.0
TDS	mg l^{-1}		< 450	450-2000	> 2000
Infiltration					
SAR= 0-3	and EC (dS m^{-1})	=	> 0.7	0.7 - 0.2	< 0.2
SAR = 3-6		=	> 1.2	1.2 - 0.3	< 0.3
SAR = 6-12		=	> 1.9	1.9 - 0.5	< 0.5
SAR =12-20		=	> 2.9	2.9 - 1.3	< 1.3
SAR = 20-40		=	> 5.0	5.0 – 2.9	< 2.9
Specific ion toxicity					
Sodium (Na)					
Surface irrigation	SAR		< 3	3 - 9	> 9
Sprinkler irrigation	mg l^{-1}		< 70	> 70	
Chloride (Cl)					
Surface irrigation	mg l^{-1}		< 140	140 - 350	> 350
Sprinkler irrigation	mg l^{-1}		< 105	> 105	
Boron (B)					
Surface-Sprinkler	mg l^{-1}		< 0.7	0.7 - 3	> 3
Miscellaneous effects					
Nitrogen					
(Total N)	mg l^{-1}		< 5	5 - 30	> 30
Bicarbonate					
(overhead sprinkling only)	mg l^{-1}		< 90	90 - 500	> 500
pH	-			Normal Range 6.5 – 8.4	

I.4 BENEFITS OF WASTEWATER REUSE AND POTENTIAL APPLICATIONS

The potential applications of reclaimed water are divided into potable and non-potable use.

Potable use of reclaimed water is also divided into direct and indirect. Direct potable use is the use of reclaimed water to augment drinking water supply following high levels of treatment. The effluent of a WWTP is sent directly into the intake of a drinking-water treatment and distribution plant. Although this process involves the most technologically advanced water treatment processes, and the water that enters in the distribution system must meet drinking-water standards, it is the one most argued against use by community citizen groups, calling it "toilet-to-tap". In fact, it is presently used only in water-critical situations. Indirect potable use is the use of reclaimed water after passing through the natural environment. The main technology involving groundwater is reservoir infiltration, in which an aquifer is supplemented with treated wastewater infiltrating from a surface basin, both as a water-supply supplement and as a safeguard against saltwater intrusion.

Non-potable use is the immediate diversion of treated effluents to:

- Agricultural irrigation
- Landscape irrigation: parks, school yards, freeway medians, golf courses, cemeteries, greenbelts and residential.
- Recreational and environmental uses: Lakes and ponds, marsh enhancement, streamflow augmentation, fisheries and snowmaking.
- Industry and urban settlements: cooling water, boiler feed, process water heavy construction, fire protection, air conditioning and toilet flushing.

The main benefits in the use of reclaimed water in irrigation are the conservation of fresh water for other uses, the potential use of plant nutrients contained in reclaimed water and the reduction in wastewater discharge to the ecologically-sensitive aquatic environment through nutrient management (Asano, 1998; Queensland Water Recycling Strategy, 2001; Mantovani et al., 2001).

In accordance with Murcott (1995) the ideal characteristics of water to be used in crop irrigation are: (a) high organic content; (b) high nutrient content (N, P, K); (c) low pathogen content and (d) low metal and toxic organic compounds contents. The value of nutrients in reclaimed water can be an important economic consideration, particularly as the cost of fertilizers increases with the cost of energy used to produce them. For irrigation application, these nutrients can be beneficial and can supply a significant portion of plant needs (USEPA, 2004). This can then reduce the amount of fertilizer that might be applied (Lazarova and Bahri, 2005; USEPA, 2004). Supplemental fertilization with specific nutrients not provided by reclaimed water may still be required depending on plant species and the recommended rates for desired results. Nutrients are immediately available to the crop, as long as they remain dissolved in wastewater and soil solution, but may be rendered less available by several soil processes. Hence, the proportions of nutrients taken up by plants are different to the proportions of nutrients applied via wastewater or fertilizers (Janssen et al., 2005).

I.5 ACTUAL SITUATION OF THE RECLAIMED WATER USE FOR AGRICULTURE AND FUTURE CHALLENGES

One-tenth or more of the world´s population consumes food grown with irrigation supplied by wastewater (Smit and Nasr, 1992). Jimenez and Asano (2004) suggest that at least 2 million hectares (ha) are irrigated with untreated, partly treated, diluted, or treated wastewater. The estimated area would be larger if the land irrigated from rivers and canals that receive wastewater were considered.

Almost all water reuse in developing countries is for agricultural purposes. In most of these cases, the farmers irrigate with raw, diluted, or partly treated wastewater. In many cities of Asia, Africa and Latin America, engineered wastewater collection systems and wastewater treatment facilities are nonexistent. In West African cities, usually less than 10% of the generated wastewater is collected in piped sewage systems and receives primary or secondary treatment (Drechsel et al., 2006). Besides crop farming, wastewater is used also for aquaculture in Africa, and in Central, South, and Southeast Asia (Bangladesh, Cambodia, China, India, Indonesia, and Vietnam) (Asano, 1998; Asano et al., 2007). The failure to properly treat and manage wastewater generates adverse health effects. Farmers and their families using untreated wastewater are exposed to health risks from parasitic worms, viruses and bacteria. Shuval et al. (1986) and Blumenthal et al. (2001) showed that wastewater use can increase the risk of intestinal nematode infections, particularly *Ancylostoma* (hookworm) and *Ascaris*, in farmers in India and Mexico. Others have shown that in Egypt, consumption of vegetables irrigated with wastewater can increase the risk of *Ascaris* and *Trichyris* infections in the general public (Khalil 1931; Shuval et al., 1984). Generally, farmers irrigating with wastewater have higher rates of helminth infections than farmers using freshwater (Trang et al., 2006). In addition, skin and nail problems may occur among farmers using wastewater (Van der Hoek et al., 2002; Trang et al., 2007).

In the developed countries, United States and Israel were pioneers in using reclaimed water for crop irrigation in the early 1900s. In the United States, the Water Reuse Association (an organization promoting water reuse research and implementation), estimates that around 10 million cubic meters per day of municipal wastewater are reclaimed and reused currently, and reclaimed water use on a volume basis is growing at an estimated rate of 15 percent per year (Water Reuse Association, 2005). Agricultural and landscape irrigation is the dominant use of reclaimed water in

California (67 percent of the total water reuse by volume). In recent years, Florida has risen to become a recognized leader in water reuse along with California in the United States. In Israel wastewater accounted for 15% of water resources in 2000 and could reach 20% by 2015 (IWMI, 2007). In Australia, over 500 municipal wastewater treatment plants now engage in the water reclamation of at least part of their treated effluent. Specific water reclamation and reuse targets have been established in major cities for irrigation purposes (Radcliffe, 2004; Anderson, 2005).

There are about 700 reuse projects in Europe (CIWEM, 2009). Reuse projects are more numerous in Southern European countries (Cyprus, France, Greece, Malta, Portugal, Spain,) than in the Northern region. The use of reclaimed water is quite different between those two regions: in southern Europe, reclaimed wastewater is reused predominantly for agricultural irrigation (44% of the projects) and for urban or environmental applications (37% of the projects); in northern Europe, the uses are mainly for urban or environmental applications (51% of the projects) or industrial (33% of the projects) (Bixio et al., 2006). Agriculture is expected to remain the largest water user in the Mediterranean countries, with more irrigation and warmer and drier growing seasons resulting from climate change (Marecos, 2007).

In Spain, 368.2 hm^3 y^{-1}, equivalent to 10.8% of the total treated wastewater volume per year, are reused in agriculture (Iglesias et al., 2010). The main projects on reclaimed water use are concentrated at the spanish Mediterranean coast and the islands. Valencia and Murcia reuse 63 % of the whole treated wastewater, and the islands (Canaries and Balearic) reuse 12.5%. Other projects are in the inland of the country such as one in Madrid and another in Vitoria which use 5 hm^3 $year^{-1}$ and 11.5 hm^3 $year^{-1}$ respectively (Iglesias, 2005).

The Segura basin (Murcia) is the only Spanish basin whose natural water resources cannot cover its water demands; for this reason, the use of treated municipal wastewater in agriculture is especially important and this drives Murcia to be a forerunner in the additional treatment and reuse of treated wastewater.

I.5.1 Research studies at field conditions

Studies on the effects of reused treated wastewater were launched initially in countries with high technological capabilities, and a certain economic level such as the USA (California, Florida, Arizona) or Israel. These were subsequently joined by the Mediterranean countries and more recently Japan and the Arab countries with economic potential. During the 1970´s, studies were initiated in forest areas, golf courses or forage crops (Bole and Bell, 1978; Burton and Hook, 1979) with the aim of increasing their biomass. In the 1980´s the treated wastewater to irrigate fruit trees was handled successfully in trees like apple (Neilsen et al., 1989a) or peach (Basiouny, 1984) and some other crops such as grapes (Neilsen et al., 1989b) cotton (Feigin et al., 1984) and some cereals, maize and alfalfa (Campbell, 1983). Furthermore, its use was extended to ornamental species (Hasek, 1986). These studies have been mainly conducted to see the effects on plant and soil, and the safety and security of this irrigation practice. Studies carried out at farm-scale had the objective to evaluate the agronomic aptitude of reclaimed water for the irrigation of different crops (horticultural crops, forage crops, fruit trees, ornamental and forest plants) under different culture systems (greenhouse, open-field) and using different water application techniques and strategies. However, few studies have evaluated the effects of treated wastewater on environmental pollution, plant physiology or crop production in field conditions. Most of these field experiments are reported in the following paragraphs.

I.5.2 Horticultural crops under greenhouse conditions

With the objective to examine the possibility of using treated wastewater for irrigation, Kalavrouziotis et al. (2005) conducted a greenhouse experiment on onion (*Alium cepa*) and lettuce (*Lactuca sativa*) grown on clay loam soil. They detected P accumulation on soil whereas Mn and Zn were accumulated mainly in lettuce roots and seeds. Another experiment with the same conditions (greenhouse, wastewater composition and clay loam soil), was conducted on Broccoli (*Brassica oleracea var. Italica*) and Brussels sprouts (*B.Oleracea var. Gemnifera*) where a significant increase in the content of some macro- and micro-elements in the soil was seen, especially for P, Zn and Cd. The heavy metal contents in the edible plant parts, and the heavy fecal coliforms and *E.coli* load of the treated wastewater constituted a high health risk factor (Kalavrouziotis et al., 2008).

In 2006, Manios et al. realized an experiment on potted tomato (*Lycopersicum Esculentum* mil Mountain Spring F1 hybrid) and cucumber (*Cucumis Sativus* L) under greenhouse conditions using reclaimed water treated at different levels (primary, secondary and chlorinated secondary treated wastewater). The primary treated wastewater showed the most significant plant development in both crops. Nonetheless, the use of un-disinfected wastewater in such greenhouse cultivations did not prove to be completely safe.

In Spain, Segura et al. (2001) presented the results of a study conducted on melon (*Cucumis melo* L.) cv. Galia grown on a sand-mulched soil under greenhouse conditions during a spring cycle (124 days). They compared the effects of applying reclaimed water versus those of using underground water commonly used in irrigation. No significant differences were found among treatments on the tested production parameters. The use of wastewater to fertilize *Cucumis melo* had positive effects on the addition of fertilizer since the application of total N and K was reduced by 40.8 and 17.8%, respectively. Microbiological analysis of fruits showed no contamination by indicator microorganisms (*E. coli*) even in fruits in contact with soil.

I.5.3 Horticultural and forage crops

In Jordan, experimental plots with three crops, alfalfa, radish and tomato, were irrigated with treated wastewater by sprinklers on a silty loam soil and no significant effects were seen on the soil and in the crops (Shahalam et al., 1998).

In the same place a long term experiment was carried out on forage crops (barley). The irrigation with wastewater proportionally increased salts, organic matter and plant nutrients in the soil as well as the whole plant biomass.

In 2005, a short study (two years) on forage crop (*Medicago sativa)* was carried out by Palacios et al., under subsurface drip irrigation (SDI) using saline and sodic reclaimed water irrigation (EC: 2.24 dS m^{-1} and SAR: 6.9). The results obtained were positively satisfactory, mainly on plant germination and final yields. Furthermore the feasibility of SDI using secondary effluent was demonstrated.

Based on these results, it can be concluded that proper irrigation management strategies and periodic monitoring of soil and plant quality parameters are required to ensure successful and safe long-term irrigation when treated wastewater are used (Mohammad Rusan et al., 2007).

I.5.4 Fruit trees

In the 80s, Förster et al. (1988) investigated the impact on the soil, the plants and the yields of different fruit trees when irrigated with wastewater from food processing industries. The main outcomes were positive in terms of no negative accumulation of harmful substances in the soil and higher yields of some crops. A recent study with saline treated industrial wastewater generated by textile firms, has demonstrated to be a valid alternative for irrigation of olive orchards in field with trickle irrigation and continuous monitoring of mineral levels (Al-Absi et al., 2009).

Another study in olive groves to see the effects on microbiological quality of soil and fruits, confirmed that under suitable conditions, low-quality wastewater can be useful as an additional water resource for olive irrigation in water-scarce Mediterranean environments (Palese et al., 2009).

In citrus trees, a municipal reclaimed water project called Water Conserv II, was developed in Florida. During ten years, irrigation water, soil and leaf samples were analyzed yearly. Fruit quality was determined before harvest. Citrus growers irrigating with reclaimed water were encouraged to use higher-than-recommended amounts of water as a means of disposal of this reclaimed source, however, this practice lead to increased weed growth and dilution of juice solids per box of fruit. Leaf boron and magnesium were significantly higher after irrigation with reclaimed water. Calcium and boron from the reclaimed water have eliminated the need for liming in the receiving soils as well as the annual foliar sprays containing boron (Zekri and Koo, 1993; Zekri and Koo, 1994; Morgan et al., 2008).

In 1997, Aucejo et al. reported boron toxicity in a citrus plantation in Valencia (Spain) irrigated with a mix of surface water, groundwater and treated wastewater. Boron pollution was attributed to industrial wastewater spills and fluorine contamination from atmospheric pollution.

Good agronomic results were found by Reboll et al., (2000) after studying for 3 years the effect of treated wastewater in *Navelina* orange trees, observing that both growth and fruit quality parameters were unaffected by the high levels of sodium, chloride and boron in wastewater. It was observed that chlorides, sodium and boron foliar concentration did not exceed toxicity levels.

Finally, another study in Florida, showed that reclaimed water alone did not provide adequate nitrogen nutrition for young grapefruit trees (Maurer and Davies, 1993).

In fruit trees, a pilot field study in coffee (*Coffea arabica L.*) irrigated with a secondary treated wastewater was conducted in Brazil. After 3 years, the study revealed that treated wastewater can be a new source of irrigation water, but new management strategies are needed to diminish sodicity risks and to sustain adequate and balanced nutritional conditions in the soil-plant system (Herpin et al., 2007).

I.5.5 Ornamental and forest plants

The ability of some Mediterranean plant species (*Olea europa, Nerium oleander, Geranium sp.*) to absorb heavy metals present in wastewater from treatment plants was researched by Drakatos et al. (2000). After a series of tests, the results showed that Geranium sp. absorbed Cu and Zn in its leaves and afforded high concentration of these elements in contrast to the other plants. The tolerance and durability of geranium under such adverse conditions offers possible options for using treated wastewater contaminated with heavy metals.

In roses, an experiment was conducted to see the effect of irrigation with secondary treated wastewater on growth, production and quality. Results showed that the visible appearance of the plants, their growth, the quantity and size of the flowering stems and their postharvest performance were not affected by the irrigation treatments (Nirit et al., 2006).

The treated wastewater reuse for reforestation with *Pinus brutia* and *Pinus marítima* was studied during three years, measuring and analyzing the mortality and the tree height increase. After three years, the treated wastewater reuse for reforestation in these species was proved successful in many regions (Kalavrouziotis et al., 2004).

I.5.6 New trends in reclaimed water research

Little is known about the combined effect of reclaimed water and different irrigation levels: The effects of rootstock variety (41B, 1103P and 110R), irrigation level (0.50, 0.75 and 1.00 of the evapotranspiration) and water quality (reclaimed versus freshwater) on water relations and gas exchange of potted Soultanina grapevines were investigated during two growing seasons. An early reduction of predawn leaf water potential (Ψ_{pd}) was detected for vines irrigated with recycled water in both seasons. However, assimilation rate (P_n) and stomatal conductance (g_s) were reduced only late in the first year in the treatment of reclaimed water at higher irrigation levels. This was consistent with the higher reduction of Ψ_{pd} at these treatments, indicating that the development of a water deficit due to salt accumulation reduced gas exchange (Paranychianakis et al., 2004).

There is insufficient information on the presence of additional heavy metal and some chemical contaminants in the reclaimed water: A general risk-assessment approach to the determination of acceptable contaminant concentrations was studied by Weber et al. (2006) Examples of some key modeling calculations were provided for three selected contaminants (chloroform, 1,1,2-trichloroethane and pyrene) during hypothetical irrigation of agricultural areas. The results of the hypothetical modeling exercise indicate that the contaminants considered in the theoretical analysis pose an acceptable risk to human health via the single exposure path considered (uptake through food grown in the irrigated soil). Finally, risk assessment of chemicals regarding wastewater reuse will, in many cases, need to be undertaken with respect to environmental organisms, as well as humans.

Few studies have combined the epidemiological component with water quality assessment: Studies are needed which consider the adaptation and partial resistance of local populations to the commonly elevated pathogen exposure in cities of developing countries (Qadir et al., 2010).

Finally, from the environmental point of view, s*ome criteria in order to asses the sustainability of water reuse proyects must be developed:* Sala and Serra (2004) established five issues to be evaluated: i) water reuse as a tool for efficient allocation of water resources; ii) ecological analysis of the cycle of the main pollutants (carbon, nitrogen and phosphorus); iii) public health protection; iv) energy balances in the municipal water cycle; and v) determination of the positive externalities. According to these criteria, these authors found interesting results related to the sustainability of reclaimed water use in the agriculture: a) The benefits for reclaimed water users are both, the water itself and its nutrients, reducing their need for external fertilizers and making important savings. In parallel, recycling nutrients means that these nutrients are not discharged. b) The use of wells in which the pumps run on gasoil is 2.6 times more expensive than using high nutrient-content reclaimed water and 2 times the cost of using reclaimed water with a low nutrient content. c) In terms of CO_2 balances, the conversion of dry farming areas into irrigation fields by using reclaimed water also doubles the atmospheric CO_2 uptake capacity.

II CHEMICAL AND MICROBIOLOGICAL STUDY OF USING RECLAIMED WATER FOR IRRIGATION OF LEMON TREES

II.1 INTRODUCTION

Citrus are of the most important crops in the world as the quantity of fresh fruit entering international trade is only exceeded by banana. The actual world production is about 98.7 million tons of fresh fruit, of which 6.5% is produced in Spain (FAOSTAT, 2008). In 2009, the Spanish production amounted to 6.4 million tons from 318,000 ha of which 8% are produced in 37,587 ha in the Region of Murcia (CARM, 2009; MARM, 2009). This Region produces more than 41% of the national lemon production. This important contribution is mainly due to the use of intensive localized fertigation techniques, where 80% of the total area is under drip irrigation (CARM, 2009). The estimated irrigation requirements for lemon trees in Murcia are 6,407.1 m^3 ha^{-1} $year^{-1}$ (CARM, 2007). However, the actual average applied amount is often less than what is required by the plant due to frequent drought conditions in the region. Water shortage impacts and the implementation of the Water Framework Directive (WFD) are pushing European farmers to adopt more efficient irrigation methods, including the use of reclaimed water.

The use of reclaimed water in agriculture is an important management strategy in areas with limited freshwater resources. Such a strategy is important because of its potential economic and environmental benefits. It is therefore necessary to initiate and support wastewater reuse projects all over the world, particularly since the population and demand for food is growing steadily and the fresh water resource will not increase.

In Murcia Region, water shortage is the main factor limiting agricultural production (Cascales, 1997). The water deficit in the Segura Basin, together with the ever-increasing demand due to the continued urban growth in the coastal zone and the major demand from intensive agricultural activity, has made it necessary to use treated wastewater for irrigation.

The aim of this work was to study the effects of reclaimed irrigation-water on lemon tree performance in the Region of Murcia. In particular, this research was to compare two sources of treated wastewater, one obtained with a secondary treatment and the other with a tertiary treatment, and to study their effects on soil chemical properties, on the leaf mineral status, crop production and fruit quality and safety.

II.2 MATERIAL AND METHODS

II.2.1 Experimental conditions

The experiment was conducted during 2005-2007 in two locations planted with lemons in the Region of Murcia. The first experimental plot was located in San Felix, a small village four km to the north of Cartagena (37º3´N, 0º58´W). The orchard size was 12 ha of the 'Fino' lemon variety grafted on 'Macrophyla' rootstock (picture 6). The trees were 7 year-old planted at 7 m between rows and 5 m between trees. The irrigation-water was supplied by drip irrigation with eight self pressure-compensating emitters per tree, each with a flow rate of 4 l h^{-1}. The second experimental plot was located in Campotejar, 7 km to the north of Molina de Segura (38º07´N, 1º13´W). At this site the orchard size was 10 ha, cultivated with the same crop (variety and rootstock), tree age, plant spacing and overall irrigation management practices as those at the Cartagena site (picture 7). The soil at Cartagena was classified as a clay loam soil (32% clay, 32% loam and 36% sand) whereas the soil at Campotejar was silty clay (48% clay, 41% loam and 11% sand).

Picture 6: View of Cartagena´s experimental site.

During the experimental period, the average annual precipitation was 285 and 287 mm and the average annual crop Evapotranspiration "ETc" was 947 mm and 903 mm in Cartagena and Campotejar respectively (table 4). In Cartagena, the used reclaimed water was derived from a secondary treatment, while that used in Campotejar

Picture 7: View of Campotejar´s experimental site.

was treated to a tertiary level and mixed with underground water of better quality. Therefore, the greatest difference between both experimental plots was the quality of water used for irrigation.

Following the common farmers practices, the irrigation doses was scheduled on daily basis from January 2005 until December 2007. The total irrigation amounts were measured with inline water flowmeters. The average amount of water applied at both locations was 571 mm y^{-1} and 545 mm y^{-1} in Cartagena and Campotejar respectively (Table 4). The fertilizers rates of N-P_2O_5-K_2O applied through the drip irrigation system were 240-90-100 (kg ha^{-1}) in Cartagena and 220-90-90 (kg ha^{-1}) in Campotejar.

