“This is an Accepted Manuscript of an article published by Elsevier in Journal of Environmental Management on 1 September 2014, available at: https://doi.org/10.1016/j.jenvman.2014.04.002 .”

Protecting effect of recycled urban wastes (sewage sludge and wastewater) on ryegrass against the toxicity of pesticides at high concentrations

Aránzazu Peña [a,e,*], Mª Dolores Mingorance [a,e], Ignacio Guzmán [b,d], Lourdes Sánchez [c],
Antonio J. Fernández-Espinosa [d,e], Benito Valdés [b,e], Sabina Rossini-Oliva [b,e]

[a] *Instituto Andaluz de Ciencias de la Tierra (CSIC-UGR), Avda. Palmeras, 4, 18100 Armilla, Granada, Spain*
[b] *Department of Plant Biology and Ecology, University of Seville, Avda. Reina Mercedes s/n, 41012 Seville, Spain*
[c] *Estación Experimental del Zaidín (CSIC), Prof. Albareda 1, 18008 Granada, Spain*
[d] *Department of Analytical Chemistry, University of Seville, Prof. García González 1, 41012 Seville, Spain*
[e] *Unidad Asociada CSIC-USE "Investigación Integral del Medio Físico y Biótico", Spain*

Abstract. Degraded landscapes, like those from abandoned mine areas, could be restored by revegetating them with appropriate plant species, after correction for acidity and improvement by adding exogenous organic material. Application of urban wastes to large areas of derelict land helps in the sustainable development of this landscape. However, the development of plant species in these soils could require in the future the management of possible pests or diseases by pesticide applications which could also affect plant yield. Therefore, ryegrass (*Lolium perenne* L.) was planted in a limed soil from the mining area of Riotinto (SW Spain), using an indoor pot experiment and the effects of amendment with sewage sludge, as well as irrigation with urban wastewater on plant uptake of the insecticide thiacloprid and the fungicide fenarimol were examined. Ryegrass biomass was reduced up to 3-fold by pesticide application. Fenarimol residues were the highest in soil, while those of thiacloprid were lower in soil and higher in ryegrass. Addition of sewage sludge and irrigation with wastewater led to a reduction of pesticide translocation to the aerial plant parts, representing a lower hazard to ryegrass quality grown in this mine soil.

Keywords: Ryegrass, Organic amendment, Water quality, Biomass production, Mine soil

1. Introduction

Mine soils suffer from high metal concentration, sometimes above the legal guidelines. For instance the Riotinto mine area has high heavy metal load making this environment inappropriate for plant establishment and growth, though different plant species have shown their ability to develop under these extremely harsh conditions (Trigueros et al., 2012; Abreu et al., 2012). Due to the degradation of this landscape, one of the strategies to cope with this situation has focused on the revegetation of the mine areas, because this will contribute with beneficial direct or indirect effects, such as erosion control, site restoration or carbon sequestration.

For successful revegetation, various measures are usually introduced oriented to the correction of soil acidity through liming or addition of exogenous organic amendments to improve soil OC and fertility. Different organic amendments of agricultural or industrial origin have already demonstrated the improvement of soil quality (Mingorance et al., 2014) allowing the development of healthy plant species (Wang et al., 2012). They also help in the development of soil aggregation, representing an early step of soil reclamation in mine waste deposits and bridging the physical and biological properties of soil systems.

---

[*] Corresponding author

Among them, land application of treated sewage sludges helps to maintain a sustainable environmental management by recycling these low-cost wastes, reducing their disposal and returning organic material, trace elements, moisture and nutrients to the soil (García-Gil et al., 2004; Mingorance et al., 2014).

However, the development of plant species in these soils could require in the future the management of possible pests or diseases by using pesticides which could also affect plant yield. The presence of organic contaminants in the soil together with heavy metals from past metallurgical activities, may impair the development of the vegetal species planted for revegetation, as well as incorporate residues of the organic pollutants into the plant. The degree of plant uptake will depend on the plant species but also on the properties of pesticides (Yu et al., 2009; Jiang et al., 2011), because the movement of non-ionic organic compounds into the roots can be considered a partition between liquid and solid phases (Sicbaldi et al., 1997) related with pesticide polarity.

In reclamation of mine soils, use of organic amendments, such as sewage sludges, has been focused on the management of inorganic pollutants, i.e. heavy metals (Alvarenga et al., 2009a). In other assays with plants, addition of organic amendments from different sources (biochars, manure, sludges) has been reported to enhance soil retention of organic pollutants (Yu et al., 2009; Hilber et al., 2009; Jiang et al., 2011), reducing plant uptake. Besides, amendments with high microbial activity, like manure or sewage sludge, apart from the benefit of recycling, may also enhance degradation processes, which further reduce pollutant plant uptake (Jiang et al., 2011; Wang et al., 2012).

In addition, in arid or semiarid regions shortage of fresh water restricts agricultural production or else plant development and yield production may be compromised. Furthermore if water is used for irrigation of a degraded soil which in principle will not be devoted to agricultural production, the selection of marginal water, such as treated urban wastewater, will provide a cost-effective and available alternative for irrigation of this impaired soil (Qadir et al., 2007). However, treated wastewater has been shown to modify in some cases the behaviour of pesticides in the soil ecosystem (Rodríguez-Liébana et al., 2011, 2014). Finally, though wastewater contains higher levels of salts and dissolved organic matter than fresh water, which may alter plant performance and yield quality (Sopper and Kardos, 1972; Bernstein et al., 2009), no reference on the effect of wastewater irrigation on pesticide plant uptake could be found in the literature.

