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Organochlorine pesticides in agricultural soils and associated biota

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Abstract The behavior of organochlorine pesticides (OCPs), such as DDTs, endosulfans, HCHs, heptachlors, drins and chlordanes, has been evaluated in the Quequén Grande River agricultural watershed including different matrices. Soil profiles from fields dedicated to soybean cultures and the associated terrestrial biota (earthworms, coleopteran larvae, collembolan and mites), stream water, suspended particle matter and surface sediment were sampled during the pre-application pesticide period. Pesticide analyses were carried out using gas chromatography with an electron capture detector. Soils samples showed a differential pesticide pattern throughout the profile. Control soils, settled 200 m from agricultural plots showed similar pesticide levels but with higher variety of compounds, denoting the historical use of OCPs in the zone. However, in agricultural plots DDTs and endosulfans constituted 90 % of the pesticides found with a further enrichment of endosulfan sulfate at depth. The relatively high p,p'-DDT levels in surface agricultural soil would be a consequence of pesticide impurities in technical acaricide Dicofol, which is widely used in the region; and it represents a new threat of fresh p,p'-DDT input into environment despite its use being forbidden. Terrestrial biota, mainly mesofauna showed high capacity to OCP accumulation, the aforementioned organisms being a good option in the monitoring of trace pesticides. Furthermore, the high OCP levels found constitute a hazard to food web since these organisms are one of the first steps in the food chain and the biomagnification is a common process with these compounds. As a result of runoff and volatilization the occurrence of OCPs in the aquatic environment was observed, draining a load of around 7.3 kg of pesticides into the river annually. As a whole, these results suggest the importance of using terrestrial biota for studying recalcitrant pesticides and a continuous monitoring is strongly advised as a first step in minimizing environmental risks.

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Introduction

Organochlorine pesticides (OCPs), such as DDTs, endosulfans, HCHs, heptachlors, drins and chlordanes, have caused great concern worldwide during various decades due to their chronic toxicity, persistence, and bioaccumulative abilities. Even though, the use of these pesticides is banned, studies around the world continue reporting elevated levels of OCPs in soils, sediments, aquatic and terrestrial biota. Therefore, there is increasing concern about bioavailability of OCPs in soil to both animals and plants. This fact is of utmost importance because the biological availability controls the extent to which toxic substances

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can adversely affect organisms and ecosystems. Many variables, including the physicochemical characteristics of pollutants, compound residual time in soil, properties of soil and environmental conditions, and species characteristics, appear to exert a substantial influence on the uptake of soil pollutants. Soil organic matter is considered to be the most important factor limiting availability and mobility of OCPs in soil. Soil moisture content and wetting/drying cycles have also been shown to disturb the uptake and extractability of contaminants (Kottler et al. 2001; Ruggiero et al. 2002). Studies have demonstrated that the aging of pollutants in soil can lead to a reduced uptake by organisms. A great variety of invertebrates live within the soil. Among them, beetles which are considered beneficial arthropods in agriculture, representing a food source for other species. Earthworms and arthropods contribute to the maintenance of the soil fertility. These non-target organisms as well as target insects can bioconcentrate OCPs from soils, due to their basic physiological similarities (Huusela-Veistola 1996; Miglioranza et al. 1999). Earthworms are organisms possessing a high ability to modify soils and plant communities (Lavelle et al. 1997; Hale et al. 2005). This process can be performed through the incorporation of surface litter, casting, burrowing and other activities, affecting physical properties (Edwards and Shipitalo 1998), soil nutrients (Edwards and Bohlen 1996) and soil biological communities (Doube and Brown 1998).

The extent to which pesticides are susceptible to being transported through and from soil, and contribute to non-point source pollution, is dependent on the processes of biodegradation and sorption which determine the longevity and mobility of the pesticide within the soil, respectively (Kookana and Naidu 1998). Pesticide leaching in soil is an integral part of the transport processes. Therefore, pesticides are transported through the soil profile in both dissolved and colloid-associated means. The subsoil represents a very different physical, chemical and biological environment, when compared to the topsoil. In particular, organic matter declines with increasing depth, resulting in reduced microbial population (Vinther et al. 2001). Moreover, several studies have shown lower pesticide biodegradation rates with soil depth (Fomsgaard 1995), as a result of the reduced microbial activity. Several authors have shown that sorption of pesticides decreases with soil depth, with the pattern governed by organic matter content (Clay and Koskinen 2003).

Organochlorine pesticides are known to accumulate in surface sediments (SS) and suspended particulate matter (SPM). It is of increasing awareness that sediments act as a major OCP reservoir and that there are a number of sites around the world where contaminated sediments represent a serious hazard (Bettinetti et al. 2003). Moreover, SPM significantly influences OCP transportation in an aquatic environment. In this way, OCPs are removed from the water column and adsorbed on the SPM due to their high affinity for organic matter, and are finally accumulated in the SS. For this reason sediment acts as an important sink of organic contaminants. On the other hand, the SS resuspension leads to sediments playing a role as a secondary contamination source to overlying water.

The aim of the present study at the Quequén Grande River (QGR) watershed was to examine spatial variations of OCP concentrations in soil profiles, terrestrial soil biota (earthworms, coleopteran larvae, collembolan and mites), stream water, SPM and SS. Potential sources, transport, and OCP fate, as well as certain governing factors, were also investigated.

