Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar
doi: http://dx.doi.org/10.5424/sjar/2012101-065-11

Spanish Journal of Agricultural Research 2012 10(1): 209-221
ISSN: 1695-971-X
eISSN: 2171-9292

Effects of fertigation with purified urban wastewater on soil and pepper plant (*Capsicum annuum* L.) production, fruit quality and pollutant contents

C. García-Delgado[1], E. Eymar[1, *], J. I. Contreras[2], and M. L. Segura[2]

[1] *Dpto. Química Agrícola, Universidad Autónoma de Madrid. 28049 Madrid, Spain*

[2] *Centro IFAPA, La Mojonera. Consejería de Agricultura y Pesca, Junta de Andalucía. 04745 Almería, Spain*

Abstract

The effects, in greenhouse conditions, of Purified Urban Wastewater (PW) from Almería (Spain), in the fertigation of pepper (*Capsicum annuum* L.) on sandy mulch soil, were evaluated. Primary, secondary (active sludges) and tertiary (Chlorination + ozonation) purification treatments were applied to wastewater. Irrigation treatments applied were PW, natural Ground Water (GW), Fertilizer PW (FPW) and Fertilizer GW (FGW). The vegetal biomass, yield and fruit quality were controlled. Heavy metals (Cr, Cd, Pb, Ni, Mn, Cu and Zn), arsenic (As) and Polycyclic Aromatic Hydrocarbons (PAH) in water, soil, leaf, and fruit were analysed. The PW presented heavy metal, As and PAH contents acceptable for its use in drip irrigation. In the soil, fertigated with PW, the concentration of heavy metals and As did not increase, whilst the PAH concentration decreased. The PW treatment supplied enough nutrients to obtain yield and fruit quality equal to that of GW with fertilization. A significant saving on N, P and K fertilizers (37%, 66% and 12% respectively) was achieved by using PW. The Cd, Pb and As contents of the fruit did not show risk for human consumption. The total PAH concentrations in the fruit were low, the highest of which was phenathrene, with no carcinogenic signification.

Additional key words: fertilizer savings; food safety; heavy metals; irrigation; polycyclic aromatic hydrocarbons (PAH); treated wastewater.

Resumen

Efectos de la fertirrigación con agua urbana regenerada sobre el suelo, la producción y calidad de pimiento (*Capsicum annuum* L.) y contenidos de contaminantes

Se han evaluado los efectos de la utilización de un agua residual urbana purificada (PW) procedente de la ciudad de Almería (España) para la fertirrigación del cultivo de pimiento (*Capsicum annuum* L.) en invernadero sobre enarenado. Al agua residual se le aplicó tratamiento de depuración primario, secundario (lodos activados) y terciario (cloración + ozonización). Los tratamientos de fertirrigación fueron: PW, agua natural de origen subterráneo (GW), agua residual urbana depurada con fertilización (FPW) y agua natural de origen subterráneo con fertilización (FGW). Se controlaron la biomasa vegetal, la producción y la calidad del fruto. En las aguas, suelos, hojas y frutos, se analizaron metales pesados (Cr, Cd, Pb, Ni, Mn, Cu and Zn), arsenico (As) e hidrocarburos aromáticos policíclicos (PAH). El PW se consideró apta para uso en riego localizado atendiendo a sus niveles de metales pesados, As y PAH. El tratamiento PW suministró nutrientes suficientes para obtener producción y calidad de pimiento iguales al tratamiento FGW. En suelo, no se observó acumulación de metales pesados ni As y disminuyeron las concentraciones de PAH. Se obtuvieron ahorros significativos de fertilizantes nitrogenados, fosfóricos y potasicos (37, 66 y 12% respectivamente) al utilizar PW. Los contenidos de metales pesados, As y PAH en el fruto no representaron riesgo para el consumo humano. En cuanto a PAH, la mayor concentración registrada fue de fenantreno, compuesto no clasificable como carcinogénico para humanos.

Palabras clave adicionales: aguas residuales tratadas; ahorro de fertilizantes; hidrocarburos aromáticos policíclicos (PAH); metales pesados; riego; seguridad alimentaria.

---

*Corresponding author: enrique.eymar@uam.es

Received: 03-02-11. Accepted: 23-12-11

Introduction

The search for new water supplies in the Mediterranean basin area is constant, due to its irrigable terrain: although it only represents 30% of cultivated land, it produces 75% of agricultural output and in many of these areas agriculture would not be possible without irrigation (Papadopoulos, 1995). Due to scarcity of available irrigation water, the use of urban wastewater has been implemented and used in Mediterranean countries (Massoud *et al*., 2003). In 2004, Spain reused 1 hm^3 day^{-1} of purified wastewater, which is estimated to be 7% (INE, 2008).

The use of Purified Wastewater for agricultural usage continues to expand due to the benefits it offers such as: a solution to irrigation water scarcity; the availability of large amounts throughout the year; the possibility to reserve better quality water for human consumption; the reduction of fertilizers needed due to the nutrients contained in this type of water; protection of the environment; the reduction of effluent waters in the surrounding area; an increase in the returns on investment made for the water's purification; regeneration of wet lands; protection of the water quality in water-bearing terrain by refilling the aquifers; avoiding marine intrusion in coastal areas and overexploitation. However, inadequate handling of fertilisation and irrigation with these types of water could supply the crop with higher quantities of nutrients and produce excessive accumulations within the plant and soil, negatively affecting the yield and production quality.

The main problems caused by the use of wastewater result from the presence of biological and chemical contaminants, most importantly those that have not been treated. These could harm the agricultural environment, as well as the health of farmers and consumers as they could cause a build-up of chemical contaminants in the soil, cause the mobilisation of contaminants from the soil to the crop due to cultivation, lead to soil salinization and cause diseases for both the farmers, who are in direct contact with the water, and for consumers if the crops have been colonised by pathogenic micro-organisms (Khan *et al*., 2008; Klay *et al*., 2010).

Inorganic chemical contamination is basically due to heavy metals, As and Na. The concern over these elements is due to the fact that they are not biodegradable. They are absorbed by the crops and they can easily accumulate in different parts of the human body, even if they are present in low concentrations, as the body has no effective elimination mechanism (Arora *et al*., 2008).

Organic contaminants that appear in urban wastewater are from diverse origins. The majority are found in the remnants of soaps, detergents, general cleaning products, pesticide residues and organic material in the stages of decomposition. There are certain groups of contaminants that, due to their chemical properties, are not very soluble in water, and as a result they appear in wastewater in very low concentrations. This is the case with Polycyclic Aromatic Hydrocarbons (PAH), which are important contaminants because they are highly toxic, and have mutagenic, teratogenic and carcinogenic properties (IARC, 2005).

The main source of PAH in wastewater occurs by it being deposited on surfaces from the atmosphere and subsequently these compounds are transferred by rain water (Blanchard *et al*., 2001). In wastewater, PAH with the least molecular weight appear in the highest concentration since their solubility decreases as molecular weight increases, and PAH with greater molecular weight are adsorbed onto particles (Charalabaki *et al*., 2005). It has been noted that sewage sludge in urban areas can contain greater concentrations of PAH compared to that from industrial or rural areas (Sánchez-Brunete *et al*., 2007).

