PHTHALATES UPTAKE BY TOMATOES AFTER BIOSOLIDS APPLICATION: WORST CASE AND OPERATIONAL PRACTICE IN GREENHOUSE CONDITIONS

Caroline Sablayrolles[1,2,4,*], Jerome Silvestre[3], Claire Lhoutellier[4] and Mireille Montrejaud-Vignoles[1,2]

[1] Université de Toulouse; INP; LCA (Laboratoire de Chimie Agro-Industrielle); ENSIACET, 4 Allées Emile Monso, F-31029 Toulouse, France.
[2] INRA; LCA (Laboratoire de Chimie Agro-Industrielle); F-31029 Toulouse
[3] Laboratoire d'Ecologie Fonctionnelle, ENSAT, Avenue de l'Agrobiopole - BP 32607 Auzeville tolosane - 31326 Castanet-Tolosan, France.
[4] Veolia Environnement Recherche et Innovation. 291 avenue Dreyfous Ducas, 78520 Limay, France.

ABSTRACT

Application of sewage sludge to agricultural land may be beneficial because it can improve the physical, chemical and biological properties of soils but it may also introduce organic pollutants in soils which could have adverse effects on wildlife and human health if these compounds enter food chain. The present study aims at evaluating the potential transfer of phthalates from biosolids to tomato plants (*Lycopersicon esculentum*) in a greenhouse experiment. Four phthalates were studied simultaneously: dimethylphthalate, diethylphthalate, dibutylphthalate and di(ethyl)hexylphthalate. Two types of experiments were carried out: aquiculture (hydroponic conditions) and soil culture. Aquiculture experiments involved (1) addition of phthalates as pure substances to the plant growth solution and (2) addition of filtrate from wastewater treatment plant biosolids to the plant growth solution. Soil experiments were carried out testing biosolid from three different origins and treatments (dried biosolids from municipal wastewater treatment plant, biosolids from municipal wastewater treatment plant composted with green wastes and dehydrated biosolids from industrial wastewater treatment plant) with application rate at 30 $t.ha^{-1}$. Phthalates were quantified by high resolution gas chromatography coupled with a low resolution mass spectrometer in single ion monitoring mode into roots, sap, leaves and fruits. The results clearly show a difference in behaviour of phthalates according to the part of the plant and kind of experiment. Two transfer pathways were identified: (1) uptake by roots and translocation (2) foliar uptake of vapour from surrounding air. The concentration of phthalates varied from non quantifiable to 174 $mg.kg^{-1}$dry matter in roots, from non quantifiable to 24 $mg.kg^{-1}$dry matter in leaves and from non quantifiable to 6.5 $mg.kg^{-1}$dry matter in fruits. Di(ethyl)hexylphthalate concentration in tomato plant was positively correlated with levels in the biosolids. Compared to the control, application of biosolids resulted in increases concentration of phthalate in plant. However, transfer percentage of di(ethyl)hexylphtalate in fruits were less than 1% even in an experiment designed to maximize transfer.

KEYWORDS: Endocrine disrupting chemicals, Transfer, Plant, Sewage sludge, Di(ethy)hexylphthalate

1 INTRODUCTION

Application of sewage sludge to agricultural land may be beneficial because it can improve the physical, chemical and biological properties of soils which may enhance crop growth [1]. Indeed, sludge application enables to recycle nutrients and to restituate organic matter to soils in order to prevent over-exploitation of agriculture. In addition the use of sludge as a fertilizer would decrease the amounts of chemical fertilizers needed in agriculture and supply micro-nutrients which are not commonly restored in routine agricultural practice. While it encourages the use of sewage sludge, the EU Directive 86/278/EEC regulates its use to prevent harm to the environment. Limit values for concentrations of organic compounds in sludge were suggested in the third draft of the "Working paper on sludge" [2].

Each year, millions of tons of phthalates esters are produced in the world for the manufacture of a wide variety of common consumer goods. Their increasing presence in the environment has prompted several countries to investigate population exposure. Phthalates are esters of phthalic acid. Although a large number of phthalates exist, only a few are commonly used and will be considered for this study (Table 1). Due to human activities, they are present in the environment in quite large quantities, since they are a group of chemicals which has been used for about the last 50 years as plasticisers agents, mainly to make polyvinyl chloride supple and flexible [3]. They are also commonly used as antifoaming agent in paper production, as an emulsifier for cosmetics, in perfumes and pesticides. In view of this widespread use, phthalates have been the subject of intensive research concerning effects on health

* Corresponding author

and the environment. These substances give great cause for concern because they bio-accumulate (accumulate in living tissues and in the food chain) and they are potentially toxic. The latter can be short-term effects (allergies, asthma, etc.) or longer term (nervous and endocrine disrupter effects, development and fertility disrupter effects carcinogenic effects) [4].

Europe, by actively pursuing a policy favouring wastewater collection and treatment, has ensured the production of clean water but also increased the biosolids production. Nowadays, 40% of biosolids are recycled biologically via land application. Phthalates are found regularly in municipal wastewater and, because of lipophilic properties, they concentrate in sewage sludge [5]. Land application limit value of 100 $mg.kg^{-1}$ dry matter for di(2-ethylhexyl)phthalate (DEHP) was envisaged by European legislation [2]. They would appear to be different potential sources for phthalates in biosolids: compounds produced and/or used industrially and abnormally present in effluents, or compounds from plastics in manufactured goods released back into wastewater, or phthalates atmospheric deposition on urban surface released by precipitation and domestic use of products containing phthalates. Phthalate levels in sludge residues varied according to wastewater treatment plant and compounds due to their different physico-chemical properties [6]. A review by Alcock *et al*. [7] stated DEHP were detected in almost all samples of sewage sludge analysed and that DEHP readily accumulates in suspended particulate material. DEHP is present in quite high concentrations in biosolids (between 4 and 170 $mg.kg^{-1}$ dry matter) [8, 9]. Sludge treatments as aerobic composting process, anaerobic process [10] and thermic process [11] have shown to reduce phthalates concentrations in sludge. Anaerobic processes were more controversial as some authors did not see any degradation [12, 13]. Soil microorganisms breakdown phthalates under aerobic conditions or the chemicals are removed by volatilisation, so they have a half-live of 50 days.