Materials and methods

Sampling area

The study area is located in the Argentine Pampean Region of, which holds a predominant 80 % of soybean production with intensive usage of agrochemicals. The OCPs have been used for many years in the QGR basin. It is located in the south of the Pampa Region of the Buenos Aires Province, covering a total area of 9.990 km^2 and is drained by several streams with a north–south orientation. During flooding events, the QGR flow rate rises to 758 m^3/s, although the mean flow rate is 12 m^3/s. The QGR drains into the Atlantic Ocean and is the most important stream of the basin. The climate is sub-humid and the mean annual temperature is 14 °C with a minimum of 7.3 °C and a maximum of 21 °C, with a mean rainfall of 891 mm for the 1960–2009 period and heavy rains during the summer months. Scarce natural vegetation covers the area between farms and stream, permitting the contaminants to reach the river.

Two agricultural plots (S1 and S2), one of them located 150 m from the river shore (S1: 38°14′38.5″S, 59°06′52.5″W) and the other adjacent to the river (S2: 38°14′51″S, 59°05′52.2″W) were sampled in June 2012, corresponding to a pre-application period. In order to evaluate pesticide dispersion, soil fauna and soil samples were also collected from a buffer zone adjacent to the QGR gully (BZ: 38°14′35.0″S, 59°06′44.5″W), 250 and 2500 m from S1 and S2, respectively. In S1 and S2 extensive agricultural activity (barley–soybean system) had been performed during many years, and at this time, they were following through with a fallow period with very little mulch on the soil to sample. BZ has a natural grass cover and was separated from S1 by a truck and a row of trees. The control area (CA: 38°11′53.93″S, 59°7′0.12″W) for sediment and stream water samples was located 5.5 km upstream from BZ (Fig. 1).

Fig. 1 Study area and samples location (modified from Lupi et al. 2015)
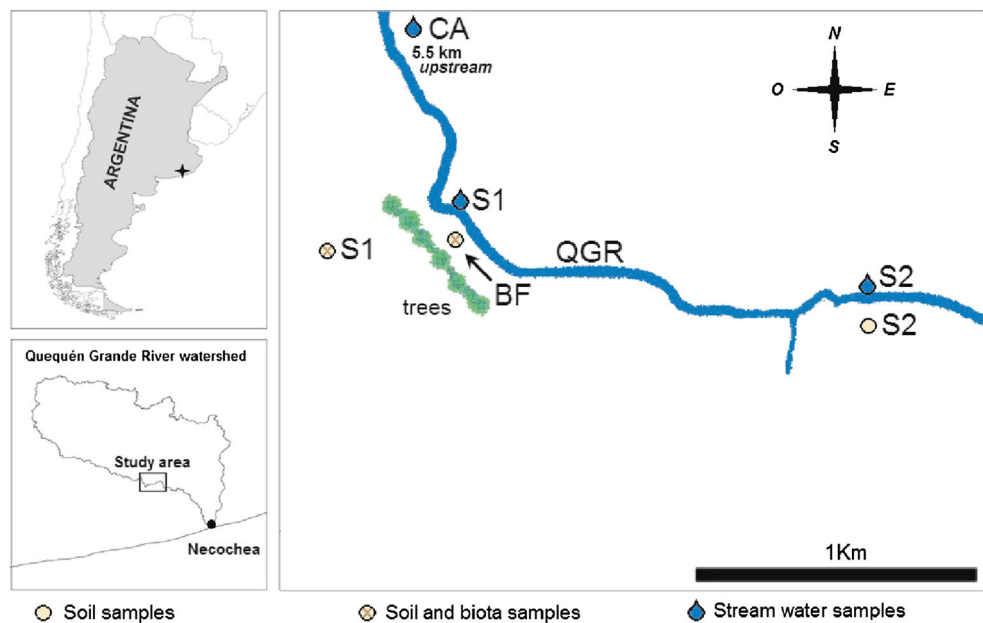
Sample collection

Three soil corers using aluminum columns of 40 × 10 cm in diameter were used at each of the sampling pots. Furthermore, stream water ($N = 3$) and sediment samples using corers of 10 × 4 cm in diameter were taken from the QGR, 100 m from the soil sampling sites. The soil columns were opened lengthwise. Subsamples were air dried until dry weight (dw) and kept frozen until their analysis.

Soil organisms were manually collected in accordance with abundance from BZ and S1. Soil macrofauna was not found in S2. Soil from the upper layer (0–10 cm) was transported to the laboratory and arthropods such as mites, collembolan and coleopteran larvae (mostly Family Scarabaeidae) were collected with the help of the Berlese funnel. From the 10–20 cm soil layer individuals of *Lumbricus* sp. in S1 and large larvae of the Scarabaeidae family in BZ, were sampled manually at the site and wrapped with aluminum. The samples were refrigerated and transported to the laboratory. Then, once the soil was removed, they were separated and freeze-stored until analysis was carried out.

Stream water samples from QGR were taken in triplicate using 1000-ml pre-cleaned glass bottles, lined with Teflon caps, at the three different sites (CA, S1 and S2). Suspended particle matter was obtained from each stream water sample by filtering through a 0.45-μm membrane filter under vacuum. Measurements of river water quality (temperature, pH, conductivity and dissolved oxygen) were performed with a Hanna 968i device (Port Louis, Mauritius).

Surface sediment samples were collected with cylindrical tubes of 10 × 4 cm in diameter at about 50 cm from the shore line. All samples were stored at −20 °C until analysis. Particle size distribution was determined according to Gee and Bauder (1986). Total organic carbon (OC) and soil pH were determined with an electrode with 1:2.5 soil–water value (Nelson and Sommers 1982).