The correct purification treatment of wastewater, along with some control over its use and quality, minimizes the health and environmental problems, even preventing their occurrence (Lubello *et al*., 2004). Hence, in 2007 a Spanish regulation was created concerning the reuse of wastewater (RD 1620/2007; BOE, 2007), which makes primary, secondary and tertiary treatments of wastewater which is going to be reused for irrigating crops mandatory, and which limits the concentrations of chemical substances and micro-organisms present in purified wastewater. One of the possible tertiary treatments is ozonization which

---

Abbreviations used: Ace (acenaphtene); Ant (anthracene); As (arsenic); BaA (benzo[a]anthracene); BaP (benzo[a]pyrene); BbF (benzo[b]fluoranthene); BghiP (benzo[g,h,i]perylene); BkF (benzo[k]fluoranthene); CF (concentration factor); Ch (chrysene); DBahA (dibenzo[a,h]anthracene); EC (electrical conductivity); FGW (fertilized ground water); Fla (fluoranthene); Flu (fluorene); FPW (fertilized purified urban wastewater); GW (ground water); IcdP (indene[1,2,3-c,d]pyrene); Naph (naphthalene); PAH (polycyclic aromatic hydrocarbon); Phe (phenantrene); PW (purified urban wastewater); Py (pyrene); RD (Royal Decree).

achieves significant reductions in the microbiological populations of wastewater. Segura *et al*. (2001) used purified ozone disinfected wastewater for the cultivation of melon and produced fruit without any microbial contamination, even in the fruit which was in direct contact with the soil.

The objective of this paper is to evaluate the suitability of Purified Urban Wastewater (PW) from Almería (Spain) for use in the fertigation of greenhouse horticultural crops by studying the water's effect on the soil-plant system, the crop yield, fruit quality and the presence of inorganic chemical contamination (heavy metals and arsenic), and organic (Polycyclic Aromatic Hydrocarbons) as crop food safety parameters using pepper (*Capsicum annuum* L.) as the model plant.

Materials and methods

Origin and treatment of urban wastewater

The PW came from the wastewater purification plant in the city of Almería (36º50'N, 2º27'W). It has been estimated that this plant treats 15 hm^3 yr^{-1}. In this purification plant the primary treatment of wastewater is carried out by decanting the solids and breaking down the fatty emulsions. The next stage is the secondary treatment (biological) by activated sludge. After these treatments, the water is sent to the tertiary treatment plant located 6 km away in Viator. In this second treatment plant, the water undergoes a chloration process with sodium hypochlorite followed by ozonation using a Tonozone T.E.F. ozonizor. The dosage used in both treatments varies depending on the microbiological contamination present in the purified wastewater to be treated. The disinfection process eliminates on average 99.96% of the total coliforms, faecal coliforms and faecal streptococcus present in the water.

Soil and growing conditions

Cultivation was carried out at the Research Station of the IFAPA (Andalusian Autonomous Government) in La Cañada (Almería, Spain), in a multi-tunnel greenhouse containing climatological equipment (air temperature and relative humidity sensor) to automatically control ventilation. The greenhouse covers an area of 800 m^2, divided into 16 plots. In August 2007, pepper seedlings plants (cv. Aifos) were transplanted in the summer-autumn cycle (175 days). The planting density was set at 2 plants m^{-2}. The experimental design was random blocks with four treatments and four replications.

The soil used for cultivation is an artificial soil called "sandy mulch" and classified by the FAO as a cumilic anthrosol. This type of soil is made by adding a 10-15 cm layer of earth with variable texture (clay to sandy loam) on top of natural soil, adding 2 cm of manure, and then covering the manure with a layer of around 10 cm of sand. Before cultivation began, a characterization analysis of the soil was carried out. The layer of sand was not taken into account in this analysis. It was a basic soil with a pH of 8.6 and 4.5% carbonates. The electrical conductivity is 1.8 dS m^{-1} and it had low organic mineral content (0.9 %).

The application of the fertilizers was by drip irrigation using a dripper for each plant with a volume of flow of 3 L h^{-1}. Two different sources of water were used for the fertigation of the crop, Ground Water (GW) as a control, and PW from the Viator (Almería) ozonizator plant. The chemical characteristics of the waters studied are shown in Table 1.

Four irrigation treatments were carried out: GW, PW, Fertilized GW (FGW) and Fertilized PW (FPW). Composition and volumes of irrigation solutions and total nutrients applied are presented in Table 2. Fertilizers used were ammonium nitrate (NH_4NO_3), potassium sulphate (K_2SO_4) and phosphoric acid (H_3PO_4). Pepper fertilization was carried out in accordance with crop extractions under local conditions (Contreras *et al*., 2006). The irrigation rates were estimated according to the ETc (Fernandez, 2000) and measures of soil matrix potential at 15 cm depth by tensiometers (Tensiometers Irrometer, Irrometer, USA) to maintain tension near 15 kPa.

Sampling and analysis of the water

Samples of GW and PW were collected every month in plastic containers to be analysed for inorganic contaminants (Cr, Ni, As, Cd and Pb), micronutrients (Mn, Cu, Zn), macronutrients (P, Ca, Mg, K) and Na. Measurements were made by Inductive Coupled Plasma-Mass Spectrometry (ICP-MS) Elan 6000 Perkin-Elmer Sciex equipped with auto-sampler AS 91 (Canada). A basic agronomic analysis was carried out to determine the pH, electrical conductivity (EC), bicarbonates, nitrates, sulphates, chlorides and ammonium in GW and PW (Table 1). The pH was determined using a micro pH 2001 Crison pHmeter (Crison Instruments, Spain); EC was determined with a 523 Crison conductivity meter (Crison Instruments, Spain), NO_3^-, $H_2PO_4^-$, SO_4^{2-} and Cl^- was analysed by ion chromatography (Metrohm Ldt, CH-9101, Herisau, Switzerland) used as solvent a solution of 3.0 mM $NaHCO_3$ and 0.8 mM Na_2CO_3; NH_4^+ was measured in UV-Vis spectrophotometer after indophenols blue colour development (MAPA, 1994); CO_3^{2-} and HCO_3^- were determined with a normalized solution of HCl (MAPA, 1994).

In parallel to this, PW and GW samples were collected in translucent 1000 mL glass bottles to determine the PAH. The PAH analyzed were: benzo[a]pyrene (BaP) and the sum of benzo[b]fluoranthene (BbF), benzo[ghi]perylene (BghiP), benzo[k]fluoranthene (BkF) and indene[1,2,3-cd]pyrene (IcdP) in accordance with the Spanish human water consumption regulation (RD 140/2003; BOE, 2003). The water samples were filtered, and a liquid-liquid extraction was made with hexane. Then the solvent was eliminated to 1 mL dissolution. The samples were injected into GC-MS/MS (Varian 3800 GC fitted with a Varian Saturn 2200 mass detector with a 1079 PTV injector, USA).