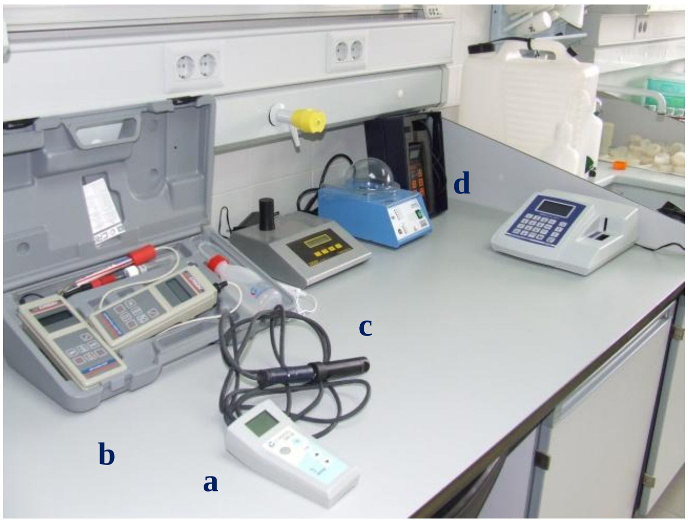
Table 4: ETc, rainfall and irrigation (mm $month^{-1}$) in Cartagena and Campotejar. The values are the monthly average from data collected during 2005, 2006 and 2007.

Cartagena	**JAN**	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**	**TOTAL**
ETc	21.78	37.15	78.48	93.50	121.96	137.94	142.54	123.81	90.81	46.19	30.38	23.40	**947.94**
Rainfall	53.67	28.27	14.27	39.33	11.73	0.67	0.13	6.73	27.60	23.13	65.53	14.67	**285.73**
Irrigation	27.64	16.03	51.86	29.07	81.85	46.16	83.98	87.50	80.36	33.16	18.44	15.41	**571.46**
Campotejar													
ETc	26.04	36.69	88.36	92.11	97.52	136.80	145.51	107.28	93.69	56.83	38.73	34.23	**903.78**
Rainfall	35.37	27.37	17.80	60.77	30.03	1.27	0.60	13.40	33.40	30.43	29.06	7.87	**287.36**
Irrigation	6.47	5.42	12.66	47.33	75.78	92.49	87.21	116.88	67.11	12.23	6.36	15.09	**545.03**

II.2.2 Water analysis

Water samples were collected bimonthly between 2005 and 2007 in order to characterize the irrigation-water quality at both locations. Four samples from each irrigation source were collected in glass bottles, transported in an ice chest to the lab and stored at 5ºC before being processed for chemical analyses. The concentration of macronutrients (Na, K, Ca, Mg), micronutrients (Fe, B, Mn) and heavy metals (Ni, Cd, Cr, Cu, Pb, Zn) were determined by Inductively Coupled Plasma (ICP-ICAP 6500 DUO Thermo, England); anions (chloride, nitrate, phosphate and sulphate) were analysed by ion chromatography with a Chromatograph Metrohm (Switzerland); pH was measured with a pH-meter Cryson-507 (Crisom Instruments S.A., Barcelona, Spain); electrical conductivity (EC) and total dissolved solids (TDS) were determined using the multi-range equipment Cryson-HI8734 (Crisom Instruments, S.A., Barcelona, Spain) and turbidity was measured with a turbidity-meter Dinko -D-110 (Dinko Instruments S.A., Barcelona, Spain) (picture 8). The microbiological quality of irrigation water was assessed by determining of the number of total coliforms, fecal coliforms and *E.coli* present in 100 ml volume.

Samples were filtered using a vacuum system through a sterile 0.45-µm-pore-size membrane filters (picture 9) (Millipore, Billerica, USA). Colony formation was gotten after incubation on top of Chromocult agar plates (Merck, Darmstadt, Germany) for 24 h. Incubation temperatures were 37 ºC for total coliforms and *E. coli,* and 44.5 ºC for fecal coliforms. Microbial counts were expressed as log cfu ml^{-1}. The helmint eggs were measured following the Bailenger´s method (Bailenger, 1979). For *E.coli* O157:H7 detection, enrichments were prepared pouring 25 ml of water samples into sterile stomacher bags and adding 225 ml of mTSB + Novobiocin (Oxoid, Basingstoke, Hampshire, UK). Once homogenized, enrichments were incubated at 37 ºC for 24 h. After incubation, enrichments were spread-plated on Sorbitol MacConkey Agar (Scharlau chemie, Barcelona, Spain) containing CT-supplement (Merck KGaA, Darmstadt, Germany), and incubated further for 24 h at 37 ºC. Presumptive *E.coli* O157:H7 colonies (colorless) were selected and stored in eppendorf tubes containing TSB + 10% glycerol at -20 ºC, before performing PCR analysis.



Picture 8: Equipments for water quality assessment: a) dissolved oxygen meter, b) pH-meter, c) turbidity-meter and, d) Multi-range equipment (EC & TDS)

Picture 9: Microbial water analyses through a vacuum system filtering a sterile 0.45-µm-pore-size membrane filters.

II.2.3 Soil analysis

Twelve soil samples were randomly collected from the top soil layer (0 to 20 cm depth), midway between emitters which are spaced 90 cm apart. This operation was repeated every three months over the experimental period (January 2005 till December 2007) and in both locations (Cartagena and Campotejar). The soil was dried at room temperature for 1 week, ground and sieved through a 2 mm nylon mesh before analysis. Correction for dry mass was obtained from a separate portion by drying at 105ºC for 24h. Organic matter (OM) and total N were analysed using an automatic micro-analyser Flash EA 1112 Series (England) and Leco TruSpec (Sant Joseph, USA). The macro-elements, microelements and heavy metals were determined by Inductively Coupled Plasma (ICP- ICAP 6500 DUO Thermo, England) after nitric-perchloric acid (2:1) digestion (Thompson, 1982). Anions were analysed by ion chromatography with a Chromatograph Metrohm (Switzerland) after using a standard soil:distilled water ratio at 1:2.5 (w:w). The pH was determined on saturated soil-paste with a pH-meter Cryson-507 (Crisom Instruments S.A., Barcelona, Spain). The EC was determined in 1:5 aqueous soil extracts and measured by multi-range equipment Cryson-HI8734 (Crisom Instruments, S.A., Barcelona, Spain). $EC_{1:5}$ was converted into EC_e using the formula $CE_e = 8.3\ CE_{1:5} – 0.9$ (Aragüés et al., 1981).

For soil microbiological analysis, another twelve soil samples were randomly taken from the soil surface within the wetted diameter below the emitter. Each sample was placed in sterile closed container suitable for isolation from environmental contaminations. Samples were transported in an ice chest to the lab and stored at 5ºC before being processed. Later on, to determine the microbial quality of soil samples, 25 grams of soil were homogenized in a 1:10 dilution of sterile 0.1 % buffered peptone water using sterile filter stomacher bags (Seward Limited, London, UK) and a stomacher (IUL Instrument, Barcelona, Spain) for 90s and plated on chromocult plates to determine the loads of total and fecal coliforms and *E.coli* as previously described. Microbial counts were expressed as log cfu g^{-1}.

II.2.4 Leaf analysis

Spring flush leaves from non-fruiting branches were sampled every three months during 2005, 2006 and 2007. Twenty leaves were randomly sampled from 12 trees at each location. Leaves were washed with a detergent (alconox 0.1%), rinsed with tap water, cleaned with a dilute solution of 0.005% HCl and finally rinsed with distilled water and left to drain on a filter paper before being oven dried for at least 2 days at 65 ºC. Dried leaves were ground and a nitric-perchloric acid (2:1) digestion (Thomson, 1982) was executed. Replicate samples (0.25 g) were digested by *Aqua Regia* acid HCl/HNO_3. The concentration of macro-elements, microelements and heavy metals were determined by Inductively Coupled Plasma (ICP- ICAP 6500 DUO Thermo, England). Anions were analysed by ion chromatography with a Chromatograph Metrohm (Switzerland) after using a standard leave to distilled-water ratio of 1:2.5

(w:w). Total N and C concentrations were measured using an automatic micro-analyser Flash EA 1112 Series (England) and Leco Truspec (Sant Joseph, USA).

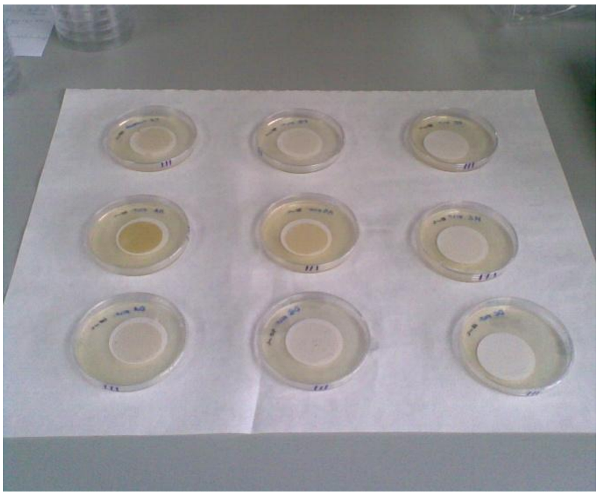
II.2.5 Fruit analysis

The parameters of fruit quality were measured on 100 fruits randomly collected during harvesting from each location (Cartagena and Campotejar) and year.

Quality Indexes: Titratable acidity (TA), pH and soluble solid content (SSC) were determined by titration of 10 ml of juice with 0.1 mol l^{-1} NaOH to pH 8.1. The pH values were measured using a pH meter and the SSC was measured with an Atago N1 handheld refractometer (Tokyo, Japan).

Microbial analyses: Peel samples were taken from the skin of the fruit by a cork borer of 7.1 cm^2. At least three peel disks from each fruit were taken and each sample was composed of five fruits. Samples were placed in a 250 ml sterile flask containing 50 ml of sterile 0.1% buffered peptone (BPW, AES Laboratoire, Combourg, France) and vigorously shaken using a IKA-VIBRAX-VXR mixer for 5 min. The peptone wash solution was diluted in sterile 0.1% peptone and plated on appropriated media. Total aerobic mesophilic bacteria were enumerated by using plate count agar (PCA) (Scharlau Chemie S.A., Barcelona, Spain) after incubation for 48 hours at 30 ºC. Total and fecal coliforms and *E. coli* were isolated by using Chromocult agar (Merck, Darmstadt, Germany) after incubation for 24 h at 37 ºC for total coliforms and E. coli and 44.5 ºC for fecal coliforms (Picture 10). Yeast and moulds were enumerated in Rose Bengal agar (Scharlau Chemie S.A., Barcelona, Spain) by spread-plating 100 µl of the appropriate sample dilution and incubated at 30 ºC for 48–72 h. Microbial counts were expressed as log cfu cm^{-2}.

Identification of Escherichia coli O157:H7 by immunochromatographic rapid test: The GLISA Singlepath® *E. coli* O157 (Merck) was used for rapid test for *E. coli* O157:H7 in fruit samples. To perform the test, samples of 25 g of lemon peel were homogenized with 225 ml of EHEC (Enterohemorrhagic *E. coli*) enrichment broth (EEB) [tryptic soy broth (TSB) + bile salts (1Æ5 g l–1) (No. 3) + VCC (vancomycin, cefixime and cefsulodin)- selective supplement] (Oxoid). Five ml of post-enrichment EEB culture were transferred to a polypropylene cap tube and placed in a boiling water bath for 20 min. After cooling to room temperature, three-falling drops (150 µl) were placed in the Singlepath test device sample port, and after 15 min, results were read and scored according to the description of the test. Samples were considered presumptive positive when red lines appeared on both test and control zones at or prior to 20 minutes.



Picture 10: Fecal coliforms and *E. coli* isolated after incubation for 24 h at 44.5 and 37 ºC, respectively.

II.3 RESULTS AND DISCUSSION.

II.3.1 Irrigation-water quality

The physical-chemical and microbiological parameters measured during three years for both sources of irrigation-water collected from Cartagena and Campotejar are depicted on table 5. The quality of the two sources was significantly different in terms of sodium, chlorine and boron concentrations as well as EC, TDS and pathogen contents (fecal coliform, *E.coli* and helmith eggs). The values of these parameters were significantly higher in the irrigation-water coming from Cartagena than that taken from Campotejar. This is due to the fact that the reclaimed water of the latter source was, on the one hand, blended with underground water of better quality and therefore this reduced its corresponding ion concentration. On the other hand, this water received a tertiary treatment which considerably decreased its pathogen content.

In both sources of irrigation-water the average sodium concentrations observed over the three years were higher than the threshold of restriction on use (>9 meq l^{-1}) recommended in the FAO-bulletin on water quality for agriculture (Ayers and Westcott, 1985). However, the corresponding concentrations of calcium and magnesium were high enough to maintain the sodium adsorption ratio “SAR” within the range of 0 to 10. This indicates the presence of a reduced sodification power, especially when the SAR and EC are compared together (Rhoades, 1982)

The average chloride concentration registered over the three years was 15.86 and 10.60 meq l^{-1} in Cartagena and Campotejar sources respectively. These values are higher than the threshold of 4.28 meq l^{-1} determined in Australia for citrus trees by Cole (1985), under warm, dry, summer conditions. This author estimated a yield decrease of about 20% for each increase of 1 meq l^{-1} of Cl^- concentration in the irrigation water above the threshold value.

The average value of the electrical conductivity over the experimental period was 2.80 in Cartagena and 2.10 dS m^{-1} in Campotejar. According to Maas (1993), salinity problems can appear when the EC of the irrigation water is higher than 2.0 dS m^{-1}.

The average level of Boron concentration of 0.96 ppm detected in the water of Cartagena was twice the average level measured in Campotejar (0.50 ppm). According to Parsons et al. (2001) a 1 ppm threshold is probably a safe upper level of B in irrigation water for citrus. Like Cl, high concentrations of B can also cause phytotoxic problems in citrus trees (Ayers and Westcot, 1985; Walker et al., 1982). In numerous studies it has been demonstrated that excess B reduces tree growth and productivity, and contributes to defoliation and leaf injury (Chapman, 1968).

The microbiological quality of the irrigation water used in Cartagena showed high levels of fecal coliforms, exceeding the limits for restriction on use recommended by the World Health Organization (>1000 cfu 100 ml^{-1}) (Cairncross and Mara, 1989) and the U.S. Environmental Protection Agency (>14 cfu 100 ml^{-1}) (Asano and Cortuvo, 1998). The fecal coliforms are a subset of coliform bacteria often used to estimate the concentration of *E.coli* in general and the presence of *E.coli* O157:H7 in particular. The latter is an enterohemorrhagic strain of the bacterium *E.coli* and a cause of foodborne illness mostly associated with eating undercooked, contaminated ground beef, swimming in or drinking contaminated water, and eating contaminated vegetables (Park et al., 1999). In our case, *E.coli* O157:H7, was not detected in any of the irrigation water samples.

Table 5: Inorganic analysis, physical-chemical and microbiological characteristics of irrigation water used in Cartagena and Campotejar. Each data represents the mean of 24 values ± the standard deviation measured on water samples collected during 2005, 2006 and 2007.

	2005			2006			2007		
IRRIGATION WATER	Cartagena	Campotejar		Cartagena	Campotejar		Cartagena	Campotejar	
Na (meq l^{-1})	16.1± 0.4	14.5±0.5	*	14.5± 0.4	13.5±0.2	*	15.9 ± 0.4	14.6±0.1	*
Cl (meq l^{-1})	16.3±3.1	13.5±0.6	*	16.2±0.3	7.6±0.3	**	15.1±0.5	10.7±1.5	**
B (ppm)	0.7±0.1	0.3±0.1	*	1.1±0.1	0.7±0.1	*	1.1± 0.1	0.5±0.1	**
Ca (meq l^{-1})	6.1± 0.6	6.1±1.2	ns	5.3±1.3	3.4±1.1	ns	5.1± 0.6	5.8±0.6	ns
Mg (meq l^{-1})	4.1±0.1	4.2±0.4	ns	4.1±0.8	3.3± 0.7	ns	3.2±0.1	3.2±0.2	ns
SAR	8.1±0.5	6.4±0.7	ns	6.7±0.4	7.4±0.8	ns	7.8±0.3	6.9±0.5	ns
NO_3 (ppm)	4.2±1.2	4.8±1.3	ns	3.8±1.3	5.9± 1.2	ns	7.5±2.1	4.2±1.5	ns
H_2PO_4/HPO_4 (ppm)	3.8±0.3	4.2±1.2	ns	3.1 ± 0.6	3.0± 0.1	ns	4.8±2.1	5.5±2.1	ns
SO_4 (meq l^{-1})	3.4±1.5	5.1±0.2	ns	3.6±1.9	5.5±1.7	ns	2.6±0.5	4.6±1.9	ns
EC (dS m^{-1})	3.3±0.5	2.2±0.2	*	2.8 ± 0.2	2.1±0.1	*	2.8±0.3	2.1±0.2	*
TDS (mg l^{-1})	2060±235	754±41	**	1589 ± 362	945±54	*	1510±254	883±110	*
Fecal coliforms (cfu 100ml^{-1})	3800±120	280±21	**	4320± 125	234±32	**	2240± 86	478±56	**
E.Coli (cfu 100ml^{-1})	820±43	92±11	**	1265±150	78±21	**	760±70	45±8	**
Helmints (eggs 10 l^{-1})	< 10	< 10	ns	< 10	< 10	ns	< 10	< 10	ns

Mean content (n=24), *, **, statistically significant at P<0.05 and P<0.01 level of significance, respectively.

II.3.2 Soil chemical composition

The results of the chemical analysis of ion concentrations in soil samples collected from Cartagena and Campotejar are shown in table 6. The concentrations of Na, K, Ca and Mg did not show significant difference between the soils of both locations and were almost constant over the experimental time period. The concentrations of chloride and boron measured in Cartagena were respectively 1.5 and 4 folds higher than those measured in Campotejar. Boron concentrations in Cartagena´s soil exceeded the recommended range of 18.5 meq l^{-1} (Asano et al., 2007) and the electrical conductivity of the soil saturated paste extract exceeded the threshold of 1.4 dS m^{-1} proposed by Maas (1993) for citrus crops. The soil nitrate concentration showed a slight increase over the experimental period and in both locations (table 6). This indicates the contribution of the reclaimed irrigation-water to the soil-plant nutrients balance and highlight therefore the potential of this water to decrease the amount of fertilizers input. This result is in accordance with other researchers who claim that reclaimed water is an important source of nitrogen for citrus trees (Zekri and Koo, 1994; Jimenez Cisneros, 1995).

It was also observed that the poor microbial quality of the irrigation water used in Cartagena´s field caused an increase in the microbial levels in soil samples collected at this location, represented by an increase in the fecal coliform loads, although always in the recommended range (<1000 cfu 100ml^{-1}) (Cairncross and Mara, 1989) (data not shown).

Table 6: Chemical ion concentration in the soil of Cartagena and Campotejar. The data contain average values derived from all samples collected during 2005, 2006 and 2007. Values represent the mean (n=48) ± the standard deviation.

Soil chemical analysis	Cartagena				Campotejar			
	2005	2006	2007		2005	2006	2007	
Na (meq l^{-1})	41.4±7.9	39.1±9.7	35.6±5.8	ns	29.2±2.7	29.6±1.6	31.1±0.5	ns
K (meq l^{-1})	11.1±1.1	9.1±0.8	9.7±0.6	ns	10.5±2.3	9.7±1.2	9.6±1.1	ns
Ca (meq l^{-1})	36.5±2.3	35.4±2.5	34.1±2.4	ns	36.4±1.9	37.8±1.5	38.7±4.1	ns
Mg (meq l^{-1})	40.1±2.4	36.5±3.9	38.4±2.8	ns	34.5±2.2	35.2±1.1	36.3±3.2	ns
B (meq l^{-1})	19.2±1.3	23.9±0.1	24.9±1.1	*	5.4±0.3	5.6±0.5	6.5±0.2	*
Chlorides (meq l^{-1})	58.1±3.1	62.9±4.7	71.1±3.5	*	38.8±2.5	39.4±5.5	52±6.1	*
Nitrates (meq l^{-1})	6.4±0.5	6.8±0.6	7.8±0.3	*	5.3±0.4	5.5±0.2	6.9±0.4	*
Sulfates (meq l^{-1})	4.3±0.5	4.6±0.7	4.2±0.5	ns	4.6±1.3	5.3±0.5	5.7±1.1	ns
ECe (dS m^{-1})	1.7±0.1	1.9±0.1	2.2±0.2	*	1.1±0.1	1.3±0.3	1.2±0.2	ns

Mean content (n=48), *, statistically significant at P<0.05 level of significance.

II.3.3 Leaf mineral analysis

The concentrations of N, Na, K, Ca, Mg and B did not show significant differences between years and between locations (Table 7). The foliar nitrogen levels were in the optimum range considered for citrus trees development (2.4-2.7 %) (Parsons and Weathon, 1996). The boron concentration was far below the limit of toxicity for citrus leaves (>260 ppm) (Embleton et al., 1973) in spite of the high boron levels observed in the irrigation water and the soil of both locations. This result is probably due, on the one hand, to water and soil pH, since boron is assimilated with difficulty in an alkaline medium (Hu and Brown, 1997) and, on the other hand, to the use of high frequency fertigation system which improve N and P fertilization and therefore mitigate B toxicity (Levy and Syvertsen, 2004). Furthermore, the lemon trees of this research are grafted on *Macrophyla* rootstock which is considered among the most tolerant rootstocks to high B (Levy et al., 1993). The leaf Cl concentration increased 40 and 30 % in Cartagena and Campotejar respectively (table 7) without showing visible symptoms. Nevertheless, long-term detrimental effects could be observed if the reclaimed water is used without implementing intermittent leaching practices to prevent the build up in the root zone.

Table 7: Leaf mineral analysis in Cartagena and Campotejar. The data contain average values derived from all samples collected during 2005, 2006 and 2007. Values represent the mean (n=48) ± the standard deviation.