The novelty of this work lies on the use of sewage sludge in mine soils oriented to reduction of environmental hazard by organic pollutants and irrigation with wastewater, the latter rarely considered to date in revegetation strategies of mine soils and never included, to our knowledge, in pesticide plant uptake. Perennial ryegrass, *Lolium perenne* (L.), which adequately develops in acid mine soils (Alvarenga et al., 2009a; b; Mingorance et al., 2014), and which has been also proposed as an appropriate species to remediate soils co-contaminated by heavy metals and organic pollutants (Chigbo and Batty, 2013), was selected as plant model species. The aim of the present work was focused on the effects of sewage sludge and wastewater treatments on plant biomass production and on the protection of plant against the toxic effect of two pesticides (an insecticide and a fungicide) with different chemical-physical properties.

2. Materials and methods

2.1. *Pesticides*

High purity standards (≥98%, Dr. Ehrenstorfer, Germany) of thiacloprid (THC), a nicotinoid insecticide and of the pyrimidine fungicide fenarimol (FEN) were used without further purification. Their octanol/water partition coefficients (log K_{ow}) are 1.26 and 3.69, and their solubility in water 185 and 13.7 mg L^{-1}, respectively (Tomlin, 2003). Stock pesticide solutions were prepared at 1000 mg L^{-1} in acetone.

2.2. *Soil, sewage sludge, liming agent and water properties*

Soil was collected from the upper layer of an acid mine waste, in the Riotinto mining area (37° 42' 4.5'' N 6° 33' 35.1'' W), located in the Iberian Pyrite Belt, which includes one of the largest deposits of pyrite (FeS_2). According to X-Ray Fluorescence analysis SiO_2 together with Fe and Al oxides represent more than 80% of the soil mineralogical composition (Rodríguez-Liébana et al., 2013). It is a very acid sandy loam soil (pH 2.4), with high electrical conductivity (EC, 1.3 dS m^{-1}) (both at 1/2.5 ratio, w/v) and low organic carbon (OC, 1.4%) content. The content of some potential hazardous metals (mg kg^{-1}) (As, 3951; Cd, 13; Cu, 694; Pb, 3976) is above the local guidelines (Mingorance et al., 2014).

The Nerva mine soil (<8 mm) was limed with 1.8% Carbocal, a residue from the sugar industry (Azucarera Ebro) which contains 83.4% $CaCO_3$. The stabilized sewage sludge (SSL), from the wastewater treatment plant of Seville (SW Spain), had an OC content (%) of 22.03± 2.94, pH 6.96± 0.01 and EC (1/10 ratio, w/v) 1.6± 1.2 dS m^{-1}. Irrigation was performed with distilled water (DW) [pH 5.3± 0.3, EC 5.0± 1.3 mS cm^{-1}, dissolved OC (DOC) 0.19± 0.03 mg C L^{-1}] or wastewater (WW) [pH 8.7± 0.3, EC 1063± 88 mS cm^{-1}, DOC 21.3± 2.8 mg C L^{-1}]. Besides, as reported by the treatment plant, WW also contained 27.3 mg L^{-1} total N and 3.8 mg L^{-1} total P, had a chemical oxygen demand of 51 mg L^{-1} and a biological oxygen demand <13 mg L^{-1}.

2.3. *Pot experiments*

Limed soil was thoroughly mixed with the organic amendment (SSL at 2%) in a mechanical shaker for 4 h. The SSL dose was selected on the basis of a previous screening with three different plant species (Mingorance et al., 2014), indicating that *L. perenne* was a species easy to cultivate with a short cultivation period and that SSL at 2% promoted plant biomass without affecting seed germination and mortality.

The experiment was carried out in the greenhouse of the University of Seville, with an average temperature of 21.8 °C and relative humidity of 67%. An indoor system was used using plastic pots containing 250 g soil. The treatments ($n = 4$) consisted in non- amended soil irrigated with DW or WW (SSL0-DW and SSL0-WW) and sludge-amended soil at 2% with DW or WW irrigation (SSL2- DW and SSL2-WW). Additionally, two treatments without pesticides (SSL0-DW and SSL0-WW) were also included to evaluate the effect of pesticides on biological plant parameters. In order to reduce soil compactness, 40 g of glass beads (4 mm in diameter) were mixed in each pot. Eleven ryegrass seeds were grown directly on each pot and irrigated every 2 days with DW for a week. Then the pots corresponding to treatments with pesticides were added with 5 mL of a solution containing a mixture of THC and FEN at 200 mg mL^{-1} in DW followed by 5 mL DW or WW, depending on the treatment. Pesticide dose corresponds to 2 L a.i. per Ha, which is approximately 3 times the recommended dose for THC. This would allow estimating a worse case scenario and quantifying pesticide residues at the end of the experiment.

Afterwards irrigation, necessary to maintain soil water holding capacity and without effluence from the bottom of the pots, was carried out by adding 10 mL water (DW or WW), every two days for 27 days.

Several parameters were measured in plants at the end of the experiment in all the treatments: germination and mortality rates, biomass production (dry weight), shoot length and relative water content. In those treatments containing pesticides, a set of soil and plant subsamples was immediately frozen and kept at their water content at —18 °C, until the measurement of soil dehydrogenase activity and the determination of pesticide residues in soil and plant.