Table 1. Characterization of ground water (GW) and purified urban wastewater (PW) used in the experiments, concentrations of inorganic contaminants and maximum concentration allowed

	GW	PW	Maximum value[1]
pH	7.1	8.3	
EC, dS m^{-1}	1.2	2.1	
HCO_3^-, $mmol_cL^{-1}$	1.2	6.6	
Cl^-, $mmol_cL^{-1}$	4.6	8.9	
SO_4^{2-}, $mmol_cL^{-1}$	4.6	5.2	
N-NO_3^-, $mmol_cL^{-1}$	0.9	0.3	
$H_2PO_4^-$, $mmol_cL^{-1}$	0.0	0.4	
N-NH_4^+, $mmol_cL^{-1}$	0.0	3.3	
Ca^{2+}, $mmol_cL^{-1}$	2.9	4.8	
Mg^{2+}, $mmol_cL^{-1}$	3.1	4.2	
Na^+, $mmol_cL^{-1}$	5.1	9.1	
K^+, $mmol_cL^{-1}$	0.1	0.6	
SAR*	2.9	4.3	
Cr, µg L^{-1}	2.455	14.22	100
As, µg L^{-1}	0.070	4.843	100
Cd, µg L^{-1}	0.004	0.040	10
Pb, µg L^{-1}	0.006	1.788	5,000[2]
Ni, µg L^{-1}	2.287	3.075	200
Mn, µg L^{-1}	28.82	13.17	200
Cu, µg L^{-1}	74.47	9.818	200
Zn, µg L^{-1}	76.23	37.76	2,000[2]

[1] RD 1620/2007 (BOE, 2007). [2] Ayers and Wescott (1985). * SAR: sodium adsorption ratio, $SAR = \frac{[Na^+]}{\sqrt{\frac{[Ca^{2+}]+[Mg^{2+}]}{2}}}$

Sampling and analysis of soil, plant and fruit

After the growing cycle (175 days), samples of the soil from each treatment and repetition at 15 cm from the plants were taken. The total content of heavy metals (Cr, Mn, Ni, Cu, Zn, Cd and Pb) and As were determined by digestion with HCl-HNO_3 (3:1) in a microwave oven (CEM MARS Xpress, USA). The dissolutions obtained were stored in polyethylene bottles at 4ºC for ICP-MS analysis. Soil and fruit PAH extraction was carried out using an accelerated solvent extractor (ASE350 Dionex) with dichloromethane:acetone 50:50 (v/v) at 100ºC, 1500 psi for 5 min. The solvent was eliminated by nitrogen flow and the residue was

Table 2. Chemical equilibria of fertigation solutions (mmol L^{-1}) of different development crop steps, volumes administrated and total nutrients (g m^{-2}) for ground water (GW), purified urban wastewater (PW), fertilized ground water (FGW) and fertilizer purified urban wastewater (FPW) irrigation treatments

	GW	PW	FGW	FPW
Crop development and flowering (56 days, 27 L m^{-2})				
NO_3^-	1.0	0.3	2.5	0.5
NH_4^+	0.0	3.3	1.5	3.5
N_{Total}	1.0	3.6	4.0	4.0
P	0.0	0.4	0.4	0.4
K	0.1	0.6	2.8	2.8
Development and fruit ripening (27 days, 24 L m^{-2})				
NO_3^-	1.0	0.3	5.1	3.1
NH_4^+	0.0	3.3	4.0	6.0
N_{Total}	1.0	3.6	9.1	9.1
P	0.0	0.4	0.4	0.4
K	0.1	0.6	4.5	4.5
Harvest (92 days, 40 L m^{-2})				
NO_3^-	1.0	0.3	7.7	5.7
NH_4^+	0.0	3.3	6.5	8.5
N_{Total}	1.0	3.6	14.2	14.2
P	0.0	0.4	1.0	1.0
K	0.1	0.6	7.8	7.8
Total crop cycle (175 days, 91 L m^{-2})				
NO_3^-	1.21	0.43	6.92	4.43
NH_4^+	0.00	4.17	5.58	8.07
N_{Total}	1.21	4.60	12.50	12.50
P	0.00	1.04	1.57	1.57
K	0.50	2.28	19.34	19.34

dissolved in 1 mL of acetone. PAHs determined were: Naphthalene (Naph), Acenaphthene (Ace), Fluorene (Flu), Phenanthrene (Phe), Anthracene (Ant), Fluoranthene (Fla), Pyrene (Py), Benzo[a]anthracene (BaA), Chrysene (Ch), Benzo[b]fluoranthene (BbF), Benzo[k]fluoranthene (BkF), Benzo[a]pyrene (BaP), Dibenzo[a,h]anthracene (DBahA), Benzo[g,h,i]perylene (BghiP). Quantification was carried out using HPLC-FL (920 LC Varian, Mulgrave, Australia) with a PAH column (100×4.6 mm, 3 µm) and as an eluyent a gradient of acetonitrile-water.

Whole plants, excluding the roots, were harvested to the end of experiment. Two plants per plot were randomly selected for destructive sampling and separated into different plant fractions: developing fruits, leaves and stem. Harvested fruits were randomly selected every harvesting, collecting 10 fruits per plot. Fresh samples for the different plant parts were dried at 70ºC to constant weight, and dry matter was determined in samples.

To analyse the heavy metals in the plant, for both micronutrients (Mn, Cu and Zn) and contaminants (As, Cd and Pb), the leaf and fruit samples were digested after the cultivation cycle in a microwave oven with a mixture of HNO_3–H_2O_2. Analysis was carried out by ICP-MS.

The bioavailability of heavy metals and Arsenic was determined by the Concentration Factor (CF) of each element. The CF is defined as the relationship as a percentage between the concentration of the element in each plant organ and its concentration in the soil $CF = 100 * C_{plant} / C_{soil}$ (Mohamed *et al.*, 2003; Cui *et al.*, 2005).

The production of mature fruit was quantified and classified by calibre categories according to the European Quality and Commercialization Norm (Commission Regulation (EC) No 2147/2002; OJ, 2002). The fruit was classified by its equatorial diameter as medium sized (50-70 mm), large (70-90 mm) and very large (> 90 mm). Fruit less than 50 mm was not taken into account.

The organoleptical quality variables of the fruit analysed were: pH, titratable acidity and total soluble solids (ºBrix) in the juice and hardness of the fruit. To determine fruit quality, pepper fruits were liquidized and turned into juice from each harvest. The content of total soluble solids from the pepper juice was determined with an Atago N1 manual refractometer, the results being expressed in ºBrix at 20ºC. The titratable acidity of the fruit was determined using a 0.1 M NaOH solution (AOAC, 1995) and expressed as mmolc of citric acid per litre of juice. The pH of the pepper juice was determined with a micro pH 2001 Crison pH-meter (Crison Instruments, Spain). The hardness of the fruit was determined with a Fruit Pressure Tester FT 327 (Facchini SRL; Italy) using a tip of 0.5 cm^2 (8 mm diam).

Data processing

The SPSS 16.0 software package was used to analyse the data in terms of descriptive statistics, standard error, univariant analysis and the Duncan's test with a 95% significance level ($p \leq 0.05$).