Leaf chemical analysis	Cartagena				Campotejar			
	2005	2006	2007		2005	2006	2007	
N (mmol kg^{-1})	1235±72	1217±92	1226±35	ns	1935±70	2000±90	2014±30	ns
Na (ppm)	6.5±1.6	5.4±2.5	6.3±2.3	ns	6.4±1.4	5.3± 0.5	6.8±1.3	ns
K (mmol kg^{-1})	201±46	230±40	225± 25	ns	212±67	226±45	215±23	ns
Ca (mmol kg^{-1})	2875±45	2810±76	2910±36	ns	2765±31	2720±70	2820±33	ns
Mg (mmol kg^{-1})	354±36	365±40	375±20	ns	304±33	295±42	325±28	ns
B (ppm)	3.9±1.1	5.1±1.4	4.9±1.2	ns	3.7±0.5	4.3±0.5	4.6±0.6	ns
Chlorides (mmol kg^{-1})	18.5±1.2	24.3±3.1	25.9±1.8	*	19.2±1.3	24.4±2.3	24.8±1.8	*

Mean content (n=48), *, statistically significant at P<0.05 level of significance

II.3.4 Yield and Fruit Quality

The total yield harvested in 2006 was 57.4 and 50.5 t ha^{-1} in Campotejar and Cartagena respectively, while in 2007 the crop production was 56.5 and 49.5 t ha^{-1} in Cartagena. This difference of 13% between locations was statistically significant. It is known that yield decreases in direct proportion to increasing salts as soil salinity increases above yield threshold of 1.4 dS m^{-1} (Maas, 1993; Mass and Grattan, 1999). Nevertheless, it is still an open question as to whether yield reduction observed is due to an osmotic effect (Cerdá et al., 1990), Cl toxicity (Cole, 1985) or both. In the present case, the increase over time of Cl concentrations observed in the soil was also reflected in the leaf mineral status but the measured leaf concentrations did not exceed the toxic level for citrus (> 200 mmol Kg^{-1}) (Embleton et al., 1973). In contrast to the results shown by Mass (1993), the absence of visible symptoms and the reduced leaf concentration of B and Cl below their corresponding toxic levels would indicate that the yield decrease could be attributable to osmotic stress rather than toxicity. Plant responses to salinity or specific ions cannot be quantified independently of other environmental conditions or stresses. The reliability of salt tolerance data depends on whether yield reductions are influenced by the interaction between salinity and various soil, water, and climatic conditions (Maas, 1993).

No significant differences were observed between quality indexes of lemon fruits obtained from Cartagena and Campotejar's fields in 2006 (table 8). However, in 2007, lemon fruits from Cartagena´s field showed higher TA and SSC than lemon fruits obtained from Campotejar's field (table 8). This difference could be the result of higher salinity levels in the soil and in the irrigation water used in Cartagena in accordance with the results of Ahmed et al. (2009). These authors deduced that salinity could increase SSC and TA of lemon fruits due to an increase in phenolic content and other organic acids. In addition, fruits from trees irrigated with reclaimed water in Cartagena had a higher SSC:TA ratio and therefore, they reached maturity standards earlier than fruits from trees irrigated in Campotejar (table 8).

Many plants adapt to salt stress by accumulating secondary metabolites, such as sugars, organic acids and proteins in plant cells, which increase quality and marketability of the product. For example, salinity stress increases the sugar and dissolved solids content of tomatoes and melons; increases the content of beneficial antioxidant compounds in strawberries; and increases the oil and lesquerolic acid in lesquerella (Dobrowolski et al., 2008).

Taking into account that the microbial quality of irrigation water applied in Cartagena and Campotejar fields was different, it could be expected that this could have an influence in the microbial quality of lemons obtained from both locations. However, the microbial load of total aerobic bacteria, yeast and moulds was very similar between both locations in all lemons and no risk of fecal contamination was observed as *E.coli* spp counts was undetected for all the samples (table 8).

Table 8: Quality indexes and microbial quality of lemon fruits obtained from Cartagena and Campotejar fields in 2006 and 2007. Values represent the mean (n=100) ± the standard deviation.

	2006			**2007**		
Quality index	**Cartagena**	**Campotejar**		**Cartagena**	**Campotejar**	
Weight (g)	148.3 ± 20.3	148.2 ± 23.7	ns	162.8 ± 10.4	136.2 ± 11.2	*
SSC (%)	8.5 ± 0.3	8.5 ± 0.2	ns	8.0 ± 0.3	6.4 ± 0.5	*
TA (%)	7.1 ± 0.3	7.0 ± 0.3	ns	6.5 ± 0.2	5.9 ± 0.2	*
Maturity Index	1.2 ± 0.1	1.2 ± 0.1	ns	1.23 ± 0.03	1.08 ± 0.05	*
Microorganisms (log cfu/cm^2)						
Mesophilic	5.3 ± 0.1	5.2 ± 0.4	ns	3.3 ± 0.3	2.9 ± 0.4	ns
Fecal coliforms	≤ 0.1	≤ 0.1	ns	≤ 0.1	≤ 0.1	ns
Escherichia coli	≤ 0.1	≤ 0.1	ns	≤ 0.1	≤ 0.1	ns
Yeast and moulds	2.2 ± 0.1	2.2 ± 0.1	ns	2.4 ± 0.2	2.0 ± 0.2	ns

Mean content (n=100), *, statistically significant at P<0.05 level of significance.

Thus, no microbial risk could be associated with the use of wastewater to irrigate lemon trees as the microbial load of the product was not influenced by the irrigation water.

II.4 CONCLUSIONS

Blending of tertiary reclaimed water in Campotejar showed better agronomic and microbiological quality than Cartagena´s reclaimed water. Therefore, mixing reclaimed water with good quality water, reduces the associated potential salinity and toxicity risks and thus increases the volume of available water for irrigation.

The high salinity, Cl and B concentration were the main restrictions associated with treated wastewater use in both locations. Although leaf toxicity levels were not observed, salt accumulation can be a decisive problem for citrus crops, particularly after long-term use.

In both locations, the soil nitrate concentration increased over the experimental time period. This indicates that the reclaimed irrigation-water can constitute an appreciable nutrient source for the plant.

The crop yield was lower (13%) in Cartagena than in Campotejar, showing that total crop production is affected by the quality of irrigation water. The absence of visible symptoms and the low leaf concentration of B and Cl below their corresponding toxic levels would indicate that the yield decrease could be attributable to osmotic stress rather than toxicity.

The fruits harvested from Cartagena in 2007 were significantly of better quality; in fact, in 2007, lemon fruits obtained from the Cartagena field showed higher SSC and TA as well as higher weight than fruits obtained from the Campotejar field.

The microbial quality of the irrigation water did not influence the safety of lemon fruits. Thus, the reclaimed water irrigation water for lemon trees did not represent a microbial risk for lemon fruit.

III INFLUENCE OF REGULATED DEFICIT IRRIGATION AND RECLAIMED WATER ON MANDARIN TREES

III.1 INTRODUCTION

The great challenge in agriculture for the near future, will be, on the one hand, the task of increasing food production with less water, particularly in countries with limited water and land resources (Fereres and Connor, 2004), and on the other hand the safe use of non-conventional water resources in agriculture, instead of potable water.

One way to optimize water resources is to employ deficit irrigation (DI) strategies and particularly the use of regulated deficit irrigation (RDI), which consists in cutting-off or reducing partially the irrigation during low water stress sensitivity periods of the orchard cycle, when adverse effects on productivity are minimized (Mitchell et al., 1986). RDI was initially designed as a tool to improve yield and control of vegetative growth (Chalmers et al., 1981; Mitchell and Chalmers, 1982). Furthermore, in the context of improving water productivity, in Mediterranean regions such as Murcia, the deficit irrigation strategies have been employed on various crops (Naor, 2006).

Under severe water shortage conditions the RDI strategies are applied using reclaimed water. This combination is prone to produce undesirable stress levels and may have negative effects on the soil agronomic characteristics; therefore, this approach requires precise knowledge of crop response to water deficit, as drought tolerance varies considerably by species, cultivar and stage of growth.

Besides of water scarcity, secondary soil salinisation from the use of saline irrigation water is a growing worldwide problem (Ghassemi et al., 1995). In fact, the major problem associated to reclaimed water use in Murcia is salinity. In this Region, 93% of the treated wastewater has an Electrical Conductivity (EC) higher than 2 dS m^{-1} and 37% has EC values higher than 3 dS m^{-1} (ESAMUR, 2005), and it is known that water with EC $\geq$ 3 dS m^{-1} requires very intensive management to control adverse salinity effects.

The investigations into reclaimed water effects in agriculture usually use non-saline reclaimed water, and in general, little is known about the interaction between deficit irrigation and saline water. For this reason, the main objectives of this study were to evaluate the combined effects of different reclaimed irrigation water qualities and the regulated deficit irrigation strategies on mandarin tree crop performance under Mediterranean climate conditions.

III.2 MATERIAL AND METHODS

III.2.1 Experimental conditions

The experiment was conducted during three years (2007/2009), at a commercial orchard located in Campotejar (Murcia) Spain (38º07´18´´N; 1º13´15´´W). The experimental plot of 0.5 ha was cultivated with 8-year old mature mandarin trees (*Citrus clementina* cv. 'Orogrande') grafted on Carrizo citrange [*Citrus sinensis* (L.) Osb. x *Poncirus trifoliata* L.] (picture 11). The plant spacing was 5 x 3.5 meters. The soil within the first 90 cm depth had a loamy texture (42 ± 1.00 % sand, 26 ± 0.67 % clay and 32 ± 0.88 % loam) with an average bulk density of 1.37 ± 0.01 g cm^{-3}.

The irrigation doses were scheduled on the basis of weekly ETc estimated as reference evapotranspiration (ETo), calculated with the Penman-Monteith methodology (Allen et al., 1998), and a monthly crop factor (Castel et al., 1987). The water was supplied by drip irrigation with three pressure-compensated drippers per tree, each with a flow rate of 4 l h^{-1} and spaced 0.9 m. The irrigation control head was equipped with two types of filters, first sand filters and then disk filters, to avoid emitters clogging.

Picture 11. Panoramic view of the experimental orchard located in Campotejar (Murcia). 0.5 ha cultivated with 8 year-old mature mandarin trees.

The irrigation control head was equipped and supplied with three water sources (picture 12); the first (TW) was pumped from the Tajo-Segura water transfer canal (EC ≈ 1 dS m^{-1}), the second (IW) was delivered by the irrigators' association of Campotejar (1≤EC≤4), this time-variable quality water depends on the proportional mix of the available water resources needed to cover the actual irrigation demand (underground water, transfer water and reclaimed water), and the third water source (RW) was pumped from the “ North of Molina de Segura” wastewater treatment plant (WWTP) (EC ≈ 3 dS m^{-1}). The WWTP apply a conventional activated sludge process followed by ultraviolet application for tertiary treatment.

Picture 12. Irrigation control-head, equipped with 3 reservoirs, 3 sand filters and 3 disk filters. 1) Mandaine experimental plot, 2) Wastewater treatment plant of the “north of Molina de Segura”, and 3) Tajo-Segura water transfer canal.

The experiment was designed to differentiate two irrigation scheduling treatments per water source and four replicates per treatment. A total of 6 treatments x 4 replicates were distributed using a completely randomized design (figure 2). In 2007, the six treatments received 100% of the water requirements and therefore no RDI was applied. In 2008, a control treatment (C) was irrigated to recover 100% ETc throughout the growing season, and a regulated deficit irrigation treatment (RDI) was irrigated to 50% ETc since the 22^{nd} of June to mid July and then was followed by only applying 30% ETc till the 13^{th} of August when the full irrigation scheduling was resumed. In 2009, the same protocol was followed except that the irrigation doses were reduced to only 50% ETc over the whole RDI period. (figure 1). The RDI period was fit to the second stage of fruit growth (González-Altozano and Castel, 1999), which extended from late June (fruit diameter 1-2 cm), till mid-August (fruit dimater of 3-4 cm). Each replicate consisted of 3 x 4 trees with the 2 central trees being used for periodic sampling. The six treatments are identified as follows, T0: TW-C, T1: TW-RDI, T2: IW-C, T3: IW-RDI, T4: RW-C and T5: RW-RDI (figure 2).

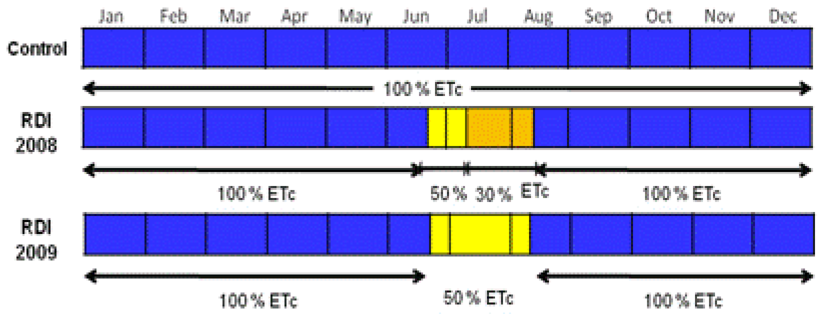


Figure 1. Irrigation scheduling: Control treatments irrigated to recover 100% ETc throughout the growing season. RDI treatments irrigated in 2008 to recover 50% ETc first during three weeks starting the last week of Jun (yellow part) and 30% next till the second week of August (orange part). In 2009, the irrigation doses were to recover 50% ETc during all the RDI period (yellow part).

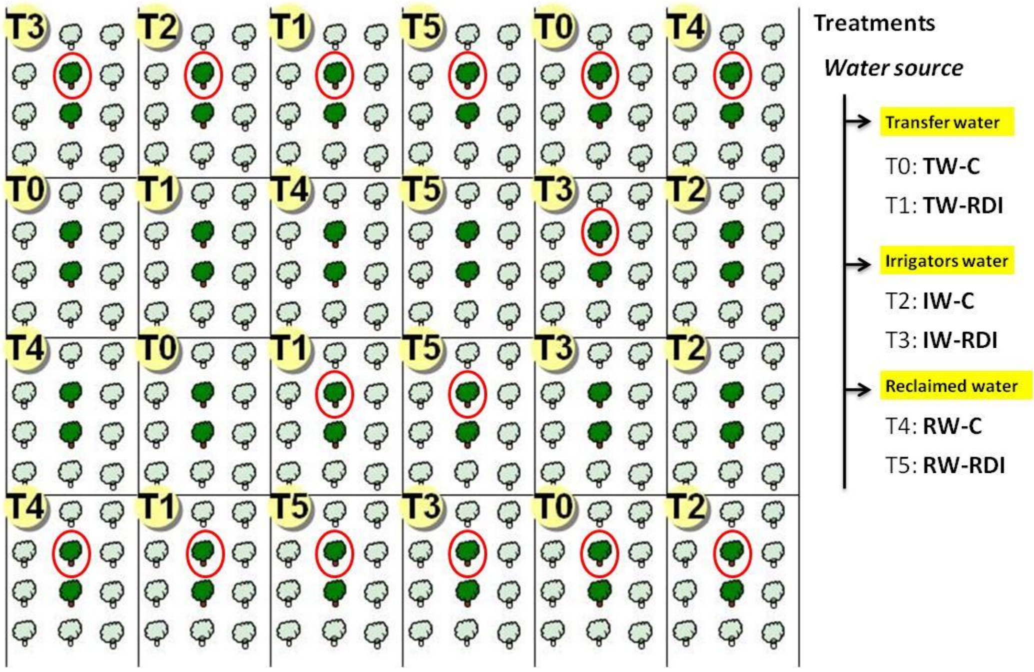


Figure 2. Experimental design: Six irrigation treatments x Four replicates were distributed using a completely randomized design. The red circles indicate the trees equipped with soil monitoring tools.

During the three seasons, the annual reference evapotranspiration (ETo) was 1299 , 1332 and 1385 mm in 2007, 2008 and 2009, respectively (table 9). The irrigation doses were applied daily from January 2007 until December 2009. The total amounts of water applied were measured with inline water flow-meters, placed on the four replicates of each treatment. The six treatments received the same amount of fertilizers which were applied through the drip irrigation system.

Table 9: Reference evapotranspiration "ETo", rainfall, irrigation (mm month $^{-1}$) and VPD (Kpa) in Campotejar. The values are the monthly average from data collected during 2007, 2008 and 2009.

2007	**JAN**	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**	**TOTAL**
ETo	45.31	59.10	104.69	92.45	170.20	185.96	199.18	157.17	103.91	71.08	59.06	50.84	**1298.95**
VPD (kPa)	12.40	14.41	20.58	14.88	36.70	40.18	43.48	36.79	21.32	15.60	14.19	13.13	**283.68**
Rainfall	35.37	27.37	17.80	60.77	30.03	1.27	0.60	13.40	33.40	30.43	29.06	7.87	**287.36**
Irrigation-C	6.47	5.42	12.66	47.33	75.78	92.49	87.21	66.88	67.11	37.23	31.36	15.09	**545.03**
2008													
ETo	56.54	53.37	122.25	135.66	131.59	172.46	179.31	173.48	120.10	77.50	59.44	50.34	**1332.04**
VPD (kPa)	14.00	10.54	27.05	30.32	25.97	34.52	43.02	45.34	33.49	19.33	15.24	13.50	**312.34**
Rainfall	3.70	26.40	0.60	3.40	67.00	44.50	4.80	0.00	48.60	32.40	32.30	6.90	**270.60**
Irrig.-C	5.27	5.27	13.17	47.40	73.73	63.20	73.73	68.46	50.03	52.66	57.93	15.80	**526.64**
Irrig.-RDI	5.27	5.27	13.17	47.40	73.73	47.10	18.28	45.21	50.03	52.66	57.93	15.80	**431.84**
2009													
ETo	59.89	61.16	96.47	123.41	163.07	188.39	197.19	170.69	109.40	91.16	74.30	49.47	**1384.60**
VPD (kPa)	14.12	13.09	18.61	22.58	31.72	47.93	50.46	46.48	28.74	23.80	24.66	14.53	**336.71**
Rainfall	31.20	4.30	84.20	25.80	2.70	0.00	0.00	3.00	66.10	8.40	2.70	77.70	**306.10**
Irrig.-C	7.43	6.22	14.53	54.32	86.97	71.73	74.32	70.09	45.14	79.02	77.02	17.64	**604.42**
Irrig.-RDI	7.43	6.22	14.53	54.32	86.97	55.31	17.75	46.37	45.14	79.02	77.02	17.64	**507.72**

III.2.2 Water analysis

Three water samples from each irrigation water source were collected monthly between 2007 and 2009 in order to characterize irrigation water quality. The samples from each irrigation source were collected in glass bottle, transported in an ice chest to the lab and stored at 5ºC before being processed. The measurements of macronutrients (Na, K, Ca, Mg), micronutrients (Fe, B, Mn), heavy metals (Ni, Cd, Cr, Cu, Pb, Zn), anions (chloride, nitrate, phosphate and sulphate), pH, electric conductivity (EC) and total dissolved solids (TDS), turbidity and microbial quality of irrigation water was made according to the water analysis methodology described in chapter II.

III.2.3 Soil analysis

Six gravimetric soil samples per irrigation replicates were collected three times per year (at the beginning of irrigation period, at the end of RDI, at the end of the irrigation period) during 2008 and 2009, from 0.2, 0.4 and 0.6 m depths at 0.1 and 0.3 m away from the emitter. Soluble-salt contents were determined in the saturated paste extract as described by Rhoades (1982). The pH and the electrical conductivity of the saturated paste extract (EC_e), were measured with a pH-meter

Cryson-507 (Crisom Instruments S.A., Barcelona, Spain) and multi-range equipment Cryson-HI8734 (Crison Instruments, S.A., Barcelona, Spain), respectively. Soluble Ca and Mg were measured using the EDTA tritation method and Na was measured using a flame photometer (Richards, 1954).

Besides of gravimetric soil samples, the soil solution was also sampled biweekly at 0.3 m depth using suction lysimeters (SDEC, Tauxigny- France) (Diameter = 63 mm) installed at 0.1 and 0.3 m away from the emitter. The suction lysimeters were installed following the procedure described by Aragües and Millan (1986).

The soil water content (SWC) was measured biweekly. The SWC was measured at 0.2 m away from the emitter and at right angle to the irrigation lateral, using the time-domain-reflectometry (TDR) probes (model 1502C, Tektronix Inc., OR.) for the top 0.1 m, and the neutron probe Troxler 4300 (Troxler, Raleigh, N.C.) from 0.2 down to 1 m depth, following a 0.1 m step.

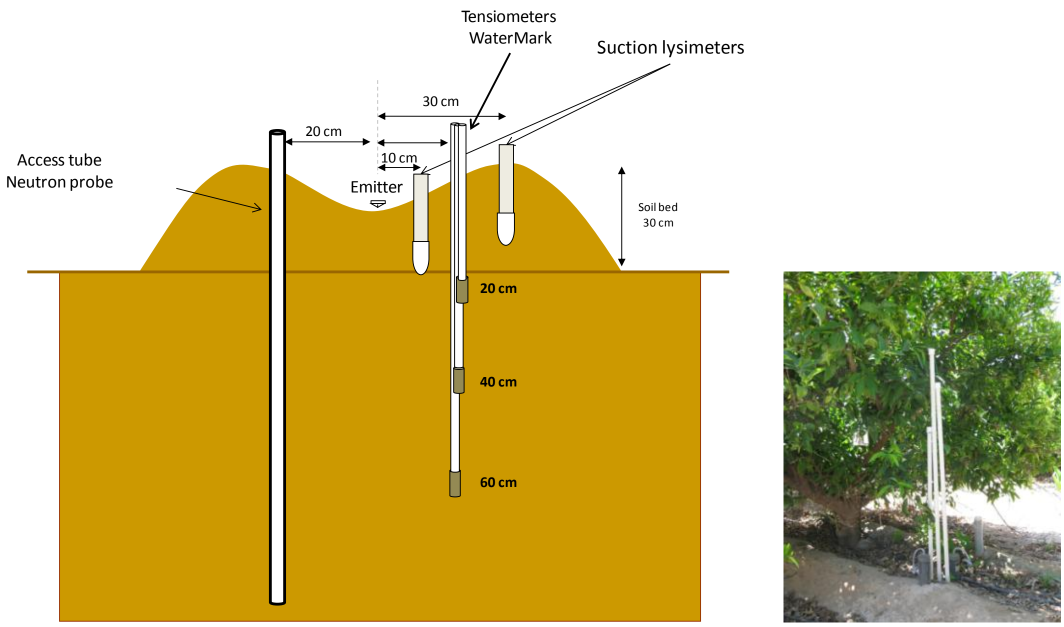
The soil matric potential (Ψ_m) was measured at 0.2, 0.4 and 0.6 m depth using granular matrix sensors WaterMark® and at 0.2 m away from the emitter.

Three trees per treatment were used to measure soil water status. In each one, three granular matrix sensors WaterMark®, two suction probes and one access tube for the neutron probe were installed (picture 13).

III.2.4 Plant analysis

Spring flush leaves from non-fruiting branches were sampled every three months during 2008 and 2009. Twenty leaves per tree were sampled in the two central trees of each replicate per treatment. The concentrations of macro-elements, microelements, heavy metals and anions were measured according to the methodology described in chapter II.

The midday stem water potential (Ψ_{stem}) was measured biweekly throughout the season and weekly during the RDI period. Two mature, fully expanded leaves from the canopy and close to the trunk, were taken from the two central trees of each replicate per treatment. The leaves were enclosed within polyethylene bag covered with aluminium foil, at least 2 h before the measurement (McCutchan and Shackel, 1992).



Picture 13. Soil measuring equipments: Three granular matrix sensors WaterMark® (at 0.2, 0.4 and 0.6 m depth), two suction lysimeters (at 0.1 and 0.3 m from the emitter) and the access tube for the neutron probe (at 0.2 m from the emitter).

The midday stem water potential was measured at solar noon (12:00 h GMT), using a pressure chamber (model 3000; Soil Moisture Equipment Corp., Santa Barbara, California, USA) and following the recommendations of Turner (1988).