Most of publications report fate, mobility and degradation of phthalates in soil [14] or in sludge amended soil [15]. Toxic effects of phthalates on crop and vegetable growth had also been studied [16]. Only a minority of the reports deal with plant uptake: carrots [17, 18], lettuce [19], barley [20, 21], radish [22]. In order to increase the knowledge on this topic, this work was carried out to evaluate potential transfer into plants of phthalates. We used such an approach successfully in the case of laurylalkylbenzene sulfonates [23] and polychlorinated biphenyls [24]. This experiment was carried out by separately (1) adding phthalates pure substances to growth nutrient solution, (2) adding biosolids filtrate to growth nutrient solution and (3) adding three types of biosolids to agricultural soil. Tomato plants (*Lycopersicum esculentum* cv) were grown in aquiculture (1, 2) to provide optimal transfer conditions [25] and in soil culture (3) to provide real experimental results, in plant containers inside a temperature and humidity regulated greenhouse.

2 MATERIALS AND METHODS

2.1. Materials

2.1.1 Plant

The experiment was conducted on tomato plants (*Lycopersicon esculentum cv*.), Rondello variety (de Ruiter seeds). This particular variety is a hybrid often used for experiments because of its high rate of germination (99%) and genetic homogeneity.

2.1.2 Containers

The containers used were 10 liters galvanised buckets to avoid problems associated with polyvinyl chloride whose plastifying agents are phthalates.

2.1.3 Aquiculture

Tomato plants were grown hydroponically on aerated, non-circulating nutrient solution. This was prepared using pure salts and deionised water: macronutrients concentrations (7 $mmol.L^{-1}$ of K^+, 5 $mmol.L^{-1}$ of Ca^{2+}, 1.5 $mmol.L^{-1}$ of Mg^{2+}, 15 $mmol.L^{-1}$ of NO_3^-, 2 $mmol.L^{-1}$ of $H_2PO_4^-$, 1,5 $mmol.L^{-1}$ of SO_4^{2-}) and micronutrients concentrations (15 $mg.L^{-1}$ of Fe, 0.49 $mg.L^{-1}$ of Mn, 0.06 $mg.L^{-1}$ of Cu, 0.11 $mg.L^{-1}$ of Zn, 0.26 $mg.L^{-1}$ of B, 0.01 $mg.L^{-1}$ of Mo). Macronutrients amounts were calculated according to the mineral needs of the plants for the duration of the experiment. The nutrient solution was replaced twice a week. Its conductivity was 2 $mS.cm^{-1}$ and the pH varied from 5.2 for fresh solution to 6.5 for spent solution.

2.1.4 Soil culture

Tomato plants were cultivated on an argilo-calcareous soil coming from an experimental station about five kilometres in the South of Toulouse (GPS Latitude 43.536° Longitude 1.498°) (Haute-Garonne, France). Soil was collected in the 0-25 cm layer of the field what corresponds to the plough layer. The soil was air-dried and sieved at 5 mm diameter to separate the fine earth which will be of use exclusively for the filling of the pot. 10 kg of soil was put in containers. The soil density was 1.5 and the soil pH was 7.6. The water capacity in pot of the soil is around 24%. Pots were watered in 2/3 of the capacity in pot to allow the oxygenation of roots. No phthalate contamination of the soil was noticed: concentrations were less than limit of quantification (10 $\mu g.kg^{-1}$ dry matter).

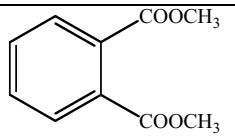
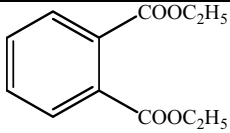
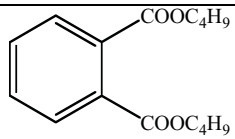
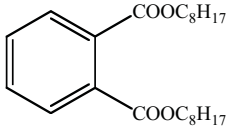
2.1.5 By-products materials

Phthaltes in pure form were introduced for the pure substance experiment: dimethylphthalate (DMP), diethylphthalate (DEP), dibutylphthalate (DBP) and di-2-ethylhexyl phthalate (DEHP) from Cluzeau Info Labo (France) (Table 1).

Biosolid A was obtained from a municipal wastewater treatment plant equipped with a separated sewer system. It was derived from an aeration tank and

dewatered by centrifugation prior to a thermal drying process. The

TABLE 1 - Physico-chemical properties and formulae of the four phthalates studied

Name	Abbreviation	Molecular weight ($g.mol^{-1}$)	Solubility ($mg.L^{-1}$ at 25°C)	Octanol-water coefficient (log Kow at 25°C)	Vapour pressure (mm Hg)	
Dimethylphthalate	DMP	194.2	4 000	1,56	$2.0\ 10^{-3}$	
Diethylphthalate	DEP	222.2	1 100	2,48	$1.0\ 10^{-3}$	
Dibutylphthalate	DBP	278.4	11	4,72	$2.7\ 10^{-5}$	
Di-(2-ethylhexyl) phthalate	DEHP	390.6	0,3	5,11	$1.0\ 10^{-7}$	

sample was collected in granular form (93% dry matter) after the drier and consisted in 10 sampling of 500 g homogenised and mixed according to the norm NF EN 12579 [26]. Biosolid B was sludge compost obtained from a composting facility processing 54% of biosolids A mixed with 23% of crushed green waste and 23% of riddling refusal in mass. The sampling of compost was accomplished following norm NF EN 12579 [26]. The acquired compost answers the French norm NF U 44-095 [27]. Biosolids C was obtained from a municipal and industrial wastewater treatment plant equipped with a semi-separative sewer system. It was derived from dewatering system prior to an anaerobic digestion and centrifugation. The sample was collected in "pâteuse" form (30% dry matter) after centrifugation and consists in 10 sampling of 500 g homogenised and mixed according to the norm NF EN 12579 [26].