2.4. *Analysis*

2.4.1. *Pesticides in soil and soil properties*

All solvents used were for pesticide analysis grade. After soil homogenisation, 10 g of each pot was extracted twice in an ultrasound bath (200 W, Ultrasons, Selecta) for 15 min with 50 mL ethyl acetate. The supernatant was filtered through a paper filter and the combined extracts were concentrated in a rotary evaporator at 40 °C. The dry extract was dissolved in 1 mL of water:acetonitrile (50:50, v/v) and filtered (0.45 mm PVDF filters, Scharlab). Pesticide residues were expressed on an oven-dried (105 °C) soil basis.

Pesticide concentrations were determined by HPLC-DAD as in Rodríguez-Liébana et al. (2013). Calibration was performed by triplicate injection of standard solutions (0.5-15 mg L^{-1}; R^2 0.999, for both pesticides). Recoveries of fortified samples (%) were 95.6± 5.0 for THC and 70.0± 5.1 for FEN.

Soil dehydrogenase activity (DHA) (García et al., 1997) and soil organic C (OC) (Mingorance et al., 2007) were determined in triplicate.

2.4.2. *Pesticides in plant*

An aliquot of 100 mg *L. perenne* was homogenised in a ceramic mortar after addition of liquid nitrogen, extracted with 2 mL acetonitrile/formic acid (0.1%) and vortexed for 1 min. Then 250 mg of a mixture of magnesium sulphate, sodium chloride (Panreac) and sodium citrate (Merck) (4/1/1) was added, the mixture vortexed (1 min) and centrifuged at 3000 rpm for 5 min. The supernatant, added with 25 mg active carbon (Agilent), was vortexed (1 min) and filtered (nylon filters, 0.45 μm, Scharlab).

Pesticides were analysed by HPLC-MS using Quattro Micro triple quadrupole (Waters, Milford, MA) with positive ion electrospray ionization (ESI). Separation was performed on an Atlantis T3 column (Waters, 3 × 150 mm, 3 mm), connected to an Atlantis T3 precolumn (Waters, 2.1 × 10 mm, 3 mm) thermostated at 30 °C at a flow rate of 0.3 mL min^{-1}. The mobile phase, a mixture of aceto- nitrile (solvent A) and MilliQ water (solvent B) both with formic acid at 0.1%, changed from 70% to 90% A in 5 min, then to 70% A in 3 min, and then hold for 7 min. The mass spectrometer was operated in multiple reaction monitoring mode, with the transitions: FEN 330.99 ⟶ 267.99, and THC 253.18 ⟶ 125.97. Recoveries (%) of fortified samples ranged from 72.3± 3.4 to 95.3± 11.3 for THC and FEN, respectively.

2.4.3. *Data analysis*

ANOVA followed by Fisher's least significant difference (LSD) test was used to compare several mean groups. All statistical ana- lyses were performed at the 0.05 significance level. SPSS 17.0 software package was used. Relationships among variables are performed by correlation or regression analysis.

Bioconcentration factors (BCF) for each treatment were calculated (Dowdy and McKone, 1997) as BCF = C_p/C_s, where C_p and C_s are the pesticide concentration in the above-ground plant part and in soil, respectively (both in mg kg^{-1}, dry weight). Relative water content (RWC) was estimated as RWC = FW$_e$—DW$_e$/FW$_e$ × 100, being FW$_e$ and DW$_e$ the shoot fresh and dry weights, respectively.

3. Results and discussion

3.1. *Plant growth indicators*

In general germination was homogeneous for all the treatments and ranged between 77 and 100%. Mortality was low (0-3%) in all the cases ($p = 0.365$), in accordance with previous assays (Mingorance et al., 2014). The high variability found in some treatments can be considered normal for the commercial seeds used in the experiment.

Ryegrass may be a plant sensitive to the addition of THC and/or FEN as can be deduced from the reduction of biomass, as well as shoot length, with the highest values corresponding to the plants growing in soil without pesticide addition (Table 1).

When considering only the pots added with pesticides, ANOVA shows that biomass and shoot length were affected by both the type of water used for irrigation and the addition of SSL ($p < 0.05$), the treatment combining SSL and WW (SSL2-WW) leading to the highest values and SSL0-DW to the lowest. These results are in agreement with Alvarenga et al. (2009a) who found that addition of SSL at 2.5% was effective in remediating a mine contaminated soil, improving soil chemical properties and allowing the greatest in- crease in plant biomass of *L. perenne*. Studies using another plant species (Jiang et al., 2011) have also shown a positive effect of organic amendments on plant elongation and biomass.

The increase of plant indicators with WW coincides with a previous report that used synthetic WW for irrigation of bermudagrass, tall fescue, Meyer Lemon and Emerald Gaiety Euonymus (Negahban-Azar et al., 2013), attributing the results to higher nutrient loading. However the synthetic WW employed had a chemical oxygen demand seven times higher than the one used in the present study and lower N and P concentrations. On the contrary, irrigation of oregano and rosemary with WW, with N and P levels similar to the one used in this study, reported no effects on biomass production (Bernstein et al., 2009). It seems that, apart from the positive effect that SSL exerts on plant development, irrigation with WW provides the plant with additional organic compounds and nutrients which may help in the success of plant establishment and vigorous development, especially with a plant species relatively tolerant to salinity (Marcar, 1987).