Results

Effects of the type of water and fertilization on the growth, production and quality of the pepper

The total biomass production of the crop is shown in Fig. 1. The production of leaves, stems and biomass, expressed as the sum of each was significantly greater

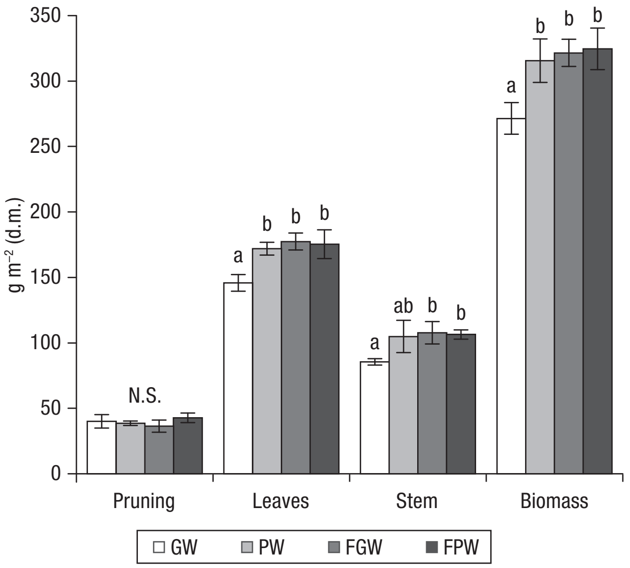

Figure 1. Generation of biomass (dry matter) divided into pruning, leaves, stems and biomass (total) at the end of the cultivation cycle of the treatments ground water (GW), purified urban wastewater (PW), fertilized ground water (FGW) and fertilized purified urban wastewater (FPW) (n = 4). Different letters indicate significantly different treatments, ^{a} being the lowest ($p \leq 0.05$). Bars indicate standard error.

in the PW treatment compared to the GW treatment, and was statistically not different to the FGW and FPW treatments. Purified Wastewater did not negatively affect crop development and achieved growth equal to the treatments with mineral fertilizers.

With regard to crop yield and fruit size (Fig. 2), the PW treatment achieved total production statistically equal to the treatments with mineral fertilization (FGW and FPW). The production of peppers classified as large exhibited significant differences. The treatments with fertilization (FPW and FGW) produced a more peppers classified as large (70-90 mm equatorial diameter) than treatments with PW and GW. In the very large category (> 90 mm equatorial diameter), the PW treatment exhibited pepper production significantly greater than with GW, and not different to FPW and FGW treatments. The production of medium sized pepper (50-70 mm equatorial diameter) did not exhibit significant differences amongst the treatments. The quality parameters pH, acidity, ºBrix in the juice and hardness of the fruit did not exhibit any differences among the applied treatments and were agree with the reference parameters (Urresterazu, 2004; Serrano & Fernandez-Trujillo, 2007) (Table 3).

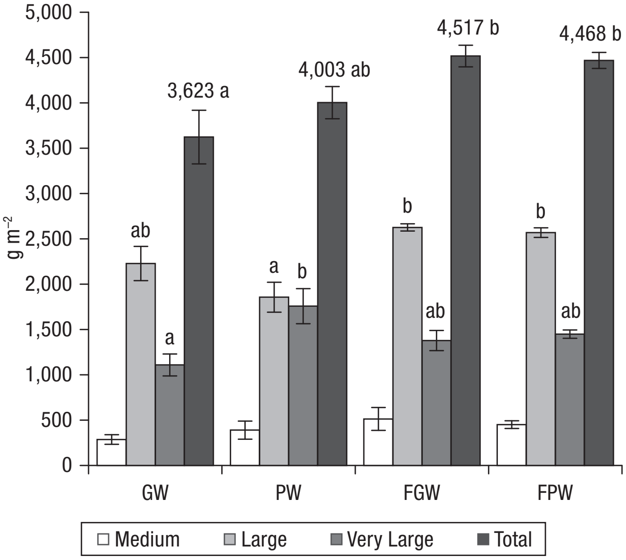

Figure 2. Production of pepper in terms of fresh weight classified according to fruit calibre, medium (50-70 mm), large (70-90 mm) and very large (> 90 mm) and total production of the treatments ground water (GW), purified urban wastewater (PW), fertilized ground water (FGW) and fertilized purified urban wastewater (FPW) (n = 16). Different letters indicate significantly different treatments for each calibre and total production, [a] being the lowest ($p \leq 0.05$). Bars indicate standard error.

Table 3. pH, acidity expressed as citric acid (g L^{-1}) and ºBrix of the pepper juice and hardness (kg cm^{-2}) of the pepper fruit for ground water (GW), purified urban wastewater (PW), fertilized ground water (FGW) and fertilizer purified urban wastewater (FPW) irrigation treatments. (n = 4)

	pH	Acidity	ºBrix	Hardness
GW	4.77	0.37	8.38	4.04
PW	4.85	0.34	8.08	4.41
FGW	4.73	0.38	8.61	3.70
FPW	4.75	0.41	9.15	3.95
Reference	$< 5^1$		$4.5\text{-}7.0^1$	5^2

[1] Serrano & Fernandez-Trujillo (2007). [2] Urrestarazu (2004).

Inorganic and organic contaminants in water, soil, pepper plant and fruit

Inorganic contamination (heavy metals and As) in water, soil, leaf and fruit

The results of the analysis for heavy metals and Arsenic in GW and PW are shown in Table 1. The micronutrients Mn, Cu and Zn appeared in higher concentration in GW, while the toxic elements Cr, Pb, Cd and As showed greater concentrations in PW.

In the soil (Fig. 3), none of the elements analysed (Cr, As, Cd, Pb, Ni, Mn, Cu and Zn) showed significant greater concentration between the irrigation treatments. Of the contaminants studied, Pb and As are found in greater concentrations, while Cd was in very low concentrations (< 0.30 mg kg^{-1}).

The elements analysed (Mn, Cu, Zn, As, Cd and Pb) in fruit and leaf are shown in Table 4. In the leaf, the results for metals and As did not show significant differences for either the micronutrients or the contaminants between the applied treatments. The metals found in greater concentrations in the leaf were Mn, Cu and Zn. The FGW treatment showed greater concentration of Cu than the reference but were not significant higher than the other irrigation treatments, although no phytotoxicity symptoms or reductions in the biomass generated or in yield were observed. The three contaminants analysed (As, Cd and Pb), appeared in very low concentrations, and in the case of As were found to be below the detection limit (< 0.01 mg kg^{-1} d.m.).

The concentrations of heavy metals in fruit were lower than in leaf and were in the following order: Zn > Mn > Cu > Cd > Pb > As. No significant differences in the content of heavy metals in the pepper fruit were observed between the treatments for Mn, Cu, Zn, Cd and As.

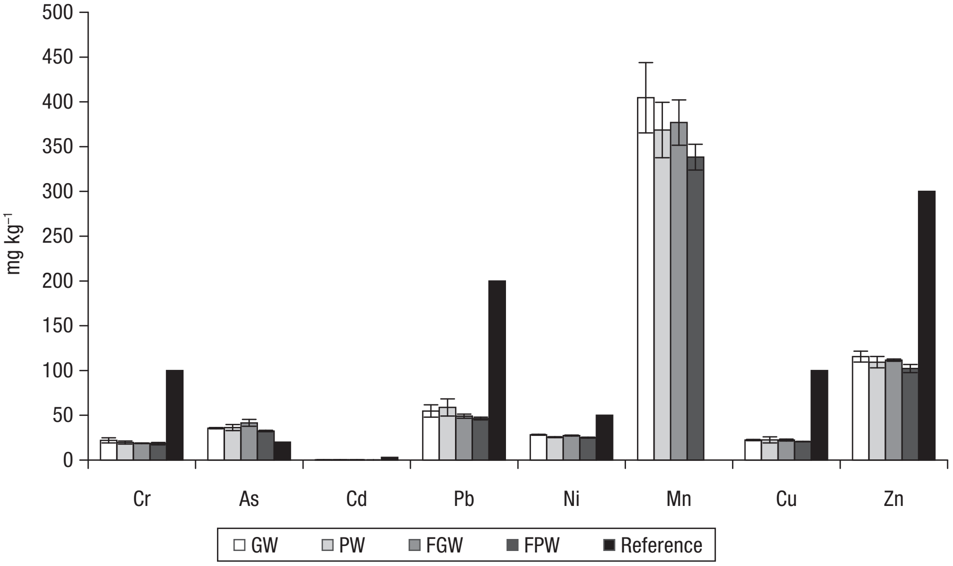

Figure 3. Total concentration of heavy metals and As present in the soil fertigated with ground water (GW), purified urban wastewater (PW), fertilized ground water (FGW) and fertilized purified urban wastewater (FPW) after the cultivation cycle and the Junta de Andalucía (1999) reference levels for contaminated agricultural soils (n = 4). Mn has not reference level. Bars indicate standard error.