Net CO_2 assimilation rate (Pn) and stomatal conductance to water vapour (g_s) were measured with a portable photosynthesis system (Li-6400, Li-Cor, Lincoln, NE, USA). Leaf gas exchange measurements were taken biweekly throughout the season and weekly during the deficit irrigation period, between 8:00 and 10:00 h GMT in daylight hours, on selected clear days. Measurements were taken in sixteen healthy and mature leaves per treatment (two leaves per tree, eight trees per treatment), exposed to the sun, in the exterior canopy positions and in the middle third of the tree.

Tree canopy height and perimeter were measured at the beginning and at the end of each season during the experimental period, in all the trees of the orchard. The canopy volume was estimated from height and diameter of the tree, measured with ranging rods in two perpendicular directions. The formula is that proposed by Hutchinson (1977), considering that the tree is shaped like a pyramid.

III.2.5 Yield and fruit quality analysis

Fruit set and fruit load were determined from four secondary branches in the 2 inner trees per each replicate (8 trees per treatment), from bloom to harvest. These branches were facing in the four directions and their basal diameter was between 2 and 3 cm. Fruit set was calculated as the percentage of fruits respect to the total initial flowers.

Yield was assessed in 8 trees per treatment. In each tree, the following measurements made were: number of fruits per tree, total kg and distribution in commercial diameters using the following classification (<44mm, <52mm, <58mm, <66mm, <70mm, <82mm and extra (>82mm) (UNECE, 2009).

Water use efficiency (WUE) was calculated for each treatment as the ratio between the annual yield and the applied water during the same period (Kijne et al., 2003).

Fruit quality was measured in 100 fruits per treatment. The parameters measured were: the peel thickness, weight, size and juice volume. A sample of 50 ml per treatment was used for the analysis of the other quality parameters: titratable acidity (TA), pH and soluble solid content (SSC). These parameters were measured according to the methodology described on Chapter II.

Vitamin C, ascorbic and dehydroascorbic acid contents were determined according to Zapata and Dufour (1992) with some modifications (Gil et al., 1999). Ten grams of frozen fruit (peel and flesh) (-80 °C) was added to 10 ml of extraction medium (0.1 M citric acid, 0.05% w/v ethylenediaminetetraacetic acid disodium salt, 5% v/v methanol, 95% water, and 4 mM NaF). The mixture was directly homogenized for 30 s and filtered through filter cloth. The filtrate was collected and centrifuged at 10500*g* in an Eppendorf centrifuge for 5 min at 2-5 °C. The filtrate was flushed through an activated Sep-Pak C18 cartridge (Waters) and was then filtered through a 0.45-μm nylon filter. Then, 250 μl of 1,2-phenylenediamine dihydrochloride solution (35 mg/100 ml) was added to 750 μl of extract for dehydroascorbic IWid derivatization into the fluorophore 3-(1,2-dihydroxyethyl) furo[3,4-*b*]quinoxaline-1-one (DFQ). After 37 min in darkness, the samples were analyzed by HPLC. Ascorbic acid and dehydroascorbic acid were quantified by HPLC (Merck, Hitachi), equipped with an L-6000 pump and coupled with a D-2500 variable-wavelength UV detector. Twenty-microliter samples were injected on a reversed-phase Kromasil 100 C18 column (250 mm × 4 mm i.d., 5-μm particle size) (Tecnokroma) with an OSD guard C18 pre-column. The flow rate was kept at 0.9 ml min^{-1}. The mobile phase was methanol: water (5/5, v/v), 5 mM cetrimide, and 50 Mm KH_2PO_4. The detector wavelength was initially set at 348 nm, and after the elution of DFQ, it was manually shifted to 261 nm for ascorbic acid detection. The vitamin C content was calculated adding the content of ascorbic acid and dehydroascorbic acid, and the results were expressed as milligrams per 100 g of fresh weight.

III.3 RESULTADOS Y DISCUSION

III.3.1 Irrigation water quality

The amount of water applied for the control treatments were 545, 526 and 604 mm in 2007, 2008 and 2009 respectively. Water saving with the RDI treatments was 18% in 2008 and 16% in 2009 (table 9). This percentage is in the range reported by González-Altozano and Castel (2003a). These authors applied RDI on the same crop in Valencia (Spain) during the second stage of fruit growth and the water savings were between 6% and 22% during the years of the experiment.

The water quality was different between each source of irrigation water. Reclaimed irrigation water (RW) showed the highest values in salinity and sodicity risk, with EC values close to 3 dS m^{-1} and SAR around 4 (figure 3), while for the Tajo-Segura transfer canal water (TW) the values of EC ad SAR were lowest (close to 1 dS m^{-1} and 1.5 respectively, figure 3). In the irrigators´ association treatment (IW), water quality resulted from the blending proportion of the different available water sources (Tajo-Segura, underground and reclaimed water). During 2007 and 2008, the proportion of reclaimed water in the “IW” treatment was very high because the amount of water granted from Tajo-Segura transfer canal was not enough to cover properly the crop water requirements. During 2009, the water transfer granted was enough to blend the different water sources to an EC suitable for the irrigation of mandarin trees (figure 3).

During the experiment, some peaks of different water quality parameters were observed in RW and IW because of the industrial spills in the WWTP and the different mix of irrigation water sources used by the irrigators´ association. The main characteristic of irrigation agriculture in Murcia is the presence of irrigation channels and drainage ditches where the reclaimed water is mixed with other water resources and stored in reservoirs. Hence irrigation water quality fluctuations are representative of agriculture water use in this region.

In general, it was observed that reclaimed irrigation water (RW) and farm irrigators water (IW) had a significantly higher concentration in Na, B and chlorides than the Tajo-Segura transfer water (TW) (figure 3). The Na concentration in RW and IW produced higher sodium adsorption ratio (SAR), and thus, in both water quality treatments there were a moderate risk of soil infiltration problems (Ayers and Wescot, 1985). During the most of the experimental period, B concentration in reclaimed water was in the phytotoxic range for sensitive crops (figure 3). Citrus are considered sensitive crops to B (Mass, 1993), as has been described in chapter I. B-toxicity is more of a concern in arid environments where salinity problems exist (Nicholaichuk et al., 1988).

The high level of salinity observed in our trials was mainly due to the high concentration of chlorides and Na (figure 3), although Ca, Mg and SO_4 were also more concentrated in RW (data not shown). In fact, it was necessary to apply an acid during the fertilization program to minimize precipitation of Mg and Ca carbonates in the irrigation system of RW and IW treatments (Pitts, 1996).

In the reclaimed water composition there was also a higher concentration of potasium and nitrates than in TW treatment (figure 3). These supplies of macronutrients (K and N) could have been used to reduce the application of fertilizers, such as it has been recommended by some authors (Lazarova and Barhi, 2005, USEPA, 2004).

In relation to the microbiological parameters measured in irrigation water, it was observed on many occasions that TW exceeds the RW microbiological values (figure 4). These results showed that open channel water distribution networks could have higher microbiological risk than tertiary treated wastewater.

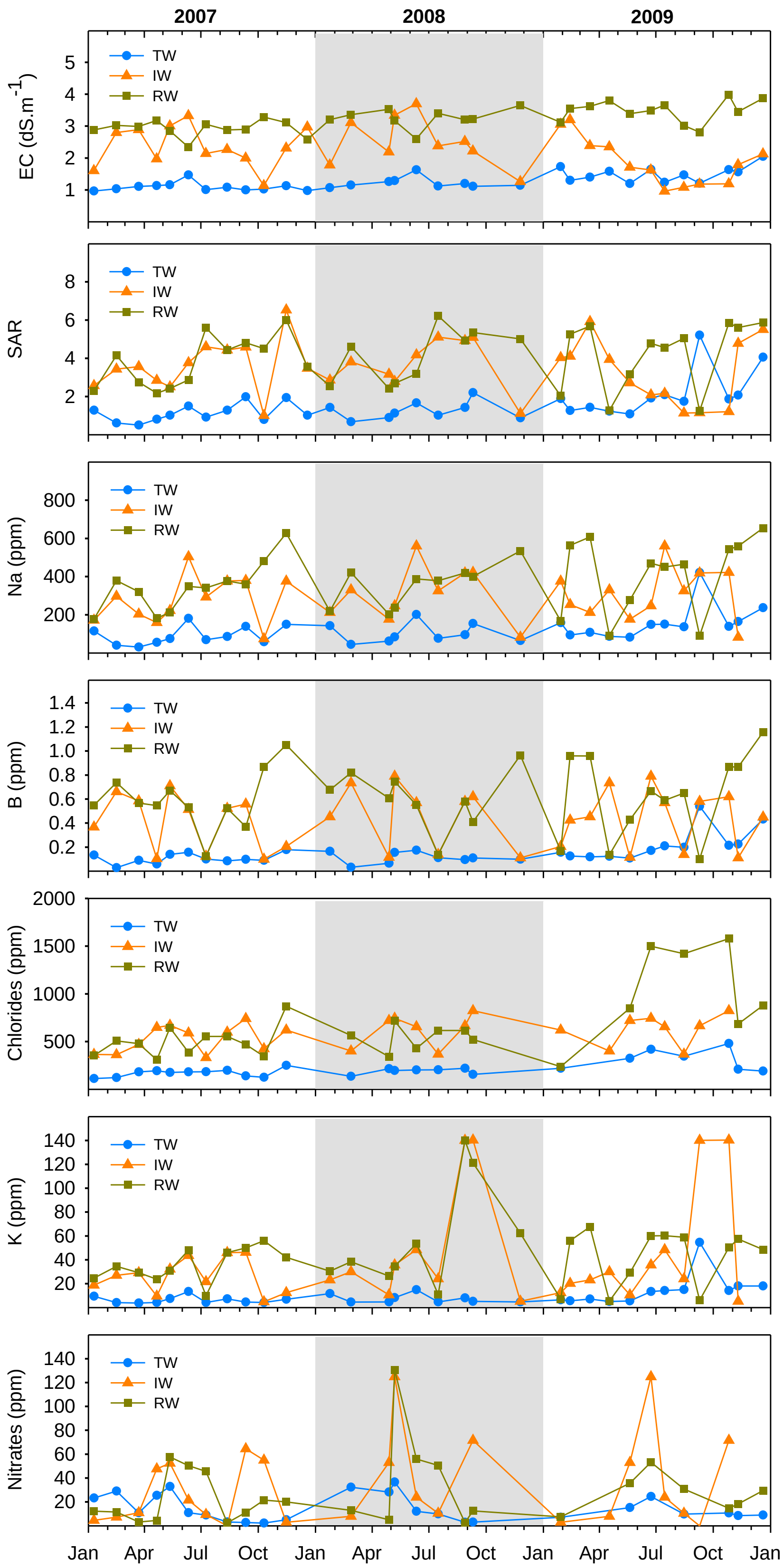


Figure 3. Evolution of monthly measured quality indexes (Electrical conductivity "EC dS m^{-1}", sodicity adsorption ratio "SAR") and chemical compositions ([Na], [B], [Cl], [K] and [NO_3] in ppm) of the tree irrigation-water sources: transfer water (TW, blue lines), irrigators water (IW, orange lines), and reclaimed water (RW, green lines) monitored over three years (2007, 2008 and 2009).

The most interesting result related to the microbiological load in the different irrigation-water sources was the high seasonal variability observed over the experimental period (figure 4). These variations were not related with some specific period of the year or to particular climatic conditions. Thus, the increase of *E.coli* or fecal coliforms resulted of some accidental and spontaneous contamination in the case of water from the Tajo-Segura transfer canal, and of some transitory pollutions in the influent of the WWTP in the case of reclaimed water. These accidental and uncontrolled contaminations support the microbiological analysis in the irrigation-water supplies must be made periodically, independently of the water source considered, to minimize negative public health impacts (WHO, 2004). The intestinal nematode eggs were always below the threshold (<1 egg 10 l^{-1}) imposed by the Royal Decree 1620/2007 that regulate the reclaimed water use in Spain. This result is in accordance with previous studies carried out in Murcia by ESAMUR (2009) which concluded that in 43 WWTP effluents analyzed, there were nematode eggs absence in 79% of the cases treated with a secondary treatment, and total absence in the WWTP effluents obtained with tertiary treatment.

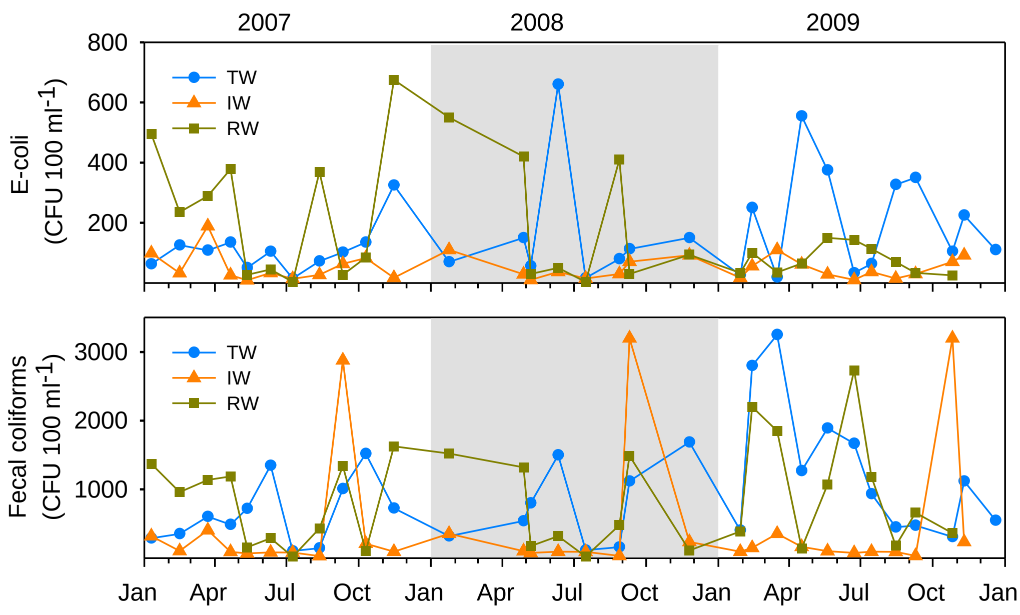


Figure 4. Evolution of monthly measured microbiological quality indexes (Fecal coliforms and *E.coli* concentrations) of irrigation-water sources: transfer water (TW blue lines), irrigators water (IW orange lines), and reclaimed water (RW green lines) over three years (2007, 2008 and 2009).

III.3.2 Soil salts accumulation

In the chemical analysis made in the soil gravimetric samples, it was observed that the electrical conductivity of the saturated paste extract (ECe) was increased significantly in the RW treatment respect to the TW treatment (figure 5A). In all saline water quality treatments considered (IW and RW) the regulated deficit irrigation strategies also increased the values of soil salinity (figure 5A), being this increase clearly higher in the reclaimed water use. Thus, the EC_e of the TW-RDI treatment reached values close to 3 dS m^{-1}, that of the IW-RDI treatment was close to 3.5 dS m^{-1} and that of the RW-RDI treatment reached the highest value close to 4.5 dS m^{-1} (figure 5A). Considering the pattern of soil EC_e, soil salts accumulation in all treatments was above the optimum threshold of 1.4 dS m^{-1} proposed for citrus crops by Maas (1993). This situation was especially problematic in the RW-RDI, which ECe level (4.5 dS m^{-1}) represents a moderate risk of soil salinisation (4-15 dS m^{-1}) accordingt to Baruth et al., 2006.

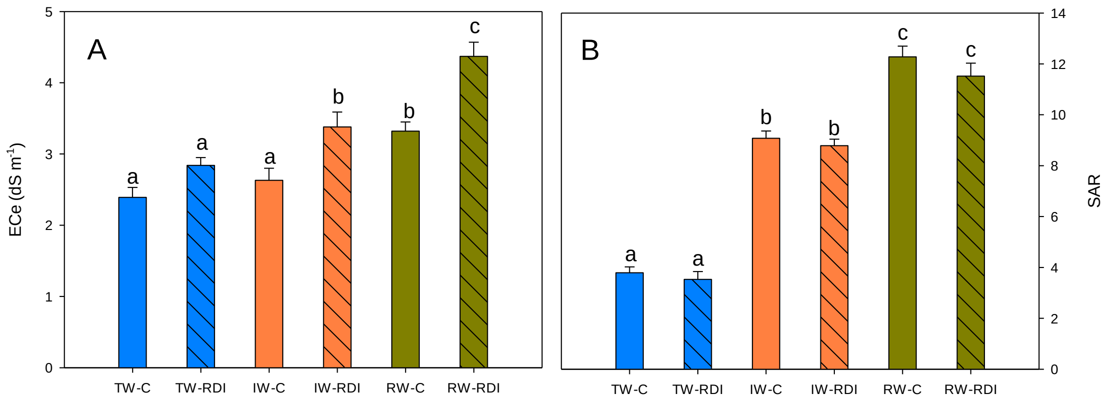


Figure 5. Electrical conductivity of the saturated paste extract (EC_e) (dS m^{-1}) and sodium adsorption ratio (SAR) measured in soil gravimetric samples obtained during 2008 and 2009. Each column is the average of the two years (three samples taken at 0.2, 0.4 and 0.6 m depths at 0.1 and 0.3 m away from the emitter, in 4 replicates per treatment). Background colors represent irrigation water sources (transfer water "TW blue colour", irrigators water "IW orange colour", and reclaimed water "RW green colour"). Background patterns represents irrigation treatment (no pattern = Control and downward diagonal = RDI). Letters in each column indicate significant differences according to $LSD_{0.05}$

Considering SAR medium level for the whole experience, there were important differences in function of the type of water source and thus, while TW treatments reached values close to 3 dS m^{-1}, IW treatments reached values close to 9 dS m^{-1} and RW treatments registered values close to 12 dS m^{-1} (figure 5B). Bresser et al., 1982, suggested that SAR values > 10 suppose a high risk of deterioration of the soil structure at medium and long term, principally caused by clay dispersion phenomena. In this parameter (SAR) there were no differences between the control and regulated deficit irrigation treatments, independtly of the type of water used (figure 5B).

Analysing the soil gravimetric samples, it was studied the evolution of EC_e throughout the experiment (figure 6). The values observed in the TW treatments was close to 2 dS m^{-1} during all the experiment, except for the data recorded at end of the regulated deficit irrigation period (August) on the TW-RDI, which EC_e value increased significantly up to 4 dS m^{-1} in 2008 and 2009 (figure 6). Similar results were observed in the IW treatments, without significant differences in the values of EC_e for all times and irrigation treatments (C and RDI), with the exception of the register measured in the IW-RDI at the end of the deficit irrigation periods in 2008 and 2009 (figure 6). The effect of RW treatments on the soil EC_e was more pronounced than that observed in TW and IW. The application of reclaimed water increased the EC_e values close to 3 and 4 dS m^{-1} in all treatments, and exceptionally the EC_e values measured at the end of the deficit irrigation period raised up to 6-7 dS m^{-1} in 2008 and 2009 (figure 6).

Taking into account the previous measurements made on the soil gravimetric samples, it was possible to conclude that the application of RDI strategies increases the accumulation of salts. This effect is aggravated when saline water (such as RW) is used for irrigation. The most important increases of soil salts accumulation were observed at the end of the deficit irrigation periods (August); later, when the irrigation deficit is ceased and the irrigation water leached down the salts (December), the values of EC_e in RDI treatments decreases to values close to those of the control treatments (figure 6). This soil recovery capacity observed in the RDI treatments in December respect to August is a relevant point to consider the importance of using an adequate irrigation management that helps to avoid the accumulation of salts in the soil, even when saline irrigation water is being applied. The leaching requirements and the appropriate crop management to avoid salinity hazards and soil degradation are provided by Ayers and Wescot (1985) when the use of low-quality irrigation-water is adopted.

Beside the analysis of gravimetric samples, the salts soil accumulation were also studied using suction lysimeters. The concentrations of (Na, Cl and B) in the soil solution were used to corroborate the soil accumulation of salts during deficit irrigation periods. The increase of phytotoxic elements (Na, Cl, B) in the soil solution of all RDI treatments, respect to the control ones, was evident for both year and water quality used (figure 7).

In 2008, the Na concentration in the RDI treatments were almost double (around 400 ppm) than in the control ones (around 200 ppm) for all type of water quality (figure 7). The Cl concentration also increased from less to more saline treatment, and thus the lowest value was measured in TW-C (around 400 ppm) and the highest was recorded in RW-RDI (around 1500 ppm), with an intermediate scale of continuous increase in function of the water applications and the use of saline water quality (figure 7). The boron concentration tendency was similar to that of Na and Cl. Under TW and IW treatments the [B] in the soil solution reached values around 0.3 ppm and 0.5 for the C and RDI treatments respectively, while under RW treatment it increased significantly respect to other treatments, reaching values of 0.4 and 0.9 ppm in C and RDI treatments, respectively (figure 7).

The evolution of these parameters in 2009 was similar to that observed in 2008; however, it was noted that during this third year of experiment the accumulation of phytotoxic ions in the soil was clearly higher under the reclaimed water treatments. For [Na], the values reached under RW treatments were 500 and 800 ppm for C and RDI treatments, respectively (figure 7). For [Cl], the highest values were reached under RW treatments again, being 1500 amd 2500 ppm in C and RDI treatments respectively (figure 7). In relation to the [B], the highest value of 1.1 ppm was recorded under RW-RDI treatment (figure 7).

At conclusion, as it was observed with the gravimetric soil samples analysis, it is possible to assume that regulated deficit irrigation strategies in combination with reclaimed water use increased the salts accumulation in soil solution. Soil accumulation of Na, Cl and B was observed mainly at the end of the water deficits periods and it was higher when saline water (RW) was used for irrigation.