2.2 Experimental set-up

48 plants were used in the experiment and Table 2 shows the plant containers that were set up. A total of 16 plants were used in the aquiculture experiment with pure substances: (1) 8 control plants were grown in a nutrient solution (2) 8 plants were grown in the nutrient solution with addition of phthalates. For the aquiculture experiment with biosolids filtrate 16 plants were used: (1) 8 controls (2) 8 growing in the biosolids A filtrate. Experimentation was also carried out in soil culture with biosolids A, B and C with 48 plants: (1) 24 controls (2) 24 growing in the biosolids-soil mixture. The pots were arranged randomly on the bench.

According to the French ministerial order of 8 January 1998, the maximum quantity authorised for land application of biosolids is 30 tonnes dry matter per ha per 10 years. For this experiment, in order to obtain clear-cut results, this quantity was applied in a single dose to the plant containers. Concerning the pure substance experiment, the dose introduced into the container's initial solution was thus 84 mg of each individual phthalate which corresponds to the maximum authorized. For the filtrate experiment, 105 g of ground-up biosolids A granules were mixed into 1 L of demineralised water. This mixture was stirred for 24 hours in a 6 L glass beaker placed on a horizontal, rotary mechanical stirrer, and then filtered on a screening column down to 32 µm in order to recover the biosolids filtrate. Phthaltes levels in the biosolids filtrate were determined: DEP < 40 $\mu g.L^{-1}$, DMP < 40 $\mu g.L^{-1}$, DBP < 40 $\mu g.L^{-1}$, DEHP=2680 $\mu g.L^{-1}$. Not all the trace or-ganic compounds present in the biosolids were found in the filtrate. However, the fractions that were present in the filtrate were also those which were most available for transfer into the plant. In the soil experiment, 110 g dry matter of biosolids was mixed with 10 kg of soil. Phthalates level in soil was lower than the limit of quantification. Levels in biosolids A were determined: DEP < 10 $\mu g.kg^{-1}$ DM, DMP < 10 $\mu g.kg^{-1}$ DM, DBP < 10 $\mu g.kg^{-1}$ DM, DEHP = 116 $mg.kg^{-1}$ DM. Levels in biosolids B were determined: DEP < 10 $\mu g.kg^{-1}$ DM, DMP < 10 $\mu g.kg^{-1}$ DM, DBP < 10 $\mu g.kg^{-1}$ DM, DEHP = 6.5 $mg.kg^{-1}$ DM. Levels in biosolids C were determined: DEP < 10 $\mu g.kg^{-1}$ DM, DMP < 10 $\mu g.kg^{-1}$ DM, DBP < 10 $\mu g.kg^{-1}$ DM, DEHP = 132 $mg.kg^{-1}$ DM.

TABLE 2 - Summary to show the various containers set up.

	Dose	Mass of DEHP in pots (mg)	Number of pots
Pure substance experiment			
Controls : tomato plant + nutrient solution	-	0	8
Tomato plant + nutrient solution + phthalates	equivalent to 30 t DM/ha	84	8
Biosolids A filtrate experiment			
Controls : tomato plant + nutrient solution	-	0	8
Tomato plant + nutrient solution + biosolids A filtrate	equivalent to 30 t DM/ha	22	8
Biosolids A experiment			
Controls : tomato plant + soil	-	0	8
Tomato plant + soil + biosolids A	30 t DM/ha	1.16	8
Biosolids B experiment			
Controls : tomato plant + soil	-	0	8
Tomato plant + soil + biosolids B	**30 t DM/ha**	0.65	8
Biosolids C experiment			
Controls : tomato plant + soil	-	0	8
Tomato plant + soil + biosolids C	**30 t DM/ha**	1.32	8

2.3. Cultivation technique

About 20 tomato seeds were germinated on pieces of polystyrene, covered with thick absorbent paper dipping into a plastic tray such that the seeds are in contact with the water in the tray bottom. The tray is then placed in a dark germination cupboard for 3 days. The germinating seeds in the tray are then put into a phytotron for 2 days. This is an enclosure lit by sodium lamps where there are controlled conditions of light, humidity and temperature: 14 hours light per 24 hours, 50% humidity in the air and a temperature of 24 ± 1°C by day and 18 ± 1°C by night. The plants then follow their development in a greenhouse where conditions are controlled as a function of the external temperature and light. Blinds, ventilation and lighting ensure optimum conditions (average temperature 24°C, 14 hours light).

2.3.1 Aquiculture

The 10 cm long seedlings are transferred into troughs containing 20 L of nutrient solution. They are wrapped in cotton wool and inserted into special holes in the trough covers, with just the root dipping into the solution. A bubbler is put into the solution to oxygenate it, with an on/off cycle of 6 minutes and 12 minutes respectively. Once the plants have attained a height of 30 cm, they are transplanted individually into the galvanized containers holding 8 L of nutrient solution, each oxygenated with an individual bubbler. This solution is topped up with demineralised water to compensate for losses through transpiration and evaporation. Once the solution conductivity was fall below half that of its original value, it is renewed. The pure substances or biosolids filtrate were introduced into these containers after 50 and 90 days growth respectively. Ten days after the introduction of the pure substances or the biosolids filtrates, the plants were sampled in order to study the fruits, the leaves and the roots.