Relative water content (RWC) is a reliable indicator of plant water status (Bradford and Hsiao, 1982) and constitutes an approach to study the treatment effects on plant water economy. Application of pesticides with or without SSL addition significantly reduced the RWC when plants were irrigated with DW ($p < 0.05$). The reduction of water availability causes significant decreases in the rate of solute diffusion to metabolic sites where intense enzymatic activity occurs. This is because water is a solvent and a medium in which diffusion of solutes and biochemical reactions take place in plant cells.

3.2. *Soil dehydrogenase and organic carbon content*

Soil DHA, which represents the overall microbiological activity of the soils, was higher in amended SSL2 than in non-amended SSL0 mine soil ($p < 0.05$), independently of the water quality used for irrigation ($p > 0.05$) (Fig. 1 left). A similar increase in enzyme activities in a co-contaminated soil after addition of an organic amendment has been reported (Doni et al., 2012), indi- cating the positive role of the amendments in neutralizing contaminant toxicity. This means that microbial activity was higher in amended soils though this activity could be mainly attributed to the transformation of labile C and N sources from the amendment.

Irrigation with WW did not significantly modify soil DHA ($p > 0.05$), contrary to recent reports which have indicated a depletion of soil enzyme activities after WW irrigation (Hernández-Soriano et al., 2009; Kayikcioglu, 2012).

Soil OC at the end of the experiment was independent of the water used for irrigation ($p = 0.150$) in each treatment but was significantly lower in SSL0 pots than in those receiving the organic amendment (SSL2) ($p < 0.05$) (Fig. 1 right). Overall, a significant correlation between DHA and soil OC ($r = 0.828$) was found which corroborated the positive role of the amendments for increasing microbial activity and, consequently, plant growing.

3.3. *Pesticides in soils*

The pesticides behaved differently being THC always found in lower concentration than FEN (Fig. 2). Residues of THC were higher in absence than in presence of the organic amendment ($p = 0.013$), but were not affected by WW ($p = 0.203$). On the contrary FEN was neither affected by the irrigation quality nor by the addition of sewage sludge ($p > 0.05$). The lack of effect of WW on the residues of both pesticides in soil agrees with previous results on Spanish or Argentinean soils which showed that the use of WW did neither affect desorption of organochlorine or organophosphorus pesticides (González et al., 2010; Hernández-Soriano et al., 2012) nor the sorption of pesticides of intermediate polarity, like FEN and THC (Rodríguez-Liébana et al., 2011; ElGouzi et al., 2012).

It is important to stress that the measured residues correlate well with data concerning pesticide sorption. FEN, a pesticide of medium polarity, was reported to be rather retained on this mine soil (distribution coefficient, K_d 6.00), while sorption of the more polar THC was found to be much lower (K_d 1.35) (Rodríguez-Liébana et al., 2013). Addition of SSL2 would reduce the presence of both pesticides in soil solution and, thus, pesticide bioavailability for biodegradation or plant uptake.

The final pesticide concentration in soil is the result of, at least, the following pathways: (1) plant uptake or accumulation, (2) degradation by soil microorganisms, and (3) abiotic loss, such as volatilization or chemical degradation (hydrolysis, photodecomposition, etc).

Plant uptake will be examined in the next section. With respect to the other processes, higher degradation rate is expected for THC in comparison with FEN (mean field half-life 7e21 d for THC and >365 d for FEN) (Tomlin, 2003), supporting the lower soil THC content in all the treatments. On the other hand, soil FEN could be lost by volatilisation at a higher rate, since its vapour pressure is various orders of magnitude higher than that of THC (0.065 and 3×10^{-7} mPa, respectively) (Tomlin, 2003). However, volatilisation seems not to have greatly diminished FEN concentration. As can be seen in Fig. 2, FEN residues in the different treatments were close to 4 mg g^{-1}, the added pesticide concentration. It has been reported that the higher the soil organic content, such as that corresponding to sludge-amended pots, the lower the volatilisation rate, though no differences were encountered.

Finally, the lower THC residues in SSL2 than in SSL0 may be additionally attributed to enhanced pesticide decomposition favoured by exogenous microbial species provided to the soil by the amendment. The higher DHA measured in the pots added with SSL (Fig. 1) further confirms this assumption.

3.4. *Pesticides in plants*

Pesticide concentrations in plant samples were dependent on the treatment. The total amount of pesticide per pot was calculated in order to assess the influence of the different plant biomass (Fig. 2, pale grey). There is a significant interaction effect for both pesticides between addition of SSL and irrigation water ($p < 0.05$). The concentrations of both pesticides in SSL0, with less vegetal development (Table 1), are correlated with plant biomass ($r > 0.95$) and lower amount of both pesticides was found for DW- than for WW-irrigated pots. When pots had been amended with sewage sludge (SSL2) the amount of both pesticides was also lower for irrigation with DW, but without significance ($p > 0.05$).

Growing on contaminated soil resulted in an accumulation of THC and FEN in *L. perenne*, because both pesticides were taken up by plant roots and translocated to the shoots. The accumulation of THC (ng pesticide per pot) was dependent on soil concentration ($r = 0.672$) (Fig. 2) but not that of the more hydrophobic FEN. Other reports concerning hydrophobic organic pollutants in soil, like PCBs, have also indicated lack of relationship between concentration in plant and soil (Weber et al., 1994). The transfer of compounds between the different soil phases is the main process that controls their mobility and availability to plants. It is possible that due to the low THC sorption on soil, almost all the measured soil THC was available for plant uptake. The more hydrophobic FEN was retained by soil to a larger extent and the measured residues were likely not in the soil solution, thus avoiding plant uptake by roots and further translocation to the aerial plant parts.