The CF order in leaf and fruit was: Cd ≃ Cu > Zn > Mn > Pb ≃ As (Table 5). The CF of Pb and As were very low (< 1%) therefore, although they were found in relative abundance in the soil, the crop did not uptake these elements. As and Pb were not bioavailable in the growing conditions. The Cd exhibited a high CF, but being found in low concentrations in the soil it did not represent risk and the final concentrations in leaf and fruit were low as has been shown. However, Cd was a highly mobile element and therefore crops which are developed under similar conditions to those in the present trial have to be controlled.

Table 4. Concentrations of Mn, Cu, Zn, As, Cd and Pb in leaves and pepper fruit (mg kg^{-1} dry matter) for ground water (GW), purified urban wastewater (PW), fertilized ground water (FGW) and fertilizer purified urban wastewater (FPW) irrigation treatments at the end of the cultivation cycle. (n = 4)

Treatment	Mn	Cu	Zn	As	Cd	Pb
Leaves, mg kg^{-1} d.m.						
GW	113.71	24.26	87.35	n.d.*	0.44	0.32
PW	114.12	24.35	87.63	n.d.*	0.44	0.42
FGW	116.89	29.25	83.14	n.d.*	0.41	0.39
FPW	115.69	24.75	82.87	n.d.*	0.52	0.42
Fruit, mg kg^{-1} d.m.						
GW	13.07	8.78	15.68	0.02	0.12	0.19^{b}
PW	12.64	7.90	13.50	0.01	0.10	0.12^{ab}
FGW	13.48	8.60	14.66	0.01	0.11	0.02^{a}
FPW	13.23	7.87	14.96	0.02	0.10	0.03^{a}

* n.d.: not detected. Different letters indicate significantly different treatments, ^{a} being the lowest ($p \leq 0.05$).

Table 5. Concentration factors (CF) of Mn, Cu, Zn, As, Cd and Pb for leaf and pepper fruit for ground water (GW), purified urban wastewater (PW), fertilized ground water (FGW) and fertilizer purified urban wastewater (FPW) irrigation treatments

Treatment	Mn	Cu	Zn	As	Cd	Pb
Leaves CF (%)						
GW	28	109	76	–	150	< 1
PW	30	109	79	–	156	< 1
FGW	32	130	76	–	145	< 1
FPW	34	120	81	–	213	< 1
Fruit CF (%)						
GW	3	39	14	< 1	42	< 1
PW	3	35	12	< 1	35	< 1
FGW	4	38	13	< 1	39	< 1
FPW	4	38	15	< 1	41	< 1

Organic contamination (PAH) in water, soils and fruits

The concentration of PAH in GW and PW was less than 0.005 µg L^{-1} for BaP and less than 0.10 µg L^{-1} for the sum of BbF, BghiP, BkF and IcdP.

As can be seen in Table 6, the total concentration of PAH (ΣPAH) in the soil before starting cultivation was significantly greater than the concentration of these compounds after cultivation for both GW and PW. Individually, the PAH which significantly reduced their concentration in the soil throughout cultivation in both the PW and GW treatments were Phe and Flu. The greatest concentrations of PAH in the soil before cultivation were probably due to the amounts of PAH in the manure used in making the sandy mulch. The largest group of PAH (Fig. 4) in both soil samples and manure corresponded to the 3 aromatic rings, mainly due to Phe. The most abundant PAHs in the soil were Phe, Flu, Fla and Py, whilst the lowest concentration showed the compounds with 5 aromatic rings, BaP, BkF and DBahA; of these, the last two exhibited concentrations in the soil lower than quantifiable limits. The main source of PAH in the soil at the beginning was manure, with PAH total concentration of 64.91 ng g^{-1}.

In the pepper fruit (Table 6) no significant differences were found in the total concentration of PAH between PW and GW treatments. As in the soil, the largest group found in the fruit corresponds to PAHs with 3 aromatic rings (Fig. 5), with Phe being found in the highest concentration in the two treatments, PW and GW. PAHs with 3 aromatic rings were found in significantly greater concentrations in pepper fruits fertigated with PW than GW (Fig. 5). In the PW treatment, Phe and BaA were found in significantly greater concentrations than in GW treatment. On the other hand, Fla and Flu were found in significantly greater concentrations in fruit with GW treatment. Naph, Ant, Ch, BbF, BkF, BaP and BghiP were not detected in pepper fruit in either of the two treatments.

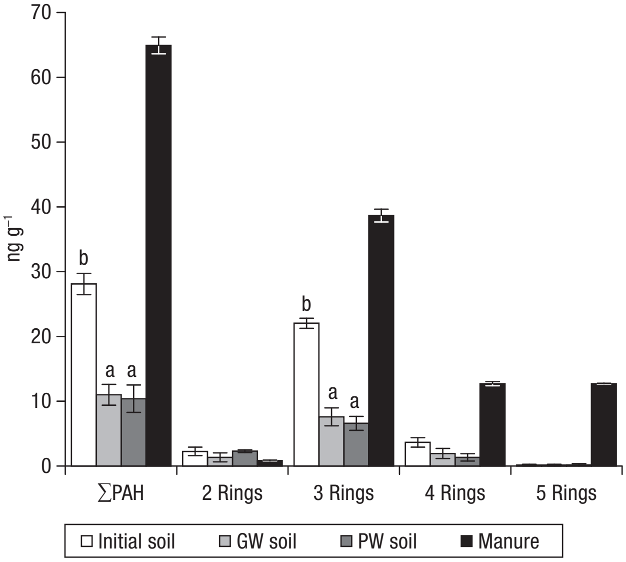

Figure 4. Total concentration of PAH (ΣPAH), 2 aromatic ring PAH, 3 aromatic ring PAH, 4 aromatic ring PAH and 5 aromatic ring PAH in the initial soil, soil fertigated with ground water (GW), soil fertigated with purified urban wastewater (PW) and manure (n = 4). Different letters indicate significantly different treatments, ^{a} being the lowest ($p \leq 0.05$). Bars indicate standard error.