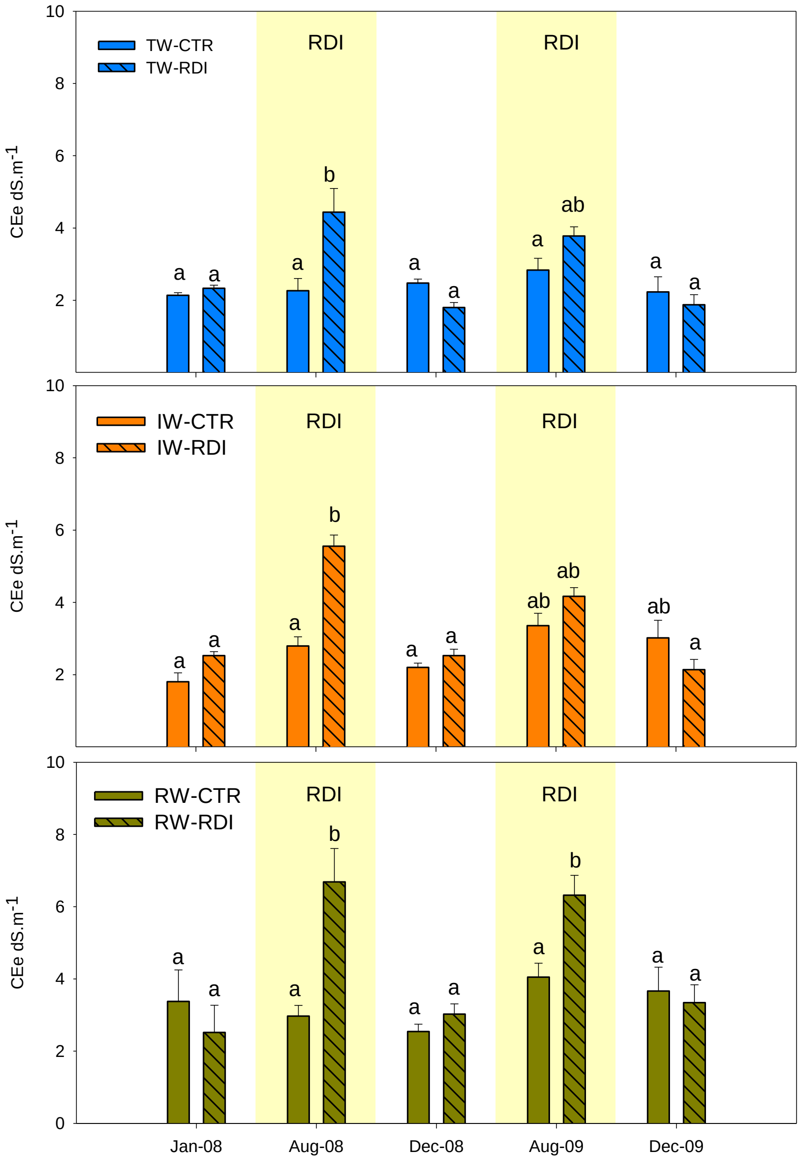


Figure 6. Evolution of electrical conductivity of the saturated paste extract (EC_e) (dS m^{-1}) measured at the beginning of the irrigation season (January), at the end of RDI period (August) and at the end of irrigation season (December) during 2008 and 2009. Each column is the average of twenty four samples taken at 0.2, 0.4 and 0.6 m depths at 0.1 and 0.3 m away from the emitter, in 4 replicates per treatment. Background colors represent irrigation water sources (transfer water "TW blue colour", irrigators water "IW orange colour", and reclaimed water "RW green colour"). Background patterns represents irrigation treatment (no pattern = Control and downward diagonal = RDI). Yellow shaded area emphasizes the values measured at the end of RDI period. Letters in each column indicate significant differences according to $LSD_{0.05}$

Although these salts were partially eliminated when the full irrigation was resumed, the increase of soil salt concentration when reclaimed water was used had a cumulative effect from year to year, and thus, the highest concentration of Na, Cl and B were registered in 2009 (after three years of experiment).

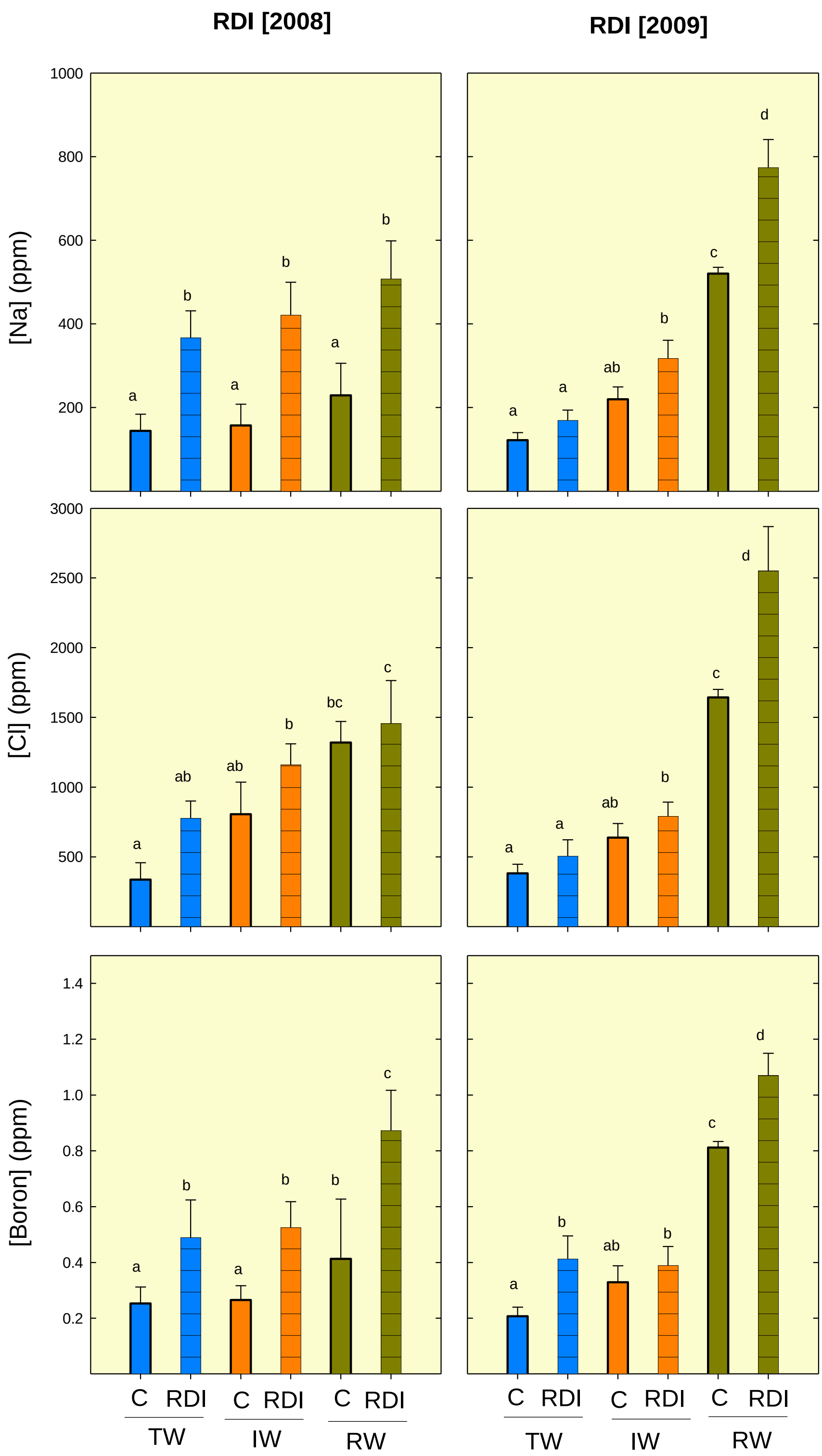


Figure 7. Sodium, chlorides and boron concentrations in the soil solution measured in 2008 and 2009 during the RDI period (late Jun to early August). Each column is the periodic average of samples collected biweekly at 0.3 m depth and at 0.1 and 0.3 m away from the emitter, in 4 replicates per treatment. Background colors represent irrigation water sources (transfer water "TW blue colour", irrigators water "IW orange colour", and reclaimed water "RW green colour"). Background patterns represents irrigation treatment (no pattern = Control and horizontal-lines = RDI). Letters in each column indicate significant differences according to $LSD_{0.05}$.

III.3.3 Soil water content

Soil water store (SWS) was maintained to field capacity (250 mm m^{-1}) under the control treatments during 2008 and 2009, indicating that water requirements of mandarine trees were completely covered (figure 8). The reduction of water application in the RDI treatments reduced the soil water content in all cases, independently of the type of water used (figure 8). However, it is important to remark that in the deficit irrigation period, the SWS for RW treatment was not depleted as much as for IW and TW treatments, especially in 2009. In this year, the SWS in the RW-RDI treatment reached minimum values of 170 mm m^{-1} while in the TW-RDI the SWS decreased to 100 mm m^{-1} (figure 8). These results are in accordance to some studies that showed that orchards irrigated with reclaimed water had higher soil water content (SWC) (Zekri and Koo, 1993). This tendency was observed during both years of experiment could be attributed to the osmotic effect arising from salt accumulation in the root zone. The greater and faster reduction of the soil water content observed in the TW-RDI treatment could be explained because these plants uptook more water in no salt stress. This is especially important when a vigorous rootstock with a high hydraulic conductance such as “Carrizo” is used (Syversen, 1981).

The values of soil matric potential (Ψ_m) in the control treatments were always maintained above -20 kpa (figure 9). For the RDI treatments, at 0.4 and 0.6 m depths the Ψ_m decreased progressively during the deficit period and reached minimum values of -130 kpa, -110 kpa and -60 kpa for TW-RDI, IW-RDI and RW-RDI respectively in the soil during 2008 (figure 9). These results are according to the data of SWS previously described. In 2009 these differences in the evolution of Ψ_m were less marked because the water stress generated was not as as severe as in 2008 (data not showed).

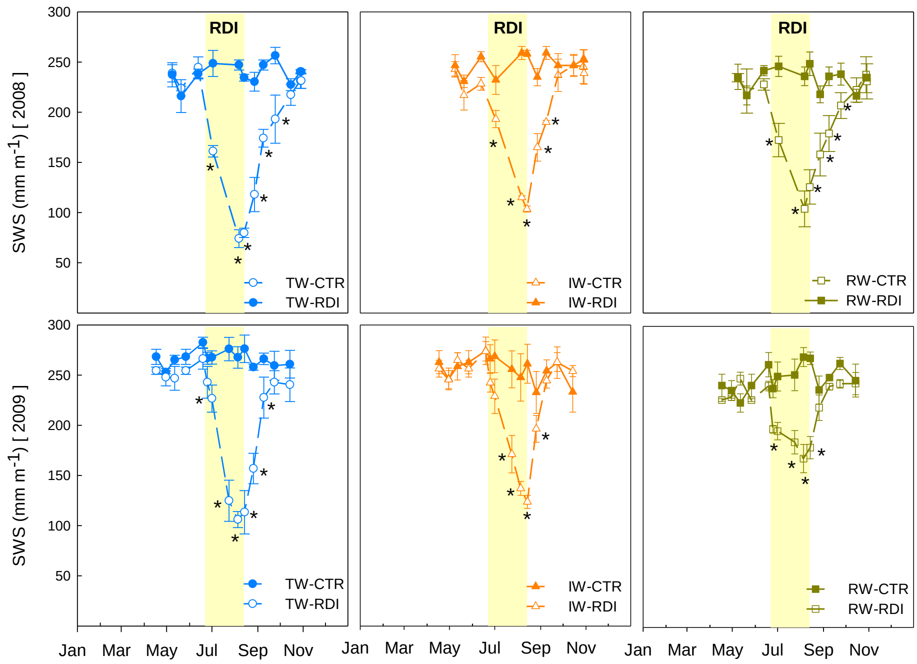


Figure 8. Evolution of soil water store measured within the top layer of 1 m depth during 2008-2009 (SWS, mm m^{-1}). Each point is the average of three measurement. Colors represent irrigation water sources (transfer water “TW blue colour”, irrigators water “IW orange colour”, and reclaimed water “RW green colour”). Filled symbols refer to control irrigation treatment and empty symbols refer to RDI treatment. Yellow shaded area emphasizes the period of RDI application. * Indicates significant differences according to $LSD_{0.05}$

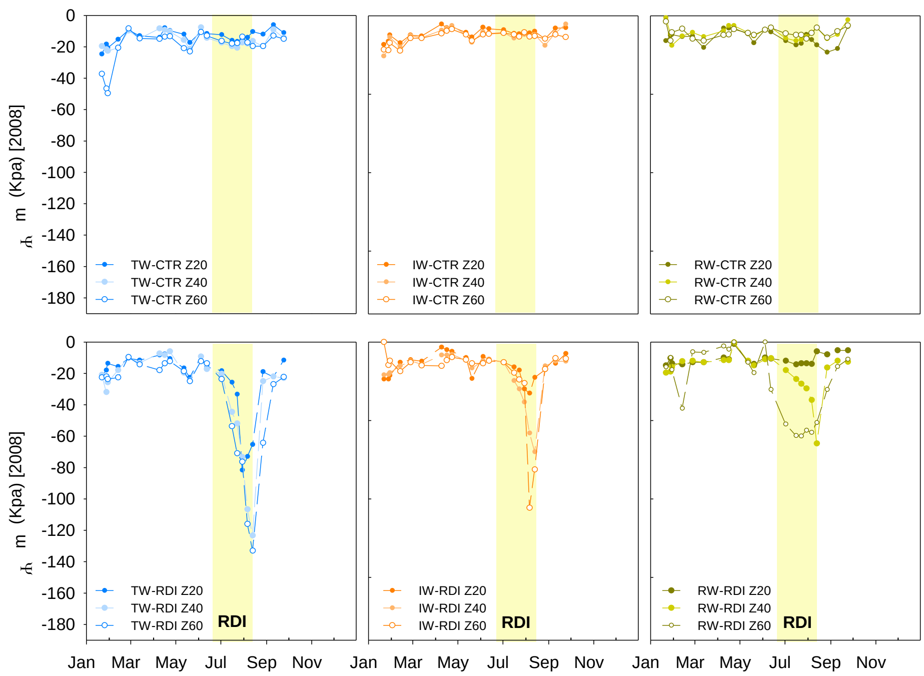


Figure 9. Evolution of soil matric potential recorded at 0.2 m away from the emitter and at three depths (0.2, 0.4 and 0.6 m) in 2008. Each point is the average of three measurement. Colors represent irrigation water sources (transfer water “TW blue colour”, irrigators water “IW orange colour”, and reclaimed water “RW green colour”). Measurements under control and RDI irrigation treatments are depicted on the upper and lower graphs respectively. Symbol fillings refer to different soil depths. Yellow shaded area emphasizes the period of RDI application.

III.3.4 Plant water relations

The midday stem water potential (Ψ_{stem}) in the control treatments was maintained at around -1 MPa during both years , although some small decreases were observed in summer because the high increase of climate demand registered in this season (figure 10). This threshold value of Ψ_{stem} for control plants (close to -1 MPa) has been previously reported by Pérez-Pérez et al. (2008), indicating that our control treatments were well irrigated.

Plant water relationships are affected by both the water availability and quality within the root zone (Paranychianakis et al., 2004). However, in this experiment, there was not a significant effect of water irrigation quality on the behaviour of leaf water status, and thus, the values of Ψ_{stem} were maintained in similar values for different water quality treatments (figure 10). These results were previously corroborated by previous studies that demonstrated no effect of reclaimed water on midday leaf water potential ((Paranychianakis et al., 2004; Walker et al., 1997).

A reduction of Ψ_{stem} was observed in the RDI treatments with respect to the values observed in control plants (figure 10). This reduction in mandarine trees has been observed previously. González et al. (2003) carried out an experiment on regulated deficit irrigation (RDI) from 1995 to 1998 in a drip-irrigated orchard on “Clementina de Nules” mandarin trees, they observed that RDI treatments always reduced the values of leaf water potential respect to the control registers, although large differences of sensitivity to water stress was reported according to the phenological stage. The best period for RDI treatments was the summer season, at initial stages of fruit growth (Stage II), because water savings were allowed while yield and fruit quality parameters were not affected.

In our experiment, the soil water deficit imposed during Stage II in the RDI treatments reduced significantly the midday stem water potential (Ψ_{stem}) at the end of this period (middle August) (figure 10). Although RDI treatments reduced Ψ_{stem} values in both years, this pattern was observed more clearly in 2008. In this year, the water stress at the end of the Stage II reached values close to -2.1 MPa while in 2009 these values were close to -1.4 MPa (figure 10). This difference was probably due to both the more severe deficit irrigation applied and the higher crop load (Berman and DeJong, 1996). In 2009, the recovery of leaf water status was quiker than in 2008, mainly due to the higher values of Ψ_{stem} reached in Stage II (figure 10).

Some studies have shown that an increased content of salts in irrigation water reduces leaf gas exchange rather than a water deficit applied (Prior et al., 1992; Paranychianakis et al., 2004; Walker et al., 1997). There have been several studies comparing stomatal conductance (g_s) and photosynthesis assimilation of CO_2 (P_n) in leaves from salinized trees with gas exchange values from non-salinized controls.

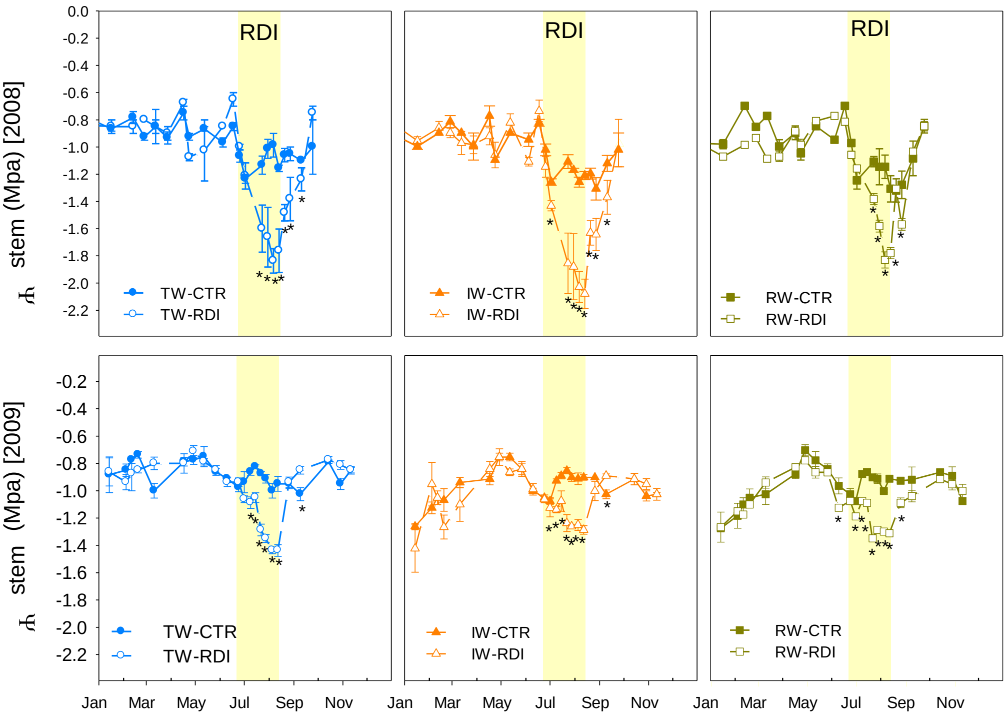


Figure 10. Evolution of midday stem water potential (Ψ_{stem}) during 2008-2009. Each point is the average of sixteen measurement. Colors represent irrigation water sources (transfer water "TW blue colour", irrigators water "IW orange colour", and reclaimed water "RW green colour"). Filled symbols refer to control irrigation treatment and empty symbols refer to RDI treatment. Yellow shaded area emphasizes the period of RDI application. * Indicates significant differences according to $LSD_{0.05.}$

It is clear that salt stress reduces water use and P_n but the underlying mechanisms are still debatable. Much of the controversy surrounding salinity-induced limitations on net gas exchange follows the same argument as the relative importance of osmotic stress vs. toxic ion stress. Osmotic stress from saline soils undoubtedly reduce water use and g_s, but the magnitude of this reduction depends on the rate at which salinity stress develops and duration over which it exits (Levy and Syvertsen, 2004). Probably due to the first reason, low salinity stress development, in our case it was not observed any differences in the behaviour of g_s and P_n between treatments irrigated with different water quality (figure 11).

The effect of regulated deficit irrigation on leaf gas exchanges was slightly modified by the type of water used. The values of g_s and P_n were not reduced by the water stress in the plants irrigated with “good quality water” (TW-RDI), however, there was a significant reduction in the values of P_n during the water stress period for IW-RDI and RW-RDI (figure 11). Patterns in g_s usually follow patterns in P_n, which has caused some researchers to mistakenly link declines in P_n to salinity-induced reductions in g_s. However, low g_s only directly limits P_n at very low leaf water potential (Farquhar and Sharkey, 1982), and in most cases, changes in P_n cause changes in g_s. In this sense, Syvertsen and Lloyd, 1994, examined effects of salinity on the relationship between photosynthesis and internal CO_2 concentrations and concluded that reductions in photosynthesis were due to a direct biochemical inhibition of mesophyll photosynthetic capacity. Thus, most decreases in photosynthesis attributable to salinity are probably caused by ion toxicity responses. This is the case of our experiment, were the reduction of $P_{n,}$ observed in IW-RDI and RW-RDI was not caused by declines in g_s (figure 11). Probably some specific ion toxicity, temporarily induced by accumulation of salts during deficit irrigation period in IW and RW, could explain this decrease of photosynthesis observed in both treatments. The possible effects on growth and yield of this photosynthesis decrease were limited, because values of P_n were recovered immediately after water deficit period (figure 11).

These results are agree with previous studies carried out in citrus trees, where the photosynthesis reduction in salt-stressed citrus leaves was associated with the specific toxicity of Cl, Na and/or Boron (Bañuls et al., 1997; Levy and Syvertsen, 2004).

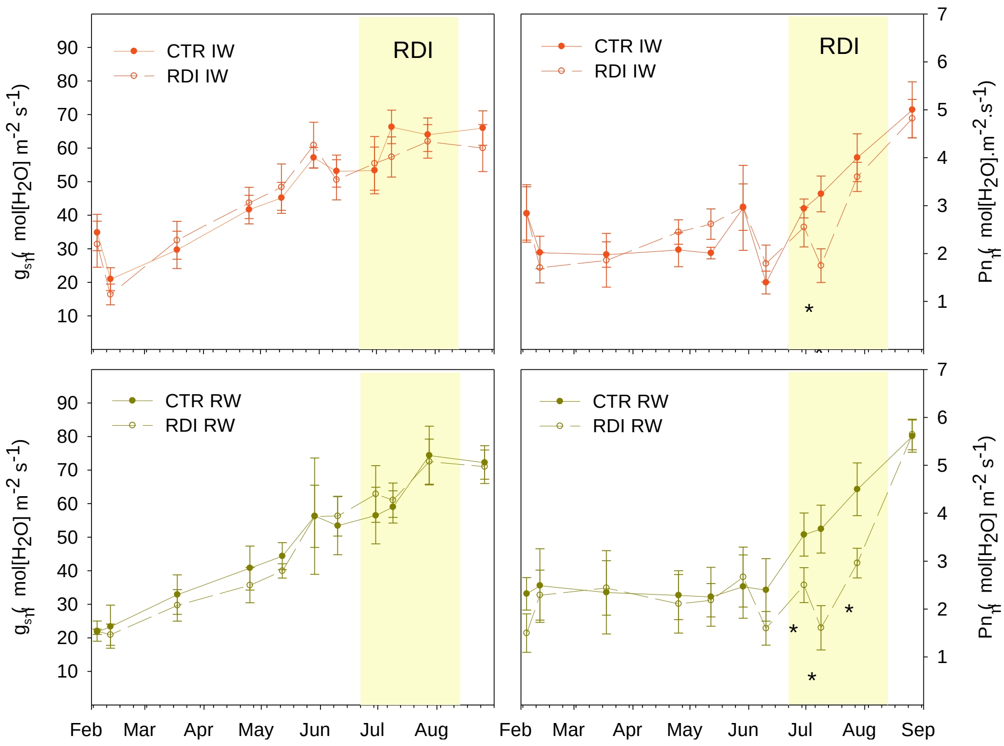


Figure 11. Net CO_2 assimilation rate (P_n) and stomatal conductance to water vapour (g_s) in 2009. Each point is the average of sixteen measurement. Yellow shaded area emphasizes the period of RDI application. * Indicate significant differences according to $LSD_{0.05}$. The treatments are represented by irrigators water (orange colour) (IW), and reclaimed water (green colour) (RW).