On the contrary when we examine pesticide concentration in shoots another picture is observed (Fig. 3). Pesticide concentration (ng g^{-1} fresh shoot weight) was also significantly affected by SSL addition and by the quality of irrigation water ($p < 0.05$), but no interaction effect was found ($p > 0.05$). Pesticide concentrations were higher for DW- than for WW-irrigation and addition of SSL led to a significant reduction in shoots. The decrease mediated by WW irrigation could be the consequence of the dissolved organic matter (DOM) content and of the composition of WW. A previous report using DOM from sludge or straw showed that the concentration in wheat of the herbicide chlortoluron, decreased after DOM addition and this decrease was dependent on the DOM composition, as shown by FT-IR spectral analyses (Song et al., 2010).

Shoot THC concentration was always *ca.* twice that of FEN ($r = 0.711$). On the other hand, though the concentration of FEN in soil did not differ among the treatments, lower shoot concentration corresponded to SSL2 irrigated with WW (Fig. 3). After addition of SSL2 FEN may have become less bioavailable and more tightly bound to soil. These results are in agreement with previous reports showing reduced pesticide uptake by plant after amendment with organic additives from different sources (biochars, manure, sludges) (Yu et al., 2009; Hilber et al., 2009; Jiang et al., 2011). The use of SSL, contrary to what occurs with biochars, could even increase pesticide degradation in soil (Sánchez et al., 2003), due to its higher microbial activity (Fig. 1), thus further reducing pesticide concentration in soil and, consequently, in plant.

Bioconcentration factors showed that in all cases THC was uptaken more effectively than FEN, in accordance with an inverse relationship of BCFs with pesticide K_{ow} values (Travis and Arms, 1988) (Table 2). The less polar a compound is, the more it will be retained on lipid material, being less mobile across the endodermis. Translocation is highly dependent on the chemical's polarity and plant transpiration. As the plant conditions were the same in all the treatments, pesticide properties had to play a role. However, because the BCFs were largely lower than 1, even lower than other values previously reported for a wide range of pesticides and crops (Travis and Arms, 1988), it can be concluded that ryegrass did neither accumulate THC nor FEN in the shoots, reflecting the low plant ability to incorporate both pesticides into the aerial plant tissues, in particular FEN. Earlier studies using ryegrass among other plant species have also provided BCFs lower than 1 for another pesticide, DDT (Lunney et al., 2004; Mo et al., 2008), being ryegrass the plant species, among the ones studied, which less efficiently uptook DDT into the shoots. BCF values for PCBs were also very low (0.0042) (Weber et al., 1994). The BCF values of both pesticides were reduced when SSL was added ($p < 0.05$), but were not affected by wastewater irrigation ($p > 0.05$) (Table 2). The effect of increasing organic addition (biochars) on BCF reduction has also been shown with chlorpyrifos, carbofuran and fipronil in different plant species (Yu et al., 2009). It appears, in agreement with an earlier study with PCBs in amended soil using different plant species (Weber et al., 1994), that pesticides represent a lower hazard to ryegrass quality after amendment with sewage sludge. The observed result seems to be related to an enhanced retention of the pesticides in the amended soil.

When considering irrigation with WW the observed trend of BCF decrease, especially in the case of THC, could be ascribed to the OC content of the irrigation water. Song et al. (2010) using wheat seedlings and DOC showed that the BCF of the phenylurea herbicide chlorotoluron diminished with increasing DOC concentration. This effect was explained because of the formation of a pesticide-

DOM complex which was too polar to pass through the plasma membrane into the root cells. A similar mechanism could be occurring in our case, being the formation of the complex dependent on the chemical nature of the pesticide and on the composition of DOM. Reduction of BCF for THC (87% reduction in SSL0 and 64% in SSL2) probably reflected a stronger interaction of THC than FEN with DOM. Finally, for both pesticides the decrease in BCF was more evident when SSL was present, likely because SSL addition would provide an additional DOC fraction which would strengthen the effect of WW.

4. Conclusions

Perennial ryegrass (*Lolium perenne*) can be adequately grown in an acid mine soil and the higher biomass increase is reached after the combined addition of sewage sludge and irrigation with wastewater. Application of THC and FEN, at concentrations higher than those recommended for agricultural use, led to a reduction of plant biomass which was compensated by organic addition, via sewage sludge amendment and wastewater irrigation. Pesticide residues in soil are the highest for FEN, a highly persistent and relatively volatile compound, which is less effectively translocated to the ryegrass shoots. On the contrary, residues of the more polar THC, which shows less persistence and volatility, were lower in soil and higher in ryegrass. Addition to soil of sewage sludge and irrigation with WW represent both a lower hazard to ryegrass quality, since less pesticide is uptaken to the aerial plant parts. The mechanisms for the observed phenomenon are different: higher soil sorption for sludge-amended pots, while possible formation of pesticide-dissolved OC complexes with less plant absorption ability, for WW irrigation. The proposed strategy of sewage sludge addition and WW irrigation, resulting in decreased pesticide concentration in ryegrass shoots, implies additional environmental benefits through disposal of organic residues produced in large quantities and which need to be recycled as well as exploitation of marginal water in a degraded site.