Table 6. Average PAH concentration (ng g^{-1}) in the manure, initial soil, soil after purified urban wastewater (PW) or ground water (GW) irrigation and fruit of pepper irrigated with purified urban wastewater (PW) or ground water (GW) (n = 4)

		Manure	**Soil**			**Fruit**		**IARC (2005)^a**
			Initial	**PW**	**GW**	**PW**	**GW**	
Naphthalene	Naph	n.d.	0.89	1.08	0.62	n.d.	n.d.	–
Acenaphthene	Ace	0.85	0.20	0.17	0.09	0.37	0.18	3
Fluorene	Flu	n.d.	1.17	1.04	0.61	n.d.	0.95*	3
Phenanthrene	Phe	27.53	18.65^b	5.56^a	6.69^a	9.91*	6.62	3
Anthracene	Ant	3.75	0.03	n.d.	n.d.	n.d.	n.d.	3
Fluoranthene	Fla	7.37	3.36^b	1.03^a	0.90^a	0.51	2.01*	3
Pyrene	Py	1.78	1.89	0.85	1.54	0.12	n.d.	3
Benzo[a]anthracene	BaA	10.92	0.99	0.28	0.10	5.80*	4.53	2B
Chrysene	Ch	n.d.	0.60	0.20	0.14	n.d.	n.d.	2B
Benzo[b]fluoranthene	BbF	n.d.	0.17	n.d.	0.17	n.d.	n.d.	2B
Benzo[k]fluoranthene	BkF	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2B
Benzo[a]pyrene	BaP	n.d.	0.10	0.09	0.12	n.d.	n.d.	1
Dibenzo[a,h]anthracene	DBahA	9.63	n.d.	n.d.	n.d.	n.d.	n.d.	2A
Benzo[g,h,i]perylene	BghiP	3.07	0.05	0.10	0.03	n.d.	n.d.	3
Total PAH	ΣPAH	64.91	28.09^b	10.39^a	11.01^a	16.71	14.30	

[a] 1: Carcinogenic to humans; 2A: Probably carcinogenic to humans; 2B: Possibly carcinogenic to humans; 3: not classifiable as to their carcinogenicity to humans. Different small letters indicate significantly different treatments for soil ^{a} being the lowest ($p \leq 0.05$). Asterisk (*) indicates significantly higher values for fruit ($p \leq 0.05$). n.d. not detected.

Discussion

Effects of the type of water and fertilization on the growth, production and quality of the pepper

The PW, FPW and FGW treatments did not exhibit differences in growth, yield or fruit quality. The better results from the PW treatment compared to the GW treatment are due to PW having greater concentrations of N-NH_4^+, P, K, S, Ca and Mg (Table 1). Because of this, a greater quantity of nutrients is carried to the plant throughout cultivation (Table 2). Mineral fertilization and water were not the only sources of nutrients; manure was also applied as an organic amendment (36% organic matter, 2.2% N, 0.8% P, 4.1% K). The mineralization of this organic material supplied nutrients to the crop slowly, which along with the nutrients contained in the PW were enough to equal in growth and production that plants treated with mineral fertilization (FGW and FPW). Manure on its own was not enough to obtain growth and yield equal to mineral fertilization, since the GW treatment produced lower yield and plant growth. Comparing the results obtained in yield with earlier studies under similar conditions (Contreras *et al*., 2006) it can be seen that production in this study is greater. The treatments without fertilization achieved a production level of 2768 g m^{-2}, well below that achieved with the PW treatment (Fig. 2).

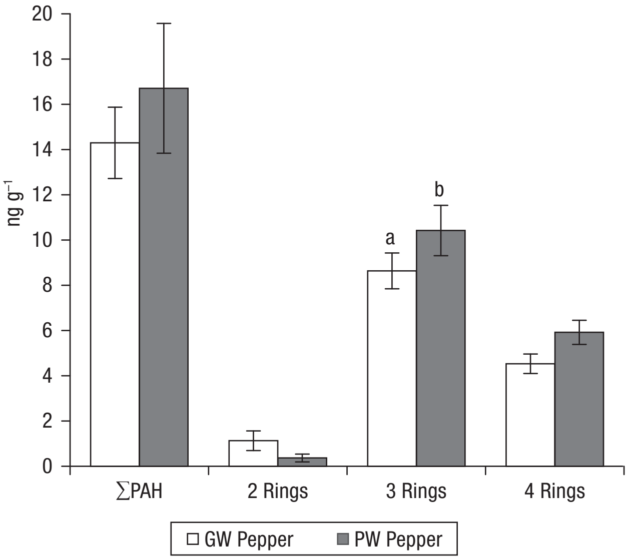

Figure 5. Total concentration of PAH (ΣPAH), 2 aromatic ring PAH, 3 aromatic ring PAH and 4 aromatic ring PAH in pepper fruit fertigated with ground water (GW) or purified urban wastewater (PW) (n = 4). Different letters indicate significantly different treatments, ^{a} being the lowest ($p \leq 0.05$). Bars indicate standard error.

The use of PW also brought about a great saving in mineral fertilizers. From the total N, P and K applied to the crop in the FPW treatment, 37% of N, 66% of P and 12% of K could already be found in the PW, whilst in GW the percentages decreased to 10%, 0% and 3% for N, P and K, respectively.

Study of inorganic and organic contaminants in water, soil, pepper plant and fruit

Inorganic contamination (heavy metals and As) in water, soil, leaf and fruit

The greater concentration of Cu, Zn and Mn in GW could be due to the continued addition throughout the years of quelated micronutrient fertilizers and their later lixiviation to the aquifer where the GW was taken from.

The low heavy metals concentrations in PW were due to a combination of several factors, the wastewater is mainly urban and the area has little industry, the effluent itself has a high pH which produces precipitation of the metallic cations (Karvelas *et al.*, 2003), and the purifying system propels the heavy metals into the sewage sludge. The levels of metals and As observed in the two types of irrigation water were lower than those permitted by Spanish legislation concerning the use of regenerated wastewater for irrigating crops by localized irrigation (RD 1620/2007; BOE, 2007).

The lack of differences of soil heavy metals and As contents between irrigation treatments indicate that neither the accumulation of heavy metals nor As in the soil could be attributed to using PW. This result was to be expected due to the low concentrations of inorganic contaminants in PW. Mn and Zn were the main metals, and their presence in basic soils carries low risk as they are micronutrients for the plants and were not found in high concentrations. The results obtained do not concur with those found in the bibliography, since there are multiple studies that observe the accumulation of heavy metals and As in soils through use of wastewater and purified wastewater (Yadav *et al.*, 2002; Kalavrouziotis *et al.*, 2008). The fact that the concentration of metals and As in PW was very low, can be due to years of application of wastewater onto the soil are necessary to produce the accumulation of metals (Klay *et al.*, 2010). According to the reference values for contaminated agricultural soils with pH ≥ 7 (Junta de Andalucía, 1999), as is the case, heavy metal contamination of the soil has not been observed. However, a slight As contamination has been detected. One has to take into account that the values in Fig. 3 correspond to the total extraction of heavy metals, and due to the soil pH (8.6) and the carbonate content (4.5%) its bioavailability will be low as showed CF. This factor were much lower than those published by Mohamed *et al.* (2003), with the exception of Cu. Pb stands out with a CF of 322 in the cited study. Khan *et al.* (2008) determined the CF in the edible parts of many vegetables cultivated on land irrigated with purified wastewater since the 1960s. For Cu and Pb in pepper fruit they obtained CF similar to those in this study, but for Cd and Zn the CF were higher. These different CF could be due to several factors, such as the different variety of pepper, the type of soil and agronomic techniques used which will affect the availability of heavy metals and As for the plant.