Organochlorine pesticide (OCPs) analyses

Extraction procedure

The OCPs analyzed included: hexachlorocyclohexanes (α-, β-, γ-, and δ-HCH isomers), chlordanes (α- and γ-isomers), heptachlor and heptachlor epoxide (Hept epoxide), p,p'-dichlorodiphenyltrichloroethane (p,p'-DDT), 4,4′-dichlorodiphenyldichloroethylene (p,p'-DDE), 4,4′-dichlorodiphenyldichloroethane (p,p'-DDD), dieldrin and endosulfans (α-, β-isomers and endo sulfate). OCPs were extracted in accordance with Metcalfe and Metcalfe (1997), with modifications of Miglioranza et al. (2003). Soil (10 g), sediment (10 g) and biota samples (between 10 mg and 3 g) were ground with sodium sulfate until complete dryness and were Soxhlet extracted (8 h) with 100 ml of hexane–dichloromethane mixture (in relation 1:1). Dried filters with SPM were directly introduced in Soxhlet extractor. Stream water samples were liquid–liquid extracted according to Gonzalez et al. (2012). For a period of 2 h the liquid samples (1 l) were shaken with 600 ml of hexane–dichloromethane mixture. As an internal standard, 20 ng of PCB#103 was added to all samples. All extracts were evaporated under vacuum to 1 ml volume. Gel permeation chromatography Bio Beads S-X3 (200–400 mesh) (Bio-Rads Laboratory, Hercules, California) was

used for separating lipids from contaminants. An additional clean-up using silica gel was made for all samples. Then, the samples were freeze-stored in 1 ml vials until chromatographic analysis.

Sulfurs were eliminated from sediment extracts by the reaction with preactivated copper particles.

Gas chromatographic conditions

All compounds were identified and quantified using a gas chromatograph, Shimadzu 17-A Shimadzu Corp., Kyoto, Japan, equipped with a ^{63}Ni Electron Capture Detector (GC-ECD) and a capillary column coated with SPB-5 [(5 % phenyl)-methyl polysiloxane, 30 m × 0.25 mm i.d. × 0.25 μm film thickness; Supelco Inc, Bellefonte, USA. 1 μl was splitless injected at 275 °C. The ECD temperature was 290 °C. The oven temperature program was: start at 100 °C and held for 1 min, followed by an increase of 5 °C min^{-1} up to 150 °C, held for 1 min, then 1.5 °C min^{-1} up to 240 °C, and then 10 °C min^{-1} up to 300 °C for 10 min. Ultra-high purity helium was used as carrier gas (1.5 ml min^{-1}) and nitrogen as make-up gas (Miglioranza et al. 2003). The standard solutions used for identification and quantification of single compounds were pesticide mixture from Ultra Scientific, RI, USA and PCB #103 from Accustandard Absolute Standards, INC, CT, USA.

Quality control and assurance

Laboratory and instrumental blanks analyzed, indicated minimal contaminations or interference in samples during laboratory handling. Pesticide concentrations were adjusted in accordance with blank results. Recoveries, calculated from spiked matrixes, were greater than 90 %. Detection limits, according to Keith et al. (1983), ranged between 0.03 and 0.05 ng ml^{-1} for HCHs and between 0.08 and 0.33 ng ml^{-1} for the rest of compounds.

Statistical analysis

Results are expressed as the mean of three independent determinations. When no detectable levels were found in all the sample determination, limit of detection (LD) was used to calculate the mean and standard deviation. To compare contaminant levels between soils, groups and depth, one-way analysis of variance (ANOVA) was used when requirements were fulfilled. Otherwise, Kruskal–Wallis non-parametric test (KW) was used. Tukey's or Mann–Whitney's U (MW) test was used for pair comparisons. The Spearman (Spr) rank-correlation test was employed to evaluate correlations. The tests were set at a 95 % confidence level and α: 0.05.

Results and discussion

Soil samples

Physicochemical properties

The OC content was higher in BZ than in S1 and S2, particularly in the 0–5 cm soil layer (3.1 and 1.6 %, respectively), although it was not significant (KW: $p > 0.05$). All of them show a decreasing content of OC throughout the profile. BZ showed a higher clay content in the 20–25 cm soil layer (MW$_{BZ-S2}$: $p < 0.1$) than S2. The soil pH increased along the profile from 7.5 to 8.5 for BZ and from 8.1 to 8.6 for agricultural plots, although a significant difference was not observed for the 0–5 cm soil layer (ANOVA: $p > 0.05$) (Lupi et al. 2015).