III.3.5 Leaf mineral analysis

Leaf Na concentration was maintained during the experiment within a normal range of accumulation (0.1-0.2 %) for all treatments (figure 12), always below toxic levels limit (< 0.5%) (Ramos, 1996). Leaf Cl concentration was also below the toxic level limit (<0.6%) (Ramos, 1996) for all treatments. The higher values of leaf Cl concentration were found in TW-C despite transfer water had the lowest Cl concentration (figure 12). It was surprising that the high accumulation of Na and Cl in the soil did not induce an increase in the leaf Na and Cl concentration; some authors has proposed that other elements presented in the soil solution when reclaimed water is used for irrigation, as Ca and K, could be very effective in reducing the transport of both sodium and chloride from roots to leaves (Bar-Tal et al., 1991; Zekri, 1993, b; Zekri and Parsons, 1990; Zid and Grignon, 1985; Zekri and Parsons, 1992).

An increase in the leaf B concentration was seen during the evolution of the experiment, reaching values over the phytotoxic limit (>100 mg l^{-1} according to Legaz et al., 1995). These high B concentration were more marked in RW treatments (figure 12), although no leaf toxicity symptons were observed. This data confirms one of the main conclusion reached in chapter II, suggesting that the high concentration of B in the reclaimed water from wastewater treatment plants in the Region of Murcia is one of the most important problem to use this type of water source in the agriculture.

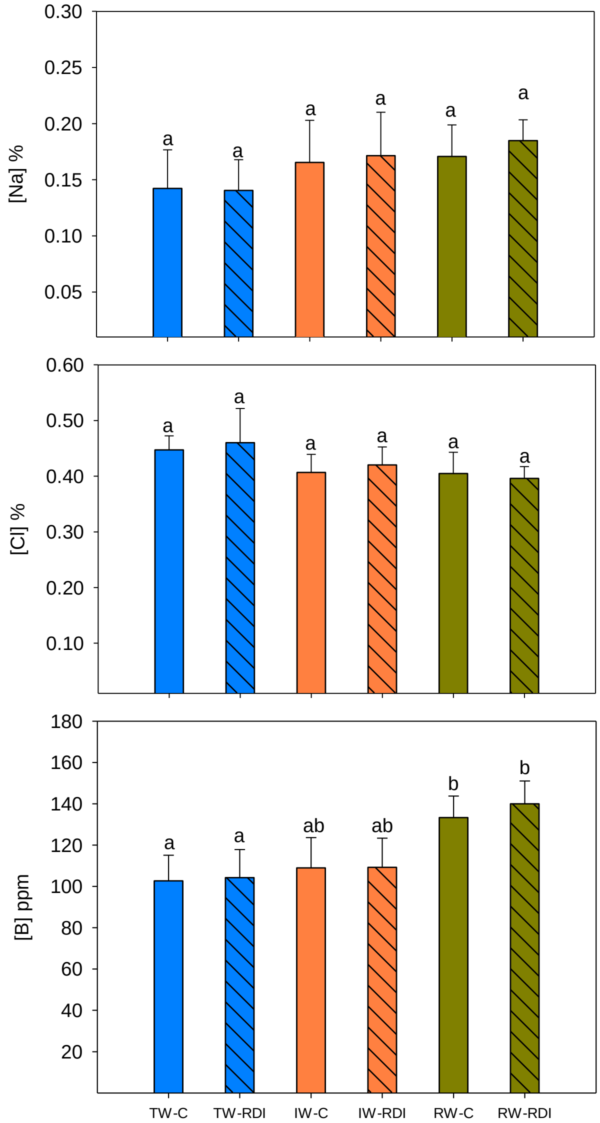


Figure 12. Biannual average of leaf mineral concentration of Cl (%), Na(%) and B(ppm) measured during 2008 and 2009. Background colors represent irrigation water sources (transfer water "TW blue colour", irrigators water "IW orange colour", and reclaimed water "RW green colour"). Background patterns represents irrigation treatment (no pattern = Control and downward-lines = RDI). Letters in each column indicate significant differences according to $LSD_{0.05}$.

It was observed that foliar nitrogen levels were, during all seasons and treatments, in the optimum range considered for citrus trees development (Parsons and Weathon, 1996). Although some researchers claim that reclaimed water is an important source of nitrogen for citrus trees (Zekri and Koo, 1994; Jimenez

Cisneros, 1995), the amount of nitrogen supplied by reclaimed water depends of the depuration treatment received in the WWTP. In this case, the water treatment process eliminated around the 90% of the nitrogen and phosphorus received in the influent. In this sense, the leaf macro-nutrients (P, K, Ca, Mg) and leaf micro-nutrients (Fe, Mn, Zn) were always in the recommended range by Legaz et al. 1995, and there are no significant differences of leaf ions accumulation between treatments (data not shown).

III.3.6 Vegetative growth, yield and fruit quality

In 2007, when the investigation started, the experimental plot had similar tree canopies between treatments (table 10). After three years applying RDI combined with different sources of irrigation water (2009), there were no significant differences in vegetative growth between the RDI and control treatments (table 10). The decrease of vegetative growth observed in response to deficit irrigation applied was not as clear as that found by other authors in different crops of citrus, presumably because their treatments were more long-term (Hilgeman and Sharp (1970) during 20 years; Levy et al. (1978) during 12 years). Other recent short-term studies in citrus trees demonstrated that RDI applied during the appropriated period, does not produce significant reductions in the vegetative growth in mature ‘Lane late’ orange trees (Pérez-Pérez, et al., 2008) and ‘Clementina de Nules’ mandarin tress (Gonzalo-Altozano and Castel, 2003).

Table 10. Tree canopy (m^3), and fruit set (%) during 2007-2008-2009. (“TW” = transfer water, “IW” = irrigators water”, “RW”= reclaimed water, “CTR”= control and “RDI” = regulated deficit irrigation).

	2007	**2008**		**2009**	
Irrigation treatments	**Tree canopy**	**Tree canopy**	**Fruit set**	**Tree canopy**	**Fruit set**
TW-CTR	8,5±0,2a	14,7±0,9c	62.5±6.5a	15,9±0,6a	33,7±4.5a
TW-RDI	-	15,6±2,3c	56.3±5.7a	14,5±0,7a	32,6±3.2 a
IW-CTR	8,9±0,3a	12,7±1b	64,6±10.3 a	16,1±1,4a	35,0±6.5 a
IW-RDI	-	13,5±1,4b	61,8±6.1a	15,9±1,4 a	36,7±6.2 a
RW-CTR	8,2±0,3a	10,1±0,5a	52,3±7.3a	13,4±0,9a	34,3±6.2 a
RW-RDI	-	11,6±1ab	51,4±7.1a	13,2±0,8a	38,2±5.7 a

Values are the mean ± SE. Letters in each column indicate significant differences according to $LSD_{0.05}$

However, the vegetative growth was affected by the type of water applied in 2008. The highest tree canopy value was measured, after harvest, in the TW treatments (around 15 m^3); the lowest tree canopy was measured in the RW treatments with values close to 10 m^3. These large differences in tree canopy size were confirmed by defoliation processes observed in the RW treatments at the end of summer in 2008, probably induced by some temporal osmotic and/or toxic effects.

An important fluctuation in the crop production was observed during the three years (table 11). The yield increase observed in 2008 respect to the crop production measured in 2007 and 2009, was mainly due to an increase in the fruit set, less pronounced in RDI treatments (table 10). This high level of fruit set was the origin of a high crop load and induced a high production in 2008 respect to the other seasons (table 11). The high crop load also was responsible of the slight reduction of fruit size observed in 2008 respect to other years, especially in the case of TW and IW treatments (table 11).

The effects of the RDI and water quality treatments on yield varied also among seasons. Thus, in 2007 and 2009, the saline treatments did not affect the crop production and the effects of RDI were irrelevant (table 11). However, in 2008, although the yield reduction only was statistically significant for the RW-RDI treatment, it was possible to observe a tendency to decrease the crop production by effect of RDI and poor water quality. Thus, in 2008, the yield recorded was 98, 92 and 83 kg/tree for TW-C, IW-C and RW-C, respectively, while in the water deficit treatments, the values were 92, 87 and 74 kg/tree for TW-RDI, IW-RDI and RW-RDI respectively (table 11). These reductions were mainly due to a decrease in the number of fruits per tree, oscillating between 998 fruits measured in TW-C and 650 fruits observed in RW-RDI (table 11).

The fruit size, expressed as fruit fresh weight, was never reduced by the effects of RDI and poor quality water (table 11), in fact, in 2008, the combined effects of both treatments slightly increased the fruit size respect to the conventional treatments (table 11). A similar result was found in fruit weight by Zekri and Koo (1993). This higher fruit size could be related to the tendency to reduce the number of fruits in the trees irrigated with reclaimed water. This “natural” fruit thinning could be one useful technique in improving tree water status and partially compensating for the negative effects of water stress on fruit growth (Lopez et al., 2006). Lower crop load can improve marketable yield under severe water stress (Marsal et al., 2010).

The water use efficiency (WUE) (Kg m^{-3}) was higher in the RDI treatments during 2008, but no differences between treatments were observed for 2009 (table 11). Overall, the present results confirm previous research by González-Altozano and Castel (1999) which showed that the summer is a suitable period for applying water restrictions in mandarine trees.

Table 11. Yield (Kg and fruits $tree^{-1}$), Fruit weight (g) and water use efficiency (WUE) (Kg m^{-3}). ("TW" = transfer water, "IW" = irrigators water", "RW"= reclaimed water, "CTR"= control and "RDI" = regulated deficit irrigation).

			Fruits $tree^{-1}$	**Kg $tree^{-1}$**	**Fruit weight**	**WUE**
2007	**TW**	CTR	289 ± 10 a	31 ±3 a	107 ± 3 a	3.3 ± 0.3 a
		RDI	-	-	-	-
	IW	CTR	315 ± 15 a	34 ± 7 a	108 ± 2 a	3.6 ± 0.2 a
		RDI	-	-	-	-
	RW	CTR	326 ± 14 a	35 ± 8 a	107 ± 2 a	3.7 ± 0.3 a
		RDI	-	-	-	-
2008	**TW**	CTR	998 ± 65 a	98 ± 9 a	98 ± 3 a	10.6 ± 0.5 a
		RDI	980±121 a	92 ± 6 ab	94 ± 6 a	12.2 ± 0.7 b
	IW	CTR	953±57 a	92 ± 9 ab	97 ± 2 a	10.0 ±0.4 a
		RDI	910±31 a	87 ± 9 ab	96 ± 1 a	11.5 ± 0.6 ab
	RW	CTR	743 ± 23 b	83 ± 8 ab	112 ± 3 b	9.1 ± 1.0 a
		RDI	650 ± 45 c	74 ± 3 b	114 ± 4 b	9.8 ± 0.7 a
2009	**TW**	CTR	618 ± 24 a	64 ± 4 a	104 ± 3 a	6.0 ± 0.4 a
		RDI	602 ± 24 a	63 ± 6 a	105 ± 6 a	7.0 ± 0.7 a
	IW	CTR	682 ± 65 a	71 ± 5 a	104 ± 2 a	6.7 ± 0.3 a
		RDI	657 ± 66 a	68 ± 8 a	104 ± 1 a	7.6 ± 1.2 a
	RW	CTR	588 ± 44 a	60 ± 5 a	102 ± 3 a	5.7 ± 0.7 a
		RDI	594 ± 41 a	60± 6 a	101 ± 4 a	6.8 ± 0.9 a

Values are the mean ± SE. Letters in each column for each year indicate significant differences according to $LSD_{0.05}$

The final effect of RDI on yield and fruit size distribution depends on both the degree and the duration of plant water stress and also the crop load. In the same way, as it has been shown in this assay, the effects of salt stress on yield depends on the interaction between salinity and various soil, water and climatic conditions (Maas, 1993) and therefore, longer term effects of the combination between RDI and different irrigation water sources in the final yield are necessary to reach .

Although some studies have shown that RDI strategies can improve fruit quality (Gelly et al., 2003; Girona et al., 2003) without affecting fruit size (Crisosto et al., 2000; Li et al., 1989); in general, quality parameters of mandarins such as peel thickness, juice volume, SSC, TA and maturity index were not affected by the RDI strategies considered in this experiment (table 12 and 13). A tendency of higher fruit weight and size were observed in reclaimed water treatments (table 11).

The fruit quality parameter more affected by the irrigation strategies was vitamin C. Although without statistically differences, in general, it was observed that RDI treatments had more vitamin C content than control treatments (table 12 and 13). In other fruit tree specie, the RDI strategies caused fruit peel stress, lowering the content of vitamin C and carotenoids, while increasing the phenolic content, mainly anthocyanins and procyanidins (Buendía et al., 2008). This demonstrates that little is known about the influence of the combined effect of reclaimed water irrigation and RDI strategies in fruit quality.

Gonzalez-Molina et al. 2008, showed that wheather conditions could determine, at least in part, differences in the contents of lemon juice bioactives. The content of vitamin C in fruits and vegetables can be influenced by various factors such as genotypic differences, climatic conditions and cultural practices (Lee and Kader, 2000). In 2009, vitamin C content decreased compared with 2008; this could be because of the lower maturity index, as it was seen in another species (Marín et al., 2004), and different wheather conditions, mainly higher rainfall (table 9).

Table 12. Fruit quality parameters measured in 2008. Peel thickness (mm), juice volume (%), titratable acidity (TA) (%), soluble solid content (SSC) (ºBrix) and vitamin C (milligrams per 100 g of fresh weight). ("TW" = transfer water, "IW" = irrigators water", "RW"= reclaimed water, "CTR"= control and "RDI" = regulated deficit irrigation).

Treatment	Peel thickness	Juice	SSC	TA	Maturity Index	Vitamin C
TW-CTR	3,1 ± 0,4 a	44,5 ± 6,3 a	12,4 ± 3,9 a	0,8 ± 0,2 a	15,2 ± 4,8 a	26,2±5,8 a
TW-RDI	2,5 ± 0,3 a	54,3 ± 7,6 a	12,9 ± 4,1a	0,8 ± 0,3 a	14,7 ± 4,6 a	28,0±3,2 a
IW-CTR	2,8 ± 0,4 a	52,1 ± 7,3 a	12,2 ± 3,8 a	0,8 ± 0,2 a	14,3 ± 4,5 a	36,1±3 b
IW-RDI	2,6 ± 0,3 a	44,9 ± 6,4 a	12,6 ± 3,9 a	0,8 ± 0,2 a	14,4 ± 4,5 a	36,1±3 b
RW-CTR	2,8 ± 0,4 a	45,5 ± 6,4 a	13,4 ± 4,2 a	0,9 ± 0,2 a	14,3 ± 4,5 a	27,7 ± 2,2 a
RW-RDI	3,0 ± 0,5 a	45,5 ± 6,3 a	13,3 ± 4,2 a	0,8 ± 0,2 a	15,0 ± 4,7 a	31,9 ± 3,1 a

Values are the mean ± SE. Letters in each column indicate significant differences according to $LSD_{0.05}$

Table 13. Fruit quality parameters measured in 2009. Peel thickness (mm), juice volume (%), titratable acidity (TA) (%), soluble solid content (SSC) (º Brix) and vitamin C (milligrams per 100 g of fresh weight).). ("TW" = transfer water, "IW" = irrigators water", "RW"= reclaimed water, "CTR"= control and "RDI" = regulated deficit irrigation).

Treatment	Peel thickness	Juice	SSC	TA	Maturity Index	Vitamin C
TW-CTR	2,5 ± 0,6 a	53,0 ± 13,6 a	11,6 ± 2,7 a	0,9 ± 0.1 a	12,8 ± 1.5 a	8,5 ± 4,9 a
TW-RDI	2,2 ± 0,5 a	53,8 ± 13,8 a	12,0 ± 3,4 b	0,9 ± 0.2 a	13,8 ± 0.6 a	10,9 ± 6,3 a
IW-CTR	1,9 ± 0,4 a	51,4 ± 13,2 a	12,7 ± 4,0 a	1,1 ± 0.1 a	11,8 ± 0.6 a	13,2 ± 7,6 a
IW-RDI	2,0 ± 0,5 a	46,4 ± 12,0 a	12,0 ± 4,9 a	1,0 ± 0.1 a	11,9 ± 0.8 a	15,9 ± 9,1 a
RW-CTR	2,4 ± 0,6 a	51,3 ± 15,8 a	11,3 ± 2,7 c	1,0 ± 0.1 a	12,1 ± 1.4 a	13,8 ± 7,9a
RW-RDI	2,5 ± 0,6 a	47,5 ± 14,8 a	11,9 ± 2,9 c	1,0 ± 0.1 a	12,4 ±1.6 a	17,6 ±6,2 a

Values are the mean ± SE. Letters in each column indicate significant differences according to $LSD_{0.05}$

III.4 CONCLUSIONS

Yield reductions were not significant between treatments, however a tendency to reduce the number of fruit was detected in the RW treatments, being this reduction more pronounced under combined conditions of reduced quality water and regulated deficit irrigation (RW-RDI treatment).

The content of vitamin C was slightly higher in fruits subjected to RDI treatments. The highest value was observed in fruits of RW-RDI treatment. This indicates that the combined effects of RDI strategies and reclaimed water can increase some fruit quality parameters on mandarin trees. Neverthelss, further studies are required to assess the long term effects of such combination.

Plant water relations and vegetative growth were not significantly affected by the use of reclaimed water. However water stress in the RDI treatments induced some reductions in stem water potential and plant growth that were immediately recovered when the water deficit disappeared.

The biggest problem of reclaimed water in Murcia is its salinity. Na, B and chloride concentration exceeded the phytotoxic level in RW irrigation-water, although no toxic visual symptoms have been seen during the three seasons.

It is important to remark that in arid and semi-arid areas, the combination of RDI strategies and reclaimed water-use can induce some problems in the long-term because of the salts and boron accumulation. A tendency was identified in terms of salts accumulation in the soil during last season in RW-RDI treatments, so intensive monitoring is needed to avoid the reduction in the agro-physical soil properties when reclaimed water irrigation is applied.

IV POTENTIAL USE OF RECLAIMED WATER WITH GIS-BASED MULTI-CRITERIA ANALYSIS

IV.1 INTRODUCTION

In the context of the implementation of the 2000/60/EC Directive (Water Framework Directive) and the recommendations of the World Water Assessment Programme (UNESCO, 2009), sustainable management of water requires an approach that, on one hand, allows control of aquatic pollution and protection of water resources, which can be achieved through the adequate provision of wastewater treatment facilities, and on the other hand, it is essential to ensure the availability of reclaimed water, in terms of quantity and quality, in order to satisfy uses like public supply and agricultural irrigation.

Over the last decade, the water management companies have invested strongly in the construction and rehabilitation of low cost wastewater treatment plants (WWTP) for small populations, in the context of the 1991/271/EEC Directive (urban wastewater treatment). According to UNESCO (2009), this kind of solution and the selective reuse of reclaimed water will constitute one of the great challenges for the integrated water management in rural areas over the next two decades. The rural areas of Beira Interior region in Portugal have several golf course projects, SPA resorts with therapeutic treatment and important agricultural activities that represent an economic gain for the region and need a considerable amount of water which is not always readily available. In the last few years, the Beira region experienced a serious water shortage period that put under threat almost all its economic activities.

The nearly four hundred small WWTP in operation in that region could contribute to satisfying the demands of these activities through the reuse of their treated effluents. Most of these WWTP are constructed wetlands (CW) that are considered a low cost technology (Vymazal and Kropfelova, 2008) for treating wastewater. Besides providing secondary treatment, CW may also be used for treating effluents from metal finishing industry, which is especially useful when the receiving streams are considered sensitive or for reuse practices (Ghermandi et al., 2005; Marecos do Monte and Albuquerque, 2010).

CW can treats contaminants such as total suspended solids (TSS), biochemical oxygen demand (BOD), nutrients (nitrogen and phosphorus), organic compounds, and inorganic constituents (such as metals) to meet regulatory targets. The two main types of constructed wetlands are surface flow (SF) and subsurface flow (SSF). SSF wetlands are designed so that water flows horizontally (HSSF) or vertically (VSSF) through the substrate and below the ground surface (USDA 1995, ITRC, 2003).

According to Angelakis et al. (1999), the reuse potential in Portugal is enormous and the estimated treated wastewater discharged into water streams would be sufficient to supply 10% of water needs for irrigation in dry years without seasonal storage. Approximately 580 Mm^3 $year^{-1}$ could be used for different purposes in rural areas. Roughly, depending on storage capacity, between 35,000 and 100,000 ha could be irrigated with recycled water (Angelakis et al, 2003). Therefore, the main reuse opportunities in the Beira Interior region would be for agricultural irrigation, landscape and golf courses and aquifer recharge. The selection of reuse options should be made based on aspects such as quantity, variability of the quality over time, reuse guidelines and regulations, climate changes, technical requirements for WWTP upgrade and reuse projects cost-effectiveness.

Many aquifers of the region are overexploited due to the increase of water demand for agricultural and landscape irrigation and SPA activities. Therefore, one possible remedy to this problem could be through aquifer artificial recharge using reclaimed water, as suggested by Bouwer (2002), either by infiltration or by direct injection. Soil aquifer treatment (SAT) would be an advantageous option because reclaimed water can be stored during times of excess and recovered in times of shortage. The big advantage of underground water storage is the prevention of evaporation losses. Groundwater recharge systems are sustainable, economic, and do not have the eco-environmental problems that dams have (Bouwer, 1999).The example of the Dan Project in Israel shows that submitting secondary effluents to a SAT system in a dune sand aquifer can result in the production of nearly potable water (Kanarek and Michail, 1996).

Adverse health effects, environmental and socio-cultural concerns associated with potable aquifer recharge need to be addressed. Human pathogens and trace organic compounds are of particular concern when groundwater recharge involves domestic water supply aquifers (Tsuchihashi et al., 2002).