Heavy metals analysis in leaf and fruit showed that PW fertigation and the use of soluble mineral fertilizers did not increase the concentration of heavy metals and As compared to the GW and FGW. In the case of Pb, the GW treatment showed a significantly greater concentration of this metal in the fruit compared to the fertilized treatments (FPW and FGW) due to possible precipitation of lead phosphates in soil of treatment with P fertilization (Hashimoto *et al.*, 2008). The foliar reference ranges for Mn, Cu and Zn are 50-250, 6-25 and 20-200 mg kg^{-1}, respectively (Mills & Benton Jones, 1996), therefore, they are within suitable levels.

Arora *et al.* (2008) determined the Mn, Cu and Zn content in different plants irrigated with wastewater or with GW, and determined that the concentration of heavy metals in plants irrigated with wastewater was greater than that found when using GW. However, all the plants showed concentrations of Cu and Zn lower than the maximums permitted by the FAO/WHO, 40 and 60 mg Kg^{-1}, respectively (Codex Alimentarius, 1984).

It must be pointed out that the results shown, correspond to concentration in dry matter and pepper fruits are consumed as fresh matter, therefore all the contaminants were more diluted and their risk was reduced. The average moisture of the peppers was 95%, so these fruits meet Commission Regulation (EC) 466/2001 (OJ, 2001), which fixes the maximum content of determined contaminants in food products (Cd < 0.05 mg kg^{-1} f.w., Pb < 0.1 mg kg^{-1} f.w.).

Organic contamination (PAH) in water, soils and fruits

GW and PW met the Spanish norm concerning water quality for human consumption (RD 140/2003; BOE, 2003) with respect to these compounds, and therefore the risk from their use in fertigation will be very low.

The concentrations of PAH found in PW were much lower than those found in the purified wastewater from the metropolitan area of Paris (France) (Blanchard *et al*., 2004) or in Venice (Italy) (Busetti *et al*., 2006). A fact that confirms that the amount of PAH in wastewater is greater in large cities due to the greater intensity of traffic on the roads and fumes from heating systems and industry (Manoli & Samara, 1999).

As with the case of heavy metals in the soil, the results found in the bibliography show accumulation of PAH in soils irrigated with wastewater (Tao *et al.*, 2004; Xiao *et al*., 2008). There are marked differences between this study and those cited because in the latter studies the soils were influenced by atmospheric contamination from the urban area as well as industry in the area which added PAH to the soil from the air. Other differentiating factors were the period of usage of wastewater as irrigation water and the wastewater treatment. In previous studies wastewater has been used over several decades and at least in the articles cited, there is no indication if purification treatments were carried out. In this study the use of wastewater has been limited to a year, and the water has undergone an exhaustive process of purification with primary, secondary and tertiary treatments. Chung *et al*. (2008) carried out a one-year trial using PW with a total PAH concentration of 1.90 µg L^{-1}. They found a certain accumulation of PAH in the soil with final concentrations between 3.48 and 6.60 ng g^{-1} depending on the crop, values within the order of magnitude of this study (11.01 ng g^{-1} for GW and 10.12 ng g^{-1} in PW). The greater values of the GW and PW soil could be due to the initial soil showed 28.09 ng g^{-1} of total PAH. Al Nasir & Batarseh (2008) found greater accumulations in soil irrigated with purified wastewater during a cultivation cycle in several plant species. In the case of pepper, the increase in ΣPAH was 60.7 ng g^{-1}.

The reduction of ΣPAH at the end of the cultivation cycle in both treatments was greater than 60%. This result means that the PW did not add PAH to the soil, and that during the cultivation period the soil microorganisms were able to degrade PAH. By group, the biggest reduction corresponds to the PAH with three aromatic rings, the percentage being 66% for the GW treatment and 70% for the PW treatment.

These discrepancies in the results for both heavy metals and PAH show the variability in the composition of urban wastewater even after having undergone a purification process. Therefore, periodic exhaustive controls of both the irrigation water and the soil have to be carried out.

The results for the concentration of PAHs found in earlier studies are very varied for plants irrigated with wastewater, from 0.0028 ng g^{-1} to 984 ng g^{-1} (Tao *et al*., 2004; Chung *et al*., 2008) depending on the crop, agronomic techniques and industrial influence. Al Nasir & Batarsech (2008) found a concentration of ΣPAH in pepper fruit of 31 ng g^{-1}, value some three times greater than the found in this study. In general, in all the studies reviewed it has been seen that PAH with greater accumulation in edible parts of the plants correspond to those with low molecular weight, and those with high molecular weight were found in very low concentrations, or concentrations below detectable limits. This tendency was also observed in the results obtained with the PW treatment, except for Naph which was not detected.

According IARC (2005), no PAH classifiable as carcinogenic nor probably carcinogenic to humans were detected in fruit. Only B[a]A presented carcinogenic significance and is classified as possibly carcinogenic to humans. Therefore, the dangers of PW for consumers are low, although due to the high variability of this type of effluent depending on where it originates from, periodic controls are necessary to evaluate possible invasion from contaminants, and in this way maintain the food safety aspect of the crop and the agro-ecosystem.

In conclusion, PW from Almería (Spain) can be used to irrigate horticultural crops with localized irrigation techniques, as long as its heavy metal, As and PAH concentrations are controlled. The effects on growth, production and pepper quality were comparable with those of the GW which is usually used, producing an important saving in N, P and K fertilization. After a cultivation cycle no accumulation of heavy metals, As nor PAH was observed in the soil; the principal source of PAH being the manure used. The concentrations of Mn, Cu, Zn, Cd, Pb, As and PAH in the fruit were not significantly different when using PW or GW and the fruits are suitable for human consumption.

Acknowledgements

This work has been funded by the *Ministerio de Educación y Ciencia* (Project INIA RTA2006-00032-00-00, European funds FEDER and the *Instituto de Investigación y Formación Agraria y Pesquera (IFAPA), Consejería de Agricultura y Pesca (Junta de Andalucía).* C. García-Delgado was funded with a *Universidad Autónoma de Madrid* FPI predoctoral grant. Our thanks for their collaboration go to Inmaculada Rivas (SIdI-UAM) for the ICP-MS determinations and Rosa Sedano (SIdI-UAM) for the GC-MS and HPLC-FL analyses.

References

Al Nasir F, Batarseh MI, 2008. Agricultural reuse of reclaimed water and uptake of organic compounds: pilot study at Mutah University wastewater treatment plant, Jordan. Chemosphere 72: 1203-1214.

AOAC, 1995. Official methods of analysis. Association of Official Analytical Chemists, 16th. Ed. Washington, DC.

Arora M, Kiran B, Rani S, Rani A, Kaur B, Mittal N, 2008. Heavy metal accumulation in vegetables irrigated with water from different sources. Food Chem 111: 811-815.

Ayers RS, Wescott DW, 1985. Water quality for agriculture, FAO Irrigation and Drainage Paper no. 29, Rome, Italy, 174 pp.

Blanchard M, Teil MJ, Ollivon D, Garban B, Chesterikoff C, Chevreuil M, 2001. Origin and distribution of polyaromatic hydrocarbons and polychlorinated biphenyls in urban effluents to wastewater treatment plants of the Paris area (France). Water Res 35: 3679-3687.