Acknowledgements

This work was supported by Proyecto de Excelencia-Junta de Andalucía (P10-RNM5814), cofinanced by FEDER funds. The technical assistance of I. Cañadas and that of A. Caballero with the graphical abstract are kindly acknowledged. Wastewater and sewage sludge were provided by Fundación Centro de las Nuevas Tecnologías del Agua (CENTA, Sevilla, Spain) and Carbocal by Juan Castellano Márquez. HPLC-MS analyses were performed by Dr. L. Sánchez at the Scientific Instrumentation Service (EEZ, CSIC) (Granada, Spain). The authors thank technicians at the Greenhouse of the University of Seville (CITIUS) for their assistance in plant cultivation.

References

Abreu, M.M., Santos, E.S., Ferreira, M., Magalhaes, M.C.F., 2012. *Cistus salviifolius* a promising species for mine wastes remediation. J. Geochem. Explor. 113, 86e93. Alvarenga, P., Gonçalves, A.P., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2009a. Organic residues as immobilizing agents in aided phytostabilization: (I) Effects on soil chemical characteristics. Chemosphere 74, 1292-1300.

Alvarenga, P., Palma, P., Gonçalves, A.P., Baião, N., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2009b. Organic residues as immobilizing agents in aided phytostabilization: (II) Effects on soil biochemical and ecotoxicological characteristics. Chemosphere 74, 1301-1308.

Bernstein, N., Chaimovitch, D., Dudai, N., 2009. Effect of irrigation with secondary treated effluent on essential oil, antioxidant activity, and phenolic compounds in oregano and rosemary. Agron. J. 101, 1-10.

Bradford, K.J., Hsiao, T.C., 1982. Physiological responses to moderate water stress. In: Lange, O.R., Novel, P.S., Osmond, C.B., Ziegler, H. (Eds.), Encyclopedia of Plant Physiology New Series, vol. 12B. Physiological Plant Ecology II. Springer-Verlag, Berlin, pp. 264-324. Chapter 9.

Chigbo, C., Batty, L., 2013. Effect of combined pollution of chromium and benzo (a) pyrene on seed growth of *Lolium perenne*. Chemosphere 90, 164-169.

Doni, S., Macci, C., Peruzzi, E., Arenella, M., Ceccanti, B., Masciandaro, G., 2012. *In situ* phytoremediation of a soil historically contaminated by metals, hydrocarbons and polychlorobiphenyls. J. Environ. Monit. 14, 1383-1390.

Dowdy, D.L., McKone, T.E., 1997. Predicting plant uptake of organic chemicals from soil or air using octanol/water and octanol/air partition ratios and a molecular connectivity index. Environ. Toxicol. Chem. 16, 2448-2456.

ElGouzi, S., Mingorance, M.D., Draoui, K., Chtoun, E.H., Peña, A., 2012. Assessment of phenylurea herbicides sorption on various Mediterranean soils affected by irrigation with wastewater. Chemosphere 89, 334-339.

García, C., Hernández, T., Costa, F., 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Commun. Soil. Sci. Plant 28, 123-134.

García-Gil, J.C., Plaza, C., Senesi, N., Brunetti, G., Polo, A., 2004. Effects of sewage sludge amendment on humic acids and microbiological properties of a semiarid Mediterranean soil. Biol. Fert. Soils 39, 320-328.

González, M., Miglioranza, K.S.B., Aizpún, J.E., Isla, F.I., Peña, A., 2010. Assessing pesticide leaching and desorption in soils with different agricultural activities from Argentina (Pampa and Patagonia). Chemosphere 81, 351-358.

Hernández Soriano, M.C., Mingorance, M.D., Peña, A., 2009. Dissipation of insecticides in a Mediterranean soil in the presence of wastewater and surfactant solutions. A kinetic model approach. Water Res. 43, 2481-2492.

Hernández Soriano, M.C., Mingorance, M.D., Peña, A., 2012. Desorption of two organophosphorous pesticides from soil with wastewater and surfactant solutions. J. Environ. Manage. 95, S223-S227.

Hilber, I., Wyss, G.S., Mader, P., Bucheli, T.D., Meier, I., Vogt, L., Schulin, R., 2009. Influence of activated charcoal amendment to contaminated soil on dieldrin and nutrient plant uptake by cucumbers. Environ. Pollut. 157, 2224-2230.

Jiang, L., Ma, L., Sui, Y., Han, S.Q., Yang, H., 2011. Mobilization and plant accumulation of prometryne in soil by two different sources of organic matter. J. Environ. Monit. 13, 1935-1943.

Kayikcioglu, H.H., 2012. Short-term effects of irrigation with treated domestic wastewater on microbiological activity of a Vertic xerofluvent soil under Mediterranean conditions. J. Environ. Manage. 102, 108-114.

Lunney, A.I., Zeeb, B.A., Reimer, K.J., 2004. Uptake of weathered DDT in vascular plants: potential for phytoremediation. Environ. Sci. Technol. 38, 6147-6154.

Marcar, N.E., 1987. Salt tolerance in the genus *Lolium* (Ryegrass) during germination and growth. Aust. J. Agric. Res. 38, 297-307.

Mingorance, M.D., Barahona, E., Fernández-Gálvez, J., 2007. Guidelines for improving organic carbon recovery by the wet oxidation method. Chemosphere 68, 409-413.

Mingorance, M.D., Rossini-Oliva, S., Valdés, B., Pina Gata, F.J., Leidi, E.O., Guzmán, I., Peña, A., 2014. Stabilized sewage sludge addition to improve properties of an acid mine soil for plant growth. J. Soils Sediments 14, 703-712.