The definition of a methodology for wastewater reuse in aquifer recharge requires the collection, processing and analysis of complex database (e.g. land use, aquifer characteristics, environmental and legal restrictions and characteristics of the reclaimed water) and tools for multi-criteria analysis. The use of Geographic Information Systems (GIS) allows the geo-referencing, organization, processing and analysis of such complex information. GIS has been used in environmental sciences for evaluating groundwater vulnerability to nitrate pollution (Lake et al., 2003), creating thematic maps for pulp mill sludge application (Ribeiro et al., 2010) and location of urban non-point pollution (Mitchell, 2005) as well as for site location of wastewater treatment facilities (Gemitzia et al., 2007) and aquifer recharge (Kallali et al., 2007).

This work aims at identifying potential sites for reclaimed water infiltration for groundwater recharge using a GIS-based multi-criteria analysis. A constructed wetland system located in the northwest part of the Beira Interior region was used as the source of reclaimed water. The work also intends to show that small wastewater treatment facilities may be used for aquifer recharge in areas of water scarcity, reducing the discharge of residuals loads to the environment and benefiting economic and tourist activities.

IV.2 MATERIAL AND METHODS

IV.2.1 Characterization of the study area

This step included the identification of the study area taking into account the location of the SPA of Cro and its protected area and the source of reclaimed water (the WWTP of Vila Fernando, Guarda, Portugal). The following digital information was used:

- Extract of Portuguese Military Maps No. 192, 193, 194, 203, 204, 214, 215, 225 and 226 (1/25000 scale);
- Map of the protected area of the SPA of Cró (1/25000 scale);
- Altimetry data (1/25000 scale);
- Orthophotomaps (photogrammetric flights of 2002, 2003 and 2004; 1/5000 scale).

The study area is located in the northwestern part of the Beira Interior region in the district of Guarda with altitudes ranging from 680 to 820 m (figure 13). The climate is continental with 780 mm mean annual precipitation. The average reference evapotranspiration (ETo) is around 700 mm and a water deficit period often extends from June till September (table 14). The mean temperature is 10.7 °C.

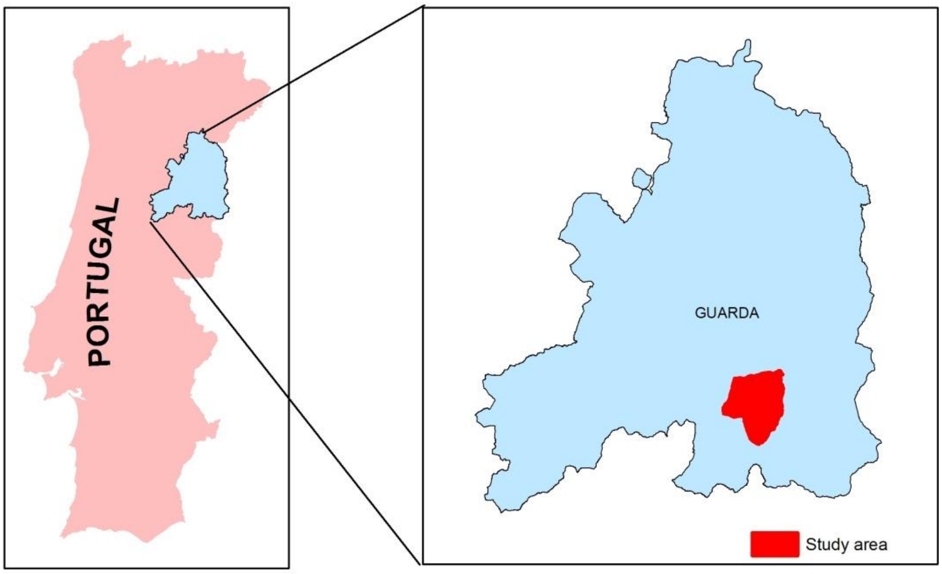


Figure 13. Location map of the study area (40º32´09´´ N, 7º16´00´´ W).

Table 14. Monthly average of precipitation (P), reference evapotranspiration (ETo) and the deficit (P - ETo) (mm month^{-1}) in the studied area in the period 1965-1994 (Cavaleiro, 2002)).

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
P	101	104	47.1	70.5	69.2	37.9	12.5	10.6	35.9	99.1	105.4	90.9	**784.1**
ETo	10.5	15.1	28.9	45.5	67.1	105.6	127.2	119.7	90	52.8	25.2	11.7	**699.8**
Deficit	90.4	88.8	18.1	24.9	2	-67.7	-114.7	-109.1	-54.1	46.2	80.1	79.1	**84.2**

Table 15. Geographic coordinates and elementary characteristics of the Cró thermal water (Cavaleiro, 2000)

Geographic coordenates			Elevation	Temperature	Conductivity	pH	Depth
	M	**P**	**(m)**	**(ºC)**	**(dS m^{-1})**		**(m)**
P1	92330	86669	683	12	0.104	6.6	7
P2	92831	86500	690	11.6	0.110	6.4	4

P1 and P2: wells

Geologically there are primitive soil formations, mainly granitic, with terciary and quaternary re-vegetation. The predominant lithology is granitic with thick and medium grain. The hydro-geological area belongs to the hydrographic catchment of the river Côa with a total area of 8,017 ha. The main characteristic of the basin is the hydro-mineral aquifer that feeds the SPA of Cró (sulphurous water). This SPA is mainly fed by two wells (P1 and P2), whose characteristics are presented in table 15.

IV.2.2 Reclaimed water monitoring

A 21 month monitoring campaign (November 2007 to November 2009) was set up in a HSSF CW located at Vila Fernando (Portugal), that started 6 months after its start-up (picture 14). The bed was colonized with *Phragmites australis*, filled with Filtralite MR 4-8mm. Its dimensions were 23 m length x18 m width and a water depth of 0.5 m. It was designed for 800 person equivalent (p.e.). The flow rates ranged from 26.5 to 49 $m^3\ d^{-1}$, the hydraulic loading rate (HLR) from 6 to 12 cm d^{-1}, the hydraulic retention time (HRT) from 5 to 9 d, the BOD_5 from 200 to 400 mg l^{-1} and the COD from 500 to 700 mg l^{-1}.

Picture 14. Bed colonized with *Phragmites australis*, in the subsurface horizontal flow of a constructed wetland (HSSF CW) located at Vila Fernando .

The campaign included the daily measurement of flow-rate (entrance of the HSSF beds) and the collection of monthly samples (a single sampling approximately at the same time) at the influent and effluent of the bed to determine pH, temperature, biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total nitrogen (TN), ammonia nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), total phosphorus (TP), total suspended solids (TSS), electrical conductivity (EC), sodium (Na), calcium (Ca), potassium (K), chloride (Cl), total coliforms (TC), fecal coliforms (FC), *E.coli* and helminth eggs (HE). In the last sampling month, the measurements included the concentration of magnesium (Mg), boron (B), cadmium (Cd), chromium (Cr), copper (Co), and nickel (Ni), lead (Pb) and zinc (Zn).

The measurements of pH, EC and temperature were carried out directly using a Tritilab TIM 900 (Radiometer, France). COD was determined with cuvette tests LCK 614 (50-300 mg l^{-1}) following the standard DIN 38409-4 and a CADAS 50 spectrophotometer UV-Vis (HACH-LANGE, Germany). BOD_5 and TSS were determined according to the 5210B, 2540D and 2540E standards, respectively, from the Standard Methods (APHA-AWWA-WEF,1999).

TN, NH_4-N and TP were obtained using the cuvette tests LCK 138 (1-16 mg TN l^{-1}), LCK 238 (5-40 mg TN l^{-1}), LCK 303 (2-47 mg NH_4-N l^{-1}) and LCK 350 (2-20 mg TP l^{-1}) following the 2.6-dimethylphenol method (TN, APHA-AWWA-WEF,1999), the standard DIN 38406-E 5-1 (NH_4-N) and the standard DIN 38405-D11-4 (TP), respectively, and the same spectrometer. For higher concentrations than the upper limits of the cuvette-tests the samples were previously diluted. NO_3-N and Cl were analyzed using a Dionex-IX120 ion chromatography (Dionex Corp., USA) according to the standard ISO 10304-1:2007.

The heavy metals (Cd, Cr, Ni, Pb, Zn, and Cu) were determined by atomic absorption spectroscopy with an electrothermic atomizer (GBC-906, Australia) following the standard ISO 15586:2003. The Na, Ca, K and Mg were analyzed with the same equipment, but using the air-acetylene flame method as described in the standards ISO 9964-1:1993 (Na and K) and ISO 7980:1986 (Ca and Mg). B was determined using a spectrophotometer UV-Vis (GBC-Cintra 40, Australia) following the Standard Methods 4500 B (APHA-AWWA-WEF,1999).

The FC, TC and *E.coli* were analyzed following the standard ISO 9308-1:2000 and the Standard Methods 9221 B e 9221 E (APHA-AWWA-WEF, 1999). EH was determined through optic microscopy (Ceti, Belgium) according to the modified Bailenger method (Ayres and Mara,1996).

IV.2.3 Identification of suitable areas for aquifer recharge

The identification of areas with potential for aquifer recharge was realized taking into account the economic, environmental and technical constraints as well as the location of the SPA of Cró and its protected area and the source of reclaimed water (the WWTP of Vila Fernando). The economic criteria included affordability and costs. The environmental criteria covered urban agglomerates, protected areas and water resources vulnerability. The technical criteria included soil and groundwater characteristics, aquifer depth, slope, land use, type of soil, soil texture, infiltration velocity and distance between the CW and the infiltration site. The statements for the election of the different variables were selected based on the procedures suggested by experts, international guidelines and technical documents (State of California, 1992; Pescod, 1992; Angelakis et al., 2003; EPA, 2006; Bixio and Wintgens, 2006; Asano et al., 2007; Marecos do Monte and Albuquerque, 2010).

Economic criteria: the economic criteria included water transfer costs from the wastewater treatment plant to the application site. As referred to EPA (2006), the transport length from the WWTP to the potential application site should not exceed 8 km.

Environmental criteria: for environmental concerns, predetermined distance-thresholds were considered to keep the potential recharge areas far enough from water supply sources (reservoirs, streams and potable wells), urban agglomerations, and natural ecological reserve. A safeguard distance of 200 m from urban agglomerations and tourist areas was respected in order to avoid direct contact with human settlements. A safeguard distance of 500 m from water reservoirs and 100 m from wells and streams (both for water consumption or irrigation) was defined to avoid their contamination by reclaimed water infiltration.

Technical Criteria: for technical reasons the following five criteria were considered:

Land use: the Corine Land Cover map (Portuguese Geographic Institute, 2009) was used to evaluate the potential land use of the studied area.

Slope: The infiltration percolation site should be feasibly constructed in slopes ranging between 0 and 12%. Higher slopes increase runoff, soil erosion and thus soil instability, with basin safety risks and increased refilling costs (EPA, 2006).

Soil texture: vadose zones should not contain clay layers or other soils that could restrict the downward movement of water and form perched groundwater mounds. To avoid soil clogging and to assure the wastewater treatment, the soil must have a very low clay fraction (EPA, 2006). For this study soils with greater than 10% clay fraction were not included.

Type of soil: the soil for wastewater infiltration should have one part of the section without soil rock, on the top. Most of the quality improvement of the reclaimed water occurs in the top 1 m of soil (Pescod, 1992). The soils with a top depth lower than 1 m were not included.

Aquifer depth: aquifers should be sufficiently deep and transmissive to prevent excessive rises of the groundwater table due to infiltration. The minimum static groundwater level accepted for reclaimed water infiltration is 5 m in order to have a sufficient vadose zone for wastewater purification (State of California, 1992; Pescod, 1992).

IV.2.4 Data analysis

The elements with restrictions on use for aquifer recharge were located and geo-referred (*e.g*. hydrographic network, water supply points and water irrigation systems, roads, urban housing areas, isolated residential areas and land slopes). Based on the collected data, some of which was confirmed in-situ through field visits, the following tasks were developed:

- Conversion of some information from analogue to digital format
- Editing and processing of digital information
- Construction of a geographic model
- Construction of new thematic maps
- Spatial analysis of the thematic maps
- Structuring of alphanumeric and cartographic information

A suitability map for aquifer recharge was generated by overlaying the eight thematic maps (digital terrain model, land use map, economic criteria buffer map, water supply sources buffer map, population buffer map, slope map, soil texture map and type of soil map) associated with the economic, environmental and technical restrictions.

The analysis of the information was carried out using the software *ArcGIS 9.1* (ESRI, USA) and the *ArcCatalog* and *ArcMap* applications, namely for the following main tasks:

- Integration and management of spatial and non-spatial data (*Raster* or *Vector*)
- Editing of both data and geographical entities
- Overlaying thematic information topics
- Spatial analysis (*Spatial Analyst*)
- Design of slope maps (*3D Analyst*)
- Definition of a protection zone on the border of a geographical entity, using the buffer application
- Query of databases according to predefined criteria
- Geo-referring of elements or entities
- Geo-processing of the information for mapping in the selected study area
- Determination of the locations with higher suitability for reclaimed water infiltration using map algebra (*Raster Calculator*).

The layers were derived from geographical data obtained from official sources or generated using satellite images and geo-statistic tools. This software analyzed each one of the 10x10 m grid cells for each one of the variables mentioned in the previous sections. If all the predefined suitability criteria were met then the particular grid cell was accepted as a potential reuse area. The result was a grid file showing the areas that met all the previously specified criteria and were suitable for reclaimed water infiltration.

IV.3 RESULTS AND DISCUSION

IV.3.1 Study area

The area selected to carry out the study is between the WWTP of Vila Fernando and the SPA of Cró intercepting its protected area as shown in figure 14, where altitudes range between 680 and 880 m. It was delimited after digitalising the Military Maps No. 192, 193, 194, 203, 204, 214, 215, 225 and 226 and geo-referencing the protected area of the SPA of Cró, having been necessary to overlap cartographic elements. The total area assessed was 13,944 ha.

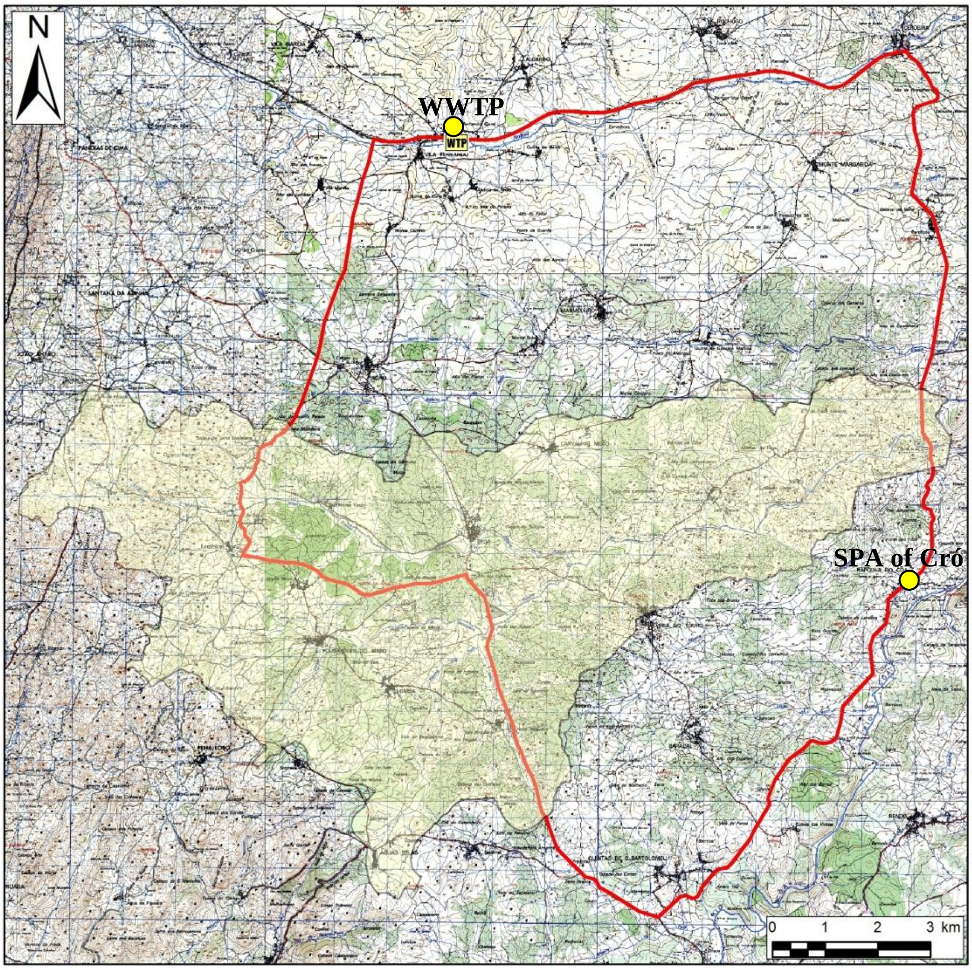


Figure 14: Total area selected within the GUARDA-Region in Portugal (red line). The green area represents the hydro-mineral aquifer delimitation.

IV.3.2 Analysis of the reclaimed water

The characteristics of the influent into and the effluent out of the CW of Vila Fernando monitored during the present work are shown in table 16. The reclaimed water does not present salinity risks for showing an EC value less than 0.7 dS m^{-1} but when this is compared to the SAR value (4.3), moderate to severe infiltration problems could be expected unless a leaching fraction is carefully applied (Westcot and Ayers, 1985). This problem is mainly related to a relatively high sodium concentration (118.7 mg l^{-1}) and low calcium and magnesium concentrations (23.6 and 0.21 mg l^{-1}, respectively).

The concentration of the phytotoxic ions (B, Cl) is under the thresholds for restriction on use (<0.7 mg l^{-1} and <140 mg l^{-1}, respectively) as suggested by

Westcot and Ayers (1985). The heavy metal concentrations (Co, Ni, Pb and Zn) are also below the toxic levels (<0.05, <0.2, <5, and <2 mg l^{-1}, respectively) but the concentration of Cd and Cr exceeds the toxic level according to the same author (>0.010 and 0.10 mg l^{-1}, respectively), althought not in the effluent.

Most of the soils of the study region are classified as poor in terms of organic matter content and values below 1% were detected in 57 agricultural parcels (Ribeiro et al., 2010), therefore the nutrient content (TN and TP) of this reclaimed water could contribute to improving soil organic carbon content (Chin-Ching et al., 2008)

The microbiological content is also high according to the health standards and regulations for wastewater reuse (WHO, 2006; USEPA, 2004), also for aquifer recharge (Royal Decree-Law 1620/2007 of Purified Water Reuse, 2007) (table 16). The average removal is around 1 log unit of total coliforms, fecal coliforms and *E.coli*. This is in accordance with results obtained by different authors working in CW operating as primary or secondary treatment and who reported average removals around 1.5 log units (Arias et al., 2003; Torrens et al., 2009). HE were not detected (<10 eggs/10 l^{-1}) in both the influent and effluent (table 16).

If the use of the reclaimed water is for aquifer recharge, the SAT system will help in the disinfection, because this treatment is effective for removal of bacteria and viruses (Asano et al., 2007). Guessab et al. (1993) observed a removal of 99.9% for fecal coliforms and a 99.9% for fecal streptococci in an infiltration-percolation system and a total elimination of helminth and cestode eggs, when using hydraulic loads of 0.23 m d^{-1}. For hydraulic rates of 0.5 m d^{-1}, using sand columns, Brissaud et al. (1991) described removal of fecal coliforms between 1.5 and 4 logs.

In the future a conservative approach will be taken, with intensive monitoring of water quality, in order to estimulate the reuse potential of this treated wastewater; this will help to verify the efficiency of the treatment processes and it will detect problems (Asano et al., 2007).

Table 16. Characteristics of the wastewater in the CW of Vila Fernando (2008-2009).

Parameters	Influent *	Effluent (reclaimed water)
Flow rate ($m^3\ d^{-1}$)	58.9 ± 26.0	-
Temperature (ºC)	15.6 - 3.8	15.3 - 3.9
pH	6.2 - 7.3	6.3 - 7.7
EC ($dS\ m^{-1}$)	0.22 ± 0.02	0.22 ± 0.02
BOD_5 ($mg\ l^{-1}$)	105.7 ± 32.1	27.4 ± 7.2
COD ($mg\ l^{-1}$)	265.2 ± 79.8	83.9 ± 13.0
TSS ($mg\ l^{-1}$)	64.0 ± 19.2	27.1 ± 18.3
NH_4-N ($mg\ l^{-1}$)	60.3 ± 5.8	54.4 ± 7.4
NO_3-N ($mg\ l^{-1}$)	1.7 ± 1.5	0.8 ± 0.5
TN ($mg\ l^{-1}$)	74.2 ± 16.1	60.7 ± 13.8
TP ($mg\ l^{-1}$)	9.5 ± 2.2	6.9 ± 1.3
Na ($mg\ l^{-1}$)	110.9 ± 14.4	118.7 ± 11.4
Mg ($mg\ l^{-1}$)**	0.23	0.21
Ca ($mg\ l^{-1}$)	19.5 ± 2.4	23.6 ± 3.1
K ($mg\ l^{-1}$)	30.2 ± 4.6	28.4 ± 5.3
Cl ($mg\ l^{-1}$)	83.7 ± 31.3	79.5 ± 32.5
B ($mg\ l^{-1}$) **	< 0.02	< 0.02
Cd ($mg\ l^{-1}$) **	0.03	0.02
Cr ($mg\ l^{-1}$) **	1.38	0.1
Co ($mg\ l^{-1}$) **	0.04	0.01
Ni ($mg\ l^{-1}$) **	0.2	0.07
Pb ($mg\ l^{-1}$) **	0.02	0.02
Zn ($mg\ l^{-1}$) **	0.02	< 0.01
TC (CFU/100 ml)	1,79 $x10^7$± 1120	1,95 $x10^6$ ± 980
FC (CFU/100 ml)	3,78$x10^6$ ± 458	6,91 $x10^5$ ± 652
E.Coli (CFU/100 ml)	5,02 $x10^6$ ± 879	1,05 $x10^4$ ± 540
HE (eggs 10 l^{-1})	< 10	< 10

* Average and confidence interval (calculated for a confidence level of 95% in the following number of samples: 21 (flow rate, temperature, pH, BOD_5, COD, TSS, NH_4-N, NO_3-N, TN, TP, Na, Ca, K and Cl), 10 (EC and TC, FC), 6 (HE, E. Coli). ** Only one measurement in the last sampling (Mg, B, Cl, Cr, Co, Ni, Pb, Zn)

IV.3.3 Suitable areas for aquifer recharge

The scale of capture of the topographic data was 1:100.000 and a digital terrain model was produced for the study area with a cell size of 10x10 m (figure 15).