Blanchard M, Teil MJ, Ollivon D, Legenti L, Chevreuil M, 2004. Polycyclic aromatic hydrocarbons and polychlorobiphenyls in wastewater and sewages from the Paris area (France). Environ Res 95: 184-197.

BOE, 2003. Royal decree 140/2003 that establish health criteria of water quality for human consumption. Boletín Oficial de Estado No 45, 21/02/2003. [In Spanish].

BOE, 2007. Royal decree 1620/2007, of 7 December, that establishes legal stage of reuse of purified wastewater. Boletín Oficial de Estado No 249, 08/12/2007. [In Spanish].

Busetti F, Heitz A, Cuomo M, Badoer S, Traveso P, 2006. Determination of sixteen polycyclic aromatic hydrocarbons in aqueous and solid samples from an Italian wastewater treatment plant. J Chromatogr A 1102: 104-115.

Charalabaki M, Psillakis E, Mantzavinos D, Kalogerakis N, 2005. Análisis of polycyclic aromatic hydrocarbons in wastewater treatment plant effluents using hollow fibre liquid-phase microextraction. Chemosphere 60, 690-698.

Chung NJ, Cho JY, Park SR, Hwang SA, Park TI, 2008. Polycyclic aromatic hydrocarbons in soils and crops after irrigation of wastewater discharged from domestic sewage treatment plants. Bull Environ Contam Toxicol 81: 124-127.

Codex Alimentarius Commission, 1984. Contaminants, Joint FAO/WHO Food Standards Program, Codex Alimentarius Vol. XVII (1st Ed.).

Contreras JI, Segura ML, Galindo P, Catalá JJ, 2006. Response of greenhouse pepper crop to fertilizer and different qualities of irrigation water. Acta Hortic 700: 203-206.

Cui YJ, Zhu YG, Zhai R, Huang Y, Qiu Y, Liang J, 2005. Exposure to metal mixtures and human health impacts in a contaminated area in Nanning, China. Environ Int 31: 784-790.

Fernández MD, 2000. Necesidades hídricas y programación de riegos en los cultivos hortícolas en invernadero y suelo enarenado de Almería. Doctoral Thesis. Universidad de Almería, Spain. [In Spanish].

Hashimoto Y, Matsufuru H, Sato T, 2008. Attenuation of lead leachability in shooting soils using poultry waste amendments in combination with indigenous plant species. Chemosphere 73: 643-649.

IARC, 2005. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 92. International Agency for Research on Cancer, Lyon.

INE, 2008. Statistical yearbook of Spain 2007. Instituto Nacional de Estadística [In Spanish].

Junta de Andalucía, 1999. Criteria and standards for declaring a polluted soil in Andalusia, methodology, sampling techniques and analysis for its investigation. Available in: http://www.juntadeandalucia.es/medioambiente/site/web/menuitem.a5664a214f73c3df81d8899661525ea0/?vgnextoid=47dbbb45c8bab010VgnVCM1000000624e50aRCRD&vgnextchannel=3259b19c7acf2010VgnVCM1000001625e50aRCRD&lr=lang_es. [01 May 2009] [In Spanish].

Kalavrouziotis IK, Robolas P, Koukoulakis PH, Papadapoulos AH, 2008. Effects of municipal reclaimed wastewater on the macro- and micro-elements status of soil and of *Brassica oleracea* var. Italica and *B. oleracea* var. Gemmifera. Int J Agr Water Manage 95: 419-426.

Karvelas M, Katsoyiannis A, Samara C, 2003. Occurrence and fate of heavy metals in the wastewater treatment process. Chemosphere 53: 1201-1210.

Khan S, Cao Q, Zheng YM, Huang YZ, Zhu YG, 2008. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. Environ Pollut 152: 686-692.

Klay S, Charef A, Ayed L, Houman B, Rezgui F, 2010. Effect of irrigation with treated wastewater on geochemical properties (saltiness, C, N and heavy metals) of isohumic soils (Zaouit Sousse perimeter, Oriental Tunisia). Desalination 253: 180-187.

Lubello C, Gori R, Nicese FP, Ferrini F, 2004. Municipal-treated wastewater reuse for plant nurseries irrigation. Water Res 28: 2939-2947.

Manoli E, Samara C, 1999. Polycyclic aromatic hydrocarbons in natural waters: sources, occurrence and analysis. Trends Anal Chem 18(6): 417-428.

MAPA, 1994. Métodos oficiales de análisis, Tomo III. Servicio de Publicaciones. Ministry of Agriculture, Fisheries and Food, Madrid, Spain. [In Spanish].

Massoud MA, Scrimsha MD, Lester JN, 2003. Qualitative assessment of the effectiveness of the Mediterranean action plan: wastewater management in the Mediterranean region. Ocean Coast Manage 46: 875–899.

Mills HA, Benton Jones J, 1996. Plant analysis handbook, 2nd ed. MicroMacro Publ Inc. Athens, GA, USA.

Mohamed AE, Rased MN, Mofty A, 2003. Assessment of essential and toxic elements in some kinds of vegetables. Ecotoxicol Environ Saf 55: 251-260.

OJ, 2001. Commission Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L 77 16/03/2001. p. 13.

OJ, 2002. Commission Regulation (EC) No 2147/2002 amending Regulation (EC) No 1455/1999 laying down the marketing standard for sweet peppers. Official Journal of the European Union L 326 03/12/2002. p. 2.

Papadopoulos I, 1995. Present and perspective use of wastewater for irrigation in the Mediterranean basin. 2nd Int Symp on Wastewater Reclamation and Reuse, eds. IAWQ, Iraklio, Greece, October, Vol. 2, pp: 735-746.

Sánchez Brunete C, Miguel E, Tadeo JL, 2007. Analysis of 27 polycyclic aromatic hydrocarbons by matrix solid-phase and isotope dilution gas chromatography-mass spectrometry in sewage sludge from the Spanish area of Madrid. J Chromatogr A 1148: 219-227.

Segura ML, Moreno R, Martinez S, Pérez J, Moreno J, 2001. Effects of wastewater irrigation on melon growth under greenhouse conditions. Acta Hortic 559: 345-351.

Serrano JM, Fernández-Trujillo JP, 2007. Influence of collection date and recirculation of nutritive solution in to pepper commercial quality. Actas de Horticultura 48: 732-735. [In Spanish].

Tao S, Cui YH, Xu FL, Li BG, Cao J, Liu WX, Schmitt G, Wang XJ, Shen WR, Quing BP, Sun R, 2004. Polycyclic aromatic hydrocarbons (PAHs) in agricultural soil and vegetables from Tianjin. Sci Total Environ 320: 11-24.

Urrestarazu M, 2004. Tratado de cultivo sin suelo, 3rd ed. Mundi-Prensa, Madrid, Spain [In Spanish].

Xiao R, Du X, He X, Zhang Y, Yi Z, Li F, 2008. Vertical distribution of polycyclic aromatic hydrocarbons (PAHs) in Hunpu wastewater-irrigated area in northeast China under different land use patterns. Environ Monit Assess 142: 23-34.

Yadav RK, Goyal B, Sharma RK, Dubey SK, Minhas PS, 2002. Post-irrigation impact of domestic sewage effluent on composition of soils, crops and ground water – A case study. Environ Int 28: 481-486.