Pesticide concentrations

Soils

The OCP concentrations ranged between 0.2 and 0.8 ng g^{-1} dw in the soil profile (Fig. 2). The higher concentrations were detected in the uppermost soil layer for the BZ, whereas S1 was detected in the 5–10 cm soil layer and for S2 in 20–30 cm soil layer, indicating a vertical transport process (a significant difference in total OCP concentrations was observed for 5–10 cm soil layer, ANOVA: $p < 0.05$, MW$_{BZ-S2}$ and MW$_{S1-S2}$: $p < 0.05$). A similar distribution pattern was observed for all the sites: endosulfans > DDTs > heptachlors ≅ γ-HCH > γ-chlordane. The low OCP concentrations found throughout the soil profile would indicate historical pesticide application, which is consistent with the legal restrictions of the country. Most of these compounds had already been banned for use and commercialization before 2012 with the exception of endosulfan product, which was forbidden a year after the sampling period. As a consequence of a recent application, endosulfans were the predominant group found in the profile. Moreover, p,p'-DDT, which is known for its high hydrophobicity and persistence was also present in all samples, despite the concentrations being lower than the endosulfans (Table 1). BZ showed a wider variety of compounds and slightly higher concentrations than S1 and S2, reflecting the historical usage of pesticides in the area. It was evident, that S1 and S2 lost soil structure due to agricultural activities (tillage and later direct seedling in comparison with BZ). As was observed in agricultural soils, an enrichment in OCP levels was also found in the uppermost layer of BZ concomitantly with higher OC content although Spr correlation was not observed (r 0.4483, $p > 0.05$). In reference to endosulfans, an
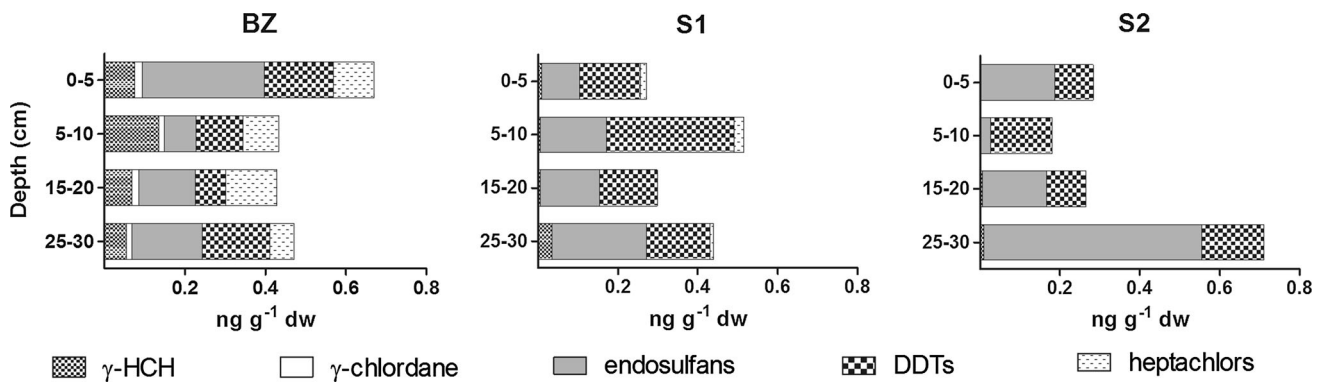
Fig. 2 OCPs (ng g^{-1} dw) in soil profiles from BZ, S1 and S2

increasing level of pesticide was found in layers from 5 cm in depth and onwards, although it was not of great significance (KW $p > 0.05$). The metabolite, endo sulfate, was the main compound found in S1 and S2 showing an increment in concentration throughout the soil profile due to the lixiviation process, enhanced by its higher solubility in relation to the isomers. BZ showed a similar behavior, in relation to endo sulfate, and also presented a decreasing α-endosulfan level throughout the profile. These results, could be a consequence of metabolism of α-endosulfan to endo sulfate. Additionally, the higher persistence of endo sulfate in relation to the α- and β-isomers leads to increasing concentrations of this pesticide after application.

Among DDTs group, *p,p′*-DDT was the principle compound found in the first three soil layers of S1 and S2, within enrichment from 5 to 10 cm, while *p,p′*-DDD was responsible for the increment in the 20–30 cm (S1-ANOVA $p < 0.05$). On the other hand, in BZ *p,p′*-DDE and *p,p′*-DDT were found mainly in the first two soil layers and enrichment was also found in the deepest one. Heptachlor and Hept epoxide were detected in some samples, but without a clear distribution pattern. However, in BZ, heptachlor was in a higher concentration in the surface layer diminishing along the profile, while Hept epoxide was only found in the deeper layers. This fact would be related to historical use of OCPs and probably a long time has elapsed since the last application. On the whole, metabolites were mainly found in deeper soil layers indicating capability of vertical transport. A recent study in an argentinean Patagonian Region, high DDTs levels in soils (1300 ng g^{-1} dw) were reported, mainly accumulated in the surface layers; although other agricultural soils showed high concentrations in the subsurface layer denoting lixiviation process (Miglioranza et al. 2013). In the present study, the median concentration of OCPs were much lower than other regions of the globe, such as in Australia (684 ng g^{-1}) (Weaver et al. 2012), Pakistan (50 ng g^{-1}) (Syed et al. 2013), India (800 ng g^{-1}) (Mishra et al. 2012), China (25 ng g^{-1}) (Yu et al. 2013).

Biota

Table 2 shows the concentrations of contaminants in different soil organisms collected in the BZ and S1. The OCP levels ranged from 9.5 to 219.8 ng g^{-1} wet weight (ww). The trend observed was endosulfans ≅ DDTs > γ-HCH ≅ Hept epoxide > γ-chlordane for the coleopteran larvae, whereas endosulfans ≅ DDTs ≅ Hept epoxide ≅ γ-HCH > γ-chlordane for earthworms. Furthermore, the total concentration pattern was M2 > L1 > M1 > L3 ≅ L2 ≅ L4 when comparing different organisms (Fig. 3). Thus, the individuals found in the 0–10 cm soil layer (M1, L1 and M2) presented higher concentrations than those found in deeper layers (L2, L3 and L4). A direct relationship was observed between OCP concentrations in organisms and soil layers, although the total concentrations in biota were one and three orders of magnitude higher than in soils, therefore showing the great capacity of these organisms to accumulate OCPs. Moreover, a similar distribution pattern of OCPs was found between biota and soils. These results indicate that these organisms are good biomonitors for the evaluation of soil contamination. Moreover, this role would be best developed by larvae, which have reduced mobility and spent long intervals (about 8 months) buried in the soil during the larval stage.

Larvae from different sites accumulated preferentially α- and β-endosulfan with the exception of L3 that accumulated 95 % of endo sulfate. This could be explained by the fact that the occurrence of endo sulfate in these organisms would be a consequence of direct incorporation from soils and/or metabolism inside them.