Mo, C.-H., Cai, Q.-Y., Li, H.-Q., Zeng, Q.-Y., Tang, S.-R., Zhao, Y.-C., 2008. Potential of different species for use in removal of DDT from the contaminated soils. Chemosphere 73, 120-125.

Negahban-Azar, M., Sharvelle, S.E., Qian, Y., Shogbon, A., 2013. Leachability of chemical constituents in soil-plant systems irrigated with synthetic greywater. Environ. Sci. Proces. Imp. 15, 760-772.

Qadir, M., Sharma, B.R., Bruggeman, A., Choukr-Allah, R., Karajeh, F., 2007. Non- conventional water resources and opportunities for water augmentation to achieve food security in water scarce countries. Agr. Water Manage. 87, 2-22

Rodríguez-Liébana, J.A., Mingorance, M.D., Peña, A., 2011. Sorption of hydrophobic pesticides on a Mediterranean soil affected by wastewater, dissolved organic matter and salts. J. Environ. Manage. 92, 650-654.

Rodríguez-Liébana, J.A., Mingorance, M.D., Peña, A., 2013. Pesticide sorption on two contrasting mining soils by addition of organic wastes: effect of organic matter composition and soil solution properties. Colloid. Surf. A 435, 71-77.

Rodríguez-Liébana, J.A., ElGouzi, S., Mingorance, M.D., Castillo, A., Peña, A., 2014. Irrigation of a Mediterranean soil under field conditions with urban wastewater: effect on pesticide behaviour. Agric. Ecosyst. Environ. 185, 176-185.

Sánchez, L., Peña, A., Sánchez-Rasero, F., Romero, E., 2003. Methidathion degradation in a soil amended with biosolid and a cationic surfactant. Use of different kinetic models. Biol. Fert. Soils 37, 319-323.

Sicbaldi, F., Sacchi, G.A., Trevisan, M., del Re, A.A.M., 1997. Root uptake and xylem translocation of pesticides from different chemical classes. Pestic. Sci. 50, 111-119.

Song, N.H., Zhang, S., Hong, M., Yang, H., 2010. Impact of dissolved organic matter on bioavailability of chlorotoluron to wheat. Environ. Pollut. 158, 906-912.

Sopper, W.E., Kardos, L.T., 1972. Municipal wastewater aids revegetation of strip- mined spoil banks. J. Forest. 70, 612-615.

Tomlin, C.D.S., 2003. The Pesticide Manual, thirteenth ed. British Crop Protection Council, UK, p. 1344 pp.

Travis, C.C., Arms, A.D., 1988. Bioconcentration of organics in beef, milk, and vegetation. Environ. Sci. Technol. 22, 271-274.

Trigueros, D., Mingorance, M.D., Rossini Oliva, S., 2012. Evaluation of the ability of *Nerium oleander* L. to remediate Pb-contaminated soils. J. Geochem. Explor. 114, 126-133.

Wang, K., Zhang, J., Zhu, Z., Huang, H., Li, T., Yang, X., Alva, A., 2012. Pig manure vermicompost (PMVC) can improve phytoremediation of Cd and PAHs co- contaminated soil by *Sedum alfredii*. J. Soils Sediments 12, 1089-1099.

Weber, M.D., Pietz, R.I., Granato, T.C., Svodoba, M.L., 1994. Plant uptake of PCBs and other organic contaminants from sludge-treated coal refuse. J. Environ. Qual. 23, 1019-1026.

Yu, X.Y., Ying, G.G., Kookana, R.S., 2009. Reduced plant uptake of pesticides with biochar additions to soil. Chemosphere 76, 665-671.

Table 1

Plant parameters measured in *L. perenne* (mean ± standard deviation; $n = 4$) growing in soils with different treatments. Different letters in the same column indicate statistical difference between treatments ($p < 0.05$).

	Dry weight (g)	Relative water content (%)	Shoot length (cm)	Germination (%)	Mortality (%)
Without pesticides					
SSL0-DW[a]	0.079 ± 0.011 a	84 ± 1 ab	16.2 ± 1.2 a	100 ± 0 ab	0 ± 0
SSL0-WW	0.054 ± 0.014 b	85 ± 1 ab	15.0 ± 2.0 a	77 ± 5 c	2 ± 5
With pesticides					
SSL0-DW	0.007 ± 0.004 e	78 ± 4 c	4.1 ± 1.1 c	89 ± 9 bc	3 ± 5
SSL0-WW	0.029 ± 0.008 c	85 ± 1 ab	11.6 ± 3.4 b	98 ± 3 a	0 ± 0
SSL2-DW	0.026 ± 0.016 cd	82 ± 2 b	7.7 ± 2.9 b	89 ± 17 bc	1 ± 3
SSL2-WW	0.066 ± 0.022 ab	86 ± 1 a	13.8 ± 2.0 a	94 ± 7 ab	1 ± 3

[a] SSL0-DW and SSL0—WW correspond to pots without sewage sludge and irrigated with distilled and wastewater, respectively. SSL2-DW and SSL2-WW describe treatments added with 2% sewage sludge with distilled and wastewater irrigation, respectively.

Table 2

Bioconcentration factors in *Lolium perenne* grown in soil treated with thiacloprid (THC) and fenarimol (FEN).