2.3.2 Soil culture

A first step consists in incubation period in order to mimic the biosolids land application before winter. Soil-biosolid mixtures were added with water in order to obtain 2/3 of water holding capacity during 17 days. Then, the 10 cm long seedlings are transferred into special holes in soil-biosolid mixtures. Transparent plastic films were placed during 15 days on each container in order to maintain tomato seedling with water saturated atmosphere. Fruits, leaves and roots were collected at 90 days after sowing.

,

2.4 Analytical procedure

All samples were carefully washed and put in aluminium foil trays into a freezer at –25°C until analyses. The analytical method is explained briefly in this paper and more details on the method development and optimisation can be found in Sablayrolles et al. [28].

The solid/liquid extraction was carried out with a Soxtec System HT2 (Tecator, France). About 2 g of lyophilised sample was extracted with 100 mL of *n*-hexane (Suprasolv, VWR Merck) for 45 minutes (30 minutes in boiling mode and 15 minutes in rinsing mode). The internal extraction standard, benzylbenzoate (1 mL at 5000 mg/L in *n*-hexane) (Cluzeau, France), was added to extraction cartridge just before extraction. Then, a rotary evaporator (Rotavapor, Büchi) and 30°C temperature controlled bath is used to concentrate the solvent down to 10 mL. Concentration of the *n*-hexane extract to 1 mL before purification is by a stream of nitrogen. Purification step was carried out with a 6 g florisil SPE cartridge (Supelco, France) rinsed with 10 mL of *n*-hexane. The 1 mL *n*-hexane extract is placed at the top of the cartridge, and a first elution with a 1-2 drops/second flow rate is carried out with 8 mL of *n*-hexane. This fraction is collected and put aside. A second elution with 5 mL of *n*-hexane/acetone (90/10, v/v) allows 100% of phthaltes compounds to be recovered. This fraction is concentrated down to 1 mL under nitrogen. A high resolution gas phase chromatograph coupled to a low resolution mass spectrometer (HRGC-LRMS) (Finnigan Trace 2000 Series) on electron impact mode with a quadruple type analyzer was used. The chromatograph is fitted with a Restek RTX-5MS capillary column (5%

diphenyl ; 95%dimethylpolysiloxane ; 30 m x 0.25 mm x 0.25 µm). A helium Alpha 2 (Air Liquide) gas vector is used, flow rate 1.2 mL/min. A 1 µL sample is injected into the split/splitless inlet in splitless mode (splitless for 1 minute, split flow: 50mL/min) at 280°C. The temperature of the HRGC-LRMS interface is 250°C and at the end, the oven temperature program chosen started at 50°C for 1 minute, followed by an increase of 20°C/min to 310°C which is maintained for 6 minutes. The full scan electron impact data is obtained as follows: solvent delay 5 minutes, electron impact energy 70 eV, source temperature 200°C, emission current 150µA, scan rate 4scan/s, detector voltage 350V. Deuterated 3,4,5,6 d_4 di-ethylhexylphthalate (DEHP-d_4) is used as the internal standard and is added to the extract (10 µL at 5 µg/mL in *n*-hexane) just prior to the analysis. Single Ion Monitoring (SIM) detection was performed (m/z: 149, 153, 167, 279, 283).

The method was validated according to the AFNOR regulation XP T 90-210 [29]. The calibration range covers concentrations from 1-10 $\mu g.mL^{-1}$ for low concentrations and from 20-300 $\mu g.mL^{-1}$ for higher concentrations. The repeatability of the analysis procedure was good: 0.2% for DMP, 3.5% for DEP, 2.4% for DBP and 0.9% for DEHP. The reproducibility of the overall extraction - purification - analysis procedure was 11% for DMP, 18% for DEP, 8% for DBP and 4% for DEHP. Recovery yield was up to 85% for each compound. The limits of quantification for the phthalates studied were 10 $\mu g.kg^{-1}$ dry matter in soil and biosolids, and 40 $g.L^{-1}$ in biosolid filtrate. A blank extract was analysed after each batch of 10 samples in order to verify the absence of any contamination which could lead to quantification errors. Phthalates concentrations in the blank extract were always less than limit of quantification.

2.5. Statistical analysis

The results are expressed on a dry weight basis. Variance analysis of data and a Newman-Keuls multiple range tests at 0.05 probability level was performed (Statistical Software, Sigma Stat 2.00). The same letter in a column means that there is no significant difference at a probability equal to 0.05. On the other hand, a different letter means that there is a significant difference between control and treatments.

3 RESULTS AND DISCUSSION

4.1. Biomass production

Dry plant matter production was presented in Table 3. It can be seen that there is a difference between the average mass of the tomato plants in the pure substance experiments and those in the biosolids ones. This can be explained by the plant's stage of development. Sampling for the pure substance experiments was undertaken when the 3rd cluster of flowers had developed (after 60 days) whereas for the biosolids experiments sampling has been possible at the 5th cluster stage (100 days). Moreover, a difference in biomass production can be observed between aquiculture (pure substance and filtrate experiment) and soil cultures. Indeed, the use of nutrient solution allows a better production. In order to compare, a ratio between the treatments biomass production (g dry matter/pot) and the controls (g dry matter/pot) was calculated. The ratio between the average mass of the control plants and the average masses produced after introduction of the pure substances is quite the same and significantly equal to one. And the same is true for the biosolids experiments. We used pure substances, filtrate and three types of bio-

TABLE 3 - Biomass production (g dry matter / plant) and mass production ratio (no unit) in each experiment.