The metabolite hept epoxide, was the only compound detected of this pesticide group indicating a probable heptachlor metabolism performed by the organisms. However, a different behavior was observed by the DDT group, where a higher proportion of *p,p′*-DDT was found in organisms than in soils. Taking into consideration the physicochemical characteristics of *p,p′*-DDT and *p,p′*-

Table 1 Pesticide concentrations (ng g^{-1} dw) in the soil profiles from BZ, S1 and S2

Site	Depth (cm)	γ-HCH	γ-Chlordane	α-Endosulfan	β-Endosulfan	Endo sulfate	p,p'-DDT	p,p'-DDE	p,p'-DDD	Heptachlor	Hept epoxide	Total OCPs
BZ	0–5	0.08 ± 0.04	0.02 ± 0.01	0.08 ± 0.07	0.01 ± 0.002	0.22 ± 0.29	0.10 ± 0.02	0.02 ± 0.03	0.04 ± 0.06	0.10 ± 0.06	–	0.67
	5–10	0.13 ± 0.04	0.01 ± 0.01	0.06 ± 0.03	0.02 ± 0.01	–	0.09 ± 0.03	0.02 ± 0.03	–	0.09 ± 0.02	–	0.43
	15–20	0.07 ± 0.11	0.02 ± 0.02	0.03 ± 0.04		0.11 ± 0.17	0.04 ± 0.05	–	0.04 ± 0.05	0.05 ± 0.07	0.08 ± 0.12	0.43
	20–30	0.05 ± 0.07	0.01 ± 0.01	0.03 ± 0.03	–	0.14 ± 0.13	0.08 ± 0.07	0.05 ± 0.04	0.04 ± 0.06	0.04 ± 0.06	0.02 ± 0.01	0.47
S1	0–5	0.01 ± 0.001	–	–	–	0.10 ± 0.08	0.13 ± 0.04	–	0.02 ± 0.01	0.02 ± 0.01	–	0.27
	5–10	0.01 ± 0.002	–	–	–	0.17 ± 0.13	0.25 ± 0.08	–	0.07 ± 0.02	0.02 ± 0.02	–	0.52
	15–20	0.01 ± 0.002	–	–	–	0.15 ± 0.13	0.08 ± 0.08	–	0.07 ± 0.02	–	–	0.21
	20–30	0.02 ± 0.04	–	–	0.02 ± 0.02	0.22 ± 0.2	0.07 ± 0.10	–	0.09 ± 0.003	0.01 ± 0.0004	–	0.43
S2	0–5	–	–	–	–	0.19 ± 0.3	0.10 ± 0.07	–	–	–	–	0.28
	5–10	–	–	–	–	0.03 ± 0.03	0.15 ± 0.04	–	–	–	–	0.18
	15–20	0.01 ± 0.005	–	0.07 ± 0.10	–	0.09 ± 0.1	0.07 ± 0.08	–	0.07 ± 0.06	–	–	0.27
	20–30	0.01 ± 0.010	–	–	0.02 ± 0.01	0.53 ± 0.6	–	–	0.16 ± 0.08	–	–	0.71

DDE, it would be expected that higher p,p'-DDE levels were to be found in organisms because of intrinsic metabolism of p,p'-DDT or direct p,p'-DDE uptake. However, the highest levels were found for p,p'-DDT. A different behavior was observed in soil invertebrates collected in rice fields from Spain. There, the coleopteran *Hydrous pistaceus* and *Helochares lividus* showed higher levels of p,p'-DDE (12–210 ng g^{-1} dw) and p,p'-DDD (1.6–26 ng g^{-1} dw) than p,p'-DDT (Pastor et al. 2004). In Argentina this pesticide has been banned many years ago, and the DDTs residues in soils would be highly adsorbed and less available; however, the use of Dicofol (acaricide) represents a fresh input of p,p'-DDT into the environment (Yang et al. 2008; Turgut et al. 2009). This acaricide has been widely used in the area; therefore, fresh p,p'-DDT (no aging) would be more available for uptake by the organisms. Kelsey and Alexander (1997) also found a higher uptake of atrazine and phenanthrene by earthworm *E. foetida* when aging was avoided.

Studies carried out in a terrestrial ecosystem undergoing intensive agricultural practices, have denoted the bioaccumulation of OCPs in earthworms (200 ng g^{-1}) and Family Scarabaeidae of coleopteran, (150 ng g^{-1}) showing a different accumulation pattern according to agricultural land use (Miglioranza et al. 1999).

The principle diet of scarab larva is graminaceous roots, although it also incorporates soil particles. In the root–soil interface, radicular exudates enhance bioavailability of contaminants leading to a higher concentration in these organisms and a possible intensification of the bioaccumulation process. A high proportion of metabolites are observed in the organisms, highlighting the capacity of biota to metabolize these compounds.

The assessment of OCPs in organisms leads to understanding the process involved in pesticide transfer throughout the food chain, and also the availability of these compounds in the abiotic matrices where the organisms live. In nature, larvae, earthworms and mites constitute a link in the transport of environmental pollutants from soil to organisms, higher up in the terrestrial food web (Rodriguez-Campos et al. 2014).