Land suitable for agriculture represents 28% of the total area and for forestry 85.2% (figure 16). The agronomic use of reclaimed water in the studied area, is not profitable because the agricultural land is scattered and has many family smallholdings. These agricultural lands are granitic zones of medium altitude, provided with aquifer resources and with two different cultivation seasons: dry winter cereals and spring/summer irrigated crops such as corn, potatoes, beans and horticultural crops.

The soil texture and infiltration velocity thematic map for the study area was defined by clipping all polygons with a very low clay fraction as the Bouwer (1999) classification (sandy-loam, loamy sand and fine sand) and an infiltration velocity less than 8 cm min^{-1} (State of California, 1992), that were assembled together and coded as 1. The rest of the polygons were coded 0. The predominant soils in the studied area are antrosol (63.8 %), rock (17%), umbrisol (12.4%) and regosol (6.8%) as can be seen in figure 17. Taking into account the variable soil texture, infiltration velocity and type of soil, the most suitable soil is regosol because it has a plagic horizon with a thickness greater than 1 m and can assure the soil treatment. Therefore all polygons with regosol soil were coded 1.

For the environmental and economic criteria, in each thematic map, the exclusion areas were coded as 0 and the inclusion areas as 1. Overlaying the economic criteria (a buffer of 8 km radius from the WWTP of Vila Fernando, coded 0) and the criteria for water supply sources (a buffer area of less than 100 m radius for streams and wells and less than 500 m radius for reservoirs, both coded 0) the potential area for aquifer recharge was delimited (figure 18).

The population agglomerates thematic map was obtained creating a buffer area within a radius of 200 m around villages, agglomeration of houses and isolated houses (coded 0) as shown in figure 19. The urban agglomerations were dispersed because of the rural character of the area.

To define the slope thematic map the study area was firstly clipped and reclassified into two categories: one with values less than 12% (coded 1) and another with higher values (coded 0). The studied area was irregular with medium hills with altitudes ranging between 680 m to 880 m to both sides of the Boi bank. The total area with slope less than 12% was 10,858 ha (figure 20).

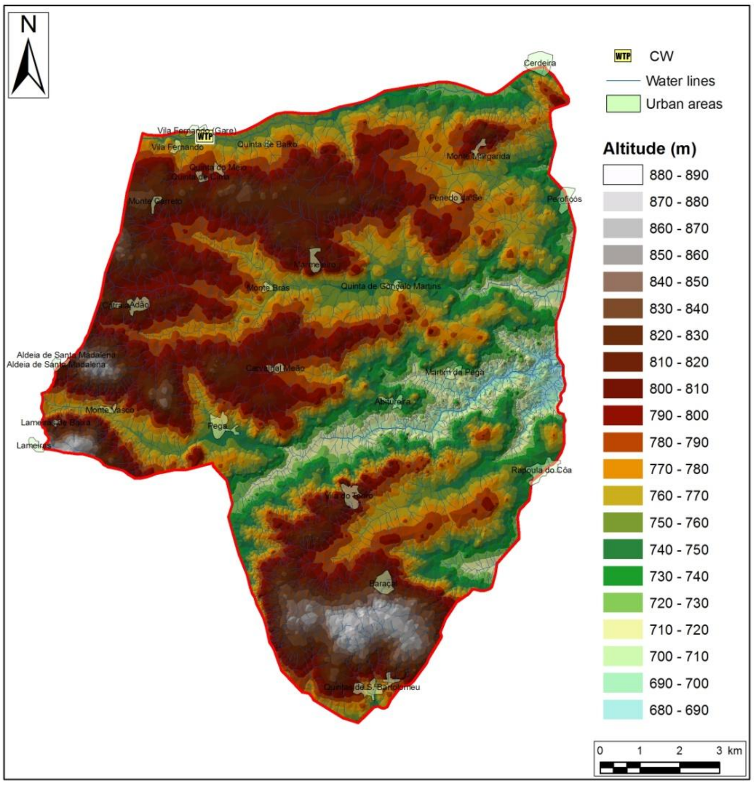


Figure 15. Digital terrain model produced for the study area with a cell size of 10x10 m

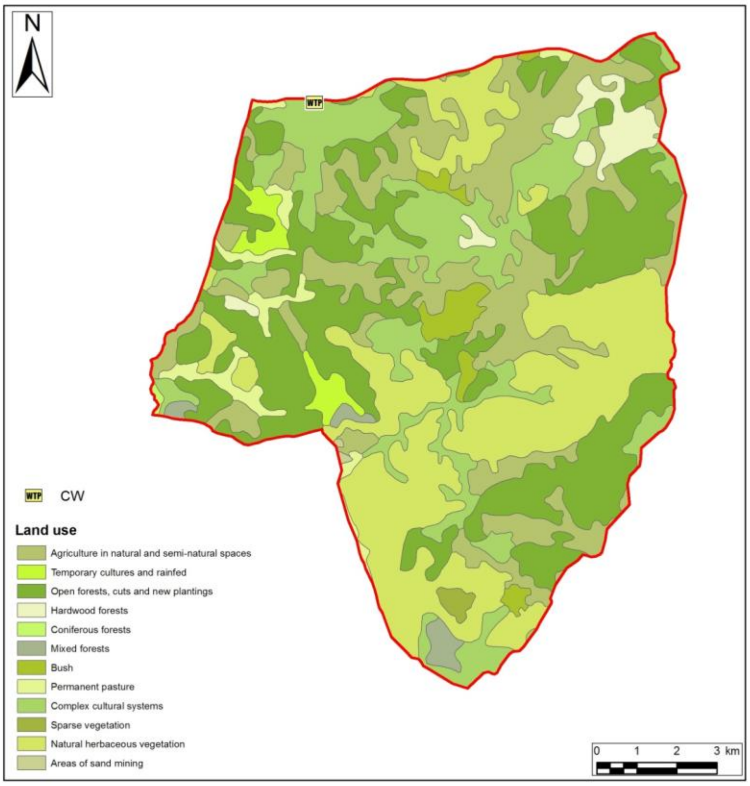


Figure 16. Land use thematic map of the study area.

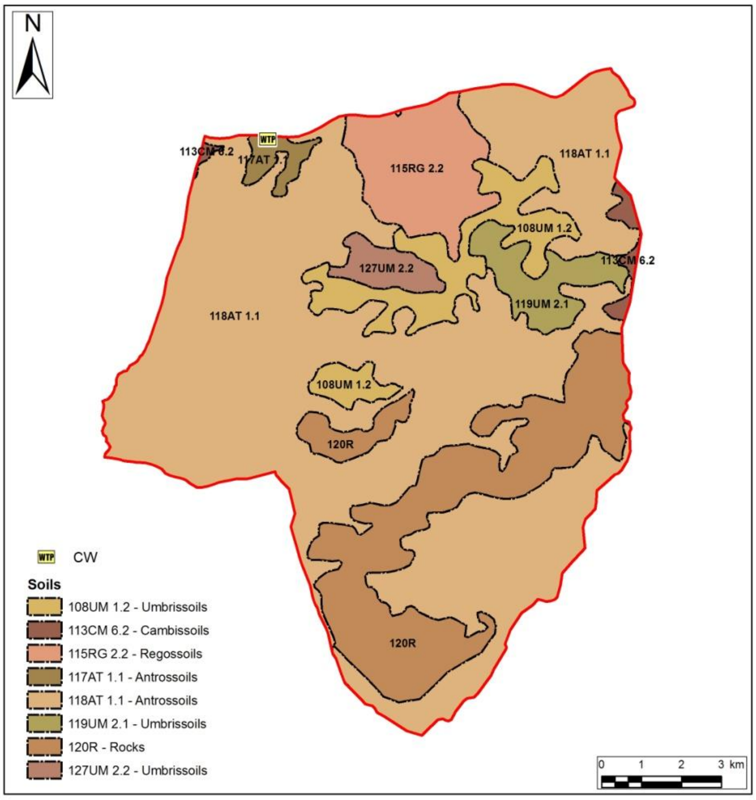


Figure 17. Type of soil thematic map of the study area.

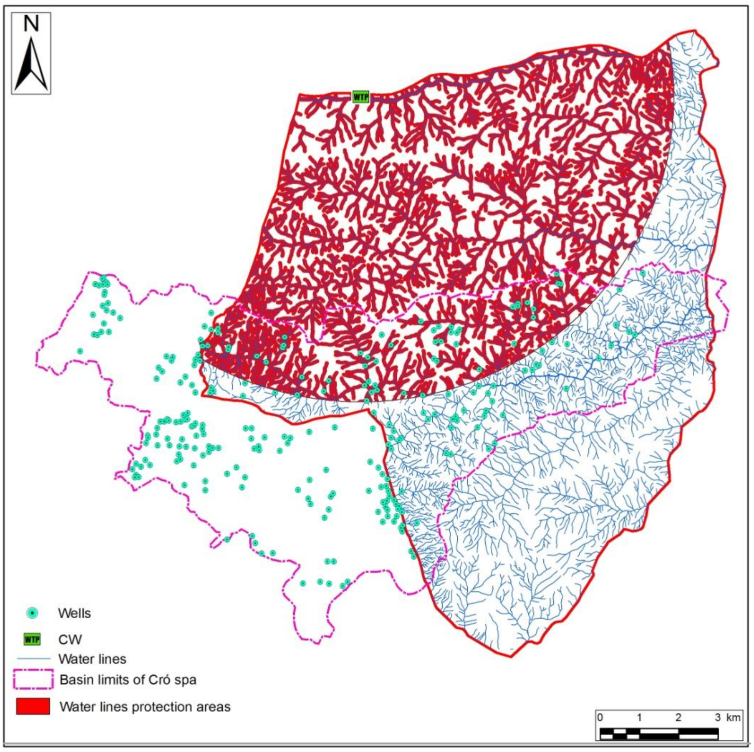


Figure 18. Potential area for aquifer recharge. It is the result of overlaying the economic criteria (a buffer of 8 km radius from the WWTP of Vila Fernando) and the criteria for water supply sources (a buffer area of less than 100 m radius for streams and wells and less than 500 m radius for reservoirs).

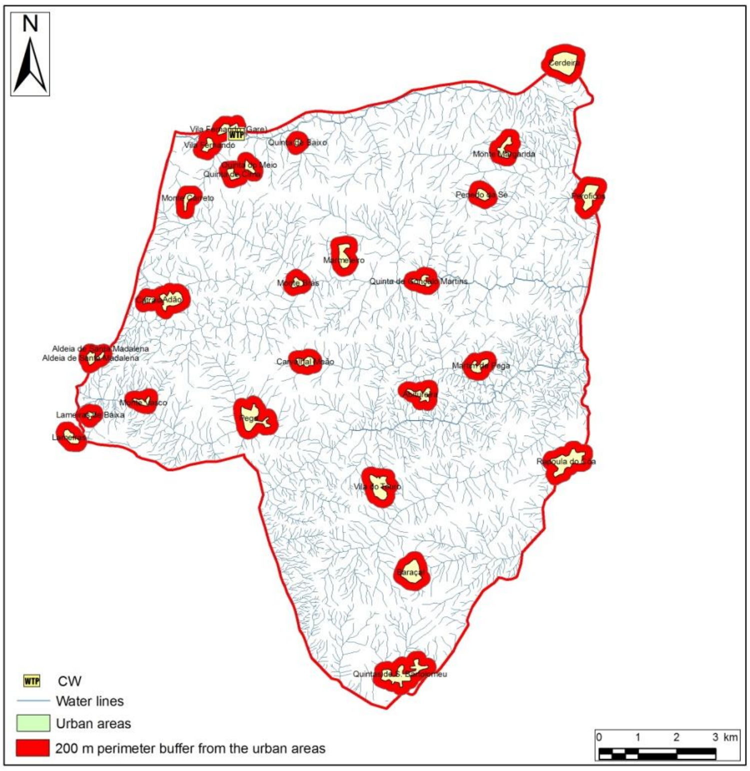


Figure 19. Population agglomerates thematic map obtained after creating a buffer area within a radius of 200 m (in red) around the villages.

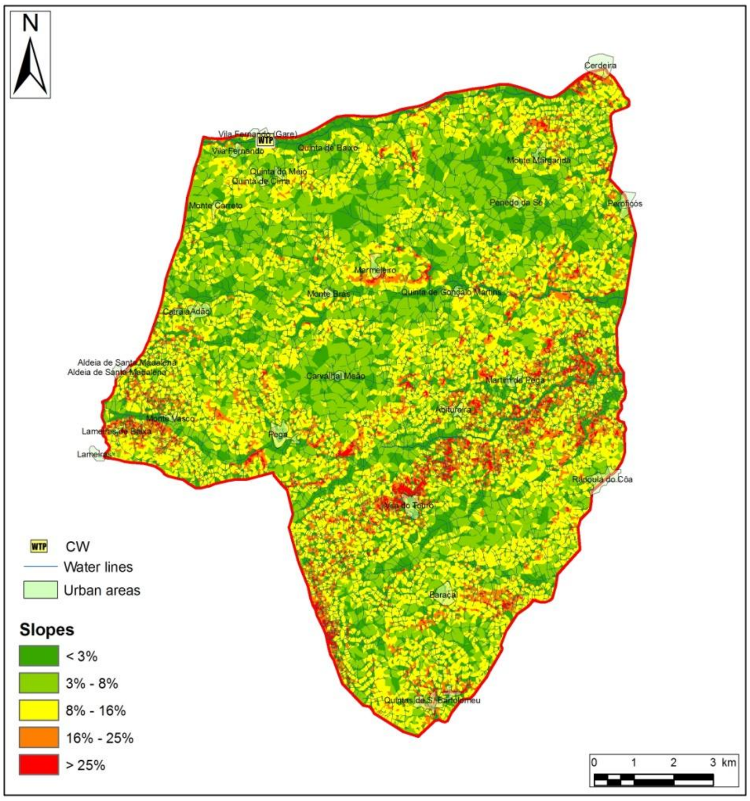


Figure 20. Slope thematic map

A GIS in *raster* format was used to create the suitability map for aquifer recharge and the results were processed according to the algebra of maps (maps overlaying for the different variables, operated in 10x10 m sized cells). The suitability map was produced from the eight thematic maps (digital terrain model, land use map, economic criteria buffer map, water supply sources buffer map, population buffer map, slope map, soil texture map and type of soil map) associated to the economic, environmental and technical restrictions (figure 21).

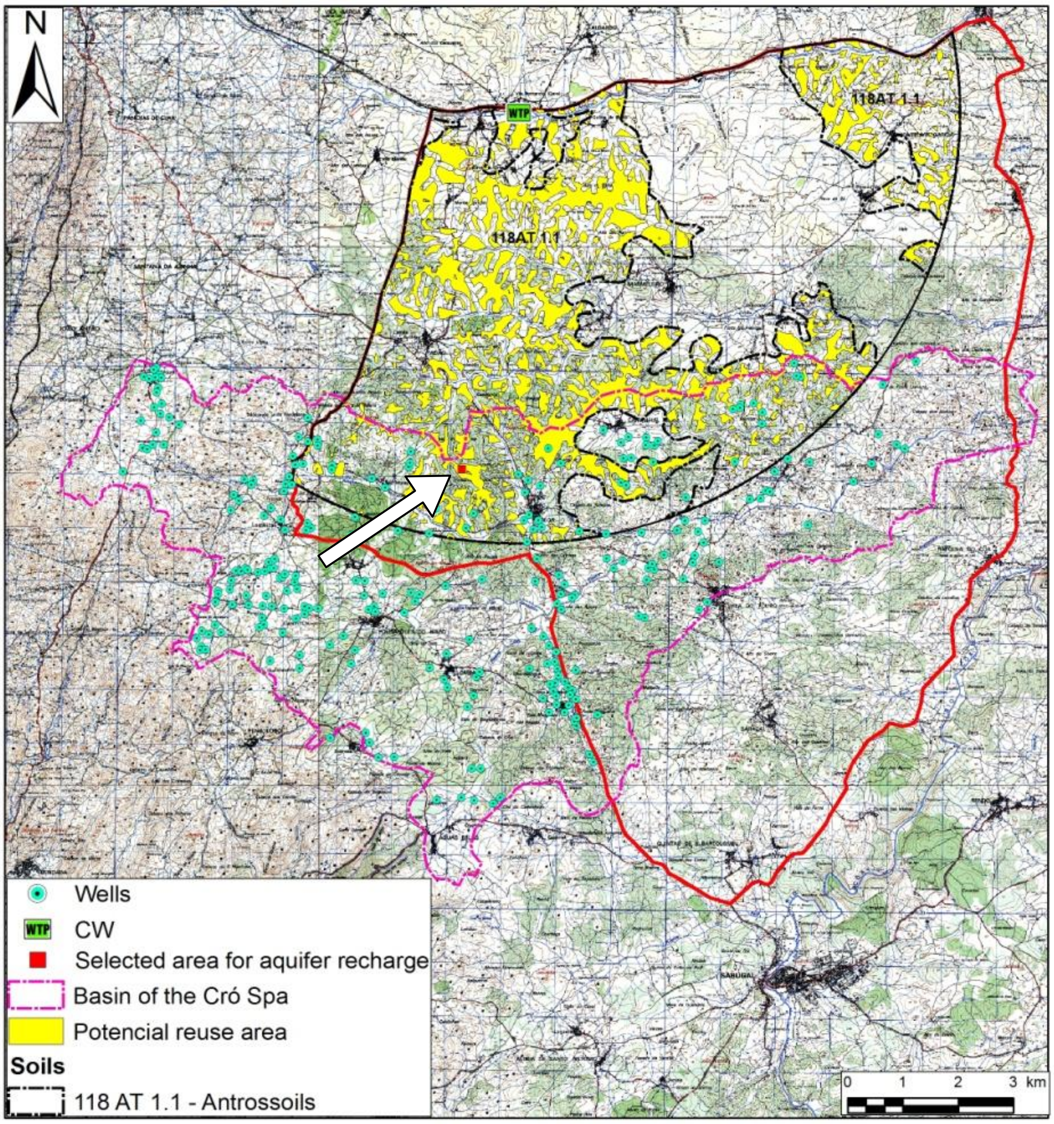


Figure 21. Final suitability map with the eight thematic maps overlaying. The potential reuse area (in yellow) and the selected area for aquifer recharge (red square indicated by the arrow).

The final potential reuse area is 1,607 ha of the total studied area (13,944 ha). The most restrictive variable was the presence of water supply sources (table 17) because of the innumerable water lines attributed to the Boi bank crossing the sub-basin.

Table 17. Suitable area for each variable.

Parameters	Area (ha)
Studied area	13,944
Area without population agglomerates	12,445
Economic restriction area	7,805
Area with slopes < 12%	10,852
Area occupied by antrosol soils	8 ,862
Area without restricted water sources	5,345
Potential reuse area	**1,607**

IV.3.4 Infiltration surface for aquifer recharge

Groundwater tables should be at least 1 m below the bottom of the infiltration basins during flooding because of the quality improvement of sewage effluent moving through an SAT system occurs in the top 1 m of soil. (Pescod, 1992). The water depth of the aquifer, in the studied area, ranges from 10 to 50 m (Cavaleiro, 2002) and the water temperature varies between 12ºC and 25ºC, because of the blend with surface water. The thermal aquifer is intercepted in wells P1 and P2 of the SPA Cró. Physichal-chemical analyses of these wells (table 15) show that the values of pH, EC and temperature are not restrictive for human use because the depth of the water table always exceeds 5 m in all the studied area.

The necessary infiltration surface was calculated according the method presented in Bouwer (2002). Because of the need for regular drying and periodic cleaning of the recharge basins or other surface infiltration systems, hydraulic capacities are best expressed in long-term average infiltration rates or hydraulic loading rates that take into account dry time. Soil clogging may cause the decrease of the infiltration rates from 1 to 0.5 m d^{-1} after 2 weeks of flooding, and if then a drying period of 2 weeks is necessary to clean the basin and restore its infiltration rate up to 1 m d^{-1}, the basin would have an average infiltration rate of 0.75 m d^{-1} during flooding period and zero infiltration during the drying/cleaning period. Therefore, the long-term average hydraulic loading rate over the flooding/drying cycle would be (0.75 + 0)/2= 0.375 m d^{-1} or 137 m y^{-1}.

Experienced operators know that different infiltration basins often show different clogging and infiltration behavior and different responses to drying and cleaning, even within the same project. For this reason, multi-basin recharge projects should be designed so that each basin is hydraulically independent and can be operated according to its best schedule. Flooding schedules typically vary from 8 hours dry-16 hours flooding to 2 weeks dry-2 weeks flooding. Therefore, SAT systems should have a number of basins so that some basins can be flooded while others are drying (Pescot, 1992). Therefore, with the maximum flow rate produced in the constructed wetland (240 m^3 d^{-1}) and the annual average infiltration rate of 0.375 m d^{-1}, 4 beds with 320 m^2 of infiltration surface each would be necessary to assure the correct operation and maintenance of the CW and the proper aquifer recharge.

Clogging of the infiltrating surface and resulting reductions in infiltration rates are the bane of all artificial recharge systems (Baveye et al. 1998; Bouwer and Rice 2001). Pre-treatment of the water to reduce suspended solids, nutrients, and organic carbon, and regular drying of the system to enable drying and cracking of the clogging layer and physical removal of the clogging layer might be necessary to minimize clogging effects. SAT improves water quality, even in conditions considered unfavourable because of the kind of soil (Oron, 2001).

The study area has approximately 10 wastewater treatment systems that represent a discharge of around 133,000 m^3 $year^{-1}$ of treated wastewater into water streams. During dry weather periods most of the streams have no flow and the discharge of those effluents may bring negative environmental impacts for the ecosystems and the downstream water uses. Therefore, the reuse of that volume of reclaimed water for aquifer recharge would provide an important source of water for groundwater. Applying the same procedure, the required infiltration area would be approximately 1 ha.

IV.4 CONCLUSIONS

GIS- based multi-criteria analysis, using technical, environmental, social and economic variables, is a good tool to assign the best use for reclaimed water in a determined region.

In the case of Beira Interior Region (Portugal), the most profitable use for reclaimed water was identified as aquifer recharge, because of the thermal water importance in the area.

From a total studied area of 13,944 ha, only an effective area of 1,607 a was available for aquifer recharge with reclaimed water. Area without restrict water sources and economical criteria were the most restrictive variables to identify this potential reuse area.

The microbiological content of reclaimed water from contructed wetland was high according to health regulations for wastewater reuse. The reuse of this water for aquifer recharge will help in the disinfection, because the soil aquifer treatment is effective to remove bacteria and viruses.

The results obtained from this work assigned a new use for reclaimed water from constructed wetlands systems, reducing effluent discharges into water streams and getting a new source of water for aquifer recharge.

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APPENDIX A

PUBLIC PERCEPTION OF RECLAIMED WATER USE

APPENDIX B

SCIENTIFIC PRODUCTION