	Roots	Leaves	Fruits
Aquiculture - Pure substance experiment (sampling at the 3rd cluster of flowers)			
Control	13.20 ± 1.34 a	26.88 ± 1.43 a	3.77 ± 0.70 a
Treatment	9.02 ± 1.59 a	27.80 ± 3.46 a	4.80 ± 0.60 a
Ratio	0.7	1.0	1.6
Aquiculture - Biosolids A filtrate experiment (sampling at the 5th cluster of flowers)			
Control	19.10 ± 3.00 b	58.72 ± 7.19 b	77.80 ± 19.39 b
Treatment	31.33 ± 2.36 b	59.41 ± 8.10 b	72.00 ± 6.37 b
Ratio	1.5	0.9	0.9
Soil culture - Biosolid A experiment (sampling at the 5th cluster of flowers)			
Control	6.96 ± 0.00 c	96.24 ± 0.05 c	22.32 ± 0.50 c
Treatment	5.24 ± 0,44 c	88.60 ± 9.38 c	20.73 ± 1.20 c
Ratio	0.7	0.9	0.9
Soil culture - Biosolid B experiment (sampling at the 5th cluster of flowers)			
Control	5.22 ± 0.23 d	84.36 ± 7.27 de	18.89 ± 3.13 d
Treatment	4.56 ± 0.58 d	60.38 ± 2.96 d	21.06 ± 0.75 d
Ratio	0.9	0.7	1.1
Soil culture - Biosolid C experiment (sampling at the 5th cluster of flowers)			
Control	5.72 ± 0.37 e	88.44 ± 7.08 de	18.86 ± 4.48 e
Treatment	3.93 ± 0.41 e	74.46 ± 6.84 de	18.66 ± 3.98 e
Ratio	0.7	0.8	1.0

TABLE 4 - Phthalate levels in tomato plants in the experiment with pure substances, with sludge filtrate and ~~with~~ biosolids. Mean level in µg.kg^{-1} dry matter after harvest. Standard deviation corresponds to 10% (corresponding to cultivation and analytical steps).

	Roots		Leaves		Fruits	
	Control	Treatment	Control	Treatment	Control	Treatment
Aquiculture - Pure substances experiment						
DMP	<10 a	<10 a	42 b	50 b	<10 a	<10 a
DEP	<10 a	<10 a	2680 c	3279 d	<10 a	<10 a
DBP	99 e	995 f	30 b	50 b	<10 a	<10 a
DEHP	150 g	173238 h	105 I	269 j	<10 a	<10 a
Aquiculture - Sludge filtrate experiment						
DMP	<10 a	<10 a	<10 a	<10 a	<10 a	<10 a
DEP	<10 a	<10 a	<10 a	<10 a	<10 a	<10 a
DBP	<10 a	<10 a	<10 a	<10 a	<10 a	<10 a
DEHP	100 b	1350 c	98 b	234 d	<10 a	10 a
Soil culture - Biosolids A experiment						
DMP	<10 a	<10 a	<10 a	<10 a	<10 a	<10 a
DEP	<10 a	<10 a	<10 a	<10 a	<10 a	<10 a
DBP	<10 a	<10 a	<10 a	<10 a	<10 a	<10 a
DEHP	90 b	272 c	1643 d	3888 e	3931 e	5578 f
Soil culture - Biosolids B experiment						
DMP	<10 a	<10 a	<10 a	<10 a	<10 a	<10 a
DEP	<10 a	<10 a	<10 a	<10 a	<10 a	<10 a
DBP	<10 a	<10 a	<10 a	<10 a	<10 a	<10 a
DEHP	90 b	488 c	1643 d	10867 e	931 f	1854 g
Soil culture - Biosolids C experiment						
DMP	<10 a	<10 a	<10 a	<10 a	<10 a	<10 a
DEP	<10 a	<10 a	<10 a	<10 a	<10 a	<10 a
DBP	<10 a	<10 a	<10 a	<10 a	<10 a	<10 a
DEHP	90 b	503 c	1643 d	23334 e	3931 f	6513 g

solids at doses of up to 30 t.ha^{-1}, which is 10 times higher than the average sludge application rate in France. It is thus interesting to note that no negative effects on the growth of the plant were apparent.

4.2 Transfer of phthalates into tomato plants

4.2.1 Accumulation of phthalates in tomato plants

Table 4 presents the average levels of phthalates found in the roots, leaves and fruits of tomato plants in the control and treatment experiments. Substantial variation in the values was observed, ranging from non quantifiable (<LOQ) to 174 mg.kg^{-1}dry matter in roots, from non quantifiable (<LOQ) to 24 mg.kg^{-1}dry matter in leaves and from non quantifiable (<LOQ) to 6.5 mg.kg^{-1}dry matter in fruits. Concentrations were comparable with those observed in tomato [28], in leaves of barley grown in soils amended with sludge [20] and in vegetables in the Netherlands [30].

The aquiculture experiments with pure substances in the nutrient solution correspond to the maximising conditions for transfer into the plant. It appears to be practically no transfer of DMP, DEP, DBP and DEHP in tomato fruits (levels under limit of quantification). However, all compounds were detected in the leaves. DMP and DBP levels in the leaves were around 50 µg.kg^{-1} dry matter, whereas DEHP levels was around 270 µg.kg^{-1} dry matter and DEP level 3300 µg.kg^{-1} dry matter. An analysis of sap sampled had not shown presence of phthalates in sap (level < LOQ, 10 µg.L^{-1}). Moreover, a contamination of leaves from the control experiment was observed. Thus, leaves contamination could be explained by a loss of DEP and DEHP from nutrient solution through volatilisation (vapour pressure of DEP = 1.0 10^{-3} mm Hg and DEHP = 1.0 10^{-7} mm Hg) [31] or presence of other sources [32]. DBP and DEHP were found in large quantities in the roots (around 1 and 175 mg.kg^{-1} dry matter) that are in contact with the nutrient solution with added pure substances. A significant difference between control and treatment was shown. This result is in convenience with physico-chemical properties of these compounds whose Kow are higher than 4.5. In the same way, Gron et al. [17] found the greatest concentrations of phthalates in the roots of their plants (3850 ± 212 µg of DEHP per kg of carrot peel).