Earthworms accumulate lipophilic substances through the epidermis and the intestine. The transference of these compounds, from the soil to the worm via the epidermis, involve two processes: from soil to pore water and from pore water to the earthworm (Šmídová and Hofman 2014). Therefore, the knowledge concerning environmental pollutants uptake in soil biota is very important.
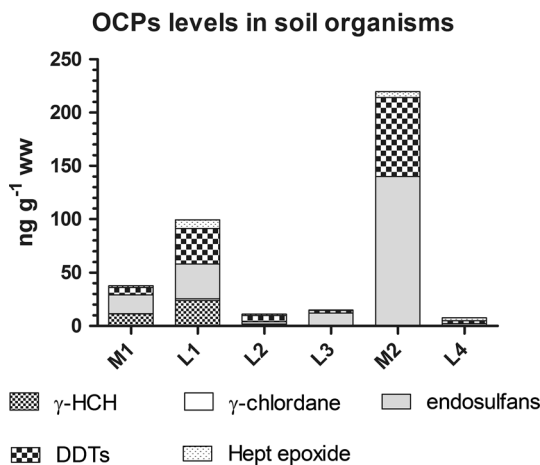
Table 3 shows the burden of OCPs in larvae from BZ. It can be observed that a bioaccumulation process is found for all compounds, which is more evident when L2 and L3 are compared as in the case of some pesticides such as β-endosulfan, γ-chlordane and p,p'-DDT. However, it is

Table 2 Pesticide concentrations (ng g^{-1} ww) in soil organisms from BZ and S1

Contaminant	BZ				S1	
	Mesofauna	Larvae (Scarabaeidae family)			Mesofauna	*Lumbricus* sp.
	M1	L1 (small)	L2 (medium)	L3 (large)	M2	L4
γ-HCH	11.40 ± 2.1	23.91	1.61 ± 1.0	0.52 ± 0.3	–	1.96 ± 0.1
γ-Chlordane	0.22 ± 0.04	1.70	0.19 ± 0.04	0.09 ± 0.01	–	0.08 ± 0.01
α-Endosulfan	8.88 ± 0.7	27.86	2.01 ± 1.4	0.52 ± 0.09	112.6	1.25 ± 1.2
β-Endosulfan	5.44 ± 0.4	4.69	0.24 ± 0.3	0.30 ± 0.03	27.4	0.87 ± 0.8
Endo sulfate	3.39 ± 0.5	–	–	10.79 ± 1.9	–	–
p,p′-DDT	5.27 ± 1.6	16.04	4.81 ± 1.4	2.20 ± 0.03	61.9	2.61 ± 2.2
p,p′-DDE	1.50 ± 0.4	17.10	1.03 ± 0.9	0.35 ± 0.1	12.3	–
Hept epoxide	1.63 ± 0.6	8.24	1.28 ± 1.3	0.33 ± 0.3	5.5	2.70 ± 3.4
Total OCPs	37.7	99.6	11.2	15.1	219.8	9.5

M1 = constituted by 50 mites (mainly oribatids) + 2 collembolan + 32 larvae (≈2.5 mm length)

M2 = constituted by 130 mites (mainly oribatids) + 12 collembolan + 3 larvae (≈2.5 mm length)

Fig. 3 OCPs (ng g^{-1} ww) in soil organisms from BZ (M1, L1, L2 and L3) and S1 (M2 and L4)

Table 3 Pesticide burden (ng pesticide) in larvae from BZ

	Larvae (Scarabaeidae family)		
	L1 (small)	L2 (medium)	L3 (large)
Individual mass (mg)	28	501	3823
Lipid (%)	2	2.9 ± 2.2	2.3 ± 0.004
γ-HCH	0.67	0.81 ± 0.49	1.97 ± 1.07
γ-Chlordane	0.05	0.10 ± 0.02	0.34 ± 0.03
α-Endosulfan	0.78	1.01 ± 0.72	1.99 ± 0.36
β-Endosulfan	0.13	0.13 ± 0.16	1.13 ± 0.12
Endo sulfate	–	–	41.23 ± 7.23
p,p′-DDT	0.45	2.41 ± 0.71	8.42 ± 0.13
p,p′-DDE	0.48	0.52 ± 0.47	1.35 ± 0.34
Hept epoxide	0.23	0.64 ± 0.68	1.26 ± 0.99
Total OCPs	2.80	5.61	57.70

important to mention the marked bioaccumulation observed for *pp′*-DDT in L2 regarding L1. Particularly, the total DDTs concentrations in larva from *H. pistaceus* and *H. lividus*, were higher than the imago of the same species (Pastor et al. 2004). As was indicated for the soils behavior, the use of Dicofol, lead to a high *p,p′*-DDT availability and consequently, more incorporation by organisms.

The burden of each pesticide, for the different larva individual body weight was plotted (Fig. 4). A lineal relationship was observed independently from the contaminant ($R^2 \geq 0.916$ in all cases). Analogously, the uptake rates observed for all contaminants had a mean value of 0.247 ng g^{-1} ww (range 0.075–0.346 ng g^{-1} ww) with the exception of *p,p′*-DDT (1.03 ng g^{-1} ww). The plot of uptake rate versus Log k_{ow} (octanol–water partition coefficient) (Fig. 5), indicates that when lipophilicity is increased, the uptake rate lowers (Spr −0.8857, $p < 0.05$).

The final adjusted values of k_{ow} were taken from Shen and Wania (2005).