	THC	FEN
SSL0-DW[a]	0.209 ± 0.061	0.013 ± 0.003
SSL0-WW	0.182 ± 0.017	0.018 ± 0.003
SSL2-DW	0.073 ± 0.026	0.011 ± 0.005
SSL2-WW	0.047 ± 0.004	0.008 ± 0.003

[a] Treatments as in Table 1.

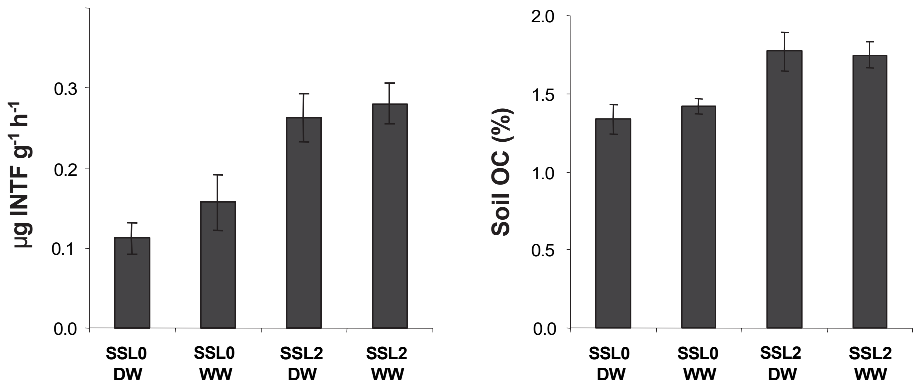

Fig. 1. Soil dehydrogenase activity (left) and soil organic C content (right) at cropping for the different treatments. Vertical bars indicate standard deviation ($n = 4$). SSL0-DW and SSL0eWW correspond to pots without sewage sludge and irrigated with distilled and wastewater, respectively. SSL2-DW and SSL2-WW describe treatments added with 2% sewage sludge with distilled and wastewater irrigation, respectively.

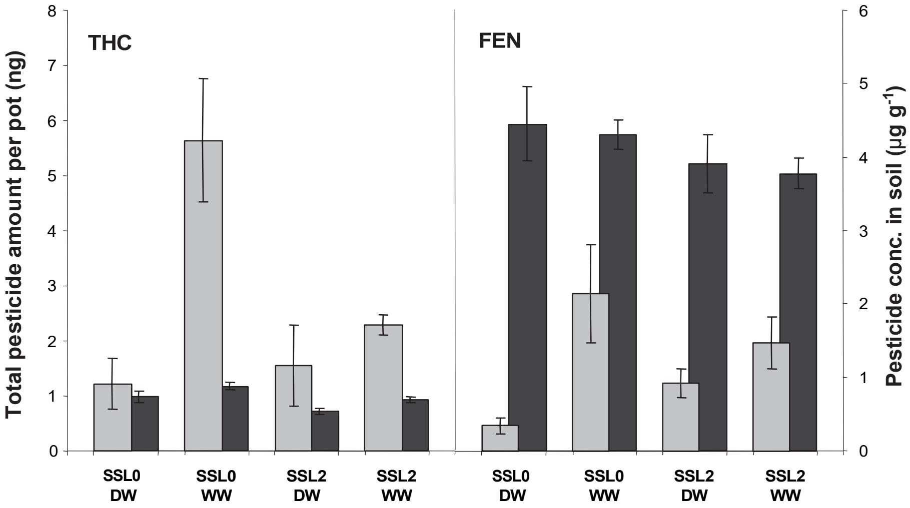

Fig. 2. Total pesticide amount in *Lolium perenne* (ng per pot) (pale grey) and in soil (mg g^{-1}) (dark grey). Vertical bars indicate standard deviation ($n = 4$). THC: Thiacloprid, FEN: Fenarimol. Treatments as in Fig. 1.

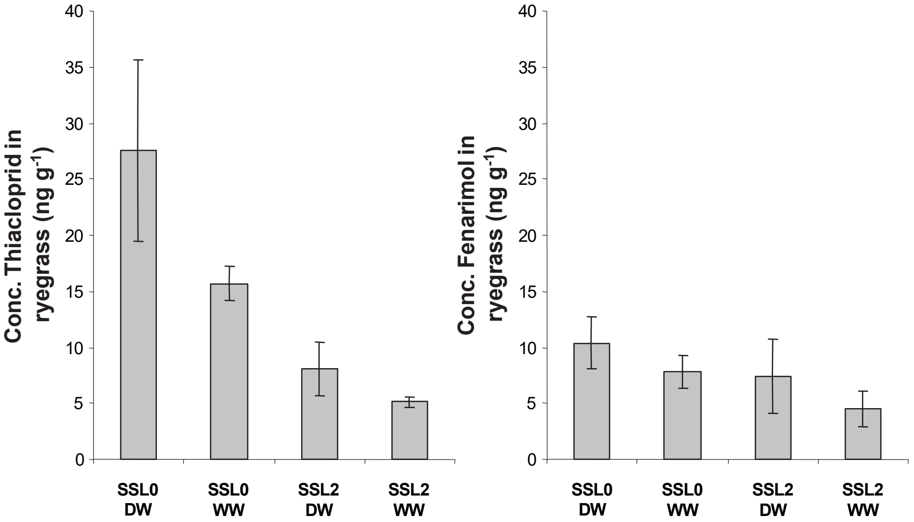

Fig. 3. Thiacloprid and fenarimol concentration in *Lolium perenne* shoots (ng g^{-1} fresh shoot weight). Vertical bars indicate standard deviation ($n = 4$). Treatments as in Fig. 1.