For aquiculture experiment with the filtrate, levels of DMP, DEP and DBP were below the quantification limits for all parts of the plant. This observation can be explained by the fact that the initial concentrations of these compounds in the filtrate were already very low. The DEHP, on the other hand, was found in roots (1.3 mg.kg^{-1}) and leaves (0.2 mg.kg^{-1}). There is a significant difference between treatments and controls. An analysis of sap sampled had shown presence of DEHP in sap. Thus, a DEHP translocation from roots to leaves seems to be a possible pathway. Several studies have also demonstrated a transfer from roots to plant [16, 19, 21]. As observed in the pure substance experiment, concentrations in roots were larger than leaves concentrations. Indeed, according to chemodynamic study of Russell and McDuffie [33], DEHP is strongly adsorbed on organic matter and is relatively immobile.

As expected levels of DMP, DEP and DBP in the soil culture experiments with biosolids were lower than the

limit of quantification in all parts of the plant, as it was in biosolids. DEHP was present in roots (around 0.3 to 0.5 mg.kg^{-1} dry matter), leaves (around 4 to 24 mg.kg^{-1} dry matter) and fruits (around 2 to 7 mg.kg^{-1} dry matter). A significant difference between control and treatment was observed. Moreover, DEHP has been detected in sap at the end of the experiment. Moreover, it seems that DEHP levels in plant were dependant with DEHP levels in biosolids: biosolids B experiment < biosolids A experiment < biosolids C experiment. This result is consistent with the work of Scheunert et al. [34]. Moreover, 74 till 94% of DEHP transferred were found preferentially in leaves. Leaves uptake of DEHP from sludge-amended soils is usually dominated by vegetative uptake of contaminated vapour from the surrounding air [17].

Distribution percentages of DEHP in tomato compartment were calculated according to equation (1).

$$\text{Distribution (\%)} = \frac{\text{mass of DEHP in a tomato compartment (µg)}}{\text{mass of DEHP into the 3 tomato compartments (µg)}} \text{ x } 100 \quad (1)$$

With:

Mass of DEHP in a compartment (µg) = DEHP concentration in the compartment (µg.kg^{-1} DM) x mass of the compartment (kg DM)

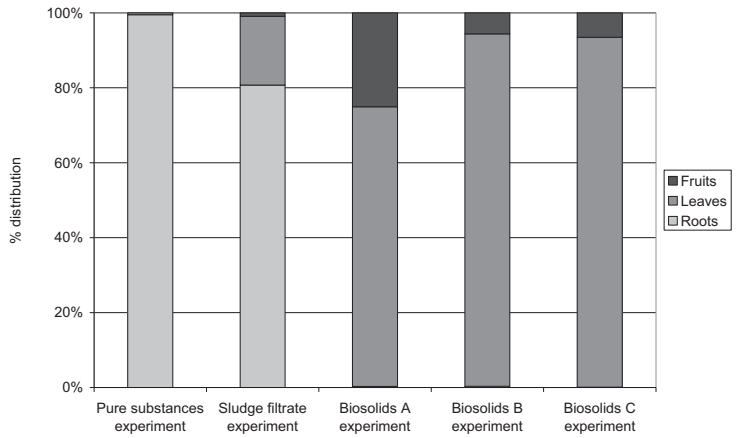


FIGURE 1 - DEHP distribution in roots, leaves and fruits of tomatoes as a function of the experiment type.

Distribution profiles in the different compartment, presented in Figure 1, clearly show a difference in the behaviour of DEHP. When introduced in the pure form, 99.5% of DEHP is present in the roots. However, in the biosolids form, it can be traced in all plant compartments. This difference could probably be explained by the presence of surfactant compounds in the biosolids, favouring the uptake of the DEHP by the plant roots. Measurements of laurylalkylbenzene sulfonates (LAS), anionic surfactants, were per-formed and were respectively 4400 mg.kg^{-1} DM in biosolids A, 1620 mg.kg^{-1} DM in biosoliods B, 13700 mg.kg^{-1} DM in biosolids C. These values are in the order of magnitude for LAS found in sludge (3 – 15 g.kg^{-1}). It seems that the greater the amount of is important, the greater the transfer taking place. This behaviour has been light up by Gunther and Pestemer [35] in pot experiments with carrots: pesticides were more easily available to plants when LAS was added.

4.2.2 Transfer percentages and bioconcentration factors of phthalates

Bioconcentration factor was expressed as the ratio of DEHP level in fruits on a dry weigh basis to the initial soil concentration (Table 5). Bioconcentration factors of DEHP in the fruits, leaves and roots ranged from 0.0007 to 1.67. Concerning biosolids-soil cultures, bioconcentration factors of DEHP in the roots were lower than those in the shoots in the same treatment, which may be partly attributed to the uptake of DEHP by the roots and then to be translocated to the shoots. It should be pointed out that the bioconcentration factors are equivalent to the present ones for radish [22].

Transfer percentages of DEHP into tomato compartments were calculated according to Equations (2-4). In

term of health risk assessment, it is interesting to see that less than 1% of DEHP initially introduced was transferred into tomato fruits, whatever the type of experimentation.