In particular, the anomalous *p,p′*-DDT behavior can be explained by taking into consideration the fact that this compound was present at a higher concentration in the soil in relation to the other pesticides and this fact was probably due to a fresh input, as afore mentioned (Dicofol). For air-breathing organisms, a very important parameter is the k_{oa} (octanol–air partition coefficient) which is used to evaluate biota-soil accumulation process. Therefore, although a suitable k_{ow} is needed for bioconcentration (log $k_{ow} > 4$), compounds with log $k_{oa} < 5.25$, are not expected to have a soil-bioconcentration factor higher than 1, due to the high rate of respiratory elimination that prevents bioconcentration (Armitage and Gobas 2007). Therefore, the high accumulation rate found for *p,p′*-DDT, being one of the
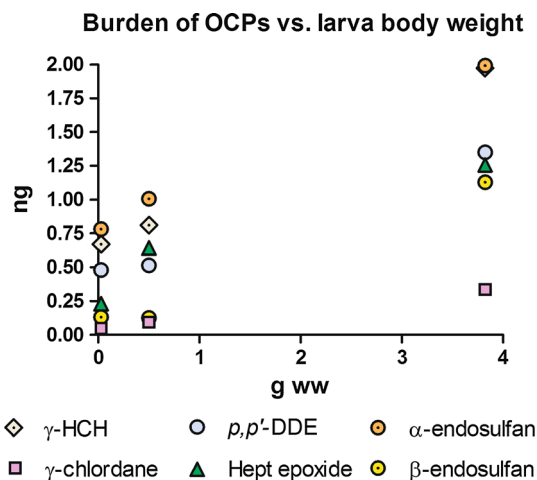
Fig. 4 Burden of pesticides vs. individual body wet weight (mean contaminant values)
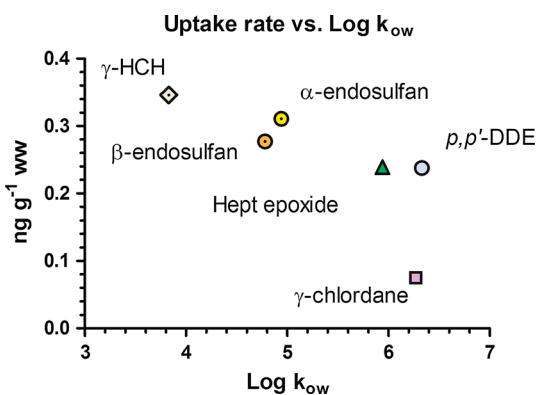
Fig. 5 Uptake rate vs. log k_{ow}

most abundant pesticides in the soil matrix, due to the fact that it is the compound with the highest k_{oa} (9.73).

Stream water

Stream water showed pesticide levels in a range of between 3 and 7 ng l^{-1} with a predominance of endosulfans (Table 4). The higher levels were found at the CA sampling site. CA was located 2.5 km upstream from S1 and S2 in order to evaluate the burden of pesticides reaching both agricultural sampling sites. Results evidence that it was also affected by agricultural pollution resulting in higher pesticide levels than S1 and S2. The ratio α/β endosulfan (7:3) usually found in technical mixture and the relation metabolite/parental could be used to understand the time elapsed after application (Weber et al. 2010). The observed metabolite/parental ratio >1 indicates a non-recent pesticide application. Furthermore, a relation α/β isomers between 2.5 and 4.5 was observed, showing an enrichment in α-isomer. This fact could be due to an

Table 4 Pesticide concentrations (ng L^{-1} and ng g^{-1} dw) in stream water, particle matter (SPM) and surface sediment (SS) of control area (CA) and in the QGR adjacent to the agricultural plots (S1 and S2)

Compounds	Stream water			SPM			SS		
	CA	S1	S2	CA	S1	S2	CA	S1	S2
γ-HCH	0.60 ± 0.28	–	0.03 ± 0.03	–	42.83 ± 74.19	26.30 ± 18.50	0.16 ± 0.09	0.19 ± 0.01	0.010 ± 0.01
γ-Chlordane	–	0.19 ± 0.08	0.03 ± 0.02	10.41 ± 1.79	25.95 ± 22.56	11.56 ± 9.44	0.013 ± 0.013	0.010 ± 0.003	0.010 ± 0.003
α-Endosulfan	2.34 ± 0.17	1.78 ± 2.07	0.61 ± 0.12	88.24 ± 7.18	134.06 ± 52.07	111.26 ± 80.47	0.15 ± 0.03	0.22 ± 0.04	0.269 ± 0.32
β-Endosulfan	0.57 ± 0.14	0.40 ± 0.38	0.25 ± 0.14	–	–	–	0.08 ± 0.05	0.02 ± 0.01	0.017 ± 0.01
Endo sulfate	3.43 ± 0.87	3.24 ± 2.25	1.71 ± 0.36	71.45 ± 940	18.68 ± 32.35	12.71 ± 22.01	0.23 ± 0.20	0.03 ± 0.04	0.14 ± 0.07
p,p'-DDT	–	–	–	90.03 ± 7.19	283.15 ± 119.16	228.20 ± 190.41	0.08 ± 0.09	0.16 ± 0.12	0.01 ± 0.01
p,p'-DDE	–	–	–	–	–	–	0.038 ± 0.001	0.009 ± 0.001	0.02 ± 0.01
Heptachlor	0.07 ± 0.09	–	–	45.08 ± 10.53	155.38 ± 65.36	104.06 ± 75.64	0.016 ± 0.007	0.008 ± 0.001	0.161 ± 0.15
Hept epoxide	–	0.16 ± 0.15	–	–	64.33 ± 101.32	–	0.26 ± 0.39	–	–
Dieldrin	–	–	–	–	–	–	–	–	0.03 ± 0.03
Endrin	–	–	–	–	–	–	0.08 ± 0.13	0.12 ± 0.10	–
Total OCPs	7.01	5.99	2.70	305.21	724.38	494.12	1.12	0.77	0.667

irreversible isomerization of β to α-isomer as a consequence of a long time elapsed from technical endosulfan application (Schmidt et al. 2014). The National Argentinean Water Board (Spanish acronym, INA) has established a value of 7 ng l^{-1} for the sum of α- and β-isomers. Endosulfan levels in the CA sampling site were close to that limit, but in the case of CA, the total endosulfan levels were the result of the sum of isomers and metabolites. Although the endo sulfate is recognized to be more persistent than its parent compound it is not included in water quality guidelines. Particularly, in this work, endo sulfate accounted for up to 66 % of endosulfans found in stream water samples. Thus, endosulfans have reached levels in stream water that were close to the limit that represents a risk to aquatic biota.

Levels of γ-HCH were also found at the CA site, although it represents only 10 % of the OCPs. Gonzalez et al. (2013), reported 10 and 5 ng l^{-1} of OCPs in July from 2007 to 2009, respectively, with a tendency of diminishing pesticide levels over the years. However, in this study, the levels found in stream water slightly surpassed that previously reported by Gonzalez et al. (2013), indicating that, in spite of these pesticide groups having been almost all banned in Argentina, they are present in the watershed at levels that can exert a sustainable influence on the aquatic environment.

The differences of pesticide levels observed between both sampling agricultural areas may be explained by the fact that a stream outflows nearby S2 and, consequently, dilution may have occurred.

Trace levels of chlordanes and heptachlors were observed in S1 indicating a non-recent use of these pesticides in the area.

Suspended particle matter

The higher OCP levels were found at S1, where soils are dedicated to agricultural activities and during that period all soils were without vegetable cover, enhancing soil erosion and consequently higher SPM reaching the stream water (Table 4). Moreover, the resuspension of sediments from S1, rich in clay content, (Lupi et al. 2015), will lead to an enhancement of pesticide levels in the water column. A similar behavior was found in S2, although the OCP levels were lower. Pesticide concentrations in stream water showed a similar pattern in relation to total OCP levels in both sampling sites. In reference to pesticide groups, a predominance of DDTs was observed in both sites followed by heptachlors and endosulfans. This fact could be related to the association of these highly hydrophobic compounds with suspended particles. A direct relationship was observed between stream water, soils and SPM, in relation to a distribution pattern of

endosulfans. Thus a predominance of α-endosulfan was found in S1 and S2, while in CA the pesticide levels were similar to those of the sum of both α- and β-isomers and endo sulfate. It is known, that the erosion process leads to an enhancement of pesticide levels in the aquatic environment. Therefore, our results support this statement. A point of concern is the very high levels of pesticides in SPM. Considering a load of 32 mg of SPM per liter of stream water, estimation could be performed in order to approximate the load of OCPs that potentially reach downstream and finally hit the coast. Therefore, the QGR with a mean flow rate of 12 m^3 s^{-1}, a load of 190 µg s^{-1} of OCPs is found downstream from the study area. This fact represents 16.5 g daily of OCPs that are transported to the coast. Therefore, it is very important to highlight that pesticides can reach lotic surface waters, which are capable of transporting high amounts of contaminants downstream and potentially cause an impact on the aquatic environment.

Surface sediments

OCP levels in SS were low (0.65–1.10 ng g^{-1} dw). CA showed higher pesticide concentrations than S1 and S2. The presence of pesticides in CA would be the result of runoff and/or atmospheric transport from other areas farther from the SS sampling site. A predominance of endo sulfate was found in CA, while in S1 and S2 the ratios isomers/metabolite were >1.

Moreover, the 20 % lower OC content and a higher temperature of water column in CA (Lupi et al. 2015) could have favored the biodisponibilization of contaminants and eventually microbial degradation. The prevalence of metabolites in CA, was also found for other compounds such as, p,p'-DDE and hept epoxide. The higher levels of p,p'-DDT were found in S1, coinciding with the values found in soils. Therefore, despite the ban on this pesticide, it is currently present in the area. As was mentioned in relation to its occurrence in soils, the use of Dicofol as secondary source of p,p'-DDT is highlighted in the sediments.

Conclusions

Current levels of OCPs in environmental matrices, such as soil profiles from an agricultural area, terrestrial biota involving coleopteran larvae, collembolan, earthworms and mites; SS, stream water and SPM were studied in the QGR watershed, during the pre-application pesticide period.

Despite their forbidden use, OCPs were found in all matrices as a consequence of their persistence and recalcitrance within the environment.

A direct relationship regarding pesticide distribution pattern was observed among soils from BZ, S1 and S2; stream water and SPM mainly related to endosulfan group which was the only pesticide being used regularly. The predominance of p,p'-DDT in SPM and in some surface soils is probably due to a higher availability of this pesticide as a consequence of a fresh application or due to its presence as an impurity in Dicofol acaricide, widely used in the region. The increasing concentration of metabolites throughout the soil profile, mainly endo sulfate, indicates vertical transport of these contaminants; therefore, a subject of great concern regarding groundwater contamination. On the other hand, a load of 7.3 kg of OCPs are annually discharged by the QGR, highlighting the role of lotic surface water as transfers of contaminants and their subsequential impact on the environment. Terrestrial biota accumulated OCPs in their tissues. Particularly the smaller arthropods and larvae (M1, L1 and M2) from BZ and S1, were the organisms that accumulated the highest OCP levels. These organisms live in the uppermost layer of soil (0–10 cm) where the highest OCP concentrations in soil were detected. Similar OCP distribution patterns were observed between organisms and soil. A direct relationship between OCP accumulation and larvae size was observed.

Results suggest the importance of using terrestrial biota for studying recalcitrant pesticides, and a continual monitoring is strongly recommended as a first preventive measure to minimize environmental risks.

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