TABLE 5 - Percentage of transfer (R) and bioconcentration factors (BCF) of DEHP in tomato fruits

	Roots		Leaves		Fruits	
	BCF (-)	R (%)	BCF (-)	R (%)	BCF (-)	R (%)
Aquiculture - Pure substances experiment						
DEHP	0.02	1.86	0	0.01	0	0
Aquiculture - Sludge filtrate experiment						
DEHP	0.006	2.38	0.0007	0.54	0.0003	0.03
Soil culture - Biosolids A experiment						
DEHP	0.002	0.01	0.03	2.97	0.05	1.00
Soil culture - Biosolids B experiment						
DEHP	0.07	0.34	1.67	10.1	0.28	0.60
Soil culture - Biosolids C experiment						
DEHP	0.003	0.01	0.16	12.1	0.04	0.85

This observation is consistent with Aranda et al. [18] and Yin et al. [36] that revels that DEHP uptake in comestibles parts of plants was very low. It can therefore be concluded that sewage sludge application and sludge compost application at 30 $t.ha^{-1}$ in the short-term is unlikely to pose significant environmental risk.

$$m_{DEHPi} = (C_{DEHPis} \times m_s) + (C_{DEHPib} \times m_b) \quad (2)$$

$$m_{DEHPf} = (m_{DEHPt} - m_{DEHPc}) \quad (3)$$

$$R_{DEHP} = \frac{m_{DEHPf}}{m_{DEHPi}} \times 100 \quad (4)$$

With:
m_{DEHPi} : mass of DEHP in pots at the beginning of the experiment (µg)
m_{DEHPf} : mass of DEHP in tomato fruits at the end of the experiment (µg)
C_{DEHPis} : initial concentration of DEHP in soil ($\mu g.kg^{-1}$ DM)
C_{DEHPib} : initial concentration of DEHP in biosolids ($\mu g.kg^{-1}$ DM)
m_s : mass of soil (kg DM) equal to 10 kg DM
m_b : mass of biosolids (kg DM) equal to 0.1 kg DM
m_{DEHPt} : mass of DEHP in tomato fruits in treatments at the end of the experiment (µg)
m_{DEHPc} : mass of DEHP in tomato fruits in controls at the end of the experiment (µg)
R_{DEHP} : Percentage of transfer of DEHP in fruits (%)

4 CONCLUSION

This paper deals with a study of phthalate potential uptake in tomato plants (*Lycopersicon esculentum*) in greenhouse conditions, with the aim of biosolids agronomic recycling. Phthalate bioavailability (DMP, DEP, DBP, DEHP) was studied in aquiculture using two types of experiments: (1) pure substances experiment and (2) sewage sludge filtrate experiment. Soil experiments were also carried out testing three types of biosolids. Transfer of phthalates was followed into roots, leaves and fruits of the tomato plant. No significant difference in growth was observed between the tomato plants among experimentations. These results show that as far as phthalate transfer is concerned, DEHP should be prioritized. For the experiments using pure substances, the roots absorb greater amounts of DEHP (99,5%) and block their transfer to the above ground parts of the plant (0.5% in leaves and 0% in fruits). In the soil with biosolids experiments, DEHP was found and traced in all parts of the plant. The concentrations of DEHP in tomato plant were positively correlated with DEHP levels in the biosolids and rate application. Two transfer pathways were identified: (1) soil-to-root and subsequent root-to-shoot translocation (2) foliar uptake of vapour from surrounding air. Transfer percentages in tomato fruits (ranging from 0.03 to 1%) were low even in pot experiment that is known to exaggerate the bioavailability of contaminants compared to field conditions.

ACKNOWLEDGMENT

This work was supported by the French National Agency for Research and Technology and by Veolia Environment Research and Innovation. We particularly thank Violaine Brochier for her help.

REFERENCES

[1] Beck, A.J., Johnson, D.L. and Jones, K.C. (1996) The Form and Bioavailability of Non-Ionic Organic Chemicals in Sewage Sludge-Amended Agricultural Soils. The Science of the Total Environment, 185(1-3), 125-149.

[2] EU (2000) Working document on sludge. 3rd draft, 27 April 2000.

[3] Staples, C.A., Peterson, D.R., Parkerton, T.F. and Adams, W.J. (1997) The environmental fate of phthalate esters: a literature review. Chemosphere, 35, 667-749.

[4] Lovekamp-Swan, T. and Davis, B.J. (2003) Mechanisms of phthalate ester toxicity in the female reproductive system. Environmental Health Perspectives, 111, 139-145.

[5] Abad, E., Martinez, K., Planas, C., Palacios, O., Caixach, J. and Rivera, J. (2005) Priority pollutants assessment of sludges for agricultural purposes. Chemosphere, 61(9), 1358-1369.

[6] Beck, A.J., Alcock, R.E., Sewart, A.P., Wilson, S.C., Wang, S.M.-J., Wild, S.R. and Jones, K.C. (1995). Long-term persistence of organic chemicals in sewage sludge amended soils: a soil quality perspective. Advances in Agronomy, 55, 345-391.

[7] Alcock, R.E., Sweetman, A. and Jones, K.C. (1999) Assessment of organic contaminant fate in waste water treatment plants. I: Selected compounds and physicochemical properties. Chemosphere, 38, 2247-2262.

[8] Fromme, H., Kqchler, T., Otto, T., Pilz, K., Mqller, J. and Wenzel, A. (2002) Occurrence of phthalates and bisphenol A and F in the environment. Water Research, 36, 1429-1438.

[9] EU (2001) Organic contaminants in sewage sludge for agricultural use. Available on http://europa.eu.int/comm/environment/waste/sludge/index.html.

[10] Marttinen, S.K., Kettunen, R.H. and Rintala, J.A. (2003) Occurrence and removal of organic pollutants in sewages and landfill leachates. The Science of the Total Environment, 301, 1-12.

[11] Bagó, B., Martin, Y., Mejía, G., Broto-Puig, F., Díaz-Ferrero, J., Agut, M. and Comellas, L. (2005) Di-(2-ethyl)phtalate in sewage sludge and post-treated sludge: Quantitative determination by HRGC-MS and mass spectral characterization, Chemosphere, 59, 1191-1195.

[12] O'Conner, O.A., Rivera, M.D. and Young, L.Y. (1989) Toxicity and biodegradation of phthalic acid esters under methanogenic conditions. Environmental Toxicology Chemistry, 8, 569-576.

[13] Ejlertsson, J., Nilsson, M.-L., Kylin, H., Bergman, A. Karlson, L., Oquist, M. and Svensson, B.H. (1999) Anaerobic degradation of Nonylphenol mono- and diethoxylates in digester sludge, land filled municipal solid waste et land filled sludge. Environmental Science and Technology, 33, 301-306.

[14] Wang, J.L., Lui, P., Shi, H.C. and Qian, Y. (1997) Biodegradation of phtalic acid ester in soil by indigenous and introduced microorganisms. Chemosphere, 35(8), 1747-1754.

[15] Matsen, P.L., Thyme, J.B., Henriksen, K., Moldrup, P. and Roslev, P. (1999) Kinetics of di-(2-ethylhexyl)phthalates mineralisation in sludge-amended soil. Environmental Science and Technology, 33(15), 2601-2606.

[16] Herring, R. and Bering, C.L. (1988) Effects of phtalate esters on plant sedlings and reversal by a soil microorganism. Bulletin of Environmental Contamination and Toxicology, 40(4), 626-632.

[17] Gron, C., Laturnus, F. and Mortensen, G. (2000) Plant uptake of LAS and DEHP from biosolids-amended soil. *ACS Symposium Series,* 772, 99-110.

[18] Aranda, J.M.,. O'Connor, G.A and Eiceman, G.A. (1989) Effect of sewage sludge on di-(2-ethylhexyl)phtalate uptake by plants. Journal of Environmental Quality, 18, 45-50.

[19] Shea, P.J., Weber, J.B. and Overcash, M.R. (1982) Uptake and phytotoxicity of di-n-butylphtalate. Bulletin of Environmental Contamination and Toxicology, 29(2), 153-158.

[20] Petersen, S.O., Henriksen, K., Mortensen, G.K., Krogh, P.H., Brandt, K.K., Sørensen, J., Madsen, T., Petersen, J. and Gron, C. (2003) Recycling of sewage sludge and household compost to arable land: fate and effects of organic contaminants, and impact on soil fertility. Soil Tillage Research, 72, 139-152.

[21] Kirchmann, H. and Tengsved, A. (1991) Organic pollutants in sewage biosolids. 2. Analysis of barley grains grown on biosolids fertilized soil. Swedish Journal of Agricultural Research**,** 21(3), 115-119.

[22] Cai, Q.Y., Mo, C.H., Wu, Q.T. and Zeng, Q.Y. (2008) Abiotic association of phthalic acid esters with humic acid of a sludge landfill. Bioresource Technology, 99, 1830-1836.

[23] Sablayrolles C., Montréjaud-Vignoles M., Silvestre J. and Treilhou M. (2009) Trace Determination of Linear Alkylbenzene Sulfonates: Application in Artificially Polluted Soil-Carrots System, International Journal of Analytical Chemistry, 2009, 1-6.

[24] Sablayrolles, C., Vialle, C., Silvestre, J., Treilhou, M., Lhoutellier, C., Brochier V. and Montréjaud-Vignoles, M. (2011) PCBs uptake by carrots after sludge composts application: worst-case and operational practice in greenhouse conditions. Fresenius Environmental Bulletin, 20, 2141-2149.

[25] Morard, P. (1995) Les cultures végétales hors sol. Paul Sabatier Univsertity Eds.; Agricole Publisher: Agen, France.

[26] Afnor (2000) NF EN 12579 Soil improvers and growing media – Sampling (in French). Afnor Eds.; Publisher: Paris, France.

[27] Afnor (2001) NFU 44-095 Organic amendments – composts with agronomic matter from wastewater treatment plant (in french). Afnor Eds.; Publisher: Paris, France.

[28] Sablayrolles, C., Montrejaud-Vignoles, M., Benanou, D., Patria, L. and Treilhou, M. (2005) Development and validation of methods for the trace determination of phthalates in sludge and vegetables. Journal of Chromatography A, 1072, 233-242.

[29] Afnor (1999).XP T 90-210. Assessment protocol of a quantitative method for physico-chemical analyses (in french).

[30] Peijnenburg, W.J.G.M. and Struijs, J. (2006) Occurrence of phthalate esters in the environment of the Netherlands. Ecotoxicology and Environmental Safety, 63, 204-215.

[31] Teil, M.J., Blanchard, M. and Chevreuil, M. (2006) Atmospheric fate of phthalate esters in an urban area (Paris-France). Science of the Total Environment, 354, 212-223.

[32] Schroll, R. and Scheunert, I. (1992) A laboratory system to determine the uptake of organic chemicals from soil by plant roots and by leaves after vaporization. Chemosphere, 24(1), 97-108.

[33] Russell, D.J. and McDuffie, B. (1986) Chemodynamic properties of phthalate esters: partitioning and soil migration. Chemosphere, 15, 1003-1021.

[34] Scheunert, I., Attar, A. and Zelles, L. (1995) Ecotoxicological effects of soil-bound pentachlorophenol residues on the microflora of soils. Chemosphere, 30, 1995-2009.

[35] Günther, P. and Pestemer, W. (1992) Phytotoxicity of surfactants to higher Plants.-in: Hall, J.E., Sauerbeck, D.R. & P. L'Hermite, 103-111.

[36] Yin, R., Lin, X.G., Wang, S.G. and Zhang, H.Y. (2003) Effect of DBP/DEHP in vegetable planted soil on the quality of capsicum fruit. Chemosphere, 50, 801-805.

Received: June 20, 2012
Revised: September 28, 2012
Accepted: October 04, 2012

CORRESPONDING AUTHOR

Caroline Sablayrolles
Université de Toulouse; INP
LCA (Laboratoire de Chimie Agro-Industrielle)
ENSIACET
4 Allées Emile Monso
31 029 Toulouse
FRANCE

Phone : +33 5 34 32 35 51
Fax : +33 5 34 32 35 97
E-mail: Caroline.Sablayrolles@ensiacet.fr

FEB/ Vol 22/ No 4a/ 2013 – pages 1061 – 1069